THE FUNDAMENTAL ROLE OF SAMPLING AND SEPARATION SCIENCES IN THE DEFINITION OF MULTITROPHIC PHENOMENA

38th International Symposium on Capillary Chromatography

and

11th GCxGC Symposium

Chairman
Prof. L. Mondello

Palazzo dei Congressi,
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ABSTRACT BOOK

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LECTURES ABSTRACTS
11th GCxGC

THE ADVANCEMENT OF GCXGC OF THE U.S. COAST GUARD ACADEMY

Glenn Frysinger, Richard B. Gaines

U.S. Coast Guard Academy, Mohegan Ave, 06320 New London, United States

The Advancement of Comprehensive Two-Dimensional Gas Chromatography (GCxGC) at the U.S. Coast Guard Academy was enabled by several key factors. These include: recognizing early the immense potential of GCxGC separations, entering a burgeoning field with many challenges and opportunities, establishing a proper balance between instrument technology development and chemical applications, encouragement and inclusion given by established separation scientists, rewarding collaborations with scientists facing “impossible” chemical separation challenges, timely funding, and an equal and supportive alliance between scientists Rick Gaines and Glenn Frysinger. The presentation will describe the challenges and successes of early GCxGC thermal rotating and cold jet modulators, GCxGC firsts at CGA including mass spectrometric detection and analysis of high boiling point compounds, and notable GCxGC contributions in the fields of oil spill and arson forensics, geochemistry, and environmental chemistry.

MOLECULAR CONNECTIVITY IN GCXGC – DEVELOPMENT OF A NOVEL STRUCTURAL ELUCIDATION TOOL

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GCxGC separations produce structured chromatograms that are characterized by the “roof-tile” effect in which compounds that share similar structural features are aligned in discernable patterns. The structured nature of GCxGC separations has been well exploited in group-type analyses (i.e. in the petroleum industry), where the knowledge of a key number of standards is used to define the rest of the analytes in their respective groups. In compound discovery applications, however, there is a need to identify the maximum number of compounds in the chromatogram in order to ascertain the presence of new or previously unidentified compounds. The primary challenge in these applications is the limited number of standards that are available for the confirmation of the many additional compounds (typically 500 to 1000 analytes) that are present in the GCxGC separations, particularly for molecules at trace level concentrations in the sample.

In this project the development of a new strategy for the identification of new analytes is presented. This method is based on the idea that the bi-dimensional separation plane in GCxGC chromatograms has the potential to be used as a structural information spectrum in which the molecular connectivity between compounds are correlated to a quantifiable “chemical retention shift” once the retention data has been normalized. A presentation of this concept will be given for a set of known standards present in the Century Mix that we have synthesized in our laboratory, and the implications for the use of GCxGC as a structure elucidation tool will also be discussed with the use of real samples analyzed by GCxGC/TOF MS.

Le.03

GCXGC IS NOT PERFECT YET (BUT IT CAN GET BETTER!)

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In the over 20 years since its invention, comprehensive two-dimensional gas chromatography (GCxGC) has firmly established itself as the most powerful chromatographic technique for the separation of very complex mixtures. The technique uses two columns of different selectivities joined in series through a special interface called a modulator. Analytes eluting from the first column are trapped or sampled by the modulator, then injected into the second column at regular intervals. Of the many approaches to modulation proposed over the years, thermal and flow modulation are emerging as the leading technologies. Commercial GCxGC systems equipped with such modulators are available form a growing number of vendors. The technique itself found numerous applications in many areas, including petroleum, environmental, biological and food and fragrance analysis.

In spite of the seemingly mature status of the technology, the adoption rate of GCxGC is not as rapid as its pioneers envisioned. Potential users are often discouraged by the high costs and the perceived complexity of the technology. In fact, mutual dependence of many parameters often makes the optimization of the separation conditions a daunting task. A change to a single parameter like carrier gas flow rate or the temperature programming rate might lead in some systems to unexpected problems related to e.g. to modulator breakthrough or peak wraparound caused by different elution temperature. In addition, the separation results themselves are often far from optimal, with peaks tailing in both dimensions and/or retention times shift from run to run. These problems might make the interpretation of the huge data sets that GCxGC can create in a short time very difficult. Chemometric techniques are particularly sensitive to sub-optimal separation results. As a result, pre-processing steps like chromatogram alignment and baseline subtraction are now considered mandatory when comparing multiple data sets.

In our opinion, rather than correcting poor GCxGC separation outcomes, we should focus on simplifying the technique and improving the separations themselves. To this end, we have developed several types of single stage modulators, which reduce the complexity of the system and make the optimization much easier. Retention time reproducibility can be greatly improved by using thermal rather than cryogenic modulation, potentially eliminating the need for chromatogram alignment before chemometric analysis. We have also investigated the sources of peak tailing for some analytes and found among others that injector backflushing can significantly reduce the severity of the problem in some cases.

Le.04

A CRITICAL ASSESSMENT OF THE CURRENT STATUS AND POTENTIAL FUTURE OF GC×GC

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Comprehensive multidimensional gas chromatography (CMDGC or GC×GC) is probably the most promising invention in GC since discovery of capillary columns more than half a century ago [1]. The approach has the potential to provide considerably more sample information in the same timeframe as single dimension GC analyses.

GC×GC has not been as widely adopted as the proponents had hoped; despite active research spanning more than 20 years after its invention and over a decade of commercially available equipment and software. There is little or no investment in GC×GC by most major GC manufacturers, and some of the smaller companies have recently reduced their investment. Why is that? There have been few or no newsworthy methods that have been identified that GC×GC can do significantly better than traditional techniques (significant enough to warrant the cost of implementation). Is there one limiting flaw of the technique or many? What is the probability that the flaws can be overcome?

The current state of GC×GC will be critically reviewed, with attention paid to market forces as well as technical aspects. Strengths and weaknesses of current modulation techniques will be discussed as well as software. Elements leading to successful commercialization of new technology will be contrasted to several familiar failed attempts. Limiting aspects of the technique as it is practiced today will be ranked with speculation on potential approaches to their improvements.

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Le.05

THEORETICAL AND PRACTICAL APPROACHES FOR PARAMETER SELECTION IN COMPREHENSIVE TWO-DIMENSIONAL GC

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Comprehensive two-dimensional gas chromatography (GCxGC) is nowadays the technique of choice for the analysis of complex mixtures. In GCxGC narrow time fractions of the effluent from the first dimension column are separated on a short and fast second column. With optimization already being complex in regular, one dimensional GC, optimization in comprehensive GCxGC gets progressively more difficult. This complexity comes from two sides:
1.There are many *input* parameters ('instrument settings') that have to be optimized,
2.There are many *result* parameters (output quality descriptors e.g. peak capacities, time, sensitivity etc.) that are affected.
In practice the above means that first the operator has to decide which quality parameters are the most relevant (e.g time and peak capacity are relevant but sensitivity is not), and then the instrument settings have to be set such that optimum performance is achieved for the relevant characteristics. In practice often a compromise will have to be made: conditions that result in optimum time, for example, are not the same as those required for e.g. maximum peak capacity. In the lecture strategies for the prediction of GCxGC retention times and peak widths will be discussed. Initially this will be strategies for isothermal operation, which will then be extended to temperature programmed operation. Pareto strategies will be applied to find the preferred conditions in situations where multiple, sometimes conflicting requirements, need to be met. The theories developed will be applied to the optimization of typical comprehensive GCxGC applications such as the analysis of fatty acid methyl esters derived from lipids, mineral oil fractions and arson materials.

Le.06

MULTI-MULTI-DIMENSIONAL GAS CHROMATOGRAPHY

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We recently introduced a new contra-directional modulation approach that enables multiplexed GC×GC separations. In such a multiplexed GC×GC experiment, two concurrent GC×GC chromatograms are produced using one detector and a single injection. We demonstrated the GC×2GC experiment for a range of complex mixtures and later modified the column assembly to permit 2GC×GC experiments. Comparison of 2GC×GC and GC×2GC will highlight the advantages and disadvantages of each methodology.
The primary motivation of our studies has been to permit concurrent acquisition of two GC×GC separations, using different stationary phases using a single mass spectrometer. To this end, we shall share recent results of 2GC-MS and 2GC×2GC-MS using contra-directional modulation, first by illustrating how multiplexed gas chromatography – mass spectrometry in combination with multiple linear retention indices provides information rich analytical results for convenient and powerful chemical characterisation of essential oils and related samples. Finally we will propose that contra-directional modulation permits ultra-dimensional separations – by demonstrating concurrent acquisition of four orthogonal retention times (or indices) in a single run.

IDENTIFICATION OF METABOLIC BIOMARKERS FOR TRAUMATIC BRAIN INJURY BY GC(xGC)TOFMS

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Traumatic brain injury (TBI) is a common cause of neurological damage and disability and it is a major cause of death and disability worldwide, especially in children and young adults. Recently, biomarkers for cellular brain injury both in blood and in CSF have been intensively searched for on order to both improve the diagnosis and the evaluation of outcome. In this study, metabolic profiles in blood for TBI patients were studied in order to find blood-based metabolic biomarkers for TBI. The study included mild, moderate and severe cases of TBI, and the samples were obtained directly after the accident, followed by daily samples for the following 7 days. Plasma samples were analyzed with comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry and GC-HRTOFMS was used for the further identification of unknown metabolites.

The metabolic profiles between the cases and controls, as well as between severe, moderate and mild TBIs showed substantial differences. The results showed also that the metabolic patterns could aid in estimating the true severity of the TBI. In addition, the metabolic biomarkers found to be associated with TBI were also strongly associated with the worse clinical outcomes. The metabolic patterns were unique to the TBI, and no strong correlation with other injuries could be observed.

Le.08

RIPENING-DEPENDENT VOLATILE PROFILING OF PINEAPPLE (ANANAS COMOSUS (L.) MERR.) FRUIT BY GC×GC-QMS AND CHEMOMETRIC DATA ANALYSIS

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Pineapples are highly valued tropical fruits appreciated by many consumers due to their exotic aroma impression. However, fully ripe fruits are exclusively available as a premium niche product, as they are to be exported by rapid cargo-planes. In contrast, the major proportion of pineapples offered on European markets is harvested at a premature green-ripe stage, since their mechanical and microbial stability allows cheaper fruit logistics by cargo ships. Consequently, sea-freighted fruits need to be post-harvest ripened for flavour development; however, differ in their volatile “profiles” compared to fruits harvested at full maturity [1].

In the presented study, HS-SPME-GC×GC-qMS profiles of four progressing post-harvest maturity stages of green-ripe pineapples were compared to fully ripe air-freighted fruits. The majority of more than 300 compounds identified were esters. The structured separation space obtained by GC×GC with clustering of compound classes as well as extracted ion contour plots of unique mass traces facilitated the identification of volatiles even though mass spectral reference data was unavailable.

Profile patterns presented in the contour plots were subsequently analyzed in an unbiased fingerprinting approach applying image processing techniques and chemometric data analysis [2]. Based on significantly different signals, Hierarchical Cluster Analysis (HCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were performed to explore interdependencies within the volatile “profiles” and discriminate the different maturity stages, respectively. Furthermore, Partial Least Squares (PLS) regression was shown to be an excellent tool to deduce those volatiles being formed during post-harvest storage. As a result of the fingerprint analysis clear-cut discrimination of differently ripened and post-harvest handled pineapple samples was possible.

The HS-SPME-GC×GC-qMS analysis of volatiles in combination with multivariate statistical data evaluation allowed the distinction between different pineapple maturity stages, hence being an excellent method to rapidly screen for metabolic changes occurring during post-harvest shelf-life of pineapple and eventually other fruits.

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Le.09

POSTMORTEM MEASUREMENT OF CADAVERIC VOCs IN HUMAN INTERNAL CAVITY GASES

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In search for minimally invasive alternatives to post-mortem autopsy, forensic radiology became a very promising approach for medico-legal investigation. Post-Mortem Computed Tomography (PMCT) is an example of a medical imaging technique that allows to access internal body parts without compromising its physical integrity [1]. PMCT is especially of prime interest for the detection of gases inside a body. On one hand, it allows to vizualize gases that can be linked to the cause of the death such as air embolism or scuba-diving accident, on the other hand, thanatology is a field of great interest to better understand postmortem putrefactive phenomena. It was recently evidenced that the appearance of postmortem gases had a specific distribution scheme [2]. Despite the fact that intestinal zones were thought to be the initial area of alteration process due to bacteriological actions and development of intestinal microflora [3], it was identified that putrefactive biological processes were starting in the hepatic and cardiac zones. Abdominal thoracic, and heart cavities were further identified as the main sites of early putrefactive processes. A better understanding of the composition of gaseous samples present in cavities of altered bodies could possibly appear to be essential in the comprehension of postmortem alteration processes and in contributing to elucidations of causes of death.
Based on our recent results of using GC×GC-TOFMS for the characterization of cadaveric decomposition VOCs [4], we investigated human postmortem gases sampled in cardiac and abdominal cavities of altered bodies sampled under PMCT supervision to target the aeric region to be punctured [5].

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MICROFABRICATED REACTORS FOR VOLATILES ANALYSIS BY GC×GCC-IRMS

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Conventional gas chromatography-combustion-isotope ratio mass spectrometry (GCC-IRMS) employs a combustion reactor in the flow path after the GC and prior to the IRMS. Our analysis of a popular commercial system shows that peaks of minimal full width at half-height (FWHH) = 1 second is limited by band broadening due to multiple changes in diameter within the flow path. GC×GCC-IRMS for high precision isotopic analysis imposes much more stringent demands because of the requirement for peak widths below 300 ms to maintain high resolution. We previously reported a prototype instrument using conventional capillaries that minimally degrade chromatographic resolution but are fragile when heated to the combustion temperature of 950°C [1]. Here we report on a microfabricated microreactor (MFMR) employing channels etched into fused silica. Two matching blank fused silica plates are coated with a photomask, exposed and developed, and etched, and once completed are mated and permanently fused. The flow channel is flared at the input and output to accept conventional capillaries that direct flow from the chromatography column into the reactor, and accept flow from the channel and direct it to the next stage. The flared ports are sealed with one of various sealants which maintain themselves leakfree to at least 70°C which they maintain when the reactor is heated, and which enables analysis of volatiles. Potential and limitations of this system will be discussed.

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CHARACTERIZING THE VOLATILES OF AGED ARTISANAL CHEDDAR CHEESE USING GCxGC/TOF MS

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There is a wealth of information in the volatile organic compounds (VOCs) in foods, which can be harnessed using a wide variety of analytical techniques for applications ranging from aroma characterization to food safety monitoring. In applying a rapid-analysis mass spectral method to the VOCs of Cheddar cheeses, we observed that the volatiles can be used to descriptively and predictively characterize artisanal Cheddars by their age, and can be used to identify batch-to-batch differences that are inherent to artisanal food production. In this work we have used comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC× GC/TOFMS) to putatively identify the characteristic volatiles of artisanal Cheddars aged 1, 2, or 3 years. The VOCs of Cheddars from a single cheesemaker were extracted using a tri-phase DVB/Carboxen/PDMS SPME fiber. GCxGC/TOF MS analysis revealed the presence of over 1000 peaks in each sample at the 50:1 SNR threshold, and a peak filtering protocol was developed to select the most relevant analytes to be used in comparative studies. The filtering process resulted in the selection of 235 compounds that included acids, esters, ketones, aldehydes, alcohols, hydrocarbons, and sulphur compounds. Relevant criteria in the peak filtering process that are of potential use in other GCxGC applications will be discussed, as well as issues related to the monitoring of individual analytes in the cheese profiles over the 3-year maturation process.

A LIBRARY OF THERMODYNAMIC DATA FOR GC AND GC×GC – PROGRESS AND OPPORTUNITIES

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There are many reasons why one would want to be able to predict retention times in GC(×GC) separations. Simulation of a separation in silico would mean that a researcher facing a new problem could describe the requirements of the separation and nature of the sample in a computer and go home while the computer optimized the separation conditions. This would save time, materials, and money for the laboratory. Simulations of separations would also permit the use of retention time(s) in GC(×GC) to aid in the identification of unknown molecules. This would add a layer of complementary information to MS information and lead to improved MS search algorithms.

In our group, we have been studying retention time prediction via modeling of the thermodynamics of a separation. Using our approaches, predictions of unprecedented accuracy have been possible (e.g.: errors of 100-500 ms in >40 min). Until recently we have been held back by two factors. The first is Time. Using traditional approaches, data could be collected at a rate of about 20 compounds per week, even with automation. The second challenge was accounting for variability between columns during manufacturing. These two problems combined meant that it would take an unreasonable amount of time to acquire useful data, and the data could only be used on a single column. This pushed the study of thermodynamics of GC into the area for oddball academics doing things with little practical use.

Using our new approaches, we are bringing thermodynamic modeling back into the realm of useful techniques. We can now obtain predictions of thermodynamic data at a rate of ~500 or more compounds per week, with higher collection rates on the horizon. We can also translate data from one column to another with little loss in accuracy. Finally, we can also predict the behavior of new molecules not in our library using QSPR approaches. A thermodynamic library and toolkit for GC and GC×GC are now closer than ever before.

Le.13

FLOW-MODULATED GC×LOW-PRESSURE-GC: A NOVEL APPROACH FOR HIGH RESOLUTION/SENSITIVITY GC SEPARATIONS

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The present contribution is focused on the exploitation of a mega-bore column (*i.e.*,10 m ×0.53 mm ID), located in the second oven of a flow-modulation (FM) comprehensive 2D GC system, operated under vacuum outlet conditions (approach herein defined as GC×low-pressure-GC). The generation of low-pressure conditions, across a mega-bore column, is an ideal situation for fast and very fast GC high-resolution second-dimension separations. Furthermore, the enhanced sample capacity of such capillaries enables a further increase in analytical sensitivity. The intra-column low pressure conditions were created by using mass spectrometry. With regards to the flow modulation conditions, it will be shown that through fine optimization, it is possible to work under MS-compatible flow conditions (*i.e.*, 5-8 mL/min), and hence 100% of the second-column effluent can be introduced into the ion source. Moreover, the appropriate choice of the accumulation loop is vital for the attainment of satisfactory peaks shapes. A series of FM GC×low-pressure-GC-MS applications will be shown in which the potential of the approach are demonstrated.

Le.14

STRUCTURAL TUNING OF IONIC LIQUIDS AND POLYMERIC IONIC LIQUIDS FOR APPLICATIONS IN COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY AND SAMPLE PREPARATION

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Ionic liquids (ILs) are a unique class of non-molecular solvents whose versatility allows them to be incorporated into various aspects of chemical analysis. ILs possess negligible vapor pressures at room temperature, have a wide range of viscosities, can be custom-synthesized to be miscible or immiscible with water and organic solvents, often have high thermal stability, and are capable of undergoing multiple solvation interactions with many types of molecules. In addition, ILs can be structurally tuned to modulate desired physio-chemical properties while retaining their unique solvation capabilities. This talk will discuss the use of ILs in sample preparation and comprehenisve two-dimensional gas chromatography. The use of ILs and polymeric ionic liquids (PILs) will be described for improving the selectivity, sensitivity, and reproducibility when used as sorbent coatings in solid-phase microextraction (SPME) and as extraction solvents in dispersive liquid-liquid microextraction (DLLME). Secondly, it will shown that the unique cation/anion combination of ILs gives rise to unique chromatographic selectivity when IL-based stationary phases are employed as the second dimension column in GCxGC separations. Using the solvation parameter model, the chemical structures of ILs can be effectively tuned to design stationary phases that exhibit high thermal stability, high selectivity, and high resolving power for complex samples such as kerosene.

TOWARD A MICROFABRICATED COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPH (μGC x μGC)

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Progress toward the development of a μGCxμGC system is described, including rapid, comprehensive two-dimensional gas chromatographic separations with microfabricated first- and second-dimension μcolumns and a microfabricated mid-point thermal modulator (μTM). Our two-stage μTM chip (0.8 cm^2) consists of two interconnected spiral etched-Si microchannels (4.2 and 2.8 cm long; 250×140 μm cross section), an anodically bonded Pyrex cap, and a 0.3-μmm-thick cross-linked wall coating of PDMS. Integrated heaters provide rapid, sequential heating of each μTM stage at > 2000 °C/s, while a proximate, underlying thermoelectric cooler provides continual cooling without consumable materials. We used a series of two etched-Si μcolumns (3 x 3 cm chips; 250×140 μm c. s.; 3-m length per chip) wall-coated with PDMS as the first dimension, and a single etched-Si μcolumn (1.2 x 1.2 cm chip; 150x50 μm c. s.; 0.5-m length) with a room-temperature ionic liquid (RTIL) or crosslinked poly (trifluoropropylmethylsilicone) (OV-215) stationary phase as the second dimension. Using the RTIL μcolumn, a C_7-C_{10} alkane separation gave modulation numbers between 2 and 5, and fwhm values of 150-680 ms among the analytes. Although a structured 17-component μGC× μGC chromatogram was generated using the RTIL μcolumn, resolution was poor in the second dimension because the RTIL was too retentive. Using the OV-215 μcolumn instead gave much better results, and a well-resolved separation of a 36-component mixture spanning a vapor pressure range of 3 orders of magnitude was generated. Current work is focused on increasing the temperature programming range using on-board μTM and μcolumn heaters. We are also exploring the use of two microsensor technologies as detectors, and early results show surprisingly rapid responses that may be suitable for use in a portable μGCxμGC system.

Le.16

INSIGHTS ON ORTHOGONALITY IN GC×GC

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The term orthogonality in GC×GC is generally defined as employing two columns that have independent mechanisms of separation (towards sample components). The two columns - dimensions - provide a two-dimensional separation. When combined with mass spectrometry, a three dimensional technique can be described.

GC and MS are easy to consider as providing orthogonal dimensions - separation in ^{1}D and mass spectral fragmentation in ^{2}D. For two columns, orthogonality is more difficult to either prove or conceptually define. A GC column only provides molecular retention based on the energy of interaction between a molecule in the gas phase and stationary phase, a measure of interaction captured in the K value - the distribution coefficient; $K = k\beta = C_S/C_M$. To have a truly orthogonal separation we might consider that a compound could have a K value between $0 < {}^2K < 1$, but also that the compound can display an independent value on the second column; $0 < {}^2K < 1$. Of course there are practical limits to these K values. Since K is related to retention, just by knowing the ^1t_R value, we cannot automatically predict its ^2t_R value. Knowledge of a compound's chemistry will provide some idea of its extent of retention on a given ^{2}D column. But orthogonality presupposes that any compound of unknown identity will have a non-predictable retention on ^{2}D.

GC×GC largely can exhibit very satisfying separations with apparently 'little correlation' using well-designed column sets and experiments. We only have access to the chemical nature of the GC stationary phase – interpreted according to its 'polarity' – and potentially to any specific molecular interactions such as shape or chiral inclusion, etc. We normally employ a polar/non-polar, or a non-polar/polar phase combination. Here, we will explore selected studies, insights and considerations of separations, to progress the understanding of orthogonality

ACCURATE ANALYSIS OF PAH, PASH AND THEIR ALKYLATED HOMOLOGUES BY SPECTRAL DECONVOLUTION OF GC/MS AND GCxGC/MS DATA

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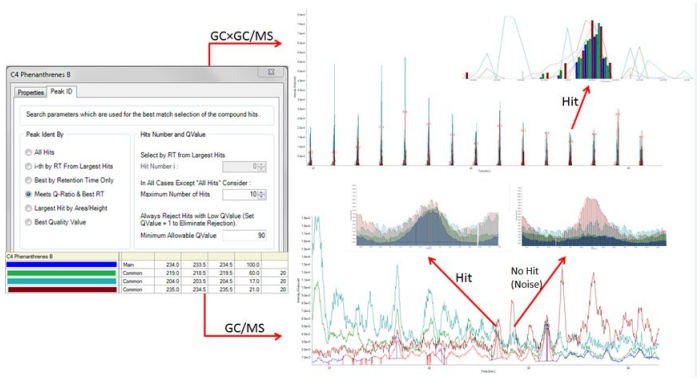
Accurate measurement of alkylated polycyclic aromatic hydrocarbons (PAH) and polycyclic aromatic sulfur heterocycles (PASH) in complex mixtures is difficult to obtain. For example, in a recent interlaboratory study conducted by the U.S. National Institute of Standards and Testing, the RSD was > 50% (n=36) for mean homologue concentrations. An extensive review of the alkylated literature, both peer-reviewed and independent agencies (U.S. EPA, ASTM, and NOAA), as well as commercial lab SOPs rely, for the most part, on GC/MS in SIM mode to detect the molecular ion (GC/SIM 1-ion detection) of C1 to C4 PAH and PASH to determine concentration. We showed, based on elucidating the electron impact fragmentation mechanisms of substituted isomers, multiple fragmentation patterns per homologue (MFPPH) are needed to correctly identify family members. Clean spectra and each homologue's retention window were obtained using automated sequential GC-GC/MS-PFPD (sulfur-specific detector) to analyze standards as well as fresh and weathered crude oils and coal tars. From this information, new GC/MS and GCxGC/MS methods have been developed that use three or more ions/alkylated PAH and as many fragmentation patterns per homologue (MFPPH) as needed to quantify PAH and PASH correctly. Comparison of GC/MFPPH and GC/1-ion analyses reveal concentrations can differ by 1000's %, with results dependent on analyst's ability to recognize homologue-specific peak patterns if the molecular ion is the sole means of allocating family membership. Spectral deconvolution of MFPPH ions eliminates analyst dependency on retention windows and peak pattern method-assignments increasing measurement precision and accuracy. SIM detection of MFPPH ions yields detection limits no different than SIM detection of each homologue's molecular ion, producing statistically the same sensitivity without sacrificing selectivity. Illustrative examples will be presented documenting these measurement attributes. The new spectral deconvolution software eliminates analyst bias, while increasing method performance.



Le.18

USE OF GCxGC-MS AND GCxGC-HRMS TO CHARACTERIZE EFFLUENTS FROM SMALL AND LARGE SCALE SEWAGE TREATMENT FACILITIES AND IDENTIFY EMERGING CONTAMINANTS

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In the modern society, large quantities of chemical substances are used, and more than 30 000 compounds are estimated to be in daily use in Europe, of which many will reach the municipal sewage treatment plants (STPs). STPs can therefore be considered as significant secondary sources for legacy and emerging contaminants to surface water. The treatment technology for large scale facilities do not differ much whilst small scale on-site sewage facilities (OSSFs) can differ greatly in design and removal efficiency. It is therefore essential to characterize effluent from facilitates of various types, identify compounds poorly removed, characterize their physico-chemical properties, and use this information to improve the treatment process. This is a challenging task that only can be solved by combining powerful analytical techniques and systematic data interpretation. Influent and effluent samples were collected and analyzed by Leco GCxGC-TOF-MS. A combined influent water sample was used to create a template for the GC×GC peak detection and data evaluation. The automatic baseline, peak find, peak and spectra deconvolution, and library search functions were applied, and the resulting peak table was used as a reference sample. The remaining samples were automatically processed to detect and integrate peaks associated with the components present in the template. Classification was performed using principal component analysis. An attempt was also made to identify compounds that were poorly removed by the STP processes. Poorly removed compounds that passed strict spectra quality cutoffs and a manual inspection were tentatively identified. The validity of these assignments were tested using a prototype Leco GC×GC high-resolution TOFMS. It was operated using very similar chromatographic conditions as the Pegaus 4D system, but at a higher resolution (> 25,000). The agreement was good between expected and observed ions (< 1ppm for intense ions,

AN INVESTIGATION OF THE VOLATILE CONSTITUENTS OF AFRICAN ORCHID SPECIES BY COMPREHENSIVE GAS CHROMATOGRAPHY - TIME OF FLIGHT MASS SPECTROMETRY

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The scent of orchids is the result of various selective pressures to attract the most efficient pollinators in the habitat [1,2]. This study has looked at a number of African angraecoid (Angraecinae) orchids of different taxa from different habitats, which are pollinated by hawkmoths, as well as taxa from other groups (Aerangidae, Eulophiinae and Polystachyinae) in similar habitats, with the aim of comparing the different volatile components and relating these variations to taxonomical, pollinator and habitat differences [3]. Samples were investigated using solid phase micro-extraction (SPME) of the headspace above the flowers, which had been heated at 40C for 30 minutes. The analysis was performed using comprehensive gas chromatography - time of flight mass spectrometry (GCxGC-TOFMS). Compound identification was achieved using library matching and by comparison of retention index (RI) with literature values. Sample analysis was repeated using gas chromatography - high resolution time of flight mass spectrometry (GC-HRT), and the accurate mass values obtained (routinely within 1 ppm of calculated formulae values) were used as a confirmation of the proposed structural formulae. To date the volatile components of only a few African orchids have been studied. This study presents data for components for a number of taxa, some of which are utilised for medicinal purposes [4]. The utility of 2-dimensional chromatograms of volatile components in classifying orchid species will be discussed, and the influence of habitat and pollinator differences on the volatiles will be illustrated in volatile maps of the different orchid taxa.

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Le.20

ANALYSIS OF TMS-SUGARS AND SUGAR DERIVATIVES IN THE WATER EXTRACT OF PYROLYSIS OILS BY GC×GC.

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During pyrolysis of biomass many organic components are formed. Both, hydrophobic and hydrophylic chemicals make up the composition of the pyrolysis oil. To characterize the volatile part of the pyrolyis oils a GC×GC analysis is routinely applied [1]. The non-volatile part of mainly sugar derivatives, can be extracted by water and next analyzed by HPLC. In this study the water extracted components, after drying, were derivatized by silylation and next analyzed by GC-MS. Complex chromatograms were obtained with many co-eluting compounds from the tri-methyl silylated (TMS) derivatives. Therefore, a GC×GC analysis was developed to create more resolution and structured information of the many chemical components. Our recently proposed experimental technique to predict and simulate the contour plot of a GC×GC column set [2] was used to find the appropriate column set for this application. The method will be explained and the results of the structured composition analysis will be shown.

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Le.21

DETERMINATION OF OCCURRENCE AND FATE OF EMERGING CONTAMINANTS THROUGH WASTE-WATER MONITORING USING GCxGC-TOFMS

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The objective of this research is to develop an analytical strategy to determine emerging compounds of concern. Wastewater samples obtained from the Pennsylvania State University wastewater treatment facility (WWTF) will be used as a control facility to refine analytical methodology. Rather than beginning with a target compound approach, a discovery analysis approach was chosen to try and determine as many compounds as possible prior to any compound list restriction. The difficulty in this approach can be the resulting complexity of the analysis. Both Comprehensive Gas Chromatography coupled with Time-Of-Flight Mass Spectrometry (GC x GC-TOFMS) analysis and Ultrahigh Performance Liquid Chromatography coupled with Time-Of-Flight Mass Spectrometry (UHPLC-TOFMS) analysis will be utilized for their inherent ability to characterize these potentially complex samples more successfully compared to other possible techniques. The ultimate goal is to determine emerging contaminants and define temporal and spatial characteristics of occurrence. Equally challenging is the need to develop and define what is to be considered “normal” so that this background can be subtracted from subsequent samples in order to develop an approach that is capable of determining when an “outlier” is detected. This presentation will address how these large data sets can be reduced to allow for more easy determination of what are outliers.
Multiple four-liter samples were gathered from stages throughout the Penn State WWTF. Samples were prepared following USEPA method 3510c, a liquid-liquid extraction. Also, soil samples were collected from the living filter (final discharge for the Penn State WWTF effluent) and extracted following USEPA 3550c, a sonification method, in order to help define environmental fate of outliers found in the treated waters. Sample extracts were introduced to the analytical systems, to identify and quantify the detected compounds

GCxGC WITH TOF-MS, FID AND SCD DETECTION FOR ANALYSIS OF PRODUCTS DURING OPTIMISATION OF DESULPHURISATION CATALYSTS USED IN CLEAN FUEL PROCESSES

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South Africa, although leading in many aspects of fuel quality improvement in Africa, is lagging major developed countries in Europe and the, USA, with fuel quality still being regulated by the Euro II fuel standard. The Clean Fuels 2 act, that will be implemented in South Africa in 2017 with bring South Africa in line with the Euro 5 fuel standard. This act is centered on the chemical constituents and elements of fuel that have an adverse effect on health and environment and will allow the application of new emission control technologies for modern vehicles. South African refineries are expected to upgrade their facilities to produce cleaner fuels. The upgrading comes at tremendous financial costs and it is essential to reduce costs as far as possible in order to minimize the cost impact on the consumer. Investments are being made in plant upgrades and improved plant refining capabilities. Optimisation of desulphurization catalysts is one of the elements that need to be addressed. Pilot plant experiments were done at the Sasol Technology R&D facilities to determine the optimum desulphurization catalyst and operating conditions for a crude oil refinery. GCxGC methods were used with TOF-MS, FID and SCD detectors for product analysis in order to evaluate the ability of the desulphurization catalysts to remove sulphur to ultra low levels and the effect of the desulphurization process on the hydrocarbon composition of the product.

PARALLEL DUAL-COLUMN GCxGC-TOFMS (2GCxGC-TOFMS) FOR ANALYSIS OF PCBS AND OTHER ENVIRONMENTAL POLLUTANTS

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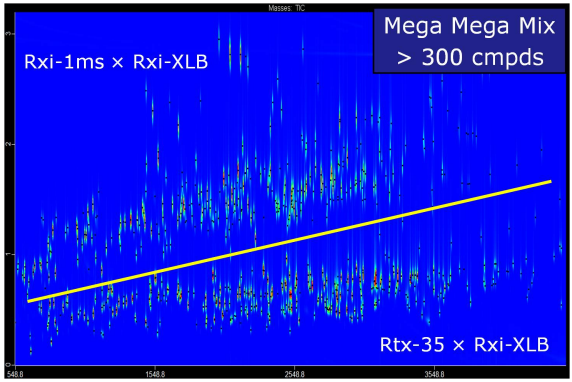
At the 10th GCxGC Symposium in May 2013, Robert Shellie of the University of Tasmania introduced what he called 2GCxGC, which involves a parallel dual-column setup (primary columns), a thermal modulator, and a secondary GC column connected to a flame ionization detector (FID). The proposed advantage of this setup is to separate compounds on primary columns of differing stationary phase, that may not be resolved on a single primary column, coupled to a single secondary column in the GCxGC configuration. One problem is that the FID does not provide additional selectivity, like a mass spectrometer. We employed 2GCxGC with a time-of-flight mass spectrometer (TOFMS) to demonstrate the concept for polychlorinated biphenyls (PCBs) and other environmental pollutant analyses. The TOFMS allows deconvolution of complex chromatograms, while simultaneously confirming the identification of particular compounds through full mass spectra. Another advantage of the technique is providing a more extensive GCxGC "fingerprint" of a particular sample, which is very important for environmental forensics work. Peak capacity is further enhanced in 2GCxGC by better filling out the chromatographic plane.



Le.24

DETERMINATION OF A NUMBER OF SEMI-VOLATILES USING ON-LINE SPE GCxGC-TOF MS

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Antwerpse Waterwerken (AWW) is for the quality of the production of her drinking water dependent on the quality of the surface water. A relatively large number of companies is pouring waste water in the Albert Channel, the source of our drinking water. As such, a large amount of unknown compounds are coming into the surface water. It is impossible of determining all the possible compounds, like pesticides, pharmaceuticals and industrial chemicals. Using our screening methodology, based on on-line SPE GCxGC-TOF MS, we first determine which compounds are present and, if the compounds encountered are toxicologically relevant and/or are frequently present, we start a quantitative validation study and measuring program of these compounds. As such, we determined in 2013 , 14 components were identified as being significantly present in surface water and, as such, need to be determined on a regular base in drinking water. A total validation study was performed and the results (bias, intra-lab reproducibility, measurement uncertainty, limit of detection) will be shown. Also the results of the measurement campaign of these compounds from 2013 will be highlighted.

COMPARATIVE DRUG ANALYSIS IN FORENSIC SCIENCE BY GC×GC-TOFMS

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Drug profiling describes the comparative analysis of seized drug samples based on chemical and physical attributes. It plays an important part in forensic science in order to identify potential links amongst seized drugs. The application of comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC-TOFMS) is thereby of great interest due to the complexity of the matrices and the low concentration of minor and trace compounds found in drug samples.

Based on different matrices and queries arising in forensic routine analysis the applicability of GCxGC in this area will be shown and successful application to the analysis of authentic samples from police seizures including ecstasy, sassafras oils and spice products will be demonstrated.

We will first present a non-targeted data analysis method for the analysis of seized 3,4-methylene-dioxymethamphetamine tablets ('Ecstasy'). Based on selected compounds discrimination of drug seizures was possible. MDMA samples resulting from laboratory syntheses were analyzed by this method in order to identify impurity compounds which are affected by modifications of the synthesis parameters.

In a next step we focused on the analysis of sassafras oils as the primary starting material in the illicit MDMA manufacturing. Discrimination of different batches of sassafras oils was possible, even in the case of significantly purified sassafras oils where characteristic compounds were absent.

The increasing popularity of herbal blends adulterated with synthetic cannabinoids give raise to the development of a method for the analysis of such products. The study focused on the identification of the chemical signature of damiana that is assumed to be frequently used as one of the herbal components in these products, in order to identify damiana in herbal blends.

Le.26

A NEW PASSIVE SAMPLER USING SILICONE RUBBER FOR THE ANALYSIS OF RIVER WATER AND DESERT SOIL BY COMPREHENSIVE GAS CHROMATOGRAPHY – TIME OF FLIGHT MASS SPECTROMETRY

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The last decade has seen increasing demands placed on regulators in South Africa to provide clean drinking water and enhance water quality. Typically large volumes of water are collected, especially where pollutants are present at trace levels. Glass sampling bottles are bulky and susceptible to breakage. We have developed a cheap and easy to use miniature passive sampler, using silicone rubber (polydimethyl siloxane (PDMS)) tubing and a stainless steel basket. Coupled to a comprehensive Gas Chromatograph – Time of Flight Mass Spectrometer (GCxGC-TOFMS) it provides excellent robustness and sensitivity, such that even pollutants at low level are easily detected and analysed. This new *in situ* sampling device was used in the watercourses around Pretoria, South Africa, and contaminants identified. A dramatic feature of the landscape of the Pro-Namib in southern Africa is the so called fairy circles – large barren patches visible in the western grasslands in which no vegetation grows [1]. Although various hypotheses have been proposed the cause of the fairy circles remains unknown [1,2]. However, chemical alteration of fairy circle soil was indicated by potting trials showing that, even in the laboratory, plants exhibited poor growth and mortality when planted in soil collected from within fairy circles [2]. This stress response was not observed for plants growing in soils collected from outside the circles [2]. The same passive samplers developed for water sampling were also placed inside and outside of fairy circles in Giribes Plain, Namibia. Low volatility complex hydrocarbon mixtures thus concentrated *in situ* and identified by GCxGC-TOFMS support a geochemical origin of the fairy circles. Unlike bulky sample containers, storage space and rugged terrains were not a concern during transportation of these small samplers. Due to their unobtrusive size and negligible mass the samplers were also easily transported by air.

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SET-UP A QUANTITATIVE SCREENING METHOD OF OXYGEN-CONTAINING COMPOUNDS IN PETROCHEMICALS BY GCxGC; FROM TUNING THE SELECTIVITY, VIA DUAL DETECTION MS / FID TO ULTIMATE IDENTIFICATION WITH ACCURATE MASS BY GCxGC-QTOF (HRMS)

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Comprehensive analysis of oxygen containing compounds in petrochemical samples has become an intensive research topic for few years. These compounds can be found in various complex matrices such as conventional and bio-feedstocks, but also as by-products in process effluents of the petrochemical industry. They are often involved in fouling build-up, catalyst poisoning, corrosion issues or failed specification, hence the need of a proper identification of these species. The label “O-compounds” can cover a wide range of organic structures such as carboxylic acids, esters, ketones, cyclic or acyclic with various degrees of unsaturations as well as combinations with other heteroatoms (e.g. nitrogen, sulfur). Unlike N- or S- heteroatom containing compounds, no robust GC-detector is specific to O-containing molecules. However, the lack of specific detection can be compensated by a highly specific separation. In that sense, an O-selective two dimensional comprehensive gas chromatography (GC×GC) methodology has been implemented with normal, reverse and hybrid configurations. An upfront fine comparison of columns selectivity, via Van’t Hoff plots, helps for choosing most promising combinations, including the new developed ionic liquid columns. As detection capabilities of FID for O-compounds, using ECN response factors, highly surpassed the ones of MS, a dual detection was implemented for combining high identification and quantification performances. Finally GCxGC-QTOF (Agilent 7200), either with EI or CI, helps for identifying remaining unknowns O-compounds for which interpretation of unit mass spectrum can be a daunting task. A high mass resolution Rs > 15 000 with a good mass accuracy < 2 ppm at fast acquisition rate (50 Hz) drastically enhances analytical possibilities. The drawback is the management of 50 GB data files.

The developed approach opened up new prospects for a generic quantitative screening of O-compounds in petrochemical samples. Identification of specific oxygenated molecules in specific effluents already gave some insights in their mechanism of formation.

Le.28

CHARACTERISATION OF ATMOSPHERIC POLYCYCLIC AROMATIC HYDROCARBONS IN SOUTH AFRICAN PLATINUM MINES

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Elevated levels of atmospheric polycyclic aromatic hydrocarbons (PAHs) may arise in underground mines due to the use of diesel machinery in confined environments. These PAHs may be in the gaseous phase or adsorbed onto the surface of diesel particulate matter (particle phase). It is therefore important to sample both phases in order to elucidate the potential PAH occupational exposure levels. To this end, a small, portable denuder device was employed consisting of two multi-channel silicone rubber traps in series separated by a quartz fibre filter. The primary trap collects gas phase semi-volatile organic compounds (SVOCs, including PAHs), whilst the filter collects particle phase analytes. The secondary trap collects any SVOCs which may have blown off the primary trap at sample volumes below that of the breakthrough volume. The low pressure drop across the denuder allows for the use of a small, battery operated, portable sampling pump which is intrinsically safe and is therefore suitable for use underground. Samples were taken at three different platinum mines at various locations in diesel shafts, as well as in non-diesel shafts. Each component of the denuder (primary trap, filter and secondary trap) was separately analysed by thermal desorption-GCxGC-TofMS. It was found that PAHs where primarily present in the gas phase, with naphthalene and the mono-methylated naphthalene derivatives being the most prevalent (0.2 - 8.7 $ng.m^{-3}$). Particle phase PAHs were present at the highest concentrations in the diesel exhaust samples, where fluoranthene and pyrene dominated the PAH profiles (0.5 – 110 $pg.m^{-3}$). The need to characterise both phases of PAHs was thus evident from this study.

CHARACTERIZATION OF FOOD PRODUCTS BY GC×GC-TOFMS: A FOOD "OMICS" APPROACH

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Gas chromatography (GC) coupled with mass spectrometry (MS) is an important tool for characterization and differential analysis and has broad applicability in the food and beverage industry. GC effectively isolates individual analytes from complex food matrices and MS provides identification of isolated analytes. As sample complexity increases and exceeds the capabilities of 1D GC, additional resolution may be desired. Comprehensive two-dimensional GC (GC×GC) is one method of separating more individual analytes within a complex matrix. In this poster, sample preparation, chromatographic, and mass spectral methods were determined to analyze a variety of food items; including hops, beer, and edible oils. In each case, HS-SPME was used to pre-concentrate volatile and semi-volatile analytes for analysis with LECO's Pegasus 4D GC×GC-TOFMS. Process changes were determined by monitoring flavor analytes from hops through a simulated boil in the beer brewing process. Distinct time-dependent trends were observed, including a decrease in essential oil levels corresponding to an increase in the duration of the boil. Characterization of beer samples was also accomplished with both chemical fingerprinting and the determination of individual analyte differences. The broad applicability of this approach is highlighted by applying similar methods to other food products. Extra virgin olive, olive, peanut, vegetable, and grapeseed oils were also characterized. Food fraud was investigated by analyzing edible oil mixtures that simulate adulteration by blending cheaper edible oils into extra virgin olive oil. The mixtures were clearly distinguished from the pure varieties by PCA with scores falling between those of the pure varieties used in the mixtures. This type of information provides food "omics" insight at various stages throughout production, including differentiation of raw materials, process changes, and finished products. These methods allowed for comparing food products by their chromatographic fingerprints, characterizing samples with the identification of individual analyte differences, and differentiating samples with PCA.

COMPREHENSIVE ANALYSIS OF POTENTIAL MIGRANTS FROM POLYOLEFINES USED FOR FOOD PACKAGING BY GCxGC-FID/MS: ELECTRON BEAM TREATMENT OF POLYPROPYLENE AS AN EXAMPLE

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Microbiological decontamination of food packaging can be achieved by treatment with electron beam (EB) irradiation. During this process part of the polymer and its additives are converted into smaller molecules. Such molecules tend to migrate into the packed food. According to legal requirements the producers have to rule out migration of toxic compounds in amounts that could endanger human health. The analytical challenge comprises a non-target comprehensive determination of all oligomers, reaction products and impurities (ORPI), respectively non-intentionally added substances (NIAS) present in the migrate at a low detection limit [1].
The main constituents of hexane extracts from polypropylene (PP) films consist of polyolefin oligomeric saturated hydrocarbons (POSH). Comprehensive two-dimensional GCxGC offers a complete separation between the homolog row of the POSH isomers and the more polar decomposition products of the additives (antioxidants, UV-stabilizers) induced by the EB irradiation, as well as the oxidation products from the polymer itself. The optimized GCxGC column set involved at polar (50 % phenyl methyl polysiloxane) first dimension separation column and a methyl polysiloxane second dimension column. The detection limit was 0.1 mg/kg PP film. GCxGC with FID was used for quantitative estimations and TOF-MS for identification. GCxGC offers the separation power needed for comprehensive analysis of potentially heath relevant migrants from polyolefins without any further pre-separation; limitations are the same as of conventional gas chromatography. Any ORPI present in polyolefins or originating e.g. from the printing ink of the labeling or the adhesives of the seals can be accessed by this powerful tool.

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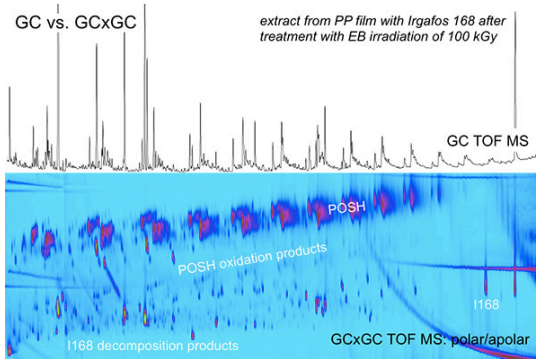

Le.31

FINGERPRINT OF VEGETABLE OILS: EXPLORING MINOR COMPOUNDS BY GC× GC WITH DUAL DETECTION

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Minor compounds of vegetable oil, which includes long and short chains alcohol esters (waxes), sterols (free and esterified), esters, terpenic alcohol esters, and fatty acid derivatives, are considered the fingerprint of different oils. This fraction is usually employed to detect frauds, in particular the addition of seed oils to more expensive extra virgin olive oil. The present work investigated this fraction by using a comprehensive GC with simultaneous dual detection (FID and mass spectrometer) for quantification and identification purposes. The fraction of interest was obtained employing a rapid solid-phase extraction (SPE) procedure to reduce manipulation and thus artefacts deriving from the traditional and tedious saponification procedure. Introducing a derivatization step before the SPE separation, free sterols were eluted in the same fraction of waxes and alkyl esters, thus allowing to explore a more "comprehensive" fingerprint of different oils in a single analysis. The separation potentiality of the GC×GC approach was investigated testing different column set and using both a cryogenic and a flow modulator.

Le.32

DEVELOPMENT OF A GCXGC-TOF/MS-BASED METHOD TO INVESTIGATE THE FATE OF 206 DIOXIN-RELATED MICRO-POLLUTANTS DURING FOOD COOKING

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Food-producing animals are exposed to various dioxin-related compounds like polychlorodibenzo-p-dioxins (PCDDs), polychlorodibenzofurans (PCDFs) and Polychlorobiphenyls (PCBs). Due to their lipophilic nature, these micro-pollutants are rapidly transferred from the environment to animal edible tissues where they are bio-accumulated, thus representing a public health risk. Only a fraction of these micro-pollutants is bioaccessible to the consumer due to technological and physiological processes applied to the food matrix before and after ingestion. Therefore, worldwide food safety agencies encourage residue chemists to investigate their fate during processes like cooking or digestion in order to upgrade their risk assessment procedures. The aim of the present paper was to develop a multiresidue method based on GCxGC-TOF/MS in order to investigate changes induced by cooking in the composition of a complex food matrix spiked with 226 dioxin-related micro-pollutants. In a first step, a GCxGC-TOF/MS method was developed to achieve a satisfactory separation of the 209 PCBs and the 17 toxic PCDD/Fs in hexane. The best GCxGC-TOF/MS conditions were determined according to peak shape (width and symmetry), peak count and resolution and enabled to separate 206 dioxin-related micro-pollutants including the 17 PCDD/Fs. Starting with meat as a model matrix, the second step enabled to set up procedures for both micro-pollutant spiking and sample preparation. The later included Accelerated Solvent Extraction (ASE), Centrifugal Evaporation and Gel Permeation Chromatography (GPC). The performance of the ASE-GPC-GCxGC-TOF/MS method was assessed in terms of recoveries, reproducibility, linearity and LODs. In the third and final step, the multiresidue method was implemented to assess the modulating influence of cooking on meat content in the 206 dioxin-related micro-pollutants. The results are discussed in light of the current knowledge about mass and heat transfer occurring in meat during cooking and about physico-chemical properties of these compounds.

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Le.33

CHARACTERIZATION OF BRAZILIAN CHARDONNAY WINES AND SPARKLING WINES USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH TIME-OF-FLIGHT MASS SPECTROMETRIC DETECTION AND STATISTICAL TOOLS

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Superior quality of Brazilian sparkling wines has been determinant of a growing share in the national market during several years and the market keeps its growing trend. They have also been recognized in the international realm through several awards in International competitions, as well as some other varietal wines, such as Chardonnay. However, only a few studies have been reported about their volatile compounds, which is one of the most important characteristics for wine acceptance. The use of headspace solid phase microextraction (HS-SPME) combined with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry detection (GC×GC/TOFMS) and the determination of odor activity value (OAV) of volatiles has shown to be an advantageous approach, as it indicated the most important odorant compounds (60 among 243) for Chardonnay wine, which were, after tentative identification, subsequently quantified. Even though sensorial analysis is of vital importance for wine aroma characterization, the use of OAV and GC×GC/TOFMS may be advantageous for the wine industry, as it provides a faster and easier preliminary approach to find out the most important odorant compounds for a varietal wine and may help quality control of fine varietal wines.

On the other hand, 78 tentatively identified volatile compounds of Chardonnay sparkling wines that were important to distinguish among sparkling and base wines could be pointed out through the application of Fisher ratio and principal component analysis to GC×GC/TOFMS data. Among these compounds some C13-norisoprenoids, esters, alcohols, aldehydes, acids, ketones and phenols were found to contribute the most for the observed dissimilarities among base and sparkling wines.

The use of strategies to facilitate GC×GC/TOFMS data treatment is an important issue to provide easier and faster ways for data interpretation, as the high amount of data poses substantial challenges that may prevent a more widespread use of this powerful technique.

Le.34

SELECTABLE GC-GC/GC×GC-SCD/Q-TOF-MS FOR IDENTIFICATION OF SULFUR COMPOUNDS IN TOBACCO SMOKE

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Over the last decade, GC×GC-TOF-MS has been widely applied to exhaustive analysis of complex samples for total profiling due to its large separation power, improved detectability, group type separation, and identification capability. High speed TOF-MS with a unit-mass resolution is considered as state-of-the-art for GC×GC because of idealist sufficient data acquisition rate of 50-100 Hz for measuring very narrow peaks. In recent years, several researchers have reported the applicability of GC×GC-high-resolution(HR)TOF-MS that allowed accurate mass detection for higher selectivity/sensitivity, and reliable identification. More recently, tandem mass spectrometry (MS/MS) with accurate mass detection has become available through recent progress in GC-Q-TOF-MS technology. Accurate masses from MS/MS product ion spectra can help verify that all fragment ions generated can be correlated to the proposed structure. However, the use of Q-TOF-MS in multidimensional GC is still quite limited.

In this study, GC×GC-Q-TOF-MS with parallel sulfur chemiluminescence detection (SCD) is applied for identification of trace sulfur compounds in a highly complex sample such as tobacco smoke. Identification is based on MS library search, ^{1}D LRI, and formula calculation based on both EI and PCI accurate mass spectra. In addition, MS/MS of the protonated molecular ion obtained by PCI provides additional information for structure elucidation. Moreover, the proposed system is configured with upstream GC-GC capability based on the selectable ^{1}D/^{2}D GC-MS system [1] using a LTM-GC technology. This offers additional power (and an extra dimension) for the selective fractionation of an extract prior to GC×GC-SCD/Q-TOF-MS analysis of incompletely resolved compounds that could not be identified previously.

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COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COMBINED WITH A FAST-SCANNING QUADRUPOLE MASS SPECTROMETER: SUITABLE FOR METABOLOMICS?

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GC×GC is an analytical technique which offers high separation efficiency and is thus an ideal platform for metabolomics analyses. Time-of-Flight-MS (TOF-MS) instruments are usually the GC×GC detectors of choice. However, recent technological improvements may allow quadrupole MS instruments to be used in an untargeted GC×GC metabolomics approach.
The performance of a GC×GC system equipped with a fast-scanning quadrupole MS instrument was evaluated. The selected column combination enabled a comparatively short analysis time, adequate ^{2}D peak widths, a relatively small difference in carrier gas velocities between both columns, and a sufficient column capacity. Hereby, about 300-350 analytes could be detected in 24h urine samples, approx. 85 % of which could be separated chromatographically. With the detector being operated in full scan mode with a scan range of m/z 60-550 and a data acquisition rate of 33.3 Hz, the vast majority of peaks were described by at least 10-14 data points. Total peak areas as well as total peak heights could thus be determined with a high precision using the TIC trace. Variation of relative fragment intensities was acceptable when the data points covering the upper peak half were considered. Apex spectra of peaks in different runs could be recorded with even higher precision. The linear dynamic ranges covered 1.0 - 2.25 orders of magnitude, depending on the analytes. The system showed excellent long-term stability with respect to retention times and overall detector performance.
Additionally, a two-stage data processing scheme was developed. Peak integration, peak annotation and export of one text file per run were done using the instrument manufacturers' software. Several self-developed R modules were used for data aggregation, denoising, alignment, drift correction, multivariate statistics and data visualization.
The system was successfully applied in several studies using human and plant matrices.

MULTIDIMENSIONALITY IN SENSOMICS: AROUND A CUP OF TEA

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Modern - omics disciplines dealing with food (foodomics, flavour metabolomics, sensomics, flavoromics [1,2]) investigate sample constituents considered collectively (primary and secondary metabolites, compounds generated by thermal treatments and/or enzymatic activity) and open interesting perspectives in the correlation between biological attributes and chemical composition.

Sensomics, in particular, focuses on revealing sensory-active compounds extending the investigation to all possible stimuli of the multimodal perception (aroma, taste, texture etc..) by comprehensively treating sample constituents and related properties (physicochemical properties, concentration in-the-matrix) together with their sensory activity (odor quality, odor threshold - OT, Odour Activity Value - OAV) [3].

Two-dimensional comprehensive gas chromatography coupled with Mass Spectrometry (GC× GC-MS), integrated with high concentration capacity (HCC) automated sampling approaches, represent a high-throughput/high-informative platform for food fingerprinting with interesting potentials in sensory characterization [4].

In the present study, a sampling design inspired by the pathways aroma compounds follow to reach the regio olfactoria (i.e. orthonasal and retronasal) has been applied to the characterization of key-active compounds responsible of tea sensory quality. The results obtained have been really straightforward and represent a bridge between high-throughput screenings with a complete and almost comprehensive profiling of volatiles related to flavour perception. In addition, most of the sensory descriptors of the product can be monitored and subsequently related with corresponding odour perceptions, by tuning the extraction capability of sampling towards a wider range of polarities and volatilities. In such a context, the information potential of each analysis increases and an almost complete sensory profile can be objectively delineated.

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METHODS FOR PEAK DETECTION IN COMPREHENSIVE TWO-DIMENSIONAL CHROMATOGRAPHY: ARE CURRENT ALGORITHMS CORRECT?

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Peak detection is one of the key issues in the treatment of data obtained from comprehensive two-dimensional chromatography. It is key for chromatographic alignment, as well as for quantification. Although the methods for peak detection in one-dimensional chromatography are well developed, this is not the case in two-dimensional chromatography. Several models have been developed to cover this objective, and they can be divided in two groups: (i) methods that use a two-step strategy [1-2] and (ii) methods that operate on the two-dimensional space and use a single-step procedure [3]. Type-i methods are based on unfolding the two-dimensional image in a one-dimensional string, applying peak detection in this one-dimensional chromatogram, and set up a decision strategy that merges the modulated peaks belonging to the same compound. Type-ii methods consider the two-dimensional GC chromatogram as a picture, and are basically based on image-analysis algorithms, with the most popular being the watershed transform. Unfortunately, the lack of bilinearity does not allow to apply multivariate techniques in peak detection (which would constitute type-iii algorithms).
Surprisingly, some of the most popular algorithms falling in category (ii) have a significant probability of failure [4]. The reason is the intrinsic intolerance to retention time variability in the second dimension (which is always present in real chromatograms). In this presentation, we will present the theory that supports why the application of the watershed algorithm has a significant probability of failure. Also, a completely different approach for peak detection in comprehensive two-dimensional chromatography will be presented [5]. The algorithm is based on Bayesian inference, and measures the likelihood of the presence or absence of a two-dimensional peak. The method is based on a type-i algorithm. It has been demonstrated that the Bayesian approach follows the chromatographers' intuition. The algorithm has been applied and tested with GCxGC data. A critical discussion of the behaviour of the existing peak-detection algorithms, their application to practical cases, and a comparison will be presented.

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Le.38

ENHANCING THE PRODUCTIVITY AND VISUALIZATION OF COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY FOR HYDROCARBON TYPE ANALYSIS

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The goal to create a more robust efficient hydrocarbon type analysis of petroleum distillates has led to recent enhancements in data processing and imaging software for comprehensive two-dimensional gas chromatography (GCxGC). Templates used to define chromatographic boundaries between different molecular types can now be adjusted either globally or regionally in an efficient manner to ensure precision of analytical results. Ideas are explored for automating the exclusion of instrument noise and other artifacts that interfere with accurate quantitative measurements of sample analytes. Expanding 3-dimensional chromatographic images into fly-by videos enables viewing of the complexity of GCxGC data in a remarkable new way.

Le.39

METABOLOMIC ANALYSES USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH HIGH-RESOLUTION MASS SPECTROMETRY (GCxGC-HRMS)

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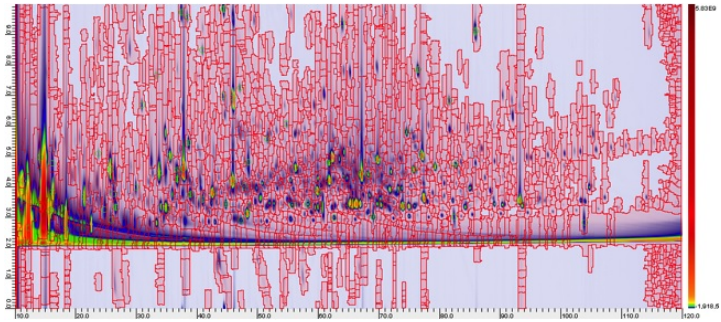
Comprehensive two-dimensional gas chromatography (GCxGC) is a powerful tool for complex metabolomic analyses. This research has developed GCxGC chromatographic and data analysis methods for metabolomic investigations with Arabidopsis thaliana. The experiments analyzed multiple Arabidopsis samples, including Columbia (Col-0) ecotype and several mutant types. For each sample, chromatographic replicates were performed using a GCxGC with a single quadrupole mass spectrometer or high-resolution time-of-flight mass spectrometer (HRMS). The chromatograms were visualized, processed, and analyzed for comparative characterizations and identification of potential biomarkers. The data processing generated a template pattern of peaks that could be reliably matched across chromatograms, used those peaks to align and composite chromatograms, and then built a feature template comprised of the reliable peaks and the 2D polygonal peak-region windows for all peaks detected in the composite chromatogram. The feature template was aligned to each chromatogram and the peak-region features used to generate a feature vector with mass spectrum for each region in each chromatogram. The feature vectors were analyzed using Fisher Linear Discriminant Analysis (LDA) to select highly discriminatory peak-regions with respect to class differences between the Col-0 and mutant samples. The spectra of these regions were matched to several mass spectral libraries for preliminary identifications. Further analyses were conducted with high-resolution mass spectrometry to indicate elemental compositions for compound identifications.



ENHANCED MULTIVARIATE ANALYSIS OF HEAD-SPACE VOLATILES FROM PARTICULATE PHASE MAINSTREAM TOBACCO SMOKE BY HS-SPME GC×GC-TOFMS

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Tobacco smoke is an aerosol containing an extremely complex mixture of chemicals.[1] It consists of liquid/solid droplets, often referred to as the particulate phase (PP), suspended in a mixture of gases and semivolatiles – the vapour phase. Tobacco smoke is formed during overlapping processes of oxidation, pyrolysis, pyrosynthesis, distillation, sublimation, condensation, filtration and elution.[2] Mainstream smoke contains over 6000 currently identified compounds[3] and some reports claim that the total number of compounds might reach 100,000.[4]

In this study, head-space volatiles from mainstream particulate phase smoke were collected on a glass fiber filter and analyzed by means of comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS). A comparison was made of six commercially available Solid Phase Micro-Extraction (SPME) fibers to collect and retain volatile substances from the smoke particulate phase. Multivariate response surface methodology was used to optimize experimental evaluation of SPME conditions.

To develop a strategy for the analysis of large numbers of samples from different cigarette types, a pixel-based software package was used to analyze and align 24 chromatograms. For multivariate analysis, the Fisher ratio (FR) and principal component analysis (PCA) were used to identify significant variations within specific classes of compounds from 2 types of cigarette differing in filter design. PCA allowed a clear differentiation of the studied cigarette types while FR analysis allowed identification of compounds that were most highly correlated with the chemical differences between the cigarette samples.

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GC×GC/QMS ANALYSIS OF POLAR COMPOUNDS EXTRACTED FROM THE BIO-OIL FROM THE PYROLYSIS OF SAWDUST

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Pyrolysis is a thermo-degradation process that can transform biomass in an important product – bio-oil – containing a large amount of many different classes of compounds with high added value. There are many studies involving the characterization of bio-oils by gas chromatography, but its complete characterization is still a challenge due the high complexity of this material. Two dimensional gas chromatography (GC×GC) is one of the best alternatives to identifying compounds in very complex samples where co-elution of similar compounds is a major problem in the analysis by one-dimensional techniques. In this paper it is studied the most polar fractions of bio-oil produced by fast pyrolysis of Lignocel BK40-90 (sawdust from forest timber). The biomass was submitted to the pyrolysis in an existing FCC pilot plant that was adapted for this procedure. The equipment consists of a fluidized bed reactor with nitrogen injection. The unit operates with continuous biomass feeding and continuous solids circulation. The produced bio-oil was submitted to an aqueous alkaline extraction extraction, isolating the acidic compounds that were analyzed by one-dimensional gas chromatography and comprehensive two-dimensional gas chromatography with quadrupole mass spectrometry detection (qMS). One hundred and thirty compounds (mainly phenols and ketones) were tentatively identified in the extract, some of them by the use of retention indexes. The main differences between chromatographic techniques were the substantial increasing in the peak capacity of GC×GC and the resolution of some co-elutions that occurred in GC/qMS. It is also possible to conclude that this extract is rich in important raw materials for the chemical industry and can be used for this end.

Le.42

RUBBER RECYCLED TIRE PLAYGROUNDS AND PAVERS: WHAT IS INSIDE?

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Elimination of discarded tires represents a major environmental management problem due to the high persistence of this material and the hazardous nature of some of its inorganic and organic components. Strategies for tire waste valorization ranges from energy production to use in asphalt and other pavement applications. However, one of the most economically interesting uses of this product is its transformation in different recycling products, including rubber mulch and paver materials [1]. These latter products are increasingly used in both open and closed environments to create colorful durable smooth flooring areas. Due to their anti-slipping nature and shock absorption capability they have become a top choice material for surfacing sport fields, fitness centers, old people´s homes and children´s playgrounds, among others. The presence of hazardous organic compounds, such as selected polycyclic aromatic hydrocarbons, vulcanization additives, antioxidants and plasticizers, in recycled rubber-based playground surfaces, has been demonstrated in a few recent studies [1,2]. In these investigations, these target compounds have been found at variable but, in most instances, environmentally relevant levels, and their capacity of mobilization by either lixiviation or evaporation has been evidenced.

This study proposes the use of comprehensive two-dimensional gas chromatography in combination with time-of-flight mass spectrometry (GC×GC-ToF MS) for the non-orientated characterization of the (semi-)volatile fraction obtained from rubber recycled tire playground and paver materials by pressurized liquid extraction with ethyl acetate [1]. More than 200 compounds have been identified in commercialized materials. The potential mobilization of these chemicals has been evaluated by comparison of these results with the profiles found in pavers collected from outdoor playgrounds in Spain.

Acknowledgments

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Le.43

ANALYSIS METHODS OF GAS-TO-LIQUIDS STREAMS: CHALLENGES AND OPPORTUNITIES

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Over the past decades analytical chemists have developed a multitude of methods to characterize hydrocarbon streams from refinery processes. The revival of the Fisher-Tropsch process led to a new range of synthetic hydrocarbon products with compositions that are a subset of conventional refinery products.
Conventional gas chromatography methods are not fully adequate to support process development of Gas to Liquids technology and gave analytical scientists the challenging task of developing a fit-for-purpose methodology. In this presentation, the development and implementation of a GCxGC method for the characterization of GTL products will be addressed. In addition, developments on other modified methods for the characterization of GTL intermediates will be shown.

IMPROVED GAS TIGHT CONNECTIONS FOR BETTER GC AND GC X GC RESULTS

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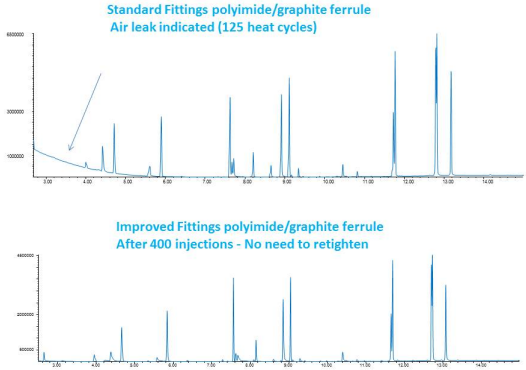
One of the most basic and yet challenging steps in gas chromatography (GC) is making and keeping reliable leak free connections. While basic for GC analysis, less robust and reliable GC column connections can be the Achilles heel for analysts using GC X GC or GC X GC-TOF MS to separate very complex mixtures.

The selection of capillary ferrule material is important in achieving leak free seals with appropriate inertness for specific applications. While soft and easy to seal, graphite ferrules are porous making them unsuitable for use in some detectors, including mass spectrometers. Polyimide/graphite ferrules are compatible with mass spec, but shrink with temperature cycling creating leaks with standard column nuts. Metal ferrules are made of stainless steel which provide unique advantages over other ferrules, but require care with regard to possible over-tightening which can break the column and cause fitting damage.

Capillary flow technology (CFT) devices help simplify the connections for GC x GC modulation, Deans' switching, and multi-channel detection. Over tightening of early design metal ferrules in CFT fittings led to column breakage and instrument downtime. Early design metal ferrules produced spontaneous breakage during operation from catalytic attack of the fused silica. Recently designed Flexible Metal Ferrules offer larger sealing surfaces making for an easier to use ferrule.

To ensure robust and reliable seals, the proper column ferrule for the application is combined with a column nut that properly seats and secures the ferrule during operation. A novel new column fitting design that extends the use of composite ferrules by addressing the limitations of polyimide/graphite ferrules is shown. By using the new fitting with polyimide/graphite ferrules, leak free connections are maintained through more than 300 heat cycles (32-325?C) without any intervention from the operator. The need to retighten polyimide/graphite ferrules due to material shrinkage has been eliminated.



ANALYSIS OF CYLCOPIA (HONEYBUSH) TEA VOLATILES BY COMPREHENSIVE 2-DIMENSIONAL GAS CHROMATOHRAPHY USING A NOVEL SINGLE-STAGE THERMAL MODULATOR

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Honeybush tea, produced from *Cyclopia* species endemic to the Cape fynbos biome of South Africa, is becoming increasingly popular as a caffeine-free health beverage. The characteristic honey-like smell of this herbal tea is one of its most important features. A better understanding of the volatile compounds responsible for the unique sensory profile of honeybush tea is essential to improve its production, yet their identification is hampered by the complexity of the sample. In this contribution, the application of headspace solid phase micro-extraction (HS-SPME) in combination with comprehensive two-dimensional chromatography (GC×GC) coupled to flame ionization detector (FID) and quadrupole mass spectrometry (qMS) for the detailed investigation of the volatile composition of *Cyclopia maculata*, *subternata* and *genistoides* infusions will be demonstrated. A novel consumable-free single stage thermal modulator was used for this purpose. The design and operation of this modulator will be addressed and evaluated. Optimization of experimental parameters included the choice of column dimensions and stationary phases in each dimension, optimal flow rate, modulation period and HS-SPME conditions for honeybush volatiles. The optimized experimental set-up utilizing an apolar×polar column combination provided significantly improved separation compared to 1-dimensional GC. Compounds were identified by comparison of retention times, retention indices and qMS data with reference standards; the majority of identified compounds were terpenoids. The modulator offered cryogen-free and highly reproducible operation. This latter aspect proved particularly beneficial as it facilitated multivariate statistical analysis of the GC×GC-FID data, which highlighted the most important differences in volatile composition between tea samples from different *Cyclopia* species.

ON-LINE LIQUID CHROMATOGRAPHY-COMPREHENSIVE 2D GAS CHROMATOGRAPHY-TRIPLE QUAD MASS SPECTROMETRY: A POWERFUL AND FLEXIBLE 4D SEPARATION-SCIENCE TOOL

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The present research is focused on the on-line combination of high performance liquid chromatography (HPLC), comprehensive two-dimensional gas chromatography (GC×GC), and triple quadrupole mass spectrometry (QqQ MS), generating a very powerful 4D separation-science tool. The selectivity of the HPLC dimension enables the separation of chemical classes, or sub-classes of compounds. Then, the whole fraction of interest can be transferred, through a syringe-type transfer device, to a programmed temperature vaporizer (PTV) injector. Each transferred fraction can then be subjected to cryogenically-modulated GC×GC. The latter is an excellent way to increase selectivity, peak capacity and sensitivity for a GC-based analysis. The analytes eluting form the GC×GC system were directed to a highly flexible and rapid QqQ MS instrument. In fact, the tandem MS system is capable of generating full scan and multiple reaction monitoring (MRM) data, in a very rapid manner. The potential of the novel LC-GC×GC-QqQ MS combination was evaluated in the analysis of a highly challenging sample, namely coal tar. The normal-phase LC step was successful in separation the following groups of compounds: I) hydrocarbons, II) aromatic compounds (with and without S), III) hydroxyl-containing constituents. Each LC fraction was subjected to a specific untargeted or targeted GC×GC-QqQ MS approach. For example, the coal tar S-containing compounds were pinpointed through MRM analysis, while for full-scan MS information was sufficient for the hydrocarbons.

ANALYSIS OF PYROLYSIS OILS BY COMPREHENSIVE GAS CHROMATOGRAPHY MASS SPECTROMETRY GCXGC-MS

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The depletion of crude oil and natural gas will lead to increasing prices during the next decades. Since these resources are not only used as feedstock for the production of fuels, but also as feedstock in petrochemical industry, there is a need to find alternative carbon based resources. Most probably petrochemical processing will transform back to a coal based industry. Several thermochemical conversion methods exist, from among pyrolysis plays an important role. Besides coal other raw materials (e.g. biomass) or waste materials (e.g. tires) may be converted to chemical feedstocks by pyrolysis. Pyrolysis provides a promising way for the production of commodity chemicals and existing technologies have to be adopted to modern requirements. To achieve this, the detailed chemical analysis of product streams is indispensable.
Liquid products from the pyrolysis of coal and other feedstocks were investigated using comprehensive gas chromatography mass spectrometry (GCxGC-MS). Several components were identified by mass spectrometry and quantified using reference compounds. In addition, several hundred to more than thousand compounds, depending on feedstock and pyrolysis conditions, were observed. Since manual identification was not feasible and quantification not possible due to the lack of reference compounds, unsupervised and supervised classification procedures based on mass spectral information were developed. The impact of the variation of experimental parameters like different feedstocks and pyrolysis temperature on the composition of the pyrolysis liquids was investigated.

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Le.48

PREDICTING THE PROPERTIES OF PETROCHEMICALS WITH PIXEL-BASED ANALYSIS OF GC×GC DATA

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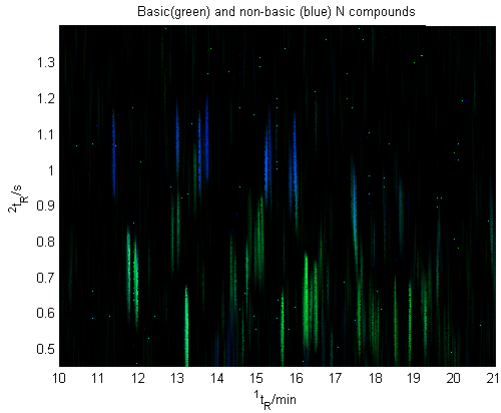
Comprehensive two-dimensional gas chromatography (GC×GC) is able to separate hundreds or thousands of peaks and produces megabytes or gigabytes of data per sample. This makes it difficult and time-consuming to extract accurate and precise quantitative information using traditional peak integration. An alternative approach to data analysis with shown utility for GC×GC data is pixel-based analysis. Here, the intensity at each ^1t_R, ^2t_R combination is used as variables and analysis is performed with multivariate data analysis techniques. However, predicting sample properties with the pixel-based approach may seem challenging: Without any feature detection and quantification, the ratio of variables to samples becomes very large, which makes the multivariate modelling more sensitive to noise. When using multivariate regression techniques such as partial least squares regression (PLSR) to predict sample properties from GC×GC data an increase in noise can cause spurious correlation between pixels that contains mainly noise and the predicted petroleum property.

Here, we present a data processing strategy for prediction of sample properties from GC×GC data using the pixel-based approach. The strategy includes preparation of a summed extracted ion chromatograms (sEICs) from the total ion chromatogram, baseline removal, retention time alignment in 2 dimensions, normalization and scaling. Specifically, the scaling to the inverse of the analytical uncertainty reduced the influence of false correlations in data due to a reduction of the noise effect. The strategy was tested on two data sets to predict from the sEICs, boiling point properties, total nitrogen and basic nitrogen content measured with reference methods. The pixels/compounds that best describes the three endpoints were detected and the compound identity confirmed.

Color plots for the chemometric models are presented and interpreted. These plots offer a unique way to understand how the chemical composition of samples determines sample properties.



PROFILING TRACE ALKYL PHOSPHATE CONTAMINATION IN CRUDE OIL AND VARIOUS PETROLEUM PRODUCT STREAMS USING GC×GC-FID/NPD

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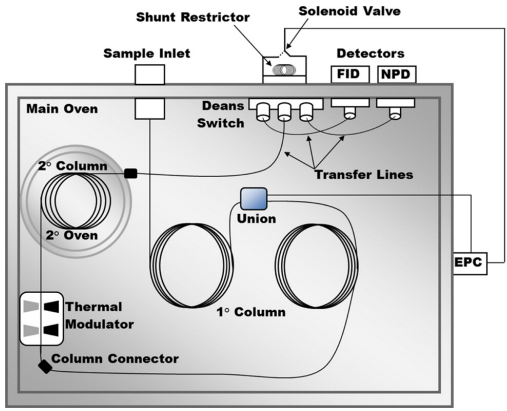
The profiling of alkyl phosphate contamination in industrial petroleum samples is of particular interest to refineries that process unconventional crude oil derived from the Western Canadian Sedimentary Basin and other similar geologies. This is due to the alkyl phosphate-based additives used during crude oil recovery processes and the subsequent contamination of the produced oil. Phosphate contamination causes numerous problems including fouling of refinery equipment, the poisoning of catalysts, and potential impacts on downstream processes or consumers if these phosphates enter product streams.

Our group has previously demonstrated the use of comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) for the quantitative speciation of alkyl phosphates in petroleum samples. Nevertheless, the use of a phosphate-selective detector such as a nitrogen phosphorus detector (NPD) would mark an improvement over the TOFMS in terms of cost and ruggedness, resulting in a detection system suitable for widespread deployment in refining environments. In previous work we identified incompatibilities between the silylation reagents required for derivatization of mono- and di-alkyl phosphates and the NPD. As a result our group has developed a method capable of speciating and quantifying alkyl phosphates using GC×GC-FID/NPD through the addition of post-column flow switching to protect the NPD from excess silylation reagents. We have also added concurrent backflushing to protect the columns and reduce instrument maintenance when analyzing heavier petroleum fractions. The resulting method presents a significant step towards a routine, robust method for profiling trace alkyl phosphate contamination in industrial petroleum samples in a production environment. Here we present the use of this method for profiling alkyl phosphate contamination in raw crude oil, desalted crude oil, and various petroleum product streams collected from an atmospheric distillation unit of a Western Canadian refinery.



PERSISTENCE OF DECOMPOSITION ODOUR IN SOIL ANALYSED BY GC×GC-TOFMS

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Cadaver dogs are commonly used to search for human remains deposited on or beneath the ground. The volatile organic compounds (VOCs) evolved from decomposing cadavers are responsible for eliciting a positive canine response. These compounds decline following extensive soft tissue decomposition or animal scavenging. It is thought that soil can act as a repository for VOCs introduced during decomposition and increase the period that a decomposition signature can be detected at a deposition site. The aim of this study was to identify the compounds retained and persistence of such compounds in decomposition soil following a period of soft tissue decomposition and simulated scavenging. Pig carcasses were used as decomposition analogues and were left to decompose on the soil surface in an open woodland research facility in Australia. The remains were artificially scavenged following three months of decomposition, and VOCs were collected onto sorbent tubes from the soil for an additional 7 months thereafter. Thermal desorption – two-dimensional gas chromatography – time of flight mass spectrometry (TD – GC×GC – TOFMS) was used to identify the decomposition VOCs. The results indicate that highly volatile compounds commonly associated with decomposition odour (such as polysulphides) rapidly diminished within 8 weeks. Principal component analysis allowed for identification of compounds exhibiting longer persistence that influenced the decomposition soil, distinguishing it from control sites where no decomposition occurred. The use of TD – GC×GC – TOFMS provided enhanced sensitivity which was required for trace volatile analysis where persistence was of interest involving a removed odour source. The instrument also provided improved peak capacity which was necessary for a complex matrix such as decomposition odour involving a large number of compounds. This study indicates the key compounds that are detectable for extended postmortem intervals in decomposition soil and may be responsible for cadaver dog alerts at sites where remains are no longer present.

KNLe.02

DISCOVERING VOLATILE BIOMARKERS OF P. AERUGINOSA ADAPTATION DURING CHRONIC LUNG INFECTIONS USING GCXGC-TOFMS AND CHEMOMETRICS

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A primary cause of morbidity and mortality for people with Cystic Fibrosis (CF) is chronic lung infections caused by the opportunistic pathogen *Pseudomonas aeruginosa*. In the lung, *P. aeruginosa* acquires mutations that allow it to persist in its new environment, including mucoid conversion, antibiotic resistance, and quorum sensing deficiency, all of which have been correlated to lung function decline. We have observed that *P. aeruginosa* mutations for these phenotypes, which are highly-conserved amongst CF-associated infections, change the *P. aeruginosa* volatile metabolome. Based on these observations, we hypothesize that suites of volatile biomarkers exist for each phenotype, making it possible to detect *P. aeruginosa* muco idy, antibiotic resistance, and quorum sensing deficiency in the lung using only the patient's breath. Our goals are to develop rapid tests to detect, track, and characterize *P. aeruginosa* infections and mutations *in situ* in order to initiate early, targeted treatment, which is essential to managing infection and maintaining healthy lung function in CF patients.
In this work we have used comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOFMS) to characterize the volatile metabolomes of 35 *P. aeruginosa* clinical isolates from CF lungs that possess hallmark mutations in antibiotic sensitivity and quorum sensing genes, *mexA* and *rhlI*, respectively. We have applied a variety of chemometric analyses to identify putative biomarkers for *mexA* and *rhlI*, and in this presentation we highlight some of the successes and limitations of the approaches we have used.

KNLe.03

ERROR ASSESSMENT IN GCxGC MODELLING AND PREDICTION

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As the comprehensive analysis of complex materials demand a high peak capacity, their chromatographic separation cannot be achieved using one-dimensional chromatography and two-dimensional chromatography offers a key advantage. In this paper we present a model relating experimental factors (column lengths, diameters and thickness, modulation times, pressures and temperature programs) with the retention and band broadening. Unfortunately, an analytical solution to calculate the retention in temperature programmed GCxGC is impossible, making thus necessary to perform a numerical integration. In this paper we present a programmed physical model of GCxGC, capable of predicting with a high accuracy retention times in both dimensions, given different conditions.

Once fitted (*e.g.* calibrated), the model is used to make predictions, which are always subjected to error. In this way, the prediction can result rather in a probability distribution of (predicted) retention times than in a fixed (most likely) value. One of the most common problems that can occur when fitting unknown parameters using experimental data is the overfitting. In our case, this problem is translated in terms of using the same conditions for both validation and training set. In order to avoid the overfitting problem, the Monte-Carlo sampling combined with crossvalidation technique is used. Another technique of error assessment used in this article is the use of error propagation using Jacobians. This method is based on estimation of the accuracy of the model by the partial derivatives of the retention time prediction with respect to the fitted parameters (in this case entropy and enthalpy for each component) in a set of given conditions. By treating the predictions of the model in terms of intervals rather than precise values, it is possible to considerably increase the robustness of any optimization algorithm.

KNLe.04

PRELIMINARY INVESTIGATION INTO THE AROMA OF ROSEMARY USING MULTI-CHANNEL SILICONE RUBBER TRAPS AND GCxGC-TOFMS

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Multi-channel polydimethylsiloxane rubber traps were used to sample the headspace of rosemary samples (two essential oils from different sources, one oleoresin and one dried herb) followed by two dimensional gas chromatography – time of flight mass spectrometry (GC x GC-TOFMS) and GC-MS analyses. The aroma of the different headspace samples were first characterised using a custom built olfactory apparatus. It was found that the oils had the finest aroma, whilst the dried herb sample had a very different aroma profile in comparison, which was evident from bubble plots of the perceived aroma of samples at different temperatures. The four rosemary samples were also heat treated to simulate cooking of food products containing rosemary ingredients, and were then reassessed to determine any changes in the aroma profile. It was found that the intense menthol and cooling aromas subsided in all the samples with heating. The use of multi-dimensional GC allowed for efficient separation of the numerous components in the headspace samples. Many isoprenoids and aliphatics were thus tentatively identified, complementing previous research on rosemary. The relative peak areas were compared to better understand the mixture that contributes to the rosemary aroma. Further studies with regards to the blending of these key components to recreate the aroma for culinary purposes are required.

KNLe.05

INVESTIGATING THE VOLATILE PROFILE OF LUNG CANCER CELL CULTURES

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According to the American Cancer Society, lung cancer is the most lethal [1], mainly due to late diagnostic. There is therefore a need for sensitive and non-invasive diagnostic methods for population screening.
A large number of diseases appear to have a volatile signature present in the breath air exhaled by patients. Indeed, analytical breath profiling offers possible solutions for early detection of different kind of lung infections [2]. For example, cystic fibrosis patients show a different volatile organic compound (VOC) profile because of the bacterial colonization that localizes in their lungs [3]. A similar behavior is observed for patient suffering from lung cancer [4]. However, the list of recognized volatile biomarkers of lung cancer is still scarce and could be improved. Indeed, the biological variability of exhaled air profile makes the biomarkers identification challenging. In the hope of contributing to a better understanding, we used GC×GC-TOFMS to investigate the VOC profile of lung cancer cells.
A SPME procedure has been developed to sample the headspace of cell cultures. After proper alignment of chromatograms, univariate and multivariate mathematical approaches were compared for isolation of potential lung cancer biomarkers and further tentative identification by GC×GC-HRTOFMS.

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KNLe.06

GC×GC, GC×GC-MS AND MINIATURISED GC×GC WITH SINGLE STAGE THERMAL MODULATION

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The development of GC modulators has been a critical topic of discussion in comprehensive two-dimensional GC for some time now. To date there are two main modulation strategies employed to achieve comprehensive two-dimensional GC, *viz.* thermal modulation and flow modulation. Cryogenic modulation is the most prevalent due to its commercial accessibility and effectiveness, however this technique often suffers from the added cost of consuming large quantities of liquid cryogen. Flow modulation on the other hand eliminates the need for liquid cryogen at the expense of experimental flexibility; often such setups are incompatible with mass spectrometric detection.

In this work thermal modulation is achieved by the application of current pulses using capacitive discharge to release trapped components from a short cold-trap placed at the confluence of the first- and second-dimension GC columns. This is in contrast to the cryogenic approach whereby compounds are trapped in a capillary by cryogenic cooling and released via the application of hot air. The resistively heated thermal modulation strategy offers reduced instrument complexity, miniaturisation of the instrument setup as well as the potential for portable GC×GC analysis. The modulator has been utilised for the separation of a number of challenging environmental and fragrance applications. Portable analysis using this technology will also be assessed.

KNLe.07

THE USE OF SCRIPTING FOR IDENTIFICATION OF POTENTIAL NOVEL POPs WITH GC×GC-ToF MS ANALYSIS

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GC×GC–ToF MS represents a valuable approach when dealing with the simultanous screening of different families of organic micropollutants in complex environmental samples. Interpretation of the large set of data generated can be a challenging task for the analyst, especially if some analytes are unknown compounds for which no MS data is available. The use of scripts allows for a fast and reproducible way of processes large complex data sets.

Recent studies have shown that some physicochemical properties can be used as predictors of potential bioaccumulative and persistent chemicals. The majority of compounds identified as POPs are halogenated. Pre-defined MS filters for the determination of halogenated compounds during GC×GC–MS analysis can provide direct information regarding both conventional and unknown POPs in the sample.

With the focus on the early identification of potential emerging POPs, this study evaluates the feasibility of using scripts during GC×GC–MS analysis to isolate organohalogens in sediments collected in Ontario (Canada). A unique script was designed to simultaneously classify peaks that contain one or more chlorine or bromine atoms.

Unknown halogenated compounds were evaluated for occurrence within the samples and HRMS analyses were used to confirm some of the most prevalent compounds as examples. Three decachlorinated dechlorane analogs ($C_{18}H_{14}Cl_{10}$), two undecachlorinated dechlorane species ($C_{18}H_{13}Cl_{11}$) and a novel mixed chloro/bromo-dibenzocarbazole ($C_{12}H_5NCl_2Br_2$) were identified in a number of the sediments that were analyzed. Relative peak abundances (concentrations) of these unknown organohalogens were in the same range or slightly higher than those detected in the samples for conventional POPs, *e.g.* PCBs. Relevant toxicological data for the novel mixed halogenated carbazole will be discussed.

CHARACTERIZATION OF BIO-OIL FROM THE PYROLYSIS OF BRAZILIAN COCONUT FIBERS BY GCXGC/TOF-MS: FRACTIONATION WITH ION EXCHANGE RESIN

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In Brazil, the coconut is cultivated mainly in the Northeast Region, and the coconut shells are normally wasted in landfills, which mean a high environmental impact. In this work, it was carried out the study of bio-oil obtained by pyrolysis from Coconut (Cocos nucifera L.) fibers by GC× GC/TOF-MS from the Aracaju city (Sergipe, Brazil). The residual fibers obtained from the shells of coconut were subjected to fast pyrolysis, producing bio-oil and bio-char. Bio-oil was submitted to a fractionation in column, using Amberlyst A-27TM ion-exchange resin as stationary phase, and the fractions obtained were characterized by GC×GC/TOF-MS. Before the fractionation, 277 compounds were tentatively identified in the bio-oil, being verified that 57% of the area on the chromatogram of bio-oil is composed by phenols, 17% by ketones and 12% by aldehydes. After the pre-treatment with the ion-exchange column, the non-polar fraction showed 252 compounds that were tentatively identified, mainly hydrocarbons (20%) and esters (14%), besides presenting some phytosterols that were not detected in the untreated sample. In the polar fraction 164 compounds were tentatively identified, which phenols correspond 50% of area, followed by aldehydes (15%) and acids (12%). The fractionation was essential for the enrichment of fractions in specified classes of compounds, specially separated in non-polar and polar. These compounds are important for the industry because hydrocarbons and esters have potential to be used as fuel, and the phenols are used as a raw material for laminate industries and manufacturing of special chemicals, as phenolic resins. This indicates that coconut fibers have the potential to be a cost-effective and promising alternative to obtain new products and minimize environmental impact.

KNLe.09

MINERAL OIL IN HUMAN TISSUES: CONCENTRATIONS, MOLECULAR MASS DISTRIBUTIONS AND STRUCTURAL INFORMATION FROM GCxGC ANALYSIS.

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Mineral oil saturated hydrocarbons (MOSH) are the most abundant contaminants found in the human body. In 2012, the European Food Safety Authority (EFSA) published an opinion on mineral oil hydrocarbons (MOH) in food, focusing on exposure and toxicology: human exposure to MOSH and MOAH (mineral oil aromatic hydrocarbons) was considered of potential concern. In this work, mineral oil occurrence was investigated in human tissues (fat tissue, mesenteric lymph nodes, spleen, liver, lung, kidneys, heart and brain). MOH were extracted using ethanol as a mediator solvent and analyzed by on-line liquid-gas chromatography equipped with flame ionization detector (HPLC-GC-FID). No MOAH were detected. Regarding MOSH, concentrations and molecular mass distribution were determined. The concentrations reached 1400 mg/kg in the lymph nodes and spleen. For a quarter of the subjects (n=37), the estimated total amount of MOSH in the body exceeded5 g. The molecular mass profile was similar for all individuals and tissues, and suggested a high uptake selectivity, evaporation and metabolic elimination, as the profile did not correspond to that of the mineral oils most commonly exposed to. MOSH in the liver and spleen were almost identical and differed from those in the lymph nodes and fat by a higher degree of metabolism.

Comprehensive two-dimensional gas chromatography (GCxGC) with FID (quantitation) and MS (structures) was used to obtain structural information about the MOSH. The MOSH in human tissues were compared to those in mineral oil products, which showed the elimination of most predominant components, particularly the n-alkanes, but no significant enrichment of isoalkanes or naphthenes.

KNLe.10

COMPARISON OF CRYOGENIC AND DIFFERENTIAL FLOW (FORWARD AND REVERSED FILL/FLUSH) MODULATORS AND APPLICATIONS TO THE ANALYSIS OF HEAVY PETROLEUM CUTS BY HT-GCxGC

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The development of new efficient conversion processes requires extended knowledge on vacuum gas oils (VGOs) and heavier petroleum cuts (T_{bp}°C=375-580, 580+). Comprehensive GCxGC benefit for the analysis of such complex samples is no longer to be demonstrated and the modulator is the key element of this analytical technique. A recent publication showed that nC68 ($T_{éb}$=641°C) and VGO resins were eluted by HT-GCxGC using a CO_2 cryogenic modulator [1]. However, it is known to present real drawbacks in term of safety, cost and time-consumption. An interesting alternative solution to cryogenic modulators is differential flow modulators. Developed by Seeley et al. in 2006 [2], this kind of modulation consists in the alternation of filling and flushing cycles of a collection channel (CC) controlled by a fast acting three-way solenoid valve. In the commercially available Agilent's version, the CC is carved in a stainless steel plate. Some improvements were made recently. First of all, it is now possible to adjust the size of the CC thanks to Agilent's Capillary Flow Technology. Moreover, Griffith *et al.*[3] evaluated on light hydrocarbons, a differential flow modulator whose flow is reversed during the flush step. Those improvements enable to reduce peak tailing and to handle significant overloading without loss of resolution. Even if it was proved that both cryogenic and the commercialized differential flow modulator are competitive in terms of peak capacity and resolution for the detailed analysis of middle distillates [4], none of the differential flow modulators has been used with more complex and heavier petroleum cuts (such as VGOs) so far. As a consequence, the optimization of HT-GCxGC methods for the detailed analysis of VGOs, using differential flow modulation is presented in this lecture. Moreover, advantages and drawbacks, in terms of peak width and symmetry, number of detected blobs, discrimination towards heavy compounds…, of cryogenic modulators, forward fill/flush with an integrated collection channel (commercial version) or an adjustable collection channel and reversed fill/flush with an adjustable collection channel differential flow modulators, are discussed.

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KNLe.11

FORENSIC CHEMICAL PROFILING OF IGNITABLE LIQUIDS WITH COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY – APPLICATION TO INTACT WHITE SPIRITS IN THE NETHERLANDS

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In recent years comprehensive two-dimensional gas chromatography (GCxGC) has become a mature analytical technique with applications in many fields. However, this powerful technique has so far not been used extensively in forensic science. The COMFOR (Comprehensive Forensics) project addresses two primary objectives.

1. The development of robust comprehensive two-dimensional chromatographic methods for the chemical profiling of complex natural materials of forensic interest.
2. The development of a statistical framework for establishing the evidential value of complex multidimensional chromatographic data.

Our initial research focuses on ignitable liquids (oil-derived products, in particular white spirits) that are frequently encountered in arson cases. Most forensic investigations of this type aim to limit (as much as possible) candidate matches to a crime scene sample. To this end, chemical profiling techniques are employed to determine characteristic features of a (natural) material. The added separation power of comprehensive 2D chromatography is ideal for forensic profiling applications. The addition of an orthogonal second dimension drastically increases the information content of a chromatographic analysis. This allows us to examine a greater number of trace compounds that relate to and are affected by the production, distribution, and storage of ignitable liquids. If these arise from random, uncontrolled processes, similarities between chemical profiles can represent high evidential value regarding the source of the material.

KNLe.12

FLOW-MODULATED COMPREHENSIVE 2D GAS CHROMATOGRAPHY COMBINED WITH HIGH-RESOLUTION TIME-OF-FLIGHT MASS SPECTROMETRY

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The combination of gas chromatography with high-resolution (HR) ToF MS generates a powerful analytical method, inasmuch that HR ToF MS provides sensitive full-spectrum data, characterized by both high resolution and mass accuracy. The presence of a molecular ion in the accurate-mass spectrum is highly desirable, because it can give a good idea on the molecular formula. Moreover, the use of EICs with narrow mass windows is a highly selective process, employed in targeted analysis, as it enables the reduction or elimination of chemical noise and matrix interferences.

The presence research is focused on a novel hyphenation, namely flow-modulation (FM) comprehensive 2D GC (GC×GC) and HR ToF mass spectrometry. In the former approach, a mega-bore column is employed in the second dimension, to exploit the vacuum-outlet conditions (GC×low-pressureGC). With regards to the ToF system used, it possesses the capability of high spectral production frequency (up to 200 Hz), good mass accuracy (< 1 ppm) and three resolution operational modes, namely unit mass, high (≥ 25,000 FWHM) and ultra high (≥ 50,000 FWHM). A series of FM GC×GC-HR ToF MS applications will be shown in which the potential of this promising approach is demonstrated.

GC×GC-TOFMS: A POWERFUL TOOL FOR HIGH-RESOLUTION MOLECULAR ORGANIC GEOCHEMISTRY AND CLASSIFICATION OF THE DEPOSITIONAL PALEOENVIRONMENT OF BRAZILIAN CRUDE OILS

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A detailed elucidation of the molecular composition of oil samples is a challenging task which involves routinely different analytical techniques (GC-MS and GC-MS/MS). Because of the restricted access to most substances present in petroleum due to the complexity of the matrix, Comprehensive Two-Dimensional Gas Chromatography (GC×GC) is ideal for the reduction of coelutions. Furthermore, its hyphenation with a time of flight mass spectrometer (TOFMS) provides both the mass spectrum of each component and reliable quantitative data. GC×GC-TOFMS extends the analytical capability beyond the presently known biomarkers, leading to the possibility of refining the application of organic geochemistry to distinguish more subtle differences in the molecular composition for a better understanding of the more complex Petroleum Systems, that represent the new frontiers of oil exploration.

The main target of this work is to show that GC×GC-TOFMS is the first analytical technique with the potential to allow a breakthrough in traditional biomarker investigation, paving the path for a true High Resolution Molecular Organic Geochemistry (HRMOG) approach. A representative number of crude oils from different Brazilian basins were selected and GC×GC-TOFMS separation allowed the determination of minor molecular composition contrasts which refined the characterization of marine and lacustrine depositional environmental and their mixtures.

One example of the importance of the HRMOG approach is the separation of C31 3β-methyl-hopane. This compound, present at trace level, is coeluting with gammacerane and the isomer C31 3β-methyl-moretane. The separation with the gammacerane can be addressed by an MRM (426>205) experiment, although for the C31 3β-methyl-moretane this is not possible, because of the same fragmentation pattern. In Figure 1, the plot reporting the non-conventional parameter based on this key component (3β-MH31/H30 %), against a traditional one, H30/St27 ααα (S+R), clearly shows the separation of 12 selected oils in 3 different depositional paleoenvironmental groups.

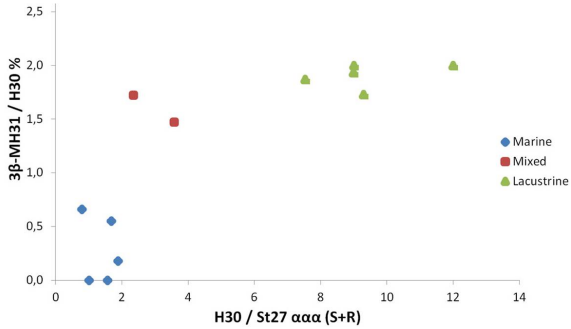

Figure 1. Depositional paleoenvironment classification of 12 Brazilian oils

KNLe.14

BIO-OIL PRODUCTION THROUGH FAST AND INTERMEDIATE PYROLYSIS OF FOREST INDUSTRY RESIDUES AND ITS CHARACTERIZATION USING GC/qMS AND GC×GC/TOFMS.

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Fossil fuels are considered an exhaustible resources and their use may also cause several well-known health and environmental impacts. Therefore, investigation of alternative and/or complimentary sources of energy is an important issue. Industrial, forestry and agricultural residues can be easily obtained and their use in different processes may result in convenient options regarding environmental and financial benefits. Pyrolysis, which can be defined as a thermal decomposition of organic material in partial or total absence of an oxidizing agent, is one procedure utilized for transforming industrial waste into chemical products or energy [1-5]. In this work, bio-oils obtained by intermediate pyrolysis (IP), fast pyrolysis (FP) and catalytic fast pyrolysis (CFP), using hardwood and softwood residues, were preliminarily studied using GC/qMS. The most promising bio-oils were further investigated using GC×GC/TOFMS. A higher yield of liquid product was observed in the FP than in the IP process: ~70% and ~50%, respectively. GC/qMS analysis indicated that ketones and phenols were the major compounds present in the bio-oils under study. The prevalence of these chemical compounds suggests the use of these bio-oils by polymer, food, and other industries. Furthermore, the catalyst ZSM-5, used on the CFP process, increased the percentage of aromatic hydrocarbons in the bio-oil, showing the potential of this type of process and residue for fuel production. GC×GC/TOFMS analysis had an important role in the study of the three most promising bio-oils, showing inaccuracies in the GC/qMS analysis due to co-elutions. Moreover, the presence of some polyaromatic hydrocarbons (PAH) provides a warning to prevent the production of toxic compounds through an appropriate process management.

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LECTURES ABSTRACTS
38th ISCC

EVOLUTION OF THREE DECADES OF SEPARATIONS RESEARCH

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Our research in separations has extended into many different areas of science and technology over the last 35 years. It began with the theory and use of pseudophases and colloids in chromatography and eventually capillary electrophoresis. It was quickly extended to chiral separations which changed the pharmaceutical industry and asymmetric synthesis. There were brief forays into bubbles, membranes and planetary/space research. These were followed by major efforts involving microbe separations, ionic liquid R & D and associated work in mass spectrometry. The highlights of this work and its impact on biomedical science, environmental science and agricultural and food science will be mentioned.

AFTER 38 MEETINGS … IS CAPILLARY GC APPROACHING THE STATUS OF MATURE TECHNOLOGY ?

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Since its introduction in 1957, capillary gas chromatography (capillary GC) has evolved into the most widely used technique for the analysis of volatile and semi-volatile organic solutes. In its evolution, the International Symposia on Capillary Chromatography (initially on Glass Capillary Columns) started in 1975 by Rudolf Kaiser, were of utmost importance. Very often, papers presented at this symposium series were a complete surprise to the participants and had a fundamental impact on the present state of the art … to mention a few: the introduction of fused silica columns, cross-linking of stationary phases, programmed temperature vaporization (PTV) injection, multidimensional capillary GC, etc. The impact on other capillary techniques was also very important, e.g. micropacked LC, capillary electrophoresis, microfabricated systems, etc.
In this contribution, an overview will be presented of the most important developments in capillary GC presented at this symposium series and illustrating, at the same time, the long and often genius work of the pioneers to present-day chromatographers.
On the other hand, capillary GC is nowadays considered as a mature analytical technique with many established applications. We asked ourselves some critical questions whether this claimed maturity is generally valid. The questions will be presented and answers, often related to new developments, will be advanced.

SEPARATION OF CHIRAL COMPOUNDS IN FOOD ANALYSIS: A CHALLENGING ISSUE FOR CEC AND NANO-LC

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The analysis of chiral compounds is a very interesting topic of research in various application fields, e.g., pharmaceutical, biomedical, environment, food chemistry etc. Therefore the enantiomers separation is a challenging issue for several separation techniques such chromatographic and electromigration ones. It is known that many compounds of natural products can be chiral with at least one couple of enantiomers. In addition two enantiomers possess very similar physical-chemical properties and just for this they can differently interact with chiral compounds present in a certain environment to produce different effects. This is the case of proteins, receptors etc. present in human body. Therefore the analysis of chiral compounds present in foodstuffs is of paramount importance for assessing their authenticity and adulteration, determination of aroma components, evaluation of processing and storage, age dating, control of fermentation processes, determination of chiral metabolites etc. Literature offers a large number of examples of applications dealing with chiral analysis carried out by high-performance liquid chromatography (HPLC), while data concerning the applicability of miniaturized techniques such as capillary electrochromatography (CEC) and nano-/capillary liquid chromatography (nano-LC/CLC) to this topic are limited. Considering the features of CEC and nano-LC/CLC (efficiency, resolution, speed of analysis, lower dilution, reduced volumes of solvents etc.) they can be advantageously used for chiral analysis.
In this communication the main characteristics and potentialities of the above mentioned miniaturized analytical techniques will be reported with special attention to the separation of chiral compounds considering the main approaches used till now (e.g., separation mechanisms, chiral stationary phases and mobile phases used). Examples of applications done in the field of food chemistry will also be discussed.

LE.04

HAND-PORTABLE INSTRUMENTATION FOR GAS AND LIQUID CHROMATOGRAPHY

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There is an increasing interest in miniaturizing GC and LC instrumentation so they can be easily taken to (and operated at) a sampling site. However, taking instrumentation from a laboratory environment for on-site analysis opens a whole new set of challenges. GC is the easiest of the chromatographic techniques to miniaturize, and various portable instruments have been reported. Adding a mass spectrometer (MS) to the GC has been considerably more challenging; however, reliable hand-portable GC-MS instrumentation is now available. Most of our recent efforts have centered on collecting target analytes from air and water, and transferring them to the injection port of the GC-MS system for analysis. Improvements in high-flow air sampling, solid phase microextraction, needle-trap and vacuum-assisted surface sampling will be described. LC has lagged behind GC in development of hand-portable instrumentation because of difficulties encountered in miniaturizing the high pressure pumping system and most common detectors (i.e., UV-absorption and MS) to acceptable size, weight, robustness and power usage. Mobile phase transport and disposal in the field is an added concern associated with portable LC. A new battery-operated (24 V DC) LC system with nano-flow pumps, stop-flow injector and on-column UV-absorption detector is currently under development in our laboratories. This system provides greatly reduced consumption of solvents compared to conventional LC systems. Each pump weighs approximately 1.4 kg (3 lbs) and can generate up to 110 MPa (16,000 psi) pressure. A major advantage of the pumping system is that it does not employ a splitter, since it was specifically designed for capillary column use. The pumps have demonstrated excellent results for flow rate calibration, percent injection carry-over and retention time reproducibility. The UV-absorption detector was found to be comparable in performance to commercially available detectors. Developments such as these will allow unprecedented detection accuracy and speed at the source of contamination, which is essential for timely response necessary to protect human health and safety.

LE.05

A HIGHLY MINIATURIZED CE-ESI-HPMS SYSTEM

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Mass spectrometry (MS) is a powerful analytical technique due to its sensitivity, versatility, and ability to provide chemical and structural information of molecules; because of this, it is often the detection method of choice for a wide range of applications. Electrospray ionization (ESI) has significantly expanded the range of mass spectrometric analysis to include biomolecules and other liquid-borne analytes, allowing integration of liquid-based separation to reduce the complexity of the materials injected into the MS at any given time. LC-MS analysis has become an incredibly powerful research tool, especially for biologically relevant analytes. However, conventional LC-MS systems are usually confined to dedicated laboratories because they are large, expensive, complex, and require significant amounts of power. LC systems are limited by the need for a rugged system of pumps, valves, and tubing, while mass spectrometers are limited by low pressure operation, which requires bulky, fragile, and expensive turbomolecular pumps. A smaller, more portable, and simpler liquid phase separation/MS analytical platform could be applied to a wide range of fields such as industrial reaction monitoring, forensics, environmental monitoring, metabolomics, and clinical diagnostics.

Our lab has previously developed technology to integrate microfluidic CE separations with ESI, resulting in high efficiency separations of small molecules, peptides, and proteins with the added benefit of MS detection. A microfluidic platform reduces sample volume, decreases analysis time, and provides flow rates more easily coupled to a mass spectrometer. Additionally, our lab has developed miniature cylindrical ion trap (CIT) based mass spectrometers capable of operating at pressures up to and exceeding 1 Torr, three orders of magnitude higher pressure than conventional ion traps; an approach we call high-pressure mass spectrometry (HPMS). Operation at high pressure reduces pumping requirements and significantly reduces the size, weight, and power of the instrument. We have recently demonstrated that we can efficiently couple microchip CE-ESI technology with a miniature CIT operating at pressures exceeding 1 Torr. Externally generated ions are transmitted into the instrument using a simple capillary interface, ion optics, and DC voltages. Data will be presented showing simple infusions and electrophoretic separations using this microfabricated CE-MS system with a focus on biologically relevant liquid-borne analytes such as amino acids and peptides.

LE.06

SUB-2 µM SILICA PARTICLES WITH INTERNAL POROSITY: CHARACTERISTICS AND ANALYTICAL IMPLICATIONS

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The use of very small (sub-2 µm) particles is essential in the increasingly popular ultrahigh-pressure liquid chromatography (UHPLC). Correspondingly, high pressures demand rigid column materials such as spherical silica particles. Small pellicular silica particles yield high column efficiencies, now shown for a variety of analytical separations across a wide range of solute types, albeit at some compromise of sample capacity. Alternatively, silica-based monolithic capillary columns, featuring both large and small pores together, have received considerable attention. We have recently introduced (Mann et al., Anal.Chem. 2013, 85, 1905) a new type of sorption materials, consisting of a spherical particle with an interconnected network of ~ 100-nm pores throughout, and featuring a large specific surface area (~200m^2/g). This material has been synthesized through a novel ultrasonic spray pyrolysis process. We estimate that such a combination of small and large pores, further supplemented with the chemical versatility of surface silanol groups for modification, may provide nearly ideal chromatographic media for biomolecular separations. To demonstrate substantially enhanced sample capacity of these materials for chromatographic enrichment of glycoproteins, we have derivatized these materials with lectins. More recently, we have evaluated the kinetic attributes of slurry-packed capillary columns through the use of zero dead-volume electrochemical detection. The reduced plate height vs. velocity curves were recorded in comparison with the pellicular materials of comparable particle size, indicating a great analytical potential for the macroporous media. Favorable van Deemter plots were obtained, with the h-value as low as 1.7 for small molecules as the solutes, for the macroporous materials. Further improvements in the column preparation are currently sought through removal of particle ‘fines” using preparative hydrodynamic chromatography fractionation of these particles, as well as through the in-situ functionalization of particles inside the separation capillaries.

BACKGROUND ELECTROLYTE ADDITIVES FOR CAPILLARY ELECTROPHORESIS OF BIOMOLECULES

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Capillary electromigration techniques can be performed by a variety of modes, based on different separation mechanisms that, in most cases, can be selected by simply changing the operational conditions, specifically the composition of the electrolyte solution (BGE). In a multiplicity of separation modes, the BGE consists of a buffered aqueous solution, which may contain one or more no-buffering additives, either neutral or charged, and can be mixed with an organic solvent. This communication discusses the influence of a variety of BGE additives that we have employed for the separation of biomolecules in capillary electrophoresis, using either uncoated or coated fused-silica capillaries. The investigated additives include buffering agents suitable to control the protonic equilibrium in a wide pH range and compounds effective at reducing untoward interactions of the analytes with a variety of active sites on the inner surface of bare fused-silica capillaries, which comprise inert siloxane bridges and ionisable silanol groups. Most of the compounds employed for this purpose act either as masking or competing agents for the active interacting sites on the inner wall of the bare fused-silica capillary, so that they are not accessible to the analytes. Others are expected to functioning as strong ion-pairing or competing agents for the interacting moieties of the analytes exposed to the BGE, subtracting their availability to the interacting sites on the capillary wall. Zwitterionic, anionic or cationic ion-pairing agents, as well as certain ionic liquids and organic solvents, have also been employed to affecting the electrophoretic mobility of analytes and, therefore, their selective separation. Also discussed is the influence of the different BGE additives on the generation of the electric double layer at the interface between the capillary wall and the electrolyte solution, determining direction and velocity of the electroosmotic flow, either in bare or coated fused silica capillaries.

LE.08

THE IMPACT OF LC-MS IN ELUCIDATING THE ORIGIN OF DISEASED CELL STATES

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The importance of advancing the frontiers of science to enable early disease detection can never be overstated. The technological outbreak of new instrumentation and techniques that has been witnessed in the past two decades has empowered researchers with an in-depth understanding of the molecular mechanisms that contribute to disease progression. Key technologies that played a decisive role in achieving such a scientific awareness include separation science and mass spectrometry (MS). In this work, we review the milestones that contributed to advancing the power of our analytical strategies, with focus on the impact of interfacing liquid chromatography (LC) to MS detection on shaping our approaches for investigating the complex milieu of biological cell states. In particular, we will address the capabilities of LC-MS for exploring the proteome, post-translational modifications and signaling pathways that lead to the progression of cancerous cell states. We will discuss our findings that resulted from the analysis of breast cancer cells (MCF-7/ER+ and SKBR-3/Her2+) in the G1 and S stages of the cell cycle, and will examine the challenges that lie ahead in deciphering the meaning of massive amounts of data that can be generated by the use of these technologies.

LE.09

HYDROGEN ISOTOPE RATIO MASS SPECTROMETRY AND HIGH RESOLUTION/ HIGH ACCURACY MASS SPECTROMETRY IN METABOLITE IDENTIFICATION STUDIES: DETECTING TARGET COMPOUNDS FOR SPORTS DRUG TESTING

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In sports drug testing, comprehensive studies on the metabolism of therapeutic agents with misuse potential are necessary to identify metabolites that provide utmost retrospectivity and specificity. By commonly employed approaches minor and/or long-term metabolites in urine might remain undetected. Hence, an alternative strategy to unambiguously identify the majority of urinary metabolites including low-abundance representatives is desirable.

Urine samples were collected for 20 days during an elimination study with an oral dose of 5 mg of 17α-C^2H_3-metandienone. The specimens were processed according to established sample preparation procedures (including fractionation and deconjugation) and subjected to gas chromatography/hydrogen isotope ratio mass spectrometry (GC/IRMS) analysis. Due to the deuteration of the administered drug, urinary metabolites bearing the deuterium label yield abundant and specific signals on the GC/IRMS instrument resulting from the substantially altered $^2H/^1H$ ratio. The sample aliquots were measured by gas chromatography/time-of-flight (GC/Q-TOF) mass spectrometry using identical GC conditions, allowing high-resolution/high-accuracy mass data to be obtained on all urinary metabolites previously identified by IRMS.

Within the IRMS chromatograms, labeled metabolites were identified up to 20 days after administration at urinary concentration down to 0.25 ng/mL. More than 50 metabolites were observed with the earlier described long-term metabolite of metandienone, 18-nor-17β-hyroxymethyl,17α-methyl-androst-1,4,13-trien-3-one, being the most prominent glucuronidated metabolite in the studied time window. In the sulfoconjugated steroids fraction, a yet unknown metabolite was observed at m/z 283.1997 comprising the experimentally determined elemental composition of $C_{20}H_{21}{}^2H_3O$.

Combining IRMS with high-resolution mass spectrometry considerably facilitates and accelerates metabolite identification of deuterium-labeled compounds in urine. Of particular relevance in doping control, the principle is applicable also to other arenas of drug research, allowing the preparation and administration of e.g. radioactively labeled substances to be omitted.

DEVELOPMENT OF NOVEL SEPARATION MEDIA FOR MICROSCALE ANALYSIS

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Carbon-based nanomaterials, including graphene, fullerene, and carbon nanotubes, have been investigated in various fields. They have large delocalized π-electrons that can form π-stacking interactions with aromatic compounds, so that the use of carbon nanomaterials in separation chemistry has been studied. To develop a novel separation medium in a capillary enabling a specific separation based on π-stacking, C_{60}-fullerene was immobilized onto the inside wall of the capillary for open-tubular capillary LC. Separation behaviors of alkylbenzenes and polycyclic aromatic hydrocarbons were analyzed using both the C_{60}-fullerene coated capillary and a common ODS column. The results suggested that π-stacking by C_{60}-fullerene strongly affected the separation.

An influence on ecosystem by pharmaceutical and personal care products (PPCPs) in environmental water has been attracted much attention, and quantitative analyses of PPCPs have been fairly required. Solid phase extraction (SPE) is a powerful technique for concentration and cleanup of environmental water samples. Since a typical hydrophobic or ionic interaction is utilized for SPE, it is difficult to achieve the selective concentration of highly hydrophilic PPCPs. In this study, a novel adsorbent for SPE was developed by using an interval immobilization technique, one of the molecularly imprinting techniques, for the selective concentration of a popular pharmaceutical, sulpiride. The adsorbent was suitable for the pretreatment column in on-line column switching SPE–LC–MS. A quantitative analysis of 10 ppt sulpiride was achieved.

LE.11

THE DEVELOPMENT AND APPLICATION OF 3D PRINTED METAL CAPILLARY CHROMATOGRAPHY COLUMNS

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The use of 3D printing technology, in both plastics and more recently in metals, has received a great deal of attention from analytical scientists and workers in the field of diagnostics and portable analytical devices. In the field of chromatography, new 3D printing technologies open up new possibilities in instrument design, including column design and fabrication, particularly in miniature and compact formats. Herein we present work carried out on the design and 3D fabrication of metal (titanium and stainless steel) capillary columns, for both particle packed and monolithic (gas and liquid) chromatography applications. Early designs included double-coiled internal formats, within 2 x 2 cm square printed metal disks, with incorporated printed column fittings, with column dimensions of 0.8 mm I.D. x 600 mm L. The presentation will include details of stationary phase packing, using reversed-phase C^{18} silica particles and subsequent column characterisation, including flow rate and temperature effects upon efficiency in isocratic and gradient mode, the latter using direct contact rapid column heating. Additionally, the formation of polymeric (PS) monolithic phases within titanium printed columns will also be presented, and their subsequent application to chromatographic separations discussed. Potential of the latter format for high temperature applications, including gas chromatography, will be included.

LE.12

NANOPARTICLE-BASED SAMPLE PREPARATION FOR SELECTIVE ANALYSIS OF OXIDIZED LOW-DENSITY LIPOPROTEINS AND PHOSPHOLIPIDS BY HPLC-MS

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Oxidized low-density lipoproteins (OxLDL) are involved in pathophysiological processes such as atherosclerosis. They may be formed under conditions of chronic hyperlipidemia which may lead to lipoprotein aggregation within the intima of the blood vessel and subsequent oxidation by reactive oxygen species (ROS). Macrophages target OxLDLs by endocytosis via scavenger receptors, which are distinct from LDL receptors. The oxidized LDL accumulates in the macrophages and other phagocytes forming so-called foam cells. They may represent the origin of plaque build-up in blood vessels. In this study, we are interested to analyze oxidized phosphatidylcholines (OxPCs) in OxLDL in the plasma. They are regarded as potential biomarkers for oxidative stress, being indicators for a mismatch of pro-oxidative and anti-oxidative processes in the body.

In our study, we used the specific affinity of anti-OxLDL-antibodies (Abs) conjugated to gold nanoparticles (GNPs) for extraction and enrichment of OxPCs via selective trapping of OxLDL from plasma combined with the sensitive detection by liquid chromatography / tandem-mass spectrometry (LC-MS/MS). In this presentation the development of the GNP-antibody conjugate will be discussed in detail. Successful bioconjugation chemistry of Abs was accomplished via bifunctional polyethylene glycol (PEG) spacer and and subsequent antibody coupling. The effect of structural parameters and the surface chemistry on the effectiveness of the immunotrapping step will be presented. The OxLDL@GNP bioconjugate was also characterized by determination of the dissociation constant K_d of OxLDL via static binding capacity measurements. The new bioconjugated immunoaffinity nanomaterial was utilized for selective extraction of OxLDL from plasma and subsequent LC-MS/MS analysis. In summary, the application of GNP-based bioanalysis for selective targeting of OxLDL and the fast and sensitive detection by LC-MS/MS offers new possibilities for targeted lipidomics in lipoproteins as well as for oxidative stress lipid biomarker screening.

LE.13

CHIP BASED MICROFLUIDIC UPLC-MS THEIR IMPACT UPON CHALLENGES IN THE DETECTION OF SMALL MOLECULES IN FOOD SAFETY

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Recent applications for the separation of residues and contaminants in the field of food safety are mainly using chromatography with columns containing sub-2 µm particles. We present several examples of the analysis of sub µg/l levels of compounds like steroids and natural toxins in different commodities. This was done by coupling conventional UPLC (column id. 2.1mm) as well as a microfluidic UPLC (id. 150µm) to the same highly sensitive triple quadrupole tandem MS system. With the availability of microfluidic UPLC it is possible to gain extra sensitivity by reduction of column diameter and efficient sampling from the nano electrospray ionization. Within this study we investigated the benefits of microfluidic UPLC applications and performed a critical comparison with the conventional UPLC methods.
The rationale behind this research originates from both a green analytical chemistry philosophy and from the limited availability of expensive standards and/or sample extract volumes. According to our microfluidic UPLC-MS/MS results, solvent use could be successfully reduced by more than 90% per run. Furthermore, the sample usage was reduced by a factor 5-10. The sensitivity increase was compound depending but between 2 and 5-fold; when taking into account that also a factor 5-10 less was injected the sensitivity on-column was increased by a factor 10-50. The most critical aspect with the microfluidic UPLC is the injection volume and corresponding injection solvent (organic strength). It can be foreseeable that for some applications this will cause chromatographic problems. Nevertheless, for the application where this was not the case excellent peak shapes and sensitivity were obtained. For the fortified samples comparable results as with the conventional UPLC applications were obtained. Based on these results, it is concluded that the 150µm microfluidic UPLC formats featuring sub-2 µm particles are a realistic option for both screening and confirmation of small molecules in food safety.

LE.14

A NEW PARTICLE FOR CHROMATOGRAPHY

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In recent years there has been a renewed interest in particle technology for both HPLC and UHPLC. Prior to this renewed interest there was very little novel development in HPLC particle technology, since the primary changes related to reducing the particle size from 5micron down to 1.7 microns. The purity of the underlying silica has become purer, for example the sodium levels have been reduced from around 1500 ppm down to 10 ppm, which has resulted in more robust chromatography, but has not intrinsically affected the structure of the particles.
We have also seen new hybrid silicas but again the particle size and particle structure has not resulted in a step change in performance from previous offerings.
The reintroduction of pellicular or core shell particles however was a great step change in particle design and brought a new interest into particles. Recent papers have shown that these core shell particles when used in the separation of small analytes benefit from their very narrow particle size distribution that leads to exceptionally well-packed columns, and also the reduced dead volume caused by the solid core.
This presentation outlines a totally new particle called a Sphere-on-Sphere particle manufactured using a one-step synthesis delivering a near monodispersed particles and core shell morphology.
The morphology of the particle has been designed to deliver the real advantages of the core-shell particles.
The total diameter of the particle and the core diameter can be controlled together with the effective pore diameters on the surface of the material. All the particles are solid and so the pores are as a result of the interconnectivity of the outer smaller spheres attached to the large single central solid core. The total particle size can be varied from 3 to 10 microns with an effective pore size range up to 1.5 microns.
Examples will be shown of these S-O-S particles used in normal phase and reversed phase separations of small molecules and also proteins. Further example will be shown of how these particles can be used as scaffold particles or carrier particles in which new, novel stationary phase can be trapped in the outer layer.

PROPERTIES OF CAPILLARY GAS CHROMATOGRAPHY USING WATER AS A STATIONARY PHASE

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The development of stationary phases for gas chromatography remains of great interest due to the significant impact that they can provide to separation selectivity and resolution. As a potential stationary phase, water offers interesting properties of being very polar, non-toxic, inexpensive and widely available. For instance, some early reports of using humidified carrier gases in packed column gas chromatography also noted that an in-situ coating of water on the surface of the stationary phase particles led to some unique separation properties observed. Based on our previous explorations in supercritical fluid chromatography, we have recently begun to explore a novel method of using a water stationary phase in capillary gas chromatography. Here we will describe this method and its primary operating considerations along with the general separation characteristics encountered when using it in some example applications.

LE.16

POLYIONIC IONIC LIQUID GC STATIONARY PHASE EVALUATIONS

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Ionic liquids are a class of nonmolecular ionic solvents with low melting points. These liquids are unique combination of cations and anions and can provide a variety of different selectivities when used as stationary phases in capillary gas chromatography. The majority of the polyionic ionic liquid phases that we have been evaluating all provide polar and highly polar selectivities similar to polyethylene glycol based our biscyanopropylpolysiloxane phases. These phases will provide unique selectivity for the evaluation of a number of petrochemical samples. The purpose of our studies is to determine the effects changing the cation and spacer groups on the selectivity and stability of the phases. Selectivity was determined and compared using various isothermal and temperature programmed test mixes. Particular cation and anion combinations appear to provide very unique selectivity by the shifting of normal alkane relative to aromatic and other chemical species. New combinations of cations and anions have been evaluated which provide unique selectivity and stability to the phases. We will also demonstrate the effects of the various functional group combinations on the overall stability of the ionic liquid stationary phases.

GC-MS OF POLYCYCLIC AROMATIC HYDROCARBONS IN HUMAN FOODS AND TOUCHABLE ENVIRONMENTS

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It is curious about how often polycyclic aromatic hydrocarbons (PAHs) can be found in our foods and ordinary touchable materials (e.g. books, newspapers and magazines). As stable chemicals with more than 200 derivatives PAHs can easily be produced in environments with organic productions and wastes[1-3]. Because some PAHs are carcinogenic, teratogenic and mutagenic (e.g. benzo[α]pyrene)[4-5], they must be known and controlled if possible. However, these remain challenging because it is difficult to analyze and identify trace PAHs[6]. This group has hence focused on the exploration of trace analytical methods, mainly focusing on solid-phase micron-extraction (SPME)-based GC-MS approaches. An ultrafast (ca. 20-30 s) approach has been invented to coat sorbents on SPME fibers. In the analysis of trace PAHs, it was found that graphene exhibited at least 100-fold higher extraction efficiency than most of the commonly used coatings, with recovery > 90%. A method of graphene-based SPME-GC-MS was thus established to analyze the trace PAHs in skewered or roasted meats, cooking smokes, evaporates from newly issued printings (e.g. newspapers, books and magazines), indoor air, and the airs of nearby gas stations and above new bituminous streets. We were shocked that new printings could release high content of PAHs, suggesting that readers should be careful while reading a new printing, better keeping it away a certain distance from you. In addition, the PAH content in roasting, skewering and baking areas was high, suggesting people should be better away the kitchens and barbecue regions. More data will be released and shared during the conference.

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LE.18

MULTILAYER INTERPARTICLE LINKING HYBRID MOF-199 FOR NON-INVASIVE ENRICHMENT AND ANALYSIS OF TRACE PLANT HORMONE ETHYLENE BY GAS CHROMATOGRAPHY

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Ethylene, with the quite simple structure and small size, is a well-known important plant hormone [1]. Non-invasive monitoring of the ethylene concentration is of utmost importance during many developmental processes of plants. However, ethylene is too volatile for quantitative retention by sorbents at ambient temperature and it is at trace level from plant samples. So the efficient enrichment and analysis of trace ethylene still remains challenging. In this work, a simple and mild multilayer interparticle linking (MIL) strategy was proposed to fabricate a novel hybrid MOF-199 enrichment coating. Strong chemical interparticle linkages throughout the coating improved the durability and reproducibility of hybrid MOF-199 coating dramatically. This coating performed a significant extraction superiority of ethylene over commonly-used commercial coatings, attributed to the multiple interactions including 'molecular sieving effect', hydrogen bonding and π-π affinity. The incorporation of multiwalled carbon nanotubes (MWCNTs) into MOF-199 further improved the enrichment capability and also acted as a hydrophobic 'shield' to prevent MOF-199 from being occupied by water molecules, which effectively improved the moisture-resistant property of MOF-199/CNTs coating. Finally, this novel enrichment method was successfully applied for the non-invasive analysis of trace ethylene, methanol and ethanol from fruit samples with relatively high humidity by gas chromatography. The detection limit was 0.016 μg/L for ethylene. It was satisfactory that trace ethylene could be actually detected from fruit samples by this non-invasive method. Good recoveries of spiked grape, wampee, blueberry and durian husk samples were obtained in range of 90.0-114%, 79.4-88.6%, 78.5-86.8% and 85.2-105% with the corresponding RSDs of 4.8-9.8%, 6.9-8.9%, 3.8-8.1% and 9.3-10.5% (n=3), respectively.

Acknowledgements
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LE.19

SOL-GEL CHEMISTRY-BASED COLUMN TECHNOLOGY FOR CAPILLARY GAS CHROMATOGRAPHY

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Sol-gel chemistry [1] offers a unified approach to the creation of surface-bonded hybrid organic-inorganic stationary phases that intrinsically provide enhanced column performance in chromatographic separations. This innovative approach to column technology can be applied to a wide range of chromatographic separation and sample preparation techniques [2]. Sol-gel column technology for capillary gas chromatography (GC) was developed by our group [3-5] utilizing the chemical ability of sol-gel precursor(s) (e.g., metal alkoxides) to undergo hydrolytic polycondensation reactions leading to the formation of a three-dimensional inorganic network structure inside a fused silica capillary where the sol-gel reactions are carried out. Additionally, if a sol-gel-active organic ligand (e.g., a hydroxy-terminated polymer) is used as a component of the sol solution, it can also take part in the polycondensation reaction providing a hybrid organic-inorganic sol-gel network. A small part of this sol-gel network evolving in the vicinity of the capillary inner walls has the opportunity to get chemically anchored to it. This surface-bonded layer of the hybrid material can serve as a stationary phase in the column after the free patches of the sol-gel network in the central region of the capillary has been removed from the capillary and the column has been subsequently conditioned. Direct chemical bonding of the stationary phase to the capillary inner walls is responsible for enhanced thermal- and solvent stability of sol-gel GC columns. An added advantage of the sol-gel column technology is that it does not involve the use of high temperature to carry out the reactions; the sol-gel reactions take place under extraordinarily mild thermal conditions – typically at room temperature. It also provides a facile pathway to integrating intrinsic properties of organic and inorganic materials offering a new opportunity to fine-tune selectivity of chromatographic separations through use of desired organic and inorganic components in the sol solution, as well as via manipulation of their concentrations in the sol solution.In this presentation, the theoretical and experimental aspects of sol-gel chemistry will be discussed in the context of capillary GC column technology. Recent experimental data from our laboratory involving both silica-based and nonsilica-based stationary phases will be presented to illustrate state of the art and potentials of sol-gel GC column technology.

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LE.20

COMBINING DRIED BLOOD SPOTS WITH STABLE-ISOTOPE TRACERS TO PROFILE DYNAMICS OF GLUCOSE METABOLISM IN HUMAN SUBJECTS

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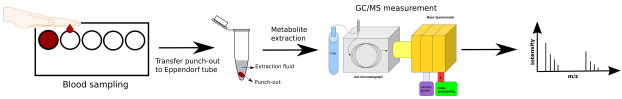
Dried blood spots have emerged as an easy sampling tool for human blood. On the other hand, stable-isotope labeling is a safe procedure to profile the dynamics of metabolism or turnover of target metabolites on a whole organism scale. Here, I will present time-resolved dynamics of glucose metabolism in blood after oral administration of 13C stable-isotope labeled glucose in human subjects. We developed a protocol to extract polar metabolites from dried blood spots and applied GC/MS to acquire MS data. To extract pure mass spectra from this complex data, we applied the ion-chromatographic deconvolution algorithm of our MetaboliteDetector software. Finally, based on the deconvoluted mass-spectra, we set-up linear equation systems to correct the data for naturally occurring stable-isotopes and determined mass isotopomer distributions (MIDs) for target metabolites. Based on time-resolved MID determination we got insights into glucose turnover, lactic acid production and Cori cycle activity on a whole organism scale.



SLIP FLOW IN TOP-DOWN PROTEOMICS

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Submicrometer particles enable higher efficiency in protein separations, and we use particles of just less than 500 nm in diameter. With reversed-phase chromatography, slip flow allows for reasonable columns length of up to 5 cm despite being packed with particles of such small diameter. The capillary end is tapered for direct nanospray into the inlet of a mass spectrometer. Using commercial nano-UHPLC and a commercial mass spectrometer, we explore column performance and applications in top-down proteomics.

LE.22

CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY WITH SHEATH-FLOW CHEMISTRY: A NOVEL TOOL FOR THE CHARACTERIZATION OF PLANT EXTRACTS

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The combination of capillary electrophoresis (CE) as a high performance separation technique and mass spectrometry (MS) as a detection method providing valuable structural information is a widely accepted tool for the analysis of a range of samples from different origins. Recently we introduced a concept adding further functionality to this well established hyphenated technique, namely the possibility to perform chemical reactions within the sheath liquid of the CE-MS interface. Thereby, we could demonstrate this concept using two different reactions, namely hydrogen/deuterium exchange and reaction with a stable free radical (DPPH$^{\bullet}$).
Whereas the first approach allows the on-line determination of exchangeable protons for analytes separated in CE-MS, the latter can be used as a fast screening tool for antioxidant activity of individual compounds. In both cases the described methodology allows rapid and information-rich analysis with minimal reagent and sample consumption to be performed. In the present work, we want to demonstrate the applicability of CE-MS with sheath flow chemistry for the characterization of plant extracts. Firstly, besides mass spectra obtained with the Q-TOF mass analyzer, additional structural information can be deducted from comparing the results from conventional sheath liquids with those from deuterated ones. Secondly, by adding sub-mM amounts of DPPH$^{\bullet}$ to the sheath liquid, antioxidant properties of extract constituents can be detected and, as a high resolution MS is employed for detection, also the products from reaction with the DPPH radicals can be identified.

LE.23

EVALUATION OF MICRO LC-MS FOR LARGE SCALE METABOLIC PHENOTYPING

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Metabolic phenotyping offers an insight into mechanisms of toxicity, responses to therapeutic intervention, and disease progression. Ultra performance liquid chromatography-mass spectrometry (UPLC-MS) is a powerful tool for metabolic profiling, with excellent separation and detection capabilities. The utility of UPLC technologies for generating large peak capacities and high-throughput methods provides the speed, sensitivity and reproducibility required for large-scale epidemiological studies. Despite the advantages UPLC offers in terms of highly efficient and very fast separations, current LC-MS methods do suffer from certain drawbacks, in particular the volume and cost of solvents required and difficulties in analysing very small sample volumes, such as those generated from rodent models or dried blood spots. For studies comprising thousands of samples, the use of 2.1 mm i.d. column formats combined with high linear velocities requires an exceptionally large volume of solvents which is both expensive and environmentally unfriendly. Reduced column diameters and scaled volumetric flow rates offer a significant reduction in solvent consumption. Until recently, however, micro LC has not been able to demonstrate the levels of robustness or throughput required for metabolic phenotyping. Here, we evaluate the applicability of micro LC for large-scale metabonomic studies. Sensitivity, robustness, solvent consumption, chromatographic performance and data quality are considered and a critical comparison of 2.1 mm, 1 mm and 150 µm column i.d. is made to determine whether micro LC is a viable approach for routine metabolic phenotyping.

LE.24

CHROMATOGRAPHIC MOLECULAR MASS ANALYSIS OF ULTRA HIGH MOLECULAR MASS POLYMERS

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Separation of ultra high molecular mass (UMM) polymers is known to have some unexpected features which can't be explained from the classical chromatographic theories. Peak shape and retention of UMM polymers depend on the mobile phase flow rate and a peak splitting is observed at high flow rates. Unusual behavior of UMM polymers is commonly explained either by a degradation of polymers during migration through the chromatographic column, or by conformational (coil-stretched) transitions of polymer molecules under the share stress caused by the flow. The performed investigation showed on the example of UMM polystyrenes that conformational transitions should be considered as the preferred source of the unusual chromatographic behavior of such analytes. The conformational transition was modeled as the reversible reaction of the first order, and both equilibrium and rate constants were calculated and discussed.

LE.25

COLLISION CROSS SECTION: A NEW STRATEGY FOR "CATCH ALL" NON-TARGETED SCREENING FOR RESIDUES AND CONTAMINANTS IN COMPLEX MATRICES

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The SANCO/11738/2013 [1] guidance document implemented in 2014 describes the method validation and analytical quality control requirements to support the validity of data used for verifying compliance with maximum residue limits, enforcement actions, or assessment of consumer exposure to pesticides in the EU. It is stated within the guidelines that “different types and modes of mass spectrometric detectors provide different degrees of selectivity, which relates to the confidence in identification” and requirements for identification are given which should be regarded as a guidance criteria for identification, not as absolute criteria to prove presence or absence of a compound. The current guideline strategies take the approach of making retention time tolerances more stringent and a ±0.2 minute retention time tolerance has been introduced. It is well recognised that retention time tolerances may be significantly impacted upon by matrix shifts and this approach may result in the requirement for very specific method and system parameters to be followed during a routine screening experiment. Alternatively, the application of a “catch all approach” enables more generic parameters to be utilised thus enabling screening to be performed more efficiently without the need to verify performance within every new matrix type, for example.
Within this study we demonstrate the potential of a multi-dimensional technique incorporating ultra performance liquid chromatography (UPLC) and ion mobility enabled QTof for trace level screening of pesticide residues in food. We illustrate the additional confidence that the use of Collision Cross Section (CCS) values derived from ion mobility drift times bring to non-targeted screening and investigate the impact on false detections whilst screening with wider tolerance parameters via validation in fruit and vegetable commodities. Additional filter based identification points will enable less stringent screening parameters and increased specificity simultaneously. The use of CCS offers the potential to reduce the initial specificity of applied screening parameters.

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LE.26

ON-LINE COMBINATION OF SAMPLE PREPARATION AND LIQUID CHROMATOGRAPHY USING COLUMN SWITCHING TECHNIQUES IN MINIATURIZED SCALE

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Miniaturization in sample preparation and chromatographic analysis is in accordance with the aims of green analytical chemistry. Over the past years several microextraction techniques emerged as alternative to conventional scale liquid-liquid extraction and solid phase extraction. Although not in the same pace, miniaturized liquid chromatography is also an emergent technique for many applications, with all major instrumentation companies currently developing commercial liquid chromatographs in such scales. In this presentation we will focus on the development and on applications of online combination of sample preparation and liquid chromatography using column switching techniques in miniaturized scale.

After defining the several miniaturized approaches (narrow-bore, micro-bore, capillary, nano, et cetera) of liquid chromatography, we will remark the importance and practical aspects of analyte focusing, which are mandatory for relative large volume injection and pre-concentration. Following this last point, we will remark the use of column switching in order to combine sample preparation with high and fast pre-column focusing.

Finally we will discuss recent developments and applications involving fully miniaturized column switching liquid chromatography, providing considerations about pros and cons of miniaturization, areas where it can be of great interest, and important aspects that will lead to the success of fully miniaturized coupling of columns.

ISOMER DISTRIBUTION ANALYSIS FOR IMPROVED HYDROCARBON MIXTURES CHARACTERIZATION

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The isomeric content and its distribution affect all the major physical and chemical properties of oils and fuels, including: combustion efficiency, octane number, flash point, viscosity, lubrication properties, solvation power, boiling points and melting points. However, since standard GC-MS can not be properly used for isomers distribution analysis in view of weakness or absence of their molecular ions, this important subject represents an untapped opportunity. The Aviv Analytical 5975-SMB GC-MS with Cold EI was used for the development of a new method of isomers distribution analysis for fuels and oils characterization. Cold EI is based on GC and MS interface with supersonic molecular beams (SMB) and on sample compounds ionization using a fly-through electron ionization ion source while they are vibrationally cold (hence the name Cold EI). GC-MS with cold EI provides trustworthy and largely enhanced molecular ions and isomeric structurally important high mass fragments for improved identification. Isomer distribution analysis (IDA) is based on their GC separation while the Cold EI provides molecular ions to all hydrocarbons isomers. The greater is the isomer branching the earlier is its elution time. Data will be shown for novel IDA applications of: A) Diesel fuel characterization for its origin. B) Crude oils source characterization via the ratio of isoprenoid isomers to linear-chain hydrocarbons. C) Engine oil cold start effectiveness linking with the degree of hydrocarbon branching. D) Forensic kerosene and diesel fuel residues source characterization via their isomer distribution. E) C-4 plastic explosives sources were characterized via its plasticizers isomers distributions. F) A new isomer based method was developed for pistol oil on human hands analysis for forensic linking between a suspect and a given fire arm. G) Cataglypghis ants communication was explored and we found that ants from different nests recognize each other via their wax isomer distributions

LE.28

SOLID PHASE MICROEXTRACTION SAMPLING AND PORTABLE GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR ATMOSPHERIC AIR MEASUREMENTS

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Volatile organic compounds (VOCs) that are emitted into the atmosphere from natural sources in marine environment and terrestrial biosphere affect significantly the global climate. They participate in many physicochemical processes. Biosphere produces and emits hundreds to thousands of biogenic volatile organic compounds (BVOCs) and studies have proved that these reactive compounds contribute significantly to the formation of aerosol particles e.g. by photo-oxidation to non-volatile species that lead to the formation and growth of secondary organic aerosols. Since aerosol particles have a clear influence on human health and atmospheric chemistry, their formation from BVOC emissions is particularly important to the climate. Portable instruments are more and more important to provide insight into the geographic variations, transport characteristics, and chemical dynamics of atmospheric aerosols and direct analytical approaches are needed for on-field environmental analysis.

In this talk, the applicability of selective solid-phase microextraction sampling including extraction step followed by the analysis with portable gas chromatography-mass spectrometry to the on-line determination of several BVOCs will be described. The results including those obtained at the Station for measuring Forest - Ecosystem Atmosphere Relationships, SMEAR II (http://www.atm.helsinki.fi/SMEAR/) will be compared with those achieved by proton transfer reaction mass spectrometer that provide long term in-situ measurements and a good time resolution.

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LE.29

FAST GC AND OPEN PROBE FAST GC-MS – APPROACHES FOR REAL TIME ANALYSIS WITH SEPARATION

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We developed a low thermal mass (LTM) fast GC module that is based on resistively heated stainless steel tube that accommodates a piece of GC column. It is located on an available detector port on the top of standard GC oven and uses its injector and detectors. An important benefit of our fast GC is easy column replacement combined with low column price since a piece of any standard GC capillary column can be used.

We combined an Open Probe inlet with the LTM fast GC and MS of GC-MS, forming a new method and device for obtaining real time analysis with separation. The Open Probe is based on a vaporization oven that is open to room air while having helium purge flow protection to eliminate air leakage into the oven and ion source.

Sample introduction into the Open Probe is as simple as: touch the sample, push the sample holder into the open probe oven for its thermal vaporization and have the results in 20-30 s.

Both Fast GC and Open Probe fast GC were coupled either with the Aviv Analytical 5975-SMB GC-MS with Cold EI, or with Agilent 5975 MSD for obtaining in-vacuum electron ionization followed by quadrupole based mass analysis.

Unlike DART, DESI and other related ambient ionization techniques, Open Probe fast GC-MS provides real time analysis in combination with GC separation, library identification, absence of ion suppression effects and uniform electron ionization response for improved quantitation.

Fast GC and Open Probe fast GC-MS will be demonstrated with several applications including hydrocarbon mixtures analysis, chemical reaction monitoring, as well as 20 s separation time and 30 s full analysis cycle time of heroin in its street drug powder and trace TNT on human hand analysis in 40 s with NIST library identification of TNT.

LE.30

THE INCORPORATION OF CALIX[6]ARENE DERIVATIVES INTO SOL-GELS FOR THE PREPARATION OF SHAPE SELECTIVE STATIONARY PHASES FOR GAS CHROMATOGRAPHY

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The detection of more micropollutants in environmental matrices is still a challenge due to their large number of congeners or isomers. The incorporation of a shape selector into a sol–gel matrix should improve the selectivity of the stationary phases toward positional isomers and pollutant congeners. Recently, some of the co-authors have developed new families of calix[6] arene-based receptors rigidified in the cone conformation by a tripodal aza-cap [1,2].
This work deals with the anchoring of calix[6]trenamide selectors (Figure 1) into a sol–gel network. An original method [3] for the preparation and coating of the stationary phase was developed to improve control over the ratio between the macrocyclic selectors and the PEG and to reduce the quantity of the macrocyclic selector used.
The new stationary phases exhibited excellent column efficiencies over a large range of temperatures and thermal stability up to 280 °C. In addition, organic compounds, including aromatic positional isomers, PCB congeners, PAHs and enantiomers, were analyzed. Then, separation factors obtained on the stationary phases with or without macrocyclic selectors are compared. The relationship between the structure and the chromatographic properties of the selectors is discussed.

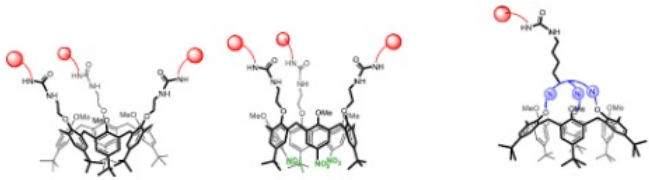

Figure 1: Calix[6]arene derivatives

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LE.31

A REVIEW OF CHROMATOGRAPHIC METHODS USED IN THE CHARACTERIZATION OF THE ELECTROLYTES IN LI-ION BATTERIES AND THEIR VOLATILE EMISSIONS UNDER NORMAL AND ABUSE CONDITIONS

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Li-ion batteries have contributed significantly to the rapid advancement and widespread use of electronic appliances, particularly in the telecommunication and entertainment industries [1]. A further field where Li-ion batteries are applied, in typically much larger aggregates, is electrical transportation. Due to the larger scale of the batteries, the hazards associated with malfunctioning or even misuse are significantly more severe, and have recently attracted great public interest due to the occurrence of a number of spectacular incidents.

A thorough understanding of the processes taking place with the highly flammable electrolyte in Li-ion batteries and an objective assessment of the hazards associated with its use requires a detailed characterization of the electrolyte in original state and under conditions of normal and abuse. While the solvent of the organic electrolyte typically is a well-defined mixture of carbonates (e.g. dimethyl carbonate, diethyl carbonate or ethylene carbonate) with the addition of a lithium salt, the degradation products formed even during normal use and to a much larger extent under abuse conditions are of sheer bewildering number: They are formed by thermal degradation, by reduction and oxidation processes at the electrodes, and by radical and onic polymerization reactions within the electrolyte solution.

To characterize this complex mixture, complementary chromatographic techniques are applied, involving headspace-GC for the volatile degradation products, GC with liquid injection for the volatile constituents of the electrolyte phase, and LC/MS and LC/IT-TOF-MS for the more polar and higher molecular weight constituents of the electrolyte phase. It will be discussed in this presentation how the characteristic fingerprint of detected compounds correlates which conditions of use of the Li ion batteries. Moreover, an outlook on the possibility of monitoring the fast process of run-away of Li ion cells under abuse conditions with high time resolution will be presented.

Acknowledgments

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THIN FILM MICROEXTRACTION (TFME)

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The ultimate goal of environmental monitoring is to perform analysis at the place where a sample is located rather than moving the sample to laboratory, as is a commonly practiced in many cases at the present time. This approach eliminates errors and time associated with sample transport and storage and therefore it would result in more accurate, precise and faster analytical data. To address this need we develop solvent-free sampling/sample preparation approaches. In one design, the SPME samplers were made by using polydimethylsiloxane (PDMS) thin-film (membrane) or various sorbents imbedded in the PDMS membrae as the extraction phase. This technique is based on a similar principle as the SPME technique with additional advantage of higher surface to volume ratio facilitating much higher extraction rates and higher sensitivities because of high volume of the extraction phase. More specifically, the development of the thin film sampler involved cutting a section of PDMS/sorbent thin-film into a specific size and shape, and mounting it onto a stainless steel wire (the handle). This technique was used as a rapid spot or TWA sampling of environmental samples. For rapid water sampling, an electric drill was used to rotate the thin-film to get higher sampling rate. Passive water sampling can be calibrated through desorption of a preloaded standard and therefore variety of the environment, such as turbulence and temperature, can be compensated for. After exposure the membrane is placed in a liner exchange system for thermal desorption and GC/MS quantification. In one studies it was found that the extraction rates of Oligochaetes (black worms, Lumbriculus variegatus) and PDMS thin-film were identical for polycyclic aromatic hydrocarbon (PAH) compounds in water, which indicated that thin-film samplers could mimic the behavior of black worms for passive TWA monitoring. Recent application of the TFME involve monitoring of skin emission and saliva determinations.

LE.33

A NOVEL DESIGN FOR VACUUM-ASSISTED HEADSPACE SOLID-PHASE MICROEXTRACTION

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We recently proposed a new headspace SPME (HSSPME) sampling procedure, termed vacuum-assisted HSSPME (Vac-HSSPME), where HSSPME sampling takes place under vacuum conditions [1]. During our initial investigations, 500 or 1000 mL sampling containers were used to extract aqueous sample volumes commonly used in HSSPME. The results revealed that applying reduced pressure conditions during the non-equilibrium stage of HSSPME sampling dramatically improve extraction kinetics for the low volatility volatile compounds [1,2]. In another report, the downsized version of the extraction device (22 mL custom-made gastight glass container) was reported and we concluded that pressure changes upon sample introduction in smaller sample containers were sufficiently low to allow efficient Vac-HSSPME [3].

In this contribution the implementation of a novel, compact and effective device for Vac-HSSPME is discussed. The proposed extraction device consists of a new O-ring sealed screw cap that provides gas-tight seal to commercially available screw thread headspace vials. The cap is made of PTFE and has a hole that can tightly accommodate a half-hole cylindrical Thermogreen septum. All operations are performed through this septum: evacuation of the device, sample introduction and Vac-HSSPME sampling. The performance of this new sampling device was tested using aqueous solutions spiked with polychlorinated biphenyls (PCBs) and parameters such as sample volume, agitation speed, extraction time and temperature were controlled and optimized. For the first time different types of SPME fibers were used and the results revealed some new and important insights on the Vac-HSSPME procedure.

Overall, the proposed design reduced the cost and enabled practical and effortless application of Vac-HSSPME. More importantly though, the successful implementation of this novel and compact design provided the first evidence for the automation potential of Vac-HSSPME.

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LE.34

APPLICATION OF IN VIVO SOLID PHASE MICROEXTRACTION FOR MONITORING OF ORGAN FUNCTION AND METABOLISM OF DRUGS DURING SURGERY

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The biochemical analysis of tissue is important part of clinical practice. However, preparation of tissue samples is very laborious and time consuming procedure. In vivo solid phase microextraction is fast and simple method due to integration of sampling, sample preparation and extraction steps. It permits extraction of wide range of small molecules and therefore can be used as alternative tool in studies of metabolic profiling. Low invasiveness and applicability of SPME for monitoring of drugs and endogenous compounds in vivo were evaluated during lung and liver transplantations in animal models.

Multivariate analysis allowed observing metabolic changes of lung during cold ischemic time, perfusion and reperfusion of the organ. It was also demonstrated that level of drugs and their metabolites can be monitored over time. The repeated analysis of the organs by standard techniques based on sample collection increases invasiveness of the analysis and carries additional risk of side effects. The small dimension of SPME probe allows obtaining good temporal and spatial resolution offering sample draw-free extraction. Based on the selected example the impairment of enzymatic properties of liver was detected in the injured organs. This finding was supported by changes in pathways of endogenous metabolites. The evaluation of biochemical profile was also performed in perfusate demonstrating potential of the approach for monitoring organ function during *ex vivo* perfusion of grafts.

LE.35

SAMPLE PREPARATION NEEDLES FOR THE ANALYSIS OF VARIOUS VOLATILE ORGANIC COMPOUNDS IN GAS CHROMATOGRAPHY

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Downsizing of sample preparation techniques has become increasingly important in the research field of separation science because of the possibility of successfully meeting many recent requirements, such as significantly enhanced sensitivity and selectivity, and more ecological and economical features. The effective hyphenation of miniaturized sample preparation to microscale separation methods has also been widely investigated. This approach makes it possible the successful on-line coupling of these techniques without the disadvantages that are typically found in off-line multistep processes used for the analysis of complex mixtures such as environmental and biological samples. Miniaturization of the sample preparation process enables to significantly enhance mass sensitivity and reduce sample size, although the appropriate optimization of the hyphenated system should be considered. Recently, novel sample preparation techniques have been developed with a specially-designed needle extraction device. A wide variety of extraction media can be used, and it is easy to handle during the extraction and desorption processes. There are additional attractive features for the automation and the coupling to typical GC instruments on-line. Fiber-packed and particle-packed needle extraction devices have been introduced as the tool of sample preparation of various volatile organic compounds (VOCs), such as aldehydes, ethylene oxide, aliphatic and aromatic solvents.

In this presentation, the development of these miniaturized sample preparation needle devices will be reviewed and several novel applications to real sample analysis will be introduced.

LE.36

DETERMINATION OF ESTROGENIC COMPOUNDS IN WATER AND FOOD SAMPLES BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AND MICELLAR ELECTROKINETIC CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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Estrogens have become one of the groups of analytes of concern in water and certain food matrices, especially in milk and milk derivatives. In the first of them, their occurrence is caused, among others, by the effluents of wastewater treatment plants or by animal wastes used in agricultural fields for fertilizing purposes. While in the second, their presence is dual: in the case of endoestrogens (i.e. estrone, (E_1), estradiol (E_2), estriol (E_3) and their methylated and hydroxylated metabolites) they naturally appear while in the case of exoestrogens (i.e. 17α-ethynylestradiol (EE_2), diethylstilbestrol (DES), dienestrol (DS), hexestrol (HEX), etc.) they are intentionally introduced in the milk cycle in an attempt to take advantage of their anabolic effects. Regarding the analytical methodologies used for estrogenic compounds analysis, solid-phase extraction and high-performance liquid chromatography has been the chosen combination in most applications [1, 2]. Nevertheless, current trends in sample preparation are moving towards the use of smaller amounts of samples and solvents. In this regard, dispersive liquid-liquid microextraction (DLLME) has become a very popular option because of it inherent advantages. Regarding the use of other separation techniques, capillary electromigration methods have also been applied for the analysis of estrogens but in a lower number of occasions. In these cases, and because they are neutral in a wide range of pH, they cannot be easily analyzed by capillary zone electrophoresis and therefore, micellar electrokinetic chromatography (MEKC) is preferred. However, the use of non-volatile surfactants in MEKC has clearly precluded its combination with mass spectrometry (MS) detection which possesses clear advantages over other detection systems. In this work, a group of 12 estrogenic compounds (E_1, 17β-E_2, 17α-E_2, E_3, EE_2, DS, zearalenone, 2-methoxyestradiol, α- and β-zearalanol, α- and β-zearalenol) have been separated and determined by MEKC coupled to electrospray ionization MS using a background electrolyte containing an aqueous solution of ammonium perfluorooctanoate as MS friendly surfactant. DLLME using chloroform and acetonitrile as extraction and dispersion solvents, respectively, was employed to extract and pre-concentrate the target analytes from different types of environmental water samples (mineral, run-off and wastewater) as well as from different dairy products (whole and skimmed cow milk, semi-skimmed goat milk and natural yogurt) after deproteinization and defatting of the samples. Calibration, precision and accuracy studies of the described DLLME-MEKC-MS/MS method were evaluated obtaining limits of detection in the μg/L-ng/L range, depending on the analyzed matrix.

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CAPILLARY AND MICRO-BORE POLYMETHACRYLATE ZWITTERIONIC MONOLITHIC COLUMNS WITH DUAL RETENTION MECHANISM FOR TWO- AND THREE-DIMENSIONAL LIQUID CHROMATOGRAPHY

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Organic polymer monolithic columns provide excellent separations of proteins and other biopolymers; however they usually show too low efficiency for separation of small molecules. The reason is low proportion of suitable mesopores in the polymeric structure. Over the last decade, various approaches were tested to improve the pore morphology in monolithic columns, by adjusting the time, temperature and porogen solvents, or by post-polymerization modification by hyper-crosslinking of the polymer surface.

We increased the size and proportion of mesopores by using polymerization of relatively polar longer-chain cross-linkers in combination with a zwitterionic functional monomer. This approach provides highly reproducible and stable (poly)methacrylate capillary or micro-bore columns with efficiencies of up to 70 000 N/m for compounds with low MW. The columns show dual retention mechanism, HILIC in aqueous-organic mobile phases with high concentrations of acetonitrile, and reversed-phase in more water-rich environment. The separation selectivities in the two modes are complementary and highly orthogonal. Off-line two-dimensional LC separations can be accomplished in alternating runs with increasing and then decreasing gradients of acetonitrile in aqueous buffers on a single column.

A zwitterionic polymethacrylate monolithic 0.5 mm i.d. micro-column in the first dimension can be coupled on-line with a short core-shell or silica-monolithic column in the second dimension in a comprehensive LCxLC setup, where subsequent HILICxRP and RPxRP 2D separations can be achieved on the same zwitterionic monolithic column. The HILICxRP and RPxRP chromatograms can be combined to provide a three-dimensional LC system. The approach was applied to the separation of natural phenolic and flavonoid antioxidants.

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LE.38

OPTIMIZATION OF COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHIC (LC×LC) SEPARATIONS

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Comprehensive two-dimensional liquid chromatography (LC×LC) is a technique with an enormous potential. The peak-production rate (peak capacity per unit time) compared quite favourably with what can be obtained with conventional one-dimensional LC. Also, LC×LC may provide separations that cannot be achieved by (a combination of) one-dimensional methods. However, without proper optimization LC×LC separations may easily turn into a “comprehensive waste of time”. To avoid such from happening, we should carefully select the conditions for the individual separations stages within the constraints of time and pressure, as well as possible constraints of the sample and the detection system. Also, we must minimizing losses in resolution (peak capacity) due to first-dimension “undersampling”, second-dimension band broadening and incompatibility of the first-dimension effluent with the second-dimension system.
Equally important are the chemical (selectivity) aspects of the separation. The two separation stages should be orthogonal for the samples under consideration and the operating parameters (as a function of time) in both dimensions must be optimized. Because these latter aspects are sample dependent, we need efficient and – ideally – automated approached.
In this lecture we will consider the optimization of LC×LC and illustrate this with practical examples.

LE.39

ORTHOGONALITY METRICS IN TWO-DIMENSIONAL CHROMATOGRAPHY: WHAT CONSTITUTES A GOOD CHROMATOGRAM?

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The term “orthogonality” usually refers to the statistical concept of spreading peaks across a two-dimensional chromatogram. However, this concept can be generalized to a onedimensional chromatogram (spreading peaks across a line chromatogram), and threedimensional chromatograms (spreading the peaks across a volume i.e. three dimensions), etc. In this talk over 20 different orthogonality metrics are calculated from two-dimensional experimental chromatograms and analyzed using statistical analysis. These metrics include the fractal dimension, nearest neighbor statistics, correlation coefficients, surface coverage, hull statistics and metrics based on information theory. The results of this analysis will be presented. Principal component analysis demonstrates that no one particular metric stands out as being better than the others. However, a correlation study between chromatograms judged by a panel of people and the orthogonality metrics show very interesting trends that may surprise you.

LE.40

FEASIBILITY OF COMPREHENSIVE TWO-DIMENSIONAL CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY (SFCXSFC) ON (HEAVY) PETROLEUM, FRACTIONS

Jan Blomberg

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Building on earlier work by Hirata *et al.*[1], in this lecture we will report on capillary SFCxSFC on petroleum products as a means to extend the boiling-point range currently covered by GCxGC. The advantage of capillary - over packed SFCxSFC as reported earlier by Thiebaut *et al.* [2] is that similar separation mechanisms/ stationary-phase combinations can be used, which, allows for easy data-merger of GCxGC and SFCxSFC results. Also, the whole range of detectors that are currently available for GC (FID, Nitrogen – and Sulphur Chemiluminescence Detection, MS) can, in principle, be used.

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LE.41

COMPREHENSIVE 2D-LC: A RAPID EVOLUTION OF A POWERFUL TECHNIQUE

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Comprehensive two-dimensional liquid chromatography (LC×LC) is a powerful method capable of drastically increasing the resolution power of liquid chromatography. In the last two decades, LC×LC techniques have experienced an ever increased interest due both to the complexity of the samples on the one hand, and to the progress in the hardware development, on the other. The term “comprehensive” means that each component in the first dimension (^{1}D) is subjected to a further second dimension (^{2}D) separation, presenting as major benefit an increased peak capacity which is multiplicative of the two single dimensions. The potential of the LC×LC is best utilized, when the separations in the ^{1}D and ^{2}D dimensions are completely orthogonal. However, in many cases such a situation is not achievable, thus significantly reducing the useful separation space.

This contribution aims to emphasize the potential of LC×LC techniques for unravelling of complex real-sample mixtures with the discussion of some selected applications and practical considerations on the most recent implementations on LC×LC software data processing.

LE.42

MULTIDIMENSIONAL LC AND COMPREHENSIVE LC X LC IN BIOPHARMACEUTICAL ANALYSIS

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Protein biopharmaceuticals such as monoclonal antibodies and recombinant proteins are currently in widespread use for the treatment of various life-threatening diseases including cancer, autoimmune diseases, diabetes and anemia. Protein therapeutics have a complexity far exceeding that of small molecule drugs, hence, unraveling this complexity represents an analytical challenge. The current lecture will highlight the power of comprehensive LC x LC in hyphenation to high resolution mass spectrometry to the characterization of protein biopharmaceuticals. Using different monoclonal antibody drugs, it will be demonstrated that comprehensive LC x LC is an excellent tool for identity, purity and comparability assessment. The utility of multidimensional LC in the determination of the pharmacokinetic properties of biotherapeutics at high sensitivity in blood plasma will be demonstrated as well. A comparison with one-dimensional LC-MS will be made.

NEW APPROACHES TO PROTEIN ANALYSIS USING MICROCHIP ELECTROPHORESIS

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Microchip electrophoresis has emerged as a powerful method for rapid analysis of proteins. In this talk we describe two new ways of using microchips for protein analysis: 1) chip-based western blot and 2) protein-protein interaction assay and screening. Of all protein assays, western blot is perhaps the most common. Despite the power of the method, it is recognized as a slow and manually intensive procedure. We have interfaced an electrophoresis microchip, operated with a sieving separation, to a capture membrane to enable rapid, automated western blots. The high speed and low sample consumption allows multiple proteins to be assayed from a single sample. With this method, up to 60 westerns can be completed in 60 min. The high-speed of separations can be used to separate short-lived species such as non-covalent complexes. This feature has been used previously to develop electrophoresis-based immuno- and aptamer assays. In this work we have examined the potential of using the method to detect protein-protein interactions in solutions. Such interactions are important in controlling cell signaling and represent an emerging type of drug target. The method allows detection of complexes and determination of binding constants. Further, when coupled to novel sample introduction methods based on droplets, it may be used to screening for modulators of protein-protein interactions. A preliminary screen of 3,443 compounds showed that electrophoresis yielded more selective “hits” than conventional protein-protein screens.

LE.44

INTEGRATED MICROFLUIDIC DEVICES THAT COMBINE ELECTROCHROMATOGRAPHIC EXTRACTION AND ELECTROPHORETIC SEPARATION

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Our group is focused on the integration of multiple processes necessary for the quantification of biomarkers. Specifically, we are combining solid-phase extraction (SPE) and microchip electrophoresis in a compact microfluidic system. We use porous polymer monolithic columns formed in situ within microdevices for affinity extraction of specific biomarkers. Similar monolithic supports are also utilized for SPE cleanup prior to separation. These columns are operated electrochromatographically, allowing for automated sample processing. We have carried out various proof-of-concept experiments in these microfluidic systems. We selectively extracted four cancer biomarkers from fluorescently labeled blood serum by using an immunoaffinity monolith; we then eluted the purified proteins and quantified them through microchip electrophoresis in comparison to standards [1]. More recently, we made SPE monoliths in microfluidic systems to carry out preconcentration and fluorescence labeling of protein samples [2]. We used voltage to drive the unlabeled biomolecules onto the reversed-phase column for enrichment, and then electrophoresed an amine-reactive fluorescent tag through the monolith to label the extracted components. Elution of the labeled sample in a different band from the unattached dye was then done under voltage control. We have further improved the formulation of the monolith for better selectivity toward target molecules and enhanced flow through the column. Ongoing studies are directed toward complete integration of the analysis steps of affinity extraction, SPE and electrophoresis in a single device. We are targeting the analysis of peptides and proteins that are associated with risk for preterm delivery in pregnancy. Our integrated microfluidic devices are well suited for small volume analysis of biomolecules linked to diseases.

Acnowledgements
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LE.45

MINIATURIZED SEPARATION SYSTEMS FOR MEASURING DYNAMICS OF PEPTIDE SECRETION

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Islets of Langerhans release a number of peptides that regulate blood glucose levels. In the past, insulin release with a time resolution of approximately 10-seconds has been measured from these cells using microfluidic systems [1,2]. We have improved on this methodology by incorporating measurement of other peptides simultaneously with insulin, as well as a perfusion system that allows for rapid changes of glucose levels to be delivered to the islets. This system is significant because a detailed knowledge of the dynamic features of the secretion patterns are critical to understanding normal regulation of blood glucose and the defects that may lead to various metabolic diseases.

A glass microfluidic device was developed that contains perfusion channels, a chamber to hold islets, reagents for the immunoassays, and channels for separation of the immunoassay reagents. Electroosmotic flow directed the immunoassay reagents, including the peptides released from islets, down a mixing channel to the separation channel. Injections were made every 12-s using flow-gated injections [1,2]. Two-color laser-induced fluorescence was required for quantitation of the different peptides and was accomplished by focusing 488- and 635-nm lasers 1-cm downstream from the injection cross. FITC and Cy5 emission were collected and split to separate photomultiplier tubes. Limits of detection of 20 nM for insulin and 3 nM for IAPP were obtained.

Secretion profiles from groups of islets were measured and provided detailed examination of release kinetics. New approaches to incorporating further numbers of peptides will also be described. The development of a system that can simultaneously monitor release of multiple peptides from islets of Langerhans will be important to produce a complete picture of islet physiology.

LE.46

LC X MULTIPLEXED CAPILLARY ELECTROPHORESIS FOR COMPREHENSIVE ANALYSIS OF COMPLEX OLIGONUCLEOTIDE SAMPLES

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Synthetic oligonucleotides (ONs) typically up to 35 bases in length are increasingly being used in a variety of biological and therapeutic applications. As quite a few therapeutically active oligonucleotides are today in the clinical trial stage, there is an increasing need for improved separation protocols. Because of the nature of ON synthesis, the synthetic impurities often include related families of fail (N-x) and adduct (N + x) sequences next a variety degradation products that can be generated under stress conditions or due to chemical and enzymatic digestions in the framework of pharmacokinetic and pharmacodynamic studies. Because of the rapidly increasing number of structural and chemical variations in ONs of certain chain length, conventional 1-D based separations are insufficient for adequate mapping off all impurities and degradants which can be generated. In this work the possibilities of comprehensive two-dimensional HPLC approaches was therefore compared with the combination of HPLC with offline multiplexed capillary electrophoresis strategies. In the LCxLC approaches hydrophilic interaction liquid chromatography (HILIC) was, amongst others, studied for use in the first dimension. IP-RPLC proved to be the most suitable for subsequent fast second dimension analysis. Offline two dimensional liquid chromatography (LC) × capillary gel electrophoresis (CGE) strategies and LC × (24) multiplexed-CGE methodologies were developed for the separation of oligonucleotides of up to 35 bases long. The figures of merit of both methodologies are compared and additional improvements of 1D and 2D LC and CE approaches, via the use of multivariate curve resolution-alternating least squares devolution strategies, are proposed.

LE.47

SINGLE PARTICLE ELECTROPHORESIS IN NANOCHANNELS

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We are developing label-free, nondestructive techniques for rapid sensing, characterization, and sorting of virus particles. Resistive-pulse sensing measures changes in ion current resulting from transit of particles through an electrically biased nanopore filled with electrolyte. To sense and characterize individual virus capsids, we have fabricated devices in plane in glass substrates. Each device contains V-shaped microchannels that are machined into the glass substrate by standard photolithography and wet chemical etching. Nanochannels are then milled into the glass substrate with a focused ion beam and bridge the gaps between the microchannels. For resistive-pulse sensing of the virus particles, we use nanochannels with two or three pores in series to track individual particles and measure physical properties of the particles, e.g., electrophoretic mobility. In other designs, we have integrated mixing on-chip and are able to monitor the assembly of single hepatitis B virus particles in real time and at biologically relevant concentrations.

LE.48

CAPILLARY ELECTROCHROMATOGRAPHY AND ITS APPLICATIONS IN METABOLOMICS AND BIOLOGICAL ANALYSES

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Capillary electrochromatography (CEC), combining capillary electrophoresis (CE) and capillary liquid chromatography (cLC), provides double separation mechanism with high efficiency, high resolution and high selectivity for both neutral and charged species. However, in practice, there were problems and difficulties associated with bubbles formation and column dry-out. These problems can be overcome by a pressurized CEC (pCEC) system, in which a mobile phase is driven by both a pressurized flow and an electroosmotic flow (EOF).

We developed a miniaturized separation platform, which consists of a pressurized capillary electrochromatography (pCEC) system and four types of capillary-based detectors, such as ultra-violet (UV) detector, laser induced fluorescence (LIF) detector, evaporative light scattering detector (ELSD) and electrochemical detector (ECD). An interface for coupling pCEC with mass spectrometry (MS) was also designed and used in metabolomics investigation on cancer. The analytical platform was applied to the separation and analyses of various samples in pharmaceutical and biological fields. We will report the advances in the miniaturization of the separation and detection modules and the coupling among the individual systems. The stability and reproducibility of the platform were investigated and will be discussed. Many examples of chromatographic separations will be reported to demonstrate the feasibility and versatility of the analytical platform.

CHARACTERIZATION OF COMPLEX MIXTURES: HIGH AND ULTRA-HIGH RESOLUTION MASS SPECTROMETRY AS DETECTOR FOR ONE-DIMENSIONAL AND COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

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Comprehensive analysis of complex matrices such requires highly selective analytical method. Gas chromatography-and comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC-MS, GCxGC-MS) are standard approaches for this task. Recently ultra-high mass resolution/accuracy mass spectrometry (HRMS), which allows the determination of elemental compositions by exact mass measurement, became available for elucidating the complexity of e.g. petrochemical samples (e.g. by direct infusion FTICR- or Orbitrap-MS measurements). However, depending on the used ionization source, the direct infusion HRMS approach usually is limited in generality (ionization selectivities/matrix effects) and isomeric compounds are not separable.

By coupling of high resolution (gas-) chromatography and high mass-resolution mass spectrometry, the knowledge e.g. on the "chemical space" in complex samples can be increased. A HR-TOFMS-system with a multi-reflection-time-of-flight technology using periodic ion focusing lenses allows the detection of gas chromatographic transients at high mass-resolution (R = 50.000) with good mass accuracy (< 1 ppm) and at a very fast acquisition rate (200 Hz). As the HR-TOFMS is capable to follow accurately very fast changing chromatographic transients it is particularly well suited for coupling to comprehensive two-dimensional gas chromatography (GCxGC, GC: Agilent Inc., USA, Modulator: Zoex Inc., USA). Furthermore a Fourier Transform Ion Cyclotron Mass Spectrometer (7T-Cryomagnet, R ~ 300.000) was coupled to gas chromatography, using APCI ionization. The two HR-MS system were applied as detectors for GC (FTICR and HR-TOFMS) and GCxGC (only HR-TOFMS). Petrochemical fractions were analyzed and the respective advantages and drawbacks of the concepts were discussed. For GCxGC the high resolution MS-mode was furthermore used to improve the selectivity of non-targeted compounds-class identification schemes (scripting, see W. Welthagen et al., J. Chrom. A 1019 (2003) 233ff). Here the high mass resolution enables an improved scripting approach, using the exact mass-values of specific fragments to suppress the accidental of matrix contribution. An important advantage of the GC-APCI-FTICR approach is the soft ionization capability and the achievable ultra-high mass resolution.

LE.50

CAPILLARY CHROMATOGRAPHY-ATMOSPHERIC PRESSURE PHOTOIONIZATION/ MASS SPECTROMETRY

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Atmospheric pressure photoionization (APPI) provides an efficient method to combine capillary liquid chromatography (capLC) or gas chromatography (GC) with mass spectrometry (MS). In APPI, a sample is vaporized by a heated nebulizer and ionized via proton transfer or charge exchange reactions using 10 eV photons. Since commercial APPI sources are designed to work with liquid flow rates on a scale of 100 - 500 µL/min, we have developed microchip [1] and capillary APPI (CPI) [2] ion sources optimized for flow rates below 10 µl/min. The APPI microchip includes a sample inlet channel, auxiliary gas and dopant inlet, vaporizer channel, nozzle, and platinum heater. Eluent, dopant and analyte are mixed and vaporized in the heated vaporizer. The chip forms a confined jet of the sample vapor, which is photoionized as it exits the chip. The narrow jet provides high ion transfer efficiency into MS through narrow sampling orifices resulting in improved sensitivity. The CPI utilizes a heated transfer capillary with a vacuum ultraviolet transparent MgF_2 window, which allows 10 eV photons enter the capillary. Liquid or gaseous sample together with dopant is introduced directly into the heated transfer capillary between the atmosphere and the vacuum of MS. Since the sample is introduced, vaporized, and ionized by photons inside the capillary, ion transmission can be maximized resulting in improved overall sensitivity. The microchip APPI-MS and CPI-MS combined to capLC or GC have successfully been applied to high sensitivity analysis of environmental, biological and pharmaceutical samples.

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LE.51

HIGH RESOLUTION GC FRACTIONATION OF COMPLETE CHROMATOGRAMS FOR COMBINED ON-LINE AND OFF-LINE ANALYSIS

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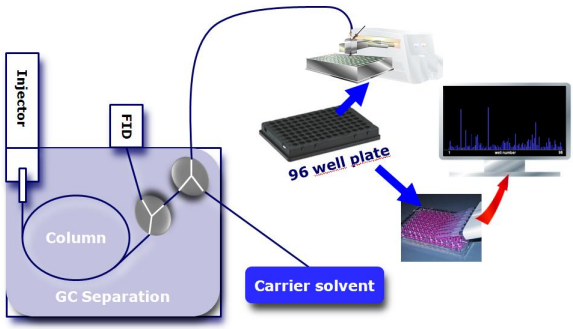
High resolution fractionation of complete GC chromatograms will be presented. The technology is very straightforward and robust, does not require sophisticated cold traps or adsorbent traps, and allows to collect large numbers of fractions during a GC run. Our new analytical technology is based on post column infusion of low boiling point solvents (e.g. hexane or acetonitrile). After exiting the column, the carrier solvent efficiently traps the eluted compounds for fractionation. Multiple fractionations allow for up concentration of the fractions collected in a fully automated fashion (i.e. software and hardware wise). A post-column split prior to addition of carrier solvent enables parallel on-line analysis by typical standard GC detectors such as FID or MS. Currently, the technology has progressed to a robust system ready for implementation in other laboratories as complete system, or as add-on to existing GC equipment. To demonstrate application areas of our technology, we performed off-line post-column analyses in environmental settings. Toxic pollutants in environmental mixtures were identified by their bioactivities towards the dioxin receptor (DR) and androgen receptor (AR) in 96 well plate format, and acetylcholine esterase (AChE) bioactivity in 384 well plate. Although gas chromatography is superior for separation of non polar and relatively volatile compounds, liquid chromatography is most often used in case of fraction collection for further off-line analysis. The current technology brings gas chromatography back into the arena of post column fractionation for off-line chemical as well as biological analysis.



LE.52

GAS CHROMATOGRAPHY-ATMOSPHERIC PRESSURE CHEMICAL IONIZATION-TIME-OF-FLIGHT-MASS SPECTROMETRY - A VALUABLE TOOL FOR METABOLOMICS

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An often used experimental strategy in metabolomics is metabolic fingerprinting delivering a “snapshot” of the metabolome of an organism, tissue, or cell with the overall aim to identify (and quantify) metabolites that differ between sample groups.
An established tool for metabolic fingerprinting is gas chromatography coupled to mass spectrometry (GC-MS) after derivatization. However, the identification of unknowns is still a bottleneck of this approach. Although comprehensive mass spectral libraries are available for GC-electron ionization (EI)-MS, they fail to identify all signals in the complex chromatograms. Atmospheric pressure chemical ionization (APCI) is used to couple GC to a high resolution TOFMS (Bruker MicrOTOF) to approach this problem. This soft ionization technique produces quasi-molecular ions that in combination with high resolution-accurate mass measurement and consideration of the isotope pattern can be used to generate a sum formula as a first step to identify unknown metabolites.
We have previously shown that GC-APCI-TOFMS is superior to GC-CI-MS because quasi-molecular ions of phosphorylated compounds and sugars were not or only to a minor extend formed by CI. In contrast, the quasi-molecular ion represented almost always the most abundant signal in the APCI spectra of 43 silylated metabolites **[1]**. However, the reproducibility of APCI without internal standards was not satisfactorily. Therefore, parameters influencing ionization were studied, such as potential matrix effects due to co-eluting compounds and the infusion of water into the ionization source. The impact on differently derivatized metabolites, e.g., methylated versus silylated, was investigated. The strongest improvement of ionization efficiency with water infusion was observed for methylated metabolites. The results of these studies will be presented together with selected examples of APCI assisted identification of unknowns in metabolic fingerprinting.

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LE.53

MONOLITHIC SILICA AND SPUTTERED STATIONARY PHASES IN MICRO MACHINED GAS CHROMATOGRAPHY COLUMNS FOR OILFIELD APPLICATIONS

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Since the late 70s, new approaches have been proposed to replace conventional gas chromatographs by silicon based micro fabricated separation systems. Performances, in terms of separation speed and efficiency, are expected to be improved with miniaturization owing to the reduction of diffusion distances and better thermal management while a miniaturized micro gas chromatograph would enable on site cycled and real time analysis for various applications requiring continuous monitoring. The main challenge consists in the reproducible and collective fabrication of micro columns.

In this communication, two types of columns preparation will be presented in order to answer this issue. One is the rerouting of the sputtering technique to coat columns with a film of silica (patent pending); the second one is the direct synthesis of a silica monolith in a column by a sol-gel process derived from classical procedure used for their preparation for liquid chromatography applications.

The parameters involved in the two approaches will be presented and the performances of columns will be commented from the points of view of separation capabilities, fabrication, ease of use and repeatability.

The separation performances of the silica sputtered micro columns coupled with thermal management will be exemplified by very fast separations of light alkanes (C1 – C10). Fast ramps of temperature can be applied during the separation thanks to a computer controlled thermal management micro system, including cooling (when necessary), heating, and temperature reading of the MEMS columns.

The potentialities of these innovative phases as an alternative stationary phase for specific applications will also be discussed (permanent gases separation, high ambient temperature separation).

WHAT CAN NANOSCIENCE DO FOR STUDIES WITH MONOLITHS?

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Monolithic structures formed from organic polymers became popular packing/supports in applications requiring high speed that result from mass transport facilitated by rapid convection. Use of functional monomers for their preparation led to monoliths with a variety of functional groups useful in numerous applications. Recently, the range of functionalities was extended by introduction of nanostructures attached to the pore surface of monoliths. For example, gold nanoparticles have proven to be excellent intermediate ligands to which selected functional molecules were attached via non-covalent interaction of gold and their thiol groups [1-5]. We now extended this approach to the preparation of capillary columns developed for the mixed mode separations. Modification of the gold nanoparticles was carried out simultaneously using pairs of thiols containing aliphatic hydrocarbon chain and hydrocarbon chain terminated with carboxylic acid group. Changes in the length of the hydrocarbon chains and proportion of these two components are a powerful means to dial the properties of the separation medium. This will be demonstrated with separations of protein mixtures in both reversed-phase and ion exchange modes. In another new application, we immobilized silver nanoparticles and used the monoliths for very efficient separation of iodine/iodide from iodinated organic compounds [6]. This monolithic column was used for the facile capture of excess of radioactive iodine isotope after labeling of radiopharmaceutical, m-iodobenzylguanidine. The high capacity of the monolithic sorbent was exhausted only after very many clean-up runs. Then, the column was easily disposed of as a closed solid radioactive waste. This approach significantly reduces the amount of hazardous liquid radioactive waste that is typically produced with standard HPLC purification methods. Finally, both gold and silver nanoparticles were also applied to monolithic thin layers enabling separations of small peptides in TLC mode followed by direct from-layer MALDI MS detection.

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LE.55

POLYMERIC MONOLITHIC MATERIALS FOR MINIATURISED AND AUTOMATED SAMPLE PREPARATION

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Polymer based monoliths were introduced about 20 years ago. The relatively simple preparation, robustness, high permeability to flow, mass transfer via convection and flexible chemistry has since seen these materials used in a range of applications such as chromatography and as supports for synthesis, catalysis and immobilized enzymes. These same properties make monolithic polymers an excellent choice as materials for sampling and sample preparation, particularly for miniaturized technologies with the potential to produce cleaner extracts and facilitate rapid sample preparation for mass spectrometry (MS).

This presentation will introduce monolithic micro-sampling devices developed for sampling and sample preparation of biological samples, including whole blood. Monolithic micro-sampling devices prepared within disposable pipette tips have been developed for in-tip blood filtration and as immobilized enzyme reactors (IMER) for protein digestion. Subsequent micro-solid phase extraction (µ-SPE) was achieved using high surface area polymer monoliths. The µ-SPE device was then directly hyphenated with both ESI-MS and nanospray-MS. Microextraction by packed sorbent (MEPS) in which the SPE phase is placed within an exchangeable needle hub integrated into an analytical syringe is also demonstrated for µ-SPE. A workflow was developed using the monolith filtration and enzyme reactor technology in combination with an at-line micro SPE-ESI-MS approach enabling both sample preparation and analysis to be completed in < 20 min, facilitating high-throughput sample analysis in a standard bioanalysis workflow. New, high surface area polymeric monolithic sorbents tailored for SPE will also be introduced and are shown to provide significant advantages over particle-based sorbents, providing greater reproducibility in the sorbent bed.

LE.56

MICROSCALE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY APPROACHES TO THE IN-DEPTH CHARACTERIZATION OF THERAPEUTIC PROTEINS

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By 2016, eight of the top ten medicines applied worldwide will be therapeutic proteins, also called biopharmaceuticals. Manufacture of therapeutic proteins is performed in bacterial or eukaryotic expression systems, requiring extensive purification of the target product. In order to ensure highest-level safety and efficacy of the drug compounds, a rigorous control of a large set of chemical, physical, and biological properties is obligatory.

Because of their high information content and versatility, characterization methods based on the hyphenation of high-performance liquid chromatography and mass spectrometry are among the most powerful analytical methods for protein characterization. Protein characterization can be performed on the intact protein level, which delivers information related to the intact protein including all its modifications at the cost of more tedious method setup and optimization. Alternatively, proteins can be enzymatically digested to obtain peptides enabling their analysis by means of generic methods, however, at the risk of losing important molecular information about the protein.

Here we report on the benefits of a generic analytical platform employing highly efficient chromatographic protein separations in combination with Orbitrap mass spectrometry as a tool for protein analysis at different structural levels ranging from intact protein over protein fragments to proteolytic digests. Peak capacities in the range of 100-1000, detection limits in the low femtomol range, and mass accuracies in the low ppm range represent characteristic performance parameters of the hyphenated systems. The technology was employed to the characterization of the monoclonal antibody Rituximab and of a recombinant protein modified with polyethylene glycol (PEG). The data obtained from different optimized workflows allow for the determination of the molecular weight of the intact proteins, the confirmation/verification of their amino acid sequence, the detection and relative quantification of oxidation variants, and the identification and evaluation of the relative abundance of various glycoforms/PEGylated variants of the recombinant proteins.

LE.57

IN-DEPTH STUDY OF THE KINETIC PERFORMANCE OF FULLY POROUS AND CORE-SHELL PACKED LC COLUMNS

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The present contribution will first briefly review the different measurement methods that are needed to make an in-depth investigation of the efficiency and the kinetic performance of packed bed columns. These method involve, apart from the "obvious" plate height measurements, also peak parking (to measure the B-term) and total pore blocking (to measure the packing heterogeneity).
Subsequently, the basics of the kinetic plot theory will be reviewed to show how any set of experimental data of plate height versus velocity can be transformed into the much more informative plot of time versus efficiency or peak capacity, and how this can be done under isocratic as well as under gradient conditions.
Subsequently, we will report on the experimental results obtained with these methods on small as well as large particle packed bed columns with different lengths and diameter. A special emphasis will be put on the difference between fully-porous and core-shell particles. Finally, the kinetic performance of the different particle types will be compared via the kinetic plot method and the differences will be ascribed to the different individual contributing factors.
A special emphasis will also be given to the contribution of the instrument band broadening and pressure drop characteristics to the observed and the theoretically achievable kinetic performance.

LE.58

OPEN TUBULAR ENZYME REACTORS AND LC COLUMNS FOR HIGH PERFORMANCE PROTEIN ANALYSIS

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Open tubular columns in liquid chromatography provide high-resolution separations of peptides as well as intact proteins, and are developed and used in our laboratory for targeted and comprehensive proteomics. Although OT columns have previously been shunned due to technical challenges (e.g. pump and detector compatibilities), OT columns are now compatible with modern commercial instrumentation, and are well suited for coupling with nanospray-mass spectrometry. Coating the capillary walls for producing polymeric layer open tubular columns (PLOT) is simple, with a very high degree of predictability. Due to their narrow inner diameter (e.g. 10 μm), they are highly suitable for analysis of small samples, and we routinely employ PLOT columns for cancer stem cell-related signal pathway analysis. For instance, PLOT columns enabled identification of key Wnt signal pathway proteins in extract corresponding to 1,000 pancreatic cancer cells [1]. We currently use long (up to 10 m) PLOT columns, which provide peak capacities and number of protein identificatations that match or surpass established approaches.

Additionally, PLOT columns are coupled with open tubular enzymatic reactors (OTERs). Trypsin, Lys-C or a combination of both are wall-immobilized to 20 μm ID polymeric layered capillaries. On-line cleaved proteins are subsequently transported to PLOT columns for separation and using a prototype system, speed, detection limits and resolution were superior compared to a more established LC-MS method for detecting a small cell lung cancer marker [2]. Recently, we have used fully automated on-line OTER-PLOT-MS with otherwise commercial instrumentation for detecting telltale Wnt pathway proteins in tumor tissues.

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EVALUATION OF GC-MS APPROACHES FOR THE DETERMINATION OF ALLERGENS IN PERFUMES AND COSMETICS.

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European legislation puts limits on the maximum levels of suspected fragrance allergens in cosmetics. The current state-of-the-art method involves GC-MS analysis, eventually using 2 capillary column of different polarity for confirmation analysis. As detector, a single quadrupole instrument is used.
The main challenge in the analysis of suspected allergens in cosmetics is the possible co-elution of target compounds with the matrix. This “matrix” includes other fragrance compounds or other cosmetic constituents such as low boiling solvents (e.g. glycols), fatty acids or detergents. It is not possible to predict separation of the target compounds from matrix compounds.
The second challenge involves the recent trend towards a more extended list of target solutes. This list includes derivatives of the original list of fragrance allergens and consequently imposes additional challenges upon selective detection and quantification.
In this presentation, a comparison will be made between single quadrupole, triple quadrupole and Q-TOF MS instruments coupled to GC for the analysis of fragrance allergens in cosmetic samples. Instrumental approaches will be compared in terms of selectivity, sensitivity and quantification.
Besides the detection, also attention will be paid to sample preparation. Especially for cosmetic products that not allow direct injection (soaps, creams, gels), proper selection of the sample introduction is needed. To this, the use of full evaporation dynamic headspace and the possibilities of automated liner exchange in combination with thermal desorption and PTV injection will be presented.

LE.60

A HELIUM GAS CONSERVING INLET FOR GAS CHROMATOGRAPHY

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The global helium shortage and price increase causes more and more analytical laboratories employing gas chromatography to re-assess their consumption patterns of this non-renewable noble carrier gas. The existing solutions to this problem include changing analytical column, migrating to another carrier gas (e.g. hydrogen) or passively reducing the helium consumption by reducing its usage during analytical runs or switching the GC or GC-MS over to nitrogen during the longer idling periods. In all these cases a considerable amount of time is spent developing new methods or wasting daily the time needed for the instruments to come back to normal operations. An innovative approach to the conservation of helium carrier gas comprises separation of the flows inside the standard split/splitless injector. Two different carrier gases are supplied to the inlet preserving the column flow with helium, while maintaining septum purge and split flows with another inert gas like nitrogen. On average, the helium consumption is reduced such that a standard cylinder of compressed helium gas can last 3-6 years vs. 3-6 months without and changes in the analytical methods or conditions. General concept and design details of this GC inlet will be presented, along with chromatography data generated by its use. In particular an extensive comparison with conventional split/splitless inlet, supplied with only Helium carrier gas, to confirm the exact preservation of retention times and the equivalence of performances in term of precision and accuracy. Additional benefits and other potential applications of supplying a GC inlet with two different carrier gases will be also discussed.

LE.61

THE FUNDAMENTAL ROLE OF SAMPLING AND SEPARATION SCIENCES IN THE DEFINITION OF MULTITROPHIC PHENOMENA

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Multitrophic phenomena can be defined as the plant responses to herbivore feeding damage by producing a volatile fraction differing not only in its quantitative composition per unit of plant biomass but also qualitatively i.e. because of the release of compounds not occurring in the volatile fraction of the intact plant [1]. Multitrophic interactions between plants and insects can therefore be monitored through chemical messages mainly consisting of volatiles. The study of the "chemistry of the interaction" implies the adoption of strategies of analysis enabling to obtain results not only analytically reliable but also biologically meaningful.
The importance of the analysis approach will be illustrated through two examples:
a) the first one describes the different toxic effect of the volatile fraction from different species belonging to the same genus on an herbivore through HS-SPME-GCxGC-qMS analysis, in particular the interaction between mint leaf beetle (*Crysolina herbacea* Duftschmid) and peppermint species (*Mentha spicata* L.*, Mentha×piperita* L.*, Mentha longifolia* L.) [2]
b) the second one is the STE-GC-MS study of the dynamics of the reaction of a plant when attacked from an herbivore: in particular the reaction of lima bean leaving leaves (*Phaseolus lunatus* L.) to the attack of Egyptian cottonworms (*Spodoptera littoralis* Boisduval).

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LE.62

NEW VACUUM ULTRAVIOLET DETECTOR FOR GAS CHROMATOGRAPHY

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Currently, the mass spectrometer represents the closest embodiment of a universal detector for gas chromatography that is capable of both trace quantitative and definitive qualitative analysis. Even so, limitations exist using GC-MS for analysis of some isomeric, isobaric, small, or labile chemical compounds. Recently, we have introduced a vacuum ultraviolet (VUV) detector, which represents the first fully universal quantitative and qualitative detector option for GC. All species absorb light in the VUV spectral range (115 – 180 nm) and their spectral signatures across this range are unique. We have demonstrated exceptional sensitivity in the low to mid picogram range for all compounds evaluated, including linear and branched hydrocarbons, polyaromatic hydrocarbons, fatty acids, and pesticides. The ability to clearly deconvolute signals of co-eluting compounds that are indistinguishable by a typical mass spectrometer is highlighted. Both the developed hardware and software offer unique advantages not previously available for aiding GC analysis. Furthermore, computed spectra (Gaussian09) can be used to study fundamental aspects of VUV electronic transitions, as well as provide useful correlations with experimental spectra. This is the first formal oral presentation of this novel GC-VUV instrumentation and application.

LE.63

ADVANTAGES OF ATMOSPHERIC PRESSURE CHEMICAL IONIZATION GAS CHROMATOGRAPHY (APGC) MS/MS FOR THE SELECTIVE DETECTION AND QUANTIFICATION AT TRACE LEVEL.

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Atmospheric Pressure GC (APGC) is a technique that has real potentials for the routine analysis of GC amenable analytes. The ionisation is a 'soft' process comparable to Atmospheric Pressure Chemical Ionisation (APCI) resulting in very low in source fragmentation of analytes. The low fragmentation rate means that both sensitivity and selectivity can be increased and in the case of multiple reaction monitoring (MRM) experiments the precursor ion selection process is simplified especially when compared to conventional Electron Ionisation (EI). Another advantage being that the APGC interface can be coupled to a mass spectrometer with a electrospray ionisation (ESI) source thus enabling a rapid changeover between GC and LC on a single instrument with no compromise in data quality.

In this work, the potential of APGC, combined with a tandem quadrupole mass analyzer was investigated for the analysis of challenging GC/MS amenable compounds, taking pyrethroids and dioxins as a case study. The formation of a highly abundant (quasi) molecular ion was our main goal.

By avoiding fragmentation, the sensitivity in MRM or SIR mode is enhanced. This technique shows high potential for the selective and sensitive analysis of POPs and fragile pesticides.

The results demonstrated that the sensitivity achieved using APGC-MS/MS is comparable with high resolution GC/MS, the standard for dioxin analysis. The soft ionization in favour of intense (quasi) molecular ions allows to secure the identification and to greatly improve the sensitivity for pyrethroids.

LE.64

CONSIDERATIONS FOR CHOOSING A DIFFERENT CARRIER GAS IN GAS CHROMATOGRAPHY

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Traditionally the carrier gas used in GC is helium. There are however increased drivers to choose a different carrier gas. This can be nitrogen or hydrogen. Especially the last years there has been a lot of discussion to use different carrier gases because of the delivery issues for helium.
For many applications one can use nitrogen, but there is an impact on the chromatography. Hydrogen is also a good alternative, as it also allows much shorter run times. There are practical issues to consider in changing carrier gas. In this poster presentation an overview of opportunities will be presented as well as the practical concerns that has to be dealt with. Changing carrier gas is one, but we do prefer the same separations.(peak elution order). That means that changing carrier gas must also followed by a change of analytical conditions, meaning not only the pressure and split/splitless settings, but also the oven temperature program. New tools are available that make the settings very easy.

EXPANDING THE IDENTIFICATION AND QUANTITATION POTENTIAL OF DIRECT-EI LC-MS INTERFACE FOR THE ANALYSIS OF COMPLEX SAMPLES

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Mass spectrometry (MS) is renowned for its impressive identification potential that has a practical implication when MS is coupled to a separation technique such as liquid chromatography (LC-MS) or gas chromatography (GC-MS). High-resolution instruments and interpretable mass spectra with library searching capability can boost identification of unknowns. Direct-EI LC-MS interface is a new technique that combines, in a single instrument, the identification advantages of library searchable, electron (impact) ionization (EI) spectra with the separation power of an LC column, without the drawbacks of matrix effects and the cost of a high-resolution instrument. Unknown identification is of increasing importance in food safety, environmental, forensic and many other applications where the complexity of the matrix is a troubling factor. The advantage of electron ionization for tentative identification of GC amenable compounds is unparalleled. However, EI is not amenable to conventional LC separation. Expansion of EI fragmentation to a wider variety of molecules separated by an LC column provides an attractive alternative to identification and offers a complimentary technique to high-resolution/high-mass accuracy LC-MS instrumentation. EI for liquid chromatography is a viable alternative to atmospheric pressure techniques. Direct-EI combines nano-scale flow-rates with the high-vacuum environment of an EI source. The liquid effluent is nebulized and vaporized inside the ion source. Once in the gas phase, an electron beam ionizes the analytes generating high quality, library searchable mass spectra. Mobile phase vapors produce background ions that typically do not impact the overall appearance of the spectrum and do not induce chemical ionization processes. Further, the simple interfacing process and the negligible solvent intake, make it compatible with any LC separation technique and with any solvent combination, including non-volatile buffers and UHPLC methods. In this presentation, the Authors will investigate the advantages offered by an MS/MS analyzer in improving identification potential and detection limits and explain the basic principles of compound identification in various applications.

LE.66

LIQUID CRYSTALS AS GAS SENSORS AND CONTROLLABLE PHASES FOR HPLC

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It is known that liquid crystal membranes can have a high degree of specificity towards molecules permeating through them. This selective behavior or permselectivity can be controlled so as to enhance the transmission of species of interest. Such systems exploit the spontaneous or induced molecular ordering exhibited by liquid crystal molecules, which encourage anisotropic molecular interactions under the influence of an alternating electric field. Differences in the steric (size and shape) and dipole-dipole (solubility) interactions between different permeants and the liquid crystal are thus enhanced, altering membrane selectivity.
Here we exploit these interactions to detect the presence of species by monitoring the dynamic optical properties of liquid crystals.
For gas applications a gas permeable membrane whose two surfaces are rendered electrically conducting; a liquid crystal material or a liquid crystal blend is bonded onto the membrane. The membrane is illuminated with a light source and the transmitted light detected by a light sensor. By applying an AC field onto the membrane the transmitted light is detected and has a unique frequency vs. intensity plot for given liquid crystal.
Passing an analyte molecule through the membrane causes a distinctive and detectable change in the transmitted optical beam. The intensity is found to be frequency dependent and is unique for different analytes.
The use of the optical properties of the liquid crystal in response to an applied field allows a number of different molecules to be identified in one sensor. The optical response converted into an electrical signal by the detector, can be split into three channels. The combination of high and low frequency response is characteristic of the analyte molecule being sensed and the DC offset is dependent upon concentration.
The same method of bonding a liquid crystal onto the membrane has been used to bond a cyanobiphenyl-mesogened liquid crystalline polymer onto 5micon silica via Surface Initiated Atom Transfer Radical Polymerisation. This bonded phase was packed into a new cross electric field capillary column (CEFCC). Varying the cross column electrical field changes the selectivity of the column. A column packed with this novel stationary phase enables shape and polarity recognition, and allows control the chromatographic separation.

LE.67

PORTABLE CAPILLARY LC: A MODULAR MEDIUM PRESSURE SYSTEM DESIGNED WITH OFF-THE-SHELF COMPONENTS

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Portable instrumentation for LC is a necessity for a number of on-site measurement areas, however, commercial portable LC instrumentation can be still considered a scarcity. We set out to rethink the so far most applied approach of designing and in-house workshop fabricating components for a portable LC, as this, unless commercialized, renders it not widely applicable. With a system as widely accessible as possible in mind, our goal was to design a portable medium-pressure LC system based almost entirely from commercial off-the-shelf components. In this work we present results from our initial investigation. The backbone of the system was a breadboard assembled modular flexible microfluidic system, used widely in microfluidics, but not as an LC system, complemented with other off-the-shelf components, including an injection valve and on-capillary detectors, all operated through a PC. The portable LC system featured five syringe pumps, 2+2 for each A and B mobile phase for gradient operation, and another one pump for sampling. The four mobile phase pumps were miniaturised syringe pumps, with 80, 20 and 5 µL syringe options, and maximum backpressure of 8.6, 43, and 106 bar, respectively, calculated from the motor mechanism maximum force and each syringe cross-section. The flow range available was considerably wider than the range desirable (for analysis sped), applicable (respecting pressure limitations) and as a compromise mostly used of ca 1-5 µL/min. The 2 A pumps and 2 B pumps were each connected with microfluidic switching valves (4 in total), and A and B streams then connected with a Y-connector, thus providing a low hold-up volume gradient formation. The stream B (usually to contain an organic solvent for gradient elution), had a microfluidic pressure sensor incorporated. The A+B mixed mobile phase was lead through a 50 mm section of 25 um i.d. capillary to a 20 nL nano-LC sampling valve, connected to a capillary LC column (100 µm i.d., 50- 250 mm length, C18 RP monolith). Detection was conducted on-column or on-capillary (100 µm i.d. PTFE-coated fused silica) with a LED-based UV-vis-NIR photometric detector [1], and/or end-column electrochemical detection (amperometric and/or potentiometric) [2-3]. The potential and experimentally determined performance were evaluated, including the gradient formation performance, and operation under pressure in a range of up to ca 100 bar. The initial test analytes included primarily charged and uncharged dyes of varying hydrophobicity. Future avenues for portable LC systems are discussed.

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LE.68

ONLINE FLUOROUS SOLID-PHASE EXTRACTION (FSPE)-LC-MS/MS FOR QUANTITATIVE DETERMINATION OF PERFLUORINATED COMPOUNDS

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An integrated fluorous solid-phase extraction (FSPE)/liquid chromatographic tandem mass (LC-MS/MS) system for the selective determination of perfluorinated emerging contaminants from water is described.
This technique uses the concept of fluorophilicity as the driving force to capture perfluorinated compounds onto the solid (perfluorinated) adsorbent. By modulating the composition of the eluent (made of water/acetonitrile mixtures to which 0.1% v/v formic acid was added, thus a fully compatible solvent with MS detection), the capture of perfluorinated molecules or their release can be easily achieved. FSPE step was online coupled to LC-MS/MS analysis by means of a multiport switching valve. Separation and quantification of target compounds was performed on a C18 column under reversed-phase conditions. The use of very similar eluents for the release of perfluorinated compounds from the fluorinated adsorbent and their chromatographic analysis is extremely advantageous for the online set-up, especially if compared to more common SPE approaches for this class of compounds (such as those based on weak anion exchangers). This is especially so in terms of automation, shorter analysis time, sample contamination and handling.
Optimization of operative conditions included: the study of wetting properties of the phase (perfluorinated adsorbents are known to be extremely hydrophobic); the choice of a proper organic modifier (based on its fluorophilicity); the minimum volume required to elute target analytes.
The method was statistically validated for the environmentally relevant perfluorooctanoic acid (PFOA) and perfluorooctanoic sulfonate (PFOS). Mass recoveries were very satisfactory.

LE.69

STRATEGIES FOR ACHIEVING UNIVERSAL RESPONSE AND CALIBRATION FOR AEROSOL-BASED DETECTORS IN LIQUID CHROMATOGRAPHY

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The response of the evaporative light scattering detector (ELSD) and the corona-charged aerosol detector (C-CAD) depends strongly on mobile phase composition, which limits the suitability of these detectors for gradient elution separations. To overcome this deficiency we have examined the use of isocratic water-rich mobile phases employed in conjunction with temperature and flow-rate gradients. It was found that eluent temperature marginally influenced the detection response. However, the response of the ELSD and the C-CAD was found to be largely dependent on temperature-induced alterations in elution band-width resulting from the temperature gradient. Compared to the ELSD, the response of the C-CAD remained relatively unaltered with flow-rate variation. Based on these findings two separation approaches were proposed to improve response homogeneity of the C-CAD. In the first approach, a temperature gradient was applied under isocratic conditions, followed by response enhancement by post-column addition of organic solvent. In the second approach, flow-rate programming was used to improve the speed of separations performed using isocratic elution coupled with a temperature gradient. The response homogeneity and the applicability of these approaches were compared to the inverse solvent gradient technique for quantitative analysis. Both the proposed approaches minimise the necessity for solvent gradients and thereby facilitate significant improvement in response homogeneity of the C-CAD. Additionally, these approaches offer flexibility in use of C-CAD for relatively unconventional LC-separation modes and thereby contribute to extending its universality.

LE.70

OPPORTUNITIES AND LIMITATIONS OF RETENTION TIME PREDICTION IN LIQUID CHROMATOGRAPHY

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Chromatographic method development is heavily reliant upon laboratory experimentation. In a typical workflow, a number of stationary phases are screened using low, medium and high pH mobile phases and generic gradient profiles. Stationary phases included in these screens are usually selected based on personal preferences or past success. The stationary phase which provides the best initial resolution of compounds of interest is then taken through a series of experiments during which the pH, gradient and sometimes temperature are optimised, typically using 'non-systematic', 'one parameter at a time' approach. This method development process usually ends when a set of conditions, which meet the resolution criteria is obtained. Although such an approach is often ultimately successful, it generates a huge amount of waste in the form of analytical runs which fail to provide the required resolution.

The objective of this work is to optimize selection of molecular descriptors (feature selection) and to establish whether it is possible to build sufficiently accurate mathematical retention models based on retention data obtained at fixed experimental conditions. Molecular descriptors and physico chemical properties of approximately 100 compounds are used to develop structure retention relationships. We employ an evolutionary algorithm to carry out data reduction and to identify significant descriptors in order to build sufficiently accurate models. In this presentation we will also describe critical factors for optimal algorithm performance

It is our intention to implement these in-silico models in our method development workflow, to reduce or eliminate the initial screening step and direct the method development activities to a chemical space where the probability of success is high.

APPLICATIONS FOR GC-HEADSPACE ANALYSIS FROM METHANE TO SEMIVOLATILES USING A DEANS SWITCH IN THE POLYOLEFIN INDUSTRY

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Headspace gas chromatography is routinely used to analyse the emissions of polyolefins. To analyse the full range of substances from methane to semivolatile hydrocarbons, cryogenic cooling of the GC oven can be used, together with polysiloxane columns. Another approach, that is better suited for quality control labs or production labs with shift operators, is to connect a PLOT column to analyse the VVOC, with a WCOT column, for the determination of semivolatiles, via a deans switch.
In the presented solution, a primary WAX column is used to protect the secondary Al_2O_3 column from water, oxygenates and high boilers. This allows e.g. to separate all hydrocarbons from C1-C6 on the Al_2O_3 column and everything from C7 upwards on the WAX column.
An additional advantage of this setup is the possibility to calibrate gaseous substances in alcoholic solutions, instead of preparing gaseous samples from gas bombs.

Presented applications:
The VVOC emitted from freshly produced material are an important parameter for a polymer producer, to avoid the formation of an explosive atmosphere in storage silos.
Depending on the application of the final product, some VVOCs have to be quantified in trace levels in virgin material. One example is 1,3-butadiene, which is used e.g. in reactive extrusion to form high melt-strength PP.
Another important parameter, that can be measured with this configuration is the total emission potential for materials intended for the interiors of cars, according to relevant standards for car producers e.g. VDA 277.

LE.72

APPLICATION OF NEW GC AND LC-MS APPROACHES TO OVERCOME CURRENT ANALYTICAL DIFFICULTIES IN PESTICIDE RESIDUE ANALYSIS

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The effectiveness of the EU enforcement and risk assessment application under Regulations (EC) No: 396/2005, 882/2004, 7882/2012 etc. rely to a large degree on the adequate performance of the food control laboratories.
A number of conflict issues between Regulations and their application in routine laboratories have been highlighted based in our experience as European Reference Laboratory. Some of these have been satisfactorily solved but others remained as being important analytical challenges. This presentation is focused in those analytical issues that remain as relevant laboratory challenges and the importance in using new mass spectrometry instrumentation to overcoming them.
The outmost of these conflicts have been selected:

- Difficulties arising from complex or “difficult” matrices that typically cause serious problems in qualitative and quantitative analysis.
- Limitations in the analytical scope. Commonly as a consequence of workflow limitations to introduce a large number of compounds full validated in multiresidue methods.

Instrumental solutions for both points are related and can be overcome by applying upgraded Mass Spectrometric based techniques. It is of great interest the use of high sensitive instruments or/and high resolution mass spectrometry.
Selected and illustrative practical cases learnt from the European Proficiency Tests performed during the last years are presented. From them a number of examples of the capabilities of updated MS approaches to solve the presented difficulties are also evaluated.

LE.73

GAS CHROMATOGRAPHY-COMBUSTION-CARBON ISOTOPE MASS SPECTROMETRY (GC-C-IRMS) FOR THE TRACEABILITY OF NATURAL COMPOUNDS

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The traceability of natural compounds is often required for food, feed and pharmaceutical natural ingredients. In fact, the origin of ingredients is often reported on foods labels for the protection of consumers, facilitating the withdrawal of foods, and providing the consumers with targeted and accurate information on the different products. The EU's General Food Law, which entered into force in 2002, made traceability compulsory for all food and feed businesses. In addition to the general requirements, sector-specific legislation applies to certain categories of food products so that consumers can identify their origin and authenticity. Thus, traceability is based on sever protocols and paperwork, but still requires analytical support, in order to unveil potential frauds.

From the analytical point of view, the assessment of the origin (geographic or source) of natural compounds can be achieved by different tools, among which the isotope abundance of ^{13}C is one of the most applied. In order to define useful parameters the isotope ratios, expressed as $\delta^{13}C$ can be determined by the use of gas chromatography-combustion isotope ratio mass spectrometry (GC-C-IRMS). The approach allows to pre-separate the components of interest on a capillary column, with the aim of directly determining the $\delta^{13}C$ value, through a combustion chamber followed by the isotope ratio MS instrument section. The instrumentation consists indeed, of a GC coupled to a mass spectrometer, *via* an appropriate interface, that enables the determination of the isotope ratios of specific elements (D/H, $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{18}O/^{16}O$ isotope).

In this presentation, GC-C-IRMS applications will be shown in relation to coffee samples of different geographic origins, fatty acids (methyl esters) of different natural sources, as well as specific examples on the characterization of various natural compounds.

LE.74

NEW DEVELOPMENTS IN GC COLUMN TECHNOLOGY

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Capillary gas chromatography columns have been around for decades but the need for improved columns continues to be a priority. The GC application field is evolving, resulting in more demanding methods, especially when highly sensitive detectors such as mass spectrometry (MS) are used. GC/MS systems are now commonly used in many analytical laboratories all over the world, and to detect low levels of compounds a clean and inert flow path is required. We at the GC columns R&D team continue to focus on inventing new technologies for the development of the next generation of GC columns.
Important attributes of GC columns are inertness, bleed and separation power. In this presentation I illustrate the development of technology for Ultra Inert Flow Path columns and supplies, and the latest development of PLOT columns with integrated particle traps.

LE.75

A NEW FOODOMICS STUDY INTEGRATING METABOLOMICS AND TRANSCRIPTOMICS PLATFORMS TO EVALUATE THE ANTICANCER ACTIVITY OF FOOD COMPOUNDS

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Nowadays, safety and bioactivity of foods and food ingredients can be investigated at molecular level integrating the results obtained from advanced omics platforms (including genomics, transcriptomics, proteomics and/or metabolomics) following a Foodomics approach [1-4].
In this work, we will present the last results obtained from a Foodomics strategy developed in our laboratory, integrating metabolomics and transcriptomics platforms, to investigate the anti-proliferative effect of dietary polyphenols against human cancer cells. The present work will give additional information on how some dietary polyphenols are able to modulate specific metabolic pathways in the cancer cells, providing new evidences at molecular level on the antiproliferative effect of this type of compounds.

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CHARACTERIZATION OF TRACE ETHYLENE GLYCOL IN INDUSTRIAL SOLVENTS AND LUBRICANTS USING PHENOL BORONIC ACID DERIVATIZATION AND MULTIDIMENSIONAL GAS CHROMATOGRAPHY

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A gas chromatographic approach is introduced for the characterization of trace ethylene glycol inindustrialsolventsandlubricants. The analytical approach employs single step derivatization technique that effectively converts ethylene glycol to the cyclic boronate ester using phenyl boronic acid as a derivatizing agent. The separation of the derivatized product was achieved by using multidimensional gas chromatography. Heavyl ubricant matrices like engine crank case oil were back-flushed to improve sample throughput and system cleanliness. Detection and quantitation of the cyclic boronate ester was conducted with mass spectrometry in selected ion monitoring mode. Propylene glycol can also be analyzed using the same approach and water does not inhibit the formation of the derivatives, most probably owing to the use of 2,2-dimethoxypropane as a solvent for the derivatizing agent.
In this lecture, the novel analytical approach, method performance, and practical applications will be presented.

LE.77

ON-LINE LC-GC: A MULTIDIMENTIONAL CHROMATOGRAPHIC METHOD TO SOLVE DIFFICULT ANALYTICAL PROBLEMS. A NEW DEDICATED INSTRUMENT THAT ENHANCES THE USE OF THIS TECHNIQUE

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The GC with capillary columns is one of the most powerful analytical techniques available today in many application fields, but in recent years it has become clear to many that a simple GC system with a single analytical column, even if combined with a selective detector, it cannot completely solve many of the problems encountered in the analysis of complex samples, especially when the components of interest are at trace levels.
In many cases a careful pre-separation of the sample is required, because the matrix may reduce or even destroy the separation efficiency of the capillary column or obscure completely the detector.
Some of the problems that can be normally found are:
- Components of interest are usually dispersed in difficult matrixes, so that many compounds can interfere with the separation or the detection
- Cross contamination as a consequence of very different concentration between target compounds and matrix components
- Lack of selectivity, detectability, reliability, accuracy and repeatability of the analysis

The on-line LC-GC can help in solving some of these problems because:
The sample is introduced into the HPLC column which allows, on one hand, to hold non-volatile substances that would contaminate the gaschromatographic column and on the other hand provides its selectivity to separate the sample into classes of compounds.
The HPLC fractions of interest are then on-line transferred to the GC and the compounds can be more easily separated by the gas chromatographic column and detected by any GC detectors, including the MS.
The system is complex and consequently to be easily operated needs to be equipped with suitable components and automated as much as possible. This is what has been done developing the new LC-GC 9000, an instrument that combines traditional components of previous instruments with modern and powerful technology in a single package.

LE.78

THE CONTINUING EVOLUTION OF MULTIDIMENSIONAL CHROMATOGRAPHY

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Multidimensional Chromatography is a technique in continuous evolution. Today it is recognized and accepted that the comprehensive version of two dimensional chromatography offers much larger peak capacities and is able to resolve compex samples where a single dimension technique does not provide sufficient resolving power. The increased resolving power has been utilized for samples of synthetic and biological origin.

In Multidimensional Chromatography, sample components are fractionated by two dissimilar column functionalities, resulting in two different retention mechanisms. In order to maximize resolution of complex samples, different retention mechanisms are required to spread the separation into an area based representation of zones.

The advantages of increased peak capacity, improved resolution and ability to handle complex matrices in an efficient manner in our opinion far surpass the dissadvantages of instrumentation complexity and time investment necessary to develop more than one separation procedure essentially at the same time.

This presentation will review the evolution of the technology, and will discuss the utilization of newer coupling approaches for the solution of difficult problems related to samples of industrial and biological origin. Various examples will be used to illustrate the concepts, and the importance of the data obtained in the context of problem solving will be discussed.

LE.79

ANALYSIS OF NUTMEG ESSENTIAL OIL AS A SOURCE OF PRECURSORS OF MDMA BY TWO-DIMENSIONAL GAS CHROMATOGRAPHY

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MDMA, or N-methyl-1-(3,4-methylenedioxyphenyl) propan-2-amine, is a synthetic substance commonly known as ecstasy, although this term is been used today to describe other substances. With a chemical structure similar to methamphetamine and mescaline, it acts as both a stimulant and psychedelic. It was first synthesized by a chemical company in 1912, but its hazardous and toxic effects caused it to be criminalized in most countries. MDMA can be manufactured from four principal precursors: safrole, isosafrole, piperonal and 3,4-methylenedioxyphenyl-2-propanone (PMK). The last three, however, can be synthesized from safrole. In this study, we analyze nutmeg (Myristica fragrans) and its essential oil as a source of safrole, with the use of two-dimensional chromatography, coupled to a mass spectrometer, CG-CG/MS. Excessive consumption of nutmeg has been already related to toxic effects due to the presence of chemical substances like myristicin and elemicida. In this context, low doses of gamma radiation were used to verify how concentrations of the MDMA precursor could vary with this kind of treatment. Four samples were prepared, being three of them irradiated in a research irradiator with cesium-137 source, at doses of 1.0, 3.0 and 5.0 kGy, respectively, with dose rate 1.8 kGy / h; the fourth one was kept as a control. The essential oil extraction was carried out by steam distillation, using the modified Clevenger apparatus. The Shimadzu MDGC system consisted of two GC-2010 gas chromatographs (defined as GC 1 and GC 2), an MS-QP2010 quadrupole mass spectrometer. The MDGC transfer device, located in GC 1, is connected to an advanced pressure control (APC) unit which supplies carrier gas (He), at constant pressure. In the first GC it was used the polar column Solgel-Wax 30m x 0,25 mm i. d. x 0,25 µm (SGE) and in the second GC the apolar column BP5 30m x 0,25mm i.d. x 0,25µ m (SGE) as columns. Mass Ion source: 230°C; interface temp: 250°C, interval scan: 40-400 *m/z*; scan speed: 2000 amu/s. The combined use of a polar column and one apolar made it possible to achieve excellent results, and safrole represented 8.72% of the essential oil composition.

LE.80

LARGE VOLUME INJECTION AND HEART-CUTTING TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY IN THE PHARMACEUTICAL INDUSTRY

Isabelle François[1], Davy Petit[1], Benny Vanneste[1], Valerie Koppen[2], Cis Van Looveren[2], Mark Bockx[2], Filip Cuyckens[2], Rudy Sneyers[2], Jeroen Peeters[2], Luc Van Grieken[2], Eddy Verhoeven[2], Gaby Torok[2]

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When using one-dimensional ultraperformance liquid chromatography (UPLC), the peak capacity and selectivity might be insufficient in order to separate all compounds. The implementation of two-dimensional (2D) LC is therefore highly beneficial in order to address co-elution issues. In the presented approach, orthogonal dimensions are combined in a heart-cutting configuration, so that no compromises towards the second dimension separation are needed allowing a relatively easy and generic approach of implementing 2D throughout various departments .

Next to co-elutions, sensitivity is another important aspect in a variety of departments of a pharmaceutical company. Despite the availability of sophisticated analytical tools and highly sensitive mass spectrometers, the development and validation of methods allowing trace level determination is a challenging task. In the present session, sensitivity requirements are addressed by the injection of large volumes. Peak distortion and interferences due to the presence of the large quantity of API are avoided by using selective enrichment on a trapping column. When using the principle of ‘at-column dilution’ (ACD), the injection solvent is furthermore not limited to weak mobile phase constituents. Furthermore, the application of the highly selective radioactivity detector allows the detection of trace levels of radioactive compounds.

In the presentation, the benefits of the indicated multidimensional approach will be illustrated with practical examples in the pharmaceutical industry.

VALIDATION OF GC-MS/MS CONFIRMATORY METHOD FOR THE EU OFFICIAL CONTROL OF LEVELS OF PCDD/F AND DL-PCB IN FEED, ANTICIPATING AMENDMENT TO REGULATION (EU) No 278/2012

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Criteria for sampling and analysis for the official control of levels of dioxins (including polychlorinated dibenzo-*p*-dioxins -PCDDs- and polychlorinated dibenzofurans -PCDFs-), and dioxin-like PCBs (DL-PCBs) in feeding stuff and in certain foodstuffs are described in Commission Regulations (EU) No 278/2012 [1] and 252/2012 [2]. At the time the abstract is written (Feb. 2014), gas chromatography high resolution mass spectrometry (GC-HRMS) is required for the confirmatory analysis of PCDD/Fs and DL-PCBs whereas MS/MS technology can only be used as screening method. However, a new amendment to part B of Annex V to Commission Regulation (EU) No 152/2009, currently amended by Regulation (EU) No 278/2012, has already been voted [3] and shall enter into force on early 2014. This amendment will allow the use of GC-MS/MS systems as confirmatory method in official control of dioxins and dioxin-like PCBs

This work aims to prove that this technology meets requirements and comply with standard criteria established for GC-HRMS. The MS/MS method has been validated for feed material of plant origin (vegetable oil) at the regulation level [4] of 0,75ng WHO-PCDD/F-TEQ/kg and 1,5ng WHO-PCDD/F-PCB-TEQ/kg. Results are also compared with the current routine confirmatory GC-HRMS method. Validation will further be extended to other matrices.

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[1] Commission Regulation (EC) No 278/2012 of 28 March 2012 amending Regulation (EC) No 152/2009 (OJ L 91, 29.3.2012, p. 8–22)

[2] Commission Regulation (EU) No 252/2012 of 21 March 2012 repealing Regulation (EC) No 1883/2006 (OJ L 84, 23.3.2012, p. 1–22)

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amending Annexes I and II to Directive 2002/32/EC (OJ L 91, 29.3.2012, p. 1–7)

KNL.02

INTERCONTINENTAL VALIDATION STUDY OF THERMODYNAMIC PREDICTIONS OF GC RETENTION TIMES

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Thermodynamic data for use in the predictive modelling of retention times has been shown to have superior accuracy in its predictions when compared to models that employ retention indices.[1] Adapting thermodynamic models to make predictions of GC×GC separations is also straightforward,[1] unlike what is observed for predicting GC×GC separations based on retention index data, for example. Thermodynamic models are also robust to changes in separation conditions such as temperature and temperature programming rate. Recent work[2] has significantly reduced the time required to collect thermodynamic data required to make these predictions from a handful of compounds per week to dozens of compounds in a morning. This opens the possibility of constructing databases of thermodynamic data that will have practical utility for gas chromatographers. Here we present the results of an inter-laboratory study spanning six research groups, four countries, and three continents that set out to validate a thermodynamic-based model using newly developed techniques to obtain the data[2] and account for column-to-column variability. The thermodynamic parameters for 91 analytes on four stationary phases will be evaluated and compared between groups. The study has been designed to also capture data on within-batch and between-batch variability of column manufacture. This study will validate the methods and indicate the actual feasibility of generating widely applicable thermodynamic libraries of GC data.

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KNL.03

UTILIZATION OF ATMOSPHERIC PRESSURE IONIZATION COUPLED TO TRIPLE QUADRUPOLE MASS SPECTROMETRY FOR THE ANALYSIS OF MIXED-HALO PLANAR COMPOUNDS

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Halogenated dioxins and furans have long been persistent organic pollutants of environmental concern that are byproducts from a variety of different sources. In many cases, it has become important to be able to detect a range of dioxins and furans at trace levels in a diverse range of sample matrices. In addition to increased sensitivity, accuracy and repeatability are important as well for dioxin analysis of environmental samples. Historically this has been nearly universally conducted with GC-HRMS systems employing double-focusing, or “sector” based instruments where specific mass-to-charge fragments are monitored for each target compound. This analytical approach, though not without its own issues, has worked well for the limited numbers of target analytes that are often measured with a method similar to USEPA 1613. When the target compound list becomes larger, or more importantly when the analysis also includes a discovery component, this technology becomes more limited.

Utilizing atmospheric pressure ionization coupled to a triple quadrupole mass spectrometer allows for trace level identification of dioxins and furans in a variety of environmental sample matrices. Using this instrumentation, a limit of detection on the femtogram levels can be reported. This technique allows for some benefits as compared to the sector-based HRMS approach, including enhanced sensitivity and the ability to monitor for more compound classes at a single time.

This presentation will demonstrate the challenges and success of using this technique for the analysis of environmental samples, including sediment, fire debris, and human serum, to demonstrate the accuracy and sensitivity of using APGC-TQS as a tool for dioxin analysis. Furthermore, identification of a variety of different halogenated dioxins and furans will be demonstrated, including polychloro-, polybromo-, and mixed bromo/chloro- congeners. Additionally this presentation will summarize the results obtained through an extensive comparison of samples analyzed using both methodologies to demonstrate that this instrumental approach is valid for the analysis of compounds of this type.

KNL.04

SOLID PHASE MICROEXTRACTION FOR IN-VIVO AND EX-VIVO ANALYSIS OF HUMAN SALIVA

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On-site sample preparation is analytical approach based on direct sampling from the system under investigation. It has the advantage of combining sampling and sample preparation in to a single step. In-vivo sampling offers many unique advantages such as being fast, minimizing the potential sources of error and eliminating risks for analytes stability. Nowadays many efforts have been made to transfer the sample preparation from in lab to on-site with better understanding of the system under study. One of them is rapid on-site sampling of prohibited substances during sport competitions. Thus on site in-vivo and ex-vivo sample preparation methods can be good alternative to classical sampling methods in doping analysis. Saliva is important as target matrix for such application, as sampling from this matrix can be performed conveniently under public supervision and the decision about the abuse can be made just after athletic competition. The stability of short-lived and matrix unstable compounds can be provided by direct and fast sampling of analytes from saliva by using solid phase microextraction (SPME). Moreover, for such analysis SPME in its thin film geometry can provide robust and convenient sampling offering in the same time faster analysis and higher extraction recovery (i.e. better sensitivity) due to large surface to volume ratio.

In this study the applicability of thin film SPME in coated blade and membrane formats will be demonstrated for saliva analysis by comparing the LC and GC based coverage of analytes with a single type of extraction phase. Due to applicability for wide range of polarity of analytes as well as thermal and solvent stability during the desorption, hydrophilic lipophilic balance particles were chosen as extraction phase and used for fast (5 min) in-vivo and ex-vivo analysis of saliva. The developed assay offers fast and reliable multiresudue analysis as an attractive alternative to the standard methods that are currently used in anti-doping laboratories.

KNL.05

ENVIRONMENTAL RISK FACTORS IN THE SUDDEN INFANT AND INTRAUTERINE DEATH SYNDROMES: AN ANALYTICAL APPROACH

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A funded research project is on-going to investigate the potential impact of endocrine disrupting compounds (EDCs) exposure on SIDS (Sudden Infant Death Syndrome) and SIUDS (Sudden Intrauterine Unexplained Death Syndrome). Literature data demonstrate the negative effect of OCPs, OPPs and xenobiotic compounds, such as Bisphenol A, on fetal growth and brain development. However, information regarding the detection of these pollutants in SIUDS and SIDS autopsy findings is still missing. The purpose of this project is to develop a sensitive method for the extraction and detection of EDCs in human fetal and newborn tissues. The results will be used to clarify whether specific classes of EDCs and other pollutants can be included as environmental risk factor for those syndromes. Twenty-three specific target compounds were selected according to the local environmental conditions of the cases under study, including organochlorine and organophosphorous pesticides used in the area of origin of the victims, an intensive agricultural region in Northern Italy. The extraction method was developed and validated on animal brain tissue in terms of accuracy, precision, LOQ, LOD and linearity and applied to human brains from SIUDS and SIDS victims. The recovery was evaluated with 7 isotopically labeled internal standards at three different concentrations, used for method validation. The analytical determination was approached by using GC-qMS, fastGC-TOFMS and GC-MSMS for comparative purposes in terms of sensitivity, repeatability and ID confirmation.A preliminary investigation was carried out to establish possible benefits in using a GC-MSMS (QQQ) in place of a conventional GC-qMS system. Despite the advantages of using GC-MS for the determination of selected EDCs, this technique may present deficiencies in trace analysis of complex matrices like biological tissues, due to the presence of matrix interferences. In particular, the specificity reached by MS/MS, using a triple quadrupole analyzer, allows to minimize or eliminate these interferences and to improve selectivity and sensitivity. In addition, TOF analyzer was used to investigate the presence of non targeted compounds non included in our list. Figures of merit and validation results will be presented.To our knowledge, this is the first study to correlate EDCs exposure with the occurrence of SIDS and SIUDS.

KNL.06

CHARACTERIZATION OF PHENOL AND ALKYL PHENOLS IN ORGANIC MATRICES WITH MONOETHYLENE GLYCOL EXTRACTION AND MULTIDIMENSIONAL GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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Phenols, cresols, and xylenols are chemicals of industrial significance. They are involved in the production of polymers, drugs, dyes, explosives, pesticides, disinfectants, antiseptics, medicinal preparations to name a few.

The use of monoethylene glycol as an extraction medium for removing phenolic compounds in organic matrices such as hydrocarbons is introduced. This single-step extraction technique was found to much more reliable than the classical caustic/acidification approaches while delivering high extraction performance. With this approach, the extraction efficiency of phenol approaches 100% while cresols, xylenols, and ethylphenols were 97% or higher.

The extraction technique was combined with multidimensional gas chromatography employing a planar microfluidic device for Deans switching. Mass spectrometry in selective ion monitoring mode for detection and quantitation was implemened to further enhance analytical system selectivity and sensitivity.

Analytical approach, method performance, and selected applications will be presented in this lecture.

HYBRID ORGANO-SILICA MONOLITHIC CAPILLARIES: OPTIMIZING SEPARATION PERFORMANCE BY TUNING PREPARATION CONDITIONS

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In the last decade, hybrid organo-silica monoliths found their application as stationary phases in capillary electrochromatography, and capillary liquid chromatography mainly due to their easy "one-pot" synthesis. This study is focused on *in-situ* preparation of monolithic stationary phases from 3-(methacryloyloxy)propyl trimethoxysilane. The process requires acid catalyzed sequential hydrolysis and polycondensation of alkoxysilane as well as polymerization of methacrylate functionalities in the presence of an initiator and a porogenic solvent. We have previously shown [1] how photo-polymerized hybrid monolithic capillaries can be prepared with differing separation properties by varying the amount of porogen and the nature of the initiator. We have since extended this concept to thermally polymerized monoliths and investigated the effect of changing the amount of: porogen (toluene), aqueous acid catalyst (HCl), initiator (azobisisobutyronitrile), polymerization time, and polymerization temperature on the separation performance. Optimized monolithic capillaries were characterized utilizing various sets of solutes in reversed-phase liquid chromatography. Resulting information about hydrophobicity, steric selectivity, extent of hydrogen bonding, and porosity serves for comparison of capillaries prepared using each polymerization approach. Comparable chromatographic properties were found for thermally and photo-polymerized capillaries with efficiency of up to 117 000 plates/m for ethylbenzene at the optimum flow rate. However, much faster light-induced conditions result in formation of monoliths with reduced average column efficiency, and poor batch-to-batch repeatability. The resulting methacrylate functionality at the pore surface provides an excellent platform for further surface modifications resulting in columns with different selectivity.

Aknowledgements
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KNL.08

COMPARATIVE PROFILING BY MICROCHIP ELECTROPHORESIS OF SERUM N-GLYCANS FROM CANCER PATIENTS

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Glycosylation of various soluble and membrane-bound proteins is impacted by the onset and progression of cancer. Quantitative analysis of these changes in the glycome may provide valuable insight for clinical diagnosis and prognosis. We are developing methods to detect cancer and pre-malignant conditions by microchip electrophoresis and laser-induced fluorescence detection of N-glycans derived from human blood serum. We designed microfluidic devices with serpentine separation channels that are 22-cm long and have optimized turn geometries to minimize turn-induced band broadening. These devices separate glycans with efficiencies and reproducibilities comparable to capillary-based platforms, but have significantly reduced analysis times and sample consumption. Separations at 1250 V/cm yielded efficiencies up to 800,000 plates and resolved high- and low-abundance N-glycans and their structural isomers within 100 s. With this platform, we determined quantitative differences among the N-glycan profiles of control individuals (age and sex matched, disease-free volunteers) and patients with late-stage recurrent ovarian cancer prior to and after an experimental drug treatment. Here, we identified several N-glycan peaks and peak combinations that distinguished the control and cancer samples with high accuracy. To improve glycan identification, we developed a strategy to neutralize the charges on sialylated N-glycans by covalent derivatization. Peaks from the electropherograms are assigned specific N-glycan structures through a correlation of migration time, molar mass, relative peak abundance, and isomeric composition. Because the neutralization reaction and fluorescent labeling are both quantitative, we are able to compare derivatized samples from ovarian cancer patients and control individuals and identify N-glycan structures that have quantitative differences among these groups.

KNL.09

MONOLITHIC THIN LAYERS FOR TLC-MALDI MS SEPARATIONS OF PEPTIDES AND FATTY ACIDS

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Porous polymer monoliths have a wide range of applications, including a variety of chromatographic modes. Aside their typical cylindrical column format, polymer monoliths have also been prepared as 12-200 μm thin layers and used for the separation of biomolecules by thin-layer chromatography (TLC)[1,2]. TLC is a rapid, facile and inexpensive separation technique. It can be the method of choice for clinical analysis in underdeveloped countries, where sophisticated instrumentation is less suitable due to requirements for qualified labor and continuous supply of electricity. It is also adjustable to 2-dimensional separations, which afford better resolution and selectivity[3,4]. Owing to its flat format, TLC can be coupled with advanced detection methods, such as matrix-assisted laser desorption/ionization (MALDI) mass spectrometry that enables the collection of molecular mass information for the identification of the separated components[4].

We have recently developed thin monolithic layers for the efficient TLC separation of biologically relevant analytes, such as peptides and fatty acids. By using a conventional MALDI instrument we detected the separated components directly from their individual spots on the developed plates. The porous structure of our monolithic layers not only enables the separation, but is also capable for efficient energy transfer from the laser beam to the analytes, in order to promote their desorption and ionization without the use of a traditional matrix. The ionization is controlled by the morphology of the porous monolithic structure. Functionalization of the pores with conductive gold and silver nanoparticles or grafting the pore surface with polymer chains consisting of polymerizable MALDI matrix eliminates the need for conventional matrixes. The advantages of this approach include simplified sample preparation and the ability to detect small molecules in size ranges currently inaccessible by MALDI due to the presence of strong chemical background signals.

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KNL.10

1.1 MICRON SUPERFICIALLY POROUS PARTICLES FOR BIOLOGICAL SEPARATIONS

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This work presents the chromatographic application of 1.1 micron diameter, wide pore, thin shell, superficially porous particles. The thin porous layer and wide pores of these particles make them suitable for biological separations. Such particle architecture has received interest in recent years for biological analysis, with commercial options entering the market. The particles presented here are much smaller than the commercially available wide pore superficially porous particles.

This work presents the chromatographic performance of in-house 1.1 micron diameter, wide pore, thin shell, superficially porous particles when packed into capillary columns. Due to their small size, elevated pressures are required for chromatographic analysis and therefore the capillary geometry is advantageous. These columns are initially characterized with small molecules to assess their baseline performance and packing characteristics. These columns are also utilized to separate biological molecules in gradient mode. Differences between particles used in these separations are discussed in light of the separation characteristics of the columns.

KNL.11

DROPLET-BASED SCREENING OF ENZYME MODULATORS USING SUB-SECOND MICROCHIP ELECTROPHORESIS

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Multiwell plate-based high-throughput screening (HTS) has emerged as a powerful tool for drug discovery and evaluation by allowing tens of thousands of assays to completed in one day. This high-throughput is achieved through use of robotic plate manipulation and liquid handling with fluorescence detection. While this method has been successful, only one analyte is typically detected and false results can occur due to interactions with indicator reactions. We have developed method to use droplet microfluidics to couple multiwell plate-based assays to microchip electrophoresis (MCE) to screen enzyme modulators. Samples contained in multiwell plates are reformatted in to droplet streams with a sample volume of 8 nL. These reformatted samples streams are coupled to a hybrid PDMS-glass microfluidic device where aqueous samples can be extracted from the droplet stream and rapidly analyzed by MCE with laser-induced fluorescence detection. The hybrid device allows for decoupling the sample extraction and separation stages to allow finer control over injection timing and volume and increase device performance. We applied this system to screen small molecule modulators of protein kinase A. For each sample in the screen, 3 droplets are generated and each droplet is injected approximately 3 times. Using a 1 second separation at 2000 V/cm, we are able to perform over 1000 injections in 17 minutes to analyze a 96 compound screen against protein kinase A (PKA). Separation resolution between the internal standard, substrate, and product is 1.2 and migration times vary by less than 2 percent over 1000 injections. We were able to identify two known PKA inhibitors from a sample set of 96 compounds and controls. While currently applied to screening applications, the device layout and operation is amenable to many applications that require rapid analysis, including coupling 2D separations and chemical sensing.

KNL.12

COUPLING OF NEW SILVER THIOLATE AND MONODISPERSE MATERIALS IN COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY FOR TRULY ORTHOGONAL LIPID SEPARATION

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A large array of liquid chromatographic (LC) methods has been used for identification and quantification of the different lipid classes, including normal-phase LC, silver-ion, and non-aqueous reversed-phase LC. However, no single analytical technology has allowed the identification and quantification of all the different lipids from either food or biological sample within a single experiment; a combination of two or more separation forms must be used, either in off-line or on-line mode.Lipid analysis is an extremely difficult task, due to the enormous number of possible fatty acid combinations on the glycerol backbone. Whenever the high sample complexity requires high separation power, ultra-high pressure LC operation mode allows more space for method optimization. The use of mass spectrometry represents an added dimension to LC separation systems, unravelling post column co-eluting components and enabling their identification. A powerful approach based on two-dimensional comprehensive UHPLC is here presented, in which high orthogonality is achieved by the coupling of silver chromatography and non-aqueous reversed phase separation in the first and in the second dimension, respectively. High accurate mass data are obtained by ion-trap time-of-flight mass spectrometry detection, along with ion fragments which render structural elucidation feasible.

HYPHENATED AND COMPREHENSIVE LIQUID CHROMATOGRAPHY ? GAS CHROMATOGRAPHY FOR THE IDENTIFICATION OF MYCOBACTERIUM TUBERCULOSIS

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Tuberculosis (TB) remains one of the world's most pressing public health problems. The World Health Organisation (WHO) estimates a global prevalence with 14 million new cases and 1.68 million deaths each year. The majority of TB cases occur in low-income countries that have poor resources in the public health care sector. Although the disease is curable, late diagnosis has serious consequences for the patient and contributes to the increase of the epidemic [1]. Current methods for identifying the mycobacteria responsible for TB, *Mycobacterium tuberculo sis* (MTB), are time-consuming, labour intensive, too expensive in terms of running costs for developing countries and lack sensitivity [2,3]. Chromatographic methods could resolve these issues at least partially.

Several chromatography-based methods for TB diagnosis have been published in literature. An HPLC method for the identification of mycobacteria based on the mycolic acid patterns was developed by the Centre for Disease Control and Prevention (CDC) already two decades ago [4]. Recently, we have developed a fully automated GC procedure based on thermally – assisted hydrolysis and methylation (THM-GC-MS) and advanced chemometrics to detect MTB [5]. Irrespective of whether LC or GC is used, due to the complexity of the samples the evaluation of potential biomarkers is extremely challenging.

The present contribution aims to combine LC and GC in parallel and in series in order to overcome the difficulties of biomarker evaluation. The in-series combination consists of the LC analysis with heart-cut or comprehensive transfer of the specific mycolic acid fractions from the LC system to the GC. In this way the strengths of the LC and the GC methods are combined resulting in better detection limits (i.e. earlier disease diagnosis) and an improved accuracy and selectivity.

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KNL.14

DETERMINATION OF ALKYL-METHOXYPYRAZINES IN VARIOUS SPECIES USING HS-SPME AND ENANTIO-MDGC-QQQ-MS

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3-Alkyl-2-methoxypyrazines, especially *iso*-propyl- (IPMP), *sec*-butyl- (SBMP) and *iso*-butyl- (IBMP) methoxypyrazines (MPs) are potent odorants in foodstuffs with intense, vegetative (green) flavors. They are not only present in vegetables like green beans, bell pepper, peas etc., but also in variants of *Vitis vinifera* (Cabernet types, Sauvignon blanc [1]) and in insects like ladybugs (*Coccinella septempunctata* and *Harmonia axyridis* [2]). The odor thresholds for MPs are very low, for example 1-2 ng/L IBMP in dry white wine [3]. Depending on the species, MPs occur in either low concentration ranges (ng/L in wine from *Vitis vinifera*) or in higher ranges (up to µg/kg in vegetables). In the case of SBMP two enantiomeric forms exist. Determination of the enantiomeric composition could provide an insight into the biosynthetic pathway of MPs.

Our proposed analytical method is based on headspace solid phase microextraction (HS-SPME) and separation of MPs by heart-cut multidimensional gas chromatography (MDGC). Using an enantioselective stationary phase in second dimension and tandem mass spectrometry (QqQ-MS) permits the (enantioselective) determination of MPs in trace levels and in complex matrices. Quantification utilizes deuterated isotopologues as internal standards in a stable isotope dilution assay (SIDA).

In addition to the quantitative determination of MPs, the differentiation of the enantiomers of SBMP in various species is presented. The results obtained indicate that the naturally occurring enantiomer is (*S*) –SBMP (> 99 %). Furthermore, we describe the presence of a dimethyl methoxypyrazine in *Harmonia axyridis* which is principally in agreement with data from literature, but differs in the assignment of the configuration [4].

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KNL.15

A DETAILED INVESTIGATION OF THE RELEVANCE OF OPERATING PARAMETERS IN THE VACUUM OUTLET GC/MS TECHNIQUE

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Among the various techniques used nowadays for fast gas chromatography, the vacuum outlet GC/MS technique probably is the best suited when conventional instrumentation is used. Vacuum outlet GC is performed by operating the separation column at reduced pressure which can be very easily realized by coupling with a mass spectrometer. Short, wide-bore columns are generally selected for expanding the vacuum throughout the entire length of the capillaries. Since the reduced pressure within the capillary leads to a reduced viscosity of the carrier gas, a highly feasible situation is achieved for fast chromatographic separation.
In order to restrict the carrier gas flow to an acceptable level for the MS, while working at column head pressures that can be precisely maintained by electronic pressure control, different types of restrictions have been developed and investigated, with the short, narrow-bore columns being most frequently selected. Under these conditions, there is no need for any modification in the injection, separation or detection part of the instrument when this fast chromatographic technique is applied [1].

In this study, various experimental setups, concerning different lengths of the analytical column and different lengths and inner diameters of the narrow-bore restrictions, have been selected and investigated. Comparison of the obtained separation efficiencies and the corresponding selectivities under different inlet pressure conditions will be illustrated, leading to a better understanding of the working principles of the technique. For the aforementioned investigations, a group of low boiling point analytes has been employed, expanding the possible application range of the technique beyond the fields where its usefulness has already been established. Such an example involves the analysis of decomposition products and gases emitted from lithium-ion batteries operated under abuse conditions. Hence, the risk assessment of unexpected chemical hazards that can occur during accidents or abuse performance is enabled.

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KNL.16

TOWARDS HIGHER PEAK-PRODUCTION RATES: SPATIAL TWO- AND THREE-DIMENSIONAL LIQUID CHROMATOGRAPHY

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Spatial chromatography is defined as a chromatographic method whereby components are separated in the space domain, with each peak being characterized by its coordinates in a plane or a three-dimensional separation body. Well-known examples of spatial LCxLC include thin-layer, chromatography (2D-TLC) and two-dimensional poly-acrylamide gel electrophoresis (2D-PAGE). Due to parallel separations in the second dimension, the analysis time is greatly reduced compared to a coupled-column multi-dimensional LC approach. As a result, spatial two-dimensional chromatography provides much higher peak-production rates. Spatial three-dimensional chromatography has the potential to offer unrivaled peak capacities and peak-production rates. In order to successfully tackle truly complex separation problems, arising in areas such as proteomics, the development of novel multi-dimensional separation technology is required, addressing both the bottlenecks of efficiency and analysis time.

The design and application of polymer-based microfluidic devices for comprehensive spatial LCxLC and LCxLCxLC chromatography will be discussed. These microfluidic devices are constructed from multiple layers of cyclic olefin copolymer (COC) substrate, each micro-machined with their specific channel layout. The proposed device for two-dimensional chromatography features a first-dimension (^{1}D) separation channel and 21 parallel second-dimension (^{2}D) separation channels orientated perpendicular to the ^{1}D channel.

Compartmentalization of ^{1}D and ^{2}D flow by a physical barrier allows for a preferential mobile phase flow path mainly restricted to the ^{1}D separation channel, with a minimal dispersion into the ^{2}D separation channels. After a ^{1}D separation is halted, a radially-interconnected flow distributor of diamond-shaped pillars with diverging side-walls introduces the mobile phase for the ^{2}D separation, allowing eluent inside the ^{1}D channel to be fractionated and simultaneously injected into the ^{2}D channels. A monolithic stationary phase was polymerized *in-situ* in desired locations via a UV-initiated polymerization reaction employing a photo mask.

Preliminary results show the separation of a mixture of dyes and a mixture of proteins, employing colorimetric and fluorescence-detection, respectively. Finally, the design of a spatial LCxLCxLC device is demonstrated, extending the 2D approach to 3D applying a ramified ^{3}D flow distributor consisting of eight generations of T-bifurcations resulting in 256 outlets.

KNL.17

SIMULTANEOUS DETERMINATION OF ALKYLPHENOLS POLYETHOXYLATES, ALKYLPHENOXY CARBOXYLATES AND ALKYLPHENOLS BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Alkylphenols polyethoxylates (APEOs) with an average ethoxylate number between 3 and 10, have been widely used in many industrial applications, mainly as non-ionic surfactants [1]. The presence of APEOs and their biodegradation derivatives have been frequently reported in literature for different fresh water ecosystems [2]. Most persistent APEOs degradation metabolites are APEOs oligomers with 1-3 ethoxylate groups, alkylphenoxy carboxylates (APECs) and alkylphenols (APs), and several studies have shown that these compounds exhibit estrogenicity; accordingly, the European Community, by means of the Directive 2003/53/EC and subsequent regulations, has restricted the marketing and the use of some of these compounds. Nevertheless, these restrictions do not cover neither the use of polyethoxylated octylphenols (OPEOs) nor the commercialization or use of goods imported from countries outside the EU. Thus, this topic is still of current concern and high-throughput analytical methods are necessary for monitoring campaigns for the evaluation of the occurrence of these contaminants.

Several methods, mainly based on HPLC-MS/MS, have been recently reported in literature [3-5] but, due to different ionization mechanisms and chromatographic behaviours of APEOs and their metabolites, two separate runs are used for their determination; hence, the separation of APEOs and their carboxylate and phenolic metabolites is a problem still unresolved. Accordingly, the objective of this study was the development of a high-throughput, single polarity switching, LC–MS/MS method for the simultaneous determination of the most environmentally relevant APEOs, APECs and APs, in which all the investigated classes of compounds were baseline resolved one from the others. The off-line and on-line SPE extraction from water samples is also discussed.

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ANALYSIS OF PRINCIPAL FLAVONOIDS IN CITRUS JUICES BY NANO-LC AND DETERMINATION OF THEIR ANTIOXIDANT ACTIVITY

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Flavonoids have been extensively studied respect to their analysis methods being HPLC the preferred one for their separation and quantification [1]. However few papers on the optimization and validation of the analysis of different mixtures of phenolic compounds in foods by using nano-liquid chromatography (nano-LC) have been so far published [2]. In this study, a rapid separation of seven principal flavanones in *Citrus* was obtained by means of nano-LC, employing a 75 μm I.D. capillary column packed with sub-2 μm particles C18 stationary phase. All compounds were baseline separated working with a step gradient elution mode in 10 min. The developed method was fully validated obtaining RSD% for intra-day and inter-day repeatability, related to retention time and peak area, all below 5.5%. LOD and LOQ values were below 3 μg/mL for all studied compounds. Good linearity with acceptable determination coefficients R^2 was also obtained. The method was applied to the analysis of studied compounds in commercial and hand-squeezed *Citrus* juices after analytes extraction and concentration by using C18 solid phase extraction cartridge. The same samples were also evaluated respect to their antioxidant potential employing two different *in vitro* tests: Folin-Ciocalteau and Trolox equivalent antioxidant capacity (TEAC) assays. Correlation among studied flavonoids concentration and antioxidant potential was studied. The optimized and validated method allows qualitative and quantitative analysis of major flavanones in different Citrus juices being suitable for their quality and nutraceutical potential evaluation. Moreover advantages of this application relies on good efficiency, short analysis time, low sample dilution and reduced consumption of reagents. For these reasons applications of this miniaturized technique to the analysis of food derived compounds with potential nutraceutical interest could be of great interest for all those working in food analysis.

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KNL.19

COUPLING GAS CHROMATOGRAPHY AND ELECTRONIC NOSE DETECTION FOR DETAILED TOBACCO AROMA CHARACTERIZATION

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Approaches for characterizing aromas rely on separation-based chromatographic methods (e. g. gas chromatography-MS), on human sniffing using an olfactory detector (ODP) or on sensor array electronic noses. Accurate aroma characterization of tobacco samples is an analyitical challenge due to the masking effect of, on the one hand, the major constituents and, on the other hand, the solvent used for the extraction step (e.g. methanol).
In order to overcome these problems, a new instrumental set-up consisting of heart-cutting via Deans-switch based on capillary flow technology and a low thermal mass GC oven in combination with an electronic nose was developed as alternative to GC-olfactometry.
The GC-E-nose was used for characterization of aroma compounds in tobacco smoke samples. Principal component analysis (PCA) and discriminant factor analysis (DFA) allowed clear visualization of the differences among different tobacco brands. The statistical evaluation showed that discrimination between control tobacco and flavoured tobacco sample became easy, even at concentrations corresponding to the human expert perception threshold. The results are promising and suggest that the GC-E-nose technology is a good approach to measure the contribution level of individual compounds to the whole smoke tobacco sample.

KNL.20

STUDY OF ODORANT COMPOUNDS FROM HEATED RAPESEED OIL USING HEADSPACE TECHNIQUES, GC-OLFACTOMETRY AND ELECTRONIC NOSE

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Omega-3 polyunsaturated fatty acids are essential for humans, but not synthesized by our body. Because of an omega-3 deficiency in the French population, nutritional and dietary recommendations were established to promote the consumption of food containing omega 3 or foodstuffs enriched in omega 3. Thus, the use of rapeseed oil for baking and frying has increased. However volatile compounds characterized by a fishy odour are formed during heating of rapeseed oil and lead to a decrease in nutritional and organoleptic properties of the oils. Consequently the monitoring of the different stages of degradation is necessary. Headspace techniques have proven to be sensitive tools to detect the formation of degradation products, such as aldehydes[1], while GC-olfactometry (GC-o), using human nose, constitutes a selective and sensitive detector of aroma active compounds.

In order to identify volatile compounds derived from lipid oxidation, several headspace techniques associated with GC-MS were compared for their sensitivity, repeatability, and for the analysis of real samples. These techniques were static and dynamic headspace, headspace sorptive extraction (HSSE), and headspace solid phase micro extraction (HS-SPME). Moreover, GC-o combined with a mass spectrometer allowed to complete the identification of odorant compounds and to associate sensory descriptors to these compounds. Finally, performances of an electronic nose based on static headspace with a fast GC-FID have also been evaluated and will be discussed.

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KNL.21

SAMPLING METHODS FOR THE DETERMINATION OF HYDROCARBONS IN ADHESION SECRETIONS OF COCKROACHES BY GC-MS

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Bionic research is an important scientific field since nature provides interesting, fascinating and often remarkable phenomena, which can be helpful for the human being. In our example studied here we look into the ability of insects to walk on walls. While geckos *(Gekkonidae)* have hairs, which are responsible for adhesion capabilities, some insects release secretions on their tarsi (feet) [1][2].

These adhesion secretions could be valuable models for novel artificial glues or adhesion materials. For this purpose, the secretions of those insects, which are able to walk on walls, are used as a model to mimic the composition of the natural product [2].

In the present investigation, the chemical compositions of adhesion secretions from *Gromphadorrhina portentosa* (Madagaska hissing cockroach) were investigated by 1D and comprehensive 2D GC-MS experiments with particular focus on a comprehensive analysis of alkane profiles. Hydrocarbon identification was based on mass spectrometric database information and specific fragmentation patterns.

Besides secretions from tarsi (feet), samples from tibia (legs) were also taken as control samples, and a further goal in our study was to elucidate differences in the chemical composition of these two different types of samples.

Due to the difficulties of sampling from a living insect and due to the low amounts of insect secretion on both tarsi and tibia, different sampling methods were investigated in order to find a method which is reproducible and efficient. Three different sampling methods were compared by measuring samples of tarsi, tibia of both genders multiple times. Solid phase microextraction (SPME) with a polydimethylsiloxane fiber (PDMS-SPME) showed higher peak intensities, whereas a self-made glass-SPME had the best reproducibility in contact-SPME sampling. Solvent sampling (rinsing) was time consuming and less reproducible.

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KNL.22

ANALYTICAL INVESTIGATIONS OF SYNTHETIC STREET DRUGS : NEW MATERIALS DEMAND NEW METHODOLOGY

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Various outlets across the globe are carrying products sold as bath salts, plant food, and jewelry cleaner. These products contain synthetic drugs, which have become prevalent in the United States since 2009 when they came from Europe.[1] The drug names (i.e. bath salts) illustrate the variety of marketing tactics that manufacturers employ to lure customers. Products exhibit colorful packaging labels and every bag states the product is “Not for Human Consumption” to allow for legal possession and consumption by circumventing potential control mechanisms.[2] Not only have these substances been sold in head shops, but they are also retailed in gas stations, adult stores, independently owned convenience stores, and online retailers as of late.[3] The internet has provided a significant means of circulating new compounds quickly and effectively. In March 2009, there were reportedly fewer than ten online vendors; by June that number had grown to dozens, with new sites opening every week.[4]

The products are mainly composed of synthetic cathinones. The cathinone backbone can be functionalized in four different places to create hundreds of possible structures and numerous compounds have already been identified in the products.[5] Three prominent compounds were temporarily categorized as schedule I drugs by the U.S. Drug Enforcement Administration (DEA) on October 21st, 2011. The banned drugs include MDPV, mephedrone, and methylone.[6] The products are sold as tablets, capsules, and powders, and modes of abuse include ingestion, inhalation, injection, and insufflation.[7] They have also been sold by independent dealers in combination with other illicit controlled substances such as ecstasy in tablets and capsules. In 2011, hundreds of calls per month were made to poison control centers across the country and there have been reported deaths in some cases, mostly due to conjugation of the drugs with other agents such as cocaine, alcohol, and MDMA.[8,9] Numerous hospitalizations have also been reported.[10] Only recently has pharmacological information come to light on the newly scheduled drugs.

Analysis of such designer drugs and the identification of individual compounds may help ban their production and abuse, but the analysis can be quite challenging. Identification of these drugs can help increase knowledge on their pharmacology to provide better treatment options and possibly decrease the number of calls to poison control centers across the country. In regards to the forensic community, crime labs already have a large workload. An efficient methodology will benefit analysts by increasing laboratory throughput.

This presentation will discuss the development of the extraction of the drug compounds from various commercial media, followed by separation using both gas chromatography with mass spectrometric detection (GC-MS) and liquid chromatography with time-of-flight mass spectrometry (LC-TOFMS). The developed chromatographic method provides qualitative and quantitative analysis of synthetic compounds in the samples based on the use of appropriate standards. A preparatory HPLC method for the fractionation of multi-component samples and the use of direct infusion MS/MS in further identification of unknown samples will also be discussed.

POSTER ABSTRACTS

NEW APPROACH IN EVALUATING THE INERTNESS OF GAS CHROMATOGRAPHIC SYSTEMS

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Known approached in controlling the inertness of chromatographic systems are based on evaluating the discrimination effects for relative areas (or heights) of chromatographic peaks of polar compounds relative to those of non-polar constituents of test-mixtures. However, not the composition of test-mixtures is the factor determining their using; rather it is the algorithm of data interpretation.

New approach implies:

1. To simplify the composition of test-mixtures;
2. To abandon the analyses of single samples.

The amount of the polar component in the probe (g) is:

$\boldsymbol{M}_i \approx \mathbf{10}^{(-i-3)}\, v_{sample}[\boldsymbol{V}_1\boldsymbol{d}_1/(V_1 + V_2)] / (R + 1)$,

where $V_1 = V_2 = 1$ mL are the amounts of mixed liquids; d_1 is the specific gravity of polar component, g/mL; v_{sample} is the probe injected; R is the split ratio (for capillary columns); i is the number of dilution, $\mathbf{p}M = \mathbf{-log}M$.

Nest step is comparing the differences in relative peak areas

$\boldsymbol{D}_i = \langle S_{1,rel}\rangle - \langle S_{i,rel}\rangle = a\mathbf{p}M_i + b$

with sums of their standard deviations, $\Sigma \boldsymbol{s}_i = a\mathrm{p}M_i + b$.

The crossing point of two regression lines ($\mathbf{p}M_{lim}$) corresponds to the amount of the analyte when the influence of the insufficient inertness of chromatographic system can be still considered as negligible:

So, the limit of the inertness of a chromatographic system is such the quantity of analyte in the probe of its mixture with conventionally inert component, when the differences in relative peak areas compared with those for more concentrated solutions exceed the sums of their standard deviations.

The examples of inertness evaluation are considered. Namely, for quartz WCOT column HP-5 MS (80 ^{0}C, test-mixture *n*-octane/1-heptanol)

parameters of regressions are:

$S\boldsymbol{s}_i = a\mathrm{p}M_i + b$: $a = 4.6 \pm 1.1$; $b = -32.2 \pm 9.5$; $r = 0.944$;

$\boldsymbol{D}_i = a\mathrm{p}M_i + b$: $a = 0.5 \pm 0.1$; $b = -2.5 \pm 0.5$; $r = 0.981$.

$\mathrm{p}M_{lim} = 7.24$, $M_{lim} = 0.06$ μg.

A.02

ANOMALOUS TEMPERATURE DEPENDENCE OF RETENTION INDICES OF POLAR COMPOUNDS ON NON-POLAR PHASES

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Temperature dependence of gas chromatographic retention indices **RI(***T*) most often is approximated with linear regression:

RI(*T*) = RI(T_0) + *d*RI/*dT* (*T* – T_0)

For most of organic compounds the temperature coefficients ***d***RI/*dT* > 0 and depend on the number of cycles in the molecule. However, at the beginning of 2000s, the examples of anomalous dependence **RI(***T*) were revealed for polar organic compounds (alkanones, alkanenitriles, nitroalkanes, etc.) on non-polar phases [1-3]. These compounds indicate the minima of such dependencies that mean changing their sign from ***d***RI/*dT* > 0 (higher temperatures) to ***d***RI/*dT* < 0 (lower temperatures).

Expanding the set of polar analytes including dimethyl formamide, dimethyl sulfoxide, alkanols, etc. confirm this effect. It means the view of the dependence **RI(***T*) is not fixed for every analyte and stationary phase, but strongly depends on its amount (*m*) injected into chromatographic column. For example, three various dependencies were observed for different quantities of dimethyl formamide $(CH_3)_2NCHO$ analysed using quartz WCOT column BPX-1:

m = 0.6 μg, ascending quasi-linear dependence, ***d***RI/*dT* > 0;

m = 4.3 μg, non-linear dependence with the minimum, $\mathbf{RI}_{min}$;

m = 17 μg, descending quasi-linear dependence, ***d***RI/*dT* < 0.

It means previously known recommendations on the interpretation of temperature coefficients ***d***RI/*dT* should be reviewed. One of the reasons of the anomalous dependence **RI(***T*) is changing the shapes of chromatographic peaks. The lower is the temperature, the higher is the asymmetry factors (*A*) of the peaks of polar compounds.

In general, the explanation of anomalous dependence **RI(***T*) is based on the theory of theoretical plates.

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A.03

GAS CHROMATOGRAPHIC RETENTION BEHAVIOR OF POLYCHLORINATED BIPHENYLS ON IL-60 STATIONARY PHASE

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Statistical studies of the relationship between the gas chromatographic (GC) retention of polychlorinated biphenyl (PCB) congeners and their structure have used topological or electrotopological descriptors in the prediction of PCB retention times or retention indices. However, the practical use of this approach is limited since accurate predictions are required due to the high number of congeners (209) and the frequent presence of coelutions. More interesting is the statistical estimation of the effects of simple structural PCB descriptors on the GC retention, which allows to compare the interactions of PCBs with different stationary phases and then to describe phase selectivity toward groups of compounds sharing a specific substructure.

Ionic liquids (ILs) have been used as stationary phases in GC because their low vapour pressure, relative low bleeding, but mainly because of their special selectivity. IL-60 was chosen in this study with the aim of describing its chromatographic interaction towards PCBs with different structures.

Programmed temperature retention times and retention indices of 60 PCBs were used as dependent variables, while independent variables were the number and position of the chlorine substitutions in the ring (used as size and shape descriptors), and the number and position of the *orto-* substitutions describing interactions between the two rings.

Retention indices data were fitted using stepwise regression (Statistica software, R=0.996). Similar results were obtained when using retention times. Significant positive descriptor contributions have been found for 2,6- , 3,4- , 2,3,4- and 3,4,5-chlorine substitutions in a ring.

Retention behavior of PCB congeners in IL-60 and in methyl polysiloxane were different, contribution of the 2,6- substitution being markedly more important in IL-60. The proposed approach can be applied to retention data from both IL-based and conventional phases to estimate and compare the contribution of the PCB characteristic substructures to their chromatographic retention.

Acknowledgments

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A.04

TEMPERATURE DEPENDENCE OF RETENTION INDICES OF SOME TERPENES AND KETONES COMPOUNDS ON APOLAR GC STATIONARY PHASES

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Retention indices are currently used for capillary gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) identification of chemical composition of complex mixtures like essential oils. The use of the retention indices permits to distinguish components possessing similar mass spectra more unambiguously, especially for isomers compounds.

The influence of the isothermal temperature and temperature programming rate on retention indices of some selected terpenes and aliphatic ketones compounds was studied. Various isothermal temperature range (100–150ºC) and different temperature programming rate (2 °C min^{-1}, 4 °C min^{-1}, 6 °C min^{-1}, 8 °C min^{-1} and 10 °C min^{-1}) were investigated.

The experimental measurements were carried out on two non-polar capillary columns: OV101 and SE30 coated with different film thicknesses (1.13 µm, 0.53 µm, 0.31 µm and 0.10 µm).

The retention indices of the terpenes and ketones compounds have been determined by the application of two new adaptation methods: A *Multiparametric least-squares regressions* iterative method based on the determination of the adjusted retention times and a C*ubic Interpolation* directly using the uncorrected retention times without dead time correction.

The retention indices calculated with a Multiparametric and a Cubic Interpolation methods were compared with those calculated by Kovàts and Van Den Dool methods. The influence of coating, film thickness and temperature, on them were investigated.

A.05

MASS SPECTROMETRIC CURIOSITY ENCOUNTERED DURING ENANTIO-GC-IT-MS ANALYSIS OF WHISKY LACTONES

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During enantiodifferentiation of whisky lactones using an ion trap mass spectrometer (IT-MS) in selected ion monitoring (SIM) mode we encountered a curiosity hampering reliable quantification. A racemic mixture (four enantiomers) showed only three instead of four peaks on m/z 99 (quantifier ion). This surprising result could not be explained and would have caused a false negative result for the missing enantiomer.
A full scan MS experiment showed a severe co-elution problem of an unknown matrix compound with the missing enantiomer. Further troubleshooting with a quadrupole MS in place of the IT-MS resulted in a chromatogram with a distorted but visible enantiomer peak in SIM mode on m/z 99. Concerning the distorted peak shape, co-chromatography with the abundant matrix compound could explain the (focused, sharpened) peak shape. Assuming that the abundant matrix compound provides a kind of "solvent trapping", such peak focusing could be explained with a solvent effect [1]. Integration of the eventually solvent focused enantiomer peak revealed the same area as the enantiomer with the normal peak shape. This supports the fact that our achiral detection environment cannot selectively discriminate an enantiomer. Considering the qualifier SIM masses (m/z 71, 41) ion ratios were correct using quadrupole MS, but were obscured with the IT-MS. Ruling out all other options for an explanation of the observed phenomenon, the most obvious explanation has to be found in the different ion sampling mechanism of the two MS instruments. However, at present the authors have no exact technical theory at hand for a full understanding of this aspect. Still, our concluding remark is that quantification with a scanning mass spectrometer could eventually give false negative results when based on SIM-mode. In our case, only the notice about the missing enantiomer peak of the racemic mixture gave us the hint to the described curiosity.

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A.06

THE IDENTIFICATION OF ALL 196 C_4-C_{30} MONOMETHYLALKANES ON OV-1 STATIONARY PHASE ON THE BASIS OF HOMOMORPHY FACTORS CONFIRMED BY MASS SPECTROMETRY

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The programmed temperature linear retention indices of all 196 C_4–C_{30} monomethylalkanes on OV-1 stationary phase were measured with an average repeatability of ±0.07 index units (i. u.). The mixture of C_9–C_{30} monomethylalkanes was prepared by methylene insertion reaction to C_8–C_{29} *n*-alkanes mixture. The preliminary identification of monomethylalkanes was performed on the basis of the dependence of homomorphy factors on the number of carbon atoms of individual homologous series of monomethylalkanes (retention indices extrapolated *with s*=0.15 i.u.). The prediction of retention of isomers with new position of methyl group beginning at higher carbon atoms number, as well as for second, third, fourth, etc., member of homologous series allowed the dependence $H_p=f(C_n)$ for first, second, third, etc., members of beginning homologous of monomethylalkane series (retention indices extrapolated with *s*=0.17 i.u.). The identification was confirmed by mass spectrometry. All gas chromatographic unseparated monomethylalkane isomers with methyl-group near the middle of molecule carbon chain were resoluted by mass spectrometric deconvolution. Obtained regular dependences $H_p=f(C_n)$ allow precise retention prediction of monomethylalkanes >C_{30}. The developed method was used for the GC characterization of monomethylalkanes in fuel diesel and exhaled breath.

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A.07

INTELLIGENT, AUTOMATED VARIABLE REDUCTION FOR RAPID AND IMPROVED CHEMOMETRIC ANALYSIS OF RAW GC-MS DATA

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The use of separation techniques like gas or liquid chromatography coupled with MS detectors (GC/LC-MS), the so-called hyphenated techniques have become very popular due to the rich multivariate data that are generated. Chemometric techniques allow for easy data interpretation. However, at data rates provided by modern instruments (>10 spectra/s), hundreds or thousands of data points are collected for an individual chromatographic peak and millions of data points for the entire chromatogram. Thus data are usually reduced prior to chemometric analysis. Most commonly, integrated peak tables, total ion currents (TICs) or extracted ion profiles are used as input variables. However, using these approaches often leads to oversimplification of the data, multivariate information is lost, and for EICs a priori information about the samples is required which poses a problem for data mining in poorly-understood systems.

The advantage of using the raw data where each signal at each scan and m/z is treated as an independent variable followed by feature ranking/selection has been demonstrated. We developed a model quality parameter called cluster resolution that lends itself to an objective and automated feature selection process. In that study it was realized that several features were selected for each compound. In as much as redundancy enforces the resulting chemometric model, over redundancy must be avoided since it has its own drawbacks.

In our current work, we introduce the unique ion filter (UIF) preprocessing algorithm for GC-MS data prior to chemometric analysis. UIF provides an intelligent, automated reduction in the number of features for subsequent chemometric study while preserving the multivariate information in the data. We have demonstrated the practicability of UIF by using GC-MS data for different octane rating gasoline samples. Our results demonstrate that UIF leads to reduction in redundant variables allow for faster feature selection and improved chemometric models.

A.08

SAME SEPARATION WITH HALF THE COLUMN: EXTENDING THE LIFETIME OF YOUR GC COLUMN WITH COLUMN TRIMMING MAINTENANCE AND METHOD TRANSLATION

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Polybrominated diphenyl ethers (PBDEs) have been found to be persistent and bioaccumulative in the environment. The technical mixtures containing penta and octa congeners were voluntarily withdrawn in the United States in 2005 and the last remaining PBDE mixture, decaBDE, should be completely phased out by the end of 2013. While these mixtures have been phased out of production and use, the concentrations in the environment have not been declining and are currently still widely monitored.

The analysis of PBDEs is challenging due to structural isomers that need to be chromatographically separated and thermally label compounds of interest that may breakdown during gas chromatography. PBDEs included in EPA Method 1614 are well resolved on a 15m x 0.25mm x 0.10µm Rtx-1614 GC column, a 5% diphenyl, 95% dimethyl polysiloxane type phase that was specifically designed to meet method resolution requirements. Using a short, thin film column also allows the elution of decabromodiphenyl ether (BDE-209) without on-column thermal degradation.

Monitoring efforts of the levels of PBDEs include a wide array of biota and environmental matrices. Non-volatile material may still persist even in cleaned-up final extracts, requiring GC column and inlet maintenance to be performed. Using a 15m x 0.25mm x 0.10µm column, how many loops of the GC column can one clip for maintenance before the Method 1614 resolution requirements of BDE 49 and BDE 71 can no longer be met? The resolution between BDE 49 and 71 must be less than 40% valley height to meet method criteria.

A.09

SEMIVOLATILE ANALYSIS USING HYDROGEN AS A CARRIER, A LOOK AT GC/MS SPECTRA, HYDROGEN VS. HELIUM

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Over the years, there has been an increase in demand for an alternate carrier gas as a helium replacement. Both nitrogen and hydrogen are potential candidates and have been discussed in detail previously. Hydrogen is generally chromatographically superior to nitrogen for GC/MS analysis but is not without concern. Hydrogen is more difficult to pump away in the MS thus one needs to be vigilant on the flow rate entering the MS. This can be partly addressed by using smaller ID columns such as 0.18mm, however, the reduced ID columns reduce sample capacity and thus will reduce the practical analyte working dynamic range. In this study, we will maintain identical conditions, where possible, to compare Hydrogen vs. Helium mass spectra for a wide variety of semivolatile compounds such as those found in USEPA 8270. Results show hydrogen and helium produced virtually identical spectra for over 90% of the analytes. Several very polar compounds showed minor ion ratios differences but continued to provide high quality matches via NIST Mass spectral library searches.

FAME ANALYSIS WITH IONIC LIQUID CAPILLARY COLUMNS

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Analyses of fatty acid methyl esters (FAMEs) are continuing to gain importance as more research is focusing on their biomedical impacts. This includes the analysis of saturated and polyunsaturated FAMEs along with the positional geometric (cis and trans) FAME isomers. Traditionally, FAME analyses have been performed using silicone polymer or polyethylene glycol based stationary phases that yield typical elution patterns. Analysts performing the task of analyzing the fatty acid composition of food have a wide variety of capillary column selectivity's available for resolving the fatty acids as FAMEs depending upon the information they require from their analyses. Nonpolar methylsilicone columns provide a boiling point separation of the FAME isomers with limited resolution of polyunsaturated isomers. Polar polyethylene glycol (PEG) columns resolve the isomers by degree of unsaturation with minimal overlap of the carbon chain lengths. The highly polar cyanosilicone columns will resolve cis and trans isomers along with possibly providing positional geometric isomer separations depending upon the column type.

New classes of stationary phases based on Ionic Liquid technology have been developed and have demonstrated to provide unique elution patterns for FAME isomers compared to the traditional silicone or polyethylene glycol based stationary phases. The two new phases are SLB-IL60 with a PEG like selectivity and the SLB-IL111 with highly polar selectivity. We will compare and contrast the selectivity of the ionic liquid phases with polymeric based phases for various FAME samples.

B.02

COMPARISON OF THE SELECTIVITY OF IONIC LIQUID STATIONARY PHASES FOR THE ANALYSIS OF PAHS

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Polyaromatic hydrocarbons (PAHs) are compounds containing two or more aromatic rings and are known for their potential carcinogenic and mutagenic properties. They are typically formed during incomplete combustion of organic matter, as industrial byproducts, and in food processing. As such, they are compounds of interest in environmental and food and beverage analysis. Many of these compounds are isomeric, and must be resolved chromatographically to be accurately quantified.

Ionic liquids (IL) are a new class of GC stationary phases that provide unique polar and highly polar selectivity with higher thermal stability compared to traditional siloxane phases with similar selectivity. Traditionally, PAHs have been evaluated by US EPA method 610 using nonpolar stationary phases operated at high temperatures. In this study, we examine the effect of selectivity of the ionic liquid stationary phases based on their chemical composition. We also examine the effects of stationary phase film thickness and column dimensions.

B.03

CYCLODEXTRIN-BASED IONIC LIQUIDS AS ENANTIOSELECTIVE STATIONARY PHASES IN GAS CHROMATOGRAPHY

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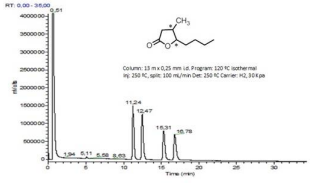
Ionic liquids (ILs) are very simple low melting point organic salts. The combination of organic cations and bulky inorganic anions provides the possibility to create tailor-made ILs derived from sugars,[1] with different properties, to be used as catalysts in asymmetric synthesis, chiral selectors in NMR studies or as stationary phases in chiral chromatographic analysis.[2]
New imidazolium and pyridinium triflate ionic liquids derived from isosorbide, isomannide and cyclodextrins were synthesized in one pot free solvent procedure. The ability to use these ionic liquids as chiral selectors was demonstrated by ^{19}F-NMR spectra and also when they are used as stationary phases in gas liquid chromatography. Some of these ionic liquids were used as stationary phases in homemade capillary columns for enantio-GC and its chiral selectivity explored in the separation of ethers, esters and lactones racemic mixtures. The permethylated 6-deoxy-6-pyridin-1-ium-a-cyclodextrin trifluoromethanesulfonate displayed good enantiomeric separations for some racemic esters and lactones, as well as epoxides. In particular, for both the racemic whiskey lactone and the high boiling point menthyl laurate, not successfully separated in a commercial cyclodextrin phase, the enantiomeric separations were achieved isothermally at 140 ºC.



B.04

REDUCED PARTICLE SIZE DISTRIBUTION BY HYDRODYNAMIC CHROMATOGRAPHY

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During column packing, smaller stationary phase particles have a tendency to segregate toward the column wall. This increases transcolumn heterogeneity and reduces chromatographic performance. Because the synthesis of fully porous particles often results in batches with a broad particle size distribution (PSD), this can be problematic. In order to help prevent this efficiency loss, methods to reduce PSD by hydrodynamic chromatography (HDC) were developed. Problems in HDC column preparation related to particle trapping and aggregation were encountered. Different frit materials and a new aqueous mobile phase method helped eliminate these issues and ensure reproducible results. Nonporous silica spheres ranging in size from 0.5 – 1.5 μm (representative of current ultra-high pressure liquid chromatography (UHPLC) packing materials) were used to characterize the system. HDC was also used to size-refine a 1.0 μm batch of bridged-ethyl hybrid porous packing material. The benefits to capillary LC columns packed with this refined material will be discussed.

B.05

GAS CHROMATOGRAPHIC STUDY OF THE LATERAL ALKOXY CHAIN LENGTH PROPERTIES OF A TWO NEMATIC LIQUID CRYSTALS

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The properties of two laterally substituted liquid crystals by gas chromatography are the subject of a comparative study. These liquid crystals be long to homologous series: Benzoic acid, 4-butoxy-,4-[[3-alkyloxy-4-[(1E)-(4-ethyl phenyl)azo] phenoxy]carbonyl]phenyl ester, referred to as ALn, where n, the carbon number in the lateral alkoxy chain, is equal to 4 or 7 carbon atoms. Their thermal properties were established with differential scanning calorimetry (DSC). The chromatographic separation abilities of AL_4 and AL_7 were studied using capillary columns. Interesting analytical performances were isomeric separation of aromatics, polyaromatics, volatils aromatics compounds and cis- and trans- isomers. Comparison between AL_4 and AL_7 is presented.

B.06

ANALYTICAL STUDY OF NEW LATERALLY AROMATIC BRANCHED LIQUID CRYSTAL USED AS STATIONARY PHASES IN GAS CHROMATOGRAPHY

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The investigation of the analytical properties of a new laterally aromatic branched nematic liquid crystal, 2-(3-hexyloxybenzyloxy)-3-n-hexyloxy-4-(4-chlorobenzoyloxy)-4'-(4-methyl benzoyloxy)-azobenzene, was carried out by gas chromatography using fused silica capillary columns. The thermal properties of liquid crystal were established with differential scanning calorimetry and polarizing microscopy. The study of the chromatographic performance of a column coated with liquid crystal in the solid, nematic and liquid state was done using a series of appropriate solutes. The mesogenic compound exhibit interesting separation for some positional and geometrical isomers in aromatic hydrocarbons, phenols, volatile aromatic compounds and polyaromatic hydrocarbons.

B.07

CONVENTIONAL AND ENANTIOSELECTIVE GC MICROFABRICATED COLUMNS VERSUS FSOT COLUMNS IN THE ANALYSIS OF REAL-WORLD SAMPLES IN THE FLAVOUR AND FRAGRANCE FIELD

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Microfabricated columns are highly attractive for gas chromatography because of the number of possible applications in particular for on-site environmental monitoring and in-field analysis. Microfabricated columns are therefore ideal for mobile micro GC instruments because of their small size and low thermal mass affording rapid temperature programming with relatively low power. Silicon micro-electromechanical system (MEMS) technologies are often adopted for microfabricated column preparation.

This study adopts etched silicon columns (equivalent, for both pneumatic impedance and chromatographic performance, to a 100 µm d_c conventional capillary column) fabricated in silicon substrates that are statically coated with a 0.1-0.2-µm-thick film of different stationary phases. Microfabricated columns 1.68 and 3.20 m long coated with conventional polysiloxane, polyethylene glycol and 30% 2,3 diethyl – 6 *t*-butyldimethylsilyl-β-cyclodextrin in PS-086. All columns showed an efficiency comparable to that of corresponding conventional columns, i.e. 10000 theoretical plates per meter for SE 52 and 7000 for CD columns respectively using hydrogen as carrier gas.

The performance of the resulting microfabricated columns were tested with a conventional GC system and their performance compared to those of conventional columns of the same length and phase ratio and under the same analysis conditions. Comparison was carried out by analyzing a set of essential oils from different plants, volatile fractions and aromas from different food sampled by HS-SPME, and enantiomer separation of racemate standards and real-world samples.

B.08

NOVEL IMIDAZOLIUM-BASED IONIC LIQUID POLYMER MONOLITHS FOR ANALYSIS OF POLAR PHENOLS IN CEC AND POLYMER MONOLITH MICROEXTRACTION

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The use of polymer monoliths for chromatography has dramatically increased since 1990s, because of their good permeability, wide pH stability, tuneability and ease of preparation, etc.. Nevertheless, the biggest problem in its application is the swelling property. Moreover, one–step preparation of polymer monoliths possessing both large through-pores and high interconnectivity of the mesopores, has always proved to be difficult [1].

The recently application of ionic liquids (ILs) as “green” solvent for separations and extractions started to attract considerable interests, due to their unique physicochemical properties [2]. Here, imidazolium-based ILs polymer monoliths were synthesized via “one-pot” approach, characterized and used in CEC and in PMME. The approach involved incorporating both ILs and hydrophilic monomers into a monolith, by copolymerization of ILs, lauryl methacrylate (LMA) and ethylene dimethacrylate (EDMA) in the presence of selected porogens, to produce mesoporous PMME monoliths or macroporous CEC monoliths inside fused silica capillary columns. A polymer monolithic column bonding with $AMIMI^+PF_6^-$ demonstrated typical hydrophobic and ionic exchange interaction properties in CEC. The EOF in this column can be controlled over a wide pH range, and the proportion of polymerization mixture, plays a critical role in monolith morphology. Compared with conventionally prepared monoliths, the ILs polymer monoliths showed better performances towards the separation of polar phenols and neutral compounds.

Another novel ILs polymer monoliths bonding with $AOCMI^+PF_6^-$ was prepared for PMME. Phenolic estrogens in food were analyzed by coupling the extraction step to CEC-amperometric detection. Results demonstrated good sensitivity, linearity and repeatability under optimized conditions. Increased porosity and stability of the sorbent, as well as the presence of the electrostatic interactions, hydrogen bonding and p-p interactions, are responsible for the improved selectivity and elution rate towards polar phenols.

Acknowledgements

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B.09

A NEW IONIC LIQUID FUNCTIONALIZED UREA-FORMALDEHYDE RESIN MONOLITH FOR HYDROPHILIC INTERACTION CAPILLARY ELECTROCHROMATOGRAPHY

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Ionic liquid (IL) functionalized monoliths have emerged as the increasingly popular option in capillary electrochromatography (CEC) [1-2]. Seeking a rapid method to fabricate IL-modified monoliths is still interesting. Herein, a rapid strategy is brought forward and a new IL-functionalized monolith has been successfully presented by a facile in-situ polycondensation of urea, formaldehyde and ionic liquid (1- acetylamino-propyl-3-methylimidazolium bromide, [AAPMIm]Br) at 65 degree centigrade in 10 minutes.

In the resultant IL-functionalized monolithic matrix, a stable skeletal microstructure with a proper permeability about 1.05×10^{-13} m^2 was presented, and a highly hydrophilic interaction mechanism could be observed when the content of ACN exceeded 20% in the mobile phase. Satisfactory column repeatability in batch-to-batch were obtained with RSD (n=3) of 2.1 % for retention time and 3.6% for column efficiency.

Besides, multiple mechanisms such as hydrophilic interaction, ion-exchange and π-π conjugation were also involved in the resultant monolith, and the mixed-mode separation mechanism of hydrophilic interaction (HI) / anion-exchange, or hydrophilic interaction / cation-exclude was well employed. Efficient separations with the high resolution of various polar analytes including phenols, benzoic acids and enkephalins have been successfully achieved in hydrophilic interaction electrochromatography (HI-CEC).

It might light a facile access to the rapid preparation of multifunctional IL-modified polymeric monoliths for the efficient analysis of polar compounds in HI-CEC.

Aknowledgements

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B.10

APPLICATION OF β-CYCLODEXTRIN MODIFIED MAGNETIC NANOPARTICLES IN MONOLITH CAPILLARY ELECTROCHROMATOGRAPHY

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Monoliths as the chromatographic separation media in liquid chromatography (LC) and capillary electrochromatography (CEC) have attracted great attention in recent years. Mixed monolithic stationary phase, which involves multiple separation mechanisms simultaneously at the same cross section of capillary, has been one of the trends in the development of monoliths.

In the paper, a novel silica monolithic stationary phase functionalized with β-cyclodextrin modified magnetic nanoparticles (CD-MNP) was prepared for capillary electrochromatography (CEC). The bare silica monolith (Silica) was prepared by a sol-gel process of cocondensation of γ-(glycidoxypropyl) trimethoxysilane (γ-GPTMS) and tetramethoxysilane (TMOS) and then modified chemically with CD-MNP to prepare CD-MNP-modified silica monolith (Silica-CD-MNP). The electroosmotic flow characteristics of the Silica-CD-MNP and Silica were studied by varying the percentage of organic modifier in buffer and buffer pH. And a strong and stable cathodic electroosmotic flow (EOF) could be observed under a broad pH range from pH 3.0 to 9.0. To evaluate the column performance, neutral compounds such as toluene, dimethylformamide, formamide, and thiourea were tested on the prepared Silica and Silica-CD-MNP. From the result shown, the obtained Silica-CD-MNP monolith possessed of obviously hydrophilic retention mechanism under ACN content more than 50% in the mobile phase. Besides, the incorporation of nanoparticles enhances column efficiency due to high specific surface area of nanoparticles and mixing separation mechanism. The highest column efficiencies up to 19 377, 11 626, 48 487 and 49 223 plates/m be obtained for toluene, dimethylformamide, formamide and thiourea, respectively. However, those of toluene, dimethylformamide, formamide and thiourea reached 40 160, 30 246, 91 710 and 76 920 plates/m for the Silica-CD-MNP columns, respectively. In addition, the Silica-CD-MNP column was applied to separation of phenols and anilines and showed great potential in the method development of polar compounds.

B.11

SYNTHESIS AND INVESTIGATION OF CHROMATOGRAPHIC PROPERTIES OF MONOLITHIC CAPILLARY COLUMNS BASED ON PENTAERYTHRITOL TETRAACRYLATE IN PEPTIDE ANALYSIS

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Nowadays there are different methods of peptide analysis, with chromatographic separations using monolithic columns among the most important methods. The aim of the study is preparation of monolithic capillary columns based on pentaerythritol tetraacrylate and optimization of their structure for high efficient peptide separation. The synthesis method was developed earlier in our previous papers [1]. The optimization parameters were chosen to be the polymerization temperature and time, composition and percentage of complex porogen which was the mixture of toluene and one of the alcohols C_5-C_{12}.

To evaluate the column efficiency Van Deemter plots were measured for each column and the parameters of respective equations were calculated. The best results were obtained using decanol in the complex porogen with polymerization time of 45-60 min. The minimal HETP values for columns synthesized in different conditions varied from 21 to 34 mkm. Monolithic columns with optimal structure were successfully applied to separation of peptide standard mixture containing five peptides: Gly-Tyr, Val-Tyr-Val, Leu-enkephalin, Met-enkephalin and angiotensin II, with molecular masses ranging from 200 to 1000.

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B.12

DEVELOPMENT OF ROBUST AND SELECTIVE STATIONARY PHASES BASED ON IONIC LIQUIDS/POLYSILOXANE FOR GAS CHROMATOGRAPHY

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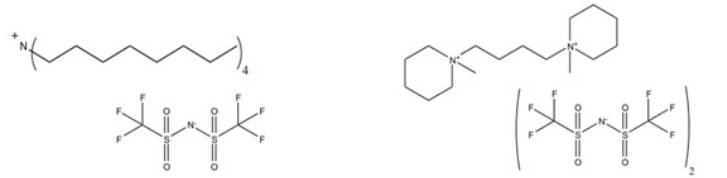
Analysis of Persistent Organic Pollutants (POPs) by gas chromatography remains a challenge due to their high number of congeners/isomers and for some of them, their high boiling point. Thus, their separation in complex matrices is difficult when using conventional stationary phases. To overcome these difficulties, a possible way consists in developing stationary phases with modulated polarity and/or extremely efficient capillary columns. Ionic Liquids (ILs) are molten salts composed of a cation, usually organic, combined with an organic or inorganic anion. Stationary phases based on ILs possessing extremely high polarity and good thermal stability up to 400°C were developed and are now commercially available [1-2]. In this work, a monocationic and a dicationic ILs, represented Figure 1, were mixed with polysiloxane OV-1701 to obtain new Lab-made capillary column for GC. The thermal stability of the stationary phase was demonstrated by cross-comparison of three methods. First, the thermogravimetric analysis on pure RTILs, then the bleeding measurement of stationary phases versus temperature program and finally the evaluation of the film coating homogeneity via the efficiency calculation. The inertness of the system was evaluated by injections of standardized test mixtures. Moreover, the durability of the phase homogeneity was assessed after a long period of storage and also during the use by regular injection of test mixtures. The interaction properties of the stationary phases were investigated by polarity calculation and by the Abraham model of Linear Solvation Energy Relationship (LSER).



Tetra-*n*-octylammonium bis(trifluoromethanesulfonyl)imide N_{8888}-NTf_2

Bis-methylpiperidinium butylidene bis(trifluoromethanesulfonyl)imide MBPPI-NTf_2

Figure 1: structures of ionic liquids studied

New stationary phases exhibited a higher selectivity than the OV-1701 for a majority of the aromatic compounds tested. In order to complete the selectivity evaluation of new phases, some complex mixtures were analyzed such as organochloride pesticides, FAMEs and PAHs.

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B.13

STUDY ON CHROMATOGRAPHIC CONDITIONS FOR INCREASING THE PEAK CAPACITY WITH A MONOLITHIC SILICA CAPILLARY COLUMN

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In the field of proteomics research, nano-flow liquid chromatography/mass spectrometry systems (nano-LC/MS) are commonly used for its high sensitivity. Detection sensitivity and quantitative capability are affected by the ionization rate of the solute components therefore the separation performance is important. However, the separation of thousands of biological components is still challenging subject for the liquid chromatography field. The separation performance in a gradient elution condition used in proteomics research is described by the peak capacity (PC). The PC is well estimated as below,

$$PC = 1 + \sqrt{N}/4 \times B \times \Delta c / (B \times \Delta c \times (t_0/t_g)+1) \quad (1)$$

$$\ln B = 0.6915 \ln(Mw) - 1.49 \quad (2)$$

where, N: the plate number, Δc: the difference in solvent composition of gradient elution, t_0: the void time, t_g: the gradient time, respectively [1]. As shown in equation 1, the plate number and the t_g/t_0 ratio largely contribute for increasing the PC. In this study, the effect of the N and the t_g/t_0 ratio for estimation and practice were compared for a high resolution monolithic silica capillary column prepared in a 0.1 mm I.D. capillary [2]. The PCs of ODS modified monolithic silica capillary columns (250mm length, 35,000 plate number) were evaluated for peptide mixtures with 0.1%TFA contained water/ acetonitrile mobile phase in 5-45% gradient condition. As a result, in the conditions of t_g/t_0 = 10, 20, 30, the PCs for each were estimated as 317, 464, 565, whereas practical PCs for each were 209 (0.66), 255 (0.55) and 293 (0.52), respectively (the bracket indicates the ratio of PC for practice to estimation). The differences of PCs between estimation and practice became larger according as the t_g/t_0 ratio increased.

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B.14

MONOLITHIC COLUMNS BASED ON ACRYLIC DERIVATIVES OF PENTAERYTHRITOL

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The nature of the stationary phase is one of the main factors influencing the elution order and the retention time of sorbates. In gas chromatography monolithic sorbents based on silica gel, DVB and DMEG [1, 2] were studied rather detailed. Comparison of the separation ability of these stationary phases showed that the most polar one was the silica monolith while the DVB monolith was the less polar with P = 16. The DMEG stationary phase was characterized with intermediate polarity with P = 29. To further study monolithic column based on pentaerythritol triacrylate (PE-3A) and pentaerythritol tetraacrylate (PE-4A) were synthesized and tested to seek for the more polar monolithic sorbents. The polarity and separation ability of new monoliths were compared to the properties of the previously studied monolithic stationary phases together with the traditional to GC stationary phases such as nonpolar poly(methyl siloxane) and highly polar polyethyleneglycol. The classification method was used suggested by Rohrschneider [3]. The method suggests determination of the chromatographic polarity of the stationary phase based on the comparative calculation of the retention data for the definite sorbates. The received data demonstrated that monolithic sorbents based on the acrylic derivatives of pentaerythritol had the larger relative polarity with P = 33 for PE-4A and P = 47 for PE-3A. However other monomers are necessary for synthesis of more polar monoliths.

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B.15

IMPROVED DEACTIVATION TECHNOLOGY FOR STAINLESS STEEL PROVIDES INERT SURFACE FOR GC ANALYSIS

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The main reason for using stainless steel tubing for GC analyses is the robustness of the material; it's virtually unbreakable and can withstand high temperatures. Within the GC, stainless steel tubing can be used for several purposes varying from guard columns, to head space transfer lines and GC plumbing for valved systems. Bare stainless steel has active sites which makes it unattractive to use for GC analysis of more (re)active compounds. Current deactivation techniques of the metal surface improve the inertness of the material, but it's still different compared to the inertness of fused silica.

By treating the stainless steel surface with the recently developed improved deactivation technology the inertness of stainless steel tubing is comparable to that of fused silica. This makes stainless steel tubing very suitable for GC analysis, even when analyzing (re)active compounds. To verify the inertness of the deactivated surface stringent testing and comparison is done by using very strong test probes. Several compound classes are used to test for different types of interactions (i.e. basicity, polarity, dipole interaction, acidity, etc.).

This poster presents results of comparisons of bare stainless steel tubing, fused silica tubing and deactivated stainless steel tubing to show the effect of the improved deactivation technology of stainless steel surfaces.

B.16

CAPILLARY (NANO) ENANTIOSELECTIVE NEAR-UHPLC/HRMS BASED ON SUB-3µm BRUSH-TYPE CHIRAL STATIONARY PHASES: AN EFFICIENT AND SYNERGIC POOL OF HYPHENATED TECHNIQUES.

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Scaling down the size of chromatographic systems is undeniably an attractive tool for "omics" sciences, pharmaceutical and food chemistry. Reducing the column diameter in the capillary (300-150 micron ID) or nano (100-75 micron ID) ranges diminishes both operational flow of mobile phase and the amount of stationary phase and sample to analyze. Moreover a miniaturized chromatographic system well matches with a direct MS detection. Compared to achiral capillary columns, the chiral ones are not present in the market and *in house* versions offer moderated efficiency. Nowadays the chiral selectors employed in Capillary/Nano-LC are mainly polysaccharide phenylcarbamate derivatives and cyclodextrins [1,2]. In 2011 we introduced the evolution of enantioseparations (namely near Ultra High and Ultra High Performance Enantioselective Chromatography) anchoring the DACH-DNB and WhelK-O1 brush-type chiral selectors on sub 3 micron silica particles. The reduced sizes of totally porous silica particles, in fact, ensure high kinetic performances of columns packed with these supports [3-5]. Here we present the first chiral capillary columns (200x0.075 mm LxID) containing the 2.5 micron Whelk-O1 or DACH-DNB stationary phases, prepared with an efficient ultra high pressure (1000 bar) ultrasonic assisted packing procedure. Enantioseparations of both chiral probes and pharmaceutical samples, carried out with MS detection using nano-spray ESI interface, showed good enantioselectivity and efficiency values. The results confirm the efficiency of the packing procedure developed for near-UHPLC supports (sub 3 micron) and its potential for future applications to UHPLC supports (sub 2 micron).

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B.17

EVALUATION OF AN IONIC LIQUID STATIONARY PHASE IN THE ANALYSIS OF BERGAMOT ESSENTIAL OIL

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Essential oils are complex mixtures composed mainly of volatile constituents, in general mono- and sesquiterpene hydrocarbons, along with their oxygenated derivatives (aldehydes, ketones, alcohols, etc.), and aliphatic aldehydes, alcohols, and esters. Essential oils are characterized by high economical importance, and are employed in a series of industrial products, from foods, cigarettes and cosmetics, to pharmaceuticals, perfumes and insect repellents [1]. In particular, bergamot (*C. bergamia)* essential oil is used for its antiseptic properties and, furthermore, is receiving renewed popularity in aromatherapy. The present research is focused on the evaluation of an ionic liquid capillary column (SLB-IL59) towards the analysis of such a complex real sample: the volatile fraction of bergamot essential oil. In the past decades a consistent number of ionic liquids have been synthesized and evaluated as stationary phase in gas chromatography. Ionic liquids are a class of organic non-molecular solvents liquid at room temperature (RTILs) that satisfy most of the requirements of a GC stationary phase, among which a high viscosity, the possibility to tune the selectivity (by changing the cation–anion combination) and a high thermal stability [2]. The SLB-IL59 capillary demonstrated to be a valid alternative as polar column in the analysis of essential oils [3].

In this work, resolution towards several volatile compounds of bergamot essential oil was calculated, a real sample of bergamot essential oil was analyzed on the ionic liquid capillary and the results were compared to those obtained with both an apolar poly (5%diphenyl/95% dimethylsiloxane) and a polar 100% poly (ethyleneglycol) stationary phase, (the most used columns in the analysis of essential oil). The results obtained confirmed the great potential of this ionic liquid stationary phase as polar column in the analysis of essential oils.

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AN ALL-IN-ONE ELECTROMEMBRANE EXTRACTION: A DEVELOPMENT OF ELECTROMEMBRANE EXTRACTION METHOD FOR THE EXTRACTION OF BASIC AND ACIDIC DRUGS WITH WIDE RANGE POLARITIES

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The simultaneous extraction of acidic and basic drugs from biological samples is a significant challenge for sample preparation [1]. Fakhari et al. reported an efficient method named dual hollow fibre electromembrane extraction (EME) for the simultaneous extraction and preconcentration of acidic and basic drugs with high log P values in a single step [2]. But, efficient and simultaneous extraction of a series of basic and acidic substances with a large polarity window by one run of EME has been problematic [1]. To jump over this difficulty, in this work a new EME setup using two auxiliary electrodes (one as cathode and the other as anode) and their circumferential hollow fibers impregnated with different organic solvent compositions from those of main hollow fibers, coupled with capillary electrophoresis (CE) was developed for the determination of five basic drugs (Methamphetamine, cocaine, methadone, buprenorphine and morphine) and three acidic drugs (Ibuprophen, ketoprofen and enlapril) as model drugs with different polarity. In the cathode side, the main hollow fiber was impregnated with pure 2-nitrophenyl octyl ether (NPOE) and used for the extraction of basic drugs with high log P values, while the auxiliary one was treated with NPOE containing 10% di-(2-ethylhexyl) phosphate, an ion-pairing agent, and applied for the extraction of basic drugs with lower log P values. And in the anode side, one of hollow fibers impregnated with pure 1-octanol used for the extraction of acidic drug of higher polarity and the other fiber soaked with 1-octanol containing 4% CTAB (cetyltrimethylammonium bromide), an ion-pairing agent, used for drug of lower polarity. Parameters affecting extraction efficiency optimized and results obtained are as follow: pH of the cathodic APs 1.0, pH of the anodic Aps 12.0, pH of the DP 6.0, 100 V potential difference, 20 min extraction time, 1000 rpm stirring rate.

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C.02

HIGH-THROUGHPUT SPME IN 96-BLADE FORMAT FOR ANALYSIS OF COMPLEX SAMPLES AND DIFFICULT ANALYTES

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Sample preparation, frequently is considered as a bottleneck of a chemical analysis. The success of the final analytical method strongly depends on understanding of the entire process of the analysis of a particular type of sample and analyte. Sampling and sample preparation using solid phase microextraction (SPME) due to the variety of available extraction phases and geometries are promising for on site, in vivo as well as high throughput laboratory analysis of challenging samples and analytes. The high throughput SPME analysis can additionally provide enhanced sensitivity without sacrificing time of the analysis if the thin film (TF) geometry is employed. Thus, the 96-thin film coated blade system compatible with standard 96- deep well plates is one of the most attractive formats of the SPME; it is easy to automate and provides enhanced sensitivity owing to high surface area to volume ratio of the extraction phase [1].
This work aims to show the applicability and capability of high-throughput SPME in 96-blade format for analysis of challenging compounds and matrices. Two applications were chosen: as the first, the performance of high throughput TF-SPME for fast high throughput analysis of doping substances in urine will be presented. Over 120 compounds varying in physicochemical properties were targeted and successfully quantified. Secondly, the investigation of quaternary ammonium compounds (QACs), which analyses are frequently affected by secondary interactions of the compounds with various surfaces during sample preparation will be presented. A fast (less than 1 min of sample preparation calculated per sample) and reliable method free of secondary interactions for analysis of alkyl and aryl quaternary ammonium surfactant homologues followed by LC-MS/MS separation and determination will be demonstrated.

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C.03

THE EXTRACTOR NAVIGLIO IN FOOD PRODUCTIONS

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For over a decade the Naviglio Extractor is a good alternative to solid-liquid extraction techniques such as maceration and percolation, as has been amply demonstrated that it is able to provide the same quality (if not more) of the extracts obtained with the traditional extraction techniques, also provides additional benefits such as significant reduction in the time of the extraction process (Ten days of maceration extraction equals one hour of extraction by the Naviglio extractor equal conditions) and a more efficient extraction. The principle on which it bases its functioning (Naviglio's Principle) [1] is studied in graduate courses in Herbal Techniques of several Italian universities. Currently, the Naviglio extractor is widely used in many fields of research and production (herbal, nutritional supplements, cosmetics, beverages). In the food sector, in particular, the Naviglio Extractor has been shown to be a viable alternative to macerate for: production of lemon liquor (limoncello) and similar liquors; and production of bitter elixir of juniper; rapid aging of wines, brandies and liqueurs ; extraction of lycopene from tomato processing waste [2-5]. Finally, have recently been found more unconventional applications of the Naviglio extractor as the rapid hydration of dried vegetables and their simultaneous aromatization [6], cleaning washers for the production of cork stoppers, cleaning the rubber polymer, the tanning of leather.

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C.04

CHIRAL AND NON-CHIRAL SEPARATION OF DRUGS WITH AUTOMATED MEPS-LVI-GC-MSD TECHNIQUE

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Introduction:
Chiral gas chromatography (GC) is advantageous by its possibility to detect very low amounts of volatile analytes. Chiral GC columns, in particular cyclodextrin columns, are easily harmed. Water based samples analyzed by chiral GC need efficient extraction methods to remove potential harming water and unwanted matrix. The automated and miniaturized form of solid phase extraction (SPE), micro extraction by packed sorbent (MEPS), is well suited for automated procedure. The elution volume of a few microliters is directly introduced into the GC-MSD system. With a PTV injector (programmed temperature vaporization injector) unusual large solvent volumes (> 10 µL) are evaporated and eliminated prior to reach the analytical GC column. Aim of the project was the development of low volume sample preparation coupled to a very sensitive enantioselective gas chromatographic method with large volume injection (LVI) technique.
Results:
Enantiomers of methylphenidate at concentrations 0.25 ng/mL are detected with LV injection (30 µL). LVI or MEPS-LVI technique is suitable for a large number of high boiling compounds. An increase in number of MEPS enrichment samplings discloses a directly proportional increase in corresponding peak area.
Discussion:
The MEPS protocol exposes efficient extraction and enrichment of different high boiling compounds and allows detection of very low analyte concentrations. Water based samples are analyzed by chiral GC with low risk of column impairment, as a result of water remove and solvent elimination in the injector. To enhance the limit of detection for a certain compound, no adjustments are necessary but increasing the number of MEPS enrichment samplings. Linear peak area increase was detected for a broad concentration range.
Conclusion:
In our laboratory we developed a new method for chiral GC separation of MEPS extracted compounds. This analytical procedure allows an automated, highly sensitive and robust detection of numerous drugs and metabolites.

C.05

ANALYSIS OF NUCLEATING AGENTS IN PLASTIC MATERIALS BY GC/MS AFTER MICROWAVE-ASSISTED EXTRACTION WITH *IN SITU* MICROWAVE-ASSISTED DERIVATIZATION

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In this work we present a novel approach for the analysis of sorbitol-based nucleating and clarifying agents (NCAs) in polyolefin materials, supporting the plastic industry with a new and powerful tool for material design and quality assurance.
Sorbitol-based NCAs are used in levels from 0.01% to 1% in polyolefins, at which they speed up crystallization and cause the formation of small spherulites, leading to clear and transparent instead of opaque and cloudy materials with good mechanical properties.
Until now, no comprehensive analytical strategies have been investigated for the analysis of these substances because of their poor solubility in any solvent and their difficult quantitative extraction out of complex polymer matrices. However, these issues could be overcome by the use of a microwave-assisted extraction with an *in situ* microwave-assisted derivatization employing a silylation reaction. Further analysis was carried out by GC/MS. This approach lead to the fast and reliable determination of a series of commonly used sorbitol-based NCAs. The sample preparation and subsequent GC/MS analysis performed remarkably well with repeatabilities ranging from 0.05% up to 4% and reproducibilities of up to 10% within a linear range of two orders of magnitude. Recoveries obtained by analysing real samples were between 93.4% and 102.8%. Moreover, the MS fragmentation pattern of silylated analytes could be clarified, leading to the explanation of unique mass spectral features. This fact also enabled to detect new species of this type of substances.
All data indicate the high suitability of this method for the reliable determination of sorbitol-based NCAs in polyolefin materials.

DEVELOPMENT OF A PRESSURIZED LIQUID EXTRACTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY METHOD TO DETERMINE FRAGRANCE ALLERGENS AND PRESERVATIVES IN BABY WIPE PRODUCTS

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Disposable baby wipes and wet toilet paper are usually used for the cleansing of baby skin. Babies are exposed several times daily to these products (up to sixteen units per day) which are mainly applied in the diaper area.

Fragrances and preservatives are common ingredients in these kinds of products. Fragrances provide nice and attractive scents and preservatives are used to prevent microbial growth because the wet tissue liquids are aqueous and the hard surface wipe (commonly cellulose) is an optimal medium for microbial growth.

An effective and simple preparation method based on pressurized liquid extraction-gas chromatography–mass spectrometry (PLE-GC-MS) has been developed for the simultaneous determination of 40 cosmetic ingredients, including the EU regulated fragrance allergens, the musk fragrance Galaxolide and 13 preservatives commonly used in baby wipes and other personal care products [1]. The efforts were focused on obtaining a homogeneous clean extract as well as a quantitative extraction. PLE was optimized by means of an experimental design and the optimal conditions were selected for validation.

Finally, after being extensively validated, the method was applied to a broad range of disposable baby wipes and wet toilet paper samples. The results proved the high use of allergens and preservatives (e.g. parabens, phenoxyethanol, IPBC) in these baby care cosmetic products.

Acknowledgements

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C.07

MULTI-VOLATILE METHOD (MVM) FOR AROMA ANALYSIS USING SEQUENTIAL DYNAMIC HEADSPACE SAMPLING WITH AN APPLICATION TO BREWED COFFEE

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Headspace gas chromatography (HS-GC) has been frequently used for aroma analysis because it is preferable to take advantage of volatility of aroma compounds. There are several established HS techniques, e.g. SHS, DHS, and HS-SPME. However, these techniques are generally more selective for more volatile and/or hydrophobic compounds. Recently, a full evaporation DHS (FEDHS) method, based on a classical full evaporation technique (FET) was demonstrated for uniform enrichment of aroma compounds in several sample types. FEDHS of 10-100 µL of samples at 80 ºC using a valve-less and short-path DHS system enables near complete vaporization and uniform recovery of aroma compounds, while leaving most of the low volatile matrix behind. However, the FEDHS method often requires large purge volume in order to remove condensed water from the adsorbent trap. Therefore, more volatile compounds which represent top-note of a sample breakthrough the adsorbent trap during the purge step.

The DHS system used in the FEDHS applications is based on a DHS module, an x-y-z robotic auto-sampler, and a short-path thermal desorption unit. This design allows the use of replaceable adsorbent traps and has the potential for sequential sampling from the same HS vial using different trapping conditions including selection of the adsorbent trap. Thus, more volatile compounds can be sampled with an appropriate trapping condition before performing the FEDHS method. In this study, we developed a novel multi-volatile method (MVM) with sequential DHS sampling (and desorbing) using different trapping conditions for analysis of aroma compounds in aqueous samples. The MVM method consists of three different DHS sampling conditions including final FEDHS step. The DHS parameters were examined with the model aroma compounds spiked in 100 µL water. The feasibility and benefits of MVM method is demonstrated with analysis of key odor compounds in brewed coffee.

C.08

STIR BAR SORPTIVE EXTRACTION OF 2,6-DICHLOROBENZAMIDE AND ITS ANALYSIS BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY: METHOD DEVELOPMENT AND APPLICATION TO GROUND WATER SAMPLES

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2,6-Dichlorobenzamide (BAM) is a prominent ground water contaminant and a metabolite of the herbicide dichlobenil [1]. Stir bar sorptive extraction (SBSE) has become a widespread analytical technique for the preconcentration of organic analytes owing to its faster analysis, higher sample throughput, lower solvent consumption, and less workload per sample [2]. Polyacrylate (PA) SBSE extraction of BAM followed by liquid desorption and analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using electrospray ionization is presented herein. The parameters influencing SBSE and LC methods were optimized to get the best results. C18 column (Agilent Technologies, 1.8 µm, 4.6 x 50 mm) was used with isocratic elution (50% ACN and 50% 5 mM aqueous ammonium acetate buffer at pH 2.4), flow rate 0.4 mL min^{-1} and injection volume 10 µl. Quantitative analysis was carried out in multiple reaction monitoring (MRM) using positive polarity. The SBSE-LD-LC-MS/MS method (as shown in Fig. 1) developed herein is fast, requires low sample volume (15 mL) and provides satisfactory figures of merit with limit of detection 0.002 µg L^{-1}, limit of quantification 0.006 µg L^{-1} and good precision (inter-day RSD below 10%). The application of the method was assessed by analyzing ground water samples collected in five different sampling sites of North Italy; obtained results were between 0.070-0.282 µg L^{-1}.

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Fig. 1. Graphical representation of SBSE-LD-LC-MS/MS analysis of BAM

C.09

ULTRASOUND-VORTEX-ASSISTED DISPERSIVE LIQUID-LIQUID MICRO-EXTRACTION COUPLED WITH LIQUID CHROMATOGRAPHY-TANDEM MASS-SPECTROMETRY FOR RAPID DETERMINATION OF FUNGICIDE CARBENDAZIM IN ORANGE JUICE

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A low solvent consumption method for sensitive determination of fungicide carbendazim in orange juice is studied. The carbendazim was extracted by means of ultrasound vortex assisted dispersive liquid-liquid micro-extraction (USVADLLME): 10 mL of sample (pH=10 with NaOH), 20 ng mL^{-1} albendazole (internal standard) and 250 µL of $C_2H_2Cl_4$ (extraction solvent) were vortexed for 30 s. The solution was kept in an ultrasound bath and the extraction was performed at 40 kHz of ultrasound frequency and 110 W for 10 min at room temperature. The sample was centrifuged at 5000 rpm for 10 min at 5 °C: the bottom phase of the chlorinated solvent was separated from the liquid matrix and brought to dryness with N_2, taken up with 50-100 µL of methanol-water (1:1), the determination of carbendazim was performed by means of liquid chromatography coupled with a tandem mass-spectrometry detector (LC-MS/MS). The Enrichment Factor is about 150-200 times and the recovery ranges from 94.70% to 100.3% with a Coefficient of Variation (CV) ≤ 14.8%. The efficiency of the liquid-phase micro-extraction process was slightly affected by the characteristics of orange samples, therefore the analytical characteristics of the method were evaluated in the linear range 0.5-50 ng mL^{-1} (R^2= 0.9993). The Limits of Detection (LOD) and Limits of Quantification (LOQ) was 0.1 ng mL^{-1} (n=10) and 0.5 ng mL^{-1} (n =5) respectively. The validation of the proposed method was according to the Commission Decision EC/657/2002 concerning the performance criteria of analytical methods and according to Document n° SANCO/12495/2011 concerning the method validation and quality control procedures for pesticides analysis in food and feed.

C.10

THERMOCHEMOLYSIS – A SIMPLE AND RAPID METHYLATION METHOD BASED ON TMAH FOR GAS CHROMATOGRAPHIC ANALYSIS OF LINSEED OIL AND AMBER

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In order to improve pyrolysis chromatographic analysis of materials that release polar functional groups e.g. carboxylic acids, a simple and rapid methylation method based on TDU-pyrolysis/ GC-MS in the presence of tetramethylammonium hydroxide (TMAH) was developed. Linseed oil was selected as test material because of its high triglyceride content comprising both saturated and unsaturated fatty acids. Pyrolysis was performed at 500, 600 and 700°C using a GERSTEL pyrolysis module (PYRO) with a heated platinum filament. The optimum pyrolysis temperature for linseed oil was found to be 500°C. The fatty acids in the linseed oil were found to have been quantitatively methylated when using a methanolic TMAH solution (~ 10 % in methanol). The use of an aqueous TMAH solution (25 wt. % in H2O) for the methylation of fatty acids was found to result in lower fatty acid methyl ester (FAME) yields, indicating that the formation of a homogeneous mixture of sample and reagent is essential. Additionally, it was evident that the reagent plays an active role in cleaving the triglycerides. Automated direct injection of the reagent into the TDU-PYRO is possible, but this function is a special adaptation. Chromatograms obtained from direct injection of 1 µL TMAH solution into the linseed oil sample show no difference compared with those obtained after manually mixing linseed oil and the TMAH solution prior to pyrolysis.

The pyrolysis- and thermochemolysis-GC-MS methods were successfully used to determine the molecular composition of Eocene amber from the Ameki formation, Nigeria. The amber was pyrolyzed at 480°C for 20 s with and without adding TMAH. Free carboxylic acids were quantitatively methylated to their corresponding methyl ester products in the presence of TMAH. Both Pyrolysis-GC-MS and thermochemolysis-GC-MS chromatograms were used to determine the structural class and botanical source of the fossilized resin. The pyrolysis products were dominated by labdane type diterpenoids and some sesquiterpenoids, which point to a conifer (gymnosperm) botanical source of the resin.

C.11

AUTOMATED EXTRACTION OF PHTHALATES IN CONSUMER PRODUCTS FOR ANALYSIS BY GC-MS

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The US Consumer Product Safety Commission's (CPSC) Test Method CPSC-CH-C1001-09.3[1], is used by testing laboratories for the analysis of phthalate content in children's toys and child care articles covered by the standard set forth in the Consumer Product Safety Improvement Act Section 108. The CPSC determined that an appropriate combination of methods of extraction and analysis is sufficient to determine the concentration of the six regulated phthalates in most consumer products. The general manual approach is to dissolve the sample completely in tetrahydrofuran, precipitate any PVC polymer with hexane, filter and then dilute the solution with cyclohexane, and analyze by Gas Chromatography-Mass Spectrometry (GC-MS).

A single robotic X-Y-Z coordinate autosampler commonly used for sample introduction in GC or HPLC can be used to perform a wide variety of sample preparation techniques using a single instrument and controlling software. Among the autosampler capabilities controlled by Maestro software are filtration and centrifugation, which can be used to generate clear filtrate from a solvent extraction of polymer. The sampler can be configured as part of a GC or LC system or can be configured as a benchtop workstation.

In this work, we demonstrate an automated extraction for phthalates in consumer products based on CPSC method CPSC-CH-C1001-09.3 coupled directly to the GC-MS to streamline the entire extraction and analysis process as well as avoid exposure to potentially hazardous materials by laboratory personnel.

C.12

SYNTHESIS AND CHARACTERIZATION OF CYANOPROPYL AND AMINOPROPYL HYBRID SILICA MONOLITHIC SORBENTS TO EXTRACT DRUGS FROM PLASMA SAMPLES BY MICROEXTRACTION IN A PACKED SYRINGE (MEPS)

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Microextraction in packed syringe (MEPS) is a recent technique for sample preparation that consists in the miniaturization of conventional SPE packed-bed devices from milliliter bed volumes to microliter volumes. It is possible to fully automate MEPS including the sample processing, extraction, and injection online. The combination of MEPS and liquid chromatography-tandem mass spectrometry (LC - MS/MS) is an excellent tool to determine drugs in biological fluids [1]. Monolithic materials show several attractive advantages including frit-free construction, easy preparation with good control of porosity and dispersive surface chemistry [2]. In this study, we prepared hybrid silica monolith capillaries with different functional groups (aminopropyl and cyanopropyl) by *in-situ* polymerization. Those capillaries were used as sorbent for MEPS to determine 16 drugs (antipsychotics, antidepressants, anticonvulsants and ansiolytics) from plasma samples by UPLC-MS/MS. The sol-gel technique involved mixing the silanes monomers with an organic solvent, in the presence of the porogenic agent. The scanning electron microscopy show that both prepared monolith possess a continuous porous skeleton and also tightly attached to the inner surface of the capillary**.** The double-pore structure of the monolithic allows low pressure to solvent permeate and high surface area. The Fourier-transform infrared spectroscopy analyses reveal successful incorporation of cyanopropyl and aminopropyl groups in the monoliths. The proposed method presented adequate analytical sensitivity to determinate drugs in plasma samples from schizophrenic patients for therapeutic drug monitoring.

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C.13

PRESSURIZED HOT WATER EXTRACTION OF ALKALOIDS FROM TOBACCO

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Tobacco is a well-known plant that occurs all over the world. It belongs to genus Nicotiana. Tobacco leaf is a complicated chemical system containing compounds such as carbohydrates and alkaloids, (e.g. nicotine, nornicotine, anatabine and anabasine), amino acids, alcohols and antioxidants [1].

The main aim of this work was extraction of alkaloids from tobacco using pressurized hot water extraction, as a reliable and simple method that fulfill the main conditions of green analytical chemistry, particularly due to non-toxic solvent [2]. Firstly, optimization of extraction conditions was performed. The optimization was made on the basis of central composite design, when the most suitable sample amount, time and temperature were investigated. Determination of extraction efficiency was carried out by gas chromatography/mass spectrometry (GC/MS). The best extraction conditions were subsequently used for determination of target alkaloids in real samples.

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C.14

DETERMINATION OF RESIDUAL SOLVENTS IN VITAMIN POWDER BY SPME GC-FID

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Manufacturing of a drug substance, excipients and/or drug products uses or produces volatile organic solvents. Since volatile organic solvents do not provide therapeutic benefit, they should be removed to the levels to satisfy product specifications. ICH (International Conference on Harmonization) published a guideline (Q3C) specifying the acceptable levels of volatile organic solvents in drug substances and drug products. The residual solvents are classified into three classes. Class 1 residual solvents are human carcinogens and environmental hazards. Class 2 residual solvents are inherently toxic so that their use should be limited. Class 3 residual solvents are less toxic than Class 1 and Class 2 solvents and their use is recommended where practical. The level of Class 3 solvents may be determined by loss on drying, while chromatographic analysis is required for the determination of Class 1 and Class 2 solvents.
The USP 467 Residual Solvents method is a compendial method for the determination of residual solvents in drug substances, excipients, and drug products. The method describes detailed procedures (A,B,C) for identification, determination, confirmation, and quantitation of residual solvents using headspace-GC/FID.
SPME (Solid Phase MicroExtraction) is a solventless technique suitable for the qualitative and quantitative determination of volatile and semivolatile analytes. The ability to extract analytes from the headspace eliminates non-volatile material from being transferred to the analytical column. Several SPME fiber choices are available, each with an affinity for specific analyte classes. Proper fiber selection leads to increased sensitivity. SPME can be performed manually (SPME sampling stand + hotplate/stirrer) or automated. This research investigated the use of headspace SPME for the determination of residual solvents in Vitamin powder and products and demonstrated that SPME coupled with GC/FID can be effectively used to quantify residual solvents in Vitamin powder.

C.15

AUTOMATED WORKFLOW FOR THE DETERMINATION OF FATTY ACID METHYL ESTERS (FAME) OUT OF FAT AND FAT CONTAINING FOOD SAMPLES USING A 90 SEC. TRANSESTERIFICATION

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The determination of fat content and composition of food samples via Fatty Acid Methyl Esters is a very common task in governmental, quality control (QC) or contract research laboratories (CRO). Most often the samples are processed manually, which is labor intensive and exposes the lab personnel to potentially hazardous chemicals.
This work presents a fully automated workflow of this procedure using an autosampler with robotic tool change (PAL RTC). This automated workflow improves process safety and minimizes handling errors. The PAL RTC was equipped with (i) an automated dilutor to handle all liquids for the reactions and the extraction and for the cleaning steps, (ii) a vortex module to provide fast mixing and extracting and (iii) a normal 10µl syringe tool to inject the sample to the GC. The software of the sampler allows overlapped sample processing, which increases the efficiency and sample throughput.
The method allows the determination of total fat content, quantitative analysis of saturated and unsaturated *cis*- and *trans*- fatty acids. The use of three internal standards allows controlling the yield of extraction, transesterification and undesired saponification. The method was applied to a number of different vegetable oils, butter and animal fats. In conclusion, this technique allows the rapid and reliable sample preparation and analysis of fat containing food samples with highest process safety.

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C.16

TAILOR MADE MOLECULARLY IMPRINTED POLYMERS FOR THE MOLECULAR RECOGNITION OF DIMETHOATE AND DELTAMETHRIN

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The extensive and uncontrolled use of pesticides as control-pest agents and their persistence up to the harvest stage can determine the presence of pesticide residues in foodstuffs, which involves serious risks to human health. Due to increasingly stringent European regulations in terms of number of compounds to trace and low Maximum Residue Limits, the development of highly selective, sensitive and reliable methodologies that enable the trace analysis of these contaminants is strongly desirable and is an emerging issue in order to ensure “Food Safety”. In the search for more selective methodologies, the introduction of “tailor-made” Molecularly Imprinted Polymers (MIPs) artificially engineered to possess pre-defined specific recognition sites for a target analyte can open up new avenues on the development of new adsorbents for solid phase extraction-based methodologies [1].

The present work addresses the development of selective MIPs for the isolation/pre-concentration of dimethoate and deltamethrin from food samples aiming to assess their suitability as adsorbents in solid phase extraction (MISPE). To achieve this goal, these selective porous materials have been synthesized and characterized using spectroscopic (FTIR, NMR) and morphological (SEM, TGA) techniques. In order to evaluate the molecular recognition of these imprinting systems several chromatographic studies were performed comprising the measurement of imprinting factor as well as affinity binding properties [2].

Acknowledgements

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C.17

TRIMETHYLSILYLDIAZOMETHANE IN METHYLATION OF ACIDIC DEGRADATION PRODUCTS OF CHEMICAL WARFARE AGENTS ANALYZED BY GC-MS

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One of the most important tasks in verification of the Chemical Weapons Convention (CWC) is precise and reliable analysis of alkylphosphonic acids (APAs), and sulfonic acids (SAs) which are the degradation products of G and V agents, such as sarin, soman, and VX. Unequivocal identification of CWC -related chemicals is required in a variety of environmental matrices including soil and water.

Direct analysis of APAs in an aqueous solution by liquid chromatography or nuclear magnetic resonance spectrometry is the most convenient choice, because the extraction with water is the easiest way to isolate these polar degradation products from various matrices. Most often, especially for on-site analysis, GC analysis is needed, however. To achieve sensitive detection and unequivocal identification at the same time, mass spectrometric (MS) identification is also required.

These acids with low pKa values and volatility are very polar, and for identification by GC-MS derivatization is need. Conversion to methyl, pentafluorobenzyl, trimethylsilyl and tert-butyldimethylsilyl esters is usually used. Instructions to perform these derivatizations are described in internationally compiled recommended operation procedures. Most of these methods are in routine use in laboratories dedicated to CWC analysis. Methylation using diazomethane is regularly considered too complicated and dangerous, however.

Methylation of APAs and SAs with trimethylsilylsdiazomethane (TMSDAM) was tested for derivatization. The derivatization conditions were optimized for a rapid GC-MS screening and identification of these chemicals. Methylation of sulfonic acids was compared to silylation. Method robustness was evaluated in official OPCW Proficiency tests. All APAs present in samples were identified. Methylation of APAs and SAs with TMSDAM suited well to the qualitative analysis in aqueous matrices with its simplicity, speed, and robustness. The method performance was excellent and the method was reliable for the detection and identification of a wide range of nerve agent markers.

C.18

DIRECT SILYLATION OF TRYPANOSOMA BRUCEI METABOLITES IN AQUEOUS SAMPLES

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Silylation is the most common derivatization method performed before GC analysis. The most commonly used silylation method is trimethylsilylation, as trimethylsilyl (TMS) derivatives have suitable thermal and chemical stability as well as high volatility. They can be easily prepared and have excellent chromatographic properties. The main disadvantage of silylation reagents is their susceptibility to hydrolysis. Recently, analytical methods with a minimal number of operations and processes involved in the sample preparation stage are preferred as these methods allow the shortening of sample preparation time, reduction of the losses of volatile analytes and the risk of sample contamination. A simple two-step method for the derivatization of polar compounds (lactate, alanine, glycerol, succinate and glucose) using hexamethyldisilazane (HMDS) and N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was developed. This method allows direct derivatization of aqueous samples without sample pretreatment. The method was used for the analysis of the metabolites of the unicellular organism *Trypanosoma brucei*. The limits of detection by GC-MS/MS analysis were in the range of 0.02 mgL^{-1} for glucose to 0.85 mgL^{-1} for lactate.

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C.19

AUTOMATED PREPARATION OF VISCOUS VACUUM GAS OIL SAMPLES FOR TOTAL SULFUR AND NITROGEN DETERMINATION USING THE AGILENT 7696A WORKBENCH

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The Agilent 7696A Sample Prep WorkBench was used to automatically dilute refinery vacuum gasoil (VGO) feedstock samples for total sulfur and nitrogen determination. The viscosity of the VGOs ranged from viscous-liquids to soft-solids at room temperature. For the viscous-liquid VGOs, the WorkBench vial heater station was used to lower the viscosity of the samples allowing precise and accurate aspiration and dispensing with the autosampler syringe. However, cooling during the transfer of vials from the vial heater to the autosampler turret caused the soft-solid VGOs to re-solidify. In order to maintain a sufficient vial temperature during the transfer, a water bath was constructed by placing the VGO sample in a 150 microliter vial insert immersed into a 1.5 milliliter autosampler vial containing water. The heat capacity of the water maintained the vial temperature and provided low enough viscosity for syringe aspiration. It was also determined that multiple small volume dilutions instead of a single large volume dilution were required to limit the cooling of the VGOs once aspirated into the syringe.
The precision in automatically transferring 40 milligrams of sample was 2.4% relative standard deviation for 5 replicate sample preparations for the soft-solid samples. The precision in total nitrogen and sulfur content using gas chromatography with chemiluminescence detection was 2.3% and 3.0%, respectively. The accuracy of the sulfur and nitrogen content were within 5% error. This application demonstrates the versatility of the WorkBench for preparing challenging highly viscous samples. The techniques developed for this application could be used for other applications in which handling of viscous samples is required.

C.20

DETERMINATION OF VOLATILE ORGANIC COMPOUNDS IN WATER BY SPME AND GC/MS: VALIDATION OF NEW ISO STANDARD 17943

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Volatile organic compounds (VOCs) are often used during the manufacturing of many different products such as petroleum products, adhesives, pharmaceuticals, paints or refrigerants. Additionally some are applied as additives, solvents or other agents. The contamination of water by VOCs is critical for health due to the fact that many of the VOCs are toxic.
Solid Phase Micro Extraction (SPME) was introduced in 1990 [1]. Since then SPME has gained broad acceptance in environmental, pharmaceutical and food analysis demonstrated by the still growing number of publications on SPME developments and applications. The prevalence of this technique was additionally increased by the automation of SPME using GC autosamplers since 1993. Another indication of the broad acceptance is the use of SPME in official methods and standards.
VOCs in water can be determined by different methods [2-5]. This work describes the details of the determination of more than 60 volatile organic compounds (VOCs) in different water matrices together with the required method optimizations. After extraction of the compounds in the headspace of the samples by SPME the analysis is conducted by GC-MS. Additionally the results from an interlaboratory trial for validation of this method as a new ISO standard will be presented.

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C.21

A NEW METHOD FOR THE DETERMINATION OF ESTROGENIC MYCOTOXINS IN WATER BASED ON DISPERSIVE LIQUID–LIQUID MICROEXTRACTION AND LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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The occurrence of estrogenic mycotoxins in river water and wastewater treatment plant effluents has been recently reported. Concentrations up to thousands ng L^{-1} were reported for zearalenone and some of its metabolites in these matrices [1,2]. Agricultural fields, livestock facilities or fields receiving livestock manure as soil amendment are potential sources of estrogenic mycotoxins for water resources, mainly by drainage and runoff [2]. A very selective and sensitive analytical method is required to reliable quantification into expected low concentrations of estrogenic mycotoxins in aqueous matrices. Thus, a simple, rapid and efficient sample preparation method based on dispersive liquid-liquid microextraction has been developed, at the first time, for the determination of six estrogenic mycotoxins (zearalenone, zearalanone, α-zearalanol, β-zearalanol, α-zearalenol and β-zearalenol) in water samples followed by liquid chromatography–electrospray ionization tandem mass spectrometry in negative mode (LC–ESI-MS/MS) analysis. Different experimental parameters were controlled and the optimum conditions found were: 100 μL bromocyclohexane as the extractant phase; 10 ml aqueous samples; 2 min vortex extraction time; centrifugation for 10 min at 3500 rpm; sample pH 4 and no ionic strength adjustment. Under the optimized conditions, the method was successfully validated showing linearity in the range of 8-1200 ng L^{-1}. Intra-day and inter-day precisions expressed as relative standard deviation (RSD) were −1 and 8–80 ng L^{-1}, respectively. This simple and economic method will be applied to analyse samples from rivers at São Paulo State, Brazil.

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C.22

MULTI-STIR BAR SORPTIVE EXTRACTION (M SBSE) USING BOTH NON-POLAR AND POLAR COATINGS FOR AROMA ANALYSIS.

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Stir bar sorptive extraction (SBSE) has been successfully applied to many areas including food analysis, odor analysis, environmental analysis, life science and biomedical analysis. However, most applications have been performed using polydimethylsiloxane (PDMS) phase due to the only available extraction phase on commercial stir bars in the past decade. The search for new coatings for SBSE is very challenging, especially if the following criteria are targeted: 1. a coating should be thermally stable to allow thermal desorption (which is the best option for hyphenation with GC), 2. the coating should give a significant improvement versus PDMS, 3. production of the coated stir bar should be possible in a very reproducible way. As reproducible coating of stir bars with more polar phases was found to be very difficult, a supporting grid was used in the development of an ethyleneglycol-modified Silicone (EG Silicone) coated stir bar. This new polar coating showed good performance for the extraction of polar solutes, but long term use also showed degradation of the coating due to friction while stirring. In order to address the lower robustness of the EG Silicone stir bar which has a much softer coating compared to a conventional PDMS stir bar, a novel SBSE procedure termed multi-SBSE (^{m}SBSE) was developed. ^{m}SBSE consists of the robust PDMS stir bar stirring at the bottom of the vial and the EG Silicone stir bar attached on the inner side wall of the vial. After extraction, the two stir bars are placed in a single glass desorption liner and are simultaneously thermally desorbed. The desorbed compounds were analyzed by gas chromatography-mass spectrometry (TD-GC-MS). Compared to conventional SBSE, ^{m}SBSE provides more uniform enrichment of a wide range of odor compounds in aqueous sample since both stir bars can complement each other, while eliminating the damage of the EG Silicone phase during the extraction. The feasibility and benefit of the method will be demonstrated with analysis of odor compounds in beverages.

C.23

PESTICIDES DETERMINATION IN THE PULP AND PEEL OF THE TAMARIND (TAMARINDU INDICA)

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Brazil has a very diverse fruit production, being one of the leading manufacturers and exporters of various native and exotic species. Within the latter variety stands out tamarind (Tamarind indica) is rich in minerals, carbohydrates, vitamins (A, B and C), potassium, iron, phosphorus and calcium. It is considered an excellent laxative and can also be used as anti-inflammatory and fever and cough depressant[1].

Originally, the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) methodology was developed for the extraction of pesticides from vegetables and fruits [2, 3]. An optimized version of QuEChERS method for simultaneous determination of 14 organochlorine pesticides in tamarind was developed using gas chromatography coupled with electron-capture detector (GC-ECD) and confirmation by gas chromatography tandem mass spectrometry (GC-MS/MS). A citrate-buffered version of QuEChERS was applied for the extraction of the organochlorine pesticides, and for the extract clean-up, primary secondary amine, octadecyl-bonded silica (C18) and magnesium sulphate (MgSO4) were used as sorbents for tamarind pulp and for peel was also added graphitized carbon black. The GC-ECD determination of the target compounds was achieved in less than 20 min. The overall average recoveries in tamarind peel and pulp, at the three tested levels (80, 98 and 196 μg/kg), ranged from 65 to 98 % with relative standard deviations in the range of 2–15 % (n = 3) for all analytes, with the exception of HCB. The mass samples were optimized achieving the best recoveries with 0.5 g of tamarind pulp and 1.0 g for peel. This methodology combines the advantages of both QuEChERS and GC-ECD, producing a very rapid, sensitive and reliable procedure which can be applied in routine analytical laboratories.

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C.24

OPTIMIZATION OF BPA DETERMINATION IN URINE SAMPLES BY SPE-GC-MS

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Bisphenol A (BPA) is a high production volume chemical. Although BPA was initially considered to be a weak environmental estrogen, nowadays it is known that this compound can stimulate several cellular responses at very low levels of concentrations.In humans, increased levels of BPA have been correlated with various diseases, health outcomes and medical conditions [1]. The identification and quantification of BPA are challenging due to the low concentrations at which the compound is typically present in human samples. Consequently, biomonitoring should be performed through highly sensitive analytical methods and exposure assessment based, besides from the free specie of the compound, also as conjugates [2].

The aim of this study was to develop a method to evaluate the presence of BPA in human urine samples.Chromatographic analyses were carried out in a Thermo GC ULTRA Mass Spectrometer equipped with a ZB-XLB (30m x 0.25mm ID, 0.25μm film). The injections were performed in splitless mode at 270ºC. Analyses were carried out in SIM mode. For extraction two different SPE cartridges were tested (Strata C18-E and Strata-X Polymeric Reversed Phase).The cartridge with higher recovery was selected.Different conditioning and eluting solutions were evaluated. For deconjugation two enzymes were tested: β-glucuronidases from *E. coli* and from *H. pomatia.* After optimization a method validation was performed. The methodology was then applied to urine sample analyses.

This methodology will enable a major advance in scientific knowledge about the health impact of exposure to BPA.

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C.25

COMPARISON OF THE EFFECTIVENESS OF DIFFERENT EXTRACTION TECHNIQUES, SPME AND QUECHERS, COMBINED WITH GAS CHROMATOGRAPHY–MASS SPECTROMETRY FOR THE ESTABLISHMENT OF THE VOLATOMIC PROFILE OF EUGENIA UNIFLORA L.

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Eugenia uniflora L. (Myrtaceae), known as pitangueira, is a fruit-bearing widely distributed throughout Madeira Island and is used in popular medicine as a diuretic, anti-rheumatic, anti-febrile, and anti-inflammatory agent and as a therapeutic agent for stomach diseases. Moreover essential oils from pitangueira leaves has been used by the cosmetic industry for its astringent properties, which are associated with its pleasant smell. Among the many attractive and desirable attributes that create demand for fruits, their characteristic flavor is the most noticeable to consumers.

In order to establish the volatile metabolomic pattern of the pitangueira fruit, pitanga, the feasibility of different high-throughput extraction techniques, based on HS-SPME and QuEChERS, was evaluated in order to test their effectiveness for the extraction of the volatile organic metabolites (VOMs) from pitanga to achieve the most complete volatile and semi-volatile signature, distinct QuEChERS parameters and different SPME fibre coatings (PA, PDMS, PEG, DVB/CAR/PDMS, PDMS/DVB, and CAR/PDMS), were tested and compared. Both the extraction techniques were followed by GC–qMS analysis, which allowed the identification of up to 35 VOMs and SVOMs, using QuEChERS technique and up to 57 VOMs by SPME, in a total of 77 metabolites, distributed by distinct chemical families: monoterpenes, C13–norisoprenoids, sesquiterpenes, higher alcohols, esters and carbonyl compounds. Mass spectra, standard compounds and retention index were used for identification purposes.

(Z)-β-Ocimene, (E)-β-ocimene, curzerene, β-elemene, and β-myrcene were found the major constituents obtained by SPME/GC-qMS, whereas by using QuEChERS/GC-qMS the most dominant VOMs and SVOMs were include (Z)-β-ocimene, curzerene and germacrone , accounting to 60% of total volatile and semi-volatile composition of pitanga.

C.26

DETERMINATION OF 1,4-DIOXANE, TETRAHYDROFURAN, AND NITROSAMINES AT NG/L LEVELS BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY WITH CONCURRENT SOLVENT RECONDENSATION – LARGE VOLUME SPLITLESS INJECTION

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N-nitrosodimethylamine is a hepatotoxic suspected human carcinogen that can enter the environment through manufacturing processes and the reaction of precursor amines with nitrosating agents in the environment. The current methodologies recommended for the analysis of 1,4-dioxane and nitrosamines in drinking waters are EPA Methods 522 and 521 respectively. EPA Method 522 is a simple gas chromatography – mass spectrometry (GC-MS) method, while Method 521 requires positive chemical ionization (PCI) using liquid methanol or acetonitrile reagent gas along with tandem mass spectrometry (GC-MS/MS). The method described here uses the same coconut charcoal sorbent solid-phase extraction tubes and dichloromethane eluent recommended in EPA Methods 521 and 522 to concentrate 0.50 L water samples to 10 mL extracts. The benefits of a combination method include fewer samples for collecting, shipping and extracting, as well as higher throughput. Because the final SPE extract cannot be concentrated via evaporation due to volatile compound loss, we employed concurrent solvent recondensation – large volume splitless injection (CSR-LVSI), which uses a standard splitless injector to deliver 50 µL extract to a pre-column connected to a typical GC column for separation and then MS analysis. This large volume injection allows for the low part per trillion detection limits needed for drinking water analysis when combined with selected ion monitoring (SIM).

C.27

STUDY OF PHOTOCHEMICAL BEHAVIOR OF PRESERVATIVES BY SPME AND MSPD GC/MS. APPLICATION IN REAL COSMETIC SAMPLES

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Preservatives are essential ingredients widely added in daily used cosmetics and personal care products (PCPs) with the primary purpose of preventing spoilage. The widespread use of preservatives in cosmetic formulations has risen scientific and social concern, as some of these compounds have been shown to have negative side effects on consumers health. However, apart from this primary harmful effect, in the case of direct exposure to the radiation coming from the sun, the photodegradation of the aforementioned preservatives may occur. The degradation induced by UV solar radiation, not only may cause the inactivation of the cosmetic ingredient, but also may produce potentially hazardous photoproducts, even directly on the skin. Although the photochemical behavior in water under different oxidative conditions have been reported for some preservatives, photodegradation studies in cosmetics are almost non-existent. Therefore, the research about the photodegradation kinetics of cosmetic preservatives, as well as the identification of their by-products chemical structures is needed. Besides, in the case of cosmetic products, the interaction between the photodegradation by-products and other formulation components, may led to the formation of new molecules with unknown toxicological properties.

The photochemical behaviour of several cosmetic preservatives, both in ultrapure water and 'on-fiber' Photo-SPME (Photo-Solid-Phase Microextraction) [1] have been performed. Preservatives such as parabens, benzoates, iodopropynyl butylcarbamate (IPBC), 2-*tert*-butyl-4- methoxyphenol (BHA) and 2,6-bis(1,1-dimethylethyl)- 4-methylphenol (BHT) has been investigated. The photodegradation of these compounds in aqueous-based cosmetics was also carried out. A selectable power UV-photorreactor was used in order to perform the trials for the required time. Gas chromatography coupled with mass spectrometry (GC-MS) has been employed to monitor the degradation kinetics of the investigated preservatives.

Different photodegradation kinetics were observed depending on the type of preservative. Several photoproducts were detected and some of them were tentatively identified by means of their mass spectra and using the information found in the literature. The formation of benzophenones and dichlorodibenzo-p -dioxin (DCDD) were detected in some of the irradiated samples. Finally, photodegradation pathways were tentatively proposed for some of the preservatives under study.

Acknowledgements

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C.28

EVALUATION OF A NOVEL, FULLY AUTOMATED, LARGE-VOLUME SOLID-PHASE MICROEXTRACTION DEVICE FOR HEADSPACE AND DIRECT IMMERSION ANALYSIS OF 16 PROMINENT POLYCYCLIC AROMATIC HYDROCARBONS IN WATER

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The new PAL SPME Arrow represents a promising alternative to traditional SPME instrumentation, offering mechanical robustness and drastically increased sorption phase volumes.
While all commercially available SPME Fiber coatings may be realized as well for PAL SPME Arrow, phase volumes of up to approx. 15µL in comparison to approx. 0,5µL with SPME-Fibers enable a surpassing performance and potentially novel fields of application.
The required changes to the utilized GC-system are marginal, allowing injector-penetration by the larger diameter of the PAL SPME Arrow (1,5mm). The specially designed tip of the new fiber thereby minimizes septum wear to levels comparable to traditional microextraction techniques.
In the presented results, we analyzed polycyclic aromatic hydrocarbons (PAH) via headspace (HS) and direct immersion (DI) analysis. PAH are known carcinogens as well as persistent and ubiquitous environmental contaminants. Novel legal regulations such as the European Water Framework Directive demand analysis of these compounds in challenging concentration ranges (170 pg/L for Benzo(a)pyrene), which are hardly achievable using available automatable sample preparation techniques.
Using DI-extraction, quantification of the prominent 16 “EPA-PAH” was possible down to the sub-ng/L range, maintaining satisfactory standard deviations.
Besides significantly prolonged fiber lifetimes, the PAL SPME Arrow is also fully automatable via the well-established PAL autosampler. Using HS extraction, analysis of six rather volatile PAH (Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene and Anthracene) is possible in the low ng/L concentration range.
Required extraction times and detection limits can be balanced according to individual demands via different extraction phase thicknesses, extraction temperatures and stirring intensities.

C.29

APPLICATION OF IN VIVO SPME TO THE DETERMINATION OF PESTICIDES IN LIVING CLAMS

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Great part of modern environmental analysis focuses on the determination of specific chemicals derived from their exploitation in different fields. Included in this group of harmful substances are: flame retardants, pharmaceuticals, pesticides. Most of these compounds and their metabolites have been proven as toxic/carcinogenic to both aquatic and terrestrial fauna, and are in general detrimental to the environment. In order to establish the pollution level of marine/river waters, it's becoming a common practice to assess the chemical composition of living organisms, which act as "bioaccumulators". Bioaccumulation is a phenomenon occurring in the biota, which consists of an increase in the chemical concentration of a xenobiotic into an organism, compared to its concentration in the surrounding habitat.

This research study presents an analytical method suitable for the determination of compounds utilized as pesticides in agriculture. Samples under investigation were living clams, collected in the area of the Strait of Messina (Sicily). Clams were divided in two groups, one kept in clean reference water (control group), one in waters spiked with appropriate amounts of pesticides. The latter were selected among those most commonly employed in the Italian agricultural practices: three fungicides, namely boscalid, captan and thiabendazole; and three insecticides, namely chlorpyriphos, deltamethrin and procymidone. *In vivo* sampling by SPME was carried out with a polydimethylsiloxane (PDMS) metal alloy fiber, 100 μm film thickness. Clams were let to stand in marine waters for a period of 48 hours, and were periodically sampled by inserting the SPME fiber in the mantle cavity. Different times of preconcentration were tested. SPME fibers were then desorbed into a Shimadzu GC-MS system, equipped with an SLB-5ms column and a dedicated mass spectral library (*Pesticides GC-MS library*, Wiley). The method was validated in terms of precision and sensitivity. Recoveries of spiked pesticides were measured, demonstrating that the method was efficient in monitoring the levels of bioaccumulation.

C.30

LIPID PROFILE OF PORTUGUESE BROWN ALGAE USING MICROWAVE EXTRACTION

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Macroalgae are nowadays used as fuel bio-resources and food sources to humans, particularly in Asian countries, due to their interesting properties such as proteins, vitamins, amino acids, trace minerals and fatty acids (FA) [1-3]. Over the past few decades, the consumption of seaweed products has increased in European countries due to the high amount of algae in European seas [5-6]. The genus *Saccorhiza*, *Sargassum*, Fucus, Cystoseira, Padina*, Halopteris*, *Cladostephus* and *Stypocaulon* are representative examples of brown algae found in the Portuguese Atlantic coast [7]. The FA composition in algae may differ significantly. Predominantly, monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) include the storage lipid fraction, although polyunsaturated fatty acids (PUFA) mainly involve the structural lipid fraction [4].

In this work the characterization of the fatty acids composition of several species of marine macro algae gathered along the NW region of Portugal during summer is reported. An advanced extraction technique, microwave-assisted extraction (MAE), was employed to extract total fat of seaweeds using petroleum ether: acetone (2:1 v/v). MAE presents several advantages when compared with the traditional Folch method due to its short extraction times and lower amount of solvents resulting in reduced costs and being an eco-friendly technique [7]. In the present study higher levels of saturated fatty acids (SFA), such as palmitic acid (C16:0), and monounsaturated fatty acids (MUFA), like oleic acid (C18:1 n-9c), were found for different brown seaweeds (*Sacchorhiza polyschides, Fucus spiralis* and *Bifurcaria bifurcata)*. Results revealed significant inter-differences between species in fatty acid profiles.

Acknowledgements

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C.31

STATIC HEADSPACE ANALYSIS OF ACETALDEHYDE IN FIVE OFFICIAL FOOD SIMULANTS OF KOREA

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Acetaldehyde is one of the substances can possibly shift to the food from the food containers and packaging made with polyethylene terephthalate that is called PET, used for the production of synthetic fibers with bottle production accounting for around 30% of global demand. Trace amounts of acetaldehyde, and to a lesser extent other aldehydes, are unavoidable byproducts in the production of PET bottles. Acetaldehyde is one of the most well-known aldehyde, occurring widely in nature and being produced on a large scale in industry. However, it must be regulated to minimize the human exposure via any artificial source such as food packaging because acetaldehyde is a probable human carcinogen. In this study, acetaldehyde analysis method was established for the five Korean official food simulants such as water, 4% acetic acid, 20% ethanol, 50% ethanol and n-heptane. The most adequate GC capillary column was PLOT U (porous layer open tubular U) column among the tested four coulmns. Static headspace method was compared to the derivatization method of pentafluorobenzyloxime (PFBO) derivative for the analysis of acetaldehyde. Given the convenience, efficiency etc, the static headspace method was better than the derivatization method. The phase ratio was optimized as 6 mL of the sample matrix in 20mL size headspace vial. The limit of quantification (LOQ) of acetaldehyde ranged between 0.098ug/mL (for the heptane matrix) and 0.50ug/mL (for the ethanol matrices) by GC-FID. The high LOQ of acetaldehyde in ethanol matrices was due to the acetaldehyde impurity in ethanol. The headspace method was precise (%RSD 0.9%~6.5% at 1ug/mL level) and accurate (Recovery 87.5%~98.5% at 1ug/mL level). The optimized headspace method of acetaldehyde is suggested as the Korean official analysis method for the measurement of the migrated acetaldehyde from food packaging such as PET.

C.32

SCREENING AND QUANTIFICATION OF ILLICIT DRUGS AND METABOLITES IN ORAL FLUID BY MICRO EXTRACTION ON PACKED SORBENT COUPLED WITH LC–MS/MS.

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Oral fluid (OF) has gained a great popularity as a valuable biologic specimen for toxicological analysis, especially in DUID investigations, because it can be easily obtained in a non-invasive and observable way and also because for most drugs there is a good correlation with degree of impairment. Limitations in OF testing are its variable viscosity, pH and flow depending on a range of physiological variables. In addition external contamination can be an issue; in these cases detection of metabolites can be crucial.
Multi-analyte procedures for screening and quantification of drugs in biological specimens are very useful because they allow analysis of several important compounds with a single sample extract injection, thus saving time and resources. For OF testing, where sample volume is limited multi analyte procedures are particularly advantageous. A number of procedures allowing the simultaneous determination of a number of illicit drugs in OF can be found in the literature especially using (ultra) liquid chromatography–spectrometry ((UP)LC–MS/MS) [1] and gas chromatography–MS (GC–MS) [2]. Most of these methods require at least 500 µL of OF and use time-consuming sample In the last years there has been an increasing interest in the development of new methodologies to address some of the shortcomings of sample preparation. In this work a procedure for the simultaneous screening and quantification of 20 illicit drugs, belonging to the classes of cocaine, amphetamines, natural and synthetic opioids and hallucinogens. The sample preparation is based on microextraction on packed sorbent (MEPS) a novel technique which is based on the miniaturization of SPE.
The method has been fully validated according to the SWGTOX guidelines.

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C.33

MICRO-SOLID PHASE EXTRACTION (µSPE) OF PESTICIDES FROM WHEAT FOLLOWED BY UHPLC-MS/MS DETERMINATION

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A common problem in the use of pesticides is the erroneous application regarding the dose, timing and frequency of application, which leads to problems both for the consumers but also for the workers in the field. Several methods are employed for the sample pre-treatment from complex matrices (Luke, QuEChERS, MSPD, ecc.). In all the cases this is the critical step of the analysis. All these extraction methods require large amounts of solvents and long working time. The purpose of this work is the development of a rapid and effective method of extraction and analysis of pesticides in wheat and derivatives. In this work a micro-solid phase extraction (µSPE) with modified tips was employed for the clean-up step; these tips are made of fiberglass functionalized with apolar chains of octadecylsilane into monolithic structure.
Pesticides belonging to different chemical classes were analyzed. In particular, the analyses were focused on the most widely used pesticides found in the wheat matrix [1]: *pirimiphos-methyl* ,chlorpyrifos-ethyl, chlorpyrifos-methyl , malathion. The determination was carried out using a liquid cromatography coupled with tandem mass spectrometry; the analytes were detected in positive ionization by means of an electrospray source.
Under the optimized extraction conditions, the method shows good recovery. Furthermore a high reproducibility intra and inter tip was also observed, and the LODs for all the pesticides was lower than the relative MRL.
This work has shown that the µ-SPE extraction procedure, coupled with LC-MS/MS analysis, allows to obtain an efficient sample clean-up with a few simple steps and with an extremely reduced solvent consumption.

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C.34

ANALYSIS OF FRAGRANCE ALLERGENS, PRESERVATIVES, MUSKS AND PLASTICIZERS IN PERSONAL CARE PRODUCTS BY MICRO-MATRIX-SOLID–PHASE DISPERSION GAS CHROMATOGRAHY-MASS SPECTROMETRY

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The Regulation (EC) No 1223/2009 establishes the rules to be complied with by any cosmeticproduct available on the market, in order to ensure a high level of protection of human health.It includes the prohibited substances, which must not be integrated in the cosmetic formulations, as well as the restrictions applied to other substances [1].
European legislation requires the monitoring of 26 fragrances considered as suspected allergens, frequently used in cosmetics and personal care products. Preservatives are added to cosmetic preparations to inhibit the development of microorganisms. Parabens are the most frequently used; these substances have restricted use according to regulations. Other two families of compounds, the musk fragrances and the plasticizers are usually present in personal care products to exhibit long-lasting odour fragrance and make the product more attractive to consumers.
An effective, simple and low cost sample preparation method based on micro-matrix solid-phase dispersion (MSPD) and gas chromatography–mass spectrometry was used for the rapid simultaneous determination of more than 70 chemical compounds commonly used in cosmetics and personal care products. The method was previously miniaturized and optimized by multivariate analysis and the final procedure requires the use of only 0.1 g of sample and 1 mL of organic solvent. The micro-MSPD method was extensively validated and it was applied to a broad range of cosmetics and personal care products (shampoos, body milk, moisturizing milk, toothpaste, hand creams, gloss lipstick, sunblock, deodorants and liquid soaps among others) demonstrating its suitability.

Acknowledgements
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BIOLOGICAL ACTIVITIES OF ALOYSIA TRIPHYLLA ESSENTIAL OIL EXTRACTED BY MICROWAVE AND ANTIOXIDANT POWER OF ITS METHANOLIC CRUDE EXTRACT

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Essential oils of various plant species are recently gaining much scientific and public interest according to their multifarious uses and diverse biological activities. Essential oils have been employed in many industries such as foods, cosmetics and perfumes. Nowadays, *Aloysia triphylla* essential oil is greatly introduced in preparation of many drugs. The objective of our work was to assess the antioxidant power of its crude extracts and to evaluate the biological activities of its essential oil extracted by microwave using as microorganisms: *Pseudomonas aeruginosa; Staphylococcus aureus; Escherichia coli; Micrococcus luteus; Fusarium oxysporum; Fusarium albedinis* and *Saccharomyces cerevisiae*. The crude extract of aerial parts of *A. tripylla* was screened for its free radical scavenging properties using methanol as solvent and butyl hydroxyl toluene (BHT) as standard antioxidant. Free radical scavenging activity was evaluated using the 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH) method. The concentration for a 50% inhibition was 13.32±0.219 µg/ml. The antimicrobial activity of the selected essential oil was determined by disc diffusion method. The paper discs (9 mm in diameter) were separately impregnated with 15 µL of essential oil or main components of essential oils and placed on the agar which had previously been inoculated with the selected test microorganism. Discs without samples were used as a negative control. Plates were kept at 4 ºC for 1h. The plates were incubated at 37 ºC for 24 h for bacteria and at 30 °C for 48 h for fungal strains. Antimicrobial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters for the test organisms comparing to the controls. The best results were obtained for microorganisms *Saccharomyces cerevisiae, Micrococcus luteus* and *Staphylococcus aureus*.

D.02

DIRECT DETERMINATION OF HALOGENATED POPs IN AQUEOUS SAMPLES BY IN-TUBE SOLID-PHASE EXTRACTION, FOCALIZATION AND GC-ECD ANALYSIS

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The quantification in aqueous samples of Persistent Organic Pollutants (POPs), anthropogenic substances widespread used and regulated by the UN Stockholm Convention, is still a demanding task. This is mainly due to extremely low concentrations at which every compound may be found.

In order to reduce contaminations introduced by traditional extraction methods, we have applied an approach based on direct trapping of analytes into short open tubular capillary columns. Two-meter long capillary traps (I.D. 320 µm, coated with 1.2-µm thick of 5%-diphenyl-95%-dimethylpolysiloxane or 14%-cyanopropylphenyl-86%-dimethylpolysiloxane) were used to extract some chlorinated pesticides (aldrin, heptachlor, heptachlorepoxide, dieldrin, endrin, 4,4-DDE, α-endosulfan) and polychlorobiphenyls (1, 15, 44, 77, 180). Every trap was installed in a GC oven and connected in series to an analytical column (25-m long, 320 µm I.D., CP-Sil 8 CB, 1.2-µm film thickness) installed in a second GC-ECD (GC-GC tandem system). The capillary trap in the first GC was quickly heated from 50 °C to 280 °C to focus the retained analytes; after, solutes were thermally desorbed and separated into the analytical column by programming the temperature of the second GC.

Extraction recovery was nearly quantitative for most of the analytes. The sorptive properties of the two stationary phases were sample volume- and velocity-independent but was slightly influenced by their polarity. The latter effect was used to investigate the possibility to fractionate the two classes of compounds. For this purpose, a capillary trap was connected in series to a second capillary trap immediately before the trapping step. Adsorbed solutes were fractioned between the two traps by eluting with water-methanol. Most of the tested compounds were retained exclusively by one of the two phases allowing for a complete fractionation.

The proposed method proved to be practically insensitive to laboratory contamination, reproducible, and suitable for POPs determination at trace level (LODs 5-50 pg L^{-1}).

D.03

PORTABLE LOW VOLUME ACTIVE SAMPLER APPLICATION FOR ENVIRONMENTAL POPS AIR MONITORING FOLLOWED BY GC/QMS ANALYSIS

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Samplers most routinely used to characterize persistent organic pollutants (POPs) in atmospheric air are high volume active samplers, whose installation requires a safely area and electricity, which restricts the application in remote areas. On the other hand, there are low volume portable samplers available in the market, but they are traditionally used for occupational studies. In the present study, analytical methodologies for the extraction and characterization of these compounds in the gas fraction of atmospheric air were developed using portable samplers with XAD - 2 adsorbent tubes and desorption with solvent. Analysis was performed by GC/qMS (Gas Chromatography with Quadrupole Mass Spectrometry) in selected ion mode for quantification of 16 polycyclic aromatic hydrocarbons (PAHs) and simultaneous scanning for identifying other POPs. The method developed for quantification of PAHs presented as figures of merit the Pearson correlation coefficients between 0.9811 to 0.9987 for the calibration curve of 15 studied compounds, while indeno(1,2,3-cd)pyrene presented just 0.9778 as correlation coefficient, in the same calibration range. The detection limits were from 0.66 to 157.72 ng m-3 while quantification limits were between 25 and 500 ng m-3 for all 16 PAHs. The coefficients of variation ranged from 2.9 to 5.7% for PAHs having up to 4 aromatic rings, and from 0.4 to 18.7% for PAH with 5 aromatic rings. Extraction recovery of all compounds was between 92 and 99%. These results indicate that the proposed method using a portable sampler was suitable for environmental application, obtaining limits close to the ones presented in the literature for the quantification of PAHs using large-volume active samplers. Summed to that, the proposed method allowed simultaneous identification of other compounds. The developed method was applied to Environmental contamination diagnostics in River dos Sinos basin, where several compounds were detected and quantified in most affected areas.

D.04

METHOD NIOSH 2549: THERMAL DESORBER TECHNOLOGY FOR HEALTH SAFETY

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Occupational Safety and Health (OSH) is an area concerned with protecting the safety, health and welfare of people engaged in work or employment. The goals of occupational safety and health programs include fostering a safe and healthy work environment. The National Institute for Occupational Safety and Health (NIOSH) is the U.S. Federal Agency for the prevention of Workplace Illnesses and Injuries.
The aim of this work is the screening of the volatile compounds according to the NIOSH 2549 method from NMAM (NIOSH Manual of Analytical Methods). This study was carried out using a system composed by two stage thermal desorber - gas chromatograph coupled with time of flight mass spectrometer (TD- GC/TOF MS).
The analytical performance has been investigated.
At first, a certain volume of sample is concentrated in a sorbent tube. This tube is heated.
The volatile compounds are then desorbed by a flow of inert gas and transferred to a narrow-bore packed trap. This last one is filled with one or more sorbent material and is kept at a low temperature. Once the refocusing step is completed, the trap is instantaneously heated providing a rapid analyte transfer into the GC-TOF MS system. Data will be reported including chromatographic parameters for VOCs and qualitative determinations.

D.05

NEWLY DESIGNED NEEDLE TRAP DEVICE FOR THE ANALYSIS OF ORGANIC COMPOUNDS WITH A WIDE RANGE OF VOLATILITY

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Sample preparation is a crucial step in the analysis of volatile organic compounds (VOCs) in exhaled breath samples. Concentrations of substances of interest, such as alkanes are as low as ppt-ppb in the samples. GC-based methods cannot be applied for quantitative analysis without sample preconcentration. In the last decades a large number of preconcentration techniques have been developed. Inside needle capillary adsorption trap (INCAT) technique is one of them.

The aim of this work was to develop a preconcentration technique, which can be used for the analysis of VOCs with different volatility in exhaled breath samples. For this reason a multibed sorbent INCAT device was developed for the sampling, preconcentration and injection of breath volatiles into the gas chromatograph. We mainly focused on the analysis of n-alkanes and monomethylalkanes as they are considered as markers of various diseases.

Acknoledgements

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D.06

REDUCED SAMPLE PREPARATION FOR FUMIGANTS RESIDUES ANALYSIS IN GRAINS

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Modern food industry heavily rely on chemicals to prevent and control stored product insect pests and assure food hygiene and security for worldwide trading. Fumigation process is therefore necessary to disinfest commodities stored in large quantity. For this purpose mainly phosphine and methyl bromide are used on large scale as fumigants, with different food exposure conditions and suitable quarantine period to minimize residues amount. In this respect, safety concerns strongly demand for high quality food contaminants free, especially pesticides and insecticides residues. This paper is focused on the optimization of a GC-TOFMS method for unequivocal quantitative determination of fumigant residues in grains, with more attention to the extraction technique, aiming to reduce the sample preparation step. The Static Headspace sampling technique has been used for phosphine determination in contaminated white rice samples.

The external standard calibration has been used to quantify the residue of PH3, preparing the standard mixtures by hydrolysis reaction of Aluminium Phosphide. A different calibration approach have been also evaluated by Multiple Headspace Extraction (MHE) with four subsequent injections, simplifying the overall workflow. The method was shown to be fast, free of interference and complying to the CODEX MRL guideline. Quantitation done with the MHE and the External Standard approaches showed consistent results, indicating the MHE as a useful and easy calibration method any time no standard material is available.

D.07

HARMFUL SUBSTANCES SCREENING IN TEXTILES BY SOLVENTLESS EXTRACTION AND FAST GC-TOFMS

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Textiles make up a very broad category of products and consumers, including children, are directly or indirectly exposed to the products' chemical content. Textile manufacturing makes use of a diverse range of process and finishing chemicals, some of which have intrinsic hazardous properties. Some of these chemicals are harmful to human health and the environment and, for example, cause allergic reactions or are persistent or bioaccumulating. Today there is no unified legislation at the EU level covering the wide range of hazardous chemicals that may be present in textile products. There are, however, a number of voluntary labels and restrictions lists used by the industry to limit chemical content in textiles.

Different classes of chemicals are of concern including perfluorinated compounds, phthalates, heavy metals, flame retardants, isocyanates, organic tin compounds, antibacterial substances, free arylamines from disperse dyes, allergenic disperse dyes. Also a few other organic compounds, such as formaldehyde, nonylphenol ethoxylate, 2-ethylhexanoic acid, urea, various glycols, dimethyl pyridines, aliphatic hydrocarbons can be found in textiles.

Purpose of this work is to investigate fast analytical methods for harmful volatiles and semivolatiles organic compounds in different type of textiles by using solventless extraction techniques as dynamic headspace and thermal desorption, coupled to fast GC-TOFMS. Green and affordable untargeted fast screening is achievable for different classes of compounds, thanks to the mass range information, sensitivity and deconvolution capability assured by the high-speed Time of Flight technology.

DETECTION AND IDENTIFICATION OF 3, 4-METHYLENEDIOXYMETHCATHINONE (METHYLONE) USING GC-MS, LC-(TOF) MS, ATR-FTIR AND FT-RAMAN IN THE FORENSIC SCIENCE LABORATORY IN SOUTH AFRICA

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3,4-Methylenedioxymethcathinone (MDMC), known as methylone, is a new analog of methcathinone, which is found also in the group of central nervous system stimulant. Street samples consisting of capsules seized by the South African police were submitted to the Forensic Science Laboratory (FSL) for further analysis. In order to detect these samples, the following techniques such as Gas chromatography-mass spectrometry (GC-MS), Liquid chromatography coupled with (time-of-flight) mass spectrometry (LC-(TOF)-MS), Attenuated total reflectance-Fourier transform infrared spectrometry (ATR-FTIR) and Fourier transform Raman (FT-Raman) were used to identify the samples. The study of the mass spectra of the samples and its acetyl derivative were indication of positive Methylone (3,4-Methylenedioxymethcathinone). The literature survey was used as a reference material.

E.02

MINIATURIZED SAMPLE PREPARATION OF 20µL BLOOD FOR PERSISTENT ORGANIC POLLUTANT ANALYSIS

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Humans all over the world are exposed to chemicals during their life time. Among the thousands of existing anthropogenic compounds are the persistent organic pollutants (POPs). This class of compounds includes polychlorodibenzo-p-dioxins/furans (PCDD/Fs), polychlorobiphenyls (PCBs), organochlorine pesticides (OCPs), and halogenated flame retardants (HFRs). Human biomonitoring of some of those toxic molecules is nowadays typically performed on relatively large samples (5-100mL blood) requiring uncomfortable and badly perceived venipunction for patients. Analysis on 20µL of blood could, by contrast, be considered as non-invasive since it simply consists in pricking the heel or finger to sample a few drops of blood from patients. However, such small biological samples, either in the liquid form or dried on filter paper (dried-blood spots (DBS)) require the development of specific miniaturized methods for reliable analysis at very low level.

We developed a method for the quantification of persistent organic pollutants (POPs), in the context of the Stockholm Convention, by miniaturized solid phase extraction using MEPS (micro-extraction by packed sorbent) and GC-MS/MS. Samples consist in 20µL of liquid serum as well as 20µL dried-blood spots. The study aims to push automation and miniaturization to its limits; indeed, a maximum of 150µL of solvents are needed for the whole procedure. Results were compared to those obtained with classical analysis procedure including GC-HRMS methodology.

E.03

SAFELY DETECT HYDROGEN LEAKS IN GAS CHROMATOGRAPHY SYSTEMS

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Hydrogen is an ideal alternative to helium as a carrier gas. Hydrogen can be used in many GC and GC-MS applications, it provides a higher efficiency, and, in most cases, separations are faster compared to other carrier gases such as helium.
Additionally, considering the shortages and ever-increasing costs for helium, hydrogen offers some desirable economic and logistical advantages. Unfortunately, the use of hydrogen as a carrier gas could also be dangerous as hydrogen is an explosive gas. Leaks of hydrogen could occur in a GC oven due to a broken column or bad connection, which might result in an explosion inside the GC oven.
This presentation highlights a multi-sensor that safely detects hydrogen leaks in GC systems. Next to the hydrogen leak detection the multi-sensor also detects Hydrogen, Temperature, Barometric Pressure and Liquid weight.
The hydrogen sensor continuously monitors the hydrogen concentration in the oven air. When the hydrogen concentration reaches the user defined hydrogen concentration of typically 25% Lower Explosive Limit (LEL), the controller will alarm the laboratory with acoustic and optical alarms and will automatically switch to an inert gas. The detection range of the hydrogen sensor is 0 – 50% LEL.

E.04

ANALYSIS OF SULFONAMIDE DERIVATIVE HOMOLOGOUS OF DODECYLBENZENE BY GAS CHROMATOGRAPHY / MASS SPECTROMETRY

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Anionic surfactants with sulfonates group are important additives in many industrial process and products. Photochemical sulfochlorination is an efficient method to transform alkanes to alkanesulfonyl chlorides which lead to the corresponding alkanesulfonates by hydrolysis[1].
This process has been used for the first time on dodecylbenzene as substrate. As the sulfochlorination reaction is not selective, all position on the alkyl chain may be obtained. Efficient separation methods, like G.C in combination with sensitive detection like EI/MS are used for the analysis of this complex mixture containing the substrate and the sulphonyl derivatives [2-3]. The major advantages of these analytical methods are their ability to separate various homologous derivatives of sulfonyl chlorides after their derivatization into sulfonamides which are thermally more stable. The mass spectrum of these the sulphonamide derivatives show strong similarities, and allow to establish their composition.

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E.05

RECENT PLOT COLUMN TECHNOLOGY DEVELOPMENT ENHANCES OPERATION WITH INTEGRATED PARTICLE TRAPPING

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Porous Layer Open Tubular (PLOT) GC columns are widely used in petrochemical and light gas applications. Many applications utilize multiple GC columns with switching valves to back flush or heart-cut sample matrix or analytes. Columns of different selectivity are used to enhance resolution and optimize operation.

Compared to Wall Coated Open Tubular (WCOT) GC columns, PLOT columns are subject to stationary phase shedding. Even with the latest in bonded PLOT column technology, care must be taken when using these columns as vibration, reverse flow and pressure changes can cause particles of stationary phases to dislodge from the column wall. These particles will then flow downstream into switching valves rotors, Capillary Flow Technology (CFT) devices and detectors causing detector spikes, damage to switching valve rotors and in severe cases can cause flow restriction. Any of these instances require system maintenance.

New development in PLOT columns with integrated particle trapping technology addresses these issues. Incorporating particle trapping capability on both ends of the column is ideal for valved applications, including back flush, and eliminates the need for attaching separate particle traps (via unions) and in-line filters to protect valve components and detectors for improved instrument uptime. This technology even expands PLOT column use for routine use with mass spec detectors. The technology enhancement is demonstrated on porous polymer, alumina and molecular sieve PLOT columns. GC/MS applications using integrated particle trap PLOT columns such as gas analysis and trace oxygenates in mixed C4 streams analysis are presented.

E.06

RECENT DEVELOPMENT IN WALL COATED OPEN TUBULAR COLUMN FOR ANALYSIS OF SULFUR COMPOUNDS USING SCD

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The analysis of sulfur containing components in different petroleum feeds and products is a true analytical challenge. Sulfur compounds are highly reactive and have adsorptive and metal catalytic properties. Furthermore, they can occur in very complex matrices and often in widely varying concentrations. Therefore, analysis of sulfur compounds requires the sample pathways including column to be inert to ensure reliable results.

Gas chromatography with Sulfur Chemiluminescence detection (GC/SCD) has become the technique of choice to identify and quantify various sulfur compounds in different petroleum samples. A well-known problem when using SCD is the effect of column bleed. This contributes to detector overloading in which hydrocarbons/aromatics as well as components of stationary phase bleed exiting the column are reduced in the hydrogen rich atmosphere which “coke” SCD reaction tubes in the burner. This inadvertent passivation of the reaction tubes in turn causes a rapid decline in the SCD sensitivity. The only solution is to replace the reaction tube, which results in instrument down time and increased cost of operation.

A recent development in wall coated open tubular columns with low bleed and exceptional inertness to sulfur compounds enables separation of a broad range of the active solutes at even low concentrations. Evolving column technology allows for not only analyzing light sulfur gases, but also extending to sulfur containing hydrocarbons out to C20. Good resolution and peak shape can be achieved. Performance for SCD demonstrates minimal column contribution to reaction tube coking which results in improved system ruggedness and greatly reduced downtime for detector maintenance.

E.07

AUTHENTICATION OF PINEAPPLE (ANANAS COMOSUS (L.) MERR.) ACCORDING TO THEIR FRUIT MATURITY STAGES BY QUANTITATIVE CHIROSPECIFIC ANALYSIS OF γ- AND δ-LACTONES

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In 2011, about 1.48 Mio. tons of fresh pineapples representing an estimated value of more than one billion Euros were imported to the EU [1]. The major proportion of pineapples is harvested at a premature green-ripe stage, and exported by cargo ships to be marketed in the lower priced segment. In contrast, due to their limited shelf-life, fully ripe fruits offered as a premium niche product are to be exported by rapid air-freight [2].

Using the stable isotope dilution assay (SIDA), HS-SPME-GC-MS (SIM) allowed quantitative chirospecific analyses of 5-alkylpentanolides (δ-lactones: δ-C8, δ-C10) and 4-alkylbutanolides (γ-lactones: γ-C6, γ-C8, γ-C10) in three progressing post-harvest stages of green-ripe sea-freighted pineapples (SF) and fully ripe air-freighted fruits (AF), respectively. Pineapples were analyzed upon arrival in Europe and after one and two weeks of post-harvest maturation, respectively, thus mimicking storage at retail and end-consumer households.

In all samples, the prevailing lactone was γ-C6. In particular, the amounts determined in AF-fruits clearly exceeded the values of SF-pineapples throughout their entire maturation period. All post-harvest stages of AF-pineapples were characterized by γ-C6 of high enantiomeric purity (85-91% (R)). Whilst SF-fruits when reaching Europe exhibited a comparable enantiomeric distribution (82% (R)), a significant decrease was observed during the subsequent post-harvest maturation (66-74% (R)). Regarding SF-pineapples, interestingly, an opposite trend was observed in the case of δ-C8, since its enantiomeric purity increased from 61% (R) to 79% (R). The results suggest different biogenetical pathways or different precursors involved in the aromagenesis of pineapple lactones. Biogenetical backgrounds and the potential of chirospecific lactone analysis for authentication of fresh pineapples are discussed.

Quantitative chirospecific analysis of γ-C6 allowed the distinction between different post-harvest maturity stages of sea-freighted pineapples and air-freighted premium fruits, respectively, hence being an appropriate tool for the authentication of pineapples according to their post-harvest procedures and fruit logistics.

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E.08

ANALYTICAL COMPARISON OF THE DETERMINATION OF BISPHENOL A IN PAPER FOR FOOD PACKAGING

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Bisphenol A [2,2-bis(4-hydroxyohenyl)propan], a potential endocrine disrupting chemical with a weak oestrogenic acitvity is used in a variety of of consumer products, including paper products to be in concontact with foodstuff and hygyienic articles [1]. Because of a variety of adverse health outcomes the European Food Safety Authority (EFSA) established a Total Daily Intake (TDI) of 0.05 mg kg^{-1} body weight (bw) [2], recently reduced to a temporary (t-) TDI of 0.005 mg kg^{-1} [3]. The main source of BPA in paper products are thermal papers, where BPA is used as a color developer. Hence the use of recycled paper in food packaging is a potential risk, because the European Commission estimates that ~30% of thermal papers enter the recycling stream [4]. Additional sources of BPA in paper products are softening agents in printing inks, resins and laquers. The main risk for consumers is due to migration of BPA from packaging to food, therefor the EFSA authorized a specific migration limit of 0.6 mg kg^{-1} food [5]. The analytical determination of BPA at low concentration levels is mainly performed by liquid-, or gaschromatography coupled to mass spectrometry (LC/MS, GC/MS), or tandem mass spectrometry (LC/MS/MS, GC/MS/MS). LC/MS enables a direct analysis, whereas GC/MS requires a derivatization, but is characterized by an increased selectivity, separability and sensitivity. The aim of the present work was to evaluate different methods to determine BPA in extracts of paper samples used for food packaging by different gaschromatographic analysis techniques (EI-GC/MS and NCI-GC/MS).

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E.09

IMMOBILIZED 3-(PERFLUOROALKANOYL)-(1 R)-CAMPHORATE COMPLEXES FOR ENANTIOSELECTIVE COMPLEXATION AND ON-COLUMN REACTION GAS CHROMATOGRAPHY

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The broad range of applications for metal-alkanoyl-camphorates currently extends from chiral selectors in enantioselective chromatography, over catalysts in asymmetric synthesis to chiral shift reagents in NMR spectroscopy, demonstrating the importance of this substance class.[1] In regard to the area of enantioselective complexation and on-column Reaction Gas Chromatography (ocRGC) we chose the metals nickel(II) and gadolinium(III) to extend the knowledge and the field of application of this derivatives.[2] OcRGC combines catalytic activity with separation selectivity in a gas chromatographic reactor in one step.[3] One of its major advantages is, that in a single run reaction parameters such as conversions and reaction rates are obtained, making time-consuming reaction progress analysis superfluous. Therefore we focused on the Danishefsky-hetero-Diels-Alder-reaction, which is as higher order reaction still a rarity in the ocRGC. In the field of enantioselective chromatography we focused on the perfluorinated substituend (R_F) and the chiral metal selector concentration on polysiloxane (n) by examining their separation abilities of various enantiomers.

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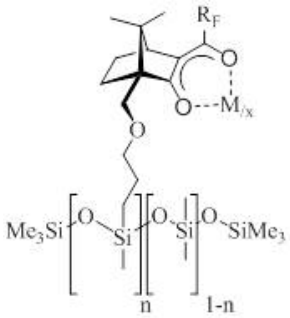

E.10

IMPROVED IMMOBILISED CATALYTIC SYSTEMS FOR APPLICATION IN FLOW-THROUGH DEVICES

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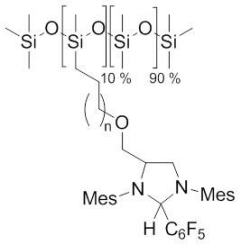
Polymer bonded catalysts and their use in heterogeneous catalysis are well known. One of the advantages of these systems is the easy removal of the catalyst from the reaction solution, which prevents undesirable side reactions like the decomposition of the products and it allows recycling of the catalyst. [1] Here we present the concept of a polymer bonded NHC-precursor (cf. adjacent structure), which is permanently immobilised onto a fused silica micro capillary and therefore suitable for application in on-column Reaction Gas Chromatography (ocRGC). [2] This concept of an integration of catalysis and selective separation in a chromatographic micro reactor system offers the opportunity of a reliable reaction screening under constant reaction conditions. [3] The synthesised and immobilised ligand system was used to investigate the influence of the spacer length systematically (n = 1, 4, 6) to ensure a sufficient flexibility of the ligand by reduced interactions with the polymer backbone. The introduced ligand system can be converted into various immobilised catalysts by using different metal precursor, thus offering an easy access to high throughput reaction screenings in a micro capillary reactor.



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E.11

HOW TO RECOGNIZE AND ELIMINATE GHOST PEAKS IN GAS CHROMATOGRAPHY

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The chromatogram is like a fingerprint. If you can read the chromatogram by looking at peak shapes, retention, base line and by comparing with “normal” situation, you have a good chance to solve problems and improve the analysis.
A ghost peak is a peak that is showing up, but is not supposed to be there. Sometimes it is referred as a “system” peak. Ghost peaks can be created in many ways. It’s a component that is added/created somewhere in the system, it is injected/trapped/focused onto the column, and will elute. Problems will escalate if a ghost peak interferes with an analyte that has to bequantified.
Sources for ghost peaks can be sample vials, gloves, syringes, reagents, carrier gas, tubing, the injection port, operation, memory effects and even the column-phase itself.
In this poster the most prominent contributions to ghost peaks will be discussed by showing practical examples and how to check for.

E.12

MESOGENIC GC CAPILLARY COLUMNS: EFFECT OF LATERALLY ALKYL SUBSTITUTE ON VANT'HOFF PLOTS AND ANALYTICAL BEHAVIOUR

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Separations on mesogenic stationary phases are based upon differences in molecular shape (length-to-breath ratio and planarity) independently on any specific energetic interactions. In this study, a homologues series of three nematic compounds termed in this work L1, L3 and L4 were studied. Thermal and analytical properties of the three GC capillary columns were determined. Characterization of the liquid crystals phases was made with proton NMR, and the nematic states were determined by DSC.

The influence of the lateral substituent on the thermal and gas chromatographic properties has been examined. The three monomeric liquid crystals have the same core structure but differ by their lateral substituent. The first goal of this study was to determine and compare the phase transitions of each LCSP by analyzing Van't Hoff plots for selected solutes probes according to the following relation: $\ln k = -H/RT + S/R + \ln\phi$. Comparison between results of transition temperature obtained by DSC and RGC were compared. A great according was found.

Analytical results were obtained by separation of homologous Ketones and alkanes then aromatics isomers. Interesting features were observed with the three columns for the chromatography of isomers with respect to the shape selectivity and polarity.

E.13

COMBINATION QUECHERS, DLLME AND CAPILLARY GAS CHROMATOGRAPHY FOR DETERMINATION OF TRICHOTHECENE MYCOTOXINS

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Trichothecenes are one of the major classes of mycotoxins, that contaminate cereal and grain crops. These mycotoxins are produced by a of different fungi and include Fusarium, Myrothecium, Spicellum, Stachybotrys, Cephalosporium, Trichoderma, and Trichothecium. Trichothecene mycotoxins are chemically belonging to the sesquiterpenoids. This group includes more than 40 different toxins.

Analytical method have been developed for the determination of 11 trichothecene mycotoxins in cereal and feed. It are T-2 toxin, T-2 triol, T-2 tetraol, HT-2 toxin, deoxynivalenol (DON), nivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, fusarenon X, diacetoxyscirpenol, neosalaniol.

QuEChERS and dispersive liquid-liquid microextraction (DLLME) was used for extraction and purification of samples. Mycotoxins were extracted from cereal and feed samples by using water and acetonitrile with added salts ($Na_2HC_6H_5O_7$. $1,5H_2O$, $Na_3C_6H_5O_7$. $2H_2O$, $MgSO_4$, NaCl). Clean up carried out with using sorbents C18 and PSA. The extract was carried out DLLME step. Different parameters of DLLME have been investigated and optimized for mycotoxins.

The samples were analyzed by gas chromatography with electron-capture detector. Chromatographic separations were performed on a capillary column with 30 m, an internal diameter of 0.32 mm and 0.25 μm film thickness. Pre-column derivatization with pentafluoropropionic anhydride with adding sodium bicarbonate was used for trichotecene mycotoxins. The detection limits were 20 mkg/kg for DON, for 3-acetyldeoxynivalenol, for 15-acetyldeoxynivalenol, for fusarenon X, 15 mkg/kg for DAS, for T-2 toxin, for nivalenol and 10 mkg/kg for HT-2, T-2 triol, T-2 tetraol, neosalaniol. Satisfactory recoveries were obtained 60–90% and precision (expressed as relative standard deviation) was 10%. Duration of the analysis was 1.0 – 1.5 h.

E.14

ON-SITE RAPID ANALYSIS OF WELL GASES FOR MUD LOGGING APPLICATIONS USING MICRO GAS CHROMATOGRAPHY

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Oil and gas exploration require the analysis of dissolved natural gas in mud samples from the well within short run times. This posters highlights the use of an Micro Gas Chromatograph for rapid, accurate mud logging analysis.
A system equipped with multiple analytical channels is used for on-line analytical testing of drilling fluid sample. Each column channel is a complete, miniaturized GC containing an electronic carrier gas control, micro-machined injector, narrow-bore analytical column and micro thermal conductivity detector (μTCD).
Gas chromatography is proven to be an accurate and sensitive technique for the characterization of individual hydrocarbon gases to combine in lithology reports for the mud logging field. Critical information is obtained for making decisions on additional drilling or production of the well. A portable field case, equipped with rechargeable batteries and carrier gas, and the instruments small form factor allows it to be transported easily to an oil rig. Moreover, its low consumption of power and operating gases facilitates integration in on-site control cabins or explosion-proof housings.

E.15

UTILIZATION OF HYDROGEN CARRIER GAS FOR HIGH THROUGHPUT GAS CHROMATOGRAPHY - HIGH RESOLUTION TIME OF FLIGHT MASS SPECTROMETRY (GC-HRT): APPLICATION COMPENDIUM

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Both the costs associated with a dwindling helium supply and the need for high sample throughput have fueled the desire to develop fast gas chromatography methods using hydrogen as a carrier gas. This poster will demonstrate the ability to utilize hydrogen carrier gas on a gas chromatograph with high resolution time of flight mass spectrometer (GC-HRT). Methods achieving fast separations for multiple application markets including, but not limited to, metabolomics, forensics, and specialty chemicals will be displayed. Derivatized NIST Human Plasma was used to develop a high speed GC-HRT method for the metabolomics application market. Various drugs of abuse including synthetic cannabinoids, cathinones, psychotropic substances such as psilocin and psilocybin were used to demonstrate capabilities in the forensic market. Polymer additives were used to represent the specialty chemical market.

Fast GC-HRT methods were developed using hydrogen carrier gas and relatively short, narrow bore GC columns (10m x 0.18mm x 0.2 micron film). Methods were developed for rapid separation of representative metabolomic, forensic, and specialty chemical markets.

NIST plasma extracts were derivatized prior to GC-HRT analysis using an optimized two-step procedure: 1) Treatment with methoxylamine hydrochloride and 2) MSTFA.

Drugs of abuse were dissolved in organic solvents and analyzed both underivatized and derivatized.

Polymer additives samples were prepared by extracting 1 gram of ground polymer material with 10 mL of dichloromethane. Extracts were filtered prior to analysis.

E.16

BACK-TRACING AN EMERGING ENVIRONMENTAL TOXICANT (HEXABROMOCYCLODODECANE, HBCD) IN ANIMAL-DERIVED FOOD CHAIN BASED ON FOODOMICS

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Human activity is the main cause of the emission of pollutants which may accumulate in environmental compartments, then in livestock tissues and subsequently in the food chain. The toxic environmental micropollutants possibly transferred to animal products are listed in the frame of the Stockholm Convention. These chemicals are particularly monitored in food products and include brominated flame retardants. Among brominated flame retardants, hexabromocyclododecane (HBCD) is a chemical of potential concern currently proposed for listing. HBCD is an emerging toxic micropollutant found in the environment and in animal tissues. Direct HBCD quantification is extremely difficult because it undergoes a rapid metabolism in biota. Based on a previous report showing the relevance of volatile compound metabolic signature in chicken liver for back-tracing a dietary exposure to rapidly metabolized xenobiotics[1], the present study investigates the relevance of this approach to evidence a previous HBCD contamination in laying hens. Three groups of laying hens (n=56) were fed a similar feed either non contaminated (control group) or contaminated during 71 days with either 0.1 µg.g^{-1} or 10 µg.g^{-1} HBCD. Animals were periodically slaughtered throughout the experiment and their liver was excised. Solid phase microextraction – gas chromatography-mass spectrometry (SPME–GC-MS) was used to determine the liver content in volatile compounds. After correcting the data from instrumental drifts by normalization methods, the use of volatile compounds enabled the differentiation of samples according to contamination level of animals. For a same contamination level, a discrimination of the samples according to exposure duration was also observed. This discrimination is improved when animals are exposed to the highest HBCD dose. The volatile compound metabolic signatures in poultry liver seem to be relevant in order to back-trace an exposure to HBCD in laying hens. In order to validate the metabolomic approach and to enable its use in the field conditions, further investigations are undertaken to determine the biochemical pathways responsible for the changes in the levels of these volatile biomarkers.

Acknowledgement

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References

E.17

DEVELOPMENT AND VALIDATION OF METHOD TO QUANTIFICATION OF NITROGEN COMPOUNDS IN DIESEL OIL BY GC/BID

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Nitrogen compounds naturally occur in petroleum samples and their derivative products. In diesel oil the nitrogen compounds can be separated into basic and neutral compounds. Pyridines, quinolines and benzoquinolines have been recognized as major classes of basic contaminants, whereas indoles and carbazoles are regarded as neutral contaminants. These substances contribute to the environmental contamination and their control is necessary because most of them are potentially carcinogenic and mutagenic. Although several methods for the analysis of nitrogen compounds in diesel oil have been described the demands for higher sensitivity and trace level analyses have increased in recent years. Therefore, searching high speed and sensitivity, a method was developed and validated for the quantification using a standard solution of ten nitrogen compounds in a new instrument, the Tracera® by Shimadzu, a gas chromatography equipped with the barrier discharge ionization detector (BID) a new detector capable of the high sensitivity detection of organic compounds. In this work the developed method was applied for the analysis of a diesel oil sample. The analytical methodology showed a high specificity and sensitivity moreover adequate precision and accuracy, as well as limit of quantification and limit of detection. The instrument showed a linear response to concentration interval in the nitrogen compounds standard. The developed method for nitrogen compounds quantification in diesel oil showed satisfactory results to the available parameters, important to the chromatographic analyzes in environment studies.

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E.18

SINGLE FAST METHOD FOR THE IDENTIFICATION AND QUANTIFICATION OF MOST COMMON DRUGS, THEIR METABOLITES AND ADULTERANTS IN BIOLOGICAL MATRICES USING GC-TOF

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The Drug abuse is the use of illegal substance or the misuse of prescription or over-the-counter drugs for at least a year with negative consequences.
Throughout the world, illicit drug use appears to be generally stable, though it continues to be rising in several developing countries. Drug abusers number is about 27 million, which is 0.6 per cent of the world adult population, but about 230 million people, or 5 per cent of the world's adult population, are estimated to have used an illicit drug at least once in 2010 (World Drug Report 2012, UNODC).
As consequence, drug screening technology has improved greatly, in order to provide better and faster quantification in biological matrices.
The aim of this work was to develop a complete analytical method using a fast Gas Chromatograph coupled with a Mass Spectrometer with Time Of Flight technology (TOF MS). This system allows a unique fast analysis (less than 8 minutes) of the most common and used drugs (Cannabis, opioids, opiates and Cocaine), their metabolites and their adulterants from biological and autopsy samples with outstanding linearity, sensibility and repeatability. With TOF MS, thanks to the full acquisition range, it's possible to identify both known and unknown compounds in complex matrix (Plasma and Urine) at very low concentrations, increasing the information about the real samples.

E.19

GC-MS ANALYSIS OF CONJUGATED LINOLEIC ACID ISOMERS IN MILK SAMPLES AND THE CHEMOMETRIC DECONVOLUTION OF THE UNRESOLVED TRIPLET

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Conjugated linoleic acid (CLA) isomers are reported to have anticarcinogenic, anti-atherogenic, anti-diabetic properties and they also improve the immune system, bone metabolism and body composition. Recent reports suggest that each conjugated fatty acid (FA) isomer has different physiological functions. The understanding of the biological role of these acids relies on their proper separation, identification and quantification in complex biological extracts.
A generally known problem of GC separation of *trans-7*,*cis-9*; *cis-9*,*trans-11* and *trans-8*, *cis-10* CLA isomers was studied by GC–MS on a 100 m capillary column coated with cyanopropyl silicone phase at isothermal column temperatures in a range of 140–170 °C. The resolution of these CLA isomers obtained at given conditions was not high enough for direct quantitative analysis, but it was, however, sufficient for the determination of their peak areas by commercial deconvolution software. Developed deconvolution procedure allowed the determination of the content of studied CLA isomers in ewes' and cows' milk samples, where dominant isomer *cis-9*,*trans-11* eluted between two small isomers *trans-7*,*cis-9* and *trans-8*,*cis-10* (in the ratio up to 1:100).

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E.20

COMPARISON OF TWO MATHEMATICAL METHODS FOR DETERMINATION OF RETENTION INDICES OF POLYCYCLIC AROMATIC HYDROCARBONS ON DIFFERENT STATIONARY PHASES IN GPC

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Various organic pollutants are analyzed by CPG on several capillary columns out of glass by polymer impregnation of the polysiloxane type OV. The purpose of the use of a binary packing material is to improve separation of alcohols, of aromatic compounds and their isomers. The retention index standards have been determined by the application of two mathematical adaptation methods, a multiparametric least-squares regression iterative method based on the determination of the adjusted retention times and a local cubic interpolation method directly using the total retention times.The polynomial interpolation method of Lagrange is very useful when the required purpose is the identification of samples with the RIS of KOVATS because it saves much time in the GC analytical use and there is no need for adjusted retention data. So, it eliminates the problem of the determination of the column dead time. Nevertheless, the measured gross retention data of the sample must be located within the field of the reference curve limited by those of four n-alkanes used as reference basic for the interpolation. Any extrapolation out this field leads to wrong results. Contrary to statistical methods, the standard deviations on parameters cannot be calculated by this very method.

E.21

CAN THE INJECTION MODE INFLUENCE THE EDIBLE OIL MINOR COMPONENTS ANALYSIS?

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The minor compounds fraction of food, despite its low amount (1-3%), can be used for indication of genuineness, especially in olive oil. This fraction includes long and short chains alcohol esters (waxes), sterols (free and esterified), esters, terpenic alcohol esters, and fatty acid derivatives, and it is considered the fingerprint of different oils. In the case of olive oils, waxes and alkyl esters, official parameters and limits are applied, so a poor measurement can lead to a misleading classification of the oil. Usually they are analyzed by capillary gas chromatography (GC) equipped with flame ionization detector (FID) for quantification purposes. However, the injection technique is the most critical step, since discrimination and thermal degradation can occur to a different extent. For this reason, the on-column injection (OCI) is the technique of choice for this kind of applications, since the sample is deposited directly into the column at the starting oven temperature, thus avoiding discrimination and limiting degradation. However, other injection techniques, namely split/splitless and programmed-temperature vaporizer (PTV) are much more versatile and thus more widespread. The aim of the present work was to compare the performance of three injection modes to analyze alkyl esters, waxes, free and esterified sterols on edible oils. Discrimination among high boiling compounds was evaluated, as well as the occurrence of degradation, especially of sterols and diterpene alcohols, phytol and geranylgeraniol, ester compounds.

E.22

ANALYSIS OF ESSENTIAL OILS OF PISTACIA TEREBINTHUS L. BY GC AND GC/MS

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Pistacia terebinthus L. is small Mediterranean trees from 2 to 6m high with pronounced resinous smell. Several studies have shown that all parts of the tree have therapeutic properties.
After the hydrodistillation of the aerial part of *P. terebinthus* (fruit and leaves), analysis of the oils were carried out on GC and GC/MS by apolar and polar capillary columns. Samples were characterized by the olefinic terpenes (70.0-91.8%). The major compounds were a-phellandrene (33.5%, 45.6%), a-pinene (14.2%, 24.5%), p-cymene (8.0%, 4.2%) and limonene (5.5%, 6.6%) respectively for leaves and fruits. The determination of amounts of limonene and β-phellandrene was made possible thanks to the separation of these two compounds on the polar column BP-20.

E.23

QUANTITATIVE ANALYSIS OF BIO-OIL OF SUGARCANE STRAW BY GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY QUADRUPOLE

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Bio-oil is a very complex mixture containing many organic compounds formed by thermal degradation of cellulose, hemicellulose, lignin and other compounds with high polarity. The main classes of compounds are aldehydes, phenols, ketones and furanones, with potential use raw material in industry. Therefore, it is necessary to know the exact concentration of these compounds in bio-oil for your application as well as for its control because they are highly reactive, unstable and environmentally important. One difficulty regarding the characterization and quantification of bio-oils is the complexity of its composition, which can be reduced by fractionation procedure, generating less complex fractions with different chemical composition. A possible alternative is to use the fractionation system which drags the components by similarity, using a pressurized solvent with high pressure in increasing polarity. This procedure facilitates the quantification analysis of compounds by gas chromatography with mass spectrometry detector in the mode selected ion monitoring (GC/qMS mode SIM).This work performed the validation of chromatography method for reference standards of the chemical classes of phenols, aldehydes and ketones in a sample of bio-oil of sugarcane straw pyrolysis and its two fractions obtained with high polarity solvent, A (obtained with acetone) and B (obtained with methanol). The compounds that showed higher concentration in the three studied samples were 2-methylphenol, 3,4-dimethylphenol, catechol, 4-methylcatechol and syringaldehyde. The fractions A and B exhibited higher concentration of this analytes compared with the original bio-oil. The compounds of fractions with different polarities allowed a better identification and quantification by GC/qMS. The method was effective and the sum of the fractions analyzed, A and B, reflected quite similar profile to bio-oil, formed by polar compounds.

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E.24

AROMA OFFICE: A NOVEL LINEAR RETENTION INDICES DATABASE FOR IMPROVED IDENTIFICATION OF FLAVOUR COMPOUNDS

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The retention time of a compound on a given column phase can be expressed on a scale based on n-alkane retention times. This produces unique retention index values for compounds and serves to standardise gas chromatographic retention data. Temperature programmed GC is used widely in laboratories and values calculated using this approach are denoted as linear retention indices or programmed-temperature retention indices. Retention indices are used widely in the flavour and fragrance field and many published data bases are available. Usually mass spectral information in addition to retention time data is available from a GC run. Either information dimension alone can often be insufficient to provide positive identification and an obvious solution here is to combine the unique attributes of both information dimensions to offer more secure identification possibilities. Modern technology can offer very reproducible retention behaviour and information rich mass spectral patterns are readily available from affordable benchtop instruments. In this regard data bases are available which list the retention index of compounds and their mass spectra in order of elution. Aroma Office (Gerstel K.K.) is an integrated software approach to automatically process retention index and mass spectral data for improved identification of flavour compounds. It contains the most comprehensive data base of flavour compounds available. This is a searchable data base with retention index information on >10,000 compounds from greater than 100,000 entries from a wide range of literature references. The program can be integrated into the Agilent Chemstation software and searches are performed using RI values and the CAS no of a candidate compound after library searching. A manual cross search for a single or limited number of compounds can be performed or an automated cross search can be performed for multiple compounds. Both use a single RI and a mass spectrum for each compound. When the chromatographic analysis is upgraded to two dimensional with heart cutting the software also offers a cross search using the two different retention index values obtained from the orthogonal stationary phases used in the first and second dimension analyses. When GC-O organoleptic evaluation is available from both first and heart cut dimensions then these signals can provide two independent complementary RI values. These can often be sufficient to propose an identification if the MS signal is very weak or even absent. Aroma Office is designed to offer significant additional identification strategies to the practicing flavour analyst.

E.25

FATTY ACIDS COMPOSITION OF THE GRAM-NEGATIVE BACTERIUM RHODOBACTER SPHEROIDES

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Bacteria have evolved mechanisms to control the formation of FAs and modify the structure of existing ones for acclimatization to different environments. These acyl chains determine the viscosity of the membrane, which in turn influences many crucial membrane-associated functions, including the passive permeability of hydrophobic molecules [1]. The variable properties that make an organism's FA composition distinctive include quantitative differences in FA content and the presence of many different acyl chains. The FA composition of *Rhodobacter sphaeroides*, a bacteria belonging to purple bacteria stain, was investigated by gas chromatography-mass spectrometry (GC-MS). *R. sphaeroides* was grown photosynthetically and polar lipids were extracted from cells and chromatophores, according to the Bligh and Dyer standard method [2]. Bacterial fatty acid methyl esters (FAMEs) were prepared by a conventional method of saponification followed by BF_3-catalyzed methylation [3]. Peak attribution was supported by mass spectra, linear retention indices and analysis of standard compounds, when available. The major fatty acids occurring in *R. sphaeroides* were 16:0, 16:1 *cis*-ω7, 18:0, 18:1 *cis*-ω9 (i.e., oleic acid) and 18:1 *cis*-ω7 (i.e., *cis*-vaccenic acid), which was the dominant FA. Along with an unknown FA, other acyl chains, of much lower intensity were 16:1 *trans*-ω7, 16:1 *cis*-ω9, and two 18:1 *trans* isomers, the first in the elution order is most likely an ω10 or 11 and the second one is presumably an ω7 or 8 isomer. The present results are in good agreement with the existing knowledge on the biosynthetic metabolism of the major FAs in *R. sphaeroides*, but the metabolic processes involving minor species are not yet established and deserve further examination.

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E.26

DEVELOPMENT OF A GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR MULTIPLE FLAVOURS QUANTIFICATION

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The chemical study of food and beverage aroma needs nowadays the accurate quantification of an always larger number of flavour compounds from different chemical classes. Generally flavours are analyzed by GC-FID [1] or GC-MS, after a procedure of purification and concentration (SPE, SPME) [2,3]. These two techniques could present low sensibility and selectivity, to avoid this problem, long chromatographic separations were generally proposed with great loss of time [4].
An answer to these problems can be given coupling gaschromatography with a triple quadrupole mass spectrometer (GC-QqQ-MS) configured in multiple reaction monitoring (MRM) mode. Compared to the single quadrupole used in SIM mode, the noise of a triple-quadrupole used in MRM mode is considerably reduced, the sensitivity increased, and the selectivity can be considerably augmented by choosing appropriate transitions. The greater specificity makes possible in many cases to resolve co-eluted peaks even using a less accurate chromatographic separation, and to save time with routine analysis.
Flavour compounds were extracted by adsorption on a SPE cartridge (ENV+) [4], eluted with CH_2Cl_2, concentrated using a Vigreux apparatus and then injected into the GC-MS/MS. The MS/MS parameters, transitions and collision energy, were studied using commercial standards, and their optimization was a compromise between signal intensity and specificity. The developed method permitted ,in wines and spirits, the quantification of 8 acids, 18 alcohols, 39 esters, 13 aldehydes, 10 ketones, 6 phenols, 12 norisoprenoids, 32 terpenols, 11 lactones and 14 sesquiterpens for a total of 163 compounds in only 40 minutes of GC run instead of the classical 90-120 minutes [4]. The number of quantified compounds is definitely higher than many classical routine methods considering also the short time of analysis. The method has shown a satisfactory reproducibility and a good linearity for all the target compounds with R^2 higher than 0.99 for all/almost all compounds. The proposed GC-QqQ-MS method is an important starting point for routine quantitative multiple aroma analysis in complex matrix like wine and spirits because of his rapidness and specificity.

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E.27

DETERMINATION OF FATTY ACID COMPOSITION OF CHEECK CELLS OF TWO HEALTHY AND TWO CYSTIC FIBROSIS CHILDREN

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Cystic fibrosis (**CF**), also known as **mucoviscidosis**, is an autosomal recessive genetic disorder that affects most critically the lungs, and also the pancreas, liver, and intestine. It is characterized by abnormal transport of chloride and sodium across an epithelium, leading to thick, viscous secretions (Wikipedia).
In this research a modified non-invasive analytical procedure was applied for the diagnosis of cystic fibrosis in children by using a validate procedure reported in literature for the analysis of acidic composition starting from of cheek cells that contain the same fatty acid composition found in blood [1,4]. The method was applied in two young CF children versus two healthy young children. Preliminary results pointed out that cystic fibrosis patients show non-normal fatty acids composition in respect of healthy subjects. This non-invasive method can be interesting for diagnose the pathology and the identification of these fatty acids can furnish new details about the typology of sickness. For the treated cases arachidonic and docosahexaenoic acids are present in negligible quantities in both cases and the differences between FC children and healthy children are negligible. Massive differences were highlighted at level of very long fatty acids (VLCA) that were not yet identified.

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E.28

CHROMATOGATE: THE PICTURE GALLERY OF SCIENCE

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SHOW THE BEST CHROMATOGRAMS AND SPECTRA FOR SCIENCE. Show your best chromatograms and spectra with all people in the world and highlight your discovery in Biology, Chemistry, Engineering, Environment, Foods and Medicine.
In this website you can show your scientific discoveries by exclusively publishing chromatograms and spectra, that are results of experimental analysis made on real samples in all areas of science for contribution to scientific discovery divulgation. No matter if chromatograms and spectra are published or not in scientific journals. After the registration you can add your best chromatograms and spectra in a very simple way.
Chromatography (LC and GC) is the most versatile and widespread technique employed in modern analytical chemistry and in the research, therefore this website wants to be the Picture Gallery of Science where research and chromatograms and spectra are linked to a discovery in all areas of science.
Our motto is: “More beautiful the chromatogram or spectra are more probable is the discovery”.

Fig. 1 – Logo for Chromatogate website (www.chromatogate.com).

E.29

FAST GC TOF-MS DETERMINATION OF SYNTHETIC CATHINONES IN DRUG SEIZURES

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Synthetic cathinones have appeared in the European and American recreational drug market since the mid-2000s. Initially they were sold as “legal highs” in the smart shops, but nowadays in Italy they are considered as drugs of abuse.
In fact all synthetic cathinones were inculded in the Table I of DPR 309/90, the Italian law that regulates drugs of abuse, under the name “stucture analogues derived from 2-amino-1-phenyl-propanone with one or more substitutions on the aromatic ring and/or on the nitrogen atom and/or on the terminal carbon atom. Hence the necessity to optimize analytical methods able to unequivocally identify and characterize the chemical structure of these molecules.
A comprehensive analytical method for the analysis of eighteen synthetic cathinones was developed by Fast GC TOF-MS. Compared to conventional GC, the use of shorter narrow bore columns and fast temperature programs allows to maintain equivalent resolution in significantly reduced analysis time, increasing laboratory productivity. The method was successfully applied to the analysis of several products that were seized on the Italian illicit market. This analytical approach was applied for the identification and quantitation of synthetic cathinones also in biological fluids. To determine synthetic cathinones spiked in urine we performed a solid phase extraction and a derivatizzation by trifluoroacetic anhydride.

HPLC ANALYSIS OF SELECTED PRIMARY AND SECONDARY METABOLITES IN NICOTIANA LANGSDORFFII: DETERMINATION OF COMMON MARKERS OF PLANT RESPONSE TO DIFFERENT KIND OF ABIOTIC STRESSES

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The exposure of plants to different kinds of abiotic stress, such as heavy-metal soil contamination, high temperatures or water deficit is a current problem, highly regarded by the scientific community, in view of pollution phenomena and climate changes, worldwide occurring [1].
The study of the stress resistance of genetically transformed plants can give a valuable contribution to a better understanding of response mechanisms to stress and their possible use in remediation strategies of heavy-metal polluted soils and as new cultivations adaptable to adverse climatic conditions.
For this reason, the model plant *N. langsdorffii*, wild type and genetically transformed by inserting of *GR* and *rolC* genes have been studied for testing their resistance towards exposure to heavy metal, water and heat stresses, which cause severe damages to plants.
HPLC analysis of plant primary and secondary metabolites is an essential tool for understanding the complex plant response induced by regulation of phytohormones and activation of metabolites involved in the response to stress [2].
Among plant primary and secondary metabolites, soluble sugars and polyphenols were elsewhere investigated as possible markers of plant response to stress [3-5] and have been therefore included in our studies [2,6]. The results showed an interesting behaviour of these metabolites in all the investigated abiotic stresses and gave important information, often complementary to phenotypic observations. These findings highlighted a key-role of HPLC metabolomic analysis in monitoring the response of wild-type and genetically modified plants to abiotic stress in order to discover a different tolerance of the genotypes investigated.

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F.02

IDENTIFICATION AND QUANTIFICATION OF PHENOLIC COMPOUNDS IN LEAVES OF CYCLANTERA PEDATA (CAIGUA) BY REVERSED PHASE HPLC ON A SEMIMICRO SEPARATION SCALE WITH PHOTODIODE ARRAY AND MASS SPECTROMETRIC DETECTION

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Phenolic compounds are secondary metabolites, which are widely distributed in the plant kingdom and therefore form an integral part of human diet. A growing interest has been addressed to this class of compounds because clinical and epidemiological studies have suggested that the regular consumption of fruits and edible plants might reduce the risk of developing chronic illnesses such as cardiovascular diseases, type-2 diabetes, and certain types of cancer. This communication reports the results of a study carried out to develop a straightforward HPLC method for the identification and quantification of phenolic compounds occurring in the leaves of *Cyclanthera pedata* Scrabs (Caigua). This is an ancient Peruvian edible plant belonging to the Cucurbitaceae family, which have an increasing commercial interest in the phytopharmaceutical market due to its anti-inflammatory, hypoglycaemic and hypocholesterolemic properties. The method employs a narrow-bore C-18 column and a semimicro photodiode array detector (PDA) cell of 2.5 μL, in conjunction with a single quadrupole mass spectrometer, equipped with an electrospray ionization source (ESI-MS). The C-18 narrow bore column is eluted by a multi-segment gradient of increasing concentration of acetonitrile in water-formic acid solution that has been optimized on the basis of the results of a study carried out to evaluate the influence of mobile phase composition and gradient shape on separation performance and detection sensitivity by ESI-MS. The identification of individual phenolic compounds has been performed on the basis of their retention times and both UV-visible and mass spectra, acquired by PDA and ESI-MS, respectively. Different organic solvents, hydro-organic mixtures, and plain water have been tested for their efficiency for extraction of phenolic compounds from leaves of *Cyclanthera pedata* either grown in a green house, under controlled conditions of humidity, temperature and exposure to light, or in an experimental field in Rome geographical area.

F.03

MULTI-DIMENSIONAL PARALLEL CAP-(NANO)-LC/UV/HRMS SYSTEM IN THE PROTEOMIC PROFILING OF BOVINE WHEY PROTEINS AND PEPTIDES

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Aim of the present work is the analysis by multidimensional Cap-(Nano)-LC/UV/HRMS of proteins and peptides from whey fraction (20% of total bovine milk proteins) in commercial milk and the study of its time-dependent degradation to better understand its impact in the health care and food industries. After casein extraction [1], whey fraction was separated from fresh skim milk and from the same milk at week IV after expiry date and then directly injected into the column, without further sample preparation. A parallel multidimensional system was developed for the Cap-(Nano) contemporary separation and identification of major and minor proteins and peptides present in the whey fraction of fresh and expired bovine milk. The analytical system used comprises a double switching valve, two trapping columns (a 5-cm long monolithic column [2] and a 5-cm long packed column with sub-3-μm RP-C18 particles), two corresponding monolithic and packed (≈ 25-cm length) capillary-(nano) columns and finally two dedicated parallel gradient elution modes. During the injection step, the trapping columns were in series connected, with the proteins and high molecular weight peptides being retained on the monolithic support (first precolumn), and the low to medium molecular weight peptides being retained on the packed silica-based support. In the successive elution step, the trapping columns were arranged in parallel. On a first gradient pump line, the mixture of intact proteins was resolved using the two in series monolithic capillary media (pre-column plus column) while, on the second gradient line, the mixture of low-medium molecular weight peptides was contemporarily separated using the home-made set of capillary (or nano) packed pre-column and column. The detection signal outputs from each line were recorded simultaneously choosing, in a first run, the UV detection for peptides and the HRMS detection for the proteins; in a second, otherwise identical run, the reversed combination was used (UV for proteins and HRMS for peptides). The parallel multidimensional approach, both in terms of columns and in terms of detectors, allows to obtain the complete UV/HRMS profile of both intact serum proteins in cow's milk (α-lactalbumin, β-lactoglobulin variants A and B, bovine serum albumin, etc.) and related low-medium molecular weight peptides.

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F.04

ENANTIOSEPARATION OF SOME FLAVANONES BY NANO-LIQUID CHROMATOGRAPHY USING DERIVATIZED -β-CYCLODEXTRIN CHIRAL STATIONARY PHASE

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Recently, miniaturization has been introduced in liquid chromatography reducing both the inner diameter of the columns and the flow rate (200 – 1000 nL/min). Therefore nano liquid chromatography (nano-LC) has been applied mainly for analytical purposes due to the several advantages which can offers over the conventional HPLC such as higher efficiency, shorter analysis time, use of minute volumes of both samples and reagents with consequent lower environmental impact and reduced costs. Furthermore small amount of stationary phase is required for the packing procedure and this is particularly relevant for expensive chiral stationary phases. In this work, the enantioselectivity of phenyl-carbamate-propyl-b-CD silica stationary phase was investigated by using nano-LC for the enantiomeric and diastereoisomeric separation of some selected flavanones, belonging to the class of polyphenols. Such compounds usually occur in fruits and vegetables and it has been reported that they possess health-related properties including anticancer, anti-inflammatory, antiviral activities. Hence, owing to the nature of the CSP's substituents, the enantioselectivity of the phenyl-carbamate-propyl-b-CD CSP was evaluated under reversed, polar organic and normal elution modes. Experiments were carried out using H2O/ACN, 100% of MeOH and EtOH/hexane/TFA as the mobile phase at different flow rates in the range 120-400 nL/min. The effect of the concentration of organic solvent on retention factors, enantioselectivity and enantioresolution of the compounds was investigated. It is worthwhile to remark that a mobile phase based on pure polar organic solvent, without any additives, enables the enantiomeric discrimination of the analysed compounds. The CSP was also successfully used for the diastereoisomeric resolution of hesperidin, naringin, two flavanone glycosides. Although good results were achieved with the use of phenyl-carbamate-propyl-b-CD stationary phase in the nano-LC system , the normal and polar organic phases were not as effective as the reverse phase conditions for the separation of this class of compounds.

F.05

IMPLEMENTATION OF COLUMN SWITCHING ON THE BASIS OF µLC-MS/MS HYPHENATION FOR ROUTINE ANALYSIS

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Miniaturized separation techniques, like µLC, can be an appropriate approach to decrease solvent consumption. Moreover, due to a high linear velocity and a reduced gradient delay volume, the cycle time can be significantly decreased, thus increasing the sample throughput. Therefore, it is important to include an automated column switching device. This is a prerequisite for µLC-MS/MS hyphenation when different columns have to be used. Conventional LC-systems offer these possibilities for a long time. In contrast, for miniaturized separation techniques, nanovolume column switching valves were not commercially available. The implementation for routine analysis depends on the influence of the extra column volume on the resulting chromatography. For this reason, comparison studies concerning efficiency need to be done.
In this study, an analyte mixture was first analyzed without any valve. Afterwards, the system design was extended by two identical valves keeping all connection tubing constant. In this way, the influence of the additional volume of the valves on peak shape can be examined. Furthermore, two valve types with different port to port volume and outer diameter were compared. These experiments were also performed on 300 µm as well as 500 µm inner diameter columns to determine which column diameter is more suitable for this application. The evaluation was carried out using UV- as well as mass spectrometry detection.

F.06

EVALUATION OF A CONCEPT COUPLING FLAME IONIZATION DETECTION WITH NANO-LIQUID CHROMATOGRAPHY

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A still unresolved issue in all areas of life science is the detection and quantification of unwanted by-products during the synthesis of pharmaceutical products. To date, there is no universal detection technique for liquid chromatography available which can be used to detect and quantify all relevant by-products. Hence, a huge variety of different detection techniques are used to determine all impurities during the synthesis of pharmaceutical products. In this context an analytical approach on the basis of the hyphenation of capillary- and nano liquid chromatography and flame ionization detection has been developed and evaluated, which allows for direct as well as universal detection of unknown compounds.
In this presentation, the system setup for coupling nano-liquid chromatography and flame ionization detection will be presented. Moreover, first results as well as current issues related to the use of nano-liquid chromatography will be addressed and discussed.

F.07

USE OF NOVEL PHENYL-HEXYL CORE-SHELL PARTICLES IN NANO-LC

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Nowadays the advantages of using core-shell materials in LC are well-known. They offer a reduced diffusion path length due to the presence of nonporous core not accessible to analytes and exhibit a narrow particle size distribution thanks to the modern technology applied. These features result in higher efficiencies and faster analysis than those obtained with totally porous packing materials. Core-shell particles have been investigated in conventional LC for both theoretical and practical studies, however just very few papers concerning their application in miniaturized separation techniques are published.
In this work, the use of novel core-shell Kinetex phenyl-hexyl particles (2.6 µm, 100 Å) in nano-LC was evaluated.
Capillary columns of different id (25, 50, 75, 100, and 150 µm) were packed for 15 cm employing the slurry packing method and were used for the separation of a model mixture containing five aromatic hydrocarbons (benzene, toluene, ethylbenzene, *n*-propylbenzene, *n*-butylbenzene) with mixtures of water/ACN in isocratic elution mode. As expected for this stationary phase, a reversed-phase chromatographic mechanism was observed obtaining the best results with 70% ACN. For each capillary id the maximum sample volume that could be injected without affecting the chromatographic performance was experimentally determined in order to evaluate columns efficiency. The column performances increased with decreasing id in terms of higher number of plates per meter at the optimal linear velocity of the mobile phase. The reduction of id resulted also in a flatter profile of the right branch of the van Deemter plot. The higher performance of 25 µm id column provided the baseline resolution of all analytes in less than 2 min.

F.08

PORTABLE MEDIUM PRESSURE CAPILLARY LC DESIGNED AS A MODULAR SYSTEM USING OFF-THE-SHELF COMPONENTS

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Portable instrumentation for LC is a necessity for a number of on-site measurement areas, however, commercial portable LC instrumentation can be still considered a scarcity. We set out to rethink the so far most applied approach of designing and in-house workshop fabricating components for a portable LC, as this, unless commercialized, renders it not widely applicable. With a system as widely accessible as possible in mind, our goal was to design a portable medium-pressure LC system based almost entirely from commercial off-the-shelf components. In this work we present results from our initial investigation. The backbone of the system was a breadboard assembled modular flexible microfluidic system, used widely in microfluidics, but not as an LC system, complemented with other off-the-shelf components, including an injection valve and on-capillary detectors, all operated through a PC. The portable LC system featured five syringe pumps, 2+2 for each A and B mobile phase for gradient operation, and another one pump for sampling. The four mobile phase pumps were miniaturised syringe pumps, with 80, 20 and 5 µL syringe options, and maximum backpressure of 8.6, 43, and 106 bar, respectively, calculated from the motor mechanism maximum force and each syringe cross-section. The flow range available was considerably wider than the range desirable (for analysis sped), applicable (respecting pressure limitations) and as a compromise mostly used of cca 1-5 µL/min. The 2 A pumps and 2 B pumps were each connected with microfluidic switching valves (4 in total), and A and B streams then connected with a Y-connector, thus providing a low hold-up volume gradient formation. The stream B (usually to contain an organic solvent for gradient elution), had a microfluidic pressure sensor incorporated. Reversed phase C18 monolithic capillary column (CapRod® Merck, 100 µm i.d., 50- 250 mm length) was connected directly to a 20 nL injector.. Dyes selected to cover a range of charge and hydrophobicity were selected as the initial test analytes. Separation were studied under both isocratic and gradient conditions. Detection was conducted on-column or on-capillary (100 µm i.d. fused silica) with a LED-based UV-Vis- photometric detector [1], and/or end-column electrochemical detection (amperometric and/or potentiometric). Experimentally determined performance parameters were evaluated and discussed, including the gradient formation performance, and operation under pressure in a range of up to cca 100 bar. The potential of the platform and future avenues for portable LC systems are discussed.

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F.09

HPLC METHOD FOR THE DETERMINATION OF THE ENANTIOMERIC PURITY OF THE ANTI-INFLAMMATORY IBUPROFEN IN BULK AND IN ITS PHARMACEUTICAL PRODUCTS.

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Ibuprofen is a non steroidal anti-inflammatory drug currently marketed as a racemate. The pharmacological activity of ibuprofen is primarily due to (S)-dexibuprofen. This has led to the introduction of (S)-dexibuprofen into clinical practice, and the chiral switching is expected to be more selective and safer. The present work represents development and validation of HPLC method for the enantioseparation of ibuprofen and quantitative determination of its eutomer (S)-dexibuprofen using (R,R)-Whelk-O 2 chiral stationary phase at 25 °C and mobile phase composed of 0.1M sodium acetate: 2-propanol (85: 15, v/v) with a flow rate of 1.0 ml/min and UV detection at 221 nm. The linearity range of the method was 5-80 µg/ml for each enantiomer. The limit of detection and quantitation for the (S)-enantiomer were 180 and 540 ng/mL, respectively. The method was applied to Laboratory-prepared mixtures of (S)-dexibuprofen and ibuprofen in different proportions. The method was also applied to ibuprofen granules and (S)-dexibuprofen tablets. The proposed method was found to be selective and accurate for monitoring the enantiomeric purity of (S)-dexibuprofen in bulk drug substance and drug products in quality control laboratory.

F.10

DETERMINATION OF BENZO[a]PYRENE IN EDIBLE OILS

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Benzo[a]pyrene (BaP) is a member of the polycyclic aromatic hydrocarbon (PAH) class and it is one of the most potent PAH carcinogens in animal experiments [1]. PAHs are ubiquitous environmental contaminants and they are formed by the incomplete combustion of organic matter and are thus generated whenever fossil fuels and they represent one of several classes of carcinogenic chemicals present in tobacco smoke. Furthermore, PAHs are present in many food, which also include edible oils [2].
The aim of the study was to develop a new method for extraction (clean-up) of benzo[a]pyrene in edible oils, especially in olive oil, that required a mixture of dichloromethane and cyclohexane as extraction solvent and polymeric-based columns (Styrene-divinylbenzene) for the gel permeation chromatography (GPC). This step allowed the removal of triglyceride from oil that eluted with void volume, while PAH were retained on column bed for more time.
The determination of BaP was carried out by liquid chromatography coupled with fluorescence (HPLC-FLD) by using reverse phase C18 column.
GPC extraction method applied assured high recoveries of BaP. The detection limit of the procedure to determine the BaP in edible oil was of 0.1 ug/kg and the recovery was 94%. Relative standard deviation was of 1.68%. The method can be also applied for determination of other PAHs in edible oils.

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F.11

CHROMATOGRAPHIC MOLECULAR MASS ANALYSIS OF ULTRA HIGH MOLECULAR MASS POLYMERS

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Separation of ultra high molecular mass (UMM) polymers is known to have some unexpected features which can't be explained from the classical chromatographic theories. Peak shape and retention of UMM polymers depend on the mobile phase flow rate and a peak splitting is observed at high flow rates. Unusual behavior of UMM polymers is commonly explained either by a degradation of polymers during migration through the chromatographic column, or by conformational (coil-stretched) transitions of polymer molecules under the share stress caused by the flow. The performed investigation showed on the example of UMM polystyrenes that conformational transitions should be considered as the preferred source of the unusual chromatographic behavior of such analytes. The conformational transition was modeled as the reversible reaction of the first order, and both equilibrium and rate constants were calculated and discussed.

COATING PROPERTIES OF A NOVEL WATER STATIONARY PHASE IN CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY

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Recently we have developed a novel separation system that features a stationary phase of pure water deposited onto the walls of capillary tubing used in supercritical fluid chromatography. The system utilizes carbon dioxide as the mobile phase, and displays a normal phase separation pattern as well as compatibility with the universal flame ionization detector. The ability of the system to separate mixtures of analytes with varying polarities (e.g. alcohols and carboxylic acids) has been demonstrated, however little is known about the mechanism of water retention on the column. This work is focused on exploring means of controlling the volume of water stationary phase that deposits on the tubing and the effect on analyte retention. Several properties have been examined, including surface oxidation, roughness and dimensions. The effects of each procedure on water retention and separations will be presented and discussed.

G.02

INVESTIGATIONS INTO A MICROFLUIDIC SUPERCRITICAL FLUID CHROMATOGRAPHY PLATFORM

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The development of a 500 µm column I.D. microfluidic platform for supercritical fluid chromatography (SFC) is appealing, amongst other things, due to ease of column installation and for a simplified interface to detection. For instance, improper fluidic connections at the 500 µm scale can have a detrimental impact of separation performance. Conversely, a microfluidic platform offers an alternative method of providing an effortless, reliable, and rugged column connection without the need of a skilled operator. Further, currently available analytical-scale SFC systems employ back pressure regulators which can make full-flow interface to detection challenging. As such, a split-flow interface to detection is commonly employed with these instruments which can introduce problems with quantitation if the mobile phase composition and/or system pressure is changed as the separation progresses (as is common with density and/or composition programmed gradient separations). To alleviate such troubles with interfacing to detection, this novel microfluidic SFC system employs a full-flow interface to detection with fully decoupled column linear velocity and system pressure.

This presentation will cover the development of a breadboard test bed for evaluating the feasibility of the 500 µm microfluidic SFC platform. Comparisons to analytical-scale SFC instrumentation with respect to peak reproducibility, system performance, column efficiency, and system sensitivity employing flame ionization detection will be included. Example separations will demonstrate the capabilities of this system.

G.03

SYSTEMATIC APPROACH TO THE STUDY OF NON POLAR LIPIDS IN EDIBLE OILS BY SUPERCRITICAL FLUID CHROMATOGRAPHY

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Supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) are powerful tools for selective isolation of key components in samples, like triacylglycerols (TAGs). In particular, ultra performance convergence chromatography (UPC^2), which uses liquid CO_2 as a mobile phase, represents an evolution of supercritical fluid chromatography, in terms of speed and reproducibility of analysis, and is enabling new ways of separating non-polar lipids, as well as phospholipids. A systematic approach is here described, for TAG separation in foodstuffs, i. e. vegetable oils, in which the parameters of pressure and temperature over a specific amount of time, impact the separation of compounds from the sample of interest. By adding of co-solvent, such as ethanol, with the supercritical CO_2, even more fine tuning of a separation was provided. Using environmentally friendly CO_2 as the mobile phase, for a greener and more selective alternative to normal phase separations.

G.04

SUPERCRITICAL FLUID CHROMATOGRAPHY: A FAST AND GREEN TECHNIQUE FOR CAROTENOID FINGERPRINTING IN FOODSTUFFS

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Since its inception, supercritical fluid chromatography (SFC) has been traditionally used for carotenoid separation. The primary mobile phase component in SFC, carbon dioxide (CO_2), in fact offers superior solubility for carotenoids and promote non-polar interactions between the analytes and the eluent, thereby reducing retention times. Additionally, the high diffusivity of CO_2 renders higher chromatography efficiency with respect to other separation techniques. While adhering to the basic principles of SFC, Ultra Performance Convergence Chromatography (UPC^2) brings in the added advantage of a reduced system volume, resulting in a greatly reduced run time, improved resolution, and increased detection sensitivity. In this contribution, we describe the separation of carotenoids in foodstuffs by (UPC^2) on HSS C18 SB stationary phase, without the need for time-consuming evaporation and reconstitution step after extraction, in a short time, and with organic solvent consumption reduced, noteworthy. A quantitative analysis of beta-carotene is also demonstrated.

SEPARATION AND CHARACTERISATION OF PURIFIED AND SURFACE MODIFIED DETONATION NANODIAMOND BY CAPILLARY ZONE ELECTROPHORESIS

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A new approach to the characterisation of purified detonation nanodiamond (DND) using capillary zone electrophoresis (CZE) has been developed. CZE has been found to offer some unique capabilities in this application, which is a subject of much interest given the rapidly growing range of applications of these unique nano-carbonaceous materials. CZE can be a quantitative tool for the characterisation of DND, providing information on sample charge, size, stability and tendency to agglomerate. Coupled with particle size and zeta potential measurements, it can provide a better understanding of the surface properties and dispersion quality of NDs in different buffer systems. CZE offers the possibility of separating different NDs, which could prove highly useful for sample fractionation in the future.

Herein, the influence of BGE conditions on electrophoretic mobility, peak shape and particle aggregation was investigated, with resultant observations supported by zeta potential and particle size measurements. Sodium tetraborate (pH 9.3), tris(hydroxymethyl)amminomethane (pH 9.3) and sodium phosphate (pH 7) were used in studying the BGE concentration effect on a commercial source of chemically stabilised DND. The BGE concentration had a strong effect on the stability of DND in suspension. The formation of aggregates of various sizes was observed as BGE concentration increased. The effect of pH on the electromigration of DND was examined using sodium phosphate (pH 8 and 10). The CZE method was subsequently applied to four different DND samples, which had undergone different routes of purification following detonation synthesis. Each sample produced a unique electrophoretic peak or profile in sodium tetraborate buffer (pH 9.3), such that the actual separation of DND samples from different sources could be achieved.

H.02

TRANSFERABILITY OF METHODS BETWEEN PLATFORMS AS AN ENTRY BARRIER IN MICROFLUIDICS: NEW OPTICAL AND ELECTROCHEMICAL DETECTION OPTIONS FOR CHROMATOGRAPHIC AND ELECTRO-DRIVEN CAPILLARY AND MICROFLUIDIC SEPARATIONS

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Microfluidics and its uptake in the analytical area has been impeded by barriers to entry that can be very high compared to classical (non-chip) methods such as capillary electrophoresis or chromatography. Those barriers can be identified as (i) substantial special equipment and skills to fabricate and use microfluidic chips, (ii) technology outcome differences at different scales resulting in some standard options being difficult to implement on the chip (such as UV-detection common in HPLC and CE), (iii) method transfer that is too often not straightforward at best, and (iv) lack of broadly available commercial chip-based analytical equipment that would allow straightforward usage without extensive specialist training but still be fully research-flexible. Such barriers impede faster and higher uptake of microfluidics. Some of the most popular impediment-lowering approaches have taken focus on low-cost fabrication technologies, paper microfluidics, or 'microfludic apps' that should be applicable widely in every lab. Our approach has been in the area (iv) namely analytical platform that allow straightforward usage without extensive specialist training are research-flexible. We have shown that in-house designed and fabricated chips can be used within 1 day in a commercial microfluidic chip based chip-CE analyzer, thus bringing chip-CE so far closest to the concepts so well proven for analytical workhorses such as liquid chromatography, namely 'put in a column, mobile phase, samples and run your analyses'. In this presentation we focus on impediments area (iii) namely method transfer: too often methods developed with CE use photometric detection, mostly rendering the method inapplicable for chip-CE and in need of redevelopment, primarily to change the detection typically to LIF. Until such times when low-cost and portable MS detection will accomplish 90+% of our needs, some of the focus needs to be turned back at 'low-impediment' detection methods, that is such that are easily transferable to the chip, and optimally are portable and low-cost. In this work we investigate novel detection options: Fluorescence detection using a new continuous plasma light source with high and equal output across the whole spectral range from deep-UV through visible to IR, compared to some standard and newer low-cost solid state light sources, and novel pulsed electrochemical detection techniques to provide both universal and sensitive multiple detection signals. The method transferability issues and detection methods will be illustrated with examples and discussed in the context of both the capillary and microfluidic chip based chromatographic and electro-driven separations.

H.03

QUANTITATIVE ANALYSIS OF UBIQUITIN DEPENDENT SUBSTRATE USING CAPILLARY ELECTROPHORESIS WITH DUAL LASER-INDUCED FLUORESCENCE

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The ubiquitin-proteasome system (UPS) is important in many biological processes, such as tumor cell growth and apoptosis [1, 2]. Inhibition of proteasome showed anti-tumor activity and proteasome has emerged as potential therapeutic target for cancer treatment [3, 4].
In this study, we developed the method for monitoring the proteasome activity in cells using capillary electrophoresis with dual laser-induced fluorescence (CE with dual LIF). Reporter genes including green fluorescence protein (GFP) fused ubiquitin substrate, RPN-1 and red fluorescence protein (Ds-red) as an internal control were transfected. Determination of GFP-RPN-1 and RFP in cell lysates were performed in an untreated capillary (75*m*m x 50 cm) and 100 mM Tris-CHES buffer (pH 9.0) containing 10 mM SDS. The fluorescence level of GFP-RPN-1 and RFP was detected, respectively at excitation wavelengths of 488 nm and 635 nm without any interference and crosstalk. Fluorescence intensity of GFP-RPN-1 was normalized with that of RFP. Proteasome activity was determined by substrate degradation using transfection of small amount of GFP-RPN-1. And three proteasome inhibitors were also characterized by substrate degradation using transfection of small amount of GFP-RPN-1. These results shows that the developed method is effective and promising for rapid and quantitative monitoring of the proteasome activity or proteasome inhibitor compared to common methods such as western blotting and pulse chase assay.

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H.04

THE PIPERAZINE-BASED DESIGNER DRUGS: THE SLOW INTRODUCTION OF CE-ESI-MS/MS TO THE FORENSIC AND TOXICOLOGICAL ANALYSES

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Designer drugs and their newly synthetized derivatives have become a serious problem across the whole world. Piperazine-based drugs (PBDs) have similar stimulant properties like the wide group of amphetamines and thus are getting more common among drug abusers [1, 2]. Because of the fact that this relatively new group of drugs have not fully understood mechanism of action, PBDs could cause many serious health problems and thus the selective and sensitive detection is strongly needed.

In this work we focused on the determination and sensitive identification of selected PBDs and their possible impurities (e.g. *N*-benzylpiperazine, BZP; 3-trifluoromethylphenylpiperazine, TFMPP or *meta*-chlorophenylpiperazine, mCPP) using capillary electrophoresis equipped with a tandem mass spectrometry (CE-MS/MS). We evaluated important parameters such as the electrolyte composition and MS conditions such as drying gas temperature, pressure and flow rate as well as the sheath liquid composition. Beside that we studied the fragmentation patterns to allow selective identification using the MS/MS analysis in the multiple reaction monitoring (MRM). Also the collision energies were determined for the studied PBDs. A clean-up step was introduced prior to the analysis using an SPE extraction method. Also the developed method was partially validated and parameters such as linearity, limits of detection and quantification and repeatability were determined. The developed method was applied to the analysis of urine samples to prove its possible employing into the toxicological and forensic analysis.

Acknowledgements

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H.05

SEPARATION OF IRON-FREE AND IRON-SATURATED FORMS OF TRANSFERRIN AND LACTOFERRIN BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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Efficient separation of iron-free and iron-saturated forms of transferrin (Tf) and lactoferrin (Lf) by using electromigration technique was a problematic task, due to their barely subtle difference in a mass-to-charge ratio. However, micellar electrokinetic chromatography (MEKC) occurred to be suitable technique for that purpose, and allowed us to separate these similar proteins. It was successfully performed in two different types of capillaries: bare fused-silica and neutral one. Neutral capillary was characterised by totally reduced electroosmotic flow, and opposite migration order of proteins. The established BGE composition for both capillaries was the same: 50 mM Tris buffer, pH 8.5, 0.2% (w/v) SDS [1].

Then, the method has been further tuned to disclose the presence of predicted monoferric forms of Tf, but previously undetected by our method. New conditions optimized by a Doehlert experimental design and surface response methodology consisted in the addition of 22.5% (v/v) methanol and increased SDS concentration up to 17.5 mM. This method allowed us to separate four different forms of Tf, including: iron-free, N-terminal monoferric, C-terminal monoferric, and differic forms, with excellent selectivity and total separation time 4 min. The experiment assuming the preparation of samples with gradually increasing concentration of iron revealed continuously changing contribution of monoferric forms, and was consistent with theoretical assumptions. Similar experiment has been conducted on-line, proving that saturation of Tf may occur directly inside the capillary.

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H.06

THE STUDY OF CYCLOFRUCTANS PROPERTIES AND INTERACTIONS IN CAPILLARY ELECTROPHORESIS

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High separation efficiency, low consumption of reagents, and wide range of available chiral selectors (CSs) predetermine capillary electrophoresis (CE) as a powerful method for enantiomeric separations. The new group of oligosaccharide-based CSs called cyclofructans (CFs) was recently introduced [1]. CFs consist of β-2,1 linked D-fructofuranose units forming 18-crown-6 ether core. Native CFs show very poor enantioselectivity for CE. The derivatization of fructofuranose hydroxyl groups can improve enantioselectivity mainly for primary amines and other, usually non polar and hydrophobic compounds [2,3].

In this work we selected three phosphate atropisomers with similar structures, namely 1,1´-binaphthalene-2,2´-diyl hydrogen phosphate (BNP), 2,2´-diphenyl-3,3´-biphenanthryl-4,4´-diyl hydrogen phosphate (VAPOL) and 3,3´-bis[3,5-bis(trifluoromethyl)phenyl]-1,1´-binaphthyl-2,2´-diyl hydrogen phosphate (BBH) as model chiral compounds for study of enantioselectivity and chiral separation abilities of native cyclofructan-6 (CF6) and isopropyl cyclofructan-6 (IP-CF6) in CE.

We used two BGEs, ammonium acetate pH 4 and sodium borate pH 10 to compare interactions between CFs in various concentrations, and studied analytes in acidic and basic pH environment. In the next step, BGE modifiers, specifically organic solvents (methanol and acetonitrile) or anionic detergent sodium dodecyl sulfate (SDS), were added to running buffers to investigate their effect on CFs interactions with analytes. Several metal ions form dynamic complexes with native CFs [4]. It can lead to stabilization of CFs core to support steric interactions between CFs and analytes. This effect was studied for selected cations, and partly confirmed for Ba^{2+} ions.

Acknowledgements

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H.07

SEPARATION OF BENZOIC ACID DERIVATIVES USING MODIFIED POLYMER CAPILLARIES FOR CAPILLARY ELECTROPHORESIS - MASS SPECTROMETRY (CE-MS)

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Capillary electrophoresis (CE) presents a powerful technique in analytical chemistry, since it provides fast analysis, high resolution and extremely small sample and solvent consumption. Typically, fused silica capillaries are employed, however, this material suffers from several disadvantages. In particular, the electroosmotic flow (EOF) is severely dependent on the pH of the background electrolyte. Resulting from the small EOF at low pH values, substances with low electrophoretic mobilities such as certain benzoic acid derivatives cannot be analysed under these conditions. However, increasing the pH results in comigration of some of the analytes, especially of positional isomers.

To overcome these problems, this study investigates polymeric separation capillaries instead of fused silica. One of the main advantages of polymer capillaries such as polyphenylsulfone (PPSU) is that the inner surface can easily be chemically modified. Among the various possibilities, sulfonation is of particular interest since it generates a relatively high and pH independent EOF. As a result, analytes with low electrophoretic mobility can be analysed at low pH values that are optimal for their separation. Under these conditions it was possible to separate several benzoic acid derivatives, including positional isomers with minimal differences in the $pK_{a(1)}$ values. The ability to separate the positional isomers of these analytes is especially important for environmental analysis of aerosols in the atmosphere, since the ratio reveals information about the origin and formation mechanism of air pollution.

H.08

COMPARISON OF RED INKJET AND STAMP PAD INKS BY MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY WITH LASER INDUCED FLUORESCENCE DETECTION

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In today's world, the vast majority of man prepare the documents using the print devices, confirming them by a signature and/or a stamp. Unfortunately, widespread availability of printers is also used by counterfeiters to perfectly imitate the entire document or just alter their parts (e. g. stamps). For this reason, scientists are constantly trying to develop newer and newer methods using more and more modern instrumental techniques.
In this study, a combination of micellar electrokinetic capillary chromatography (MECC) with a highly sensitive laser induced fluorescence (LIF) detector was tested in regards to possibility of various ink differentiation. Due to the fact that the most important stamps are made with red inks, the study focuses on inks of this color. The separation process was conducted in a polyimide-coated fused silica capillary (ID 50 µm, 60 cm total / 50 cm effective length) in background electrolyte consists of 40 mM sodium borate buffer, 20 mM sodium dodecyl sulphate (IV) and 10 % *v/v* acetonitrile with 30 kV applied. The optimized temperature of storage and capillary were 10 and 25°C, respectively. The samples were prepared using 15 dots (ø 0.5 mm) of printed or stamped area, extracted in 35 µL BGE and diluted with 35 µL of water. The MECC separation of main printing ink components by the proposed method showed excellent precision (repeatability < 0.7%, reproducibility < 2.5%). It was applied to group identification and differentiation of red printing and stamp inks of different brands and batches.
It was demonstrated that differentiation can be performed effectively using only the migration times and proportion of peak areas. The results showed that the proposed procedure can be valuable for an objective examination of the red stamped questioned documents.

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H.09

DETECTION OF miR-124 AS PROSTATE CANCER MARKER BY CAPILLARY ELECTROPHORESIS

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MicroRNAs (miRNA) are currently very promising markers of numerous diseases including diabetes, neurodegenerative diseases and cancer. Besides the diagnostic potential of these small non-coding RNA molecules, they may be utilized in gene therapy.
Isolation by magnetic particles is nowadays proven to be an elegant and simple method for extraction of target molecule from complex biological mixture and therefore it is highly beneficial also for isolation of miRNA from the interfering matrix.
In this work, a detection method for analysis of miR-124 (5-UAA GGC ACG CGG UGA AUG CCA), a prostate cancer marker, was developed. Capillary electrophoresis with light emitting diode induced fluorescence detection (CE-LEDIF, exitation – 400 nm, emission - 520 nm) was utilized to optimize the fluorescamine labeling procedure. Detection limits of this derivatization approach reached 10 nM (RSD 12.3%) of miR-124 standard and the calibration curve exhibited good linearity expressed as R^2 = 0.9988. The separation was carried out in 20 mM sodium borate buffer (pH 9.2) using separation voltage of +20 kV and sample was injected by 2 psi for 5 s.
Finally, streptavidin-modified magnetic particles were coupled to the DNA probe complementary to the target miRNA (5-Biotin-TGG CAT TCA CCG CGT GCC TTA) using the streptavidin-biotin interaction. Targeted miRNA was hybridized with the probe for 40 minutes in the hybridization solution (0.2 M sodium phosphate + 0.6 M guanidinium thiocyanate + 0.15 M Tris-HCl (pH 7.5) + 0.5 M NaCl). By utilization of these magnetic particles, miR-124 was extracted from the sample and subsequently eluted by the elevated temperature. Single-stranded miR-124 molecule was fluorescently labeled by fluorescamine which interacts with primary amine groups and the derivatized miRNA was analyzed by developed CE-LEDIF method.

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H.10

DETERMINATION OF DIFFERENT ANTIBIOTICS IN PHARMACEUTICALS BY CAPILLARY ELECTROPHORESIS

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Antibiotics are used for the treatment of many bacterial infections. This group of compounds has been analysed successfully by thin-layer chromatography and high-performance liquid chromatography (HPLC) using isocratic and gradient elution, but these methods are time consuming. Interest in antibiotics mainly lies in the analysis of of pharmaceuticals counterfeit. Capillary electrophoresis has become popular in recent years mainly because of its ability to separate charged and neutral species with high efficiency. For example, micellar electrokinetic chromatography (MEKC), one of the modes of CE, involves the use of surfactant solutions such as sodium dodecyl sulphate (SDS). Selectivity in MEKC separations can be enhanced by adding modifiers to the system.

A simple, rapid and accurate method has been developed for effective separation and simultaneous determination of a) fluoroquinolones, sulphonamides (lomefloxacin, danofloxacin, enoxacin, ciprofloxacin, levofloxacin, enrofloxacin, pefloxacin, sulfadimethoxine, sulfamerazine, sulfadiazine and sulfachloropyridazine), b) nitrofuran antibiotics (furazolidone, nitrofurazone, furaltadone), c) metronidazole, d) chloramphenicol, e) penicillin antibiotics (amoxicillin, oxacillin, ampicillin, cloxacillin, penicillin g, dicloxacillin) in pharmaceuticals by CE (CZE and MEKC) with UV detector.

All experiments were performed with a system of capillary electrophoresis Kapel 105M with water-cooling and a UV detector (Ampersend,Russia). Data were collected with the “MultiChrom” (version 3.x) and “Elforan” (version 3.1.0) chromatographic data system. A 60/50 cm (effective length) × 50/75 μm I.D. uncoated fused-silica capillaries were utilized.

The running buffers are a) 25 mM phosphate buffer (pH 8.5), b) 10 mM sodium tetraborate, 40 mMSDS, 10 % acetonitrile, c) 10 mMsodium tetraborate,50 mMSDS, 10 % acetonitrile, d)10 mM sodium tetraborate, 40 mM SDS, 10 % acetonitrile, e)10 mM phosphate buffer (pH 7.0), 30 mM SDS. The capillary was conditioned at the beginning of each day with 0.1 M NaOH for 5 min, followed by water for 5 min and a running buffer for 10 min. In order to equilibrate the capillary and minimize hysteresis effects, the capillary was flushed with water for 1 min and the running buffer for at least 3 min between analyses. The buffer was refreshed after eight analyses. Sample introduction was made at the positive side using the pressure of 30 mbar for 10 s. The high-voltage power supply was set to 25 kV. Capillary temperature was kept at 20 °C (35 °C to fluoroquinolones), and the compounds were detected at a) 280 nm, b) 362 nm, c) 312 nm, d) 220 nm, e) 210 nm.

The method was successfully applied to the determination of certain antibiotics in pharmaceutical tablets and liquids. Excipients present in pharmaceutical and degraded products from different stress conditions did not interfere in the assay. Concentration range of antibiotics is 1-2000 $mg \cdot g^{-1}$ for solid samples and 0.001-0.20 % for liquid medicines.

H.11

CHROMATOGRAPHIC AND ELECTROPHOTROPHORETIC DETERMINATION OF NEUROTRANSMITTERS, AMINO ACIDS, ANTIOXIDANTS, SUGARS USING LIGAND-EXCHANGE PROCESSES

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The main problems of electrophoretic determination of bioactive substances are absence of the chromophore groups, low values of dissociation constants (sugars), lack of stability during the analysis (ascorbic acid, polyphinoles, caterhins). Using additives able to form specific complexes with analytes (such as macrocycles, cyclodextrines, surfactants, ionic liquids, metal cations and et.al.) can improve the separation selectivity, decrease the detection limits in capillary electrophoresis (CE). Promising in this direction could be the method of ligand-exchange capillary electrophoresis with UV detection based on the on-line formation of labile complexes "analyte - Me +" between analyte molecules containing OH^-, NH_2, $COOH^-$ functional groups and metal-complexant (Cu^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+}) having different electrophoretic mobilities and absorbtion in UV-light.

We have established factors that provided the determination of amines, amino acids, sugar, that contain functional groups capable to complexion with metal cations in ligand-exchange capillary electrophoresis (LECE): the nature of metal-complexant (Cu^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+} and e.g.), the nature of counter-ion (SO_4^{2-}, Cl^-, CH_3COO^-), characteristics of the analytes (pKa value, capability to chelate formation and e.g.).

It was showed that the use of ligand-exchange processes in capillary electrophoresis with UV detection allows determination of analytes without absorption in the UV region (aliphatic amino acids, biogenic amines and sugars) with detection limits 5-10 mg/l, decreases the detection limits for those that UV absorb (tryptophan, tyrosine, histamine) and influences on the selectivity of their separation.

It was investigated the possibility of simultaneous electrophoretic determination of organic ligands (sugars) *by direct detection* in the form of complex *sugar-Cu2+* and inorganic cations Na+, K+ *by indirect detection* in biological samples. Comparative analysis of methods of ligand exchange capillary electrophoresis with direct UV detection and capillary electrophoresis with indirect detection in efficiency, time analysis, and detection limits was performed. Ligand exchange capillary electrophoresis has provided higher efficiency than zone variant and ligand exchange chromatography, this compensate the lack of separation selectivity in comparison with chromatographic methods.

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H.12

INVESTIGATION THE POTENTIAL OF USING HYPERBRANCHED POLYMERS POLY (ETHYLENIMIN)S AS COMPONENTS OF CHROMATOGRAPHIC AND ELECTROPHORETIC SYSTEMS

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In recent years interest in dendritic polymers has been increased due to the use of these materials in chromatographic and electrophoretic separation methods as stationary and pseudostationary phases. The interest in dendritic macromolecules originates from their unique chemical and physical properties, such as micelle's like structure, ability to encapsulate small molecules, multivalency. It is makes these materials promising for use in separation methods. In this study we have investigated the potential of use of watersoluble hyperbranched polymers *core-shell type*, poly(ethylenimine) functionalized with oligosaccharides (maltose, lactose, maltotriose), as a stationary and pseudostationary phases in chromatographic and electrophoretic determination of bioactive substances and in on-line preconcentration techniques in CE. These polymers have different molecular weights (5 and 25 kDa) and degree of modification with oligosaccharides (PEI-OS).

The influence of PEI-OS on efficiency and separation selectivity of bioactive substances in CE was investigated. Dependence of electrophoretic and chromatographic parameters and characteristics of investigated analytes (migration and retention factors) on the size of the core and the degree of modification of polymer core-shell was revealed for both macromolecular (proteins) and low molecular weight compounds (amino acids, vitamins).

The variant of capillary electrochromatography (CEC) with dynamic and covalent modifications of the surface of fused-silica capillary with positively charged hyperbranched poly (ethyleneimine) decorated with maltose shell (PEI-Mal) was developed. We have performed columns with PEI-Mal covalently bonded to the interior of a capillary, etc. *PLOT*-column. High reproducibility of migration parameters of proteins (RSD%: 0,5-1,8) on prepared columns was obtained. It was discovered the possibilities of different variants of *on-line* concentration in CEC. The comparative data of detection limits, efficiency and separation selectivity of proteins by CEC with synthesized PLOT-dendritic and PLOT-methacrylate columns and by CZE was obtained. It was shown that in CEC with dendritic columns the separation of analytes is more selective and has a better reproducibility of the migration parameters of analytes.

We have also investigated the possibilities of using the PEI-OS as components of chromatographic systems in high-performance thin-layer chromatography (HPTLC) using model mixture of hydrophilic analytes (*water-soluble vitamins and amino acids*). We have optimized modification conditions for both mobile and stationary phases with PEI-OSs. It was shown that by adjusting the pH of the mobile phase, we can change the type of interactions in the systems such as "*polymer-vitamin*" or "*polymer-amino acid*", thus affecting the separation selectivity. The obtained increase in efficiency up to 2-5 times for amino acids (lysine, tryptophan, glutamic acid) and riboflavin-5-phosphate solution can be successfully used in on-line concentration of these analytes in the real objects in HPTLC.

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H.13

ELECTROPHORETIC DETERMINATION OF TRACE AMOUNTS OF BIOACTIVE SUBSTANCES IN COMPLEX BIOLOGICAL MIXTURES

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Although CE holds great promise as the method of choice for high resolution of biological and environmental samples, it suffers from poor concentration sensitivity, particularly when UV detection is used. Diagnostically important analytes found in biological matrix at 10^{-9} – 10^{-12} M. On-line sample preconcentration is a useful technique in CE to improve the concentration sensitivity.

The development of different on-line preconcentration approaches (stacking with reversed migration micelles, stacking with high conductivity matrix, field-enhanced sample injection, head-column field-amplified sample stacking, stacking with high salt, sweeping, sweeping with addition of β-cyclodextrin, sulfo-β-cyclodextrin and 18-crown-6 into the sample matrix) permits us to improve the sensitivity for the trace analysis of ionic (antioxidants of polyphenolic type, biogenic amines and proteins) and neutral (steroids) analytes by different modes of capillary electrophoresis: zone (CZE) and micellar (micellar electrokinetic chromatography (MEKC)) modes with normal and reversed polarity capillary electrophoresis with UV detection.

It was observed, that dynamic preconcentration techniques has provided 20-fold increases in detection sensitivity for steroids in MEKC by stacking and 120-fold - by sweeping with the use of β-cyclodextrin in sample matrix. It was developed the determination of steroids in biological fluids (serum and urine) by MEKC with *on-line* concentration (sweeping) with detection limit for about 3 ng/ml (S/N=3) and method for the analysis of proteins in urine using CZE (LOD 10 μg/ml).

We have developed a novel *on-line* concentration technique in MEKC with reversed polarity for catecholamines and their metabolites with 18-krown-6 (4mM) and cyclohexilamine (5 mM) in the sample matrix. It was achieved 900-1500-fold sensitivity enhancement factor. Developed *on-line* concentration technique in ligand-exchange CE allows to decrease detection limits of analytes without absorption in the UV region (aliphatic amino acids) at 20-30 folds. The use of on-line concentration variant with polymer solution PEO allows determination of albumin in real biological systems with a detection limit of 15 and 10 μg/ml, respectively.

We have investigated the potential of using hyperbranched polymers polyethylenimins (PEI), functionalized with oligosaccharides as a stationary phases in electrophoretic separation of bio-active substances. PEI coating of capillary column reverse the direction of the EOF at acidic pH (2.2) and perform a good conditions for *on-line* preconcentration - the large volume sample stacking (LVSS) without polarity switching.

We have compared three sample stacking methods, including LVSS, HC-LVSS, FESI with LVSS. It was founded that using a combination of focusing principles of sample stacking with electrokinetic injection (FESI) and LVSS to analyze proteins resulted in a 1100-fold improvement in sensitivity. The LOD was achieved about 0,1 μg/ml for analytes.

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H.14

PARTIAL FILLING AFFINITY CAPILLARY ELECTROPHORESIS INTEGRATED WITH ADSORPTION ENERGY DISTRIBUTION CALCULATIONS IN BIOMOLECULAR INTERACTION STUDIES

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Biomolecular interactions play an important role in physiological and pathological phenomena at molecular level, and many instrumental techniques and tools are employed in exploring them. Biosensors, capillary electrophoresis and nano liquid chromatography enable small sample and reagent consumption and label-free analysis, both of great advantage relative to more traditional approaches such as ELISA and affinity chromatography. Moreover, these methods enable the determination of interactions' strength as affinity constants, partition coefficients, retention factors and reduced mobilities. Because partial filling affinity capillary electrophoresis (PF-ACE) does not experience interferences in UV detection as ACE does, it can be used even without the sample purification step so long as the components migrate separately. Numerous polymeric and copolymeric coatings developed have offered an excellent opportunity to manipulate an electroosmotic flow and to overcome one significant disadvantage of PF-ACE in biological studies, namely the unwanted adsorption of positively charged compounds, such as proteins and peptides, onto the uncoated capillary inner wall.

In this study PT-ACE was employed for elucidation of the strong binding site interactions determined in a narrow concentration range. With the help of adsorption energy distribution (AED) calculations, even small differences in the binding process, such as those between dermatan sulfate and two isoforms of apolipoprotein E, could be distinguished. Microscale thermophoresis, quartz crystal microbalance and molecular dynamics simulation calculations provided excellent supportive and complementary insight into the interaction mechanisms. PT-ACE combined with AED calculations can readily be exploited in other biomolecular interaction studies.

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H.15

ASSESSMENT OF RECOMBINANT HUMAN INTERLEUKIN-11 IN BIOPHARMACEUTICAL FORMULATIONS BY CAPILLARY ZONE ELECTROPHORESIS METHOD

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Introduction: Interleukin 11 (IL-11) is a multifunctional cytokine, which modulates the proliferation, differentiation and maturation of various types of hematopoietic cells. Recombinant human interleukin-11 produced by recombinant DNA technology (rhIL-11), expressed in *Escherichia coli*, Oprelvekin, consists of a 177 amino acids, with a molecular mass of 19 kDa and isoelectric point of 11.7. It is clinically used for the prevention of severe chemotherapy-induced thrombocytopenia and to reduce the need of platelet transfusions in patients with nonmyeloid malignancies. The aim of this study was to assess the content/potency of rhIL-11 in biotechnology- derived medicine, by a validated capillary zone electrophoresis (CZE) method, evaluating correlations with a reversed-phase liquid chromatography method.

Methods: The validated CZE method was performed using rupatadine fumarate, as internal standard. A fused-silica capillary, (50 µm i.d.; effective length, 40 cm) was used maintained at 25ºC; the applied voltage was 20 kV. The background electrolyte solution consisted of 50 mM disodium hydrogen phosphate solution at pH 3.0. Samples were injected using the pressure mode at 50 mbar for 45 s, with PDA detection at 196 nm.

Results: The method was linear over the range of 1.0-300 µg/mL (r^2=0.9992), and the limit of quantitation was 1.0 µg/mL. The specificity and stability-indicating capability were established in forced degradation studies, which also showed that there was no interference of the excipients. Moreover, method validation demonstrated acceptable results for the accuracy, precision and robustness. The proposed method was applied for the quantitative analysis of rhIL-11 in biopharmaceutical formulations, giving values between 90.20 and 106.43 % of the stated potency. The results were compared to those of a validated reversed-phase liquid chromatographic method showing a higher mean difference of the estimated content/potencies of 0.42%, with significant correlation as calculated by ANOVA (p>0.05).

Conclusion: The results demonstrated the potential of the CZE method, as an alternative to current methods, which can be applied for quantitative analysis of rhIL-11 during the biotechnology process, to monitor its stability and to assure the quality and therapeutic efficacy of the bulk and finished biological medicine.

Acknowledgments

FAPERGS, CNPq.

H.16

INFLUENCE OF THE COMPOSITION OF THE ELECTROLYTE SOLUTION ON PROTEIN MOBILITY AND ON CAPILLARY ELECTROPHORESIS ANALYSIS OF THE SYNTHETIC THERAPEUTIC POLYPEPTIDE CALCITONIN

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This communication reports the results of a study performed to evaluate the interactions between proteins and ionic species in solution that may affect the electrophoretic mobility of these biomolecules. The investigation has been conducted with standard proteins and with synthetic human calcitonin, a 32-amino acid linear polypeptide hormone, which is currently employed for the treatment of chronic bone disorders, such as osteoporosis and hypercalcemia. The study covers a series of background electrolyte solutions (BGE) composed of phosphate, acetate, trifluoroacetate, chloride or formate salts of N,N,N'N'-tetramethyl-1,3-butanediamine (TMBD) that has been proven to be effective at preventing protein-capillary wall interactions in bare fused-silica capillaries. It is reported that several ionic species used as the components of the BGE may interact with proteins to different extents, depending on the chemical nature of both the protein and the ionic species in solution and on pH and concentration of the electrolyte solution. Such interactions are expected to influencing both charge and molecular size of the solubilized protein and, therefore, it charge-to-mass ration and, consequently its electrophoretic mobility. In addition, the components of the electrolyte solution affect the electric double layer at the interface between the capillary wall and the electrolyte solution with consequent variations of the electroosmotic flow. As a consequence, the migration behavior of proteins and their resolution are affected by both the variation of the electroosmotic flow and of the electrophoretic mobility of proteins, resulting by their interactions with the components of the electrolyte solution. It is shown that besides affecting the apparent mobility of the analytes, the appropriate selection of the concentration of TMBD in the running electrolyte can be useful to modulate the resolution of a given separation, such as the degradation products present in an aged sample of synthetic human calcitonin for therapeutic use, separated with BGEs of different compositions.

INSTANT CONNECT GAS SAMPLING VALVE MODULE INTRODUCING A NEW FLEXIBILITY IN GAS SAMPLING FOR GC AND GCMS

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Injection through a sampling valve is one of the most reliable and wide-spread technique to inject a gas or a vapor sample into a gas chromatographic system. Nowadays development of this technology relies on the attempt of different Valve suppliers to improve material reaching higher temperature and/or longer life time of mobile parts, reduce internal volumes for improving chromatographic performance and reduce overall valve size. This paper describes a new modular concept that instrument manufacturer has undertaken to integrate latest valve technology, into a handling, plug-in module, full incorporating heating control of the valve, miniaturized pneumatic circuits for carrier gas supply and split as well as valve backflush to the vent. This modular design allows a new level of instrument flexibility, where inlets and/or detectors selection is based on the application in use, and can be changed in a matter of few minutes by the operator when a new analytical need or application requires different injector and/or detectors.

On top of improved technical performance in terms of injection repeatability and stability, this work shows the simplicity and flexibility in configuration setting provided by this Instant Connect modular design.

Without further hardware complexity the gas sampling valve module can be set to back flush to vent undesired part of sample, therefore offering an easy and integrated set-up for more complex analysis. Data showing performance of this solution are illustrated and discussed.

I.02

SNIFFLES – A PORTABLE MS-BASED SNIFFER INSTRUMENT

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MS techniques have been increasingly deployed in security sniffing applications. MS is a non-intrusive high-resolution technique able to detect single atoms and complex molecules through their charged species (ions) or fragmentation pattern. The technique is capable of detecting an extremely wide range of substances rapidly, with high accuracy and with a stand-off capability - critically it is able to detect trace levels below parts per million. Once the MS fingerprint of an unknown substance is measured it can be compared online with a database of known substances and rapid identification can be made on the spot in real time.
The research will develop a universal gas sensor using modular technologies to function as an artificial sniffer that will detect a range of (semi)volatile substances. The technology will complement trained sniffer dogs that are currently used.
The technology proposed is based on linear ion trap mass spectrometry (LIT MS). The LIT MS has a mass range larger than other comparable MS techniques. Additionally, methods for miniaturisation and modularisation will be applied to allow reduced vacuum demand and upgradeability. Miniaturisation will be made possible through improved designs based on results from modelling, implementation of novel manufacturing techniques and improvements in the MS drive electronics and vacuum system. These advances will bring benefits including reduced acquisition/operating costs, greater mobility, user friendliness and flexibility.
Sniffles has the potential to have a significant impact on national security and border control and enable exploitation of international markets. A successful project outcome will demonstrate an automated portable MS-based sniffer instrument, tested and evaluated for a range of security applications and markets by end-users.

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I.03

GAS CHROMATOGRAPHY–POST-COLUMN FRACTIONATION

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Gas chromatography is superior for separation of many compounds classes. These often non-polar and relative compounds are, however, still separated by liquid chromatography when fractionation and further analysis is required. In this contribution, a new technique will be presented for post-column fractionation of complete GC chromatograms in 96 or 384 well plates. This new analytical technology is based on post-column infusion of solvents with a low boiling point, e.g. hexane, acetonitrile, in the GC oven. Prior to infusion, the solvent is pre-heated. Directly after infusion, a transfer capillary runs outside the GC oven, where the solvent condensates while trapping compounds eluting from the GC capillary column. The transfer capillary is connected to a fraction collector. After evaporation of the solvent, the dried fractions can be used for further analysis.

The demonstrated technology is very straightforward and does not require sophisticated traps and allows the collection of large numbers of fractions during a GC run. Also, the technology has demonstrated to be robust and is ready for implementation in other laboratories. The application has been shown in environmental settings. Toxic pollutants in environmental mixtures were identified by their bioactivities towards the dioxin receptor and androgen receptor in 96 well plate format, and acetylcholine esterase bioactivity in a 384 well plate.

I.04

MAXIMIZING EFFICIENCY IN UHPLC-MS/MS METHOD DEVELOPMENT FOR MULTI COMPONENT ANALYSIS

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With the development of high sensitive and ultra-fast LC-MS/MS instruments, the triple quadrupole technology has found its way into a huge area of applications. Nowadays, it is the method of choice for trace level analysis and identification of various compounds. The steadily increasing number of applications in different fields like pharmaceutical, environmental, food, forensic and clinical analysis demand fast and efficient development of new LCMSMS methods. The basis for stable generation of high quality data is a well optimized chromatographic separation. Fully automated optimization of the UHPLC/HPLC method in combination with automated MS optimization for MRM and Interface parameters are the perfect platform for the generation of new triple quad MS methods. Here, we report a fast procedure for LCMSMS method optimization for multi component analysis. Choosing the best UHPLC/HPLC column and composition of eluents are often the most important but time-consuming steps during method development. We used Shimadzu´s Method Scouting System in combination with the ACE Excel Method Scouting Column Kit in order to elucidate the best UHPLC/HPLC parameters for the analysis of different compounds. The Method Scouting System allows the combination of up to 6 HPLC columns with up to 16 different eluent combinations, resulting in the investigation of up to 96 different combinations, which requires only a fraction of the time required by traditional approaches. The MS control software LabSolutions offers the possibility to select MS parameters like precursor ion selection, collision energy and fragment optimization for MRM via flow injection analysis in an automated way while the Interface Setting Support Software is a useful tool to find the most suitable Interface settings. All steps combined lead to generate a fully automated final method for the analysis of multi components.

TOWARDS INNOVATIVE MICRO MACHINED GAS CHROMATOGRAPHY COLUMNS FOR OILFIELD APPLICATIONS

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Since the late 70s, new approaches have been proposed to replace conventional gas chromatograph apparatus by silicon based micro fabricated separation systems [1]. Performances, in terms of separation speed and efficiency, are expected to be improved with miniaturization owing to the reduction of diffusion distances and better thermal management. Moreover, such a fully miniaturized micro gas chromatograph would enable cycled and real time analysis for various applications requiring continuous monitoring (oilfield, environment, safety…). The main challenge in miniaturized gas chromatography consists in the reproducible and collective fabrication of micro columns. Indeed, the critical step of stationary phase insertion is hardly compatible with miniaturization and mass production. In this work, two types of columns preparation were developed in order to answer this issue. One is the rerouting of the sputtering technique to coat columns with a thin film of silica [2]; the second one is the direct synthesis of a silica monolith in a column by a sol-gel process derived from classical procedure used for their preparation for liquid chromatography applications. The parameters involved in the second approach were studied and the performances of these silica monolithic columns were evaluated from the points of view of separation capabilities, sol-gel process conditions, ease of use and repeatability. Due to a good permeability and a large specific area, the silica monolithic columns exhibited a higher chromatographic performance than silica sputtered and conventional WCOT columns, with a strong retention which allowed very fast separations of light hydrocarbons at high temperature.

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I.06

USE OF HYDROGEN FOR IN SITU CONDITIONING OF MASS SPECTROMETER SYSTEM

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The addition of hydrogen to detector system flow can help maintain or recover response in a mass spectrometer (MS) or gas chromatograph/mass spectrometer (GC/MS) system. Hydrogen conditions and cleans one or more components of the MS, such as the source. The use of a modified EPC module in the Self-Cleaning Ion Source automates and accurately controls hydrogen addition off-line, when the system is not analyzing samples, or on-line, when the system is actively analyzing samples. Off-line conditioning recovers MS performance without the need for system vent and manual cleaning of components. On-line, continual conditioning maintains source performance during analytical performance prolong time between of line cleaning. Application of *in situ* conditioning to the analysis of polycyclic aromatic hydrocarbons (PAHs) GC/MS and GC/MS/MS addresses issues analysts have reported with poor peak shape, variation in peak response, and non-linear calibration curves.

I.07

AN INNOVATIVE INJECTOR ALLOWS HELIUM CARRIER GAS CONSERVATION IN ANALYTICAL GAS CHROMATOGRAPHY

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The global helium shortage and price increase cause more and more analytical laboratories employing gas chromatography to re-assess their consumption patterns of this non-renewable noble carrier gas. The existing solutions to this problem include changing analytical column, migrating to another carrier gas (e.g. hydrogen) or passively reducing the helium consumption by reducing its usage during analytical runs or switching the GC or GC-MS over to nitrogen during the longer idling periods. In all these cases a considerable amount of time is spent developing new methods or waiting the instruments to come back to normal operations. A new, innovative injector is presented which allows conservation of helium carrier gas. While preserving the analytical GC column flow with helium, it maintains the septum purge and the split flows with another inert gas like nitrogen - even during the analytical run. On average, the helium consumption is reduced such that a standard cylinder of compressed helium gas can last 3-6 years vs. 3-6 months without any changes in the analytical methods or conditions. This new patented technology for saving Helium will be presented, along with the data validating its use with different sample introduction techniques.

I.08

AUTOMATED, UNATTENDED SAMPLE DERIVATIZATION APPROACH PRIOR TO GAS CHROMATOGRAPHY ANALYSIS

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Many analytical procedures require the implementation of derivatization reactions prior to the GC or GC/MS injection. Derivatisating a sample prior to the analysis may be a problematic and time consuming activity; in many sample preparation workflows for GC analysis the necessary reagents must be added to the sample and the derivatization reaction can require agitation or sample heating for a significant amount of time. When all these steps are manually executed the laboratory throughput is limited. The possibility of performing the sample preparation automatically, by means of the same robotic sampler used for sample injection into the gas chromatograph, allows running a quantitative workflow for GC or GC/MS in unattended way. In this poster an automated and reliable approach to derivatization prior to GC analysis is presented which can be applied to a different range of food analysis for the automation of all the steps of the sample preparation process and injection of the sample into the GC. Examples will be shown on the potential automation of the derivatization and subsequent injection and GC analysis of melamine and its derivatives in powdered milk.

DEVELOPMENT AND VALIDATION OF A TGA-GC-MS METHODS FOR THE THERMAL CHARACTERISATION OF ULTRA-THIN POLYMERIC FILMS

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The present work is devoted to study the thermal stability of different random and block copolymers, widely employed to generate templates for nano-patterned materials, through a combination of TGA and TGA-GC-MS techniques.
In a TGA-GC-MS configuration, the TGA operates as thermal treatment equipment, since the thermo-balance provides, among other things, with precise and reproducible heating conditions. Hyphenation with the gas chromatographic mass spectrometric equipment permits to add the structural information obtained by the mass spectrometer about the products that evolve from the TGA furnace and the time evolution of these species thanks to the chromatographic separation obtained by GC. In this way it is possible the separation of the evolved gas mixture into single components and to distinguish among fragments having the same m/z ratio but that deriving from different molecules.
In particular the thermal stability was studied by the analyses of the evolved gas from TGA that was transferred to a GC-MS using an automatic interface that permits a repetitive pulsed transfer of known amounts of the evolved gas, with the desired frequency, in the injector of the GC-MS system.
The above reported technique was used to obtain an optimised and validated method that results adequate to characterize the thermal behavior of macro system as the bulk materials, micro system as thick films (few mm of thickness) and nano system as ultra-thin film (few nm of thickness).

J.02

MULTIDIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY-OLFACTOMETRY WITH INTEGRATED PREPARATIVE FRACTION COLLECTION FOR THE SEPARATION AND CONCENTRATION OF TRACE HIGH-IMPACT ODOUR COMPOUNDS.

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Volatile extracts of food are complex mixtures of compounds and present challenges when analysed using Gas Chromatography-Odour Port (GC-O) techniques. Some compounds can be detected reliably on the Odour Port but are below the limits of detection (LOD) of the GC detectors and therefore, cannot be identified. Increasing the amount injected onto the GC column to improve detection can exceed peak capacity and decrease resolution of other compounds. Multidimensional (MD) GC has been used for many years to increase peak capacity and resolve co-eluting peaks [1], while preparative (p) GC has been used to collect fractions so as to concentrate specific analyses before re-injection into a GC [2]. Instruments previously designed to combine both MDGC and pGC have required the manual removal of the collected material and reinjection by an automated thermal desorption system [3]. Here we describe a MDGC system with integrated, automated preparative fraction collection and desorption.

The system comprises an Agilent 7890A GC with two low thermal mass (LTM) ovens, which can be used for one dimensional GC-Mass Spectrometry (MS) and Olfactometry (O), to identify odour-active areas of the chromatogram. To identify and quantify co-eluting peaks or analytes below LOD, an Agilent capillary flow technology (CFT) Deans switch is used to heart-cut the peak or area of interest to a SIM Ice Cube cryo-trap. The cryo-trap has a large volume, and multiple heart cuts can be collected before thermal desorption onto the second column and detection at MS and O.

The presentation will show the concentration effects (and reproducibility) of the system using test mixtures of volatile compounds, as well as real food systems.

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J.03

DETERMINATION OF VOLATILE CHEMICAL WARFARE AGENTS AND THEIR HYDROLYSIS COMPOUNDS BY SELECTABLE ONE-DIMENSIONAL OR TWO-DIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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In the case of chemical warfare/terrorism, it is important to identify causative toxic substances. Volatile chemical warfare agents (CWA's) such as nerve gases and blister agents are unstable in water and body fluids, resulting in hydrolysis to produce stable compounds. We have used gas chromatography-mass spectrometry (GC-MS), but, because of sample matrix interference, GC-MS technique are suffered from low sensitivity. Here, we developed methods for determination of volatile CWA's themselves and their hydrolysis compounds by selectable one-dimensional or two-dimensional (^{1}D or ^{2}D (^{1}D/^{2}D)) GC-MS. Nerve gases (sarin, soman, tabun, cyclohexylsarin, VX, RVX) and blister agents (mustard gas, nitrogen mustard 1, 2, 3) were extracted with dichloromethane from on-site samples. CWA hydrolysis compounds (alkylmethylphosphonic acids, methylphosphonic aicd, thiodiglycol, ethanolamines) were extracted from aqueous samples. The extracts containing hydrolysis compounds were dried up, and incubated with MTBSTFA to convert to *tert*-butyldimethylsilyl (TBDMS) derivatives. Brief ^{1}D/^{2}D GC-MS condition: 1st dimension, DB-5; 2nd dimension, DB-17; heart cut time, time of the target elution; 1st column back flush time, time of the target elution or internal standard elution; scan 13 scan/s, *m/z* 50-400. Under the ^{1}D GC-MS condition, from the spiked complex samples such as gasoline and perfume, CWA's could not be clearly identified. Instead, under the ^{1}D/^{2}D GC-MS conditions, the target CWA's were separated from the matrix components, and their mass spectra were nearly identical to those of the authentic compounds. Under the ^{1}D GC-MS conditions, from the solid phase extracts of the spiked urine samples, TBDMS derivatives of the hydrolysis products could not be clearly identified. Instead, under the ^{1}D/^{2}D GC-MS conditions, the base line separation between the target TBDMS derivatives and interfering compounds was attained, and their mass spectra were nearly identical to those of the authentic compounds.

J.04

MINERAL OIL MIGRATION FROM PACKAGING INTO DRY SEMOLINA AND EGG PASTA UNDER ACCELERATED CONDITIONS

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Most foodstuffs are provided with a packaging which carries out several important functions; however, the transfer of undesirable compounds can occur. In particular, cardboard packaging represents an important source of food contamination with mineral oil, especially when recycled fibers or mineral oil based printing inks are used.
In this work MOSH (mineral oil saturated hydrocarbons), MOAH (mineral oil aromatic hydrocarbons), and DIPN (diisopropyl naphtalenes) migration from packaging into dry foods has been monitored under accelerated conditions.
In particular, semolina and egg pasta, of the same small size, were packed in recycled and virgin paperboard boxes and in plastic film bags and wrapped in aluminum in order to eliminate the external environment influence and to minimize a possible escape of a part of the contamination, which was therefore forced to migrate into food. Accelerated migration kinetics were studied at 40 °C (for virgin and recycled paperboard) and at 60 °C (for plastic film) up to 30 days. Furthermore, tests using MPPO (modified polyphenylene oxide - Tenax®) as food simulant were performed according to Reg. EC 10/2011, UNI-EN 1186:2002 and EN 14338:2003.
Migration from packaging into dry food was calculated in two different ways: the direct migration was calculated from the mineral oil contamination found in pasta samples (after subtracting of base contamination), while the indirect migration was calculated comparing the mineral oil content of the paperboard before and after the exposure.
Pasta, packaging material and Tenax® were conveniently extracted and analyzed by using an on-line liquid-gas chromatographyc system (LC-GC) equipped with a flame ionization detector (FID).
The data obtained under accelerated conditions by using pasta and Tenax® were compared with data obtained monitoring mineral oil migration from packaging into pasta during shelf-life (up to 2 years).

J.05

MULTI-DIMENSIONAL GAS CHROMATOGRAPHY WITH CAPILLARY FLOW TECHNOLOGY FOR THE ANALYSIS OF SELECTED OXIDATION INHIBITORS IN TRANSFORMER OILS

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A multidimensional gas chromatography (MDGC) method has been developed for the analysis of 2,6-ditertiarybutyl-para-cresol and 2,6-ditertiary-butyl phenol in transformer oil. These compounds are added to transformer oils to prevent radical auto-oxidation of hydrocarbon species in the oil, prolonging the transformer's life. Therefore these compounds are required to be routinely monitored to ensure the reliability of the transformer. Currently ASTM methods are used (2668 and 4768), which analyze these compounds by infrared (IR) spectrometry or single dimensional GC with a prior extraction process. These tests can obtain the desired results, but lack either selectivity or reproducibility. For example IR spectrometry cannot distinguish between the inhibitors and single dimensional GC lacks reproducibility due to extensive sample preparation steps. By utilizing the advantages of MDGC, selectivity and reproducibility are enhanced in a single, simple analysis. By utilizing a second dimension column, resolution between the oil matrix and the analytes of interest is improved thereby eliminating the need for sample preparation. Finally, a backflush mode was employed to preserve the first column, helping to improve system reliability. Overall the method outlined represents a quick, robust, and reliable means to determine amounts of oxidation inhibitors present in used transformer oils.

J.06

TWO-DIMENSIONAL ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY SYSTEM TO DETERMINE DRUGS IN PLASMA SAMPLES FROM THE SCHIZOPHRENIC PATIENTS

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Schizophrenia is a relatively common, chronic, and frequently devastating neuropsychiatric disorder, affecting approximately 1% of the world´s population. Apart from antipsychotics, most schizophrenic patients also makes concomitant use of other classes of drugs such as antidepressants, anxiolytics and anticonvulsants, in order to reduce the symptons associated with this condition. A rapid, sensitive and automated two-dimensional ultra performance liquid chromatography–tandem mass spectrometry method was developed to determine antipsychotics, antidepressants, anxiolytics and anticonvulsants in plasma samples. A hybrid organic–inorganic silica monolith capillary with cyanopropil functional groups (45 × 0.53 mm) was prepared and used in the first dimension for trace enrichment. Monolithic materials presented several attractive advantages including frit-free construction, easy preparation with good control of porosity, and satisfactory loading capacity. Cyanopropylsiloxanes exhibit both polar and polarizable characteristics. Protein precipitation of 100 µL of plasma with acetonitrile was used as a single-extraction procedure. After two-dimensional LC, all drugs were quantified by electrospray ionization-triple quadrupole mass spectrometry by selected reaction monitoring in the positive mode. Using hydrid monolith cappilary (1D), drugs were selectively isolated from plasma samples and the impurities were eliminated simultaneously with no matrix interference in the LC-MS/MS system. The proposed automated method has adequate analytical sensitivity (LOQ < 10 ng mL^{-1}), and selectivity for simultaneous determination of drugs in plasma samples from schizophrenic patients for therapeutic drug monitoring.

ON-LINE COUPLED LC-LC (Ag^+)-GC-FID AND GCxGC-MS: CHARACTERIZATION OF POLYOLEFIN OLIGOMERS MIGRATING INTO FOOD

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Polyolefins, like polyethylene or polypropylene, are one of the most important packaging materials in contact with food. They contain oligomers of low molecular weight (≤ C35), which can migrate into food. Online coupled LC-GC-FID is fairly common regarding the analysis of mineral oil contaminants migrating from recycled cardboard. These MOH (Mineral Oil Hydrocarbons) are separated by normal-phase HPLC in MOSH and MOAH (mineral oil saturated/aromatic hydrocarbons). The fractions of interest are transferred into GC for analysis [1]. The MOSH fraction is also used to analyse polyolefin migrates and can contain unsaturated hydrocarbons (one or two double bonds), which are hidden under the unresolved hump in GC. Especially, the POH (polyolefin oligomeric hydrocarbons), also known as POSH (polyolefin oligomeric saturated hydrocarbons), consist of a large proportion of monounsaturated species up to 40% (≤ C35). This seems problematic according to the toxicological evaluation of these contaminants. Thus, it is necessary to develop an automated method for quantification of olefins in the so-called saturated hydrocarbons and MOSH fraction, respectively. The separation of these olefins is achieved by a silver-impregnated HPLC column. It is integrated in the LC-GC-FID system with a specific valve shift and the “saturated” hydrocarbon fraction is eluting trough this second HPLC column after the first preseparation. The olefins are trapped on the second column and only the saturated hydrocarbons pass. A more polar mobile phase is used to elute the olefins from the silver-impregnated column. Consequently, a transfer of two well separated fractions directly into GC is facilitated to quantify saturated and monounsaturated hydrocarbons separately. The presentation provides information about the impregnation of a commercially available HPLC-column with silver ions and the identification of substances by GCxGC-MS after LC-LC (Ag^+) preseparation. The amount of saturated hydrocarbons and olefins (≤ C35) in extracts of different polyolefin samples, recycled cardboard and contaminated food will be presented and discussed.

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J.08

APPROACHING THE IDEAL FORENSIC GC-MS

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Advanced forensic applications were explored using the Aviv Analytical 5975-SMB GC-MS with cold EI. This unique GC-MS is based on interfacing the GC and MS with supersonic molecular beams (SMB) along with electron ionization of vibrationally cold sample compounds in SMB in a fly-through ion source (hence the name Cold EI). The 5975-SMB provides much faster analysis, increases the range of compounds amenable for analysis and improves sample identification. Several forensic applications will be demonstrated including: A) Very fast universal GC-MS analysis method was developed for the analysis of illicit drugs in under 3 minutes total analysis cycle time, based on column flow programming from 1 to 32 ml/min. B) The analysis of labile peroxide explosives TATP and HMTD along with all other organic explosive will be shown with dominant molecular ions for the peroxide explosives. C) C-4 plastic explosives source was characterized via its plasticizers isomers distributions. D) A new method was developed for pistol oil on hands analysis for forensic linking between a suspect and a given fire arm. It is based on oil hydrocarbons isomers distribution characterization. E) Isomer distribution analysis was applied for fuels and oils characterization for improved arson investigations. F) Nonoxynol-9 condom spermicide oil analysis was achieved with vastly improved sensitivity, enhanced molecular ion and oil isomers characterization. G) Chemical characterization of Cannabis seeds was demonstrated via few marker compounds. H) Largely improved unknown sample identification was achieved via the combination of enhanced molecular ions, extended range of compounds amenable for GC-MS analysis and the TAMI software that generates elemental formulas from single quadrupole MS data. We believe that the combination of the above mentioned improvements as demonstrated in the above applications implies that GC-MS with Cold EI is a step closer to the ideal forensic GC-MS.

J.09

THE FIRST EXPERIENCE WITH ANALYSIS OF PERFUME

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Nowadays, perfumes are produced from natural or synthetic substances. The most of perfumes contain a lot of compounds. This research deals with the analysis of volatile organic compounds in natural perfumes such as Praga Alchymica, which is produced by Rafaella. Rose Natura, Rose Taif and Rose Josefina are additional samples. These are the starting materials for the manufacture of perfumes. For analysis of volatile substances headspace and gas chromatography-mass spectrometry (GC/MS) have been used as these are frequently used methods for perfume analysis. The results indicate that nature of pure perfume like Rose Natura and Rose Taif has different compounds without 1-linalool as both of them have different smells. Moreover, on the aforementioned basis such information can be used for determination of basic compounds in mixed perfume like Rose Josefina. The same substances from Rose Taifa and other aromas were measured in this perfume. Praga Alchymica was mixed from Rose nature and unknown flavor. This research also proved that for small amount of samples it is better to use GC/MS compared to headspace GC. Research will also entertain using “heart-cut” MDGC system with using chiral columns in second dimension.

J.10

IMPROVING PROTEIN SCORE IN BOTTOM UP PROTEOMICS: NEW MULTIDIMENSIONAL PROTEIN IDENTIFICATION APPROACH COMBINING ORGANIC MONOLITHIC AND REVERSED PHASE PRE-COLUMNS

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Proteomics studies play a central role in the discovery of disease biomarkers and drug targets. Bottom-up proteomics is the most mature and most widely used approach for protein identification and characterization. On-line nano/capillary-scale reversed-phase LC–ESI–MS^n provides high-resolution separations of peptide digests. By bioinformatics tools the resulting peptide masses are matched to the calculated ones generated by "in silico" cleavage of proteins. Practical limitations occur when protein identification starts from very complex peptide mixtures or from low-abundance peptides. In order to improve the throughput of bottom up approach, we developed a new on-line capillary multidimensional system using two chemically different pre-columns: one polymeric methacrylate-based monolithic support and the other C18 stationary phase packed with silica microparticles. The peptides were resolved on reversed phase stationary phase in capillary column format. In detail, the trapping step involves both pre-columns connected in series and the low to high molecular weight peptides were fractionated in two portions: the monolithic pre-column keeps peptides with medium-high molecular weights while the RP packed pre-column holds peptides with low masses. In elution step, the capillary C18 packed column will be coupled alternately with the two pre-column to resolve the on-line split samples. The two runs can be performed by using different gradient profiles specifically optimized for each sub-sample (low masses and medium-high masses). This new capillary chromatographic system ensures a protein score gain up to 30 % compared with a conventional pre-column/column approach.

J.11

AUTOMATED DEVELOPMENT OF THE COMPREHENSIVE COMPOUND DATABASE FOR TARGETED MRM-BASED METABOLOMICS USING GC-MS/MS TECHNOLOGY

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Gas chromatography coupled to electron impact (EI) quadrupole mass spectrometry (GC-MS) is currently one of the most developed and robust metabolomics technologies. This approach allows for simultaneously measurements of large number of chemically diverse compounds including organic acids, amino acids, sugars, sugar alcohols, aromatic amines and fatty acids. Untargeted GC-MS profiling based on full scan data acquisition requires complicated raw data processing and sometime provides ambiguous metabolite identifications. Targeted analysis can provide better specificity, increased sensitivity and can simplify data processing and compound identification. Wider application of targeted GC-MS/MS approach in metabolomics studies is hampered by the lack of extensive databases of MRM transitions for non-derivatized and TMS-derivatized endogenous metabolites.

Selection and optimization of the single reaction monitoring (SRM) and multiple reaction monitoring (MRM) conditions for quantitation of large number of cellular metabolites in GC-MS studies is a lengthy and complicated process. Automation of this process can significantly reduce method development time and makes it feasible to develop quantitative methods for several hundred metabolites and use this strategy for metabolomics applications. AutoSRM software (Thermo Fisher Scientific) combined with triple quadrupole GC-MS system provides an integrated automated workflow for precursor and product ions selection and optimization of the SRM transitions. Optimized transitions can be easily exported into master compound database and subsequently imported into analytical method editor. We have developed large compound database using AutoSRM software and used this database to generate analytical and processing methods for MRM-based metabolomics study. Methods were tested and validated using complex mixtures of metabolite standards and biological matrices, including Arabidopsis tissue extracts.

J.12

MULTI-DIMENSIONAL GAS CHROMATOGRAPHY FOR THE CHARACTERIZATION OF ORYZA SATIVA L. VOLATILES UNDER DIFFERENT SOILS AND CO_2 CONCENTRATIONS

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Rice crops are the main staple for about half of the world's population. The study of the environmental consequences of the interactive effects of land-use practices (water regime, fertilizer placement and type, soil type (sandy and clay soils), microbial activity) climate (temperature), and atmospheric composition of CO2 by the production and emissions of gaseous NOx (NO, N2O), NH3, CO2, CH4, and volatile organic compounds (VOCs) is a demand in face of more intensive use of existing agricultural land. VOCs emitted by rice crops may also play an important role in its susceptibility and defence against insects.

Emission of volatile compounds emitted by the rice variety Oryza sativa were studied under different conditions, with and without enhanced carbon dioxide concentrations and two different soils, loamy sand and silty clay. The VOCs emission were measured in open field and open chambers, in 2 irrigated rice (Oryza sativa) fields at Salvaterra de Magos (Lisboa e Vale do Tejo region, Portugal), in replicated plots by means of Active Sampling on Tenax TA Sorbent tubes and analyzed by one dimensional gas chromatography/ mass spectrometry (1D-GC/MS) after thermal desorption (TD). Due to the higher environmental background and the trace amounts of VOCs emitted by rice no qualitative information was possible to extract from the 1D-GC data. In order to characterize the volatile composition O. sativa saples were randomly collected and submitted in the laboratory to SPME, steam distillation extraction (SDE) and silica monolith sorptive extraction (MonoTrap) in order to extract the Headspace components. 1D-GC and GCxGC coupled with a flame ionization detector (FID) and with a quadrupole mass spectrometer (qMS) has been used to analyze rice volatiles. Detection and identification of volatile compounds such as green leaf volatiles, terpenes, monoterpenoids and sesquiterpenes at trace amounts were achieved.

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DEVELOPMENT OF A MULTIPRESERVATIVE METHOD BASED ON LIQUID CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE-MASS SPECTROMETRY FOR COSMETIC ANALYSIS.

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To guaranty product integrity, protecting as well as consumer health, preservatives are essential ingredients used in all cosmetic formulations. The vast family group of cosmetic ingredients are subjected to several restrictions according to the European Cosmetic Products Regulation [1], where limitations, requirements, label warnings, and the maxima permissible concentrations are indicated. Thus, as a part of a quality control procedure, analytical methods for the determination of this diverse group of cosmetic additives in the wide variety of marketed personal care products are required. Methods for preservatives analysis in cosmetic samples are mainly focused on parabens determination, whereas analytical methods for the analysis of more than one class of preservatives are still a field under development. Liquid chromatography (LC) coupled to UV/Vis detector is the most commonly used technique to separate and determine cosmetic preservatives in official and non-official methods reported in literature. Recently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been described for isothiazolinones analysis in cosmetics and household products [2]. Although in less extent, gas chromatography (GC) coupled to mass spectrometry (MS) detectors has been recently applied for the analysis of multiclass preservatives in cosmetic products [3]. However, most phenolic additives require a derivatization step. In the present work, a sensitive and selective methodology based on liquid chromatography-triple quadrupole tandem mass spectrometry (LC-TQ MS) is proposed for the simultaneous analysis of different classes of preservatives including parabens, isothiazolinones, 2-tert-Butyl-4-methoxyphenol (BHA), iodopropynyl butylcarbamate (IPBC), triclosan, triclocarbán, chlorophene, and climbazol among others, in cosmetic products. Considering the broad variety of highly complex matrices, sensitive detection of the target analytes could be achieved working in the selected reaction monitoring (SRM) mode. Selected transitions were optimization for each target analyte. After chomatographic performance evaluation, the developed method was validated in real samples and applied to the analysis of different cosmetic and personal care products.

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J.14

INTACT PROTEIN LC-MS, HOW TO OVERCOME THE CHALLENGES?

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Mass spectrometry based protein characterization finds two major applications in both top down proteomics and the analysis of recombinant proteins, antibodies and other biotherapeutics within biopharma laboratories. However, analysis of high molecular weight proteins remains challenging. The methodology should be chosen to minimize common problems in protein analysis such as degradation, poor recovery, broad elution profiles, poor ionization efficiency, ion sensitivity, and resolution. Here we compared different prototype column-spray interfaces to see where the best performance for the above challenges is found. Mixtures of various proteins were used to represent top-down proteomics and monoclonal antibody (MAb) samples were used to represent the demands for a typical biopharma analysis. The column-spray interface for peptide separations was modified to create prototypes more suited for intact protein separations. A porous particle, a solid core particle and a monolithic structure were used to determine separation efficiency. The optimal chromatographic conditions for these prototypes vary and have to be matched to the mass spectrometric detection. The sample degradation was evaluated by temperature variation. The influence of post column volume on recovery and the sensitivity were assessed by comparing the prototypes with traditional setups. Proteins have a slower diffusion rate compared to peptides and small molecules. This predominantly affects the separation efficiency in terms of peak width. Gradient steepness influences peak width, but has to take sample complexity into account. The more complex samples had to be separated with shallower gradients to prevent coelution of peaks. Between the three prototypes it was found that the particle based stationary phases showed a higher loadability compared to the monolithic stationary phase, which depending on the protein could be a factor 5 difference. The peak capacity on the monolith was higher due to peaks typically eluting in half the peak width. The integration of column and sprayer minimizes the post column volume which will further reduce peak broadening and allows the MS to detect peak with higher intensity. In the case of protein separations, sensitivity is not only increased through minimized dispersion, but minimizing the losses from e.g. wall interaction between the protein and the transfer tubing as well. The integrated design of column and sprayer features the smallest possible surface area and therefore added to the sensitivity. The recovery from the monolithic columns was also found to be slightly higher, which is attributed to the non-porous nature. The exact level of improvement of the total system is still to be determined.

J.15

CHARACTERIZATION OF THE PROTEOME OF POMEGRANATE FRUIT BY NANOLIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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The fruit of pomegranate *(Punica granatum L.)* has a high antioxidant content, and its recognized pharmaceutical properties have partially been attributed to polyphenols [1]. However, information on genome sequence of pomegranate are lacking, therefore only a few and limited studies have been devoted to its proteins, which represent ca. 120 g kg^{-1} of the seeds. The aim of the present work was to characterize the *P. granatum* aril proteome by a shotgun proteomic approach using nanoliquid chromatography-high resolution tandem mass spectrometry. The employment of a high resolution mass spectrometer such as the hybrid linear ion trap Orbitrap allowed to identify several proteins in a single run without any prefractionation step. To increase the number of identified proteins, two distinct sample preparation protocols were employed, i.e. a standard extraction based on one of the classical protocols reported in literature and a protein capture based on the combinatorial hexapeptide ligand library technology, which equalize protein concentration, thus allowing to identify also the low abundant proteins. Because pomegranate is a non-model plant species, only a few protein sequences are included in the most diffused protein sequence databases. To improve both the number of identified proteins and data reliability, identification was performed integrating the results obtained with three distinct plant protein databases, since the majority of proteins could only be attributed by homology with other plant species. Nevertheless, many proteins had assigned only one unique peptide, because of the phylogenetic distance of pomegranate from the main model plants. After manual revision of the identified proteins to eliminate the redundant or ambiguous identifications, a list of 1488 proteins was obtained, only six of which belonging to pomegranate species. To author's best knowledge, this is the first work aimed to the proteomic characterization of *P. granatum*.

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J.16

STRATEGIC UTILIZATION OF GAS CHROMATOGRAPHY WITH BOTH NOMINAL AND HIGH RESOLUTION TIME-OF-FLIGHT MASS SPECTROMETERS FOR METABOLOMIC STUDIES

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Metabolomics has emerged as a critical tool for effective life sciences research. Out of all "omics" research, it is closest in proximity to system phenotype and therefore provides quick insight into system perturbations from drugs, environmental factors and gut microbiota. Challenges associated with metabolomics include the overwhelming number, diversity and concentrations of metabolites in both plants in animal species. The main objective in this study was to develop an effective workflow for the identification of dysregulated metabolites and to correlate these compounds to unique biological processes or diseased states. The main bottleneck in such studies is identification and structural characterization of the dysregulated compounds. This is a daunting task regardless of the instrumental technique utilized for data acquisition. While no single instrument is capable of fully profiling the metabolome, we have developed a workflow that utilizes nominal and high resolution time-of-flight (HRT) instrumentation for confident identification of metabolites. This workflow includes data acquisition using both EI and CI-HRT to obtain comprehensive profiles of derivatized blood plasma samples, as well as, statistical analysis of raw data to identify differentially expressed metabolites. Mass spectral data was collected using a Pegasus HT, Pegasus 4D and Pegasus HRT. HRT data was collected at a resolution of 25,000 and chromatographic parameters were adjusted to maximize the number of metabolites identified. EI-HRT data was collected using a source temperature of 250°C, mass range 30–600 m/z and acquisition rate of 6 Hz. CI-HRT data was collected with a source temperature of 200°C, mass range of 60–1000 m/z using reagent gas consisting of 5% ammonia in methane. Excellent mass accuracy values (MA < 1.0 ppm) allowed for confident elemental composition determinations for molecular, fragment and adduct ions.

J.17

GENERAL WORKFLOW FOR IDENTIFICATION OF UNKNOWN COMPOUNDS IN FORENSIC,PETROLEUM, FOOD, ENVIRONMENTAL, AND BIOLOGICAL MATERIALS: THE GAS CHROMATOGRAPHY - HIGH RESOLUTION TIME OF FLIGHT MS ADVANTAGE

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In this study, a relatively simple workflow for confident compound identification was developed and tested on a variety of samples. This workflow included a combination of hard and soft ionization methods coupled to high performance time of flight mass spectrometry, as well as, effective data processing for characterization of samples. In general, GC-TOFMS profiling of materials is an effective way to characterize petroleum, food, environmental and biological samples. It is fast, robust, reproducible and provides excellent chromatographic resolution. High resolution time-of-flight mass spectrometry (HRT) provides additional benefits such as robust formula determinations for fragment, quasi-molecular and adduct ions, as well as, increased selectivity resulting in reduced background interferences. HRT data is acquired over a large mass range and therefore can be probed multiple times via targeted or untargeted processing methods. Various data types were initially searched against accurate and well established nominal mass libraries (e.g., NIST 11) to arrive at potential hits for compounds. These results were corroborated using formulae generated from the high resolution, accurate mass data. For compounds that were not present in the commercially available MS libraries, this unknown compound identification workflow was employed. Where possible, standards were analyzed for unequivocal confirmation of chemical formulas and their proposed structures.

J.18

ISOLATION OF BIOFLAVONOIDS FROM CITRUS BY-PRODUCTS BY MEANS OF MULTIDIMENSIONAL LC PREPARATIVE SYSTEM COUPLED WITH A MASS SPECTROMETER

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It has always been paid particular attention to the analysis of natural compounds. However, standards of many molecules that characterize the natural matrices are often not commercially available. Then the use of preparative HPLC can be found necessary and useful for the isolation of natural standards (carotenoids, flavonoids, oxygen heterocyclic compounds, fatty acids, anthocyanins, ecc.) from natural matrices. Often the use of a single preparative column not guarantee to obtaining pure standards, and it is therefore necessary to further purify the standard thus obtained. The complexity of the matrices sometimes is a limitation for monodimensional analytical preparative approaches because of the limited peak capacity of a single column driving to coelutions. An off-line LC-LC technique, which involves the transfer of one or more unresolved fraction from a first to a second dimension, has already proven its utility in the analysis of natural matrices for separations that require very high efficiencies; this technique could be considered the most suitable approach for this purpose. Furthermore the use of a mass spectrometry detector allows to develop a method to increase the precision and efficiency of high throughput purification strategies for life science and pharmaceutical discovery. The instrumentation that was used for this research allowed the coupling of two preparative LC columns with different retention characteristics, and the detection system was a combination of UV/Vis photodiode array and LC2020 mass spectrometry detectors. The two preparative HPLC dimensions were connected by using three controlled two-position valves, placed between the two LC systems. This system is useful to simplify high throughput mass directed auto purification. The scope of the present project is to obtain pure bioflavonids from Citrus by-products (like waste waters). The standards isolated would represent a useful tool for the Citrus industry, in order to re-evaluate their side-products. This approach would lead to the reduction of the costs related to waste management and to an increased profit.

J.19

THE ON-LINE COMBINATION OF LIQUID CHROMATOGRAPHY-LARGE VOLUME INJECTION-COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY-TRIPLE QUAD MASS SPECTROMETRY: A POWERFUL FOUR-DIMENSIONAL SEPARATION-SCIENCE TOOL

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The present work is focused on the on-line combination of high performance liquid chromatography (HPLC) and comprehensive two-dimensional gas chromatography coupled by triple quadrupole mass spectrometry (GC×GC-QqQ MS), and it's application to the detailed qualitative and quantitative analysis of complex samples. The selectivity of the HPLC dimension enables the separation of chemical classes, or sub-classes of compounds. Then, the whole fraction of interest can be transferred, through a syringe-type transfer device, to a programmed temperature vaporizer (PTV) injector, which is part of a cryogenically-modulated GC×GC system. The use of the GC×GC instrument is an excellent way to increase the selectivity, the peak capacity and the sensitivity (through solute band re-concentration) of a GC system. Finally the triple quadrupole mass spectrometer employed was capable of generating full scan and multiple reaction monitoring (MRM) data simultaneously, and in a very rapid manner. Thus, it is possible to attain targeted and untargeted data in the same analysis. The potential of the novel automated four-dimension system was demonstrated in the analysis of a highly-complex real-world sample.

J.20

ANALYSIS OF LOW-VOLATILITY COMPONENTS BY A NANO-LC SYSTEM COUPLED TO AN ELECTRON IMPACT MASS SPECTROMETER

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The present research is focused on the potentiality of a nanoLC system coupled to an electron impact mass spectrometer. Thanks to the reduced amounts of effluent eluted by a nano-LC column, the liquid phase can be directly introduced into the mass spectrometer through a fused silica capillary tube of internal diameter smaller than 30 µm [1]; the analytes can be then converted into very small aerosol droplets and vaporized reducing the probability of thermal degradation also for thermolabile compounds; finally the molecules in the gas phase can be ionized and detected without any interference due to the presence of liquid mobile phase. This was also achievable thanks to the MS vacuum pump capabilities, able to work with carrier flows up to 10 ml/min. Such a system represents a new and useful tool for the separation and identification of non volatile and thermally unstable molecule in the range of 400-1000Dalton, most of them not suitable for a GC-MS system. The project implied the injection in the system of pure compounds, commercially available standard or fractions isolated from real matrices, in order to register their mass spectra in a personal database, that can be used for identification purposes in real samples. Molecules of different chemical classes were chosen to test the performance of the new instrumentation: tocopherols, flavonoids, polymethoxyflavons, besides an aromatic hydrocarbon mixture also used to test the chromatographic efficiency. The results have showned the proper functioning of the whole chromatographic system and the opportunity to obtain good quality EI-spectra of compounds not available in the commercially EI-library.

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J.21

CHARACTERIZATION OF SECONDARY METABOLITES OF CITRUS PLANTS BY USING GAS CHROMATOGRAPHY HYPHENATED TO CARBON ISOTOPE RATIO MASS SPECTROMETRY (GC-C-IRMS)

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The *Citrus* industry processing waste can still arouse economic interest if reused, and introduced on the market, through the production of nutraceuticals and cosmeceuticals. The characterization of such matrices has consequently, became important also in the scientific research field. The Gas Chromatography hyphenated to Carbon Isotope Ratio Mass Spectrometry (GC-C-IRMS) is considered very useful in quality control of matrices of high economic interest. Such analytical approach, indeed, is of choice in determining the botanic and geographic origin of the matrices, because carbon isotope ratio is linked to biosynthetic pathways of each plant specie and related to plants CO_2 fixation, subjected to the environmental changes and latitude. The present research focuses on the characterization of the secondary metabolites coming from the biogenetic mevalonate pathway, linked to the terpenes formation, in *Citrus* plants, by using the GC-C-IRMS investigations. The obtained $\delta^{13}C$ values related to several species of biological *Citrus* peel oils, have permitted to create a database of authenticity and genuineness, useful in the comparison of the carbon isotope ratio of terpenes, extracted from the *Citrus* processing waste products. In order to evaluate the carbon isotope ratio related to secondary metabolites of each *Citrus* specie, the use of an internal standard (i-std), chosen from the volatile compounds of interest was mandatory. The *Citrus* species investigated are those mainly used by the transformation industry to produce juice and essential oils (lemon, sweet orange, mandarin, bergamot etc.). The results are applied to identify the origin (geographic and botanical) of side-product and by-products, and determine the authenticity of *Citrus* derivatives.

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J.22

RAPID ISOLATION OF HIGH SOLUTE AMOUNTS BY USING AN ON-LINE FOUR-DIMENSIONAL PREPARATIVE SYSTEM: NORMAL PHASE-LIQUID CHROMATOGRAPHY COUPLED TO METHYL SILOXANE-IONIC LIQUID-WAX PHASE GAS CHROMATOGRAPHY

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This study reports the recent evolution of a multidimensional GC-GC-GC preparative system, now combined with an on-line LC pre-separation step, operated under normal phase conditions. The four-dimensional instrument can collect sample components with a concentration lower than 10%, in a short time period, while maintaining a high level of analyte purity. The LC dimension allows: I) the injection of higher sample amounts, compared to straight GC injection; II) a polarity-based pre-separation, leading to the GC injection of simplified sub- samples, and thus reducing the possibility of co-elutions; III) to eliminate the essential-oil “matrix”, replacing it with the LC mobile phase (the GC system is protected from potential contamination); IV) the LC mobile phase is of much lower viscosity with respect to a pure, or highly-concentrated essential oil, avoiding difficulties in the syringe sample withdrawal process, prior to GC injection. System optimization was performed by using standard solutions; additionally, a very complex sample, namely vetiver essential oil, was subjected to the preparative process, with the scope of isolating two low-amount constituents, namely α-amorphene and β-vetivone. These latter two sesquiterpenoids, which accounted for 1.7 and 4.0% of the sample (considering the volatiles), respectively, were successfully collected at the milligram level, in a one-day work period, with a purity degree in excess of 90%.

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J.23

CONSTRUCTION OF A VAPOUR PHASE FOURIER-TRANSFORM INFRARED SPECTROSCOPY DATABASE FOR THE CORRECT IDENTIFICATION OF COMPONENTS CONTAINED IN COMPLEX MIXTURES

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Fourier-transform infrared (FT-IR) spectroscopy plays a crucial role as detection tecnique in gas chromatography because of the unique structural information that can be extracted from IR spectral data. In fact sometimes mass spectrometry fails in the recognition of those isomers, which have the same basic molecular structure and thus the same fragmentation pattern. In this concern, such information is an additional valuable support, to mass spectral and retention data, for the unambiguous identification of unknown compounds. Last but not least, the non-destructive nature of IR detection makes the analyte available for other purposes such as fraction collection or other detection techniques.

The present research is focused on the evaluation of a novel vapour phase FT-IR spectral database, containing more than 1000 pure spectra, belonging to the flavour & fragrance field. The database was evaluated by using a multidimensional gas chromatography system, an approach that enabled the injection of higher sample amounts, thus avoiding problems related to the low IR sensitivity.

J.24

COLLECTION OF ENANTIOMERIC PURE COMPONENTS BY USING A MULTIDIMENSIONAL GAS CHROMATOGRAPHIC PREPARATIVE SYSTEM

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The collection of pure components from complex samples can be considered a hard task, especially at milligram level in a reasonable time. Recently a heart-cut multidimensional GC-prep system based on the hyphenation of three chromatographic dimensions, equipped with stationary phases characterized by different selectivities, have proven to be suitable to collect in a very short time chemicals from real samples in a one-day work time characterized by a degree of purity higher than 95% [1].
The present research reports the use of the system in order to demonstrate the suitability of this approach for the collection of enantiomeric pure components from different sample. The system was thus equipped with a cyclodextrin based stationary phase in the third chromatographic dimension with the aim to separate the enantiomers of components purified on the first (apolar) and second (medium polarity) GC columns. The collection station connected to the third GC dimension allowed the collection of different enantiomers components in the same run due to the presence of a 10-position carousel.

Acknowledgments
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J.25

DEVELOPMENT OF AN ANALYTICAL STRATEGY FOR THE IDENTIFICATION OF POTENTIAL ANTIMICROBIAL PEPTIDES FROM PROTEINS OF THE FISH MUSCLE BY NANOHPLC-MS/MS

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Nowadays, food protein and peptide are receiving great attention because in the last years the new field of food-derived bioactive peptides has come out. In order to investigate the presence and activity proteomics and peptidomics approaches have been recently applied in the study of bioactive peptides in several food matrices, combining powerful analytical techniques such as high resolution mass spectrometry and several bioinformatics tools for the identification of proteins, peptides and their main functions. This study presents a comparison and evaluation of four different experiment for the identification of sarcoplasmic and myofibrillar fish peptides. In particular, we investigated the effect and efficiency of protein purification and precipitation and UFM isolation to provide an univocal experimental pipeline also suitable in different fish samples and applications.The final comparison and evaluation of the methods was essentially based on the number of protein, medium size peptide and potential bioactive peptide identifications, obtained by digestion with trypsin, by nanoHPLC coupled with a high-resolution Orbitrap LTQ-XL mass spectrometer. In particular we tested the use of ultrafiltration membrane with a molecular weight cut-off of 3000Da. Data analysis has shown that the experiment which there is neither precipitation and ultrafiltration step performed better in identification of a larger number of peptides and potential antimicrobial peptides (AMPs) with 473 and 398 total identified peptides and 44 and 18 AMPs for sarcoplasmic and myofibrillar extracts, respectively. The experiment proved to be an effective protocol, faster and more straightforward with respect to the other three tested workflows. The developed strategy could be also useful for other food matrices and could provide information about food quality and safety control.

J.26

ATMOSPHERIC PRESSURE GAS CHROMATOGRAPHY-MASS SPECTROMETRIC PROFILING OF VOCS IN FRUITS

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Gas chromatography coupled with mass spectrometry (GCMS) is a well-established analytical approach for volatile compounds analysis and metabolomics. The most widely used ionization technique in GCMS is electron ionization (EI), which produces library searchable spectra dominated by fragments. The molecular ion in EI spectrum is often of very low abundance or absent. Many plant terpenoids have same molecular formula and shows matching fragmentation pattern when using EI, therefore any minor dissimilarity in the relative abundances of the masses in a spectrum lead to a false search result in NIST. Also lack of molecular ion information can give incorrect compound identification, if using spectral matching alone. Alternative approaches, such as the chemical ionization (CI), can be optimized to provide a molecular ion with reduced fragmentation, but with the serious drawback of a major loss of the sensitivity.
Atmospheric pressure gas chromatography coupled with mass spectrometry (APGC-MS) is an ionization technique that generates a spectrum conserving the molecular ion species with minimal fragmentation; additionally the system offers high mass accuracy, which is extremely useful in structure elucidation of unknown volatiles. We are establishing an analytical method employing APGC-MS for VOCs in fruits like Grape and Strawberry. In first phase we have done optimization of instrumental parameters using mixture of pure reference standards of fruit aroma compounds, this also aided in understanding ionization patterns of VOCs using APGC-MS since there is no database(like NIST MS) is available for primary identification of the compounds. Using injections of pure reference standards we started developing in-house APGC-MS library of the fruit VOCs. Parallelly, efficiency of thermal desorption system (TDS) for the extraction of VOCs also has been tested.

QUANTITATIVE DETERMINATION OF VOLATILE COMPOUNDS OF CHARDONNAY WINE USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY AND EVALUATION ON CONTRIBUTION TO THE WINE AROMA

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Volatile compounds play a significant role to wine aroma and the presence, absence or different proportions of these compounds can influence in wine quality and consumer acceptance. The quantitative evaluation of volatile compounds of Chardonnay wines using headspace solid phase microextraction (HS-SPME) combined with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry detection (GC×GC/TOFMS) and the determination of odor activity value (OAV) of volatiles were studied for the first time. The use of GC×GC/TOFMS for the analysis of Chardonnay wine of Serra Gaúcha region, located in the southernmost state of Brazil, resulted in the tentative identification of 243 compounds, showing the superior performance of this analytical technique for this specific varietal wine, considering that the number of compounds usually separated by 1D-GC for this type of wine is lower, around 60 compounds. Furthermore, 42 compounds co-eluted in the first dimension and 34 of them were separated in the second dimension, while the others 8 compounds were resolved by spectral deconvolution. The calculation of OAV allowed the determination of the volatile compounds that presented the greater contribution to wine aroma. Ethyl octanoate, ethyl hexanoate, ethyl butanoate, and b-damascenone showed the highest OAV, although other 43 compounds showed also potential to contribute to wine aroma. Figures of merit of the method were: recoveries from 92.4 to 102.6 %, repeatability from 1.2 % to 13.4%, LOD from 0.001 mg L^{-1} (ethyl isovalerate and hexanoic acid) to 2.554 mg L^{-1} (ethyl 3-hydroxybutanoate), LOQ from 0.003 mg L^{-1} (ethyl isovalerate and hexanoic acid) to 7.582 mg L^{-1} (ethyl 3-hydroxybutanoate). The combination of GC×GC/TOFMS for quantitative analysis of Chardonnay wines along with the OAV have shown to be advantageous and may facilitate the implementation of a quality control for the wine industry through the analysis of the most important odorant compounds identified for each varietal wine.

K.02

IDENTIFICATION OF IMPORTANT VOLATILE COMPOUNDS TO DIFFERENTIATE SPARKLING WINES FROM THEIR BASE WINES USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH TIME-OF-FLIGHT MASS SPECTROMETY AND CHEMOMETRIC TOOLS

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Sparkling wines, produced by the Traditional method, are prepared by double fermentation followed by aging of the wine with the yeast in the bottle. The first fermentation transforms grape must into base wine, which undergoes a second alcoholic fermentation inside a sealed bottle by adding a suspension of yeasts and sugar. The main changes in the volatile profile of base wines and their corresponding sparkling wines were investigated for the first time using headspace solid phase microextraction combined with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry detection (GC×GC/TOFMS) and chemometric tools. Fisher ratios obtained with chromatographic area counts helped to reduce the number of tentatively identified volatile compounds by GC×GC/TOFMS from 241 to 119, which were considered to be the main responsible for differences between base and sparkling wines. Principal component analysis explained 93.1% of the total variance related to the 78 compounds selected among the 119. Representative compounds of each chemical class that were appointed by statistical analysis as the volatile components that contributed the most to the differences observed among base and sparkling wines were: C13-norisoprenoids (TDN, vitispirane and β-damascenone), esters (laurate, 2-hydroxybutanoate, decanoate, 2-hydroxypropanoate, pentanoate ethyl esters), alcohols (4-butoxyl butanol, 1-propanol, methionol), aldehydes (3- phenyl-2-propenal, nonanal, undecanal), acids (acetic, 2-ethyl hexanoic, butanoic), ketones (acetoin, diacetyl), and phenols (4-vinyl guaiacol, 4-ethyl-phenol). On the other side, some of the acids, aldehydes, ketones and alcohols have been reported as a negative contribution to wine aroma and monitoring their presence may be important for future quality control of sparkling wines and also for the improvement of vinification process. GC×GC/TOFMS superior resolution power was demonstrated for these sparkling wines, as it provided separation of twenty co-eluted volatile compounds that may be important to wine aroma.

K.03

PULSED FLOW MODULATION GC × GC-MS WITH COLD EI

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Comprehensive two dimensional GC×GC-MS significantly improves GC separation. However, with thermal modulation it suffers from complexity and high cost of purchase and cooling gases. In addition, with standard electron ionization the molecular ions are often weak or absent particularly in complex hydrocarbon mixtures analysis. We developed a pulsed flow modulation (PFM) technique for obtaining GCxGC-MS with supersonic molecular beams (SMB) (also named Cold EI) based on a quadrupole mass analyzer and without any added gas. Sample compounds which elute from the first GC column are temporarily stored in a fused silica transfer line and are periodically injected by ~25 ml/min helium gas pulse into the second column. After the pulse, 20 ml/min He develops the chromatography in the second column for a few seconds. PFM is simple to construct, does not require any added gas and the injection time can be tuned, hence GCxGC-MS with Cold EI is enabled with quadrupole MS, and the increased PFM flow rate is handled by the nozzle chamber differential pumping. We combined PFM-GCxGC with the Aviv Analytical 5975-SMB GC-MS with Cold EI. PFM-GCxGC-MS with Cold EI combines the improved separation of GCxGC with Cold EI benefits of enhanced molecular ions and mass spectral isomer and isotope information for the provision of ultimate sample information. Unique to PFM-GCxGC-MS with Cold EI is that as the second dimension elution time is increased the observed molecular ion mass is reduced for easier GCxGCxMS like identification. Examples of PFM GCxGC-MS with Cold EI will be shown with JP8 jet fuel and in the universal analysis of pesticides in agricultural products. The presentation will demonstrate how PFM-GCxGC-MS with Cold EI provides ultimate level of information in hydrocarbon mixture analysis and enables a new and powerful method of universal pesticide analysis that can serve as an alternative to GC-MS-MS.

K.04

SCREENING METHODOLOGY FOR THE DETERMINATION OF UNKNOWNS IN DRINKING, SURFACE AND WASTE WATERS: VALIDATION AND RESULTS

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GCxGC-TOF MS is a powerful technique, not only for the quantitative determination of known compounds, as has been shown in previous occasions, but also for the qualitative analysis of unknowns (general unknown screening). Water pollution monitoring is very important in the safe production of drinking water. In our case, we start from surface water, which is then purified. Especially the sources of waste water should be characterized. During a campaign in 2013 (six monitoring moments), we listed the waste water sources along the Albert Channel and analyzed these samples both with GCxGC-TOF MS and LC-HR MS. From these results, it can be concluded that a waste water treatment plant in Riemst is responsible for the majority of compounds, present in the surface water. Compounds, like methyl-1H-benzotriazole (industrial chemicals), venlafaxine and metformin (pharmaceuticals) and chloridazone (pesticides) were encountered. A comparison will be made with the presence of these compounds in surface water (daily monitoring at two locations) and the final drinking water. Also the way in which the enormous amount of data are handled, is of utmost importance. A comparison between the use of statistical techniques like multivariate data analysis (PCA) and more traditional way of working with databases will be made. A validation study, using spiked samples, was done in order to determine the correct parameters and to estimate the measurement uncertainty of the method.

K.05

EXTRACTION AND ANALYSIS OF FATTY ACIDS FROM CYANOBACTERIA USING GC × GC-TOFMS

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Cyanobacteria grow in freshwater bodies when they are provided with suitable growth conditions such as nutrients, temperature and light. Algae biomass is known to contain a large amount of lipids such as saturated and unsaturated fatty acids. In this study fatty acids from algal cells were extracted using a newly developed extraction protocol using ionic liquid enhanced by direct transesterification at an elevated temperature. The identification and quantification of fatty acids was performed using gas chromatography coupled to a time-of-flight mass spectrometer (*GCxGC-TOFMS*). The extracted fatty acids were dominated by those with carbon chain of C16 and C18; [ie. 7-hexadecenoic acid (C16:1) and hexadecanoic acid (C16:0) for C16, whereas C18 includes γ-linolenic acid (γ-C18:3); linoleic acid (C18:2); linolenic acid (C18:3); 6,9,12,15-octadecatetraenoic acid (C18:4); oleic acid (C18:1) and octadecanoic acid (C18:0)]. The obtained fatty acids composition was then compared with that obtained by organic solvent extraction using a mixture of chloroform and methanol. Statistical evaluation was performed using one-way *ANOVA* and found that there was no statistically significant difference ($P = 0.908$) between the two extraction methods, a finding which indicates the usefulness of ionic liquid as a solvent to replace volatile organic solvent to minimize environmental pollution.

K.06

EXPLORING BACTERIAL FATTY ACIDS USING GC × GC AND MDGC TECHNIQUES WITH MS DETECTION

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Fatty acids (FA) are essential components of most living cells and cellular fluids, which comprise high complexity in their structures. The analysis of bacterial FA derived from phospholipids using chromatographic techniques is very helpful for taxonomic classification of microbes and biomass extracted from these complex matrices, as well as to provide insights into biochemical processes. Today, large databases have been developed containing the bacterial FA profiles of over hundreds of bacteria for agricultural, environmental and clinical fields. However, such databases are largely limited to reference FA profiles from single cultured organisms grown on solid media and using 1D GC / GC-MS methods. It is most probably impossible to determine the whole-community FA in complex matrices impacted by multiple bacteria, especially for unique FA such as cyclopropyl, anteiso, and chiral FA. To solve this problem, comprehensive two-dimensional gas chromatography (GCxGC) coupled with simultaneous flame ionisation and mass spectrometry (MS) detection can be applied to aid tentative identification of the bacterial FA classes in complex matrices such as in the heterogeneous soil ecosystem. Moreover, trends of minor amounts of unique or unusual FA components can be identified using GCxGC technique with high resolution MS to confirm the empirical formulae of the components. This presentation will present such solutions for the profiling and identification of bacterial FA.

K.07

ON-LINE ANALYSIS OF SULFUR COMPOUNDS IN COMPLEX HYDROCARBON MATRICES USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY FID/SCD

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On-line comprehensive two-dimensional gas chromatography (GC×GC) coupled to a sulfur chemiluminescence detection (SCD) technique has been applied for fast and accurate sulfur compound analysis in a pilot plant setup. Increasing oil prices are driving the industry towards the use of increasingly heavier feeds in steam cracking. However, heavier fractions contain more sulphur compounds (thiols, thiophenes, (di-)benzothiophenes) than the classically used lighter feeds, affecting the steam cracking process, particularly coke formation. Obtaining comprehensive experimental data on the decomposition and the role of the petroleum sulphur containing constituents is the primary objective. On-line sulfur detection is a difficult task because of the adsorption of sulfur compounds on metal surfaces (tubings), as well as due to the absorption in the GC system and capillary column. For these reasons all the metal tubings were replaced by sulfur inert coated tubings (Sulfinert®, Restek®), larger volume injections and special columns for low level sulfur analysis (fused silica column) were used. On-line system using a Thermo Scientific Trace GC×GC equipped with two capillary columns (Rtx®-1 PONA and SGE BPX-50), an FID detector and together with an Agilent 355 Sulfur Chemiluminescnce dual plasma detector is presented. The GC×GC-SCD settings were optimized for trace level (ppm) sulfur analysis in hydrocarbon matrices and used to understand to which degree sulfur compounds are decomposed in a steam cracking coil as well as which products are formed starting from the original compounds. Both a simultaneous FID-SCD and a separate “FID and SCD” configurations were tested. Although the simultaneous FID-SCD configuration is faster, separate configuration produces a more sensitive SCD analysis. Level of detection and quantification for the separate configuration were determined at 11 and 35 mol-ppb of sulfur, respectively. The method provides excellent quantitative measurements with a relative standard deviation of 10%.

K.08

COMPARISON OF ODOUR SOURCES USED FOR VICTIM RECOVERY DOG TRAINING

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Victim recovery dogs are trained on a variety of odour sources which can include soft tissue, blood, bone and/or soil surrounding a decomposition source. The chemical profile of these sources has not been fully characterised and therefore the impact of using one source over another for training purposes is unknown. The aim of this study was to compare the odour profile from two sources; the headspace from human analogues (porcine remains) and the soil beneath these remains. The odour profile obtained from both matrices was analysed using two dimensional gas chromatography – time of flight mass spectrometry. This technique is ideal for analysing complex mixtures such as decomposition odour due to its enhanced sensitivity and ability to separate a large number of compounds. Many characteristic decomposition odour compounds were identified in both matrices whereas compound class substructure differed between the matrices. The odour profiles were complementary and recovered different subsets of the overall decomposition odour profile. The compounds identified in each matrix provides valuable information regarding the use of different training aids for victim recovery dogs and can aid in determining the key compounds to which the dogs alert.

GCxGC-TOFMS QUANTIFICATION METHOD FOR MIDDLE DISTILLATES USING VISUAL BASIC SCRIPT

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The subject of this presentation is the development of a GCxGC-TOFMS method for the complete quantification of petroleum middle distillates. The analysis method was developed on the basis of different common fuels from light to heavier middle distillation fractions, namely jet fuel, diesel fuel (blended with 7% biodiesel) and light heating oil. The physical properties of middle distillates, like the cetane number, CFPP or cloud point are caused by the chemical composition and regulated by DIN EN 590, what makes a precise knowledge of the composition necessary. A well-established method for the determination of aromatic hydrocarbons is described in DIN EN 12916, an HPLC method that provides a rough separation between mono-, di- and polyaromatics. Comparatively, a complete quantification of all components can be achieved with GCxGC-TOFMS due to its inherent features, like the classification of several thousand different compounds can only be achieved due do the structured chromatograms and the fragmentation pattern that provide the possibility of using Visual Basic Scripts as an analytical tool [1,2] But also this technique poses some difficulties, e.g. for TOFMS an interpolation of response factors is needed for the group-type quantification. For this, well-separated and recognizable internal standards according to the different compound classes are needed. The results of the here presented quantification method have been compared to common petrochemical methods.

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K.10

COMPREHENSIVE CHARACTERIZATION OF MIXED-HALOGEN DIOXINS AND FURANS GENERATED IN FIRE DEBRIS USING GCxGC-TOFMS

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Large scale fires such as the Plastimet fire (Ontario) and The World Trade Centers (New York City) have raised concern about long term exposure of firefighters to the combustion byproducts of brominated flame retardants (BFRs). An increase in cancer rates among debris-exposed first responders have been associated with each incident. In response, this study focuses on the investigation of the role mixed halogenated planar compounds, generated during the combustion of BFR-containing products, play in the toxicity experienced in first responders. The emphasis of the project is placed on the generation of polyhalogenated dibenzo-p-dioxins and dibenzofurans in residential or commercial fire situations. Comprehensive two dimensional gas chromatography (GCxGC) has been chosen as the initial analytical technique for characterization of fire debris samples due to its advantages in enhanced peak capacity and decoupled separation mechanisms. Therefore, isobaric compounds may be better resolved from one another, and more importantly from the matrix. A GCxGC-TOFMS method has been developed for characterization of polyhalogentated dibenzo-p-dioxins and dibenzofurans in fire debris samples. Data from a simulated burn study show differing patterns of dioxins and furans depending on the type of fire (i.e. electronics rich vs. household simulation). An electronics rich fire generated a range of bromo/chloro dibenzofurans (PXDFs), while polybrominated dibenzofurans (PBDFs) dominated as the main halogenated furan species in both the electronics rich and household simulation fire. GCxGC-TOFMS analysis has also identified a series of mixed halogen species for which there are no commercially available standards. In addition to GCxGC-TOFMS analysis for initial exploratory work, atmospheric pressure ionization GC (APGC) -triple quadrupole mass spectrometry (TQS) is being utilized for high sensitivity targeted analysis of the samples to aid in the identification of species without standards, as well as to probe the dioxin content, expected to be present in the samples at trace levels.

K.11

THE POTENTIAL OF TWO-DIMENSIONAL GAS CHROMATOGRAPHY (GCxGC) COUPLED WITH ACCURATE-MASS TIME-OF-FLIGHT MASS SPECTROMETRY (GCxGC-Q-TOF MS) FOR THE CHARACTERIZATION OF COMPLEX PETROCHEMICAL MIXTURES

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Recently, ToF-MS systems have become available that can generate accurate-mass spectra at rates sufficiently high to keep up with the high-speed second-dimension separations of GCxGC. Here we will report on the combination of GCxGC with the newly purchased Q-ToFMS system. Together with new in-house developed software this simplifies MS-data analysis including data filtering and confirmation of structure identification through library searches. This tool opens new opportunities for problem solving and high end research.

K.12

CHARACTERIZATION OF SEBUM LIPIDS USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COMBINED WITH RAPID-SCANNING QUADRUPOLE MASS SPECTROMETRY

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Mass spectrometry (MS) coupled with separation by gas chromatography (GC) is a very powerful tool for identifying unknown volatile compounds. However, the presence of co-eluting compounds can lead to misidentification and quantitative errors. To minimize such an issue, the separation power of GC can be enhanced by using a comprehensive two-dimensional GC (GC x GC) method. In the latter, two capillaries with distinct selectivity (positioned together in a single, or individually within two GC ovens) are connected in series with a transfer device, defined as modulator. The occurrence of peak overlapping is then greatly reduced since this undesirable chromatographic feature would require similar elution times on both columns. The combination of a third MS dimension with a GC x GC system generates the most powerful tool available today for analyzing volatile compounds. Comprehensive 2D GC has been applied successfully in many research areas. Historically, its initial and most common application focused upon petrochemical samples, one of the most complex sample-types known to analytical chemists. GC x GC has also been widely employed for unravelling complex food matrices, in many instances for the analysis of fatty acid methyl esters (FAMEs). In the present investigation, a series of hair sebum FAME samples were subjected to GC x GC analysis, using a rapid-scanning quadrupole mass spectrometer as detection system. The unexpected complexity of the sebum lipid profiles were entirely deciphered through the three-dimensional method. Reliable peak identification was achieved by exploiting the formation of group-type patterns and a novel GC x GC-MS FAME spectra library. Finally, data processing was carried out with the support of a recently developed comprehensive chromatography software.

K.13

MONODIMENSIONAL (GC-FID AND GC-MS) AND COMPREHENSIVE TWO-DIMENSIONAL (GC×GC) GAS CHROMATOGRAPHY FOR THE ASSESSMENT OF VOLATILES AND FATTY ACIDS FROM RUTA CHALEPENSIS L. PLANTS

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Ruta chalepensis L. belongs to the family Rutaceae and it's widespread in the Mediterranean area. This plant has a solid tradition in ethnomedicine, because of various biological activities. Besed on previous reports, the main volatile constituents of *Ruta chalepensis* L. are 2-undecanone and 2-nonanone, while only a few is known about fatty acid composition. The study was directed toward the determination of volatiles and fatty acids, in samples of *Ruta chalepensis* L. obtained from different organs of plants harvested in different periods. GC-FID, GC-MS and GC×GC advanced techniques, with the support of dedicated mass spectral databases provided with Retention Index (RI) information were applied to the determination of both volatiles and fatty acids. Samples were extracted by hydrodistillation and underwent methylic transesterification in order to be transformed into the correspondent fatty acid methyl ester (FAMEs). The monodimensional analysis by GC-MS-RI confirmed that 2-nonanone and 2-undecanone are the predominant components in all the plan parts, followed by esters and monoterpenes. A different distribution of the main comopounds in the various plant parts depending on the life cycle of the plant (vegetative or reproductive stage) was observed. The multidimensional GC×GC analysis allowed for a complete screening of the fatty acids. The results indicated *R. chalepensis* as a good source of fatty acids from the w_3 and w_6 series. In both cases, essential oil or lipidic extract, many compounds were determined for the first time.

K.14

IMPROVED CIS/TRANS FATTY ACID ANALYSIS OF EDIBLE OILS AND FATS USING COMPREHENSIVE GC×GC–FID

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The determination of the fatty acid composition of edible oils and fats (as their methyl esters or FAMEs) is routinely performed in numerous laboratories all over the world. The level of detail that is required depends on the intended use of the data. For label-claim purposes a distinction between saturated, mono-unsaturated and poly-unsaturated fatty acids generally suffices. Studies on the health impact of fat consumption, on the other hand, require detailed information on chain length distributions, number and positions of double bonds as well as double-bond orientations (*cis vs. trans*). The standard method for *trans*-FAME analysis is capillary GC on a 50 to 100 meter highly polar cyanopropyl column (*e.g.* CP-Sil 88). With this technique *trans* an alysis is possible in relatively 'simple' samples such as untreated or mildly processed vegetable oils and fish oils. For more complex samples, more sophisticated methods, such as combined HPLC with silver-ion columns (Ag^+-HPLC) and GC, are needed. Such systems provide a good separation, but only at the expense of an increased complexity and longer analysis time. In this contribution we will demonstrate the powerful characteristics of comprehensive GCxGC with FID detection for *trans* fatty acid analysis of commercial fat blends and processed oil samples. The comprehensive GCxGC system applied combines two widely used columns in FAME analysis: the 100% methyl-silicone column in the first dimension and the cyanopropyl column in the second. The quantitative results are compared with results obtained by the current standard GC methods.

K.15

ENHANCED SCREENING OF ENVIRONMENTAL POLLUTANTS IN COMPLEX MATRICES BY GCxGC-TOFMS WITH VARIABLE-ENERGY ELECTRON IONISATION

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Two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOFMS) can provide highly sensitive detection and confident mass spectral identification of pollutants within complex environmental extracts. Nevertheless, the identification of individual compounds may be hindered by weak molecular ions or when similar mass spectral characteristics are evident across entire chemical classes. Select-eV ion source technology aims to combat this problem by allowing both hard and soft electron ionisation with no inherent loss in sensitivity. Select-eV provides enhanced molecular ions whilst retaining structurally-significant fragment ions, delivering both confident compound identification and increased selectivity. We show the potential of this technology for the analysis of both target pollutants and unknown chemicals in two different, complex extracts. The routine monitoring of water quality is now a requirement of environmental legislation, such as the EU's Water Framework Directive. Often the cause of a poor water quality status is unknown and extensive investigative monitoring is needed to determine what chemical maybe responsible. Passive sampling devices (e.g. semi-permeable membrane devices (SPMD), LDPE and silicone rubber) are often used for this purpose. The samplers were deployed for several weeks in a polluted river course in the UK to effectively sequester large volumes of water and provide a concentrated, representative extract for analysis by GCxGC-TOFMS with Select-eV. In a second study, explanted silicone breast prostheses obtained from patients over a wide age range were collected. Silicone oils in the prosthesis extracts were removed using a multi-step extraction procedure and the resultant extracts were analysed by GCxGC-TOFMS with Select-eV. This novel approach aims to better estimate the overall body burden of bio-accumulative substances and how this changes over time of exposure. This presentation shows the suitability of this novel analytical platform for environmental investigations, using both target-focused studies as well as non-targeted routines for screening for the presence of emerging contaminants.

K.16

DEVELOPMENT OF GC×GC-TOFMS METHODOLOGY FOR DIOXIN ANALYSIS IN A DEVELOPING ECONOMY

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Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) constitute three classes of structurally related chlorinated aromatic hydrocarbons. Due to their hydrophobic character and resistance to metabolic degradation these substances exist as complex congener mixtures in the environment. South Africa faces the challenge of establishing a dioxin capability for monitoring the environment and quantifying these complex congener compounds in environmental samples since the composition of these samples is different from many northern hemisphere samples due impart to different climatic conditions, geology and source contributions. Alternative methods of analysis for PCDD/Fs and PCBs have been investigated using comprehensive two-dimensional gas chromatography coupled with time of flight mass spectrometry (GC×GC-TOFMS) [1-2]. There are numerous challenges that have to be overcome to ensure separation of the PCDD/F and dioxin-like PCB compounds from each other and from matrix interferences and to allow accurate quantitative measurement below 500 fg for 2,3,7,8-TCDD as mandated in US EPA Method 1613 [3]. To establish a dioxin capability, the National Metrology Institute of South Africa (NMISA) undertook a study to develop and validate a GC×GC-TOFMS method for the analysis of the 17 WHO PCDD/Fs. A multi-step temperature programmed GC method was optimised, harnessing simultaneously the added selectivity of GC×GC, and the enhanced sensitivity afforded by the narrow peaks obtained from the modulation [4]. This is specifically for application in developing countries where access to gas chromatography - high resolution mass spectrometry (GC-HRMS) and highly skilled personnel is limited. Validation of the method included extraction and clean-up of South African soil samples using Total Rapid Prep™ System (FMS, Watertown USA) [5]. Direct comparison against results obtained using GC-HRMS provided further confirmation and good agreement was observed. The limit of detection (LOD) and quantitation (LOQ) for the method was determined and preliminary South African sample results are discussed.

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K.17

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHIC PROFILE OF VOLATILE COMPOUNDS RELEASED FROM CHESTNUT AND CHERRY WOODS INTO AN HYDROALCOHOLIC SOLUTION

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Volatile composition of wood is variable depending on several factors such as its botanical origin and/or toasting level. Wine characteristics may be improved by wood contact as wood components, including volatile compounds, are transferred to wine. On the search for alternative wood species with potential interest for oenological purposes our research is to evaluate the volatile composition of chips from chestnut and cherry as an alternative to oak wood, aiming to assess their impact on wine sensorial profile. The aim of this study was to evaluate the release of volatile compounds from chestnut and cherry woods chips with two different toasting levels (untoasted and medium toast). In order to avoid the matrix effect, an hydroalcoholic solution that mimics table wine was used. Twelve grams of each wood sample were extracted with 250 mL of the model wine solution during 35 days, in the dark at room temperature. Comprehensive two-dimensional gas chromatography–time of flight mass spectrometry (GC×GC-ToFMS) combined with headspace solid phase microextraction (HS-SPME) using a DVB/CAR/PDMS fiber was used, in order to search for differences between the two wood species under study, using the potentiality of this analytical technique. High sensitivity and chromatographic resolution of GC×GC-ToFMS allowed to establish a fingerprint profile from the different woods and toasting degrees, and to in-depth search for analytes that may be related to botanical species and to follow the toasting effect, since more information is obtained than by 1D-GC/MS.

Acknowledgments

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K.18

OPTIMIZATION OF A GCxGC-FID ANALYSIS METHOD TO QUANTIFY 8-HYDROXY-2'-DEOXYGUANOSINE

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8-Hydroxy-2'-deoxyguanosine (8-OHdG) is considered an important biomarker in oxidative DNA damage and it is associated to several carcinogenic and pathological diseases. 8-OHdG is generated from oxidative stress reactions and its trace amounts monitoring is normally performed by gas and liquid analytical methods. Two-Dimensional Gas Chromatography (GC× GC) is a highly sensitive method which has been applied in several studies where target analysis is aimed. The possibility to obtain an isolated peak in the two-dimensional chromatogram space, is an important advantage of bidimensional methods, especially when complex biological samples are analysed. After a derivatization step, 8-OHdG is GC chromatographed as a silylated derivative in an apolar column at a high eluting temperature. One of major problems in GC× GC analysis of 8-OHdG is the optimization of the modulation period and also the hot pulse time period in order to allow the compound release from the first to the second dimension column. Since 8-OHdG derivative elutes at a high eluting temperature there is a need to adjust properly the hot pulse time, among other chromatographic parameters, in order to avoid peak broadening and thus lack of sensitivity. This work aimed the analysis of 8-OHdG derivative using different modulation and hot pulse time periods. The best results were obtained when a 6 seconds modulation period with a 0.75 sec hot pulse was chosen for the analysis. Although eluting at a high temperature, the peak presents a good symmetry that will allow future quantitation analysis using GC×GC with FID detection.

K.19

CHARACTERIZATION OF PERSISTENT ORGANIC POLLUTANTS IN SUSPENDED SEDIMENTS BY THERMAL DESORPTION COUPLED TO GC×GC-TOFMS

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The objective of this study was to better understand contamination sources to the Niagara River by providing a comprehensive upstream/downstream comparison of the contamination profile over the past decade, while screening for emerging persistent organic pollutants (POPs). Archived samples of suspended sediment collected annually in January from 2005-2014 as part of Environment Canada's Niagara River Upstream/Downstream Monitoring Program were analyzed by thermal desorption coupled to comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS). For each analysis, a 10 mg sample was placed into a quartz tube plugged with glass wool and desorbed directly to the GC inlet using a pyroprobe 5200 (CDS Analytical); GCxGC-TOFMS analysis was performed on a Leco Pegasus 4D. This technique eliminated the need for traditional wet chemistry which is necessary for liquid injections. It also enabled the detection of a wide variety of POPs simultaneously in one chromatographic run, rather than the four separate experimental analyses routinely required by Environment Canada's monitoring program. Additionally, this non-target approach revealed a number of chemicals in these samples not currently on the list of monitored compounds. This poster will describe temporal trends in POPs from Niagara River suspended sediments over a 10 year period, and highlight the occurrence of a few classes of compounds not routinely monitored in this river.

K.20

EVALUATION OF GC×GC-QMS AND GC-GC-QMS FOR THE COMPLETE CHARACTERIZATION OF DIFFERENT GRADES OF GAHARU OIL

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Gaharu (or agarwood) oil is a highly valuable resinous oil extracted from the heartwood of *Aquilaria* species. Today, this aromatic oil has grown to be one of the most valuable traded essential oils, reportedly worth billions of US dollars annually. Interestingly, the composition and price of the oil varies considerably with the grade of the oil, however despite its high economic value, there are few detailed reports on the precise chemical composition of this oil and the different grades of oil. Challenges arise due to the diversity and complexity of the oils, in which gaharu oil formation is due to fungal or parasitic infection of the host tree in the wild, and exact metabolic pathways that lead to the formation of the resin are complex and remain largely unexplained. Hence, profiling of these oils is of great importance for bioanalysts to explore the distinctive interaction of microbial communities with the *Aquilaria* tree in a natural ecosystem, that produce these mixtures of compounds. Preliminary gas chromatography coupled with quadrupole mass spectrometry (GC-QMS) analysis of these oils clearly illustrates the complexity of these samples, where positive identification of many overlapped peaks is impossible. This has highlighted the requirement of an improved method that affords a much greater separation space for untangling phytoconstituents within these complex samples. Hence, the work presented will focus on complete characterisation of these oils using a series of multidimensional high-resolution GC techniques. The potential of comprehensive two-dimensional GC coupled with various MS approaches (GC×GC-QMS/QTOFMS) and heart-cut multidimensional GC coupled with QMS (GC-GC-QMS) for the study of these plant secondary metabolites will be discussed. The current work also aims to discover and identify the marker compounds that lead to the differentiation of the grades of Gaharu oils.

SI-MICROFABRICATED COMPREHENSIVE TWO-DIMENSIONSAL GAS CHROMATOGRAPHY WITH MICROSENSOR ARRAY DETECTION

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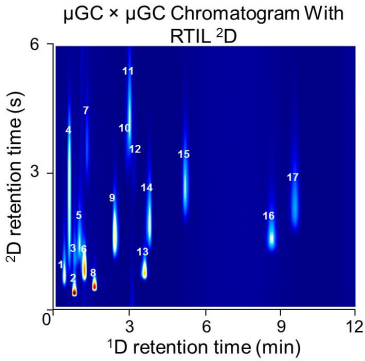
Initial performance tests of a Si-microfabricated μGC×μGC microsystem with microsensor array detection are described. The system includes etched-Si μcolumns (first- and second-dimension) and thermal modulator (μTM), as well as planar-Si 4-sensor chemiresistor array detector employing sorptive nanoparticle interface layers. The 1st dimension consisted of two μcolumns (each: 3 × 3 cm footprint; 3-m length; 250×140 μm i.d.; bonded Pyrex cap) with a crosslinked PDMS phase. The 2nd-dimension μcolumn (1.2 × 1.2 cm footprint; 0.5-m length; 150´50 μm i.d.) was coated with a trigonal-tricationic room-temperature ionic liquid (RTIL) or a crosslinked poly(trifluoropropylmethylsilicone) (OV-215) phase. The 0.8-cm^2 μTM chip has a series of two spiral μchannels (4.2 and 2.8 cm long; 250×140 μm i.d.), a bonded Pyrex cap, and a crosslinked PDMS phase. On-chip heaters provide heating of each modulator stage at ~ 2400 °C/s, and a thermoelectric cooler beneath provides constant cooling to a minimum of ~ -20 °C when the heaters are off. With the RTIL-coated second dimension mcolumn, deposited following a salt pretreatment, a mixture of C_7-C_{10} was tested isothermally and with a modest temperature program (in GC oven). Modulated fwhm values ranged from 150-680 ms (M_n = 2-5). In general, however, the RTIL phase was too retentive for polar analytes, giving fwhm values as high as 2.7 s for heptanal. An alternative pre-treatment method did not resolve this problem. Using the OV-215 μcolumn, good results were achieved, and a 36 compound mixture was separated in 22 min with fwhm values ranging from 90 to 478 ms, and breakthrough (minor) of only the most volatile analytes. The FID was replaced with a microsensor array, with peak widths increasing slightly depending on the sensor coating. Current efforts focus on expanding the analyte range accessible by independently programming the temperature of the μcolumns and μTM as well as alternative/additional microsensor options.



K.22

STUDY AND CHARACTERIZATION OF THE CHEMICAL COMPOSITION OF BIO OILS OBTAINED THROUGH CATALYTIC PYROLYSIS OF SUGAR CANE BAGASSE VIA GC/MS AND GCxGC/TOF-MS

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The increasing demand for renewable fuels and low costs have created processes capable of transforming biomass into substitute products to fossil fuels. Pyrolysis is a viable alternative that converts lignocellulosic residue into products that can become fuels. Furthermore they can also be used as raw material for the polymer industry and fine chemistry. However, the bio oil is highly oxygenated and presents low energetic density and low chemical stability. In order to minimize these issues processes must be carried out that reduce the presence of these compounds. A methodology with potential to reach this objective is pyrolysis, which uses catalyzers, that improves the quality of the bio oil and also slows the process using lower temperatures and smaller time reactions. The products of the biomass pyrolysis are generally a complex mixture of components, which makes it hard to identify when using the classic unidimensional chromatographic technique GC/MS. Therefore, a more sensitive and efficient technique is required such as GC x GC/TOF-MS. This work investigated the effects of the CaO, ZnO and FCC catalyzers in the pyrolysis of the sugarcane bagasse. The mixtures of bagasse/catalyzer were prepared in the proportion of 10% (m/m) of catalyzer. The obtained bio oils were analyzed through GC/MS and GC/TOF-MS in order to identify their composition. The analyses through GC/MS and GCxGC/MS presented differences amongst the generated products. The use of catalyzers provided the possibility of altering the proportion of the oxygenated compounds of the products from the pyrolysis of sugarcane bagasse and reducing the concentration of carboxylic acids suggesting that the use of catalyzers reduces the acidity of the product. The employment of catalyzers specially the FCC reduced the content of aromatics showing itself to be efficient in the degradation of lignin which is the component of the lignocellulosic biomass more thermally stable.

K.23

STUDY AND CHARACTERIZATION OF THE CHEMICAL COMPOSITION OF BIO-OILS OBTAINED FROM DIFFERENT SOAPS FROM MACAUBA FRUIT OILS VIA GC/MS AND GCxGC/TOF-MS

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Pyrolysis of triglyceride materials is not well established as other biomass source. A natural source of a composite mixture of triglycerides (TGs) is the oleaginous plants. Here it was studied the oils from Macauba fruit(Acrocomia *sclerocarpa* M), whose are located mainly in the centre of the country and, in particular, in the state of Minas Gerais. In the present work it was carried out the pyrolysis of different soaps from Macauba fruit oils using different basic reagents. It was investigate the influence of this reagents in the in the yield and composition of the pyrolysate, the bio- oil. The oils studied were characterized from their physicochemical properties and the chemical composition of the oils in terms of their methyl esters derived from fatty acids, which was carried out in accordance with international standards methods. The samples were prepared using the oils of the pulp (OP), nut (OA) and a mixture(1:1)(MIX) from Macauba fruit, which were mixed stoichiometrically with various reagents with basic character such as Ca $(OH)_2$, NaOH, KOH and ZnO to obtain their respective soaps. These were subjected to thermal analysis (TGA and DTG) under inert atmosphere (N_2) to obtain the optimum cracking temperature of samples to carry out the pyrolysis. Then, samples were pyrolyzed in a tubular furnace at 500ºC and 600ºC for 60 min. The condensed fraction, bio-oil, was collected and analyzed for its characterization by GC/MS and GCxGC/Tof- MS. GC/ MS and GC x GC/Tof-MS analyzes showed that the bio-oils obtained have a very complex and similar profile having in their composition the presence of aliphatic and cyclic alkanes, alkenes and cycloalkenes, aromatics, and other oxygen-containing compounds such as alcohols and ketones. These preliminary studies show that the bio-oil obtained in these conditions were very promissory to be used as an alternative fuel.

EVALUATION OF DIFFERENT COLUMN COMBINATIONS IN GCxGC-TOF MS ANALYSIS OF ORGANIC ACIDS IN HUMAN URINE

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The effect of different column combinations on the GCxGC-TOF MS analysis of organic acids in human urine was studied. Urine sample after ethoximation, acidic extraction and trimethylsilylation was analysed on six column sets: two conventional non-polar/polar sets BPX5/SolGel-WAX and BPX5/SLB IL-59, two "reversed-type" column combinations BPX50/BPX5 and SolGel-WAX/BPX5, one midpolar/polar BPX50/SolGel-WAX and one polar/midpolar SLB IL-59/BPX50. To establish the mass loadability, two different internal diameters (0.10 and 0.25 mm) of secondary column were used. To assess the performance, sixteen metabolites spread across the two-dimensional chromatogram were chosen (Fig. 1). Among these metabolites there were cases of partly coeluting peaks in the first and/or second dimension. The degree of orthogonality was evaluated using conditional theory approach [1], which considers quantitative distribution of peaks in the entire 2D separation space and off-diagonal correlations between two separation mechanisms. To determine the mass loadability of D2 column, second dimension peak width and asymmetry of deuterated methylmalonic acid was evaluated. Neither polar/non-polar nor polar/midpolar column combinations led to a useful separation. Using the wide-bore thicker film secondary columns improved orthogonality of non-polar/polar sets, whereas decreased orthogonality of midpolar/non-polar set. The best separation was obtained using the set BPX5/SolGel-WAX with internal diameter of the D2 column 0.25 mm (orthogonality 69.17 %, deuterated methylmalonic acid peak width 4 σ 0.11 s, asymmetry 1.12).

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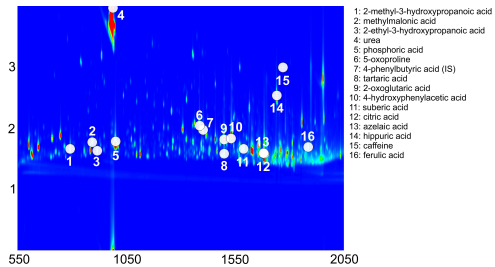

K.25

ADVANCED SCRIPTING FOR AUTOMATED PROFILING OF TWO-DIMENSIONAL GAS CHROMATOGRAPHY-TIME-OF-FLIGHT MASS SPECTROMETRY DATA FROM COMBUSTION AEROSOL

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Multidimensional gas chromatography is an appropriate tool for non-targeted and comprehensive characterization of complex samples generated from combustion processes. Especially the particulate emission is composed of a large number of compounds such as condensed semi-volatile organic compounds (SVOCs). However, the complex amount of information gained from such comprehensive techniques is associated with difficult and time consuming data evaluation. Because of this obstacle two-dimensional gas-chromatography is little used in the field of aerosol science. To cope with this, advanced scripting algorithms based on knowledge based rules (KBR) were in house developed and with the usage of the LECO ChromaTOF® scripting feature applied to GCxGC-TOFMS data. Previous reported KBR and newer findings were considered for the development of those algorithms. The novelty of the presented advanced scripting tools is a very selective search criteria for data screening which is primary based on fragmentation patterns and the presence of specific fragments. Combined with “classical” approaches, based on retention times, a fast, accurate and automated data evaluation method was developed,which was qualitative and quantitative evaluated for Type 1 and Type 2 errors. The applicability was further tested for filter samples obtained from ship fuel combustion. Major substance classes like polycilc aromatic hydrocarbons (PAH), alkanes, benzenes, ester, ethers etc. can be targeted. This approach allows the classification of about 75% of the peaks of interest within real samples. Different conditions of combustion like fuel composition and engine performances could be clearly characterized and differentiated.

K.26

DEFINITION OF QUANTIFICATION AND DETECTION LIMITS FOR GC×GC PART II: EXPERIMENTAL DATA

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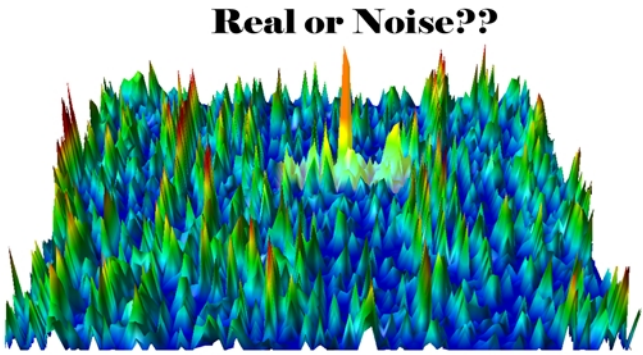
Some of the most powerful techniques for the analysis of complex samples are the comprehensive multidimensional separations (e.g. GC×GC, LC×LC, etc.). Comprehensive two-dimensional gas chromatography (GC×GC) is the most well-established of these techniques and has been applied in many different fields (e.g. enviromental, food, metabolomics, etc.) with excellent results. This technique offers lower quantification and detection limits (LOQ and LOD) than one-dimensional techniques; however, new sources of error introduced by the modulation process are inherent to multidimensional separations. These new errors fundamentally change the calculation of LOD and LOQ for these types of separations. Theoretical results were shown in previous work; in this contribution the concepts were applied to experimental data from both univariate (e.g. FID) and multivariate (e.g. TOFMS) detectors on a GC×GC system. These experimental results confirmed the theoretical results obtained in the first work. To make the results transportable to any result where quantification using a two-step algorithm is emloyed, these are expressed in terms of the dimensionless paramenter of signal-to-noise ratio of the base peak (S/N_{BP}). The need to consider the effects of tailing peaks in both ^{1}D and ^{2}D are highlighted in this work.



K.27

CHARACTERIZATION OF IONIC LIQUID COLUMNS FOR USE IN GC×GC SEPARATIONS

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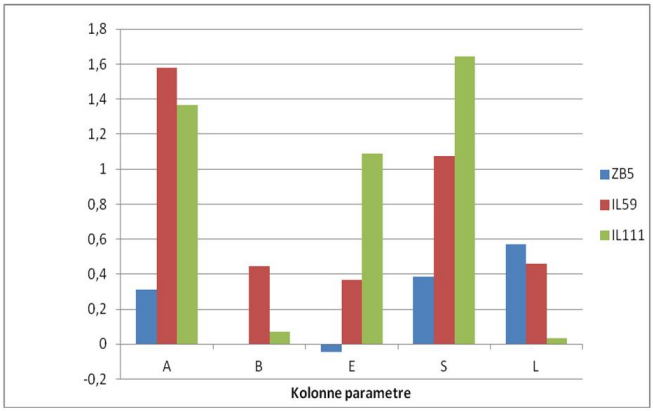
Having access to GC columns where the stationary phases have different selectivities can be helpful when doing GC, and is essential to GC×GC. The selection of practical stationary phases for GC have long been limited to silicones with limited polarity and variation in selectivity, and the more polar polyethylene glycol (PEG). Recently, ionic liquid (IL) columns have come to be an alternative that offers a much wider variety in polarity and selectivity. However, a wider array of stationary phases also complicates column choice, so tools to assist this selection is needed. One such tool is the Abraham solvation model, which can be used to classify and quantify the interactions between GC columns and analytes. It quantifies the interactions between column and solute by dividing them into 5 terms: *A* describes hydrogen bonds where the column is the base, *B* describes hydrogen bonds where the column is the acid, *E* describes interactions between lone pairs and polarizable molecules, *S* describes dipole-dipole and dipole-induced dipole interactions, and *L* describes non-polar interactions (see figure). We determined the Abrahams solvation parameters for several IL columns over a temperature range. As the relative retention times in GC×GC can be predicted from these parameters for the two columns, this allows for prediction of the retention patterns for different samples. This can be used as a guide for selecting the columns that provide the best separation. In addition to being more polar than even PEG columns, the IL columns show exiting selectivities, with one of the acting as a hydrogen bond acid, something that very few GC other GC columns can do. Furthermore, the parameters can be interpreted, and the differences and similarities between different selectivities can be investigated in regards to the chemical structure of the ionic liquids.



K.28

PIXEL-BASED INVESTIGATION OF THE BEHAVIOR OF HYDROCARBONS DURING PETROLEUM HYDROTREATMENT

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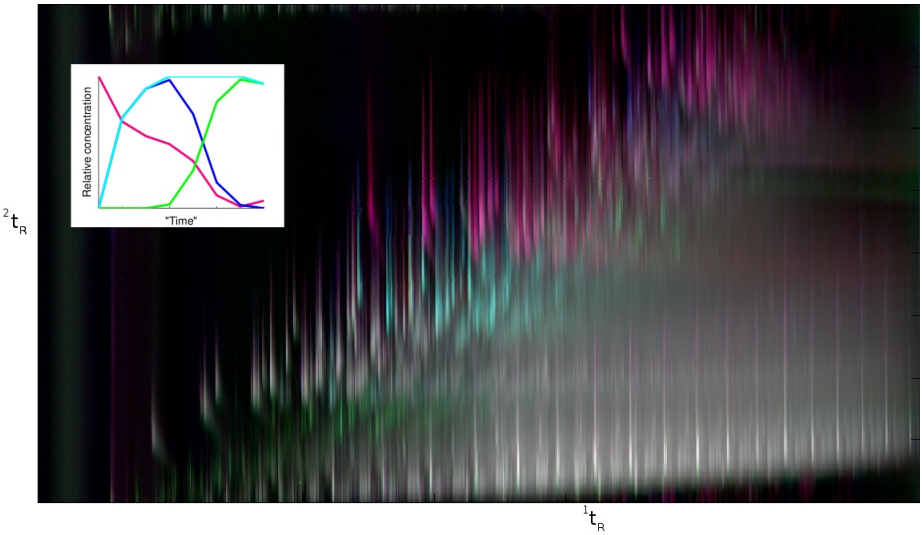
Data from comprehensive two-dimensional gas chromatography (GC×GC) can be analyzed by multivariate statistical methods using the intensity at each retention time pair as variables. As these variables represent pixels in a color plot, this can be called pixel-based analysis. The data must be processed to remove non-sample variations before analysis. Eight samples from a hydrotreatment experiment were investigated with GC×GC-FID. Several causes for non-sample variation were identified and handled: The background was modeled and subtracted from the chromatograms, the intensities were log transformed to remove the undue influence of random variation in the intensity of the most intense peaks, and the intensities were scaled with the relative standard deviation of replicate chromatograms of identical samples. After removing non-sample variation, the intensities were modeled with archetypal analysis, revealing the behaviors of all hydrocarbons during hydrotreatment without quantifying any compounds (see figure). This approach provides an overview of the behavior of many compounds in complex samples without any identification or quantification steps.



K.29

EXHALED AIR ANALYSIS FOR EARLY DETECTION OF LUNG CANCER

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The curability of lung cancer is highly dependent of the early diagnostic [1]. However, lung cancer is typically silent early in its course. Thus, the majority of patients are diagnosed at an advanced stage, resulting in poor prognosis [2]. There is, therefore, an urgent need to develop earlier diagnostic screening tests allowing detection of lung cancer at a more curable stage. Since Pauling's early chromatographic separation on breath specimen, many studies have focused on lung cancer exhaled biomarkers identification. The volatile organic compounds (VOC) content of the gaseous phase was typically analyzed using GC-MS. A limited number (15 to 30) of VOCs (mainly alkane and benzene derivatives) has been identified by this method as part of a lung cancer VOC profile [3]. However, the complexity of exhaled breath VOC profile requires the use of comprehensive two dimensional gas chromatography coupled to time of flight mass spectrometry (GC×GC-TOFMS) to go deeper in analyte separation and identification. In this work, we used TD-GC×GC-TOFMS to compare breath VOC profiles between patients diagnosed with lung cancer and healthy controls. The separation was followed by a non-targeted approach for data processing. Supervised multivariate and univariate statistical approaches were used to identify potential biomarkers. These candidates were, then, compared to the ones previously reported in the literature using a classical GC approach.

References

[1] C. Henschke, D. Yankelevitz, D. Libby *et al.*, New Engl J Med (2006)
[2] P.J. Mazzone, Breath Res (2008)
[3] H. Chan, C. Lewis, P. Thomas, Lung Cancer (2009)

K.30

PIXEL-BASED CHARACTERIZATION OF PETROLEUM FEEDS

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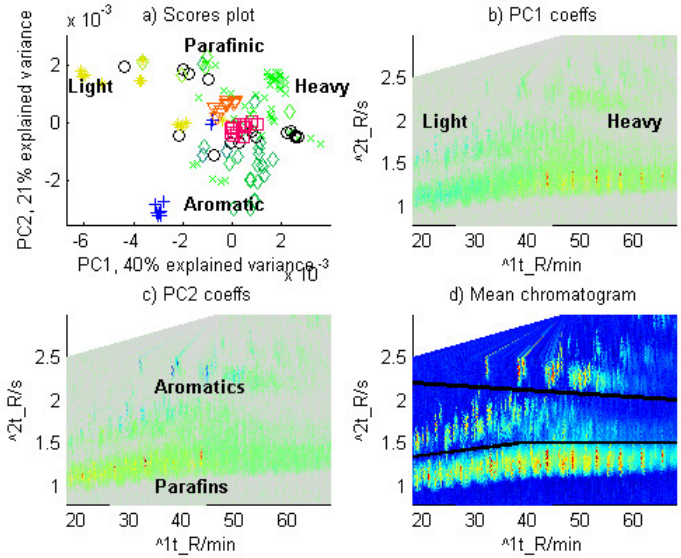
Data from comprehensive two-dimensional gas chromatography (GC×GC) can be analyzed by multivariate statistical methods using the intensity at each retention time pair as variables. As these variables represent pixels in a color plot, this can be called pixel-based analysis. The data must be processed to remove non-sample variations before analysis. 75 different petrochemical samples were analyzed with GC×GC-ToF-MS. These were mostly light gas oils, light cycle oils and kerosenes, and mixtures of such samples. Several causes for non-sample variation were identified and handled: The extracted ion chromatograms were filtered to reduce noise and background, the chromatographic shift was estimated and the chromatograms aligned, and the chromatograms were scaled according to the relative standard deviation of replicate chromatograms of identical samples. After removing non-sample variation, the chromatograms were analyzed with principal component analysis to characterize the samples and describe how they were different. The first PC separated the samples based on volatility, while the second PC described their parafinicity/aromaticity (see figure). Together, these two PCs described kerosenes (yellow in subfigure a) as light and parafinic, the gas oils (green in subfigure a) as heavy and parafinic, light cycle oils (blue in subfigure a) as aromatic, and placed the mixtures between these groups. Further PCs detailed the volatility differences and parafinicity/aromaticity. This approach is useful in quickly getting an overview of the most important difference between complex samples without the need for manual identification and quantification steps.



K.31

PIXEL-BASED ANALYSIS OF THE KINETICS OF PETROLEUM DESULFURIZATION

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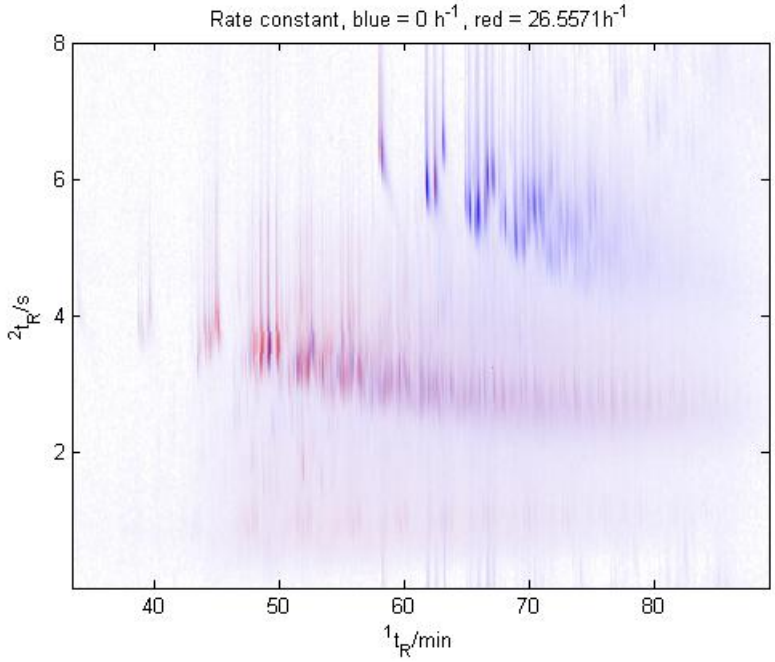
Data from comprehensive two-dimensional gas chromatography (GC×GC) can be analyzed by multivariate statistical methods using the intensity at each retention time pair as variables. As these variables represent pixels in a color plot, this can be called pixel-based analysis. The data must be processed to remove non-sample variations before analysis. Eight samples from a hydrodesulfurization experiment were investigated with GC×GC-SCD. Several causes for non-sample variation were identified and handled: Spikes (single pixels with much higher intensity than their neighbors) were found and removed, the background signal was modeled and subtracted from the intensities, and chromatographic shift was estimated and the chromatograms aligned. After removing non-sample variation, the development of intensity at each ^1t_R, ^2t_R pair (pixel) was modeled with a first order kinetic model. In this way, the kinetics of many species was determined without quantifying any of them. The result can be presented as a color plot (see figure), making it easy to obtain an overview of the kinetics of all compounds in the samples. This approach can be used to determine the kinetics of many compounds in complex samples without any identification or quantification steps.



K.32

DETAILED VOLATILE ORGANIC COMPOUNDS ANALYSIS BY SPME/GCxGC COMBINED WITH HIGH-RESOLUTION MASS SPECTROMETRY

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Solid phase micro extraction (SPME) is a well-known preparation technique that can easily and effectively extract volatile organic compounds from liquid, solid and gas samples. Additionally, comprehensive two-dimensional gas chromatography (GC´GC) is a well-known technique for separating large numbers of chemical components within a given sample. For this work, we measured alcoholic beverages using a SPME/GCxGC/high resolution high resolution time-of-flight mass spectrometry (HRTOFMS) system for the detailed analysis of volatile organic compounds. The addition of the HRTOFMS as the GCxGC detector further enhances the analysis technique by providing accurate mass measurements for the detected ions. As a starting point, we measured two tequila samples using a SPME/GCxGC/EI method. The anejo tequila had 409 chemicals detected in the 2D TIC with 236 of them (57.7%) identified with match factors of over 700, which is typically sufficient for reliable chemical identification. As for the gold tequila, 291 chemicals were detected with 141 of them (48.5%) identified with match factors of over 700. As an example, one of the compounds identified during the NIST search, linalool, is a naturally occurring monoterpene alcohol that is found in many plants. This compound is widely used as a flavoring agent for many kinds of foods. The molecular ion for linalool was not observed in the EI mass spectrum. However, the fragment ion resulting from the dehydration of the molecular ion ($[M-H_2O]^+$) was observed and showed a mass accuracy of 1.57 mDa compared to the calculated value for $C_{10}H_{16}$ while using an external one-point drift compensation for the mass calibration. A full range of compounds detected in the samples will be presented to show that the SPME/GCxGC/HRTOFMS analyses produce normal EI mass spectral fragmentation patterns and high mass accuracy information, which help to further confirm the identity of unknown compounds through elemental composition calculations.

K.33

NEW METHOD TO SIMULATE A GC×GC CONTOUR PLOT OF A COMPLEX SAMPLE. EMPIRICAL PREDICTION OF CONTOUR PLOTS BASED ON SINGLE COLUMN GC-MS ANALYSIS.

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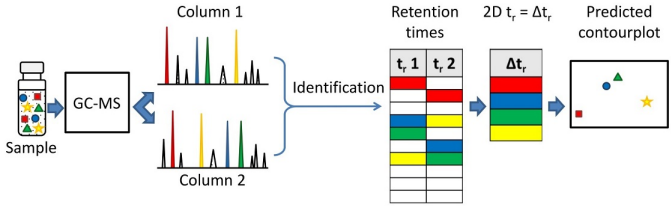
Many ^{1}D-^{2}D column sets are available to optimize the analytical method, but it is laborious and time consuming to test all possibilities. Here, a new prediction model is proposed to simulate the contour plot of a defined column set. By simply analyzing the sample on each of the two different columns by GC-MS, it is possible to construct a simulated contour plot. The retention times (t_r) of identical components are used to calculate the t_r difference (Δt_r). Next, Δt_r is plotted versus the ^{1}D retention time of the first column.
This application was tested for a sample of a lignin oil. The comparison of the predicted and measured contour plot of the sample on several column sets will be shown. Finally, the GC×GC analysis on a polar-apolar column set (Rtx-1701; 14% cyanopropyl versus Sil-5 MS; 5 % phenyl) was found the best to characterize the various chemical classes and functional groups. By using the prediction model fast insights and good impressions of the contour plots were obtained.



K.34

IDENTIFICATION OF FUELS CONTAINING FAME IN ANALYSIS OF FUEL SPILLS USING 2D-GCxMS

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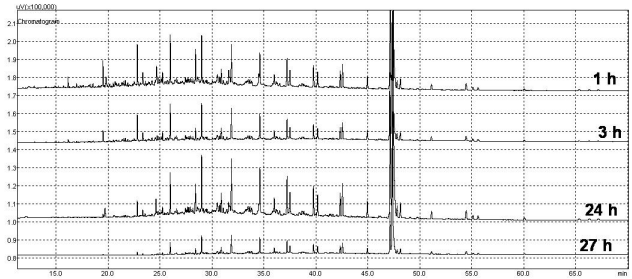
One of the most promising alternative fuels at the present moment is biodiesel consisting on the mixture of fatty acids monoalkyl (methyl or ethyl) esters (FAME) from vegetable oils and animal fats due to its renewable character and environmental-friendly properties. Fuel blends containing FAME are used in different countries, and FAME contents should be controlled both at everyday monitoring and in the extraordinary situations such as fuel spills. The aim of the present work is development of the chromatographic method allowing monitoring of the fuel profile in the spill on the water surface for the unambiguous identification of the fuel type together with the possible spill time. The FAME mixture was obtained from the rape oil, the diesel sample was the commercial fuel. All the chromatographic experiments were performed using the 2D-GCxMS system Shimadzu 2010 Plus equipped with the Deans switch between two chromatographic columns. The first dimension column was equipped with FID, the second dimension column was equipped with MS detector. 2D-GCxMS method allows the investigator to separate the compounds of the petroleum and vegetable origin even when they are coeluted in the one-dimention method. This fact makes possible the identification of the fuel blends even containing the minor amounts of FAME in petrodiesel. Five types of fuel blends (B20, B10, B5, B2.5, B1.25) were spilled in the model experiments on the water surface, and changes in fuel profiles were monitored depending on time as it is shown in the Figure. Also the characteristic ratios were determined for all the fuel blends at all the control times.

Fig. 1. Changes in the chromatographic profile of the B20 fuel blend depending on the spill time.



K.35

CEMS: CONSENSUS ESTIMATION OF MULTI-DIMENSIONAL SHIFT, A NEW TOOL FOR ALIGNING GC×GC CHROMATOGRAMS

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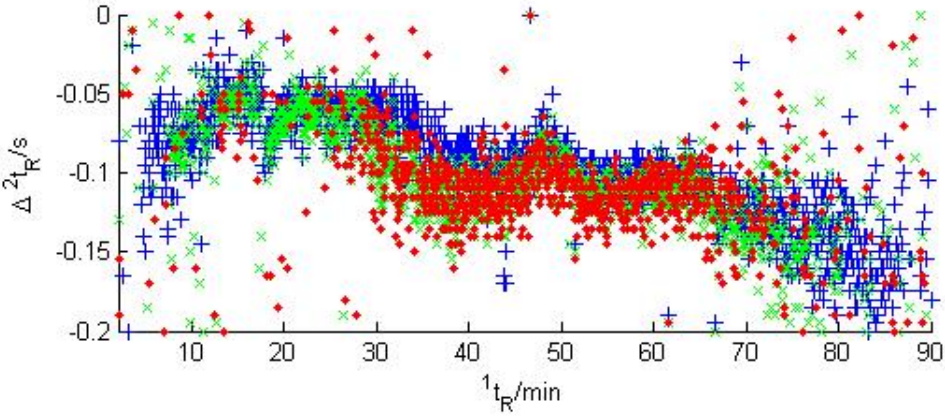
Retention time shifts complicate the analysis of chromatographic data. This is particularly problematic for comprehensive two-dimensional chromatography, where shifts can not only occur along two time axes, but may be correlated between the two chromatographic directions. In this study, a novel approach to correct for shifts in comprehensive two-dimensional gas chromatography (GC×GC) was developed: The Consensus Estimation of Multidimensional Shift (CEMS). CEMS uses Peak Alignment by Fast Fourier Transform (PAFFT) to determine the optimal shift for each 2D chromatogram and mitigates possible mistakes by using neighboring values to find a consensus correction value. This approach is valid as closely eluting compounds will exhibit similar shift patterns. We show that different hydrocarbon compound classes have similar 2D shifts (see figure, which shows the ^2t_R shift as a function of ^1t_R for alkanes (blue pluses), monoaromatics (green ×'es) and diaromatics (red dots). We also show CEMS to be fast and effective in removing 2D shifts from several sets of GC×GC chromatograms.



K.36

CARBOXYLIC ACIDS CAN BE USED FOR CHEMICAL FINGERPRINTING OF CRUDE OIL USING GC×GC-MS AND CHEMOMETRIC DATA ANALYSIS

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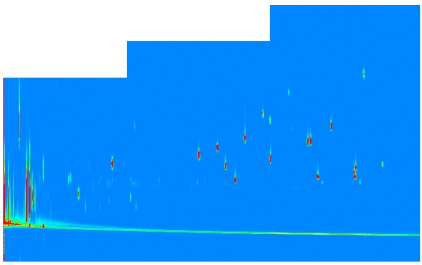
Crude oil carboxylic acids have been an overlooked group of compounds with respect to chemical fingerprinting of crude oils. Most frequently used constituents for oil spill identification have been individual hydrocarbons including *n*-alkanes (C_8-C_{40}), volatile BTEX, the EPA priority parent polycyclic aromatic hydrocarbons (PAHs) and alkylated homologs (C_1-C_4) of selected PAHs. Naphthenic acids (including both aliphatic and aromatic acids) are found in crude oil. These acids can also contribute to a significant percentage of weathered oil, as they are produced during microbial degradation of hydrocarbons. Furthermore, aromatic acids have been found by Malmquist et al. and Boll et al. to be produced from biodegradation of alkyl substituted PAHs. The recalcitrance towards microbial degradation makes the persistent acids well suited for oil spill identification. The aim of this study has, therefore, been to isolate aliphatic and aromatic acids from crude oil, analyze them with comprehensive two-dimensional gas chromatography-mass spectrometry (GC×GC-MS), and apply chemometric data analysis to compare crude oils from different sources. This is done to investigate whether this compound group has a potential to be used for oil spill identification. Here, we present a GC×GC-MS method for chemical fingerprinting of crude oil. Carboxylic acids have been isolated from 21 crude oils by strong anion exchange (SAX) solid phase extraction (SPE) and silylated using the derivatization agent BSTFA before GC×GC-MS analysis. Isolation of acids has been tested on different SPE columns on a mixture of 19 known acids to obtain retention of aliphatic and aromatic mono- and di-carboxylic acids. Finally, a method for pixel based analysis of entire chromatograms will be tested. The figure shows a GC×GC-ToF MS chromatogram of 19 acids (aliphatic and aromatic mono- and di-carboxylic acids) derivatized with BSTFA. Column combination was BP5 (25m×0.22mm×0.25µm) × BP50 (1.5m×0.1mm×0.1µm).



K.37

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY FOR THE ANALYSIS OF IRRADIATED SPICES

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Rosemary, thyme and black pepper (in ground form) were packaged under 100% N_2 (modified atmosphere packaging; MAP) or ambient air (aerobic packaging; AP). The packaged samples were gamma-irradiated at 7 kGy, 12 kGy or 17 kGy. Non-irradiated (0 kGy) samples were used as contols. After the irradiation process, the essential oils of the spices were obtained by hydrodistillation. Combined effects of irradiation and packaging on essential oil composition were obtained and contrasted by using comprehensive two-dimensional gas chromatography (GC×GC); GCxGC analysis of essential oils was conducted by using a GC coupled with an FID with modulation performed using a longitudinally modulated cryogenic system (LMCS). The identification of the unknown peaks were accomplished using a GC with a Q-TOFMS detector and an LMCS. A significant difference was observed in the region of oxygenated compounds, with three peaks noted after irradiation of air packaged samples at all doses by using GC×GC-FID. The observed peaks were then identified by using GC×GC-Q-TOFMS as 4-(1-methylethyl)-1,3-cyclohexadiene-1-methanol, cuminaldehyde and 2-caren-10-al. These peaks did not significantly change in essential oils of samples packaged under 100% N_2, compared with non-irradiated controls Thus, it was concluded that irradiation caused the formation of new compounds or promoted oxidation of the essential oil, and that these changes are limited when O_2-free packaging is employed. The use of GC×GC for detection of irradiated rosemary, black pepper and thyme was successful, and it provided both better separation and improved presentation of the total volatile oil composition compared to 1D GC. In conclusion, comprehensive two-dimensional gas chromatography exhibits excellent potential for the comparison of irradiated spices with their non-irradiated counterparts, and MAP suppresses the effects of oxidation of the spices, according to their volatile profiles.

K.38

QUALITATIVE AND QUANTITATIVE STUDY OF ORGANIC COMPOUNDS IN CRUDE OILS WITH/ WITHOUT CATALYST TREATMENT BY USING COMPREHENSIVE TWO-DIMENSIONAL GC - HRTOFMS

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Petroleum products are produced through several purification processes from crude oil. Qualitative and quantitative analysis of organic compounds in purified oil are important to confirm the effect at each purification step. In particular, identification of organic compounds in crude oil and its desulfurized oil are very important. However, it is difficult to analyze small amount of organic compounds by using conventional gas chromatograph-mass spectrometer (GC-MS), because chromatographic separation is insufficient due to large amount of chemical matrices. Recently, comprehensive two-dimensional gas chromatograph (GCxGC) - time-of-flight mass spectrometer (TOFMS) is using to get the high chromatographic resolution. In this study, we attempted to identify organic compounds in crude and its desulfurized oil by using GCxGC-high resolution TOFMS (GCxGC-HRTOFMS) which has both of high chromatographic separation and high mass resolving power. At the result, it was clear that 2D maps of LGO (light gas oil) and LCO (light cycle oil) were different. LGO contained large amount of chained hydrocarbons and thiophenes. On the other hand, LCO contained thiophenes, indenes and naphthalenes. In contrast, desulfurized LGO and desulfurized LCO showed similar 2D maps except for peaks of chained hydrocarbons. The quantity of thiophenes and naphthalenes has decreased in both of desulfurized oils but indenes were characteristically observed. This result suggested that indenes were produced from thiophenes and naphthalenes by the catalyst reaction. In addition, amount of benzothiophene homologues (BTs) and dibenzothiophene homologues (DBTs) were dramatically changed before and after catalyst treatment. As a result, 97% of BTs and DBTs in LCO were removed in the catalytic desulfurization process. It was suggested that analysis method by using GC×GC-HRTOFMS has ability to do qualitative and quantitative analyze of organic compounds in crude oil without any pretreatment. Therefore, GC×GC-HRTOFMS is one of the good systems for the process management of purification in the petroleum industry.

K.39

GCxGC-TOF MS ANALYSIS OF PETROCHEMICAL SAMPLES IN "REVERSED SETUP": FINE TUNING OF THE SEPARATION AND DATA PROCESSING STRATEGIES

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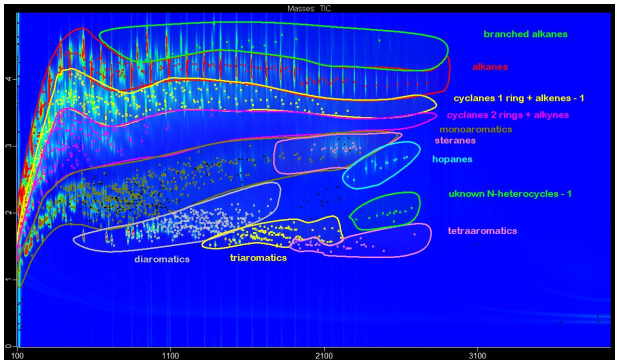
Petrochemical samples have always been a challenge for GC separation. Because of their complexity, the separation by one separation mechanism (one column) does not bring sufficient resolution of the sample. GCxGC, on the other hand, utilizes two columns with different selectivity of stationary phase, and therefore the sample „dimensionality" can match with separation „dimensionality" which results in dramatically increased separation power. The aim of this study was to optimize the GCxGC parameters for three different types of samples (i) crude oil, (ii) vacuum light gas oil (fraction: 370-430 °C) and (iii) vacuum residue (fraction +550 °C). For the analysis of high boiling samples such as vacuum light gas oil and vacuum residue, the temperature stability of the selected columns is very critical. In GCxGC, second column is exposed to higher temperatures in the modulator and secondary oven than the first column. From this point of view, the use of polar column (which usually has less temperature stability) for the first dimension is more convenient. Besides this, the reversed column set-up has been shown to provide better separation of saturated from unsaturated and cyclic structures. In this study, the following column set has been used: 1st dimension: Rxi-17Sil, 15 m x 0.25 mm, 0.1 µ m film, max. programmed temperature 360°C (Restek, USA); 2nd dimension Rxi-5HT, 1 m x 0.25 mm x 0.1 um, max. programmed temperature 400°C (Restek, USA). Using a relatively short first dimension column with thin film resulted in "reasonable" elution temperatures even for high boiling fraction. At the same time efficient separation of the structural groups has been achieved in the second dimension. Non-typically both columns were of same internal diameter, which is beneficial for the separation efficiency, as the same optimal flow can be used for both first and second column.

Figure below shows the optimized separation of crude oil. In this poster some aspects of fine tuning of some GCxGC parameters such as secondary oven offset and hot pulse for modulation will be discussed. Also the application of advanced software features such as classifications or data summary tables in this practical application will be demonstrated.



K.40

ANALYSIS OF ELECTRONICS WASTE BY GCxGC COMBINED WITH HIGH-RESOLUTION MASS SPECTROMETRY: USING EXACT MASS INFORMATION TO EXPLORE THE DATA

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Comprehensive two-dimensional gas chromatography (GCxGC) in combination with high-resolution mass spectrometry (HRMS) is a powerful tool for the analysis of complex mixtures. However, new software tools are required to facilitate the interpretation of the rich information content in GCxGC/HRMS data sets. In this work, we analyzed a dust sample collected from an electronics recycling facility by using GCxGC in combination with a new high-resolution time-of-flight (TOF) mass spectrometer. A composite mass spectrum was created by summing the mass spectra for all components in the GCxGC/HRTOFMS analysis. Halogenated contaminants were readily recognized by their mass defects. The mass defect plots facilitated rapid identification of families of compounds that differ by the number of chlorine and bromine substituents. This approach also helped guide the analysis of the chromatographic data. Mass chromatograms were generated based on specific ions identified in the plots as well as regions of the plots predominantly occupied by halogenated contaminants. Tentative identification of specific contaminants was aided by database searches and elemental composition determinations from the exact-mass data. Software tools that incorporate nontraditional Kendrick mass defect plots greatly enhanced the interpretation of the GCxGC-HRTOFMS data. The details of these results will be reported for this presentation.

VOLATILE SECONDARY METABOLITES FROM ORGANIC APPLES BY HS-SPME IN COMBINATION WITH COMPREHENSIVE TWO-DIMENSIONAL GC-MS

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Trentino Alto Adige is a small region in northern Italy that produces about 1.5 M of tons of apples per year corresponding to about 15% of total EU production [1]. The high production density brings a burden for soil exploitation. In the last decade efforts have been done to make apple production more eco-sustainable by adopting organic farming practices. Organic farming is a production system which avoids or excludes the use of synthetic preparations – artificial fertilizers, pesticides, growth accelerators and fodder additives [2]. Furthermore, recent works showed a positive willingness to purchase organic apples and consumer preference for apples organically rather than conventionally produced [3-5] providing important information about market opportunities. Despite this, many producers are afraid about lower sensory quality of organic fruits. Instrumental and sensory analyses were applied to investigate the impact of organic farming on apple quality. Fruits from several apple varieties grown, in different parcels of the same field, using organic and integrated production systems were compared. Here we present preliminary data on volatile secondary metabolites obtained by HS-SPME in combination with comprehensive two-dimensional gas chromatography coupled to a quadrupole MS (HS-SPME GCxGC-MS). Data analysis of the 63 most abundant identified peaks shows statistical significant differences in volatile secondary metabolites emitted by apples grown under organic production compared to the traditional one. Fig 1 shows separation of the 2 theses (bio: Organic farming; trad: integrated production system). For example apples grown under organic practice emit more α-farnesene than conventionally produced fruits.

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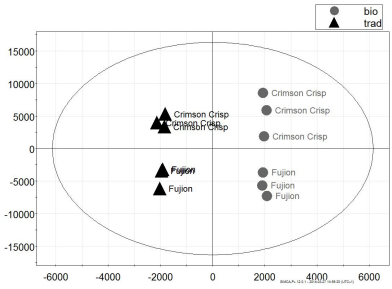

K.42

TRAPPIST BEERS: EXPLORING THE CLOAK OF SECRECY BY GC×GC-TOFMS

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Among the thousand of existing types of beer are the Trappist beers. They are famous for their special taste and for their great quality. Because they are brewed in respect to ancestral monk traditions, they are often rare and difficult to find on the market, which further enhance their image of high end product. Today, only ten abbeys provide Trappist beers in the world: 6 in Belgium, 2 in the Netherlands, 1 in Austria and 1 in USA. All together, these 10 abbeys produce 29 different types of beers. Even though basic ingredients – malted barley, hops, water, yeast – involved in the preparation of the beer are the same, the typical nature of these beers arises from an historic brewing know-how used to transform raw materials in aromatic and tasty beers. Aromas in beers are known to orientate mainly from malted barley [1] (due to barley itself as well as the thermal treatment during malting), hops [2], yeast [3-4], and to develop during maturation and aging [5-6]. In the quest for a better understanding of the ancestral processes, we started to investigate the volatile organic compound (VOC) composition of this family of beers, by mean of GC×GC-TOFMS, in the hope to highlight differences and common typical patterns among samples. For the first part of this study, two Trappist abbeys were selected and the VOC profiles of their beers were characterized. The Chimay's and the Rochefort's beers were sampled by solid phase micro-extraction (SPME) and analyzed by GC×GC-TOFMS. This sample batch represented replicates of 6 different beers, 3 from each abbey. Various statistical approaches have been tested and applied to differentiate volatile makers of each beer. We hope to be able to link some selected volatile markers to specific transformation steps of raw materials, to eventually better learn from the monk's secret recipes.

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QUALITY ASSURANCE SCREENING FOR COMPREHENSIVE TWO-DIMENSIONAL CHROMATOGRAPHY

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Comprehensive two-dimensional chromatography produces data that is rich with chemical information. However, data from comprehensive two-dimensional chromatography is large, highly complex, and challenging to analyze. Due to its large peak capacity and sensitivity, comprehensive two-dimensional chromatography can produce data with thousands of detected analytes. Data visualization, processing, and analysis techniques have been developed widely for comprehensive two-dimensional chromatography and are available in commercial software for automated peak detection, compound identification, target analysis, group analysis, non-target analysis, and comparative analysis. Although these software tools are generally effective, the problem of assuring the quality of complex, automated analyses for large data sets, with hundreds or thousands of analytes, is difficult. Moreover, comprehensive two-dimensional chromatography often is applied to particularly critical or difficult problems, in which cases, quality assurance is especially important. For these reasons, tools for rapid screening of data analysis with comprehensive two-dimensional chromatography are of great value. A new informatics framework and associated tools now support rapid and effective quality assurance (QA). QA Screen TM guides users through a sequence of tightly integrated visualizations that highlight pertinent aspects of the data and analytical results. Based on the screening, the analyst can confirm acceptable results, make notes, reject unacceptable results, and/or reprocess data. The workflow in of QA Screen comprises two steps. 1. The analyst defines the QA Configuration, which specifies the QA Features (e.g., blob/peaks, compound groups, areas), the QA Criteria for those features, and the QA Views for assessing the QA Criteria. The QA Criteria are expressions of chemical logic, written in CLIC TM, that describe constraints on attributes of the QA Features (e.g., retention indices, mass spectral match factors, qualifier-ion ratios) that are indicative of the quality of the QA Features. The QA Views are synchronized visualization interfaces (e.g., image, 1D slice profiles, apex or blob spectrum, selected ion chromatogram, etc.) that enable detailed examinations of a QA Feature. 2. After a chromatogram has been processed, the user invokes QA Screen. The QA Screen displays table(s) of QA Objects with the evaluations of their QA Criteria. The user can sort and filter the tables, e.g., to show only the objects that don't meet various QA Criteria. The user navigates among QA Objects, e.g., by selecting an object or using navigation tools to move forward and backward in the table. When a QA Object is screened, its QA Views are displayed and the user can switch between and navigate within the QA Views. During QA screening, the user can reprocess the data (e.g., perform local blob detection or edit a graphic object). For each QA Object, the user can set the QA Result, such as approved or failed, and can add other comments. After the QA Screen is completed, the user can generate a QA report.

K.44

UNTARGETED METABOLOMICS STUDY OF THE PLANT-PATHOGENIC FUNGUS MAGNAPORTHE ORYZAE BY GC x GC x QTOFMS

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The rice blast fungus *Magnaporthe oryzae* is a widely spread plant pathogen that causes significant losses of rice production. The precise regulatory mechanisms that allows for the efficient growth of the fungus within its host remain unknown. Characterization of the metabolome of the non-pathogenic mutants could thus help to better understand metabolic strategies employed by *M. oryzae* to colonize rice cells and would shed light on the challenges faced during growth in host cells. In this study, we have compared metabolomes of a wild type strain of *M. oryzae* Gny11 to non-pathogenic mutant strains. GC x GC methodology combined with an accurate mass high resolution MS was employed to ensure precise identification of the metabolites. This analysis was performed in order to take the first steps towards identifying and characterizing biochemical pathways essential for rice blast disease.

K.45

ANALYSIS OF TRENTO DOC SPARKLING WINES USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY TOF-MS WITH HS-SPME

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The characteristics of the foam and aroma are considered among the most appreciated attributes for the final quality of sparkling wines. The aroma of sparkling wines is due to dozens of varietal and fermentative compounds, while an essential contribute of aroma is due to several classes of volatiles that develop from precursors during wine aging or are released due to yeast autolysis. Trento DOC (PDO) is the name of the first Certified Brand of Origin for an Italian sparkling wine produced by the traditional method in which a second fermentation in the bottle was followed by aging of wines with lees for at least 15 months before the disgorging. Trento DOC wines can be aged on lees up to 10 years, and these premium wines are expected to improve as the years go by, with excellent vintages and premium reserve put on the market after 5-10 years. The aim of this study was to explore the volatile profiles of Trento DOC sparkling wines with HS-SPME followed by GCxGC-TOF-MS and to compare these products with a selection from another premium Italian sparkling wine, the "Franciacorta" DOCG. The application of the GCxGC protocol provided us with the most comprehensive description of the volatiles, highlighting the huge complexity of the aroma of Italian premium sparkling wines. With this untargeted metabolomic approch, more than 1500 features were found, many of which remain so far unidentified. Among the identified compounds, several compounds were observed here for the first time in these wines. Some differences in the composition betweeen the two data sets were found. The wines belonging to the Trento DOC were found to have on average an higher amount of terpenic compounds and of some C6 compounds while Franciacorta wines were richer in lactones, C13 norisoprenoids, sulfur compounds and a sesquiterpene gamma eudesmol. This research was funded by AGER, Agroalimentare e Ricerca, cooperative project between grant-making foundations under the section "wine growing and producing", Project "New analytical methodologies for varietal and geographical traceability of oenological products" contract n. 2011-0285.

K.46

CHARACTERIZATION AND QUANTIFICATION OF ESSENTIAL OILS BY GC AND GC×GC WITH TOFMS AND FID

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In this poster, GC and GC×GC data for essential oils are presented to demonstrate the benefits of a comprehensive second separation dimension. One dimensional GC separations with long columns and slow temperature programs to chromatographically isolate each component are commonly paired with FID detection to determine quantitative area percent information for the components of an essential oil sample. Even with the long analysis time, typical in the industry, co-elutions were observed in these data leading to some inaccuracies in the area percent values when single peaks were comprised of multiple analytes. With TOFMS and related deconvolution tools, many co-elutions were mathematically resolved and relative area information was determined. Without calibration, the area percent values within a sample were difficult to obtain due to variations of analyte response with MS detection. The addition of a secondary separation dimension with GC×GC-FID chromatographically separated many of these co-elutions in the second dimension and provided more accuracy to area percent determinations. As this relieved the requirement for all analytes to be completely resolved in the primary separation dimension, a reduction in overall analysis time was achieved with a simultaneous improvement in the isolation of individual analytes. The structured nature of the 2D separation space and the distinct visual chromatograms also provided better characterization capabilities of the essential oils. Analyte characteristics could be deduced based on an unknown analyte's elution relative to the structured bands of analytes with similar functional groups and properties. The distinct visual chromatograms also provided improved chemical fingerprinting for rapid characterization and differential analysis of samples. Methods are reported and examples of these benefits are highlighted in this poster.

EVALUATION OF A SINGLE-STAGE CONSUMABLE-FREE MODULATOR FOR GCXGC: ANALYSIS OF POLYCHLORINATED BIPHENYLS, ORGANOCHLORINE PESTICIDES AND CHLOROBENZENES

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Comprehensive two-dimensional gas chromatography (GCxGC), is a separation method recognized as offering far greater peak capacity than conventional one-dimensional separations. Today, the most frequently used GCxGC systems require consumables such as liquid N_2 for the trapping function of the modulator. Although these systems are recognized as being very effective, their initial and running costs are a hindrance to more widespread use. A new, single-stage thermal modulator for GCxGC that requires no consumables for operation has been developed to overcome these problems. The device traps analytes through the use of a specially prepared coated stainless steel capillary compressed between two ceramic cooling pads. Analytes are thermally released from the trap into the secondary column via resistive heating. To evaluate this system, we compared the performance of our device to that of an industry leading modulation system. A routine accredited method (E3487) for the analysis of polychlorinated biphenyls, organochlorine pesticides and chlorobenzenes was used with both devices for the study. Performance characteristics such as repeatability, peak heights and detection limits were compared. The single-stage consumable-free device performed comparably to or better than the industry leading system. Operational aspects of the single-stage device, as well as well as a complete review of the performance comparison will be presented.

K.48

EVALUATION OF A SINGLE-STAGE CONSUMABLE-FREE MODULATOR FOR GCxGC: REPEATABILITY AND REPRODUCIBILITY

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Comprehensive two-dimensional gas chromatography (GCxGC) is a separation method receiving increased attention in recent years due its superior peak capacity and separation power. Today, the most popular commercially available systems require consumable materials such as liquid N_2 for the trapping function of the modulator. We have developed a single-stage, thermal modulator for GCxGC that requires no consumables for operation. Analytes are trapped, focused and desorbed from within a specially prepared stainless steel capillary trap. Desorption is completed using a capacitive discharge power supply to resistively heat the trap while the cooling function is performed by compressing the steel capillary between two passively or actively cooled ceramic pads. As part of a major goal to evaluate all performance characteristics of the device, we have investigated the repeatability of the system. Replicate analyses of diesel have been performed to compare the within-day repeatability, day-to-day reproducibility, as well as the between-trap reproducibility. The results are very promising, with no shifts in retention times observed for both within-day and day-to-day comparisons. Eliminating or significantly reducing retention time shifts vastly simplifies the advanced statistical analysis of data without the need for alignment procedures. It is also highly beneficial for routine analysis and quality control applications. Both an overview of device operation and the results from this study will be presented.

K.49

WHEN SINGLE DIMENSION GAS CHROMATOGRAPHIC SEPARATIONS FAIL: EXPLORING REAL WORLD APPLICATIONS FOR COMPREHENSIVE TWO-DIMENSI0NAL GAS CHROMATOGRAPHY (GC×GC)

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One of the benefits of GCMS as an analytical tool is its ability to provide data which can be effectively searched against established libraries. This requires resolved or well-defined analytes, or the detection, *inter alia*, of lower abundance analytes which elute under highly abundant analytes. The potential for long analytical runs exists as a solution but these often fail due to retention mechanisms in GC. An approach which addresses this issue is comprehensive two-dimensional gas chromatography (GC×GC) where orthogonal selectivity between two phases facilitates separation of co-eluting analytes. This poster will demonstrate several applications in which single dimension gas chromatographic separations fail to provide resolution of components of complex mixtures. In many instances, time of flight mass spectrometry, coupled with mathematical deconvolution algorithms has been successfully utilized to extract pure spectra for co-eluting analytes in a complex chromatogram. However, there are instances when math alone cannot solve these challenging problems. One common example is a so called perfect co-elution in which the peaks of two or more analytes apex at the exact same retention time. This leads to a mass spectrum composed of more than one analyte which cannot be mathematically resolved. Instances such as these provide an opportunity for GC×GC to demonstrate the separation power needed to successfully isolate and identify components that are often missed in one-dimensional GC separations. Examples will be highlighted for food flavor and fragrance, metabolomic, and petrochemical application markets. The advantage in number of compounds detected and overall quality of their mass spectral library similarity scores as compared to one-dimensional methods will be clearly demonstrated.

K.50

AROMA-IMPACT OF VOLATILE COMPOUNDS IN BANANA TERRA SPIRIT

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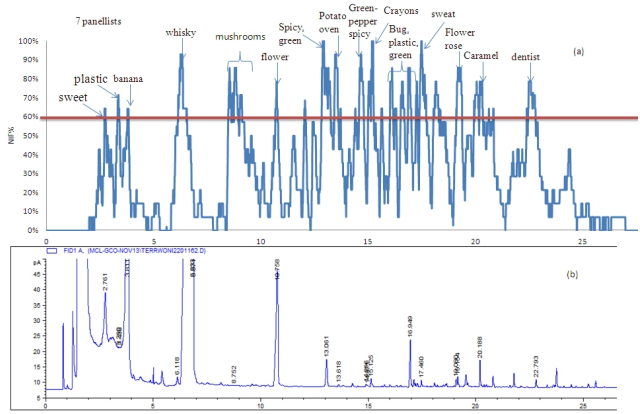
Banana (*Musa* spp.) is an important food crop, which grows extensively in tropical and subtropical regions. However, losses in fruit production represent a significant cost in the market; developing alternative products for banana is an imperative. Considering the nutritional value and its aroma quality, manufacturing of banana-based beverages is of interest to the industry. The abundant quantity of sugar in banana fruit suits the preparation of fermented-distilled beverages (spirits). This work aims to characterize the aroma compounds of banana *Terra* spirit using multidimensional gas chromatography (GC) in a multi-hyphenated system – *i.e.* coupled to flame ionization detection (FID), mass spectrometry (MS), and olfactometry (O). Solid-phase microextraction (SPME) was used to isolate the headspace aroma compounds of the banana spirit. The detection frequency (DF) technique was applied, and aroma regions detected in 1D GC separation with over 60% NIF value were screened as the target potent odor regions in the samples. GC-O analysis enabled identification of 15 aroma-impact regions, such as comprising spicy, whisky, fruity, and others. Using a polar/non-polar phase column set, the potent odor regions were further subjected to MDGC separation with simultaneous olfactory and mass spectrometry (MS) detection for identification of individual resolved aroma-impact compounds. Compounds were tentatively identified through mass spectral data matching and retention indices in both first and secondary dimensions. This is the first such study that reveals important knowledge about the major aroma compounds that contribute to banana spirit.



Aromagram showing the most important odoriferous regions for banana spirit (NIF≥60%) and chromatogram (b) with the corresponding retention times (min).

K.51

EXPLORING THE MOST SUITABLE GC×GC COLUMN COMBINATION FOR VEGETABLE OIL MINOR COMPOUNDS

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Minor compounds of vegetable oil can be used as an indication of genuineness, especially in olive oil. This fraction includes long and short-chain alcohol esters (waxes), sterols (free and esterified), esters, terpenic alcohol esters, and fatty acid derivatives are considered the fingerprint of different oils. Most of them are a class of very homogenous compounds which frequently coelute in monodimensional gas chromatography analysis. Analytical determination is a critical step, since discrimination and thermal degradation can occur not only at the injector, but also during the GC analysis if compounds elute at too high temperatures. Therefore, the separation potentiality of the GC×GC approach was investigated testing different column sets, namely orthogonal and reverse –type, using both a cryogenic and a flow modulator with simultaneous dual detection (FID and mass spectrometer). The fraction of interest was obtained employing a rapid solid-phase extraction (SPE) procedure to reduce manipulation and thus artifacts deriving from the traditional and tedious saponification procedure.

K.52

DUAL SECOND DIMENSION COLUMN-DUAL DETECTION IN TWO-DIMENSIONAL COMPREHENSIVE GAS CHROMATOGRAPHY (GC×2GC-MS/FID): INCREASED INFORMATION IN OPTIMIZED SEPARATION CONDITIONS IN METABOLOMICS

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Two-dimensional comprehensive GC-MS (GC×GC-MS) is the most advanced GC platform, among other, presenting a great potential for metabolomic studies because of its uncomparable separation power, sensitivity and possibility to obtain structured 2D patterns that can be adopted for. GC×GC-MS profiles can successfully be used for samples cross-comparisons to provide more extensive information than with 1D-GC-MS enabling to run simultaneously sample profiling and fingerprinting. However, the great diversity of chemical properties and the wide concentration ranges of these compounds in tissues and biological fluids is a significant challenge since methods need to be robust, reproducible, accurate and informative to enable samples to be reliably compared. In this perspective, the system configuration is a critical but challenging aspect requiring a careful tuning of columns diameters to avoid 2D column overloading and to improve quantitation accuracy and response linearity over a wider range of concentration [1]. This study investigates the advantages of a GC×2GC system in the metabolite profiling of urine samples from murine models of diet-induced metabolic derangements, characterized by hyperlipidemia, impaired glucose tolerance and insulin resistance. [2]. The system consists of a conventional first dimension column (^{1}D - 30 m x 0.25 mm ID) coupled to two second dimension columns of variable lengths (2D-FID 1.6 m x 0.1 mm ID and 2D-MS 1.8 m x 0.1 mm ID) and combined with parallel MS and FID detection. In particular, male C57BL/6J mice were maintained on control rodent diet or high-fat high-fructose diet (HFHF, 45 kcal% Fat and 24 kcal% Fructose) for 22 weeks and urine samples were collected at different steps of the study. Our preliminary results show that urine sample profiles offer pivotal and comparative data on the presence and on the relative distribution of early markers of metabolic disease. Besides, samples collected at the end of the experiments provide information on the global impact of the dietary manipulation on the systemic metabolism. Experimental results emphasized the advantages of the adopted configuration in terms of quantitation accuracy and precision in targeted profiling, maximization of the informative potentials due to the increased ^{2}D column loadability and selectivity, reliability of untargeted fingerprinting performed by template matching approaches [3] on dual patterns.

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K.53

ENHANCING THE SECOND DIMENSION SEPARATION POWER IN DIFFERENTIAL FLOW-MODULATED COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

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Nowadays most of GC x GC systems are equipped with thermal modulators, where the modulator unit is a cooled zone trapping the effluent from the first column. The other modulation technique which is yet rather underrepresented in the literature is the flow modulation technique. Flow modulation only requires a switching valve, some fittings and an auxiliary carrier gas flow so this technique do not require additional consumables and freezing of the analytes thus there is no need for supply of cryogen. This makes flow modulation a straightforward cost efficient alternative to thermal modulation. The limitation of the technique is the complex optimization. Unlike thermal modulation where the modulation period can be easily changed, detailed optimization is required for the first and the second column flow, the modulation time, the collection time, the injection time and the oven heating rate for each column combination. Our goal was to examine the relationship between these parameters and develop a differential flow modulated GC x GC method for the detailed characterization of diesel oil samples. Our experiments show that consideration of the coherence of parameters enables extensive method development by flow modulated GC x GC. Lower heating rates and longer modulation times are obtainable by using columns of smaller internal Diameter in the first dimension with decreased flow rates. Elongation of the modulation period ensures the application of lower second column flow rates. These conditions significantly enhance the second dimension separation efficiency which is the main driving force of a method development in GC x GC.

K.54

ANALYSIS OF ENDOCRINE DISRUPTORS COMPOUNDS IN VEGETABLES USING SPME AND BIDIMENSIONAL GAS CHROMATOGRAPHY

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Some organic micro-contaminants are often found in foods which can cause dysfunction in the endocrine system of humans and animals and are therefore classified as endocrine disruptors compounds (EDC). Phthlates and alkylphenols are classified as EDC. Food contamination by phthalate can occur due to migration of these compounds present in packaging for food. Alkylphenols are commonly present in pesticides and the contamination can occur due to the use of such compounds in the cultivation of vegetables. The EDC are present in low concentrations in foods and the development of highly sensitive analytical techniques is required for its quantification. This study describes the development of a new method for analysis of EDC, phthalates and alkylphenols in frozen foods wrapped in plastic packages. The analyses were performed by solid phase microextraction (SPME) using comprehensive two-dimensional gas chromatography with flame ionization detector. To determine the EDC 10.0 g of sample was transferred to a beaker containing 40 mL of water, which was stirred at room temperature for 30 min. An aliquot of 15 mL was transferred to SPME vial in which the extraction was made by direct immersion of a polyacrylate fiber. For this, extraction temperature of 65 °C and extraction time of 30 min were used. The validation was performed following EURACHEM recommendations. The developed method shows high separation efficiency and peak resolution. The limits of detection for the studied compounds ranged from 0.07 to 0.3 μg L^{-1}. The precision was evaluated in intra- and inter-day assays at concentrations of 1.0 and 5.0 μg L^{-1}. The analytical curves showed that the residues were homoscedastic and independent with normal distribution for all compounds.

K.55

GC×GC-TOFMS IN THE CHARACTERIZATION OF BIO-OIL FROM PYROLYSIS OF AGROINDUSTRIAL RESIDUES: (1) MANGO

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Cultivation of fruits is one of the main activities of the agricultural business in Brazil [1]. For instance, production of mango fruits in Brazil occupies the seventh position in the world. However, the residuals after processing the fruits is equivalent to 30-60% of the raw product, which is commonly discarded environment [2]. One interesting alternative for the reduction of the solid residues is the generation of bio-oils through pyrolysis process [3], which can be used as bio-fuel or as raw material for other chemical products [4]. The present study aims the evaluating the potentialities of producing bio-oil from pyrolysis of industrial residues of the endocarp of mango fruits. The bio-mass was subjected to pyrolysis in a homemade reactor (5 g sample, flow rate of N_2 of 1 mL/min, heating rate of 100 °C/min) in temperatures of 450 °C, 550 °C and 650 °C. In the considered range of temperatures, the maximum efficiency (38.8%) was achieved in the temperature of 650 °C. The chemical composition of the bio-oil volatile fraction in the temperature of 650 °C was analyzed by GC×GC-TOFMS technique. In the identification of the compounds, it was used the Kratz retention index combined with the mass spectrum and elution order of the compounds. In the study, 114 compounds were identified in the bio-oil and classified as phenols, alcohols, carboxylic acids, aldehydes, ketones, sugar derivates and hydrocarbons. The major compounds were 2(5H) furanone (9.4%), 2-furancarboxaldehyde, 5-(hydroxymethyl)-(7.5%), phenol (6.5 %) and 2,6-dimethoxy-phenol (5.7%). The identified products have recognized industrial importance which indicates their potential use as sources of such materials.

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K.56

ANALYSIS OF NITROGEN-CONTAINING COMPOUNDS IN COAL TAR WITHOUT PRE FRACTIONATION BY GC×GC/TOFMS

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The GC×GC/TOFMS has shown great skill in analyzing complex mixtures such as fossil fuels, especially for compounds at very low concentrations. The analysis of nitrogen compounds in fossil fuels (coal and crude oil) is a major challenge for analytical chemistry due to its environmental and technological importance and also their diversity of concentration in the matrix. In this work it is studied the basic and neutral nitrogen compounds from a coal tar derived from fast pyrolysis of Brazilian coal (Parana state, southern Brazil) using GC×GC/TOFMS without prior fractionation. Typically these compounds are analyzed after complex processes of sample treatment, making the process expensive and time consuming. The methodology used here consisted of chromatographic injection of the diluted sample using a conventional columns set (OV5/DB17) and data analysis using ChromaTOF and Excel™ softwares. Some basic compounds (pyridines and quinolines) and neutral ones (carbazoles and indoles) were found with good chromatographic separation, good spectral similarity and excellent signal/noise ratio. The tools of spectral deconvolution and the dispersion graphics allowed greater security on the identification and separation of compounds.

GC×GC-TOFMS IN THE CHARACTERIZATION OF BIO-OIL FROM PYROLYSIS OF AGROINDUSTRIAL RESIDUES: (3) COCONUT FIBERS: INFLUENCE OF TRIGLYCERIDES ON THE COMPOSITION OF BIO-OIL

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Brazil is the fourth largest producer and consumer of coconut, generating in its coastal cities, a lot of waste (coconut husk) after the removal of water (its largest and most consumed product), causing a huge environmental problem due to its persistence and slow degradability. There are few studies for the reuse of this waste and the pyrolysis appears as an efficient alternative to its thermodegradation and the production of compounds with higher added value. The aim of this study was to evaluate the influence of triglycerides of the coconut fiber in the yield and composition of the bio-oil obtained by fast pyrolysis of this biomass. The pyrolysis was conducted before and after the extraction of the fat material from the fibers (by soxhlet extraction with hexane, ethyl acetate and ethanol) and the bio-oils were chromatographically analyzed. After, the biomasses were pyrolyzed in a fixed-bed reactor following parameters described on a previous paper [1]. The bio-oils were analyzed by GC×GC-TOFMS, using dispersion graphics, retention indexes (LPTRI) and comparison of mass spectra and retention times (^{1}D and ^{2}D). More than 150 compounds were identified in the bio-oil before extraction, with a clear predominance of the fatty acids and esters being the other compounds classified in phenols, ketones, aldehydes with traces of hydrocarbons. For the bio-oil after the soxhlet extraction, 102 compounds were identified and distributed into phenols, ketones and aldehydes with only 0.1% of free fatty acids. Beside this, the chromatographic profile and the similarity between spectra were greatly superior after the extraction. It is possible to conclude that the bio-oil of coconut fibers is a rich and important raw material for the industry and its recovery can be improved in the absence of glicerides in the biomass, which could produce free fatty acids and esters.

Reference

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K.58

CHARACTERIZATION OF VOLATILE FRACTION OF GREEN MATE (YERBA MATE) AND MATE TEA BY GC-qMS AND GCXGC qMS

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The purpose of this work was to study the effect of the roasting of dried green mate (yerba mate) leaves on the Volatile fraction. Mate (*Ilex paraguariensis* A. St.-Hil), is a shade-tolerant tree from South Brazil, Missiones, Argentine, east of Paraguay, and from some isolated populations in Uruguay. The consumption of mate in Southern Brazil is approximately 1.2 kg per person per year. Mate is prepared as an infusion of the triturated and dried leaves of *Ilex paraguariensis* St.-Hil. (Aquifoliaceae). People drink this infusion due its diuretic, anti-inflammatory and stimulant properties. The ingestion of *I. paraguariensis* can also contribute to the increase of antioxidant defense against free radical action. The mate samples were placed in a 2 L round bottom flask with distilled water in a Clevenger apparatus. The steam distillates were extracted with hexane, and analyzed by GC × GC/qMS (Shimadzu QP2010 Plus system) equipped with a modulator ZX1-GC × GC (Zoex, Houston, TX, USA). This modulator utilizes liquid nitrogen (Linde Gases, Porto Alegre, RS) for the coldjet (which is continuous) and gaseous nitrogen for the hot jet (activated only for a short period of time).The chromatographic separation in the first dimension was performed in a non-polar column OV-5, 5% phenyl–95% methylpolysiloxane (60 m × 0.25 mm i.d. × 0.10 μm film thickness — Ohio Valley Specialty Company, USA). In the second dimension it used a more polar column DB-17, 50% phenyl and 50% methyl-polysiloxane (2.15 m × 0.18 mm ×0.18 μm, J&W Scientific, Agilent Technologies, USA). Kovats retention indexes were obtained through the co-injection of the sample with a homologous mixture of n-alkanes (C_9H_{20}–$C_{25}H_{32}$), and calculated using the Van den Dool equation. The major compounds identified in the green mate (Yerba mate) volatile fraction were limonene, linalool and geranyl acetone. The major compounds identified in the mate tea volatile fraction were trans-geranyl acetone, limonene and beta-E-ionone.

Reference

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K.59

GC×GC-TOFMS IN THE CHARACTERIZATION OF BIO-OIL FROM PYROLYSIS OF AGROINDUSTRIAL RESIDUES: (4) COFFEE GROUND

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Coffee is the most important agricultural product and the most consumed beverage in the world. The residues obtained during its preparation (coffee grounds) are nutritionally rich containing many bioactive compounds with antioxidant properties and, due to the intense use coffee, the production of residues is in tons and do not have a noble use, being considered a waste product. Few studies have been developed in the characterization of this material, despite its great production in all the world. [1,2] The goal of this work was to study the thermodegradation of coffee grounds (from Coffea arabica L.) by pyrolysis as an alternative for using this biomass as a renewable energy source or raw material for industry. The bio - oil was produced by fast pyrolysis in a fixed bed reactor. The bio-oil was fractionated by pressurized solvents and analyzed by GC/qMS and GC×GC/TOFMS using some tools as Retention Indexes and Dispersion Graphics beside the informations of the CromaTOF software (retention times, areas, similarities, unique masses, …) for the identification of the constituents of the bio-oil. It was used one and two dimensional gas chromatography, with a clear advantage for the second technique, in order to characterize this material. Many classes of compounds were found in the fractions of bio-oil, mainly hydrocarbons, phenols, nitrogen compounds and other oxygenated compounds. The spatial classification on dispersion graphics had favored the identification and classification of compounds according their number of carbons, polarity and molecular weight. It was concluded that the fractionation process facilitated the identification of the constituents, in particular after the isolation of the fatty compounds (acid and esters). Fractions of high industrial interest were found, as hydrocarbons, phenols and ketones, indicating the potential use of this residue with a view of industrial application.

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K.60

GC×GC-TOFMS IN THE CHARACTERIZATION OF BIO-OIL FROM PYROLYSIS OF AGROINDUSTRIAL RESIDUES: (2) GUAVA SEEDS

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The use of biomass as an energy source is growing around the world. This material is used in the generation of high value products using different technologies including biochemical, mechanical and thermochemical processes. This work is focused on the pyrolysis, a thermochemical process that promotes the degradation of biomass into more simple organic compounds in the absence of oxygen. The aim of this work was to use the guava seeds for the bio-oil production by fast pyrolysis and the subsequent identification of the main compounds using one-dimensional gas chromatography with mass spectrometry detection (GC/qMS) and the comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry detection (GC×GC/TOF-MS). Guava seeds were provided by a juice industry (POMAR, located in Aracaju, Sergipe, northeast of Brazil). To obtain the bio-oil, 5 g of the dried seeds were exposed to a temperature of 600 ºC with a constant nitrogen flow of 1 mL/min in a fixed-bed pyrolysis reactor. The obtained bio-oil was submitted to simple decantation with the aid of addition of dichloromethane to better separate the organic phase (bio-oil) of the aqueous phase. The two dimensional technique (GC×GC/TOF-MS), as was expected, allows to identify a greater number of compound with higher sensibility and resolution if compares to GC/qMS. Many hydrocarbons, ketones, acids, alcohols and phenols were found between the major compounds. The retention indexes (LPTRI) and the dispersion graphics, constructed using the retention times (^2t_R versus ^1t_R) were also important for completing the identification and classification of the compounds. This study indicated that this agro-industrial residue is a potential source of important raw material for many different uses as food, pharmaceutical and cosmetic industry and the GC×GC allowed solving many misidentifications that could be provided by one-dimensional gas chromatography.

K.61

CHARACTERIZATION OF A NEW REVERSED-FLOW MODULATOR FOR GCxGC

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A new second generation flow modulator is described that while more complex in design shows performance benefits over the original. In the standard flow modulator, flow from the pressure control modulator directs or "injects" contents of the collection channel into the second dimension column in the same direction as flow from the first column was used to fill the channel. In other words, analytes in the channel continue to flow in the same direction as they entered when injected. The second generation design reverses flow direction in the channel during the inject part of the modulation cycle. This is accomplished by use of a channel fill restrictor. The channel fill restrictor can be either vented external to the GC or interfaced directly to a second FID as a monitor. The advantages of the reverse-inject flow modulator include improved peak symmetry, ability to handle a higher concentration range of analytes, better definition of analytes in 2D images, expanded GCxGC modulation periods, and a wider range of column dimensions. Use of the new reverse-inject flow modulator will be demonstrated in comprehensive and non-comprehensive modes. The performance will be characterized for complex mixture analysis and parameter optimization will be discussed.

K.62

AUTOMATIC COMPOUND DECONVOLUTION AND ALIGNMENT IN COMPREHENSIVE GAS CHROMATOGRAPHY

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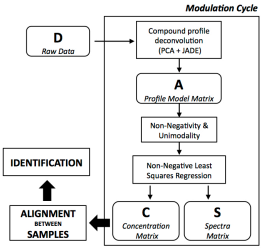
In this study we present a new method to automatically process a complete comprehensive double gas chromatography - mass spectrometry (GCxGC-MS) experiment. This processing involves compound deconvolution, compound alignment across samples and compound identification. The method deconvolves all the compounds of each sample in the experiment in a first step called factor extraction. An algorithm based on independent component analysis (ICA) is used to perform a true deconvolution of the mixture of compounds in data. Compound profile models are extracted by non-negative unimodal independent component analysis. Non-negative and unimodality constraints are applied to the ICA outcome to obtain chemically meaningful results in its application to chromatographic data. Concentration and spectra matrices are determined by non-negative least squares. For each modulation cycle, three steps are applied to resolve the mixture: (1) Estimation of number of independent components with a principal component analysis (PCA) analysis. (2) Compound profile model extraction using non-negative unimodal independent component analysis. (3) Profile and spectra extraction by non-negative least squares regression. In a second stage, the factors (compounds) found in the different samples are aligned. The algorithm groups the extracted factors across the different samples according to their distance in the retention time and their correlation distance between their spectra. Finally, the aligned factors are identified by comparing their spectra against a reference database such as the Golm metabolome database (GMD). Also, data can be statistically analyzed to retrieve significant variations of the deconvolved compounds between the existing classes or conditions in the experimental design.



K.63

COMPREHENSIVE MATERIAL EMISSIONS PROFILING BY TD-GCxGC-TOF MS

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Exposure to air pollutants has for many years been recognised as a major cause of health problems. Historically, pollutants from vehicle exhaust and the burning of fuels were of primary concern, but as urban air quality has generally improved, attention has shifted to vehicle interior air quality (VIAQ). Emissions of volatile and semi-volatile organic compounds from car interiors can have an adverse effect on VIAQ, raising concerns for passenger health and safety. As a result of these concerns, VIAQ is of growing importance to the automotive industry, culminating in the development of harmonised methods to quantitate the release of chemicals from materials used in car manufacture. Methods generally specify the use of environmental chambers, vapour sampling onto sorbent tubes and analysis by thermal desorption (TD) with GC-MS. However, the broad range of sample types and the presence of target compounds at ultra-trace levels, often within complex matrices, provide a challenge to analytical chemists. Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOF MS) offers a potential solution by combining enhanced chromatographic separation with exceptional sensitivity. Furthermore, the revolutionary Select-eV ion source technology extends the capabilities of GCxGC-TOF MS by enabling both hard and soft electron ionisation with no loss in sensitivity. The availability of full-fragmentation spectra for confident library matching is complemented by the ability of Select-eV to enhance the diagnostic ions that define compound identity and distinguishing between similar compounds. Fragment ions that ordinarily dominate high matrix samples become greatly reduced, enhancing selectivity and further improving detection levels. This holistic approach to material emissions profiling, combining TD-GCxGC-TOF MS with Select-eV ionisation technology, provides both enhanced separation and extended limits of detection for confident identification of a wide range of target compounds in a single analysis.

K.64

SELECT-EV: INCREASING DIMENSIONALITY IN GCxGC-TOF MS

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Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOF MS) offers greatly enhanced peak capacity, through the coupling of two columns of different selectivities, as well as highly sensitive detection and definitive mass spectral identification of trace-level analytes. Despite this increased separation capacity, the identification of individual compounds in complex samples may be further complicated by weak molecular ions or when similar mass spectral characteristics are evident across entire chemical classes. Select-eV is a new innovation in ion source technology which solves this problem through the ability to switch between hard and soft electron ionisation with no inherent loss in sensitivity. Select-eV offers a wide, tuneable range of ionisation energies without the requirement for source switching or additional reagent gases. The use of soft electron ionisation enhances the intensity of molecular and structurally-significant fragment ions, magnifying differences between isomeric spectra and, by consuming less instrumental dynamic range, a wider concentration range of analytes can be supported in any single analysis. This poster provides an introduction to Select-eV technology, as applied to GCxGC-TOFMS analyses within a range of applications; from crude oil fingerprinting to environmental monitoring.

K.65

COMPREHENSIVE TWO-DIMENSIONAL GC FOR THE ANALYSIS OF LOW-MOLECULAR-WEIGHT OXYGENATES IN THREE DIFFERENT MATRICES FROM A PETROCHEMICAL PILOT PLANT USING A SINGLE CALIBRATION

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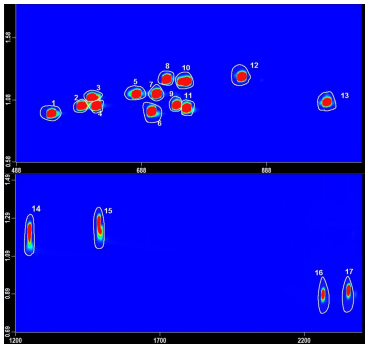
A method using comprehensive 2DGC with flame ionisation detection was developed to quantify 17 low-molecular-weight oxygenates in three different matrices, namely water, oil and gas, using a single calibration. The method was required for the pilot-plant experiments of a chemical process unit. From an analytical perspective, the first task was to find a suitable analytical method with sufficient selectivity and sensitivity to analyse the selected oxygenates at low levels in the presence of high levels of hydrocarbons. The second was the accurate quantitation of oxygenates in the water, oil and gas fractions, using the same instrument and calibration. Both these requirements were met by using comprehensive 2DGC in the inverse configuration and calibrating the detector with the number of moles injected versus response.
The method was successfully applied for the characterisation of the reactor product stream of the chemical process unit and made it possible to determine the fate of the selected oxygenates after passing through the reactor. The development of the method and some of the results are described in this paper.



K.66

CHARACTERIZATION OF ORGANIC SULFUR COMPOUNDS IN FOSSIL FUELS USING FLOW-MODULATED COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY

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The main forms of organic sulfur compounds (OSC), in fossil fuels such as coal and petroleum, are thiophenes linked to aromatic rings and defined polycyclic aromatic sulfur heterocycles (PASH), which consist primarily of alkylated benzothiophenes and dibenzothiophenes. The presence of OSC in these matrices is undesirable, due mainly to the release of sulfur oxides into the atmosphere during burning processes. In general, identification of these compounds requires various steps of isolation and fractionation, mainly due to co-elution of these compounds with polyaromatic hydrocarbons (PAH). From the analytical point of view, there is no known method that is fully satisfactory for the separation of PASH and PAH in these matrices. Furthermore, there is the problem of the large number of isomers of alkylated PASH, which makes the separation of these compounds even more than an analytical challenge. The circumvention of such a challenge is important so that the monitoring of recalcitrant OSC in final products can be made possible.

The objective of this study was evaluate the potential of flow-modulation (FM) GC×GC, added to the selectivity of tandem mass spectrometry (MSMS), in the direct injection of fossil fuel samples, allowing a qualitative characterization and the PASH target determination.

The use of FM GC×GC-MSMS, applied to fossil fuel OSC analysis, has shown that the determination of PASH is possible without the pre-fractionation step, and it can represent an analytical advance in the petrochemical area.

K.67

DETAILED ELUCIDATION OF THE UNSAPONIFIABLE FRACTION OF LIPIDS BY USING ENHANCED PEAK CAPACITY CHROMATOGRAPHY AND HIGH-RESOLUTION MASS SPECTROMETRY PROCESSES

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The present research is focused on the generation of a detailed qualitative and quantitative profile of the unsaponifiable lipid fraction belonging to milk and human plasma, by using data derived from comprehensive two-dimensional gas chromatography (GC×GC), with dual quadrupole mass spectrometry/flame ionization detection (quadMS/FID). The structure of many constituents (particularly sterols) was confirmed by using GC-high resolution time-of-flight MS. The GC×GC column set used consisted of a low-polarity first dimension, and a medium-polarity secondary one, both characterized by a high thermal stability. The use of dual detection enabled the simultaneous attainment of both mass spectral information and relative % data. The complexity of the fingerprint, generated by the unsaponifiable fraction, fully justified the employment of the two-dimensional GC technology. However, it was two other GC×GC characteristics that contributed most to the attainment of promising results, namely sensitivity enhancement and the formation of group-type patterns. Furthermore, GC coupled with high resolution time-of-flight (HR ToF) MS was used to increase the reliability of identification of several unsaponifiable lipid constituents.

The synergism between both high-resolution chromatography and mass spectrometry processes enabled the attainment of a more-in-depth knowledge of the unsaponifiable fractions of lipid samples.

K.68

HIGH TEMPERATURE GC×GC FOR THE THERMODESORPTION ANALYSIS OF POLYMERS

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Thermodesorption-GC-MS is a routine test for polymeric materials, especially if their intended use is inside a car. Besides this main application it also used as a sample preparation free test to investigate the oligomeric part of the polymer, semivolatile additives or degradation products. While 1D-TD-GC-MS is suitable for most routine tasks, a higher peak capacity is especially useful for very complex materials like compounds of different polymers or recycled materials. To be able to use high thermodesorption temperatures, to increase the range of potential analytes, the suitability of a high temperature column combination for TD-GC×GC-MS was investigated.

K.69

TWO DIMENSIONAL GAS CHROMATOGRAPHY AS A TOOL FOR IDENTIFICATION OF MINOR FATTY ACID METHYL ESTERS OF PALM AND PALMIST OILS

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Vegetable oil such as palm oil (obtained from the fruit mesocarp of Elaeis guineensis) is an essential nutrient and an important source of energy, while the palmist oil (obtained from the kernel of the same fruit) is used to treat skin irritations and cutaneous eruption in new born babies in Cameroon. Comprehensive two-dimensional gas chromatography with time of *flight* mass spectrometry (GCxGC-TOFMS) and gas chromatography coupled to mass spectrometry with flame ionization detector (GC-MS-FID) were used to determine the fatty acid methyl esters (FAMEs) in palm and palmist oils. Five samples of palm oils obtained from three different countries (Cameroon, Nigeria and Ghana) and two palmist oil samples from Cameroon were used in this study. The FAMEs were obtained by esterification of free fatty acids. Most of the FAMEs were identified by NIST version 05 library spectra search and by single or mixtures of standard injection.

Quantitative and qualitative variation was determined by investigating the composition of the FAMEs in palm oil. The major compounds as determined with LECO Pegasus 4D GCxGC-TOFMS and GC-MS-FID were hexadecanoic acid methyl ester (27.4-37.0%), 9-octadecenoic acid methyl ester (22.1-43.0%), 9-12-octadecadienoic acid (8.5-13.4%) and stearic acid methyl ester (7.5-13.7). FAMEs present in small amounts that can differentiate palm oil from different origins include 11-octadecenoic acid methyl ester, tetradecanoic acid 12-methyl-, methyl ester, 8,11-octadecadiynoic acid methyl ester, 2-pentenoic acid, 2-methoxy-3-methyl , methyl ester, 2,6-dimethyl-8-oxoocta-2,6-dienoic acid methyl ester, 9,12,15-octadecadienoic acid (Z)-methyl ester that could only be identified by the two dimensional GC. The palmist oil showed little variation in composition of the major FAMEs (both with two dimensional and conventional GC-MS-FID) with high levels of dodecanoic acid methyl ester (35.2-40.1%), 9-octadecenoic acid methyl ester (14.0-17.6%), hexadecanoic acid (Z) methyl ester (11.2-15.0%) and myristic acid methyl ester (15.1-17.8%). However, qualitative and quantitative variations were noted when minor FAMEs were considered. Compounds such as boronic acid methyl ester, propanoic acid, 3-hydroxy methyl esterdecanoic acid, 2-oxo-methyl ester can be used to differentiate the two palmist samples when using the two dimensional GC. In general few FAMEs could be detected when the GC-MS-FID was used (usually less than 10) while this number was more than double when using the two dimensional GC. When applying two dimensional GC methods to investigate vegetable oil samples, not only the sensitivity is higher than the one obtained with GC-MS-FID, but also many minor fatty acids methyl esters can be identified.

K.70

DETAILED LIPID CHARACTERIZATION OF LEMON SEED OIL BY USING ADVANCED CHROMATOGRAPHY METHODS IN COMBINATION WITH MASS SPECTROMETRY

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Citrus seeds are one of the principal residues in the production of essential oils and fruit juices. Such residues are a potential source of valuable by-products and their utilization can significantly decrease problems related to disposal. Thus, the chemical characterization of these waste products becomes very important. It is in such a context that the present research work can be placed, inasmuch that lemon seed oil was subjected to detailed analyses, highlighting the lipid composition. Different chromatography-based methods coupled to mass spectrometry detection were used to accomplish such a goal. Specifically, non-aqueous reversed phase high performance liquid chromatography (NARP-HPLC), in combination with atmospheric pressure chemical ionization mass spectrometry (APCI MS), was used for (i) triacylglycerol elucidation, whereas flow-modulated comprehensive gas chromatography (FM GC×GC), coupled to triple quadrupole mass spectrometry (QqQ MS), was employed for the investigation of the (ii) fatty acid profile and (iii) unsaponifiable fraction, after methyl ester and trimethylsilyl ether derivatization, respectively.

K.71

THE OFF-LINE COMBINATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY: A NEW POWERFUL APPROACH FOR HIGHLY-DETAILED ESSENTIAL OIL ANALYSIS

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The present contribution is focused on the off-line combination of high performance liquid chromatography (HPLC) and comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry (GC×GC-quadMS), and its application to the detailed qualitative analysis of two genuine Citrus essential oils, bergamot and sweet orange. Specifically, a silica column was exploited for the separation of the essential oil constituents in two groups, namely hydrocarbon and oxygenated compounds. After, each HPLC-fraction was reduced in volume, and then subjected to cryogenically-modulated GC×GC-quadMS analysis. The volatiles were separated on a normal-phase GC×GC column train set, and identified through database matching and linear retention index information. The concentrated HPLC fractions gave origin to unexpectedly-crowded chromatograms, due to two fundamental GC×GC characteristics, namely the enhanced separation power and sensitivity. The results attained were particularly stimulating with regards to the oxygenated compounds, namely those constituents which contribute most to the essential oil aroma, and are of more use for the evaluation of quality and genuineness.

ANALYSIS OF THE EFFECT OF GRAPE WITHERING ON AROMA PROFILE OF WINE USING HS-SPME AND GCxGC TOF-MS

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Drying of grapes and subsequent wine-making are processes that characterize some premium quality Italian wines including in particular Amarone, a "Passito" dry red wine produced by a traditional, slow drying grape process. During this process, depending on the occurrence of favourable seasonal conditions for a noble rot infection, a positive impact on volatile compounds can develop[1-2]. In recent years a more modern drying approach has been suggested, with the aim of faster and more strictly controlled drying of grape. The drying conditions are expected to lead to different wine composition and the aim of this study was to evaluate the differences in wine volatile compounds produced from a) traditional and b) modern processes applying 2 different analytical approaches, SPME followed by GCxGC TOF MS and a classical approach with SPE followed by GC MS [3]. The wines produced from two different grapevine cultivars, Sangiovese and Corvina, with two different grape drying systems (slow vs. accelerated) were analyzed. The profiles of volatile compounds in wines were, as expected, quite different and the sample were clearly separated by PCA. The compounds that characterize the wines obtained by traditional drying system were well distinguishable and some of them were produced by Botrytis cinerea such as 1-octen-3-ol, p- cymene, 4-terpineol, benzaldehyde, phenylacetaldeide. The concentrations of fatty acids and ethyl esters were higher in the wine produced by faster drying system as already reported in the literature [2], as well as the 3- hydroxy ethyl butyrate, phenyl ethyl acetate and linalool. The preliminary results of this survey calls for further metabolomic and transcriptomic analyses of grapes, in order to better understand how the variability of the grape metabolism induced by different drying conditions can influence the aroma of Amarone wines.

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GC×GC-TOFMS FOR BIO-OIL CHARACTERIZATION: SWOT ANALYSIS

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Solid biomass is extensively studied as a renewable energy resource to substitute fossil fuels. Flash-pyrolysis of biomass is a thermal process that uses lignocellulosic materials such as wood chips and sugar cane bagasse to produce a liquid named bio-oil, characterized by a complex chemical composition, mainly in terms of oxygenates. Regarding the product quality, the high oxygen content of bio-oils appears as a problem for its stability and corrosiveness. Therefore, the chemical characterization of the bio-oils is important to optimize the processes involving the biomass transformation, its upgrading and to suggest a proper use of the produced fuel. GC×GC-TOFMS is currently a vastly adopted analytical approach for the investigation of bio-oil. Despite the resolving power, group-type separation and reliable quantification, the GC×GC-TOFMS has some threats that must be taken into account (e.g. linearity, repeatability). This study aims to evaluate the Strengths, Weaknesses, Opportunities and Threats (SWOT) of a GC×GC-TOFMS method for the analyses of bio-oils. The bio-oil samples analyzed, produced from different biomasses (palm oil process waste, pine wood chips and sugarcane waste), were provided by Cenpes/Petrobras. In optimizing the GC×GC-TOFMS method for bio-oil composition elucidation, different parameters were evaluated. Chromatographically, the primary and secondary columns, the oven temperature programs and the modulation period have been evaluated. From the detection point of view, a fine tuning of the detector voltage has been carried out in order to detect compounds at trace level and avoiding the saturation issues related to the use of a micro-channel plate (MCP). Standard solutions at different concentrations (0.05 and 200.0 ng μL^{-1}) have been injected for achieving the optimal instrument calibration. Repeatability of both standards and bio-oils (spiked with standards) injections have been investigated. SWOT analysis of a GC×GC-TOFMS method for bio-oil is mandatory to understand the factors that may affect the characterization of the bio-oil composition.

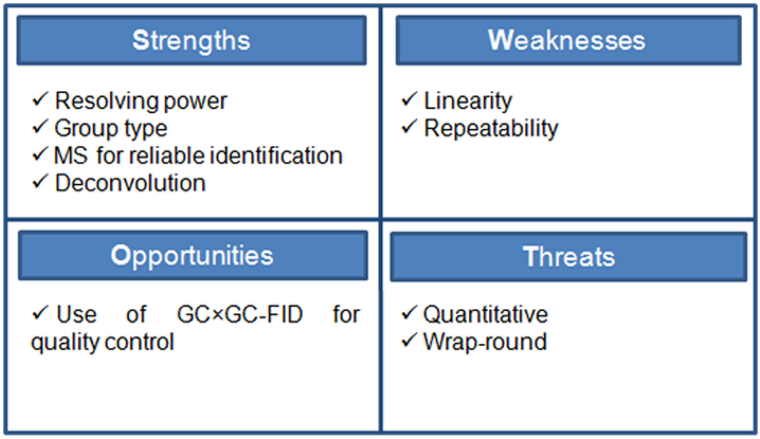

Figure 1. SWOT analysis of a GC×GC-TOFMS method for bio-oil characterization.

K.74

INTERFACING GCxGC WITH ICP-MS FOR ELEMENT-SPECIFIC SCREENING OF ENVIRONMENTAL CONTAMINANTS

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Environmental contaminants, e.g. brominated flame retardants, occur in highly complex mixtures of varying composition. Recent studies predict compounds with different elemental composition in the environment that have never been measured and some of them are only present at very low concentrations [1]. Comprehensive gas chromatography (GCxGC) is a powerful tool to enhance analytical selectivity and increase resolving power allowing for the identification of a larger number of chemicals. However, element specific identification at trace level is difficult. Previous work has shown that 1 D GC - inductively coupled plasma (ICP) MS is a highly sensitive technique for the determination of brominated compounds in samples [2]; however, to our knowledge GCxGC-ICP-MS has not been tested before. Here we demonstrate that interfacing GCxGC to high resolution ICP-MS is a promising tool for the detection of trace level environmental contaminants in complex matrices. The method offers element-specific scanning, e.g. for Br, Cl, P, and I-containing compounds. The feasibility of this method was evaluated using different matrices including house dust (NIST Standard Reference Material (SRM) 2584), mussel tissue (NIST SRM 2977), and tissues from marine sponges. Compounds containing the target elements were detected and selected Br and Cl-containing compounds were identified by comparison to standard mixtures. While all samples contained Br-containing compounds, P- and I-containing compounds could only be detected in some of the analyzed samples. The sensitivity of the method varied between single elements. For example, the detection limit for polybrominated compounds determined using individual congeners of polybrominated diphenyl ethers (PBDEs) ranged from 0.07 – 0.23 pg. Chlorinated compounds, e.g. polychlorinated biphenyls (PCB 153), were hardly detectable below 500 pg. Of particular interest was the detection of numerous I-containing compounds which are difficult to identify by usual GC-electron ionization MS methods.

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ENCAPSULATION PROPERTIES AND DIGESTIBILITY OF OSA-MODIFIED POTATO STARCHES

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Encapsulating properties and digestibility of OSA-modified potato starch (MS) derivatives for the coating of caraway essential oil (EO) by spray-drying were evaluated. The hydroxypropylated starch (P-$HP_{0.2}$) was prepared by etherification of native starch (PN) with propylene oxide. Modification was accomplished by the addition of different amounts of OSA by esterification with octenyl succinic anhydride into PN and P-$HP_{0.2}$.

The encapsulation efficiency ranged from 20% (PN) to 88% (P-$OSA_{0.020}$). The P-$HP_{0.2}$ ability to entrap EO increased ~twice (38%) compared with PN. There was good correlation between total EO retained and OSA content added to the PN (r=0.995) and P-$HP_{0.2}$ (r=0.934). To evaluate the changes in EO composition taking place during processing, volatiles from spray-dried products were isolated by hydrodistillation procedure and analysed by GC-FID and GC´GC-TOFMS LECO Pegasus 4D. Limonene (35%) and carvone (62%) were the major compounds among 25 identified in pure EO used as the initial material for encapsulation. The compositions of pure and encapsulated in different starches oil were quite similar, some slight changes in the percentages of some individual volatiles were observed.

Digestibility of MS derivatives was determined according to the method of Miller et al. [1] with slight modification including an oral step. The main indicator to characterize the digestibility of each starch fraction was DE [2]. In general, PN starch was the most digestible, the modification with propylene oxide reduces the digestibility and the addition of the highest food grade OSA content was the most effective. The P-$HP_{0.2}$-$OSA_{0.020}$ was the most stable to enzymes and the lowest digestible matrix.

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K.76

DETERMINATION OF PROCESS-INDUCED TOXICANTS AND ODORANTS IN FOOD BY MULTIDIMENSIONAL GC TECHNIQUES HYPHENATED WITH OLFACTOMETRY AND MASS SPECTROMETRY

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The assessment of the dual impact of heating treatments on food safety and aroma is a key issue. The objective of the present paper was the determination of process-induced polycyclic aromatic hydrocarbons (PAHs) and odor-active compounds with cooked meat as food model.
PAHs were analysed by accelerated solvent extraction - comprehensive bidimensional gas chromatography - time-of-flight mass spectrometry (ASE-GCxGC-TOFMS). Odor-active compounds were determined by dynamic headspace-GC-eightbooth olfactometry (DH-GC-8O) and DH-multidimensional GC hyphenated with olfactometry and mass spectrometry (DH-GCGC-O/MS). For PAH determination, the GCxGC conditions consisted in a combination of a primary apolar BPX5 column and a secondary polar BPX50 column, and a modulation period of 5 s. In terms of linearity, recovery rate and limit of quantification, the ASE-GCxGC-TOFMS method was found consistent with the multi-residue determination of 17 PAHs in cooked meat.
For aroma compounds, multi-booth olfactometry using eight sniffers revealed major meat odoractive compounds. A home-made heart-cut GC-GC-MS/O enabled to resolve the co-eluting odor zones with high odor-activity. Finally, these developments of multidimensional approaches were used to investigate and compare the balance between 17 PAHs and 68 odor-active compounds generated with different cooking techniques.

K.77

FLASH PYROLYSIS COUPLED TO GC-TOFMS, GC×GC TOFMS, AND GC-HIGH RESOLUTION TOFMS FOR CHARACTERIZATION OF PETROLEUM PRODUCTS

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Petroleum is the most complex matrix in nature, constituted by many thousands of compounds, and presents an analytical challenge. For comprehensive analysis we need a chromatographic separation with high peak capacity and for mass spectrometry analysis we need high resolving power and high mass accuracy. As the world's remaining deposits of petroleum become heavier it's important to understand the chemical nature of heavy crude oil and its fractions. The analysis of high boiling point constituents of crude oils adds a new dimension for the complete characterization of crude oil. In this study we evaluated the use of a pyrolysis probe (Py) coupled to gas chromatography (GC and GC×GC) time-of-flight (TOF) and GC high resolution time-of-flight (HRTOF) mass spectrometry (MS) for the characterization of crude oils and asphaltene fractions. Typically we run the crude oil samples in two steps of thermal desorption (350 ^{o}C and 500 ^{o}C). After that we pyrolyze the residue at 800 ^{o}C. Isomeric ratios of methyldibenzothi ophene, alkylnaphthalenes, and alkylphenanthrenes were evaluated, and may reflect the diversity of organic source input, thermal maturity, biodegradation level and depositional environments. Preliminary results show compositional differences among crude oil samples. The comparison of pyrolysis products of asphalthene samples shows compositional similarity with the pyrolysis of the residue indicating that the residue obtained after thermal desorption at 500 ^{o}C is constituted mainly by asphaltene. The pyrolysis of asphaltenes from distinct crude oil samples shows distinct composition in terms of light and heavy aromatic hydrocarbons and alkyldibenzothiophenes. The use of Py-GC×GC-TOF MS is crucial for the determination of isomeric composition and Py-GC-HRTOF MS analysis with high resolution and mass accuracy complements the identification by unequivocal chemical formula assignment.

K.78

IDENTIFICATION OF VOLATILE SULPHUR COMPOUNDS OF COOKED HAM BY GCXGC-TOFMS, GC-MS/8O AND GC-GC-MS/O

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The role of sulfur compounds in the aroma of cooked meat is well-known, yet volatile sulfur compounds (VSC) in cooked ham are poorly described in the literature. These compounds are difficult to analyze, especially in this complex matrix where they are present at trace levels. Furthermore, VSC are very difficult to extract and to detect due to a strong retention by the matrix and a high chemical reactivity during all analytical steps. Hams were investigated using different types of extraction and detection methods in order to achieve the most exhaustive identification of VSC. These methods were: solid phase micro-extraction coupled to the comprehensive bidimensional gas chromatography-time of flight mass spectrometry (SPME-GCxGC-TOFMS), dynamic headspace coupled to gas chromatography-mass spectrometry (DH-GC-MS) or a specific extraction of thiols with mercury salts prior to GCxGC-TOFMS analysis. The different analytical approaches have led to a reliable identification of 39 VSC [1]. The most efficient method of detection and identification was SPME-GCxGC-TOFMS. In addition, olfactometry analyses [2] were performed using an eight way olfactometer (GC-8O/MS) and a home-made heart-cut GC-GC-O/MS device was used to resolve the co-eluting odor zones. These analyses revealed that 7 out of 39 VSC identified by mass spectrometry were perceived during olfactometry. The study demonstrated the key role of VSC in cooked ham aroma, especially 2-methyl-3-furanthiol and methyl 2-methyl-3-furyl disulfide that possess a strong “meaty” odor similar to the odor of cooked ham.

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K.79

TIME-RESOLVED BREATH GAS ANALYSIS USING NEEDLE TRAP MICRO EXTRACTION AND COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY FOR SIMULTANEOUS PTR-QUAD-MS

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Analysis of breath gas is a non-invasive tool for the medical diagnosis of diseases. Further, the metabolic profile of breath biomarkers and their changes in concentration can possibly verify the progress of a disease. The objective of this study was to develop and validate an off-line method based on needle trap micro extraction (NTME) and comprehensive two-dimensional gas chromatography – time of-flight mass spectrometry (GCxGC-TOFMS) for the monitoring of rapid changes in the composition of exhaled breath originating from metabolic stress. Influencing factors such as sample humidity, sample volume and adsorbent material were examined critically. It could be demonstrated that sample humidity strongly influences NTME and thus, needle trap devices consisting of a copolymer of methacrylic acid and ethylene glycol dimethylacrylate are not suited for the analysis of volatile organic compounds in humid gaseous samples such as breath gas. Needle trap devices containing Tenax, Carbopack X and Carboxen 1000, on the other hand, show a breakthrough of highly volatile organic compounds at high sample volumes under humidified conditions. Recent implementation of NTME simultaneously to a proton-transfer reaction - quadrupole mass spectrometry (PTR-Quad-MS) approach enabled the monitoring of changes in volatile organic compound profiles of end-tidal breath during a metabolic challenge. Correlations confirmed the applicability of the developed method. Although breath gas sampling remains challenging, NTME – GCxGC-TOFMS holds great promise for the screening of volatile organic compounds originated from metabolic stress. The combination of off-line and on-line analytical techniques has diagnostic potential for the monitoring of early breath biomarkers.

K.80

HIGH CONCENTRATION CAPACITY SAMPLING AS A FURTHER ANALYTICAL DIMENSION WITH HIGH INFORMATION POTENTIALS: EXTRA VIRGIN OLIVE OIL CLASSIFICATION

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Olive oil is presently the only food product whose sensory attributes are officially regulated and sensory assessment has to be carried out through rigorous protocols by highly and continuously (permanently) trained panelists [1-2]. On the basis of the presence/absence and the intensities of specific defects and/or positive attributes, defined by smelling and tasting, Virgin Olive oil (VO) is classified into three categories namely Extra Virgin Olive oil (EVO), VO, and *lampante* oil [3].

Volatile compounds play a crucial role in defining olive oil sensory quality and strong efforts have been dedicated to unravel the composition of this informative fraction as well as to understand correlations with quality attributes. The potentials of HCC-HS (High Concentration Capacity Headspace) sampling have here been evaluated on the basis of a previous study, where the most informative compounds, present in the “blueprint” of specific defects (or combination of defects) were adopted to discriminate and classify EVO and *lampante* oils [4]. In particular, key-odorants and other informative components (located by a supervised approach, i.e., Partial Least Squares-Discriminant Analysis PLS-DA) have been extracted and their distribution within samples successfully characterized thanks to the combination of effective and selective sampling by HCC and D-HS techniques.

Out of the sample preparation techniques investigated, Headspace Sorptive Extraction (HSSE) with differently polar and/or apolar extraction polymers, has shown to be really effective because of the high concentration factors, providing highly representative profiles as well as analyte recoveries suitable for reliable quantitation (precision and accuracy) of potent odorants in sub-trace amounts.

The possibility of extending the analytical investigation to a broader range of chemicals, will also be discussed in view of the potentials offered by multidimensional separation, by two dimensional comprehensive GC, that improves fingerprinting sensitivity and effectiveness of sample classification.

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K.81

THE USE OF PLOT COLUMNS FOR THE ANALYSIS OF LIGHT HYDROCARBONS USING FLOW MODULATED COMPREHENSIVE GCXGC

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Comprehensive gas chromatography or GCXGC is normaly associated with the analysis of liquid samples with carbon numbers ranging from C5 to over C30. The unique characteristics of differential flow modulation makes it possible to separate light hydrocarbon streams beginning as low as methane. Such streams cannot be analyzed using thermal modulation techiniques. In this work, a Capillary Flow Technology (CFT) differential flow modulator is coupled with appropriate column sets for separtion of C1 to C7 hydrocarbons including configurations where either the first dimension or second dimension column is a PLOT.

The design and operation of the CFT flow modulator will be described. Its performance for separation of light streams will be demonstrated with selected applications. Application examples will include: C4 isomers, C1 to C6 hydrocarbons and aromatics, BETX in methanol, C1 to C3 alcohols, and synthetic cracked gas.

Recommendations for best column sets for a given application and optimized operating conditions will be summerized.

K.82

CONTINUOUS WATER INFUSION ENHANCES ATMOSPHERIC PRESSURE CHEMICAL IONIZATION FOR DERIVATIZATION GAS CHROMATOGRAPHY COUPLED TO TIME-OF-FLIGHT MASS SPECTROMETRY IN UNTARGETED METABOLOMICS

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Metabolic fingerprinting requires powerful strategies for the identification of metabolites that distinguish among sample classes and puts high requirements on quantitative precision. In a recent study [1], we could demonstrate the complementarity of GC-APCI-TOFMS and comprehensive two-dimensional gas chromatography (GC?GC)-electron ionization (EI)-TOFMS for metabolic fingerprinting of *Escherichia coli* strains. It included the successful identification of discriminating metabolites missed by the latter technique [2], which was achieved based on the accurate mass of the quasi-molecular ($[M+H]^+$) ions. In the present study, for maintaining stable ionization conditions, we introduced water from the top into the APCI source to ensure a well-defined humidity, which has emerged as critical factor influencing reproducibility [1]. We have evaluated both, methyl chloroformate (MCF) and oxime trimethylsilyl (TMS) derivatives of metabolites since these derivatization strategies are most commonly pursued for GC-MS based metabolome analyses.

Water infusion at a flow-rate of 0.4 mL/h yielded a median 13-fold increase in intensity of the $[M+H]^+$ ions of 20 MCF-derivatized metabolite standards through suppression of in-source fragmentation. Furthermore, performance in the detection of 1.17- to 3.5-fold changes in metabolite concentration, which was assessed by spiking a metabolite standard mixture into a human serum extract, was improved in case of MCF derivatives, with a 1.33-fold change having been discriminated against background. On the other hand, TMS derivatives were not significantly affected by infusing water, neither in their fragmentation patterns nor with regard to the detection of differentially regulated compounds.

As a proof of principle, we applied GC-APCI-TOFMS to the analysis of MCF-derivatized metabolites in pancreatic cancer cell extracts. Water infusion led to a distinct increase in abundance of the $[M+H]^+$ions of detected metabolites and improved reproducibility of peak areas, which altogether almost doubled the number of identified significantly regulated (false discovery rate

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K.83

EXPLORING THE AMYLOID BETA (ABETA) DEPENDENT METABOLIC FINGERPRINT OF HIPPOCAMPAL NEURONS BY TWO-DIMENSIONAL COMPREHENSIVE GAS CHROMATOGRAPHY: POTENTIALS IN DRUG DISCOVERY STUDIES

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Two-dimensional comprehensive GC-MS (GC×GC-MS) represent the most advanced GC platform with great potentials in metabolomic studies due to its increased separation power, sensitivity and structured 2D patterns that can be adopted for samples informative features location and cross-comparison. The resulting chromatographic profiles, in fact, processed by suitable chemometrical approaches, enable to contemporarily run profiling and fingerprinting characterization of complex samples [1]. In the present study, we investigate the potentials of a GC×2GC system equipped with a conventional first dimension column (^{1}D - 30 m x 0.25 mm ID) coupled to two second dimension columns of variable lengths (^{2}D FID 1.6 m x 0.1 mm ID and ^{2}DMS 1.8 m x 0.1 mm ID) and parallel dual detection by MS and FID in the metabolite profiling of neurons treated with Amyloid Beta42 Peptides (ABeta42), aiming to investigate the existence of metabolic changes occurring during early stage of Alzheimer Disease (AD) onset. AD is the most common cause of dementia; it is considered the consequence of several cellular degenerative processes that primarily affect brain networks related to memory and cognition, such as the hippocampus. The accumulation of oligomers of Amyloid Beta peptides (ABeta), such as ABeta42 produced by the proteolytic processing of the amyloid precursor protein (APP) is one of the initiating event that triggers the progressive dismantling of the synapses, neuronal circuits and networks. Here, through the use of Microelectrode Arrays (MEAs), we have first characterized the neuronal firing inhibition induced by ABeta42 and then propose to investigate if the same peptides are also able to modify the neuronal metabolite fine print. Afterwards, as also DNA methylation/demethylation has a critical role in the regulation of gene expression, we aim also to understand if it can be related to AD for a possible relationship between these latter, excitability and metabolomic profiles of neurons. In this project, newly designed chemical entities will be tested to investigate the interplay between DNA methylation/demethylation on neuronal excitability and metabolic profile. By this way we will hopefully provide new molecular tools for an improved diagnosis, prognosis, and therapy. Preliminary results emphasize the potentials of GC×2GC-MS in terms of: (a) enhanced information sensitivity, being limited the amount of biologic material available for each sampling, (b) accuracy and reliability of the resultant metabolic fine-print thus enabling reliable and consistent evaluation of drug interaction with living cells and,(c) information potential that is extended to a large number of chemical features thus providing an almost complete picture of metabolic changes undergone.

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K.84

TEA (CAMELLIA SINENSIS L.) VOLATILES PROFILING BY HEADSPACE - TWO-DIMENSIONAL COMPREHENSIVE GAS CHROMATOGRAPHY - MASS SPECTROMETRY: FLAVOR COMPOUNDS QUANTIFICATION CHALLENGES

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Tea infusions are the most consumed beverages around the world, prepared by water infusion of dried leaves from *Camellia sinensis* and consumption is associated with several health benefits. Teas are classified into three major categories: unfermented green teas, semi-fermented oolong teas, and fully-fermented black teas, with the latter accounting approximately 80% of the total world tea production [1]. The sensory quality of tea is undoubtedly a key-factor affecting consumer preference, it includes color, strength, taste and aroma [1]. While phenolic compounds (mainly glycosides) and xantines conditions tea color and taste, volatile compounds are fundamental for its aroma. More than 600 volatiles have been identified in fully fermented black tea, and 41 of them have been identified as key contributors to the black tea aroma [2]. Green tea, on the other hand, is characterized by a less complex volatile fraction of about 200 volatiles, 30 of them contributing to its distinctive aroma [3]. This study reports the results of a systematic investigation on the effectiveness of different sampling approaches by miniaturized extraction devices, based on either sorption and adsorption polymers, hyphenated with a two-dimensional comprehensive gas chromatography mass spectrometry analytical platform. A selection of different high concentration capacity (HCC) sample preparation devices for Solid Phase Microextraction (SPME), Stir Bar Sorptive Extraction (SBSE) and Headspace Sorptive Extraction (HSSE) together with Dynamic Headspace (D-HS) techniques have been investigated to provide information useful for fingerprinting of tea dried leaves and resulting infusions. In the present study, volatiles and semi-volatiles contributing to define fermented and green teas aroma have been successfully characterized thanks to the combination of effective and selective sampling by HCC and D-HS techniques, high separation and detection power of GC×GC-MS and suitable data elaboration (i.e., Comprehensive Template Matching Fingerprinting- CTMF) [4]. Within the sample preparation techniques investigated, HSSE and SBSE have shown to be really effective for tea quantitative profiling because of both their high concentration factors and highly representative profiles, which cover most of the highly sensory-informative chemicals contributing to the final aroma perception.

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K.85

CHARACTERIZATION OF THE VOLATILE PROFILE OF BRAZILIAN MOSCATEL SPARKLING WINES USING SOLID PHASE MICROEXTRATION AND GAS CHROMATOGRAPHY

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The production of Moscatel sparkling wine is a growing market in Brazil. Moscatel sparkling wines are known for their typical aroma and the Brazilian ones have been calling the international attention for a while and in the last year (2013), they won 13 gold medals in 14 international competitions [1]. Solid phase microextraction (SPME), one-dimensional gas chromatography (1D-GC) and comprehensive two-dimensional gas chromatography (GCxGC) with mass spectrometric detector has been used to qualitatively and semi-quantitatively characterize the volatile compounds of 21 Brazilian Moscatel sparkling wines (52.4% of the wineries that produce their own wine, corresponding to 25.3% of the total number of wineries of the South region). Results have shown a similar volatile profile for all sparkling wines investigated by 1D-GC and GCxGC and their major compounds were the following: 3-methyl-1-butanol, hexanoic acid, ethyl hexanoate, linalool, hotrienol, 2-phenyl ethyl alcohol, nerol oxide, diethyl succinate, α-terpineol, ethyl octanoate, octanoic acid, decanoic acid, ethyl decanoate. Results of principal component analysis applied to the compounds that presented higher Fisher ratio have pointed out ways to discriminate among sparkling wine samples due to the presence of specific compounds, which may be considered potential candidates of markers for variety [2], region [3] or for quality control of wine production [4] in future studies. Several co-elutions found in 1D-GC were resolved by GCxGC, while the number of tentatively identified components was four times higher in the two-dimensional technique when compared to 1D-GC. The higher selectivity and peak capacity of GCxGC/TOFMS helped to separate volatiles that are reported as important to wine aroma, as well as to distinguish among Moscatel sparkling wines.

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K.86

EVALUATION OF A SECOND GENERATION CAPILLARY FLOW TECHNOLOGY (CFT) BASED FLOW MODULATOR FOR COMPREHENSIVE GCXGC AND GCXGC-MS

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Modulators based on differential flow have been introduced as alterative to thermal modulators for comprehensive GC [1]. Although their excellent performance have been demonstrated and similar results were obtained compared to thermal modulators for fatty acid methyl esters and petrochemical application [2-4], it is also observed that they have a number limitations. Using the Agilent capillary flow GCxGC modulator, the selection of column dimensions is relatively limited and due to the very high flow rate in the second dimension column, flow splitting is needed for coupling with MS.

As suggested by Luong et al [5], a reversed flow modulator (with reversed flow injection) could be an interesting alternative offering a broader range of column dimensions, more freedom in operation conditions and a broader second dimension "space".

In this work, a second generation type flow modulator was evaluated and a number of applications demonstrate the performance and flexibility in combination with FID and MS detectors.

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COMPREHENSIVE HPLC ANALYSIS OF ALKALOIDS AND PERMITTED ADDITIVES IN DARK CHOCOLATE PRODUCTS

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A comprehensive two-dimensional LC method for the simultaneous determination of polyphenols, methylxanthines, sweeteners, flavouring substances, as well as some common preservatives has been developed. All these compounds can be found in a number of food products and beverages, namely dark chocolate, green tea or red wine, and need to be monitored for quality control purposes. Instead of employing various methods for the different groups of substances, this multidimensional approach allows for screening of all the compounds of interest in one single run, by using a combination of two reversed phase analyses with the largely different selectivities of an ODS column in the first and an amide type bonded phase in the second dimension.

The comprehensive LC x LC technique allows for all the eluent from the first-dimension to be introduced into the second much faster analytical run. The entire sample is subjected to a two-dimensional analysis, eliminating the risk of sample losses during fractionating or trapping the sample before introducing it to a second separation. The double separation was acquired by a single detector. Comprehensive chromatographic data was generated by mathematic data manipulation. This online 2D approach offers enhanced resolving power compared to a one dimensional run by employing two successive separations, with different selectivities in a single analysis. It also results in enhanced identification power due to the formation of 2D chemical class patterns.

K*.02

EVALUATION OF SUB-2 μm PARTIALLY AND TOTALLY POROUS PARTICLES AS D2 IN LC X UHPLC FOR THE SEPARATION OF MILK PEPTIDES

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Milk is a rich source of bioactive peptides with several healthy properties. These compounds are released after proteolytic processes that occour in milk after expiration date. This matrix is highly complex and, in order to obtain good separation before the mass spectrometer source and prevent competition for ionization, high peak capacity is essential. A two-dimensional comprehensive LC × UHPLC platform was developed for the separation of peptides after IV week from the expiration date. In this approach we evaluated the employment in the second dimension (D^2), of both sub-2 μm partially (core-shell) and totally (monodisperse) porous particles columns. High peak capacity values, with respect to a conventional monodimensional approach, were obtained. A ten port-two position high pressure switching valve was used for the online continuous transfer between first and second dimension, on the detection side, both diode array detector (DAD) and a hybrid ion trap-time of flight (IT-TOF) mass spectrometer were employed in series. This platform represents a powerful tool for the identification and profiling of milk peptides, and allows to promote this “waste product” as an important source of bioactive compounds for the development of nutraceutical products and functional milks.

K*.03

DEVELOPMENT OF AN OFF-LINE COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY FOR TRIACYLGLYCEROL ANALYSIS IN FISH OIL

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An off-line comprehensive two-dimensional liquid chromatography (LC×LC) system for the analysis of triacylglycerols (TAGs) in fish oil was developed. In the first dimension (^{1}D) a Ag-coated cation exchanger (250 x 4.0 mm, 5 um) was employed with a linear gradient of butyronitrile (BCN) in hexane (Hex) at a flow-rate of 0.8 mL/min. In the second dimension (^{2}D) a shell-packed C18 column (150 x 4.6 mm, 2.7 um) was used under gradient conditions with a mobile phase composed of acetonitrile (ACN) and isopropanol (IPA) at a flow-rate of 1.0 mL/min. The primary column eluate was fractionated every 5 min prior to be injected onto the secondary column. All fractions were concentrated to dryness under nitrogen stream and subsequently dissolved in 1 mL of acetone. More than 250 TAGs were identified in the menhaden oil sample investigated by combining retention data with atmospheric pressure chemical ionization (APCI) MS spectra information. The combination of the two different stationary phases allowed to attain high orthogonality along with clearer MS spectra.

K*.04

CONTINUOUS VS. SEGMENTED SECOND DIMENSION SYSTEM GRADIENTS FOR COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY OF SUGARCANE (SACCHARUM SPP.)

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Sugarcane is any of several species of the Saccharum genus belonging to the Poaceae family. The sugarcane cultivation has been relevant for the Brazilian economy since interspecific crosses allowed the development of modern varieties with improved agronomic traits such as disease resistance and high sugar production. The metabolic composition of sugarcane comprises a large amount of bioactive compounds and especially polyphenols.
In this contribution, a comprehensive two-dimensional liquid chromatography system in combination with photodiode array and mass spectrometry (LC×LC-PDA-MS) detection was developed. To tackle such a task a micro cyano and a partially porous octodecylsilica columns were employed in the first and in the second dimension, respectively. The choice of the cyano column over other reversed phase columns tested for the first dimension separation was due to its lower correlation selectivity with respect to the secondary octodecylsilica column. Even using RP mode in both dimensions, a satisfactory degree of orthogonality was achieved by the employment of different approaches of gradient elution mode in the second dimension. By means of the investigated set-up, a number of 38 polyphenolic compounds were detected and among them 24 were positively identified by means of complementary data gathered by PDA, MS and an in-house database.

K*.05

INCREASE THE SEPARATION POWER OF TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY THROUGH THE USE OF SHIFTED GRADIENTS FOR POLYPHENOL DETERMINATION IN RED WINE

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The polyphenol analysis in real world-samples, such as wine, can be so complicated a task to overwhelm the separation capability of any one-dimensional liquid chromatography (LC) technique. Here we describe a novel system for comprehensive two-dimensional liquid chromatography (LC×LC), fully automated, in which on-line coupling of the two separation dimensions was achieved by means of two 6-port, two-position switching valves. A cyano and a partially porous octodecylsilica columns were employed in the first and the second dimension, respectively in combination with photodiode array and mass spectrometry (LC×LC-PDA-MS) detection. A cyano (250 mm ×1.0 mm, 5 µm d.p.) was used for the first dimension separation, interfaced to a secondary C18 column (30 mm × 4.6 mm, 2.7 µm d.p.) packed with fused-core particles. The performance of conventional, full-in-fraction and shifted secondary gradients was compared, aiming to tune the selectivity and increase the peak capacity of the RP-LC×RP-LC method. The separation capabilities of the developed approaches tested allowed the analysis of such a complex natural sample without any pre-treatment, by effectively reducing the interferences coming from the matrix. The selectivity and sensitivity attainable in the multiple reaction monitoring operation make analyte quantification more robust.

K*.06

THE ON-LINE HYPHENATION OF COMPREHENSIVE LIQUID CHROMATOGRAPHY TO TRIPLE QUADRUPOLE MASS SPECTROMETRY FOR THE ELUCIDATION OF THE CAROTENOID CONTENT IN FOODSTUFFS WITH HIGH ORTHOGONALITY AND SENSITIVITY

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The high complexity of many food samples places a great demand in terms of both separation capabilities, and specificity of detection. Here we describe a novel system for comprehensive two-dimensional liquid chromatography (LC×LC), fully automated, in which on-line coupling of the two separation dimensions was achieved by means of two 6-port, two-position switching valves. High orthogonality was achieved by using a micro-bore cyano column (250 mm ×1.0 mm, 5 μm d.p.) for the first dimension separation, interfaced to a secondary C18 column (30 mm × 4.6 mm, 2.7 μm d.p.) packed with fused-core particles. The hyphenation to a triple quadrupole mass spectrometer generates a powerful analytical system, capable of extremely high-resolution power, as well as targeted and untargeted analysis, simultaneously. The so-called *selected reaction monitoring* (SRM) mode in fact enhanced selectivity, reducing sample consumption and the need for tedious clean-up procedures, specifically for beta-carotene quantification in a red pepper extract.

FAST AND ON-SITE NATURAL GAS ODORANTS ANALYSIS USING MICRO GAS CHROMATOGRAPHY

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Natural gas is a widely used source of energy; it's a colorless, odorless, flammable gas and therefor odorized for safety reasons. The actual location where the gas is odorized is country depended. This could vary from during production, at the country border or at different stages in the distribution network. Multiple, relative costly component can be used to odorize the natural gas. To prevent both 'under' and 'over' odorization results in a demand for a fast and accurate method for odorant level quantification.

A natural gas suppliers association for an European-based country uses a Micro Gas Chromatograph for on-site analysis of both tetrahydrothiophene (THT) and tert-butyl mercaptan (TBM). This association is responsible for periodic odorant characterization and quantification for over 350 distribution points across the country's entire natural gas network. Instead of taking a sample and bring it to the lab, which can take up to a few days before the result is known, the natural gas is directly analyzed using a Micro GC mounted in an off-road vehicle.

The Micro GC's shoe-box size dimensions and low carrier gas consumption enables easy implementation in process applications and mobile laboratories. Direct, on-site analyses secures the integrity of the sample. Moreover, it leads to fast availability of the odorant's concentration levels. In case of the Micro GC analysis, results are known within 90 to 120 seconds run time. Out-of-spec values can directly be communicated and corrective actions can be taken accordingly.

L.02

NATIONWIDE SURVEY OF NITROSAMINES BY SPE OPTIMIZATION WITH FULLY AUTOMATED GC-EI-MS/MS METHOD

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This report is about the first nationwide survey on nitrosamines in Korea by a sensitive electron impact ionization GC-MS/MS method. In the past, the main analysis NDMA and other nitrosamines has been based on chemical ionization. This method provided sensitive and reliable data. However, an easy EI method can be applied with sufficient sensitivity due do the advanced ion focusing and timed selected reaction monitoring (timed SRM) technology. In this study the ionization methods EI, CI, and a sensitivity optimization were tested. For sample preparation commercial SPE cartridges were validated. The optimized method was applied to 76 samples from nationwide drinking water treatment plants.

For instrumental analysis a Thermo Scientific TSQ 8000 GC-MS/MS system was used. An automated step by step SRM program was applied for a CI and EI method, and the sensitivity was compared. After SRM optimization the instrumental detection limits had been tested. For the SPE validation various cartridges had been tested using the same preparation procedure. Treated water samples were collected from 76 drinking water treatment facilities in Nov. 2013. The sample volume used was 500 mL.

The nitrosamine sensitivity in CI and EI was similar. In terms of convenience of the operating method EI was adopted. The AutoSRM method setup was 60% faster than the conventional MRM setup. The instrument detection limit of NDMA was 0.3 pg. The IDL of other nitrosamines were in the 0.3 - 0.5 pg range.

The SPE cartridge validations showed drastic differences in recoveries: 1) carbon particle leaks (A brand), 2) too strong binding (B brand) and 3) available values without particle leak (UCT). Finally, the optimized SPE using UCT cartridges and the GC-EI-MS/MS method was implemented. Calibration curves showed good linearity above R^2=0.997. MDLs, LOQs and QC test were assessed using different spiking samples with 1.0 ng/L nitrosamine mixtures with RSDs better 20% (n=10). In the survey water samples NDMA and NDEA were detected near PQL values.

The results of this study indicate that the current EPA method 521 based on CI should be reassessed. EI ionization deserves to be an option for the official method.

L.03

RAPID AND RELIABLE METHOD FOR ANALYZING ACARICIDES USING CARBOGRAPH 1 AS ADSORBENT AND ANALYSIS BY GAS CHROMATOGRAPHY-ION TRAP MASS SPECTROMETRY

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The use of acaricide to combat different diseases due to parasitic mites such as Varroa jacobsoni and Ascophera apis, is a common practice in honey cultivation. In particular, amitraz, bromopropylate, coumaphos, tau-fluvalinate and fipronil are the most common acaricides used by the beekeepers worldwide for increasing the production. On the other hand, the presence of the these substances in honey decreases its quality and could affect the human health. So, it is important to set up a very easy, reliable and rapid analytical method for determining such compounds in different bee's products (e.g., honey, molasses, royal jelly).
The method is based on a solid-phase extraction (SPE) and analysis using Carbograph 1 as sorbent. The SPE procedure allows efficient recovery ranging between 85% and 107% with a relative standard deviation (RSD) ≤ 7 for the standard solution and between 85% and 98% with a RSD ≤ 9 for samples spiked with 10 ng mL^{-1} of each acaricide. The adsorption isotherms and breakthrough curves for Carbograph-1 sugary solution are reported. Gas chromatography coupled with ion trap mass spectrometer detector (GC-IT/MS) was used for analysis. The instrumental analytical protocol has been found to yield a linear calibration in the range 0.010-100 µg mL^{-1} with r^2 values ≥ 0.987. The Limits of Detection (LOD) in GC-IT/MS (SIM mode) vary between 1 ng g^{-1} and 10 ng g^{-1} (RSD $\leq$ 9) whereas the Limits of Quantification (LOQ) range between 2 ng g^{-1} and 30 ng g^{-1} (RSD $\leq$ 11); the intra-day and inter-day repeatabilities calculated as RSD for honey samples, were below 8% and 12%, respectively. The analytical method developed has been applied to several commercial bee-made products.

L.04

FULLY AUTOMATED SPE/GC/MS-ANALYSIS OF Δ9-TETRAHYDROCANNABINOL (THC) AND ITS METABOLITES IN SERUM

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This poster presents a fully automated analysis system for the determination of ?9-tetrahydrocannabinol (THC) and its metabolites in blood serum. Automation is based on the GERSTEL MultiPurpose Sampler (MPS) equipped for solid phase extraction (GERSTEL SPE) and a module for automated eluate evaporation (GERSTEL MultiPosition Evaporation Station, mVAP).
A validated, semi-automated analysis method used for routine analysis was transferred and automated using the described system. Improvements were realized such as a reduction of the sample volume used from 1 to 0.5 mL serum and the use of smaller 1 mL format SPE cartridges. The analysis method has been validated according to GTFCh guidelines (Society of Toxicological and Forensic Chemistry). Limits of quantification below 1 ng/mL for THC and THC-OH, extraction efficiencies between 70 and 93 % and relative standard deviations between 3.3 and 10 % were achieved. The SPE system performs sample preparation in parallel with the chromatographic run, enabling the GC/MS-system to operate at maximum productivity and full capacity.

L.05

THE APPLICATION OF PDHID FOR THE DETERMINATION OF SELECTED MINOR COMPONENTS IN GASES

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The pulsed discharge helium ionization detector is a very sensitive, universal detector. The ionization source is based on a pulsed discharge in helium, creating a helium continuum with energy ranging from 13.5 to 18. eV [1]. The principal mode of ionization is photoionization making it in addition a nondestructive detector which allows using it in connection with other detectors if necessary [2]. PDHID is suitable for the analysis of organic and inorganic compounds giving it a broad range of applications [3]. One of the main applications is the analysis of impurities in gaseous samples such as the determination of impurities in hydrogen produced from water electrolysis.

Among the most common electrolysis technologies is the alkaline based electrolysis. The department of inorganic chemistry in ICT has developed a demonstration unit of water electrolysis. The unit is based on alkaline electrolysis with. A microporouse membrane is used as a separating membrane. This membrane is a necessary component of the unit, allowing separating the produced gases thus avoiding the risk of creating an explosive mixture. Working temperature of the unit is 35ºC.

From previous analyses carried out in our department via GC-FID and GC-TC it is known that the major contaminants in the produced hydrogen are oxygen and water vapour. Using PDHID it was not only possible to determine the remaining concentrations of these two major compounds but, in addition, even some other small molecules were found and determined.

Probably the most important step of the whole analytical process of determining extremely low quantities of small-molecular substances is the securing of tightness of the whole sampling way. Results are given on connection of various sampling devices with PDHID.

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Acknowledgements

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L.06

MULTI-COMPONENT QUANTITATIVE ANALYSIS OF PHARMACEUTICALS IN THE ENVIRONMENT BY UHPLC-MS/MS WITH ONLINE SPE

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Pharmaceuticals comprise a group of emerging contaminants which have received considerable attention in recent years. Many common drugs can be found in the environment and sometimes even in drinking water. These drugs and their metabolites get into the waste water through excretion via the urine or feces and may reach surface water, groundwater and also drinking water after the passage in the sewage treatment plants. So far, conventional sewage treatment plants are failing to eliminate the Biodegradable substances completely.

Many of these compounds are ubiquitous, persistent and biologically active with recognized endocrine-disruption functions. Paying attention to the hazardous nature of these compounds, there is a need to provide fast and sensitive multi-residue methods that are able to analysis multiple classes of compounds within one analytical procedure. Highly sensitive triple-quad-MS systems are suitable tools for the analysis of residues in ground-, surface- and wastewater, but development of a simple, fast and reliable method for simultaneous measurement of trace levels of various different classes of analytes in complex matrices is a challenge.

This study describes a novel multi-residue UHPLC-MS/MS method that utilizes an online SPE enrichment of the various compounds followed by a fast and optimized chromatographic gradient which results in excellent ng/L detection levels.

With online SPE no further sample pre-treatment is necessary but the transition of the low pressure online SPE part of the analysis to the high pressure analytical part is difficult. Using the benefit of the modular design of Shimadzu's Nexera X2 combined with the high speed values for MRM recording and the fastest polarity switching time of 5 ms on the Shimadzu LCMS-8050, the difficulties of analyzing various classes of compounds in different polarities during one single analysis in sufficient sensitivity could be overcome.

L.07

ANALYSIS OF DOPING AGENTS USING ULTRAFAST LC-MS/MS WITH SCHEDULED MRM

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Faster, higher, further - doping accompanies sports already for many centuries. But as it was not possible to detect the illegal substances at the time, the first doping case was discovered in 1812 - only because the culprit was caught in the act.
In general, doping refers to the use of banned performance-enhancing drugs, or the use of banned methods to improve performance. But doping not always means improvement of performance. In horse racing for example terms such as negative doping, that is doping to defeat, are an issue.
In the past the attitude “Allowed is, what is not found” predominated. Nowadays improved analytical methods allow the detection of even the slightest traces of doping agents in blood and urine. Thus, the analytical possibilities of the different labs are crucial for the detection of a substance.
Here we show the advantage of an ultra-fast MS technique with excellent sensitivity when analyzing horse doping agents. Real samples from a horse doping laboratory were tested for various steroid hormones, in the form of free steroid or steroid esters, as well as prohibited neuroleptics, benzodiazepines and opioids. The samples were analyzed with Shimadzu’s triple quadrupole mass spectrometer LCMS-8050 coupled to a NEXERA X2 UHPLC. Due to the high scanning speed of the LCMS-8050 it is possible to obtain data in outstanding quality and with no loss in sensitivity even with a large number of MRMs. This could be shown impressively based on experiments performed during application development. In further measurements, the reproducibility was examined as a measure of good data quality, while a Synchronized Survey Product Ion Scan was carried out simultaneously using a standard solution containing doping agents.

L.08

MEASUREMENT OF TRACE LEVEL DECHLORANE FLAME RETARDANTS IN FOOD AND FEED BY GC-MS/MS

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Dechlorane or Mirex was extensively used as a pesticide but also as an additive flame retardant in the USA during the 1960s and the 1970s. After its ban, other related compounds such as Dechlorane Plus (DP), Dechlorane 602, Dechlorane 603, Dechlorane 604, and Chlordene Plus (CP) became candidates to replace Mirex due to their similar properties [1].
The environmental occurrence of dechlorane-related compounds was first reported in 2006 in North America when DP was detected in air, sediment and fish samples from the Laurentian Great Lakes [2]. Quite recently, their presence in significant amounts in environmental samples from Canada was also reported [3]. The presence of those compounds in the environment is of concern and so far, no data are available on the route of exposure to human. As first part of the study, we reported levels of Dechloranes in human serum from France [4], and suggested that possible routes of exposure such as food consumption should be investigated.
The aim of the second part of the study is to develop a method based on GC-MS/MS to measure Dechlorane 602, 603, 604, DP, CP, and Mirex in feed/food samples collected during continuous EU monitoring for dioxins. We optimized the MRM transitions and validated the method to be able to report first data of levels of Dechlorane in foodstuffs. These data can be crossed with food habits to estimate a human dechlorane daily intake.

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L.09

ARSON INVESTIGATION: THE VALUABLE USE OF TARGET ORGANIC COMPOUNDS IN FORENSIC ANALYSIS

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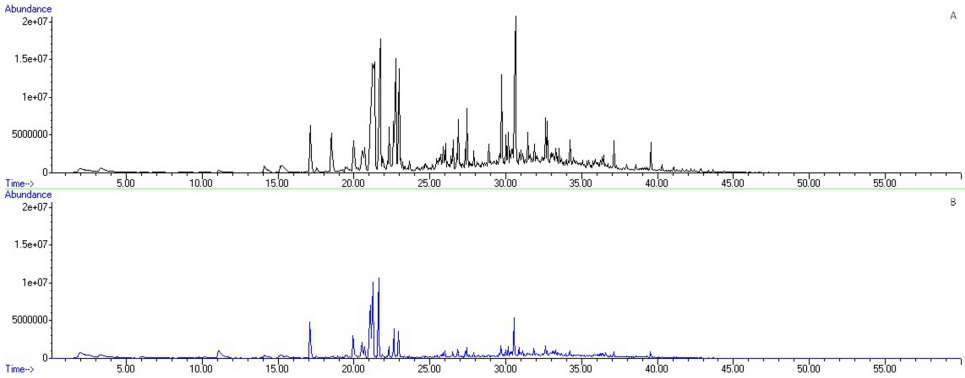
Arson is one of the most difficult crimes to solve by forensic investigators because determination the presence of accelerants in fire debris from suspected arson fires is a challenge to many forensic scientists. Due to the lack of physical evidences, chemical analyses have been made to screening the presence of accelerants on fire residues.

The most commonly used accelerants are liquid, such as gasoline, petrol, diesel and kerosene. These ignitable liquids have characteristic chemical compositions which allow the use of target compounds in identification of accelerants in fire debris.

This project, involving industry, have been studying the criteria for identification the presence of accelerants in fire debris and the target chemical compounds and also the influence of environmental conditions and time.

Different chemical profiles have been identified in different ignitable commercial products, potential fire accelerants. Time delay was studied, showing to be a crucial factor between fire and sample collection. Matrix effect was also studied, demonstrating that matrix can influence the success of identification of accelerants in arson analysis.

Figure shows the influence of time in arson analysis (A – Sample collected two days after the fire; B- Sample collected thirty days after the fire).



L.10

OPTIMIZATION OF HEAD SPACE SOLID-PHASE MICROEXTRACTION CONDITIONS FOR DETERMINATION OF HYDROGEN SULFIDE

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Both wastewater treatment and waste management are associated with odor emissions, being the presence of sulfur compounds (eg, hydrogen sulfide and mercaptans) one of the causes. In this context there is a demand for technologies that are effective in their quantification. The aim of this study was the development and validation of a methodology based on a head space solid phase microextraction (HS-SPME) coupled with gas chromatography- flame photometric detector (GC-FPD) to analyze H2S in a wide range of concentrations.

A sample bag was used to store a small quantity of pure H_2S and standard solutions were mixed in 600 and 300 ml glass flasks closed with Suba-Seal rubber septa [1],[2].

A 75-mm Carboxen-polydimethylsiloxane fiber coating fiber was used and some variables (extraction time and desorption time) were considered in order to establish optimum conditions. The samples were injected in splitless (0.3 min) mode in a GC 2010 Plus A from Shimadzu with a S filter Sulfur, the column temperature program was from 60ºC (2min) increased by 15ºC / min to 100ºC. The injector temperature was held at 250ºC, and the detector at 260ºC.

The method developed would give important contributions both in the measurement of emissions and in the study of new and effective technologies for the treatment of gaseous emissions, namely by biofiltration.

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L.11

LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY-BASED METHOD TOWARDS THE COMPREHENSIVE ANALYSIS OF MIGRATION OF PRIMARY AROMATIC AMINES FROM FOOD PACKAGING

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A liquid chromatography-Orbitrap-full scan-high resolution mass spectrometry (LC-HRMS) method was devised and validated for the determination of migration levels of primary aromatic amines (PAAs) from food contact materials. Recently, our research group successfully proposed DESI-Orbitrap-HRMS methods to control safety of food plastic materials [1]. PAAs, which are toxic compounds and suspected human carcinogens, can migrate from plastic multilayer laminated films and colored materials into foods and can be found as residuals from incomplete reactions, as by-products or degradation products. To protect consumer health, EU legislation has established that plastic materials and articles shall not release PAAs (detection threshold: 0.01 mg/kg of food or food simulant) [2]. Currently the determination of PAAs is based on a colorimetric measurement [3] or on more accurate and selective targeted LC-MS/MS approaches [4]. Taking into account the advantage of specificity of accurate mass and the intrinsic limitation of multiple reaction monitoring acquisition mode, that does not permit retrospective screening analysis, we exploited the potential of LC coupled to Orbitrap-full scan-HRMS without any pre-treatment step for the rapid screening analysis of 22 PAAs released from food packaging.

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L.12

UTILIZATION OF GAS CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY IN BOTH TARGET AND NON-TARGET ANALYSIS OF PESTICIDE RESIDUES IN DIFFICULT MATRIX - TEA

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Tea (canelia sinensis) is one of the oldest agricultural products and one of the most consumed beverages worldwide. The health effect examination of tea has been performed already more than 4000 years ago and positive influence on different diseases were reported. The latest scientific studies then recently discovered components mainly responsible for the health benefits that have traditionally been attributed to tea (catechins).

The increasing consumption of tea demands the producers to intensify its production, while pesticides are one of effective tools for control of tea-pests. Although the usage of pesticide preparations significantly helps to eliminate the losses in production, the consumer's exposure to used pesticide residues and hence potential health risk should be considered. The effective control of residue is thus needed, however, tea represents a very complex matrix containing high amount of matrix constituents, such as caffeine, pigments and polyphenols, making from a multiple pesticide screening an analytical challenge, namely if a large volume injection (LVI) is applied.

Although modified Quechers method recently published substantially simplified the sample preparation and effectively removes some of matrix components. The high selectivity of pesticide detection is still highly required.

The HRT instrument utilizes a unique multi-reflecting time-of-flight mass spectrometry based on the Flight Folded Path (FFP^{TM}) technology represents a method that enables achieving of the mass resolution up to 50.000 FWHM with mass accuracy measurement of < 1ppm (routinely achieved), along with extremely high data acquisition speed (up to 200 spectra/second).

The method comprising the large volume injection of Quechers extracts, gas chromatography with ultra-high resolution mass spectrometry (FFP^{TM}) was used within this case study. The power of used GC-MS approach with enabled highly selective detection of analytes and their identification using elemental composition and isotopic fine structure. In this contribution, targeted and non-targeted screening of multiple (>110) pesticide residues spiked into black tea extracts will be demonstrated.

PERSISTENT ORGANIC COMPOUNDS IN CAIMAN YACARE EGGS: VALIDATION OF A METHOD BASED ON SOLID–LIQUID EXTRACTION WITH LOW-TEMPERATURE PARTITION (SLE-LTP).

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This work presents the validation of a method based on solid-liquid extraction followed by low-temperature partition (SLE-LTP) for the determination of organochlorinated pesticides (p,p'-DDT, p,p'-DDD, p,p'-DDE, o,p'-DDT, o,p'-DDD, o,p'-DDE, α-endosulfan, β-endosulfan and endosulfan sulfate) and PCBs (congeners 28, 52, 101, 118, 138, 153 and 180) in the eggs of the Pantanal caiman (*Caiman yacare*), from Brazilian Wetland (Pantanal, Mato Grosso, Brazil). The optimized extraction method (Sousa et al., Microchemical Journal 114 (2014) 266) includes12 mL of extractor solvent, 5 min of vortexing time, 5 min of centrifugation time and 12 h of freezing time. PCBs and organochlorinated pesticides were determined using gas chromatography (GC) with electron capture detection (ECD), PCB 209 was used as internal standard. The method limits of detection (LOD), determined from the extracted matrix-based calibration curve, ranged from 5.64 ng g^{-1} (PCB 180) to 12.26 ng g^{-1} (o,p'-DDT), while the method limits of quantification (LOQ) ranged from 8.50 ng g^{-1} (PCB 180) to 27.01 ng g^{-1} (o, p'-DDT). All these values were reported on liofilized eggs base. The method presented recovery range of 61±6% (o,p'-DDD) to 121±20% (o,p'-DDT), and relative standard deviations (RSD) of less than 18%, for all compounds. All pesticides presented significant matrix effects, because this all quantifications were done using extracted matrix-based calibration curves. Apart from some minor pros and cons, the method is fast, efficient, robust and not expensive with adequate performance to the aimed application. This method have been applied to an environmental study to evaluate the potential of caiman eggs as POPs contamination bioindicator for the Brazilian Pantanal region.

M.02

A TUCKER MODEL BASED APPROACH FOR ANALYSIS OF COMPLEX OIL BIODEGRADATION DATA

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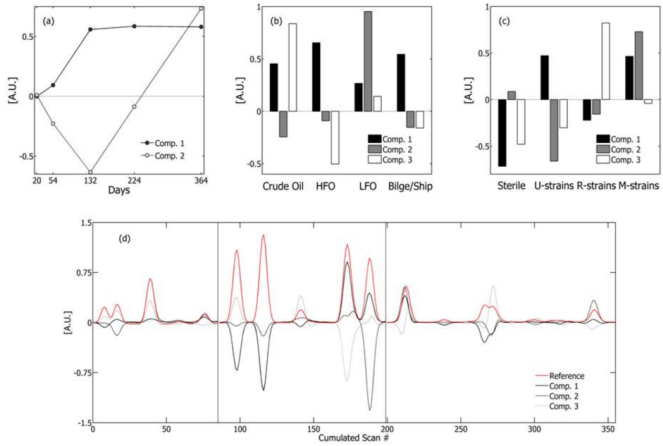
A novel method based on gas chromatography – mass spectrometry in selected ion monitoring mode (GC-MS/SIM) and Tucker models is developed to evaluate the effects of oil type, microbial treatments and incubation time on the biodegradation of petroleum hydrocarbons. The data set consists of sections of the m/z 180, 192 and 198 GC-MS/SIM chromatograms of oil extracts from a biodegradation experiment where four oil types were exposed to four microbial treatments over a period of one year. The chosen sections, which are specific to methyl-fluorenes, phenanthrenes and dibenzothiophenes were combined in a 4-way array (incubation time × oil type × treatment × combined chromatographic retention times) that was analyzed using both principal component analysis and the Tucker model. Several conclusions could be reached: the light fuel oil was the least degradable of those tested, 2- and 3- methyl isomers were more easily degraded compared to the 4-methyl isomers, the mixture of surfactant producers and PAC degraders provided the most effective degradation and the largest part of the degradation occurred between 54 and 132 days (see Figure)



M.03

ANALYZING THE GCMS AMENABLE COMPOUNDS IN THE EU WATERFRAMEWORK DIRECTIVE

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The EU water frame work directive poses stringent new detection limits for a list of priority substances in various watermatrices. Many of those substances are GC amenable and can be analyzed with different injection and detection techniques. The compounds have been divided into different classes, according to the standard analytical workflow in the environmental lab and the analytical method for each class is described, together with the current detection limits and showing results in various surface water samples. The volatile compounds are analyzed using headspace GC, with the MS in SIM mode. All compounds are analyzed using three different SIM ions which are monitored continuously for their ratio. A brief discussion on other volatile pollutants, that are not included in the directive is included in this section. For the semivolatile compounds a triple quadrupole MS is being used in MS/MS mode. All compounds are measured using two transitions of which the ratio is monitored continuously. For the semivolatile analysis six compound classes are discussed: polycyclic aromatic compounds, pesticides, phenols, short chain chlorinated parafins, organotin and polybrominated diphenyl ethers. All compound classes pose their own chromatographic challenges and some solutions will be presented.

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M.04

VOCS MONITORING IN AMBIENT AND POLLUTED AIR BY MOBILE LAB EQUIPPED WITH MINI TD AND TRANSPORTABLE GCMS

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GC and GC/MS analysis of VOCs can be performed in the lab after samples are gathered from the field.
However, since most samples originate outside the lab, the majority of VOC emissions testing is performed by mobile labs equipped with transportable instruments to ensure fast response and accurate on-site results.
One of the most important environmental mobile lab tasks is to identify and quantify the wide range of VOCs in air pollutants. In general, most of these pollutants come from stationary or organized emission such as petrochemical plant, vehicle exhaust, and accidental or unorganized emission, for example, solvent leakage during the transportation.
Sample preparation is a critical step in the field analysis process, with an impact to data quality, test accuracy, and analysis speed. The 7667A mini Thermal Desorber is used in this work to on-site analyze environmental VOCs providing truly mobile features including a much smaller size, lower power consumption, ease of installation.

FAST GCMSMS ANALYSIS OF 76 VOC COMPOUNDS USING HEADSPACE-TRAP SAMPLING

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The analysis of regulated volatile organic compounds in drinking and waste water was shown to be compatible with fast GCMS when a cold trap was mounted at the top of the column directly under the injector [1]. Here the method was adapted to a tandem GCMS. The triple quadrupole detector was run in mrm and /or pseudo mrm depending on the spectra of the target compound. The latter describes a situation where precursor (Q1) and product ion (Q3) was set to the same m/z with collision energy CE=0. As an example the first eluting compounds which are Dichlorodifluoromethane, Chloromethane, vinyl chloride, Bromomethane and Chloroethane were run in that mode. Those compounds have low m/z main electron impact fragments like m/z= 85, 50, 62,94, 64 , respectively. A total number of 52 volatile organic compounds which mainly show m/z higher than about 100 in EI were run in traditional mrm conditions. Product ion scans for different CEs were done to optimise transitions and CE. The sensitivity in mrm mode was considerably higher than SIM modes except the compounds measured in pseudo mrm where the sensitivity was comparable .
The split ratio was 5:1 and the linear velocity was set to 45 cm/sec (He). The GC oven temperature started at 40 °C, 5 min and then ramped with 50 °C/min to 120 °C, 30 °C/min to 170 °C, 60 °C/min to 220 °C. The total run time was below 10 minutes for all 76 compounds. Calibration curves were done between 5ng/L and 1 mg/L and real world samples from river water were quantified.

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M.06

AUTOMATED AND HIGH-THROUGHPUT QUANTITATIVE ANALYSIS OF PCB'S (DIOXIN-LIKE) IN DRINKING WATER BY MTX-MFX-SOLID PHASE MICROEXTRACTION COUPLED TO FAST GAS CHROMATOGRAPHY-ELECTRON IONIZATION/MASS SPECTROMETRY. VALIDATION AND PROFICIENCY TESTING

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The Italian Legislative Decree No 236/88 implementing Directive 80/778/CEE sets out a series of analytical profiles for water relating to the quality of water intended for human consumption . In previous studies the use of solvents and/or clean-up steps are often reported to extract and eliminate most of the interfering compounds. Accordingly, we developed a procedures where the properties of Head Space (HS)-Solid Phase MicroExtraction (SPME) technique as well as the automation of the preparation procedure by new robotic system called MTX-MFX, through change of the fibers and syringes , allowed a friendly use of Fast GC-MS/EI apparatus with a number of advantages including reduced analysis time and greater reproducibility. As, this increased versatility of MTX-MFX allows approaches in fully automated mode, The purpose of this work was to optimize an analytical procedure fully automated by the construction of the calibration curve for the validation of the method, by preparing and analyzing the standard solutions to the preparation and analysis of proficiency testing for the verification of the validation in short time by use of single GC/MS apparatus with consequently more sensitivity power and high discrimination, than other techniques for routine employed in environmental laboratories. The resulting calibration curves were linear, in the investigated range for all the considerated analytes, with correlation coefficients >0.999. The RSD resulted $\leq 10\%$, for some analytes also $< 5\%$. Together with the use of validated methods, proficiency testing is an essential element of laboratory quality assurance [2]. The automation of the preparation procedure with MTX-MFX, that allowed the change of the fibers and syringes by robotic device, allowed a friendly use of Fast-MS apparatus with a number of advantages including reduced analyst time both for routine analysis and method development, and greater reproducibility.

HS-SPME-GC/MS ANALYSIS OF VOLATILES FROM EUCALYPTUS WITH DIFFERENT SUSCEPTIBILITY TO THE EUCALYPTUS WEEVIL GONIPTERUS PLATENSIS ATTACK

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Eucalyptus plantations and its products (e.g. production of pulp and paper) are an important economic resource in Portugal. The Gonipterus is a genus of weevils in the Curculionidae family. Among them, the Eucalyptus Weevil (Gonipterus platensis), native from Australia, has become a major alien pest in most Eucalyptus plantations around the world where it became the most severe defoliator with a strong economic impact.
In order to study potential chemical factors that may mediate host tree selection, the leaf volatiles of different Eucalyptus, 3 hybrids of E. Globulus plus one E.nitens, with different, susceptibility to the attack of Gonipterus, were studied. The volatiles from each sample were extracted by solid phase microextraction (SPME), using a 100 um Polydimethylsiloxane (PDMS) fiber and analysed by Gas chromatography (GC-FID) and Mass Spectrometry (GC/MS). The compounds separation was performed on non polar 5% Phenyl 95% Dimethylpolysiloxane capillary columns with 25 m x 0.25 mm i.d (ZB-5ms and ZB-5 from Phenomenex). The film thickness was 0.23 um for GC/MS and 1.0 um for GC-FID. The volatiles identification was performed by GC/MS and linear retention indices (LRI) calculated according to van den Dool and Kratz. 71 compounds were detected in the volatile fractions emitted by the Eucalyptus leaves, being 49 identified. The results show that samples belonging to more susceptible trees emitted qualitatively and quantitatively more compounds. By means of a principal component analysis it was possible to visualize a separation between the sample provenance driven by alpha-pinene, limonene, 1,8-cineol and viridiflorene.

Acknowledgements
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M.08

DETERMINATION OF MUSTY/EARTHY ODORS IN DRINKING WATER USING DYNAMIC HEADSPACE/P&T-TIME OF FLIGHT GC/MS

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The musty/earthy odor compounds associated with drinking water are mainly related to two substances: Geosmin (trans-1,10-dimethyl-trans-9-decalol) and 2-MIB (2-methylisoborneol). Some species of algae and bacteria produce naturally odour chemicals inside their cells, Geosmin and 2-MIB are common odourous compounds. The aim of this work was to develop a simple, fast and sensitive analytical method for the determination of earthy and musty odor organic compounds in drinking water using a Dynamic Headspace /Purge&Trap-Gas Chromatograph-Mass Spectrometry system.
The water sample is directly placed in a standard 20 ml vial, heated and purged with a flow of an inert gas for a defined time. The inert gas sweeps the sample and transfer the compounds in a cold focusing trap where they are concentrated.
Finally, the trap is heated up and desorbed in backflush by the carrier gas.
The extraction parameters are investigated. All data will be reported including chromatographic parameters.

DETERMINATION OF UV FILTERS BY GAS CHROMATOGRAPHY COUPLED TO TANDEM ION TRAP MASS SPECTROMETRY IN NATURAL WATERS FROM SÃO PAULO STATE (BRAZIL)

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UV filters are organic compounds with high molar absorptivity in the UV range, they are used in sunscreen products. Because of their effectiveness against the harmful effects of ultraviolet radiation, have been added not only to sunscreen products but also to the formulations of many everyday products. Therefore, UV filters are introduced into the environment in significant amounts, both directly, and indirectly [1]. The concern about the presence of such substance in the environment resides in the ability to act as endocrine disruptors. Bioassays in vitro and in vivo show that the substances benzophenone-3 (BP-3), ethylhexyl methoxycinnamate (EHMC), ethylhexyl salicylate (ES) and octocrylene (OC), exhibit estrogenic activity [2]. Despite the evidence, there are few studies on the occurrence and fate of this type of substance in the environment and none refers to the Brazil. Thus, this study aimed to: investigate the occurrence of these substances in natural waters of an Hydrographic Basin at São Paulo State, Brazil. The use of sensitive and selective analytical technique, assisted by a well optimized extraction procedure was necessary for the monitoring of UV filters. For this purpose we used solid-phase extraction (SPE) followed by gas chromatography-tandem mass spectrometry (GC-MS/MS). The analytical method was showed recoveries at four fortification levels between 62 and 107%, with relative standard deviations below 14%. The detection limits ranged from 7.6 to 24.1 ng L^{-1}. Water samples were collected (March 2013 to February 2014) in Araraquara, Bauru, Bueno de Andrada, Jau, São Carlos and Trabiju in São Paulo, Brazil. All UV filters studied were detected in the samples studied, however, only the BP3 in measurable concentrations.

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M.10

GAS CHROMATOGRAPHY A USEFUL TOOL FOR THE VERIFICATION OF THE BIOMETHANISATION PROCESS

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Municipal landfills, the water treatment stations, the anaerobic lagoons or deep lagoons, produce tremendous amounts of biogas that escape into the atmosphere. If it is not used and valued, this biogas can affect the air quality and act as greenhouse gases and thereby impacting the climate change. On the other, biogas is very rich in energy and can be operated to produce electricity and heat, and thus meet the energy needs of rural and remote areas, while reducing odors, the environmental pollution and greenhouse gas emissions. Processing of municipal waste by anaerobic digestion can be a solution to the mentioned problems. In order to make this process more efficient, this technology has been developed in recent decades. In the present work, the biomethanisation processes that transform the municipal wastes into biogas of energetic value are presented and their advantages and disadvantages are discussed. The national strategy for the operation and development of biogas for energy use would contribute to reduce the emission of toxic air pollution and greenhouse gases while providing another renewable energy resources. Organic waste disposal in a big waste landfill has been selected for this investigated. After anaerobic digestion and purification, the produced biogas has been analysed using gas chromatography to check the purity of produced biogas.

M.11

PERSISTENT ORGANIC POLLUTANTS IN SOILS OF GUYU (SOUTHEAST CHINA) BY GC–MS–MS–SRM

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The present study was conducted to investigate PAHs, PCBs and OCPs levels in soils taken from fields surrounding small workshops for e-waste recycling activities in Guiyu, one of the largest e-waste recycling sites in China. After extraction procedures with an accelerated solvent extractor (ASE 350, Dionex) and clean-up (EPA Standard Method 3620C) (USEPA, 2007), samples were analyzed by GC–MS–MS–SRM (TSQ Quantum XLS, Thermo Fisher Scientific). Concentration of acenaphthylene, fluorene, phenanthrene, anthracene, pyrene, benzo[a] anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo [a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[ghi]perylene ranged from 17.65 to 236.38 µg/kg, with a mean value of 98.84 µg/kg. Sample soils in this study are characterized by a dominance of high-molecular-weight (HMW) PAHs indicating pyrogenic sources. PCBs were detected in all samples with total concentrations ranging from 1.83 to 493.71 µg/kg, with a mean value of 62.86 µg/kg; 12 dioxin-like PCB concentration ranged from 1.21 to 24.64 µg/kg with mean concentration of 9.29 µg/kg, contributing 13.8–34.63% of the total. OCP concentration ranged from 0.63 to 117.3 µg/kg with an average of 18.82 µg/kg. The dominant OCPs were DDTs and their metabolites (DDT, DDE and DDD). As general overview, POP contamination in Guiyu decreases after the rigid intervention of Ministry of Environmental Protection of China beginning from the year 2000 if we compare our results with large-scale of sampling time and sites in Guiyu.

M.12

AUTOMATED, RAPID AND RELIABLE DETERMINATION OF DISSOLVED GASES IN WATER BY STATIC HEADSPACE - GAS CHROMATOGRAPHY

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Hydraulic fracturing is a well stimulation technique in which typically water is mixed with sand and chemicals and injected at high pressure into a wellbore to create small (< 1mm) fractures in order to maximize fluid removal and well productivity. The technique makes accessible big amounts of formerly non-accessible hydrocarbons.
The dissolved gases have become a controversial environmental and health matter with some countries completely banning the practice.
Public outcry over preservation of water quality has led the U.S. EPA and other state agencies to investigate the impact of hydraulic fracturing on the quality of environmental waters. In this poster we present a high throughput test method for the quantitative determination of dissolved gases in ground, waste and drinking waters that employs robust, automated and relatively inexpensive instrumentation like static headspace and gas chromatography with flame ionization detection.

M.13

CHROMATOGRAPHIC ANALYSIS OF A FUNGICIDE IN SOIL ON GRAPES GROWING AREA

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The accurate detection of pesticide is crucial to ensure the safety of the food product and the environment. Behaviour of azoxystrobin fungicide was studied in vinegnard grapes when applied 250 $g.ha^{-1}$ (recommended dose) in the grapes growing state of Médéa (Algeria), namely Benchikao, in the year of 2011. A total of two sprays were given at an interval of about 7 days. The quantitative evolution of fungicide in soil is studied under the conditions natural fields. The three soil samples analyzed in this work are: The first is taken from near the first row and the other near the second row of vineyard that was used to quantitative analysis of residues in grapes. A third sample floor is taken away from the processing zone, the latter is regarded as a sample uncontaminated (white), located approximately 100 m from the treated area.

The persistence of the fungicide in soil samples was conducted using accelerated solvent extraction "ASE" and gas chromatographic analysis (GC / ECD) analysis. The results of the chromatographic analysis showed the persistence of the pesticide after 6 weeks of treatment. Which an amount of 1.36 $mg.kg^{-1}$ for soil samples collected at the foot of the first row and 0.55 $mg.kg^{-1}$ in soil collected at the foot of the second row of the vineyard has been confirmed. For the analysis of control soil (uncontaminated) we were surprised to find a number of 0.63 mg/kg of the azoxystrobin. This can be explained by the transport phenomena created by climatic conditions field especially wind exposure.

M.14

EVALUATION OF QuEChERS APPROACH FOR THE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS

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Polycyclic aromatic hydrocarbons (PAHs) are mainly produced as by-products from the incomplete combustion of organic matter and fossil fuel. They constitute ubiquitous environmental contaminants and are of main concern since their toxicities have been widely demonstrated (carcinogenic and/or mutagenic properties notably).
Regarding aquatic environment, they have been included in the list of priority substances as defined by the European Union Water Framework Directive. Their monitoring is therefore mandatory to assess the quality of aquatic media. The PAH are hydrophobic component so are mostly adsorbed onto sediments.
Extraction using pressurized fluid is the most popular method for sediment sample treatment. It is very efficient for extracting the target analytes but also all the other potentially interfering components that might be present within the matrix. Moreover, this technique is relatively expensive since dedicated equipment is needed.
The QuEChERS approach, developed initially for the analysis of pesticides in fruits and vegetables, can be regarded as an alternative technique to simplify this step. It is easy to handle, relatively cheap and with low solvent consumption. Therefore, this technique has been evaluated in this study for the extraction and sample treatment of PAH in sediments. The analysis of PAH was then performed by gas chromatography coupled to mass spectrometry.
A QuEChERS type method and purification protocol based on the approach generally carried out for fruits and vegetables was applied. An extraction with vortex agitation using acetonitrile was performed followed by a purification step by dispersive solid phase extraction. Extraction parameters such as agitation time were investigated. Influence of water addition to sediments was determined as a particularly significant parameter on recoveries of PAH. The purification step was also evaluated with the test of several phases.
Finally, the optimized protocol was tested on several sediments and on reference certified materials. Overall, in most cases, the QuEChERS based approach appeared suitable for the analysis of PAH in sediments.

MULTIRESIDUE DETERMINATION OF UV FILTERS IN SURFACE WATERS BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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In the last years, both public institutions and researchers are paying increasing attention toward the so-called “emerging contaminants”. Among them, there is the commercial category of the UV filters, which include various and heterogeneous chemical classes. UV filters are present as ingredients in personal care products to protect skin and hair from the negative effects of sunlight; furthermore, some UV filters are also used in plastic products and packaging, paints, glasses, textiles, to prevent yellowing and degradation of polymers and pigments [1]. Because of their widespread employment, these compounds can enter the aquatic environment from recreational activities (such as sunbathing and swimming in seas, lakes and rivers), industrial wastewater discharges and wastewater treatment plants [2]. Currently, the EU Regulation permits the employment of twentysix compounds, within certain limits, in cosmetic (sun-screen) products.

The aim of this work was to develop and validate a highly sensitive multiresidue method based on solid phase extraction (SPE), employing graphitized carbon black as sorbent material, followed by ultra high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) for the analysis of sixteen UV filters and their environmental degradation products with a wide range of physicochemical properties. After a thorough investigation of SPE and UHPLC-MS/MS conditions with electrospray source operated in polarity switching mode, the methodology permitted the enrichment and the simultaneous determination of the compounds, selected among the most commercially used. Recovery above 70% were obtained for all the analytes from lake water samples. A survey on few water samples from three lakes in the area of Rome showed that some of these UV filters are present in small amount.

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STRUCTURE AND THERMAL REACTIONS OF PETROLEUM ASPHALTENES: A PY-GC STUDY

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Asphaltenes are high-molecular weight species present in crude oils which influence their migration, combustion and processing properties and current refining practice produces oils with higher levels of asphaltenes. These heavy fuel oils find application in industrial boilers and furnaces as well as in marine engines where increasing asphaltene content results in increased smoke emissions. Atmospheric soot has been shown to be the second most important cause of global warming, in addition to having significant impacts on human health.

Knowledge of the chemical structure of petroleum asphaltenes is therefore required; in this study pyrolysis was carried out in a microflow cell reactor with MS and AED detection: an SGE pyrojector was coupled to a CDS 5200 series pyrolysis unit. The resulting Py-GC-MS chromatograms of petroleum asphaltenes at 600C contained alkane/alkene pairs, alkanoic acids, and alkyl aromatics and heterocycles; pyrolysis at 900C gave similar products but also evidence of secondary reactions. Py-GC-AED with sulphur detection identified numerous alkylated benzothiophens and dibenzothiophens.

These results are consistent with an 'archipelago' rather than an 'island' structure for petroleum asphaltenes; The former are made up of linked small aromatic and naphthenic clusters and their sulphur isosteres with substituent alkyl groups, some in long chains, with the building blocks held together by bridging alkyl and naphthenic groups. During pyrolysis and combustion these linking groups are broken to yield radicals which are then 'capped' by hydrogen transfer, hence producing the low MW compounds detected by capillary GC-MS. The radicals resulting from hydrogen donation cross link to form a carbonaceous residue and soot.

N.02

CONTINUOUS MONITORING AND CALORIFIC POWER CALCULATION OF NATURAL GAS WITH STANDALONE MICRO-GC FULL MEMS BASED

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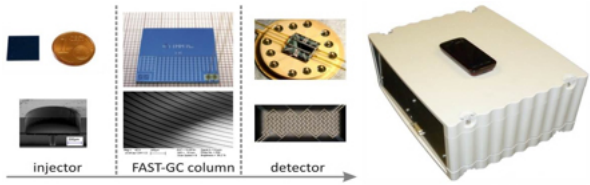
Natural gas is formed by a mixture of gaseous, main component is methane (CH4), but also contains heavier HC: ethane (C2H6), propane (C3H8), butanes (C4H10), pentanes (C5H12). Percentages of CO2 and gases such as nitrogen and oxygen are present.
The conventional measuring systems are gas chromatographs with TCD detector. A complete analysis requires at least 3 analytical columns:

1. He, H_2, O_2, N_2, CH_4, CO
2. C1-C3, CO_2
3. C4-C10

The main goal of this work is to have a single MEMS column to share CO_2 – N_2 – CH_4 and C2-C6 in only one analytical run on chip silicon technology.
The instrument need to work continuous in a remote location with a large range of ambient condition between -10 to +50 °C.
A full MEMS based micro-GC, with his small-sized, is the best solution to control temperature of the “oven” easily, with a heating and Peltier plate; the temperature for this analytical method is between 10 and 15 °C, that means we have to heat in winter and cold down in summer time. With same analytical performance would therefore be the ideal solution for an effective continuous sampling of the above-mentioned parameters, for quick and easy control analysis and calorific power calculation.
A pre-series of a miniaturized instrument formed of micro processed components in silicon chips had been realized, able to allocate in reduced dimensions the injector, the column and the detector, and able to minimize energy, flows and gas consumption, are installed on Natural gas secondary distribution cabinet.
The MEMS elements allow to separate and quantify the various components of the natural gas, including CO_2 and N_2 in a single run. An impressively short analysis time and better separation power than the ones obtained with the conventional instruments had been obtained for HC, CO_2 and N_2.
The control of temperature is very smart and precise.



Overview and micrographs of the MEMS devices (left) and complete assembled prototype (right)

N.03

CHROMATOGRAPHIC DATA ANALYSIS: THE PIXEL-BASED APPROACH

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The pixel-based approach for signal processing of chromatographic data represents an alternative to feature detection and extraction, and curve resolution techniques. Instead of providing a list of features or peaks that can be analysed by multivariate methods, the intensities are used directly as variables in the multivariate analysis. This avoids the need for identification and quantification of peaks, which is prone to errors and in some cases hard to automate. In this poster, we present a short tutorial on how to use the pixel based approach for the analysis of chromatographic data. The tutorial provides a description of each individual step: How to (1) set up the experiment, (2) do the chemical analysis, (3) identify and remove instrumental artifacts, (4) focus the analysis on relevant information, (5) find the optimal signal processing steps in an automated and objective way, (6) perform multivariate data analysis, and (7) interpret results from the analysis. GC-MS data of petroleum biomarkers (steranes) for oil spill identification are used to illustrate the use of this approach for the analysis of complex mixtures of chemicals.

DETERMINATION OF THERMAL DEGRADATION PRODUCTS OF HINDERED AMINE LIGHT STABILIZERS USING HPLC-QTOF-MS

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Plastic materials are getting increasing importance due to their wide range of applications. For the extension of their lifetime, different types of stabilizers are used. In order to prevent degradation of plastic materials by UV-radiation, hindered amine light stabilizers (HALS) can be added into the polymer. Nevertheless there could be a depletion of initially used stabilizer and it has to be clarified if there is still a stabilization potential of their degradation products.
Previous work allowed the separation of different HALS with reversed phase chromatography at pH values around 11 coupled with quadrupole - time of flight (Q-TOF) detection [1]. In the present work six different hindered amine light stabilizers were treated under thermal conditions at 100 °C for one hour or were aged in methanol for one day in a drying oven.
Structure elucidation of degradation products was accomplished by liquid chromatography-mass spectrometry. To identify incurred degradation products an Agilent 6510 Q-TOF mass spectrometer equipped with an electrospray ionization (ESI) source was operated in the positive ion mode. A general overview on the most important species formed during degradation is shown in this work. The results indicated that typical degradation products of HALS have still a potential for protecting plastic materials during expose to light.

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N.05

NEAR REAL-TIME PROCESS CONTROL USING MICRO GAS CHROMATOGRAPHY – FAST, ON-LINE ETHANE, PROPANE and BUTANE PRODUCT ANALYSIS

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This poster highlights the use of Micro GC instrumentation for fast, on-line analysis of liquefied ethane, propane and butane process streams. Results are calculated by the instrument's on-board data handling and calculation module and delivered to plant processing systems in an unattended manner.

The ethane stream analysis involves the detection of nitrogen, methane, carbon dioxide, ethane and propane, with trace detection of hydrogen sulfide and methanol. The micro GC uses a single column channel to analyze all components of interest in 180 seconds. Auto ranging feature on the micro thermal conductivity detector (μTCD) enables automatically switching from ultra-high sensitivity (low ppm range detection) to normal settings (percentage level detection). As a result trace level component and matrix composition analysis is performed in a single analytical run.

For liquefied propane and butane (LPG) product characterization, a single channel Micro GC was used for alternating analysis of the two streams. All compounds of interest (ethane, propane, butanes, and pentanes) were analyzed in 60 seconds. This resulted in a near real-time data transfer to the plant process control system for faster trend analysis and better informed decision making.

N.06

EXPANDING ANALYTICAL CAPABILITIES FOR LABORATORY GC THROUGH THE USE OF AN EXTERNAL ISOTHERMAL ZONE

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Complex GC applications can require using multiple valves for column switching, heart cutting, and multi-dimensional analysis. Similarly, columns used for certain application have temperature limitation for packing materials or stationary phase films. The addition of a large valve oven to the 7890B GC provides an external isothermal zone addresses these requirements. Capable of supporting a combination of up to six (6) column mandrels of valves the large valve oven provide thermal regulation up to 300 °C. When applied to Refinery Gas Analysis (RGA) the new system provides stable response for oxygen (O2) and hydrogen disulfide (H2S) with profiling of hydrogen, hydrocarbons (C6+ as backflush), and permanent gases in eight (8) minutes using micropacked columns, and seventeen (17) minutes using standard packed columns. The additional capacity for multiple valves and columns in a single heated zone also allows for the analysis of reformulated fuels per ASTM D3606, D4815 and D5580.

N.07

COMPARISON OF THE MULTI-MODE AND COOL ON-COLUMN INLETS FOR EN 14105

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European Standard EN 14105:2011 is used to determine the free glycerol and residual mono-, di- and triglyceride contents in fatty acid methyl esters in biodiesel by gas chromatography with flame ionization detection. The method specifies the use of cool on-column (COC) inlet in order to ensure unbiased sample introduction onto the analytical column. According to the method, the chromatographic system must provide a relative response factor of no greater than 1.8 for 1,3-glyceryl dinonadecanoate (Di C38) relative to glyceryl trinonadecanoate (Tri C57). A similar method, ASTM D6584, was recently demonstrated to have equivalent performance using the Agilent Multi-Mode Inlet (MMI) when compared to COC. The advantage to using MMI for this application includes the ability to backflush the retention gap and inlet to eliminate carryover. As an additional benefit, the MMI requires less maintenance compared to COC.
In this study, the use of the MMI will be demonstrated for EN 14105:2011 for determining free glycerol and residual glycerides in a biodiesel sample. Performance using the MMI will be compared to COC in achieving the method performance criteria. Key aspects including method optimization and method robustness will be presented in detail.

N.08

CHARACTERIZATION OF DIESEL FUEL BY COMPOUND SPECIFIC STABLE CARBON ISOTOPIC ANALYSIS

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Compound-Specific Stable Isotope Analysis (CSIA) is a highly efficient technique in the field of forensic environmental investigations. This technique combines conventional gas chromatography (GC) with isotope-ratio mass spectrometry (IRMS) through a chemical reaction interface which quantitatively converts organic compounds to a common molecular form (for example CO_2) for stable isotopic measurement. The unique isotopic fingerprint of the environmental pollutants can be used to trace the origin of a contamination.
Our goal was to develop a stable carbon isotopic fingerprinting method to characterize unique diesel fuel features beneficial for the differentiation of diesel fuels originating from different sources e.g. different suppliers. The stable carbon isotopic ratios ($\delta^{13}C$) for n-alkanes in each investigated fuel sample were determined therefore. Accurate measurement of the stable isotopic composition of an individual compound requires baseline separation from any other coeluting compounds. The baseline separation of n-alkanes in a diesel sample is impossible with a conventional GC separation hence selective chemical separation methods are required in this case. We investigated two different approaches to separate the n-alkane fraction in diesel fuel based on urea adduction process.

N.09

EVALUATION OF THE DEMULSIFICATION OF HEAVY CRUDE OIL EMULSIONS BY GC/QMS

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Ionic liquids are organic salts that generally are liquid at room temperature. The difference in ionic chain generated by various combinations of cations and anions give rise to ionic liquids with different properties [1].This peculiarity has sparked interest for its application in the destabilization of emulsions and upgrade of crude oil [2-5].Formation of stable emulsions in crude oils is a challenge for the petroleum industries and their destruction has been object of many studies, sing different forms of demulsification, including microwave, sonication and others. The aim of this work was to investigate the effect of five ionic liquids – $[C_4mim]^+[NTf_2]^-$, $[C_8mim]^+[NTf_2]^-$, $[C_{12}mim]^+[NTf_2]^-$, $[C_4py]^+[NTf_2]^-$ and $[C_8mim]^+[OTf]^-$ on destabilization of crude oil emulsions and extraction of naphthenic acidic compounds from oil. The set of operating conditions studied were the heating type (conventional and microwave), ionic liquid concentration (between 0.74 to 8.9 mmol/g emulsion), size of alkyl chain, type of cation and anion, composition of the aqueous phase, mode of microwave irradiation, effect of ionic liquid dissolved in ethanol and effect of reducing the viscosity of the oil phase on process efficiency. After synthesis and destabilization of crude oil emulsion, oil phase was collected, derivatized and analyzed via GC/qMS. Through the chromatographic analysis of samples before and after demulsification, it was verified a meaningful reduction on number of naphthenic acids, from monocyclic to tricyclic, on the demulsificated phase by the combined ionic liquid and microwave action. This reduction indicates the effective partitioning of acidic species into the aqueous phase. The information gathered in this work opens up perspectives for using the microwave technology in the future of oil industry to achieve simultaneously two highly relevant objectives: breaking of emulsions and removal of acidic species of the oil phase.

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N.10

ULTRAFAST SIMULATED DISTILLATION WITH A LOW THERMAL MASS GC SYSTEM

Juan Aybar[1], Roger Firor[2], Luca Godina[1]

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High ramp rate temperature programming in GC systems can be powerful tool to shorten analysis times. System specifications tend to emphasize very high rates, in many cases above 1000 º C/min for resistively heated columns. In practice, however, rates above 250°C to 300 °C seldom have any real benefit. Of equal importance, the often overlooked cool down rate to an equilibrated start temperature must be considered since this parameter is critical for maximum sample throughput. Also, in order to take full advantage of high temperature programmed rates, optimization of column dimensions for a given analysis must be carefully investigated. These and other considerations will be investigated and discussed in this work using Ultrafast Simulated Distillation as the example application. A Low Thermal Mass (LTM) system integrated into a 7890B GC is used for the experiments. This system combines fast programming, fast cool down, and low power consumption to provide near real time simulated distillation results as per ASTM D7798.

N.11

AROMA ANALYSIS OF BATH TISSUE

Richard Borders

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Characterization of odors and malodors is often difficult due to the complexity of the volatiles from the sample and low concentration of the odorants. Roswell Analytical purchased a Gerstel ODP-3 Olfactory Detection Port and installed it on an Agilent 5973N Gas Chromatograph/Mass Spectrometer (GC/MS). This allows the effluent from the capillary column to be split between the mass spectrometer and the ODP-3 giving the capability to smell compounds while simultaneously recording the mass spectrum (Figure 1).
This poster will focus on the odorants responsible for odors in one lot of bath tissue. This odor is probably caused by exposure to higher than normal temperatures during drying. The three main odorants found are octanal, hexanal, and nonanal. The odorants found support formation by oxidative processes associated with overdrying of pulp [1].

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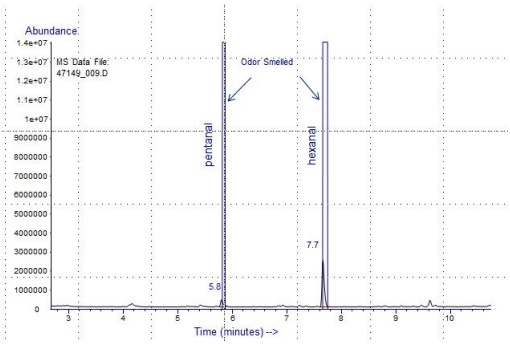
Figure 1 Example of aromagram of a simple sample consisting of the GC/MS data (black) and odor data (blue). Olfactometry data includes the time an odor is perceived along with a sensory description.



NANOHPLC-MS/MS ANALYSIS TO STUDY THE BIO-NANO-INTERACTIONS BETWEEN PEGYLATED LIPID NANOPARTICLES AND BIOLOGICAL FLUIDS

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When nanoparticles (NPs) enter a biological environment, medium components, especially proteins, compete for binding to the NPs surface, leading to development of a new interface, commonly referred as the "protein corona"[1]. Unfortunately, opsonins are also adsorbed. These proteins are recognized by macrophages with rapid clearance of the NPs from the bloodstream. PEGylation is the most efficient antiopsonization strategy [2]. Linear chains of PEG, grafted onto the NP surface, are able to create a steric hinderance, resulting in a significant inhibition of protein adsorption and less recognition by macrophages. However, an excessive PEGylation can lead to less efficient binding with protein targets and to a strong inhibition of cellular uptake. To reach a compromise in this question, referred as the "PEG dilemma", we employed a multi-component (MC) lipid system and, in particular, we investigated the protein corona of MC, MC-PEG1k, MC-PEG2k and MC-PEG5k liposomes. NanoHPLC-MS/MS analysis allowed us to accurately determine corona composition showing that apolipoproteins are the most abundant class in the corona and that increasing the PEG length reduced the protein adsorption and consequently the liposomal surface affinity for apolipoproteins. Since the abundance in apolipoproteins, we tried to exploit the "protein corona effect" giving CL-HP complexes to prostate cancer PC3 cells that express high level of scavenger receptors class B type 1(SR-BI) receptor in order to evaluate the cellular uptake efficiency of the four systems. Biological experiments demonstrated that MC-PEG2k is a good compromise between anti-opsonization strategy and active targeting.

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O.02

ISRR ELECTROPHORESIS AND SDS-PAGE PROTEIN ANALYSIS FOR GENETIC AND PROTEIN CHARACTERIZATION OF THE IN VITRO REGENERATED ENDANGERED PILOSOCEREUS ROBINI

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Pilosocereus robini is a rare species with many reported medicinal activities which is experiencing sudden population collapse [1]. Identifying and developing effective conservation and management strategies to halt the forestall extinction of this species is crucial. A successful in vitro propagation system of P. robinii has been developed. The plants were acclimatized in the greenhouse at 100% survival rate. The fidelity of the in vitro raised plants to the mother P. robinii was investigated using PCR-ISSR markers for DNA fingerprinting and SDS-PAGE protein analysis which are two major applications of capillary electrophoresis. Where, the regenerated explants on MS medium supplemented with TDZ were the highest in inducing new specific marker bands. Sh6 ISSR primer showed the highest polymorphism value, 81.8% with 33 total amplified fragments, while Sh3 ISSR primer showed the lowest value with polymorphic percentage of 14.3%. Furthermore, SDS-PAGE protein analysis showed no variation in protein pattern of the studied treatments. On the other side, HPLC analysis of the in vitro plantlets extracts was done to assess their phytochemical profile. The HPLC analysis has shown that 2ip based treatments were the highest in organic acids accumulation, while the phenolic constituents' accumulation was found to reach its peak in the BA based treatments.

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O.03

DETERMINATION OF $^{13}C/^{12}C$ RATIOS OF ENDOGENOUS URINARY AICAR

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AICAR (5-Aminoimidazole-4-carboxamide 1β-D-ribofuranoside) is prohibited in sport according to rules established by the World Anti-Doping Agency. Doping control laboratories identify samples suspicious of AICAR abuse by measuring its urinary concentration and comparing the observed level to naturally occurring concentrations. As the inter-individual variance of urinary AICAR concentrations is large, this approach requires a complementary method to unambiguously prove the exogenous origin of AICAR. Therefore a method for the determination of carbon isotope ratios (CIR) of urinary AICAR has been developed and validated.

Concentrated urine samples were fractionated by means of liquid chromatography for analyte clean up. Derivatization of AICAR yielding the trimethylsilylated analog was necessary to enable CIR determinations by gas chromatography-combustion-isotope ratio mass spectrometry. The method was tested for its repeatability and stability over time and a linear mixing model was applied to test for possible isotopic discrimination. A reference population of n = 63 males and females was investigated to calculate appropriate reference limits to differentiate endogenous from exogenous urinary AICAR. These limits were tested by an AICAR elimination study.

The developed method fulfills all requirements for adequate sports drug testing and was found to be fit for purpose. The investigated reference population showed a larger variability in CIR of AICAR as for endogenous steroids. Nevertheless, the calculated thresholds for differences between AICAR and endogenous steroids can be applied straightforward to evaluate suspicious doping control samples with the same statistical confidence as established e.g. for testosterone misuse. These thresholds enabled the detection of a single oral AICAR administration for more than 40 h.

Determination of CIR is the method of choice to distinguish between an endogenous or exogenous source of urinary AICAR. The developed method will enable investigations into doping control samples with elevated urinary concentrations of AICAR and clearly differentiate between naturally produced/elevated and illicitly administered AICAR.

O.04

REALCOW, A WARPING APPROACH FOR A SYSTEMATIC DETERMINATION OF MARKERS IN GC-MS DATA

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GC-MS is now widely used in metabolomics but the comprehensive determination of markers remains challenging. The development of tools for an automatic search of markers requires breaking down several barriers including noise filtering, signal warping and normalization. The present paper introduces a set of tools for an automatic processing of GC-MS data and a comprehensive determination of distinctive biomarkers. On the top of that, the toolkit includes a novel alignment approach which was upgraded from the Correlation Optimized Warping (COW) method for correcting local distortions. The performance of the toolkit was assessed on a dataset corresponding to the profiling of the volatile compounds of 117 samples of three vegetable oils with a GC-MS system intentionally submitted to instrumental drifts [1]. According to multifactorial analyses, no discrimination of the three oils was observed on unaligned raw data. In contrast, a satisfactory discrimination was achieved when the COW method was implemented on the ionic signal (IONCOW) but the alignment remained insufficient to qualify for a systematic determination of distinctive biomarkers due to local distortions. In order to refine the alignment, a new algorithm dubbed RealCOW (Realigned COW) was developed. Its implementation enabled a suitable correction of distortions which were shown to resist to IONCOW and therefore a significant improvement of oil discrimination. Furthermore, the RealCOW method enabled to unmask more than 256 relevant markers in the dataset, whereas only 23 were manually pointed out by an expert. The relevance of these 233 unmasked markers and the way they were evidenced by the RealCOW algorithm are discussed.

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O.05

METABOLOMICS MEETS HISTOLOGY - METABOLIC PROFILES OF DIFFERENT BRAIN REGIONS IN MICE DESCRIBE THE BIG PICTURE

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The goal of *metabolomics* is the systematic identification and quantification of metabolites. Metabolites are small molecules covering a wide range of physicochemical properties, sizes, polarities, etc. and are involved in biochemical reaction networks. The structural diversity is a big challenge for the analytical platform. Here, we will present a gas chromatography-mass spectrometry – based workflow to analyze the metabolic phenotype of brain regions in mice. This protocol can be used for comprehensive metabolic studies in brain tissue to add a new layer of information explaining histological findings.
The dissected brain tissue is directly snap frozen to keep the actual “snapshot” of the metabolic phenotype. To prevent enzyme activity, tissue homogenization and two-phase metabolite extraction are also performed at cold temperatures. A two-step derivatization (methoxyamine and MSTFA) enables gas chromatographic separation, followed by electron ionization – mass spectrometry to detect the extracted analytes. Furthermore, the obtained data set was analyzed using MetaboliteDetector [1]. This software package detects all single ion chromatographic peaks in raw GC-MS data and performs a metabolite identification step. Therefore, we are using an in-house mass spectral library to provide unambiguous and reliable results. Both, known as well as unknown compounds are used for statistical analyses to find significant differences.
I will present results for selected metabolites, e.g. the neurotransmitters dopamine and γ-aminobutyric acid, in combination with specific metabolite patterns that are unique for every brain region. Moreover, we will present the metabolic profile of neurotoxin-lesioned mouse brains and relate these findings to histological assessments of neuronal injury.

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O.06

STEREODYNAMICS OF EM12, LENALIDOMIDE AND CAPTOPRIL DISULFIDE: FROM ENANTIOMERIZATION TO COMPLEX ISOMERIZATION

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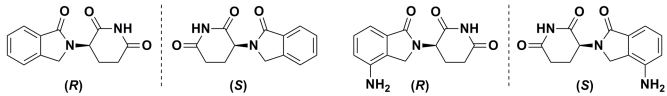
For pharmacokinetic studies and research a profound knowledge of the enantiomerization barriere and its effects are essential. Based on the well-investigated enantiopure or racemic drugs tailored therapies could be evolved and further developed. Important representatives of this class are EM12 (left) and Lenalidomide (right).



These phthalimodone derivatives are both structurally related to thalidomide and are highly interesting drugs. Very recently Lenalidomide attracted great attention as anti-tumor and immune-modulating drug in the therapy for multiple myeloma.[1] EM12 and Lenalidomide contain a stereogenic carbon in the piperidine-2,6-dione moiety, which is prone to interconversion, based on its keto-enol tautomerization abilites. We used dynamic electrokinetic chromatography (DEKC) in combination with direct calculation methods [2] to determine the enantiomerization barriers of EM12 and Lenalidomide. Futhermore the isomerization process of Captopril disulfide was analyzed by HPLC, followed by computer simulations. The computer simulation is a beneficial tool to get a better knowledge of the coupled interconversion of substances.

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O.07

COMPREHENSIVE ANALYSIS OF NAPHTHOYLINDOLE-TYPE SYNTHETIC CANNABINOIDS BY GC-MS/MS USING SIMULTANEOUS EI-SCAN, MRM AND PRECURSOR ION SCAN MODE

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Recently, the abuse of synthetic cannabinoids (SCs) in the form of so-called herbal smoking powders has become increasingly common. The detection and identification of designer drugs normally uses either GC-MS or LC-MS. However, for a class of compounds such as the SCs that is undergoing rapid chemical evolution, detection of new analogs is limited by the lack of available standards. In this study, a GC-MS/MS method for the identification of naphthoylindole type SCs (NISCs) is described.

Twenty-one standards of NISCs and extracts of 'herbal' products containing NISCs were analyzed using a GCMS-TQ8030 (Shimadzu Corporation). Data were acquired using simultaneous EI-scan; MRM and precursor ion scanning with MSMS utilizing the two fragments arising from fission of the ketone moiety. Mass shifts in the CID-induced fragmentation of the indolyl-carboxyl precursor allowed the assignment of the R1 and R2 substituents while fragmentation of the precursor attributable to the naphthylcarboxyl precursor allowed assignment of the R3 substituent. MRM transitions (naphthylcarboxy > naphthalene) were monitored to identify the R3 substituent for thirteen different substituents. Another set of MRM transitions (indolylcarboxy > indole) were monitored to determine if R2 was methyl (yielding a product ion of m/z 158) or hydrogen (m/z 144). R1 was inferred by the mass lost from the indolylcarboxy precursor in each case.

All of 21 NISCs standards showed two EI fragments attributable to the two alternative positions for fission of the ketone. Precursor-scanning of the product ions attributable to the indole (m/z 144) or 2-methylindole fragment (m/z 158) allowed assignment of the R1 substituent. Similarly, decarbonylation of the napthylcarboxy fragment allowed assignment of the substituent on the naphthalene ring. A GC-MS/MS method using simultaneous scan, MRM and precursor ion scanning provided an effective method for the detection and structural assignment of a number of NISC analogs.

O.08

COMPREHENSIVE AUTOMATION OF THE SOLID PHASE EXTRACTION (SPE)-GC/MS ANALYSIS OF OPIOIDS, COCAINE, THEIR METABOLITES AND 7-AMINOFLUNITRAZEPAM FROM BLOOD SERUM AND OTHER MATRICES

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The analysis of opioids, cocaine and metabolites from blood serum is a routine task in forensic laboratories. Commonly the employed methods include many manual or partly-automated steps like protein precipitation, solid phase extraction, evaporation and derivatization preceding GC/MS or LC/MS analysis.
In this study a comprehensively automated method is compared with a validated, partly-automated routine method. The automation relying on a MultiPurposeSampler MPS after manual protein precipitation includes the solid phase extraction, evaporation of the eluate, derivatization and injection into the GC/MS. A quantitative analysis for almost 170 serum samples and more than 50 samples of other matrices like urine, different tissues and heart blood on cocaine, benzoylecgonine, methadone, morphine, codeine, 6-monoacetylmorphine, dihydrocodeine and 7-aminoflunitrazepam was conducted with both methods proving that the analytical results are equivalent even near the limits of quantification.

O.09

NARROW-BORROW RP-HPLC OF N-ACYLHOMOSERINE LACTONE (AHL) QUORUM SENSING SIGNALING MOLECULES RELEASED BY GRAM NEGATIVE BACTERIA

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Most bacteria release extracellular molecules into the surrounding medium to self-regulate expression of specific sets of genes in response to their own population density, once a critical concentration ("quorum") of the signalling molecules has been reached. The process is known as "quorum sensing" (QS) and is used by bacteria to monitor population density and to change bacterial gene expression in order to compete and persist in nature or to colonize a particular environment. QS is also involved in inter-species communications, like that of higher plants with bacteria.

In Gram negative bacteria, QS is mediated mostly by N-acylhomoserine lactones (AHSs). The chemical structure of AHLs comprises a homoserine lactone moiety linked to an acyl side chain, varying in length and nature of the substituent (i.e. oxo or hydroxy group). In addition, the acyl side chain can vary with regard to the presence of double bonds; although most AHLs have fully saturated acyl side chains. This communication reports the results of a study performed to identify the N-acylhomoserine lactone signalling molecules released by several nitrogen-fixing bacteria such as *Azospirillum brasilense*, *Herbaspirillum seropedicae*, *Burkholderia ambifaria,* and *Gluconacetobacter diazotrophicus,* which are of interest in plant microbiology to study pathogenic or symbiotic interactions of bacteria with plant hosts. The AHLs, extracted from cell-free spent culture supernatants of the selected bacteria, have been separated ad identified by HPLC-ESI-MS, using a narrow bore reversed phase column. The HPLC method has been developed by a Quality-by-Design approach using the chromatographic modelling software DryLab® to optimize gradient time, column temperature, composition and pH of the eluent, flow rate, and start and end concentration of the gradient. The optimized method has been validated in terms of linearity of calibration graphs, limits of detection, limits of quantification, repeatability and accuracy, which has been evaluated by a recovery study.

O.10

DEVELOPMENT OF A DERIVATIZATION METHOD FOR THE DETERMINATION OF PSYCHOTROPIC SUBSTANSES USING CE-LIF TECHNIQUE

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Most psychotropic drugs analysis schemes are currently based on high-performance liquid chromatography (HPLC). However in recent years, the capillary electrophoresis (CE) - extremely powerful technique - increasingly become an alternative for HPLC separation due to its simplicity, high separation efficiency and less consumption of a sample and reagents [1]. The development of efficient separation and determination of psychotropic substances using a capillary electrophoresis coupled with laser-induced fluorescence (LIF) detection is an ongoing challenge for analytical toxicologists in forensic sciences [2]. However, only a minority of analytes is a naturally fluorescent, thus most often a derivatization of the analytes must be performed prior to the analysis. Additionally, this modification of the analytes most often increase selectivity and sensitivity of a method.

The aim of this study was to develop, optimize and validate a derivatization procedure and separation method for the determination of bezylopiperazine (BZP), ephedrine (EPD), α-metylobenzyloamine (α-MBA) and 7-amino-clonazepam (7-CLO). As a the best derivatizing agent fluorescein isothiocyanate (FITC) was chosen. The most efficient background electrolyte for CE analysis was found to be 25 mM borate buffer with addition of acetonitrile (20%). The CE-LIF method was validated by assessing the following parameters: linearity, accuracy, precision, recovery and relative process efficiency.

Acknowledgments

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O.11

INVESTIGATION OF SOLID PHASE MICRO EXTRACTION AS AN ALTERNATIVE TO DRIED BLOOD SPOT

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There has been a growing trend in bioanalysis toward the utilization of micro extraction techniques for sample isolation and transportation. Not only is there an interest in cost reduction associated with sampling, but also ethical advantages and improving sample retention using microsampling techniques. Major focus has been on utilizing dried blood spot (DBS) media as an inexpensive alternative to terminal blood draws. Though DBS does offer benefits towards cost reduction, shipping and sample storage, it is not without limitation specifically with respect to blood hematocrit levels. The purpose of this study is to explore the utility of solid phase micro extraction (SPME) as an alternative sampling device to DBS media cards. Bio-SPME, as described as functionalized particles bound to a core fiber substrate, enabling direct micro sampling of biological matrices without the need for additional sample treatment.

A simple set of model compounds is used to explore extraction efficiencies, detection limits, binding issues, and also hematocrit impact differences between DBS and Bio-SPME sampling techniques. Blood samples with ranging hematocrit levels are extracted using both techniques for comparison of analyte detection and overall sample cleanliness. Parameters such as extraction conditions, desorption solvent optimization and desorption times are detailed. Results demonstrate the capability of analyzing sub ng/mL concentration levels of mixed drugs in whole blood using both techniques. Whole blood samples extracted using the Bio-SPME technique exhibited increased analyte response, while demonstrating significant reduction in detected endogenous matrix as compared to DBS.

O.12

STUDY OF INFLUENCE OF DERIVATIZATION CONDITIONS ON STRUCTURE AND STABILITY OF NUCLEOSIDE DERIVATIVES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Metabolomics is now fast growing area of life-science research. Metabolite concentrations provide useful information about the physiological state of a cell or organism and their responses to environmental influence or disease. Gas chromatography-mass spectrometry (GC/MS) is one of the most widely used methods for metabolite detection and identification. In case of polar metabolites a derivatization is required.

Nucleosides are the basic building blocks of nucleic acids and play an important role in purine–pyrimidine metabolism. N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) is common derivatization reagent used in the GC/MS analysis of nucleosides. However, derivatization with BSTFA could lead to a mixture of trimethylsilyl (TMS) derivatives as it has been shown during our research (derivatization of 9 nucleosides was investigated). Unsatisfactory chromatographic properties of some TMS derivatives also complicated determination of nucleosides.

We have used other chemical derivatization techniques in an attempt to solve these problems and find an optimum derivatization method. Nucleosides contain hydroxyl and amine groups. Nucleosides first were silylated with BSTFA in pyridine solution. After pyridine and BSTFA elimination respective derivatives were reacted with N-methyl-bis(trifluoroacetamide) (MBTFA) exchanging the TMS group on the amine group for a trifluoroacetyl (TFA) group. Using two step derivatization O-TMS-N-TFA derivatives were obtained. Derivatization procedure for nucleosides using MBTFA was investigated and respective conditions were optimized. The structures of the derivatives were confirmed by interpreting their electron ionization mass spectra. Pyridine and reagent were replaced for a more volatile and inert solvent to prevent contamination of the ion source and pre filter of mass spectrometer and increase column lifetime. Besides that it allowed to decrease concentration detection limits 100-1000 times due to off-line large sample injection of such solutions as it was shown by us.

The obtained results can be used for identification of various nucleosides and similar substances in biological sample.

O.13

LC-MS/MS METHOD FOR THE DETECTION OF NEW PSYCHOACTIVE SUBSTANCES (NPSs) IN HAIR

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In the latest years, many NPSs from several drug classes have appeared in the illicit drug market. Their identification in biological fluids is of great concern for forensic toxicologists. Analytical methods were developed for their identification in biological matrices, such as oral fluid [1], plasma [2], urine [3]. Head or body hair is a useful alternative biological matrix, allowing the determination of drugs that accumulate in keratinized tissues. So far few studies deal with the determination of NPSs in hair [4]. We describe a validated multi-analyte method for the determination of NPSs, pertaining to different chemical classes (synthetic cannabinoids, synthetic cathinones, ketamine, piperazines and amphetamine-type substances ATS), in human hair using LC-MS/MS. We focused on a sample preparation able to extract the different classes of NPSs. About 30 mg of hair were decontaminated and incubated for four hours under sonication in different conditions depending to the analytes to be extracted: a) with 300ml of HCOOH 0.1% for cathinones, piperazines and ATS; b) with 300ml of MeOH for synthetics cannabinoids. 10 ml of the extracts were analyzed in ESI-UPLC-MS/MS in MRM mode. The LLOD was was 2 pg/mg for most of the analyzed cathinones and 10 pg/mg for synthetic cannabinoids. The method was linear in the range from LLOQ (20 pg/mg) to 1000 pg/mg and showed acceptable precision (%CV < 15%) and accuracy (%E).

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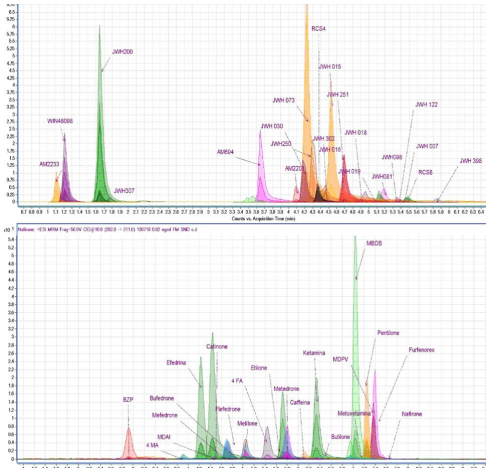

O.14

DETERMINATION OF BILE ACIDS IN HUMAN PLASMA BY HOLLOW FIBER LIQUID PHASE MICROEXTRACTION USING LCMS-IT-TOF

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Bile acids (BAs) are produced in liver derived from cholesterol. Cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA) and ursodeoxycholic acid (UDCA) conjugated with glycine and taurine are the most abundant bile acids in humans. Various methods using (LC–MS/MS) with or without derivatization were developed to the analysis of free and conjugated bile acids using liquid–liquid extraction (LLE), solid-phase extraction (SPE) and sample dilution [1]. Nevertheless, these extraction procedures show drawbacks in time consuming, matrix effect and ion suppression. Hollow fiber based liquid-phase microextraction (HF-LPME) is a technique based on the use of porous and hollow polypropylene fibers that combines sample clean-up, extraction and concentration [2]. The aim of this study was to develop a method to analyse bile acids in plasma by HF-LPME of two phases using LCMS-IT-TOF. A 2^3 two-level full factorial design (FFD) showed that the desirable conditions for the analyses were 60 min extraction time and pH 1.0. Table 1 shows the merit parameters of the optimized two-phase LPME procedure. The developed method was applied to the analyses of bile acids in human plasma for assessing exposure to hepatotoxic compounds. HF-LPME-LCMS-IT-TOF method proved to be simple, cheap, very lower consume of organic solvent. It offers good linearity and precision being thus suitable for biomonitoring purposes.

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Table 1. Merit Parameters of bile acids analysis in serum using HF-LPME - LCMS-IT-TOF.

Compound	Linearity	Dynamic Range	LD (nmol L^{-1})	LQ (nmol L^{-1})	Repeatability % CV	
					25.0 µg L^{-1}	100.0 µg L^{-1}
UDCA	R^2=0.99837	12.75 a 509.0	2.549	12.75	4.4	3.6
HDCA	R^2=0.99837	12.75 a 509.0	2.549	12.75	4.1	3.6
GCDCA	R^2=0.99909	11.13 a 445.0	2.226	11.13	3.3	3.6
CA	R^2=0.99782	12.25 a 489.0	2.449	12.25	5.3	2.1
TLCA	R^2=0.99957	10.35 a 413.8	2.069	10.35	3.9	4.4
DCA	R^2=0.99725	12.75 a 509.0	2.549	12.75	11.9	8.3
LCA	R^2=0.99278	13.29 a 531.0	2.658	13.29	5.2	6.6

Table 2. Serum bile acids determinations by HF-LPME-LCMS-IT-TOF.

Bile acids	Control		Exposed	
nmol L^{-1}	Mean ± SD	Min - Max	Mean ± SD	Min - Max
UDCA	60,05 ± 64,41	1,34 - 232,01	94,71 ± 85,50	18,18 - 276,10
HDCA	134,98 ± 73,01	34,35 - 232,24	124,21 ± 153,22	39,51 - 533,48
GCDCA	1076,73 ± 585,18	331,19-2079,00	1556,47 ± 1059,38	561,01 3580,66
CA	36,98 ± 26,88	3,72 - 69,75	149,53 ± 147,21	69,84 - 537,87
DCA	171,97 ± 73,96	76,55 - 309,51	196,10 ± 171,10	2,04 - 631,37

O.15

THROUGHOUT INVESTIGATION OF FUSED-CORE STATIONARY PHASES COUPLED TO ION TRAP-TIME OF FLIGHT MASS SPECTROMETRY DETECTION FOR A COMPREHENSIVE ELUCIDATION OF THE TOTAL LIPID PROFILE OF HUMAN PLASMA

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Biological and metabolomic (proteomic, lipidomics) studies are receiving a great deal of attention, since they are capable to provide new insight into the biological networks in which these molecules are involved, and a more comprehensive view of the molecular basis and disrupted pathways in several pathologies. Lipid metabolism is of particular interest due to its high concentration in central nervous system (CNS); their importance in cell signalling and tissue physiology is demonstrated by many CNS disorders (e.g., Alzheimer's, Parkinson's, Multiple sclerosis, and schizophrenia) and injuries that involve deregulated metabolism, especially for phospholipids (PLs). On the other hand, total cholesterol, HDL-cholesterol ("good cholesterol"), LDL-cholesterol ("bad cholesterol"), and triglycerides (TAGs) together make up the lipid profile in plasma, which is used by physicians as an indicator of the risk of coronary heart disease by blockage of blood vessels. In this research, we are assessing mono-dimensional and two-dimensional LC/MS methods for lipid determination in human tissues, from both healthy people, and patients with known or unknown diseases. The goal is to develop fast, reliable, and reproducible methods to detect qualitative and quantitative differences in the lipidic profile, aiming finally to provide new bio-markers, in the case of known diseases, or help to characterize unknown diseases. Step-by-step implementation of our platform include: column evaluation (selectivity, resolution, robustness); method development (temperature, UHPLC conditions); detection (tandem MS experiments to increase the identification power and achieve more confident elucidation).

O.16

DETERMINATION OF ANDROGENIC ANABOLIC STEROIDS (AASs) IN NUTRITIONAL SUPPLEMENTS BY LC-HRMS

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All layers of population, especially sportsmen at all levels, from amateurs to professional athletes, are often tempted to take dietary supplements in order to improve their performance. The easy availability of supplements through several web sites has contributed to the spread of these products. During recent years it has been reported that products marketed as dietary supplements contain non-labeled substances, like clenbuterol [1] and anabolic androgenic steroids (AASs) [2]. A sensitive method for the identification and quantification of about 30 AASs, including esters and clenbuterol, in traces in dietary supplements by Liquid Chromatography High Resolution Mass Spectrometry (LC-HRMS) in APCI mode was developed and validated. One gram of dietary supplement was added with testosterone-D3 as internal standard, dissolved in methanol, evaporated to dryness, diluted in sodium hydroxide and extracted with a mixture of pentane/ethyl ether, 9:1. The organic phase was evaporated, the residue was reconstituted in 100 µL of of a mixture methanol/water/formic acid 6:4:0.03 and10 µL were directly injected in the LC–HRMS system. The method was fully validated. LODs obtained for AASs varied from 1 to 25 ng/g and LOQ was 50 ng/g for all analytes. The method was linear for all the compounds in the ranges from the LOQ to 2000 ng/g, with correlation coefficients always higher than 0.99. Accuracy (intended as % E) and repeatability (% CV) were always lower than 15 %. Good values of matrix effect and recovery were achieved. The method was applied to the analysis of 30 dietary supplements. Many AASs were detected, often in combination: androstenedione was detected in nine supplements, DHEA in 12 samples and methandienone in three samples, stanozolol in one supplement, testosterone in seven samples and testosterone esters in 4 of them.

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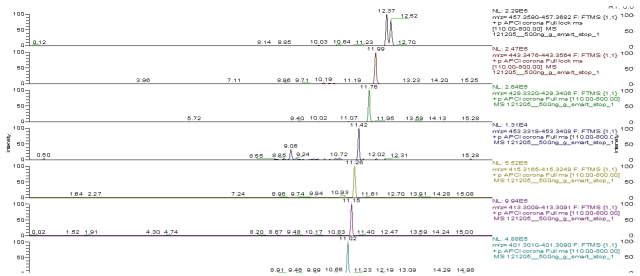

O.17

AN UNEXPLORED STRATEGY BASED ON SEMI-AUTOMATIC MEPS PROCEDURE FOLLOWED BY UHPLC-PDA AS HIGHLY SENSITIVE AND SPECIFIC METHODOLOGY TO QUANTIFY THE URINARY LEVELS OF LEUKOTRIENE B_4

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Leukotriene B4 (LTB4) is a potent chemotactic agent generated enzymatically in leukocytes from arachidonic acid *via* the 5-lipoxygenase pathway is regarded as an important mediator in several pathological processes such as inflammatory and allergic responses. Elevated levels of LTB_4 have been found in various body secretions associated with several inflammatory conditions including chronic bronchitis and asthma. Due to the very low concentration of endogenous LTB4 in the biological fluids, it is challenging to develop a highly sensitive and selective method.

Therefore, in this study an ultra-fast, selective and sensitive analytical procedure based on semi-automatic microextraction by packed sorbents (MEPS) technique, using a new digitally controlled syringe (eVol®) in tandem with ultra-high pressure liquid chromatography (UHPLC), was developed and validated to evaluate the occurrence and levels of endogenous LTB_4 in urine of endogenous LTB4 levels in healthy subjects and a chronic bronchitis and asthmatic population. Important parameters affecting MEPS performance, namely the type of sorbent material, number of extraction cycles (extract–discard) and elution volume, were evaluated. The optimal experimental conditions among those investigated for the quantification of LTB_4 in urine samples were as follows: PGC sorbent, 10 extractions cycle (10 × 250 µL of sample) and LTB_4 elution with 100 µL of acetonitrile.

Under optimized conditions good results were obtained in terms of linearity within the established concentration range with correlation coefficient (r^2) value higher than 0.996, with a residual deviation for each calibration point below 10 %. The limit of detection (LOD) and limit of quantification (LOQ) obtained were 0.37 and 1.22 ng/mL, respectively. Precision was lower than 7 %. Typical recoveries ranged between 73 and 86 % (RSD< 9 %). The applicability of the proposed analytical procedure in urine of asthmatic patients revealed the presence of the target analyte in concentrations ranged from 2.5 to 9.42 ng/mL.

O.18

INTEGRATION OF SOLID PHASE MICROEXTRACTION, MASS SPECTROMETRY AND METABOLOMIC DATA AS A POWERFUL STRATEGY FOR IDENTIFICATION OF URINARY VOLATILE METABOLITES AS POTENTIAL CANCER BIOMARKERS

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Non-invasive diagnostic strategies aimed at identifying biomarkers of cancer are of great interest for early cancer detection. Urine is potentially a rich source of volatile organic metabolites (VOMs) that can be used as potential cancer biomarkers. Our goal was to develop a generally reliable, rapid, sensitive and robust analytical method for screening large numbers of urine samples, resulting in a broad spectrum of native VOMs, as a tool to evaluate the potential of these metabolites in the cancer diagnosis.

To investigate urinary VOMs as potential cancer biomarkers, urine samples from 33 cancer patients (14 leukaemia, 12 colorectal and 7 lymphoma) and 21 healthy individuals (control group, cancer-free) were analyzed. Solid-phase microextraction (SPME) in combination with GC-qMS-based metabolomics was applied to isolate and identify the volatile metabolites. This procedure provides a potential non-invasive method for early cancer diagnosis as a first approach. Important SPME experimental factors that influence the extraction efficiency (fibre coating, extraction time and temperature of sampling) were optimized using a univariate optimization design. The SPME fiber coated with CAR/PDMS afforded the highest extraction efficiency particularly when the sampling is performed at 50 ºC for 60 min.

Up to 82 VOMs belonging to distinct chemical classes were identified in the control and oncologic groups. Benzene derivatives, terpenoids and phenols were the most common classes found in the oncologic group, whereas ketones and sulphur compounds were the main classes that were isolated from the urine headspace of healthy subjects. The positive rates of 16 patients among the 82 identified were found to be statistically different

O.19

A POWERFUL STRATEGY BASED ON MICROEXTRACTION BY PACKED SORBENT COMBINED WITH UHPLC-PDA FOR ANALYSIS OF RISPERIDONE, CLOZAPINE AND THEIR ACTIVE METABOLITES IN HUMAN URINE

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Since their introduction in 1950's, antipsychotics have become widely prescribed in clinical psychiatry as a first-line treatment for schizophrenia, bipolar disorder and psychotic depression. With more than 35 antipsychotics currently available worldwide, this drug class has rapidly gained importance in both clinical and forensic settings. Since initial studies demonstrated an association between clinical response and blood levels of psychotherapeutic drugs, measurement of serum levels of these therapeutic drugs has become routine in many laboratories. In fact, therapy personalization can reduce side and toxic effects, thus avoiding unnecessary and expensive hospitalizations and drug administrations [1]. On the other hand, the potential abuse and their involvement in intoxications and suicides are of considerable interest in forensic toxicology that focuses on medico-legal aspects of chemical exposure and toxic injury [2]. Usually, antipsychotics are administered in oral doses of only a few milligrams per day that are widely metabolized in the liver. As a result, the concentration of these therapeutic drugs and their metabolites in human specimens is very low, which complicate their detection. For this reason, the clinical and toxicology services constantly need to develop advanced analytical methods, often based on innovative combinations of modern instrumentation and alternative biological matrices.

The current research study is dedicated towards the development and validation of a novel sensitive, fast and accurate approach for the simultaneous determination of Risperidone, Clozapine and their active metabolites in human urine. The new approach offers decreased sample preparation and analysis time as compared to traditional methodologies. This approach was based on microextraction by packed sorbent (MEPS) combined with ultra-high performance liquid chromatography (UHPLC) equipped with a photodiode array (PDA) detection. Important factors affecting the performance of MEPS such as the type of sorbent material, number of extraction cycles, sample volume and sample pH were studied.

O.20

SIMULTANEOUS QUANTIFICATION OF FLUOXETINE, CLOMIPRAMINE AND THEIR ACTIVE METABOLITES IN HUMAN URINE SAMPLES BASED ON MICROEXTRACTION BY PACKED SORBENT FOLLOWED BY UHPLC-PDA ANALYSIS

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Depression is one of the most prevalent psychiatric disorders in our society and epidemiological evidences exist suggesting a substantial incidence increase of the disease in recent years. Over the last decade, the consumption of drugs used to treat depression has exponentially increased in worldwide. According to Intercontinental Medical Statistics Health (IMS Health), antidepressants, the third most used therapeutic class worldwide, had in 2000 an increase of 18%, representing 4.2% of global pharmaceutical market. Social disadvantages, economic crisis period and adverse changes in socioeconomic status are factors that can propitiate the increase of psychiatric illness cases and consequent increase in the antidepressants drugs consumption.

Due to the wide variety of antidepressants currently available, this drug class has rapidly gained importance in both clinical and forensic fields. On the other hand, the potential abuse of these drugs by the population has contributed to the increase in suicide cases, which has particular interest in forensic toxicology.

Typically, the patients are treated with low doses of antidepressants per day that are mainly metabolized in the liver. Consequently, the concentration of these therapeutic drugs and their metabolites in human specimens is very low, which complicate their detection. Therefore, there is the need for the development of advanced analytical methods based on recent technology that ensure a more rigorous control of these substances in biological matrices.

In this work, a microextraction by packed sorbent (MEPS) combined with an ultra high performance liquid chromatography (UHPLC) coupled to a photodiode array detector (PDA) method is proposed for simultaneous determination of fluoxetine and clomipramine and their active metabolites in urine samples of patients.

Acknowledgements

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O.21

ESTABLISHMENT OF THE SALIVA VOLATOMIC PROFILE AS AN EXPLORATORY AND NON-INVASIVE STRATEGY TO FIND POTENTIAL BREAST CANCER BIOMARKERS

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Breast cancer (BC) is a major health problem that affects quality of life in many developed countries of the world. BC is the most common malignancy in women and the second most common cause of cancer-related mortality. A sensitive assay to identify volatile organic metabolites (VOMs) as biomarkers that can accurately diagnose the onset of BC using non-invasively collected clinical specimens is ideal for early detection and diagnosis, to predict outcomes of the pathology, and help in surveillance for disease recurrence.

Saliva is potentially a rich source of VOMs that can be used as potential cancer biomarkers. Therefore the aim of this study was to establish the saliva volatomic profile between BC patients and healthy individuals, and to explore the VOMs as potential biomarkers in BC diagnosis at early stage. Solid-phase microextraction (SPME) using CAR/PDMS sorbent was selected as a powerful and highly sensitive strategy to isolate VOMs from saliva. Combined with GC-qMS, was used to obtain metabolomic information patterns of 25 BC patients and 20 healthy individuals.

Up to 79 VOMs were identified in BC and control groups. Ketones and sulphur compounds were the chemical classes with highest contribution for both groups. Results showed that positive rates of 20 VOMs among the total of 79 detected were found to be statistically different ($p < 0.05$). A significant increase in the peak area of 2-pentanone, in metabolomic profile of cancer patients relatively to controls was observed. In average, statistical significant lower abundances of dimethyl disulphide was found in cancer patients. Multivariate statistical methods (PCO and SLDA), were used to gain insight into the metabolomic differences between healthy and patients.

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O.22

URINE VOLATOMICS FOR THE EARLY BREAST CANCER DIAGNOSIS

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Cancer is currently one of the most prevalent diseases, presenting increasing trends that follow the continuous rise in life expectancy. Breast cancer, in particular, despite the many efforts made in the last decade, continues to present high mortality rates, mainly because there are still missing effective tools for its timely diagnosis. Although breast cancer develops in a relative asymptomatic way till a dangerous stage is reached, there are necessarily changes in the concentration of certainly body metabolites than could be assessed for an effective early diagnosis. Regarding this, urine, as a mirror of whole body metabolism, is certainly a promising matrix to assay, given its many advantages, including its non-invasiveness and easy collection. Its potentiality for cancer biomarker research is being explored under different approaches. Here we described the characterization of urinary volatile organic compounds (uVOMs) using headspace solid-phase microextraction (HS-SPME) coupled to GC-qMS to characterize the volatomic profiles of breast cancer patients. This strategy focused only in the uVOMs simplifies the matrix characterization, shortening the work necessary to obtain different cancer volatomic fingerprints. The application of this approach to large sets of patients will enable us to start building reliably metabolic profiles able to be used in the early non-invasive breath cancer diagnose.

Acknowledgements
The authors acknowledge the Portuguese Foundation for Science and Technology (FCT) through the MS Portuguese Networks (REDE/1508/RNEM/2005) and Pluriannual base funding (QUI-Madeira-674) and BPD 66177/2009 fellowship given to JP.

O.23

ANALYTICAL METHOD OF VOLATILE AROMATIC COMPOUNDS IN MECONIUM BY HEADSPACE-SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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Meconium is the earliest stools of newborn. It starts forming from the 13th week of gestation in intestinal contents and accumulates until birth. Usually, it is expelled by the newborn between 24 first hours after birth. This complex matrix has the advantage to integrate a large period of exposure of the foetus to xenobiotics compare to infantile urine or blood. This matrix was used in the *PENEW*[1] project to determine a correlation between congenital malformations and foetus exposure.

This work presents the analytical consideration for the quantification of volatile organic compounds (VOCs): BTEX (benzene, toluene, ethylbenzene and xylenes) and chlorinated solvents (trichloroethylene and tetrachloroethylene) in meconium. This biological matrix is very viscous and sticky, difficult to manipulate and to homogenize. Analyzes were then carried out by HeadSpace Solid Phase MicroExtraction (HSSPME) with Gas Chromatography coupled to Mass Spectrometry (GC/MS).

Several extraction parameters were optimized (fiber type, incubation time and temperature of fiber, use of salt). Internal calibrations performed in water and in matrix were compared to determine if quantification in water was possible with the correction of isotopic labelled internal standards. Importance of internal standard for each target molecules was particularly verified with a comparison between calibrations performed with specific internal standards and calibrations carried out with a single internal standard for all compounds.

It was determined that analyzes of meconium should be carried out with an internal calibration in matrix for BTEX and chlorinated solvents with specific internal standards. The limits of quantification were established between 80 pg/g and 120 pg/g of meconium.

The method was applied to 40 meconium samples in which all target compounds were determined.

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O.24

UNCERTAINTY OF BLOOD ALCOHOL CONCENTRATION (BAC) RESULTS AS RELATED TO INSTRUMENTAL CONDITIONS: OPTIMIZATION AND ROBUSTNESS OF BAC ANALYSIS PARAMETERS

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Analysis of blood alcohol concentration is a routine analysis performed in many forensic laboratories. This analysis commonly utilizes headspace-sampling, followed by gas chromatography combined with flame ionization detection (GC-FID). Studies have shown several “ideal” methods for instrumental operating conditions, which are intended to yield accurate and precise data. Given that different instruments, sampling methods, application specific columns and parameters are often utilized, it is less common to find information on the robustness of these reported conditions. A major problem can arise when these “ideal” conditions may not also be robust, thus producing data with higher than desired uncertainty or inaccurate results.

The goal of this research is to incorporate the principles of quality by design (QBD) in the development of BAC instrument parameters, thereby ensuring that minor instrumental variations, which occur as a matter of normal work, do not appreciably affect the final results of this analysis. This presentation will discuss both the QBD principles as well as the results of the experiments, which allow for determination of “ideal” instrumental conditions. Additionally, method detection limits will also be reported in order to determine a reporting threshold and the degree of uncertainty at the common threshold value of 0.08g/dL. Finally, differences between pressurized loop headspace systems and volumetric headspace systems will be discussed, comparing and contrasting these two different types of analytical instruments.

O.25

QSAR APPROACH TOWARDS RIBONUCLEOTIDE REDUCTASE INHIBITORS: HYDROXAMIC ACIDS

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Ribonucleotide reductase (RR) is a key enzyme, which plays a major role in the DNA synthesis and repair in all dividing cells.[1] Hydroxamic acids are a group of weak organic acids having the general formula RC(=O)N(R')OH, have shown promising RR inhibitory property with antitumor activity. Quantitative structure-activity relationship (QSAR) for Hydroxamic acids (HAs) as ribonucleotide reductase using the molecular descriptors by partial least square (PLS) regression was performed. The cross-validation Q^2 cum values for the optimal QSAR model of Hydroxamic acids obtained is above 0.620 (remarkably higher than 0.500), indicating good predictive-abilities for $log1/IC_{50}$ values of Hydroxamic acids. The resulting QSAR model shows that $log1/IC_{50}$ values of HAs are mainly governed by lipophilicity, CLOGP, Total number of valence electrons, NVE, molar volume, Vx, energy of highest occupied molecular orbitals, E_{HOMO}, energy GAP, E_{HOMO}- E_{LUMO} and chemical hardness, n.

O.26

GCMSMS ANALYSIS OF BENZODIAZEPINES USING ANALYTE PROTECTANTS

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Benzodiazepines are psychoactive drugs commonly used as sedatives and in the treatment of anxiety, seizures and insomnia. Benzodiazepines are challenging to analyse with GCMSMS as they are bases and hence are frequently trapped on active sites in the GC inlet liner, column, etc. The basic nature of these compounds can lead to poor peak shape, poor linearity of calibration curves and reduced sensitivity. In this work diazepam, lorazepam, lectopam and nitrazepam were analysed by triple-quadrupole GC-MS/MS both with and without the presence of analyte protectant (sorbitol). The effect of analyte protectant for the benzodiazepine class drugs was investigated and indeed all three factors were significantly improved by the use of the protectant. The results are discussed in terms of peak shape, sensitivity, reproducibility and linearity.

O.27

CHARACTERIZATION OF THE DESIGNER DRUG BK-2C-B BY MEANS OF GC-MS, GC-MS AFTER DERIVATIZATION WITH 2,2,2-TRICHLOROETHYL CHLOROFORMATE, LC/HR-ORBITRAP-MS, AND NMR

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An increasing number of new amphetamine-related designer drugs, including cathinone derivatives, are appearing on the recreational drug market [1,2]. They can be purchased over the Internet through many websites which sell them cheaply as “legal” alternatives of controlled drugs. Obtaining the structural characterization of these new drugs has been one of the recent goals of forensic toxicology laboratories.

We describe the analytical characterization of the designer drug BK-2C-B, contained in a seized tablet. This seizure occurred, to the best of our knowledge, for the first time in Europe. The analytical techniques employed to this end include Gas Chromatography – Mass Spectrometry (GC-MS), without or with derivatization with 2,2,2-trichloroethyl chloroformate, Liquid Chromatography – High resolution/High Accuracy Orbitrap® Mass Spectrometry (LC-HRMS), and Nuclear Magnetic Resonance (NMR).

GC-MS analyses led us to obtain highly informative EI mass spectra, particularly after the derivatization of BK-2C-B with 2,2,2-trichloroethyl chloroformate. Moreover, the application of LC-HRMS, allowing for accurate mass measurements at 100.000 resolving power, greatly enhanced analytical capabilities in structural characterization of this new designer drug. The employed LC-HRMS analytical strategy is based on accurate mass measurements of MH^+ ionic species in full scan conditions; study of MH^+ collision-induced product ions; comparison of experimental and calculated MH^+ isotopic clusters; examination of the isotopic fine structure of the M+1, M+2, M+3, M+4 isotopic peaks relative to the monoisotopic (M+0) peak. In this case the contribution of ^{79}Br and ^{81}Br isotopes produced a characteristic isotopic cluster. Lastly, NMR spectra allowed to obtain useful information about the position of substituents in the designer drug.

The combination of all these analytical techniques allowed the full characterization of the seized psychoactive substance, in spite of the lack of a reference standard.

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O.28

DETERMINATION OF RESIDUAL SOLVENTS IN PHARMACEUTICAL PRODUCTS USING STATIC HEADSPACE AND TIME OF FLIGHT GC/MS SYSTEM ACCORDING USP

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The identification and quantification of residual solvents in pharmaceutical products is performed according the USPmethod. It is possible to apply the method to all drug substances, excipients and products. Levels of residual solvents need to be monitored and controlled for a number of reasons including human and environmental safety. This is why the USPmethod requirements are designed to ensure that the potential presence of residual solvents is reduced to relatively low concentrations.
In particular the USPdescribes the use of static headspace sampler coupled with a GC-FID system in order to recognize and quantify organic volatile impurities. This paper shows the benefits obtained using the Headspace technique coupled with a GC/TOF-MS system capable of analyzing all classes of compounds in a single analysis reducing drastically the time analysis. Data will be reported including chromatographic parameters, qualitative and quantitative determinations. In addition, alternative approaches to conventional analysis will be provided.

DETERMINATION OF VETERINARY DRUG RESIDUES IN MILK BY GC/MS

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Amitraz, *N'*-(2,4-dimethylphenyl)-*N*-[(2,4-dimethylphenyl)-imino]methyl-*N*-methyl-methanimidamide, is an acaricide used to control the infections produced by the mite on the cows. Its use can cause the presence of residues in milk. For this reason, the analysis of amitraz residues in milk has received special attention in the scientific bibliography as opposed to milk analysis. On the other hand, amitraz is a very labile pesticide whose degradation products contain the 2,4-dimethylaniline moiety. Thus, the analysis of all the residues containing this substructure is advisable. In this context, the aim of this work has been to develop a method to determine the amitraz total residues in milk.

Samples were hydrolysed in acidic condition and then add alkaline solution and n-hexane to extract 2,4-dimethylaniline. After centrifugation the upper layer of n-hexane was added anhydrous sodium 1g to remove water, and then determined by gas chromatography mass spectrometry.

Column: TG-5MS (30 m × 0.25 mm × 0.25 μm);

Column temperature: 50°C(1 min), 20°C / min to 250°C (10 min);

Injection mode: splitless, splitless time of 1min; injection volume: 1 μL;

Inlet temperature: 260°C;

Carrier gas: helium (99.999%), constant flow mode, 1 mL / min;

MS ion source temperature: 300°C, transfer line temperature: 300°C;

Selected ion monitoring mode, m / z = 106,120,121, quantitative ion 121.

Quantitation was performed by external standard method according to peak area of quantitation ion. When confirmed, according to the fragment ions and their abundance ratio as a basis for positive discrimination.

Spiked experiments were done to validate the method. The results showed that the average recovery was 73.8-81.7%, RSD values of five parallel measurement ≤ 8.28%, the lower limit of the method for the determination of 2ng / g. This methodology is easily accessible to the basic instrumentation of many laboratories.

P.02

PYROLYSIS GC-MS OF FRACTIONATED TOBACCO EXTRACTS FOR THE IN-DEPTH STUDY OF AROMA COMPOUNDS

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Analytical pyrolysis combined with gas chromatography-mass spectrometry (Pyro-GC-MS) is believed to be the most effective technique to study smoking processes of tobacco. In this study a comparative study of the composition of tobacco smoke condensates and tobacco leaf pyrolysates was made. Tobacco smoke condensates were obtained through the use of a calibrated smoking machine and the analyses of the condensates were done by GC-MS. Analytical pyrolysis was done on a filament type pyrolysis unit that was hyphenated with a GC-MS instrument and very similar chromatographic profiles were obtained for both techniques.
A more in-depth analysis of the components that are responsible for the formation of typical tobacco smoke aromas was set up by Pyro-GC-MS of so-called 'fractionated' tobacco extracts. These fractionated samples were obtained by extraction of a single leaf type tobacco, followed by semi-preparative liquid chromatography (Prep-LC). Different fractions were collected, dried and submitted to Pyro-GC-MS. Because of the complexities of the pyrograms, multivariate statistical analysis was used for the differentiation of the extracts. It is illustrated that this approach is very suitable to correlate the impact of different classes of compounds in the tobacco to the typical aroma compounds in the smoke.

P.03

A FULLY AUTOMATED METHOD FOR SIMULTANEOUS DETERMINATION OF AFLATOXINS AND OCHRATOXIN A IN DRIED FRUIT BY PRESSURIZED LIQUID EXTRACTION AND ON-LINE SOLID PHASE EXTRACTION CLEAN UP COUPLED TO ULTRA HIGH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Mycotoxins are natural foodstuff contaminants mainly produced by fungi of genera *Aspergillus*, *Penicillium* and *Fusarium*. These mycotoxins can be found on a wide range of agricultural commodities such as cereals, dried fruits, nuts, coffee, cocoa, spices, oil seed, dried peas and fruits [1]. The growing concern over food safety necessitates more rapid and automated procedures to take into account the constant increase in the number of samples to be tested and capable to reduce as much as possible the humans contact with these highly toxic compounds. For these reasons, analytical methods that are fast, sensitive, solventless, inexpensive and suitable for automation are required [2]. In the present study, a fully automated method for simultaneous determination of aflatoxins B1, B2, G1, G2 and ochratoxin A using pressurized liquid extraction (PLE) and on-line solid phase extraction (on-line SPE) clean up, followed by ultra high liquid chromatography tandem mass spectrometry in dried fruit was developed. The extraction was carried out through an ASE 200 and all parameters that affecting on extraction efficiency, such as temperature, pressure, solvent extraction mixture, number of cycles and flush volume were carefully investigated and optimized using an experimental design. Furthermore sample preparation was done by an automated on line SPE in order to reduce the time and cost of analysis. On-line SPE was performed by loading 50 mL of extract at pH 3 through a C18 cartridge. MS/MS was carried out with an H-ESI interface operating in positive mode and registering two selected reaction monitoring (SRM) transition for each analyte. Under optimized condition, recovery ranging from 81 to 103% and relative standard deviations is lower than 10% and method detection limits are in the range 0.01–0.20 mg/Kg. To prove the applicability to real samples the developed methods was successfully applied to the analysis of retail dried fruit products with quantitative results comparable to the immunoaffinity chromatography (IAC). The procedure, based on the extraction by pressurized liquid extraction (PLE) and on-line solid-phase extraction (SPE) clean up of the PLE extracts and subsequent analysis by ultra high liquid chromatography tandem mass spectrometry, reduce the sample preparation time, solvent consumption allow a minimum contact with samples potentially toxic.

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P.04

DEVELOPMENT OF SPME-LC-MS/MS METHOD FOR CONCOMITANT EXTRACTION OF ROCURONIUM BROMIDE AND TRANEXAMIC ACID FROM PLASMA

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Rocuronium bromide and tranexamic acid are standard medications used during liver transplantation. However, analysis of concentration of these drugs, and rocuronium bromide in particular, remains problematic due to their physicochemical properties. Rocuronium bromide is a quaternary ammonium compound and therefore it tends to stick to various surfaces including labware and parts of the analytical instruments. Moreover, it is not stable in collected plasma samples and for this reason current sample preparation approaches consider acidification of plasma after sample collection. This modification of the matrix destroys interaction with transporting proteins and consequently precludes determination of binding parameters of the drugs.

The current work describes novel approach for simultaneous extraction of tranexamic acid and rocuronium bromide from plasma samples and their further determination by LC-MS/MS. The proposed method requires minimum sample handling, no use of ion pairing agent, no matrix modification and/or derivatization procedure. A weak cation exchange (WCX) coating was chosen as the best extracting phase for selected drugs, which guarantees a good recovery, minimum carry-over, reusability and reproducibility. SPME procedure met all Food and Drug Administration acceptance criteria for bioanalytical assays at three concentration levels, for both selected drugs. Post-extraction addition experiments showed that matrix effect was less than ±3% indicating excellent sample clean-up. In addition, the 96-blade format of SPME system provides many advantages like high throughput analysis for up to 96 samples in 35 min (22 s/sample), a small amount of plasma sample required, and simple sample preparation protocol, all of which shows a promise for possible on-site application in hospital to monitor concentration of the drugs in close to real time.

P.05

IDENTIFICATION AND QUANTIFICATION OF PHENOLIC ACIDS IN DURUM WHEAT BY RP-HPLC ON A SEMIMICRO SEPARATION SCALE WITH PDA AND ESI-MS DETECTION

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Durum wheat is one of the most important cereal crops used in the Mediterranean-type temperate zones whose consumption might reduce the risk of developing chronic diseases, such as cardiovascular diseases, type-2 diabetes, and certain types of cancer. These health benefits have been partly attributed to the occurrence of phytochemicals with antioxidant activity, which include phenolic acids (PAs) belonging to hydroxycinnamic acids and hydroxybenzoic acids derivatives. They occur as soluble free acids, soluble conjugates PAs that are esterified to sugars and other low molecular mass compounds, and as insoluble bound PAs that are mostly ester-linked to cell wall polymers such as polysaccharides and lignin. This communication reports the results of a study performed to identifying and quantifying PAs occurring as soluble free, soluble conjugated and insoluble bound compounds, which were separately extracted from wholemeal of durum wheat of different genotypes cultivated in three different Italian regions over three consecutive crop years. The three forms of PAs were independently identified and quantified by HPLC, using a narrow bore reversed phase column and a semimicro photodiode array detector (PDA) cell, in conjunction with a single quadrupole mass spectrometer, equipped with an electrospray ionization source (ESI-MS). The method was validated in terms of linearity of calibration graphs, limits of detection, limits of quantification, repeatability and accuracy, which was evaluated by a recovery study. Our study has evidenced that RP-HPLC on a semimicro separation scale offers both economical and environmental benefits, while maintaining separation performance and reliability of traditional HPLC methods, allowing the accurate estimation of the individual PAs occurring as soluble free, soluble conjugated and insoluble bound compounds in wholemeal of durum wheat. The variations in the content of the three forms of PAs in durum wheat as a function of genotype, cultivation region and crop year are discussed.

P.06

HARVESTING TIME INFLUENCES THE YIELD AND OIL COMPOSITION OF THYMUS VULGARE L. SSP. VULGARE

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Thyme herb, essential oil (EO) and extracts are valuable sources of natural functional ingredients for using in foods as flavourings, preservatives and antioxidants, and for a non-food applications, as well. This study was aimed to evaluate the biological and chemical properties of *Thymus vulgaris* ssp. *vulgare* (TVV) grown in Lithuania at different harvesting phases (seasonal variation). Crop yield depended on plant growing phase and varied between 2.2-5.1 t/ha(fresh) and 0.4-1.7 t/ha(dried). The total productivity of EO from fresh TVV varied from 3.8 to 20.1 dm^3/ha, the maximum yield being reached just before flowering; after drying EO slightly reduced and was 3.1-19.8 dm^3/ha. More than 60 constituents were identified in thyme EOs by GC-FID and GC-MS. It is evident that TVV grown in Lithuania depends to thymol chemotype, which constituted 58.0-67.7% (fresh) and 56.8-71.2% (dried). The amount of thymol precursor *p*-cymene increased from 5.2 to 12.6%, while γ-terpinene decreased 17.3→4.9% during all vegetative growth. All these compounds exhibit important biological activities (e.g. antimicrobial, flavour, antioxidative), therefore, their content defined the quality of thyme. Other quantitatively important components were myrcene, linalool, *(E)*-sabinene hydrate, carvacrol and β-caryophyllene. The biochemical composition of thyme was also investigated and the amount of dry soluble solids, vit C, carotenes, nitrates and total sugars were determined in TVV at different growth stages. Determination of optimal harvesting period would increase the grower's ability to control crop yield and EO quality and would be the most important factor in further commercialization and application of such thyme products for industrial uses in food and agriculture, the environment and in medicine for human health.

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P.07

ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN FOODS BY GC/FID: 1 - BEER SAMPLES

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The polycyclic aromatic hydrocarbons (PAH) are a group of over a hundred different compounds consisting of only carbon and hydrogen atoms having two or more condensed aromatic rings. The PAHs are environmental pollutants found in air, soil, water and sediment, usually as contaminants in complex mixtures of two or more compounds, resulting for incomplete combustion of carbon-containing materials such as oil, wood or coal. Humans can be exposed to PAHs through different routes. For non-smoking people, the major routes of exposure to PAHs are the food and inhaled air. Food can be contaminated in three main ways: through the PAHs in air, water or soil; during processing of industrialized foods and in food preparation at home. The objective of this research is the detection of PAHs in different foods and, in this first part, the goal is to verify the presence of PAHs in beers commercialized in the Brazilian market. Several brands of beers from Vale do Taquari Market (Rio Grande do Sul State, Brazil) were studied: pilsen (6), malzbier (2), smoked (2), porter (1) and Pale Ale (1). Samples were extracted using solid phase extraction (SPE) associated with C18ec cartridges of Octadecyl silica stationary phase. All analyses were performed using an HP 6890 Series gas chromatograph equipped with a flame ionization. The experiments evidenced the PAHs presence in all beer types and brands. Naphthalene, 1-methyl naphthalene, acenaphthene, acenaphthylene, phenanthrene, anthracene and pyrene were found in pilsen beers at concentrations oscillating from 1 to 298 μg kg^{-1}. The two malzbier brands had naphthalene and acenaphthene at concentrations ranging from 1 to 4.6 μg kg^{-1}. Naphthalene, acenaphthene and pyrene at concentrations between 0.6 and 6.4 μg kg^{-1} were found in smoked beers. The Porter and Pale Ale beers exhibited acenaphthene in a concentration ranging from 5.2 to 6.6 μg kg^{-1}.

P.08

ANALYSIS OF LIGNIN-DERIVED AROMATIC SUBSTANCES IN AGED WINE DISTILLATES

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Ageing is one of the most important factors determining quality of wine distillates – main raw material for the production of brandy. Identification of distillates is complicated by a rich chemical composition, resulted from the interaction between the components of the wine distillate and the oak barrel. In the present work, the relationship between the age of wine distillates as the main indicator of their quality and the accumulation of lignin degradation products, such as aromatic aldehydes and acids, has been studied. The wine distillates from different producers and with ageing times from 3 to 50 years have been investigated by capillary zone electrophoresis under the following conditions: silica capillary with an effective length of 60 cm and an internal diameter of 75 μm, voltage of 25 kV, and detection at 210 nm. In order to optimize the separation efficiency, concentration of sodium tetraborate and organic modifiers, such as methanol and acetonitrile, were varied. The total duration of the analysis was about 13 minutes which is much less than duration of HPLC analysis for the same product. The total amount of oxidation products of sinapil alcohol (sum of sinapaldehyde, syringaldehyde, and syringic acid), and coniferyl alcohol (sum of coniferaldehyde, vanillin, and vanillic acid) increased with increasing ageing time. It has been shown that aromatic acids are the main quality markers for distillates aged 10-30 years. Total amount of syringic and vanillic acid increased with increasing ageing time. Distillates with age more than 30 years had more complex electrophoretic profiles than younger ones, having unidentified peaks. The presented analysis method is time saving, costs and reagent efficient. Besides, it provides a good separation efficiency, and it is ideally suited for the rapid analysis of aged wine distillates and brandies for determination of their overall quality, approximate ageing time and signs of counterfeiting.

P.09

USING TDU-PYROLYSIS-GC-MS TO INVESTIGATE AGED WHISKEY SAMPLES AND THEIR OAK BARRELS

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Pyrolysis GC-MS was used to profile residual solids after drying aged whiskey samples. The samples in question were both 20 years old from the same unaged parent distillate but matured in the very different wood species of Quercus Robur and Quercus Alba. Fractionated pyrolysis chromatograms generated at 450°C were obtained for both the different whiskey residues and samples of the respective wood species. The whiskey residues showed differences in peak pattern profiles and the same differences were observed between each residue and it's originating wood. Pyrolysis GC-MS could be applied to whiskey maturation investigations and can help to establish a link between the spirit non-volatile fraction and the type of wood used for maturation.

P.10

FAST GC-MS/MS ANALYSIS OF MULTICOMPONENT PESTICIDES RESIDUE (>300) IN FOOD MATRICES USING UF MS TECHNOLOGY

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The determination of pesticide residue in food prepared by the well-established QuEChERS method is mainly done by GCMS/MS and LCMS/MS using multiple reaction mechanisms (MRM). The selectivity of tandem MS detectors is often preferred because samples prepared by QuEChERS contain large matrix signals which may interfere with target peaks. To reduce analysis time regarding GCMS the use of narrow bore capillary columns have become a powerful tool. This approach reduces analysis time drastically while mainly maintaining the chromatographic resolution. As the sample capacity for 0.1 mm inner diameter capillary columns is reduced in comparison with standard columns in this work a RTX-5 15m, 0.15 mm, 0.15 mm was used as capacity with respect to matrix is larger compared to the ones with 0.1 mm I.D.. Using the selectivity of the tandem MS a large number of compounds can be measured. Regarding the detector part the system must be able to follow sharp increases of signals as the peak widths at half height (FWHM) in fast GC with narrow bore columns are expected to be down to about 0.5 s. Therefore fast MRM switching modes are needed with no interfering cross talk. Each pesticide was measured with one quantifier and two qualifier transitions to ensure necessary selectivity. The method was adapted to QuEChERS extracts from apple and tea. The run time was below 12 minutes to screen all compounds (>300). The limit of quantification (MRM) was below 0.1 ppB. Linear calibration curves were done between 0.5 ppB and 100 ppB using triphenylphosphate (TPP) as internal standard.

P.11

THE USE OF LINEAR RETENTION INDICES IN PESTICIDE RESIDUE ANALYSIS USING A TANDEM GCMS WITH MRM DATABASE

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Linear retention indices have been used in different analytical fields. This approach has been applied with GCMS full scan data as an additional filter for identification beside the similarity search with library spectra. This is helpful when coelutions are observed or different target compounds have similar electron impact spectra. Beside that benefit linear retention indices can also be used for determining expected retention times for a specific stationary phase after injection of an n-alcane mix. The retention times of target compounds are calculated from the retention times of the n-alcanes and known retention indices of targets and the relevant n-alcanes. In pesticide analysis the most common phase of the capillary columns used is RTX-5 or similar. Therefore indices for that phase were used for the pesticide data base which contains more than 450 compounds. The advantage over retention time registered data bases is that unlimited target compound retention times are updated from one injection of n-alcanes while the GC method including the column head pressure remain unchanged. For applying this procedure a standard mix containing 24 alcanes (Cn) was injected starting from n=10 to n=33 with delta n = 1. The difference of predicted values to real peak positions were smaller than 0.1 minute for a multicomponent mix of 200 pesticides. Retention indices were registered in addition for a RTX-200 phase. Then two columns were used (RTX-5, RTX-200) simultaneously mounted into the MS detector. When selectivity due to large matrix signals was not sufficient on the RTX-5 phase even in MRM mode the sample was injected into the RTX-200 column. Identification and quantification was done using this method for different QuEChERS matrices.

P.12

ASCORBIC AND ERYTHORBIC ACID DETERMINATION IN FOOD: THE USE OF EXPERIMENTAL DESIGN TO DEVELOP AN UHPLC-UV METHOD

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Ascorbic acid and its sodium and calcium salts are used as preservative antioxidants in food without limitation (quantum satis) and are classified in the Europe (Reg CE 1129 2011) as E 300, E 301 and E 302 respectively.
Erythorbic acid (isoascorbic acid) and its sodium salt have the E315 and E316 European classification with a specified limit of addiction (500 mg/kg) for meat products and 1500 mg/kg for frozen fish.
Therefore is essential to distinguish between ascorbic and isoascorbic acids in their determination. One of the most diffused HPLC-UV method uses metaphosphoric acid as extracting media and diluted phosphoric acid as mobile phase: is simple and effective must it lacks in robustness. An experimental design approach was used to develop a rapid, robust and likewise simple and effective UHPLC-UV method to determine these two preservatives.
The effect of four factors, column temperature, acetonitrile percentage, flow and injection volume, on the resolution and asymmetry of the ascorbic and isoascorbic peaks (response variables) was evaluated using a fractional factorial design. Considering this, a 2^{4-1} factorial design was chosen, which involved fourteen experiments, in triplicate, carried out in random order and five center points to estimate the experimental error. By using this design, the four factors were tested at two different experimental levels and regression algorithms have been used to simulate the experimental responses and to search the optimal settings of the experimental factors.
The optimized UHPLC-UV method was applied in the determination of ascorbic and isoascorbic acid in different food samples.

P.13

AUTOMATED DETERMINATION OF TOTAL FAT, SATURATED FAT, MONOUNSATURATED FAT, AND TRANS FAT CONTENT IN FOOD SAMPLES

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Determination of total fat, saturated fat, monounsaturated fat and trans fat content in food samples is necessary for complying with Food and Drug Administration labeling requirements. A typical procedure for saponification of the sample involves refluxing with sodium methoxide in methanol, followed by a second reflux with boron trifluoride in order to esterify the free fatty acids. Prior to injection into the gas chromatograph, the fatty acid methyl esters (FAMEs) must be extracted from the reaction mix and the extract dried prior to injection. The reflux times are typically an hour. After the sample is prepared, the round bottomed flask and condenser must be cleaned. This process is laborious and time consuming, which limits sample throughput.
A single robotic X-Y-Z coordinate autosampler commonly used for sample introduction in GC or HPLC can be used to perform a wide variety of sample preparation techniques using a single instrument and controlling software. The current version of GERSTEL Maestro is capable of interfacing directly to a CEM Discover SP-D microwave.
In this work, we demonstrate an automated saponification/esterification sample preparation using a GERSTEL MPS autosampler coupled to a CEM Discover SP-D microwave. The use of the microwave, in place of refluxing, allows a significant reduction in the time required for the saponification/esterification reactions, such that the entire sample preparation procedure can take place within the timeframe of the gas chromatographic run. This allows efficient overlap of the sample preparation and sample analysis times for maximum throughput. The autosampler is coupled directly to a GC-FID to streamline the entire extraction and analysis process as well as avoid exposure to potentially hazardous materials by laboratory personnel. Several food types were used to demonstrate this process.

P.14

QUALITATIVE AND QUANTITATIVE ANALYSIS OF VOLATILE COMPONENTS IN PITURANTHOS SCOPARIUS BY GC-MS AND MHE-GC-FID

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*Piturantho*s *scoparius*, named locally “Guezzah”, is an umbelliferous plant native to North Africa and used in folk medicine by applying it in poultices on the head against headache. In contrast to other umbellifers, it is much less known to the western world and consequently, its chemical composition only began to gain interest in the research area. In comparison to more common umbellifers (e.g. fennel, anise etc.), it has a lower percentage of essential oil, albeit with a distinct chemical profile. Studies on the chemical composition of the essential oils of the genus *Piturantho*s are limited. The composition of the essential oil proved to be affected also by processing and handling conditions of the plant before hydrodistillation. GC-MS analysis of the essential oil revealed some distinct differences between the distillates of fresh and dried *Piturantho*s plants. The direct quantification of volatile compounds in the dried plant material was achieved by using multiple headspace extraction (MHE) coupled to GC-FID. The content of volatile compounds in the plant material proved to be relatively high compared to typical essential oil yields obtained by hydrodistillation. As we found out, the sample preparation (dismembration) of samples played a crucial role in the dynamics of analyte desorption from sample matrix. Hence, the slope of MHE curves was also affected by the sample preparation step. MHE is therefore the appropriate approach for quantification of such problematic sample matrices.

P.15

COMPOSITION OF FATTY ACIDS FROM MONOVARIETAL OLIVE OILS PRODUCED IN BRAZIL

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Consumption of olive oil is increasing in Brazil, but still most of the demand is supplied by imports, which reached 50000 tons per year. EPAMIG (Agricultural Research Company of Minas Gerais) has been working in the adaptation of traditional cultivars and in the breeding of new ones. Extra virgin olive oils from European monovarietal cultivars Arbequina, Alto Douro, Negroa and Brazilian bred Maria da Fé, Grappolo 541 and Grappolo 575 were obtained from plants cultivated in Minas Gerais State, Brazil, by pressing in a Oliomio 100M system. Fatty acid methyl esters (FAME) were obtained by Hartman-Lago derivatization [1] and analysed by gas chromatography in an Agilent 7890A system fitted with a capillary cyanopropylsilicone column (60 m X 0.32 mm X 0.25 μm). Oven temperature program ranged from 100ºC (3 min) to 150º C at 50ºC/min, from 150ºC to 180ºC at 1.0ºC/min and from 180 to 200ºC at 25ºC/min. Injector was operated at 250ºC in split mode (1:50). Detector (FID) was kept at 280ºC. For the identification of FAME, a NU-CHECK standard mixture was injected. A significant difference (p

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P.16

STEROLS PROFILE IN MONOVARIETAL OLIVE OILS PRODUCED IN BRAZIL

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One of the legacies of the Portuguese colonization in Brazil was the habit of consuming olive oil. However, almost all olive oil consumed is imported. EPAMIG (Agricultural Research Company of Minas Gerais) has been developing a germplasm bank for adaptation and breeding of olive varieties. Monovarietal olives bred by EPAMIG (Grappolo 541, Grappolo 575 and Maria da Fé) as well as European varieties (Alto Douro, Negroa and Arbequina) from the germplasm bank were cold pressed on site to produce extra virgin oils. The sterol profile was analysed and compared to International Olive Council (IOC) and Brazilian standards. The unsaponifiable matter was obtained according AOCS Official Method Ca 6b-53 [1] and the sterol fraction was isolated by preparative thin layer chromatography. This fraction was analysed by gas chromatography using an Agilent 6890N GC fitted with a methyl silicone (25 m X 0.32 mm X 0.17 μm) capillary column. Oven temperature was raised from 260 to 290ºC at 3ºC/min. Injector and detector (FID) were kept at 300ºC. A solution of the sterols fraction was injected in split mode (1:20). A significant difference ($p<0.05$) was observed among the varieties tested for total sterols content, which ranged from 1002 to 1178 mg/Kg. Higher sterols content was observed for Alto Douro and Negroa varieties. The apparent content of β-sitosterol ranged from 93.1 to 95.0%, campesterol varied from 2.6 to 3.1%, and stigmasterol from 1.0 to 2.1%. The larger variation was verified for 5-avenasterol, from 7.6 to 12.9%. For the Arbequina oil, however, 5-avenasterol, from 7.6 to 12.9%. For the Arbequina oil, however, 5-avenasterol ratio reached 22%, which is in agreement with literature results from oil of this variety cultivated in Europe. No discrepancy was observed to IOC and Brazialian standards.

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INFLUENCE OF DIETARY SUPPLEMENTATION WITH CONJUGATED LINOLEIC ACID AND PLANT ANTIOXIDANTS ON THE FORMATION OF HYDROXYLATED FATTY ACID IN ITALIAN HAM

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Ham quality depends on the food technology as curing, smoking or salting, ripening and conservation techniques, but also on factors strictly related to the quality of raw material, which is function of pigs rearing system [1,2].
During cured ham processing, adipose tissue lipids are subjected to both lipolysis and oxidation. While a small amount of oxidation products is required to get the typical aroma of dry-cured ham, an excessive oxidation leads to off flavor. Especially, the development of oxidized fatty acids represents a noticeable problem during the production of dry cured ham due to unpleasant off flavor [3].
In particular, the lipid oxidation play a key role when newest strategies in meat producing domesticated animals have aimed at changing the fatty acid composition by bringing the polyunsaturated/saturated fatty acids ratio of meat closer to the recommended value (>0.7). Unfortunately, the PUFA enrichment of meat is restricted by the resulting decrease in lipid oxidative stability [4]. Anyway, the dietary supplementation with a proper amount of antioxidants could provide a good alternative to enhance the oxidative stability of meat with a high content of PUFA [5,6]. Some plants, as oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*) characterized by high phenolic compounds content, had demonstrated an interesting delaying activity to meat lipid oxidation when they are included in the animal feed [7–9].
In view of these evidences, the present work was aimed to characterize the oxidized fatty acid fraction in Italian ham obtained from animal feed with different diets: control, diet enriched with oregano and CLA, diet enriched with rosemary and CLA.
The different dietary trials did no affect the qualitative composition of hydroxilated fatty acid fraction in ham. The most abundant peaks in all the TMS derivatized sample were identified as the fraction of C18 hydroxy fatty acids.

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DETERMINATION OF BENZO(A)PYRENE IN MICROCRYSTALLINE WAXES USED AS FOOD ADDITIVE

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Waxes derived from petroleum are hydrocarbons of three types: paraffin, semi microcrystalline and, microcrystalline. Microcrystalline waxes are a mixture of solid, saturated hydrocarbons mainly branched and characterised by carbon numbers predominantly in the range between C41-C51; they are used as food additive particularly as surface treatment agent on non-chocolate confectionery, chewing-gum, some fruits and decorations, coatings and fillings.
Commission Regulation (EU) No 231/2012 and Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2000) established physical and chemical specifications for microcrystalline waxes to use in food. Furthermore, Commission Regulation posed a limit for benzo(a)pyrene (BaP) content (< 50 μg/kg). However, the only official method on this matrix, was published by JECFA and it uses an unspecific spectrofotometric detection (all polycyclic aromatic hydrocarbons (PAHs) extracted are considered) after a quite laborious, time and solvent-consuming method.
The aim was to develop a rapid and straightforward method to evaluate BaP in microcrystalline waxes. A preliminary step using liquid-liquid partition was necessary to remove the bulk of saturated hydrocarbons. Wax sample was dissolved in organic solvent, namely cyclohexane, and BaP was extracted with dimethyl formammide (DMF)/water 9/1 (*v/v*). A mixture of water and hexane were added to the residual DMF/water in order to change the coefficient partition of BaP and extract it in hexane. The latter was the most suitable solvent to carry out the final enrichment step by direct-immersion solid phase microextraction (SPME), employing a particular fiber, namely a carbopack Z/polydimethylsiloxane (PDMS). The prevalent sorption mechanism of the specific fiber depends on the extraction conditions, in a non-polar solvent the effect of carbopack Z is predominant, which is particularly prone to the extraction of planar compounds by π- π interaction. The analytical determination was carried out with gas chromatography-mass spectrometry (GC-MS) in single-ion-monitoring (SIM) mode with isotope dilution calibration. The method was validated in term of linearity, accuracy, repeatability, limit of detection and quantification.

P.19

DETERMINATION OF 2-AMINOACETOPHENONE IN RIESLING WINES BY SPME-GC-MS

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The unpleasant odor quality of an off-flavor is mostly caused by only one, sometimes several volatile compounds with very low odor threshold values and the typical unpleasant odor taint of the spoiled food product. 2-aminoacetophenone is an aroma compound which causes the untypical aging off flavor in *Vitis vinifera* white wines.[1] Wines showing this note, described as acacia blossom, naphtalene note, furniture polish, fusel alcohol, damp cloth, have caused a considerable amount of rejection during 1980s in Germany. UTA can be developed in young wines within a few months after the end of fermentation.[2] It can be realized organoleptically in wine at 0.5 to 1.5 μg/L. Tryptophan (Trp) and its metabolites, especially the phytohormone indole-3-acetic acid (IAA) are considered to be potential precursors of 2-aminoacetophenone (2-AAP). Ultrasound assisted headspace solid phase microextraction (UAE-HS-SPME) and direct immersion solid phase microextraction (DI-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) were tested and optimized for determination of 2-aminoacetophenone in Riesling wines fermented by different yeast strains. Divinylbenzen/Caboxen/Polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm fiber was used. Both methods, DI-SPME and UAE-HS-SPME are quantitative (recoveries in the range 39-63% and 59-83%, repectively) and sensitive (limits of detection were 0.01 μg/L and 0.03 μg/L, repectively). Ultrasonic assisted headspace SPME showed significantly reduced time of extraction. 2-AAP was detected only in two samples below odour treshold.

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QUANTITATIVE ANALYSIS OF α- AND β-IONONE IN WINE USING HS-SPME-ENANTIO-MDGC-MS-MS

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α- and β-ionone resort under the C_{13}-norisoprenoids and are aroma compounds reminiscent of violets, raspberries and floral flavors. They occur naturally in a diverse array of plants, especially raspberries, tea and tobacco [1]. α- and β-ionone can also be present in wine in concentrations up to 2 µg/L [2], in particular cases 60 µg/L [3]. Their presence significantly impacts wine aroma as a result of their very low perception threshold in wine (3 µg/L for α-Ionone [3]). Since the reliable quantification of α- and β-ionone in wine at low concentrations could be hampered by severe co-elutions due to the wine matrix, a method has been developed which enables the interference-free analysis of α- and β-ionone at a concentration level as low as 0.1 µg/L.

The method consists of a headspace solid phase microextraction (HS-SPME) followed by heart-cut multidimensional gas chromatography (MDGC) coupled with a triple quadrupole mass spectrometer (QqQ-MS). Tandem mass spectrometry (MS-MS) in order to improve the specific detection of α- and β-ionone was used. Furthermore, the stable isotope dilution assay (SIDA) was applied for quantification, using isotopic (deuterated) internal standards. These techniques (MDGC, MS-MS, SIDA) are prerequisites for the reliable quantification at low-µg/L concentration levels in complex wine matrices.

In the case of α-ionone, two enantiomeric forms exist. In order to differentiate these, an enantioselective separation column was used in the second dimension (^{2}D). The determination of the distribution of the α-ionone enantiomers allows the detection of wine adulteration by α-ionone supplementation. In those cases the ratio of (*R*)- and (*S*)-α-ionone is different compared to authentic wines.

This novel method combines MDGC with enantioselective separation in ^{2}D, MS-MS and SIDA and allows high-throughput analyses for routine application.

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P.21

THE VOLATILE CONSTITUENTS OF THE ESSENTIAL OIL OF HYPERICUM PERFORATUM L. FROM ALGERIA

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The volatile constituents of the essential oil of Hypericum perforatum L., growing in middle of north Algeria, extracted by hydro-distillation have been analysed**.**A total of 77 volatile compounds were identified and 2-Methyl-octane (30,67%), a-Pinene (23,01%) , b-Bisabolol (8,84%),b-Caryophyllene (7,20%) were the major components of the essential oil.

Antimicrobial activity of essential oils was evaluated against Gram-positive bacteria (Bacillus subtilis (B. subtilis), Staphylococcus aureus (S. aureus) and Gram-negative bacteria (Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa) bacteria, fungi (Candida albicans (C. albicans), Aspergillus flavus (A. flavus), Fusarium sp)) , showed that B. subtilis bacteria were more inhibited by the essential oil tested. A strong activity was also observed on fungi (C. albicans).

The antimicrobial activity of essential oils of Hypericum perforatum L. were tested at various concentrations (0.5-20 mg/mL) and their antimicrobial potency was assessed by the minimal inhibitory concentration (MIC (mg/mL).

The results showed that the essential oil had a great potential antimicrobial activity against all micro organisms.

These studies confirm use of Hypericum perforatumL as plant medicinal and can be used in pharmaceuticals and natural therapies of infectious diseases for humans.

P.22

GC AND GC/MS ANALYSIS OF ESSENTIAL OILS OF EUCALYPTUS VIMINALIS LEAVES, TWIGS AND FRUITS OBTAINED BY HYDRODISTILLATION AND MICROWAVE-ASSISTED HYDRODISTILLATION

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Two different extraction methods were used for a comparative study of *Eucalyptus viminalis* leaves, twigs and fruits essential oils: microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD). The leaves were rich in essential oil (1.83% and 1.6%) than the twigs (0.77% and 0.20%) and fruits (1.6% and 0.13%). The essential oils were analyzed by capillary GC and GC-MS. The compounds were identified according to their retention indices and mass spectra (EI, 70eV). A total of sixty-seven compounds were identified representing over than 85% of the oils in each sample. The microwave-assisted hydrodistllation essential oils were riche in oxygenated components. The main components in the essential oil of leaves were 1,8cineol (52.0% and 65.3%) and α–pinene (14.2% and 15.1%) respectively in oils extracted by HD an MAHD. In the twigs oils the major compounds were p-cymene (36.9%) and α–pinene (14.0%) in the HD essential oil and p-cymene (43.7%) and 1,8 cineol (12.5%) in the MAHD oil. The composition of essential oils of fruits obtained by HD or by MAHD was also characterised by p-cymene (39.3% and 24.6) and 1,8 cineol (12.0% and 11.0%). The results obtained allowed a reliable differentiation between the essential oils obtained by the two extraction methods.

The antioxidant activity of the methanolic extracts of *Eucalyptus viminalis* leaves, was investigated by free radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). All samples showed a relatively weak antioxidant activity in comparison to that of the synthetic antioxidant (BHT).

P.23

ESSENTIAL OIL FROM PEELS AND LEAVES OF BRAZILIAN CITRUS SP. AND ITS BEHAVIORAL EFFECT IN MICE

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Essential oils (EO) from *Citrus sp.* (Rutaceae) are extracted from peels, flowers and leaves by cold pressing, hydrodistillation or steam distillation. Several studies have shown that the presence of certain chemical constituents may provide the EO with its therapeutic properties. [1]. EO of 15 samples of peels and leaves of Brazilian *Citrus sp.* were analyzed by gas chromatography with mass spectrometric detection (GC/MS), including determination of the percentage of enantiomers [2]. EO of *Citrus sinensis* (L.) Osbeck and *Citrus aurantium* L. are rich in limonene and linalool and for this reason were selected for behavioral tests in mice. Mice (n=12) were placed in an inhalation chamber during 30 min, in an atmosphere saturated with 10% EO (in Tween 80 v/v). Headspace GC/MS was performed with samples of mice plasma after their 30 min period inside the inhalation chamber [3]. Behavioral tests (light-dark, locomotor activity and tail suspension) with mice were also carried out after their 30 min inside the inhalation chamber. Results obtained were the following: (1) area percentage of myrcene, limonene, linalool and linalyl acetate ranged from 45 to 97% of the EO composition; (2) the enantiomeric excess (e.e.) *in EO was*: (+)-(*R*)-limonene (60-99%), (-)-(*R*)-linalool (59-78%) and (+)-(*S*)-linalool (76-99%) (3) behavioral tests were indicative of anxiolytic and sedative effects of the EO inhaled; (4) myrcene, limonenne and linalyl acetate were detected in the plasma of mice.

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USING GC/MS ANALYSIS AS EVALUATION TOOL TO STUDY THE IMPACT OF STORAGE CONDITIONS ON ANTI-OXIDANT ACTIVITY OF VOLATILE COMPOUNDS FROM SANTOLINA CHAMAECYPARISSUS

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In pharmaceuticals, plant extracts are especially relevant due to the use of their active compounds for medicine development and as source to obtain adjuvant.
The growing demand for medicinal species indicates the emergence of a market with high potential for consumption, requiring a consistent and readily available supply of high quality raw material. So, the post –harvesting process of medicinal plants has great importance in the production chain, because of its direct influence on the quality and quantity of the active ingredients in the product sold.
Santolina Chamaecyparissus, is one of the endemic plant growing in Algeria.
In this work, flowers of santolina C. used in Algerian folk medicine were collected from East of Algeria location and dried and stored with traditional process during three years. Many laboratories investigated the relationship between essential oil composition and biological activity. Volatile extracts of the stored plants were compared with those of fresh ones.
The GC and GC /MS analysis showed that the main compounds in the oils extracted by steam distillation were: a-pinene (8.62%), p-Cymene (18.58%), beta-ocymene(8.94%), a-Humulene (41.19%), linalyl isovalerate (9.17%), Artemisia ketone (2.02%) and guaiol (8.47%). The comparison of their chemical composition showed quite remarkable differences.
The total terpens of the extract was essayed through an in vitro model such as antioxidant capacity by radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl(DPPH)assay.
The fresh essential oil of santolina C. showed a better antioxidant capacity (IC50: 629,19 mg/L) than the stored ones.

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SPME-GC/MS ANALYSIS OF VOLATILES IN BANANA´S WINES PRODUCED BY DIFFERENT ALCOHOLIC FERMENTATION

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Banana is a tropical fruit widely consumed throughout the world, which present an attractive flavor and has nutritional value. Their aroma is one of the most significant factors, which determines the character and the quality of bananas. It is an important fruit crop grown in Brazil, however fruit production losses represent a significant cost to the economy. The manufacture of beverages based on banana becomes attractive because of the fruit's abundance, relatively high concentration of fermentable sugars, and provision of yeast growth.

In this study, the effects of filtration, enzymatic treatment and strain of yeast were investigated during the production of banana wines. For all the kinetic parameters, ethanol yield, efficiency and productivity were significantly different ($p<0.05$). The most optimum results were shown employing filtration, enzymatic treatment and commercial moist yeast in banana must, which achieved a maximum ethanol yield (85.97 % and 86.39 %) and efficiency (97.98 %). Two selected strains of *Saccharomyces cerevisiae* UFMGA-1007 and UFMGA-1031 were also tested. It was developed a method using solid phase microextraction with gas chromatography/ mass spectrometry (SPME-GC/MS) to evaluate the effects of alcoholic fermentation of different banana wines on the volatile organic compounds (VOC) profile. Twenty-two compounds of distinct chemical classes were analyzed including alcohols, esters, organic acids, aldehydes, ketones, among others. The VOC concentrations were related to the fermentation condition. The values of higher alcohols per 100 mL of anhydrous alcohol in the banana wines ranged from 353 to 1017 mg. The banana wines studied showed a similar composition to other alcoholic beverages and showed significant differences in the profile of VOC of beverages produced by using different fermentation parameters.

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DETERMINATION OF FATTY ACID COMPOSITION IN SICILIAN EXTRA VIRGIN OLIVE OILS BY MEANS OF 1H NMR SPECTROSCOPY AND GAS CHROMATOGRAPHY

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The fatty acid composition in extra Virgin Olive Oils (VOOs) is the major factor influencing their chemical and physical properties and consequently their overall quality in terms of organolepetic and nutritional factors. In particular, health effects have been attributed to certain fatty acids such as oleic and linolenic acids [1]. Indeed a balanced proportion of fatty acyl chains in the diet is of primary importance for human health.
As it is well known, the composition of fatty acids in extra VOOs changes from cultivar to cultivar. Our aim is to characterize the individual composition allowing the identification of the geographical origin of each cultivar. In doing so it is possible to determine the extra VOO authenticity and to prevent the adulteration of high-value extra VOOs [2].
We use two different but complementary experimental techniques such as Nuclear Magnetic Resonance (NMR) spectroscopy and Gas Chromatography (GC) in order to determine the fatty acid composition of several Sicilian extra VOOs.
The big difference between the two techniques is that for GC experiments there is the need to "extract" the portion of interest within samples whereas for NMR experiments samples were only diluted in deuterated chloroform before the acquisition of the spectra. GC experiments were performed with a GC Shimadzu 2010 with FID detector. NMR experiments were conducted with a Bruker Avance 700 MHz spectrometer by means of the experimental setup known as High-Resolution Magic Angle Spinning (HR-MAS).
The synergic use of both techniques has allowed obtaining the fatty acid composition of different extra VOOs produced in Sicily in order to safeguard both producers and consumers by commercial frauds. For example, the development of precise experimental protocols would allow the protection of those extra VOOs accredited by European certificate such as *Protected Geographical Indication* (PGI) or *Protected Designation of Origin* (PDO).

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LOW-LEVEL BENZENE MONITORING IN BABY FOOD BY NEW GENERATION OF STATIC HEADSPACE AUTOSAMPLER COUPLED TO FAST GC-TOFMS

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It's demonstrated that the combination of benzoate salts with ascorbic acid produces low levels (ng/g) of benzene. Benzoates are widely used as food preservatives and especially beverages, like fruit-flavored soft drinks, suffer potentially for benzene formation. Benzene contamination was recently found also in benzoate free foods, especially in carrots products for infants. The chemical process that leads to benzene formation is still unclear: heat-treatments seem to have a key role, promoting the benzene release from suspected precursors (beta-carotene and terpenes).

Due to benzene high carcinogenic activity, its presence in food and beverages is of great concern. In particular, contamination in baby food is even more critical, since infants are more exposed to toxicity than adults.

The limits of benzene in soft drinks and food matrix are not officially established. Therefore the maximum contaminant level (MCL) for drinking water is adopted as quality standard, according to the US Environmental Protection Agency (EPA) limit of 5 ppb or to the European Union limit of 1 ppb.

The new generation of Static Headspace Autosampler coupled to FastGC-TOFMS system, is proposed for benzene contamination assessment in carrots based baby food. Besides a quick sample preparation offered by the static headspace technique, lower detection limit and outstanding repeatability are achieved.

Fast GC allows to complete the chromatographic run in 10 minutes, maintaining the required separation power and improving the sample throughput. The use of Fast GC enhances the potential of High Speed TOF-MS technology, in which the acquisition rate can be increased without sacrificing sensitivity or losing spectral information. Benzene confirmation is possible by mass spectra library matching without the need to previously select diagnostic ions. The obtained results confirm that the described system offers the specificity and sensitivity required to detect benzene at low levels in complex food matrices.

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USING CHROMATOGRAPHIC TECHNIQUES TO UNCOVER ANCIENT DIETS: IDENTIFYING THE CONTENTS OF LUSITANIAN AMPHORAE FROM PAX JULIA REGION

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Eating and drinking is normally much more than just sustaining life. “You are what you eat” - food choices are not normally just personal choices, resulting from social conditioning, religious beliefs, gender and ethnicity.

Despite the fact that there is some written information concerning food and drinking in the Roman world, there were regional differences that are not reflected in those sources.

Archaeological data together with chemical analysis of organic residues recovered from pottery artefacts, can provide information on the diet of a region’s inhabitants during a chosen period of time. Organic residues recovered from pottery shards can be used as fingerprints (biomarkers) of the food products or other materials that were once inside those containers. The recovered residue is usually a complex mixture of compounds, not only due to the diversity of materials once contained in the ceramic, but also due to the chemical transformations occurring during the cooking processes and digenesis due to burial.

Gas chromatography and liquid chromatography coupled with mass spectrometry (GC/MS and LC/DAD/MS) have been used to identify the compounds present in the organic extracts of the pottery artefacts. Further isotopic analysis of the fatty acid residues by GC/IRMS is required to obtain more detailed information on their source.

In this work, organic residues recovered from several fragments of Lusitanian amphorae gathered in São Cucufate and Monte da Cegonha were analysed by GC/MS, LC/DAD/MS and GC/IRMS. The archaeological sites of São Cucufate and Monte da Cegonha, located in the municipality of Vidigueira, South Portugal, were agricultural centers from the Pax Julia region. Both villae show Roman occupation from the 1st century AD to the 4th century AD.

This analysis allowed the identification, among other compounds, of fatty acids (palmitic and stearic acids), steroids (cholesterol and beta-sitosterol) and resin sealants. The data suggests that Pinaceae resins were used to make the amphorae sealants and that both animal and vegetable oil residues were present in the same amphorae, suggesting either multiple uses or presence of composed aliments as animal sauces (garum). Information regarding the making of the pitch sealants can also be inferred from the chemical profile of the samples.

Acknowledgments

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STATIC HEADSPACE SAMPLING FOR DITHIOCARBAMATE PESTICIDES RESIDUES IN VEGETABLES ACCORDING TO METHOD EN 12396-2

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Dithiocarbamates are common fungicides widely used in agriculture. In mammals dithiocarbamates typically show low acute toxicity, but after prolonged exposure the toxicity significantly increases. The European Standard EN12396-2 describes a method for the determination of dithiocarbamate residues in non-fatty foods, based on the reaction with tin-(II)-chloride and hydrochloric acid and consequent release of CS2. The CS2 amount, proportional to the concentration of fungicides, is analyzed by GC coupled to electron capture or MS detector. A new approach based on the use of Static Headspace technique coupled to GC-TOFMS is described for the determination of dithiocarbamate residues in fruit and vegetable samples, in compliance with the method EN12396-2. The sample is heated and shaken in presence of HCl and SnCl2 into a closed headspace vial and, after the incubation time, an aliquot of the headspace is automatically transferred to the GC-TOFMS system for CS2 analysis. The obtained results confirmed the effectiveness of the approach for fruit and vegetable extracts (pear and spinach). In the concentration range suggested by the EN12396-2, the method exhibited excellent linearity, assuring a reliable quantification in real samples. The SHS automation guaranties high precision with RSD<1.1%. A limit of detection of 0.2 ppb for CS2 was extrapolated considering a signal 3 times the noise: this limit corresponds to a concentration of 0,68 ug/Kg of dithiocarbamate pesticides in the sample. Besides, TOFMS allows to easily recognize and confirm CS2 peak according to the full mass spectrum conveniently matched with the NIST library.

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GC-MS ANALYSIS OF FLOWER SCENT ISOLATED FROM TROPICAL PLANTS

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The analysis of volatile compounds from tropical plant flowers has important applications in ecological chemistry, in the study of plant-insect relationships, biochemistry, and plant physiology. The chromatographic profiles of flower volatiles may inspire the creation of new perfumes, but fundamentally, constitute the basis to discern the biogenesys of these compounds, their different functions in the flower (pollinator attraction, herbivore repellence, other). In this work, HS-SPME was used to isolate flower volatiles followed by GC-MS analysis [1]. Various fiber coatings were compared (PDMS, PDMS-DVB, CAR-PDMS), as well as the flower volatile sampling conditions (time of day, temperature, fiber exposition time). Superior reproducibility, ruggedness, and amount and number of isolated components were obtained with PDMS-DVB-coated SPME fibers [2]. Chromatographic analysis was performed on capillary columns with orthogonal polarity (DB-WAX, DB-5MS, 60 m). The mass-selective detector was operated under the full-scan, SIM, and full-scan+SIM modes. Tandem mass spectrometry (GC-QQQ-MS) was employed in the detection of alkaloid traces. Compound identification involved the use of mass spectra (EI, 70 eV), linear retention indices obtained in both columns, and standard compounds. Despite the presence of common flower compounds (ocimenes, benzaldehyde, methyl benzoate, benzyl benzoate, linalool, terpineol, methyl salycilate, farnesol) in the different species studied [*Sansevieria guineensis* (Mother-in-Law´s Tongue, Asparagaceae Fam.), *Coffea arabica* (Coffee, Rubiaceae Fam.) [3], *Moringa oleifera* (Moringa, Moringacea Fam.), *Erythroxylum novogranatense* (Coca, Eritroxilaceae Fam.), *Persea americana* (Avocado, Lauraceae Fam.), *Passiflora edulis* (Passion fruit, Pasifloraceae Fam.), *Petrea volubilis* (Albiflora, Verbenaceae Fam.), and others], each flower emits its unique combination of volatile compounds, which changes during the day, before and after pollination. Specific, “diagnostic” substances, were found, such as isothiocyanates in moringa flowers, or bovolide, emitted by coca flowers only in the morning; 3-octanone, 3-octanol and 1-octanol in albiflora; methyl salicilate, which appears in passion fruit flowers only after their pollination.

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ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN FOODS BY GC/FID: 1 - SMOKED SAUSAGE

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Polycyclic Aromatic Hydrocarbons (PAHs) are a group of organic compounds, which represent an important class of chemical carcinogens in foods and in the environment. PAHs occur as contaminants in different types of foods, mainly due to environmental pollution and some types of processing. The curing, drying and roasting are examples of processes that contribute to the formation of PAHs in foods such as meat products, cheeses, coffee, soda, sugar and others. The objective of this research is the detection of PAHs in different foods and, in this second part, the goal is to verify the presence of PAHs in artisanal smoked sausages. The smoking process in sausages is made by burning organic matter, usually wood or charcoal. A combination of factors such as incomplete combustion, high temperatures and the amount of air in the environment can promote the formation of PAHs. The sausages have a great amount of fat in its composition, which facilitates the deposition of PAHs, since these compounds possess high lipophilic affinity. These compounds also exhibit photosensitivity, which reduces their concentration in food due to decomposition by light exposure. However, the degradation is not complete, leading to a decrease of their concentration up to a constant value. The objective of this study was to evaluate the presence of PAHs in samples of smoked sausage handmade. Twelve samples of smoked sausage, handcrafted produced, obtained in commerce of Vale do Taquari, Rio Grande do Sul, Southern Brazil, were analyzed. The PAHs extraction was carried out with 20 g samples of sausages through the saponification with KOH in methanol. The chromatographic analysis was performed using a gas chromatograph with flame ionization detector. The analysis results indicated the presence of fluorene, phenanthrene, anthracene and pyrene in samples of smoked sausages with concentrations ranging from 0.05 to 5.7 mg kg^{-1}.

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MODIFIED QUECHERS EXTRACTION, SIMPLE SILICA CARTRIDGE SPE CLEANUP, AND HYDROGEN CARRIER GC-TOFMS WITH A NOVEL SELECTIVITY GC COLUMN TO DETERMINE THE EFSA PAH4 IN MATE TEAS

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Polycyclic aromatic hydrocarbons (PAHs) are toxic compounds found in some foods, especially those that are smoked, roasted, grilled, or dried during preparation. Teas, including yerba mate, contain PAHs, sometimes at relatively high levels. While classic sample preparation methods such as Soxhlet and Pressurized Fluid Extraction (PFE) yield excellent quantitative results for PAHs in tea, Soxhlet has high solvent use and PFE requires expensive capital equipment. The more simple QuEChERS procedure requires little solvent and no expensive equipment, so it is a natural choice for consideration as a replacement extraction method for PAHs in tea.
We used a modified QuEChERS procedure for extraction of PAHs from teas. Acetonitrile, the classic QuEChERS solvent, was inefficient at extracting PAHs from the complex tea matrix, but hexane:acetone (50:50) gave quantitative recoveries. A simple silica cartridge cleanup with one elution solvent was used to clean extracts, including the removal of chlorophyll that can foul GC inlets and columns. A novel GC stationary phase with selectivity towards PAHs separated EFSA PAH4 benz[a]anthracene, chrysene (triphenylene was separated), benzo[b] fluoranthene (separated from other benzo fluoranthenes), and benzo[a]pyrene under hydrogen carrier GC-TOFMS conditions. A candidate NIST SRM mate tea was analyzed for PAHs with our method in addition to characterizing other teas.

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SCREENING OF THE VOLATOMIC PROFILE OF DIFFERENT PASSIFLORA L. SPECIES THROUGH HEADSPACE SOLID PHASE MICROEXTRACTION TANDEM WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

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Passiflora L. (Passifloraceae), known as passion fruit, originated from tropical and warm climates of South America, is a fruit-bearing widely distributed throughout Madeira Island. As bioactive food, is used in popular medicine as a diuretic, antioxidant, sedative, antihypertensive and anti-inflammatory agent. They constitute a powerful source of carbohydrates, flavonoids, alkaloids, ascorbic acid, carotenoids, vitamins, minerals and terpenoid compounds. In addition, it is a good source of nicotinic acid, riboflavin and a fair source of mineral matter.
To gain insights on the aroma composition of *Passiflora* L. varieties, the volatile metabolomic pattern of six passion fruit varieties produced at Madeira Island - yellow, purple, tomato, banana, orange, lemon and pineapple, was investigated through isolation of the volatile organic metabolites (VOMs), by HS-SPME followed by identification using GC–qMS analysis.
HS-SPME technique was optimized in terms of fibre coating, extraction time, extraction temperature and sample amount, in order to achieve the best extraction efficiency. The best result was obtained with 5 g of sample, using a DVB/CAR/PDMS fibre for 40 min at 40 ?C under constant magnetic stirring (800 rpm).
After optimization of the extraction methodology, all the passion fruit samples were analyzed with the best conditions that allowed to identify about 70 volatile metabolites. The major compounds identified in the *Passiflora* L. samples investigated were ethyl hexanoate, ethyl butyrate, β-pinene, cis-β-ocimene, cis-3-hexenyl acetate and hexyl hexanoate.
This study showed that each cherimoya cultivars have 30 common metabolites, corresponding to different chemical families, namely terpenes, esters, alcohols, fatty acids and carbonyl compounds. The semi-quantitative results were then submitted to principal component analysis (PCA) in order to establish relationships between the compounds and the different passion fruit species under investigation

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EVALUATED OF BARRIER DISCHARGE IONIZATION DETECTOR FOR THE GAS CHROMATOGRAPHY DETERMINATION OF TERPENOID COMPOUNDS

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The detector is an essential supporting device for the gas chromatograph and has played a critical role in the development of the technique as a whole. The Barrier Discharge Ionization Detector (BID) is constructed of two main sections, the plasma generation section and collector section. The plasma generation section contains three electrodes, and generates non-equilibrium plasma from the electrical discharge of the dielectric barrier by applying high voltage to the central electrode. The metastable condition of the helium generated from the non-equilibrium plasma ionizes the sample using the photon energy emitted when it returns to the ground state. These ions are accumulated and amplified by the collector and are output as a voltage value to the computer [1]. Essential oils are concentrated natural plant products which contain volatile aroma compounds. The oils usually consist of a complex mixture of tens to hundreds of low molecular weight terpenoids [2]. The performance of this new GC detector was evaluated by the limits of detection (LODs), the determination of a series of volatile compounds including trans-nerolidol, α-humulene, β-cariophyllene, α-bisabolol and α-pinene, and the analysis of a real essential oil sample. Preliminary results showed that it provided slight higher LODs and detectability than those obtained by GC with flame ionization detector (FID) the most commonly used detector.

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CHEMICAL COMPOSITION AND FUMIGANT TOXICITY OF ALGERIAN CORIANDRUM SATIVUM SEED ESSENTIAL OILS AGAINST SITOPHILUS GRANARIUS

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The chemical composition of essential oils obtained by hydro-distillation from *Coriandrum sativum* seeds growing in Sétif region (Eastern Algeria), was investigated by high resolution capillary GC and GC/MS. Retention indices and mass spectra were used to identify a total of 17 compounds. The major constituents were linalool (73.1%), p-mentha-1, 4-dien-7-ol (6.5%), a-pinene (3.4%) and neryl acetate (3.2%). The fumigant toxicity of this essential oil was assessed under laboratory conditions against the adults of *Sitophilus granarius*, an important stored-product beetle observed in grain storage facilities in different countries. A filter paper treated with 1, 5, 10, 50, 100 and 500mL of test essential oil in 50 mL acetone was placed in the bottom cover of a plastic bottle of 1L. The insects, fifty adults per bottle, were exposed for 1-5 days. Controls received 50 mL acetone. Cumulative mortalities were determined 24, 48, 72, 96 and 120 hours after treatment. All treatments were replicated five times. Concentrations of coriander seed essential oil of 5 and 10mL/L air showed 51 and 70% mortality after 120h fumigation. Concentrations of 50, 100 and 500mL/L air gave 50, 70 and 100% mortality after 72h fumigation. The results on *sitophilus* mortality indicate that these natural constituents may find potential application as useful and biodegradable grain protectants.

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GC-MS/MS ANALYSIS OF PESTICIDE RESIDUE IN GREEN TEA EXTRACTED BY QUECHERS WITH ACETONITRILE AS FINAL SOLVENT

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QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) is a well known approach used for the extraction and clean-up of pesticide residue in various matrices. This procedure involved an initial step when a few grams of the sample are extracted with acetonitrile followed by a clean-up step (with dispersive-SPE) used to remove, to a certain extent, unwanted matrix compound (such as pigments, sugars, organic acids). Typically, the final extract ends up with the pesticides in acetonitrile. Acetonitrile is a polar solvent and can be problematic in GC-MS. Poor focusing of chromatographic peaks and high expansion coefficient are issues that need to be addressed when acetonitrile is used as a solvent for GC-MS analysis. To overcome this, an additional step can be added to the QuEChERS method where acetonitrile is replaced with solvents that are more amenable to splitless injections in GC-MS.
This work shows the results of the analysis of 21 pesticides in green tea using acetonitrile as final solvent and splitless GC injection. The compounds analysed are representatives of various classes of pesticides, such as carboxamids, OC, OP, pyrethriods, aromatic, phenylamides. The aim of this study was to assess the chromatography, repeatability, robustness and linearity of these compounds when using acetonitrile and splitless injections. Two different injector designs were used in all these experiments, the first optimized for conventional Band Liquid Formation injection while the second one designed for Thermospray injection. GCMS experiments were performed with Thermo Scientific TRACE™ 1310 GC coupled with a TSQ 8000 triple quadrupole GC-MS/MS analyser. Excellent chromatography for most compounds analysed was observed. A comparison review of results with a critical assessment of the used injector techniques is further discussed.

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ANALYSIS OF POLYCHLORINATED BIPHENYLS AND ORGANIC CHLORINATED PESTICIDES IN MUSSELS BY GC-ECD

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Gas chromatography with electron capture detector (GC-ECD) and capillary column was used to analyze qualitatively and quantitatively polychlorinated biphenyls (PCBs) and organic chlorinated pesticides (OCPs).
The PCBs and almost of the OCPs are persistent organic pollutants (POPs). They have been produced and used for industrial and agricultural purposes for a long time and a large scale. These compounds have been identified as contaminants in almost all components of the global ecosystem including air, water, soil, fish, wildlife and human adipose tissue. Long-term exposure PCBs and OCPs has been correlated with severe injury to the nervous, endocrine, reproductive, and immune systems in birds, fish and mammals.
In 2001, several countries, including Algeria, signed the Stockholm convention under the United Nations Environment Program to implement measures in order to reduce and eliminate the release of persistent organic pollutants into the environment, including bans on production, import, export, and use of certain POPs.
The current work was initiated to assess the levels, distribution, and sources of PCBs and OCPs in Algiers Bay. Thus, mussel samples were collected from Tamentfoust (ex. La Perouse) and Ain Chrob (ex. Surcouf) located in the Eastern coastal side of the Algiers bay. Before GC-ECD analysis of PCBs and OCPs, the biological samples were microwave extracted and purified. The results obtained show that although the ban of the target compounds, they are still remained in the marine environment.

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CHARACTERIZATION OF BIOACTIVE MOLECULES IN EXTRA VIRGIN OLIVE OIL AND IN OLIVE BY-PRODUCT BY MEANS OF HPLC COUPLED WITH PHOTODIODE ARRAY AND ELECTROSPRAY IONIZATION MASS SPECTROMETRY DETECTION

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During olive oil production a large amount of by-products, like olive pomaces and mill wastewaters, are generated from industrial processing. This causes serious environmental problems for the disposal of the generated by-products. As a consequence, an increasing interest arises from the research of a valid alternative for the aforementioned problem through the valorisation of by-products as a source of potentially bioactive molecules.
Specifically, polyphenolic compounds, are well known to exert positive health effects thanks to their anti-oxidant, anti-inflammatory and anti-microbial activities.
The aim of this research was the development of a method for the investigation and quantification of tocopherols and polyphenols (flavonoids, lignans, phenolic acids, secoiridoids and derivatives) in extra virgin olive oils, olives and in olive by-products.
Separation of phenolic components was performed by high performance liquid chromatography, on a 150 x2.1 mmI.D. Ascentis Express F5 Fused Core column, with a particle size of 2.7 μm. This kind of column provides more than twice the speed and efficiency of traditional columns at half the backpressure of sub-2-μm columns.
Polyphenol identification was achieved through HPLC-ESI-MS, whereas the employment of PDA detection enabled their quantitative assay. Linearity, recovery and precision were also evaluated.

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BOTANICAL ORIGIN IDENTIFICATION OF FOOD TANNINS USING COMBINED PROFILES OF MINOR SUGARS AND SIMPLE PHENOLS

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Several papers investigated the complex chemical and technical properties of the family of tannins. In particular, they studied their role in wine stabilisation phenomena, the peculiar ability to modify the beverages sensory perception (*e.g.* astringency, colour and flavour), but also the beneficial health effects (e.g. antioxidant and antimicrobial activity, possible uses as anti-cancer or against cardiovascular diseases) [1,2]. They are traditionally classified as hydrolysable tannins (gallotannins, ellagitannins and flavonoids) [3], that are mainly derived from several plant material [4,5], and condensed tannins, that are generally extracted from grape tissues [6]. O.I.V. identifies the tannins produced from different botanical sources as juice and wine clarification adjuvants (Recueil des methods Internationales d'analyses, 2013), while the European Authorities recognise them as flavourings and food ingredients (EU Regulation No. 2232/96, EC No 1334/2008).

This paper investigated the possibility of assessing the botanical origin of commercial tannins (N=109 samples; 10 botanical origins: Grapes, N=44; Oak, 23; Gall, 11; Tree fruit, 8; Chestnut, 6; Quebracho, 5; Tea, 5; Acacia, 4; Tara, 2 and Officinal plant, 1) on the basis of the minor sugars (by Ionic chromatography) and simple phenols profiles (UHPLC-coulometric electrochemical detection).

The O.I.V. approach [7,8] permitted to correctly reclassify roughly 80% tannins to the relevant botanic categories, while our approach achieved more than 90% correct results, also allowing to trace 6 new typologies, not considered before by O.I.V..

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CHARACTERIZATION OF VOLATILE COMPOUNDS IN TRUFFLE INFUSE-OILS BY HS-SPME-GC-MS ANALYSIS

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As is well-known, the culinary and commercial value of truffles is mainly due to their sensorial properties, such as their aroma, the quality of which clearly gives economic value to this edible fungus. Some culinary preparations are made with this fungus, such as truffle-infused oils. The adulteration of these products must be controlled and prevented due to the high economic cost of natural truffles. The aim of this study is to characterize the volatile organic compounds of some truffle-infused oils, in order to try to determine their authenticity.

Truffle-infused oil samples were purchased in local (3 samples) and foreign markets (4 samples) and one black truffle-infused oil (Tuber Melanosporum) sample was prepared in our laboratory to have a positive authenticity control. The same black truffle used to make the oil control was also analysed to look for its volatile composition. Samples were stored at 4ºC and the analysis was done in 24-48 h. In our study, volatile compounds were extracted (two replicates of each sample) by headspace-solid-phase-micro-extraction, and separated and identified by gas chromatography-mass spectrometry (HS-SPME-GC/MS) analysis [1-9]. The extraction method was based on SPME headspace; a 50/30 µm divinylbenzene / carboxen / polydimethylsiloxane coating (DVB-CAR-PDMS) and a 100 µm polydimethylsiloxane coating (PDMS) fibers were applied. Extraction was carried out in 20 mL glass vials, which were closed with PTFE/Silicone septa, using 2 g of sample for 30 min at 50 ºC, to avoid oil oxidation. GC-MS analysis was performed with a Zebron ZB-WAX Plus (30 m x 0.25 mm x 0.25 µm) and a Zebron ZB1 (30 m x 0.25 mm x 0.25 µm) capillary columns and oven programmed temperature. The SPME-extracted volatiles were directly desorbed (10 min) into the split-splitless injector at 250 ºC. The mass spectrometer was operated in EI+ mode. Wiley, NIST'08 and proprietary database libraries were used for mass spectra identification of volatile compounds found in the different studied samples. Some considerations about product authenticity were given, comparing commercial samples with the laboratory truffle-infused oil control sample.

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STUDY OF THE CAROTENOIDS ESTERS IN DIVERSE CITRUS CULTIVARS BY MEANS OF HPLC WITH PHOTODIODE ARRAY AND ATMOSPHERIC PRESSURE CHEMICAL IONIZATION MASS SPECTROMETRY DETECTION

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Citrus products are one of the most widely consumed fruit products worldwide. Oranges, which stand out among *Citrus* products, usually contain an intricate pattern of carotenoids including different carotenes and xanthophylls as well as different geometrical isomers. Carotenoids are usually present as free carotenes or, in the case of the xanthophylls, can be esterified with fatty acids. The study of the native carotenoid composition is challenging since carotenoid esters may have a remarkable impact on their stability and bioavailability and moreover they could be used as markers of food authenticity.

The aim of this work was the characterization of carotenoids esters in different *Citrus* cultivars. The native carotenoid profile of several mandarin (n = 7) and oranges (n = 22) cultivars has been studied. The qualitative and quantitative determination of the carotenoids has been carried out on a YMC C_{30} column, in combination with diode array (DAD) and atmospheric pressure chemical ionization (APCI) mass detection, under positive (+) and negative (-) ionization modes. It was possible to identify a typical profile of free carotenoids, mono- esters and di- esters in the analyzed oranges juices. In general, the mono- esters represented the most important fraction in quantitative terms. As expected, mandarin was characterized by the presence of mostly β-cryptoxanthin, both free and esterified with fatty acids.

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IMPROVED DETERMINATION OF PERSISTENT ORGANIC POLLUTANTS (POPS) IN FATTY FOODS AND BEVERAGES USING QUECHERS EXTRACTION/CLEANUP AND GC/MS

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Persistent organic pollutants (POPs) are harmful man-made compounds that are not easily degraded by chemical, biological, or photolytic processes. Therefore, they persist in the environment for long periods of time. POPs encompass compound classes such as dioxins, polychlorinated biphenyls (PCBs), chlorinated pesticides, and polyaromatic hydrocarbons (PAHs). Many of these compounds are lipophilic, and bioaccumulate in the fatty tissues of living organisms. Moving up the food chain, their concentration increases as they pass from one organism to another [1]. As a result, these compounds can end up in animal-based foods such as fish, meat, and milk. The analysis of POPs in fatty foods is challenging, because fats often get co-extracted with the target analytes, causing interference and/or sensitivity issues during analysis. Work done recently by Sapozhnikova and Lehotay on extraction and analysis of POPs in catfish, found zirconia-coated silica effective in background removal, while obtaining good recovery and reproducibility [2].

An additional source for PAHs in food may be the grilling process, because PAHs can be formed during the grilling process when the flames containing the PAHs come into contact with the meat and the PAHs get absorbed into the food. The amount of PAHs in grilled food products varies greatly with the type of meat, the cooking temperature, and how long the meat is cooked [3]. Extracting PAHs from complex matrices like animal meat that contain fats, lipids, muscles, and proteins can involve multi-step extraction procedures that are time-consuming and complicated.

In this work, the QuEChERS method (dispersive SPE), developed by Anastassiades and Lehotay [4], using two new zirconia-coated silica adsorbents was applied for the extraction and cleanup of PAHs from raw salmon and grilled burger as well as for PCBs from cow's milk. For GC analysis of these compounds capillary columns were especially selected to provide the optimum selectivity, temperature range and analysis time. The use of the zirconia-coated silica resulted in higher removal of matrix components by an improved recovery for the POPs.

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ANALYSIS OF PAHS IN OLIVE OIL USING A NEW DUAL-LAYER SPE CARTRIDGE

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Olive oil can become contaminated with Polynuclear Aromatic Hydrocarbons (PAHs) through exposure of the olives to pollution in the environment. Concerns over exposure to these compounds have resulted in European Union Commission Regulation No 835/2011. This regulation sets a maximum limit for PAHs in edible oils of 2 ng/g benzo[a]pyrene alone, and 10 ng/g total for the sum of benzo[a]pyrene plus three additional PAHs.

Trace analysis of PAHs is commonly done by either GC-MS or HPLC-FLD. Oily/fatty samples present an analytical challenge due to the heavy matrix effects often encountered. In the case of GC-MS, fatty matrix can cause contamination of the GC inlet, column and detector. In the case of HPLC, matrix can build up on the column, resulting in loss of chromatographic efficiency and/or an increase in system backpressure. Various cleanup techniques exist for fatty samples, and some can be time consuming and expensive.

In this work, a new SPE cartridge containing two different layers of adsorbent was evaluated for the simultaneous extraction and cleanup of PAHs from olive oil. The layers consist of Florisil and a mix of zirconia-coated silica and C18 modified silica. Olive oil sample was loaded directly onto the SPE cartridge, followed by elution of the PAHs with acetonitrile while fatty matrix remained bound to the adsorbents. The resulting extract was concentrated, and analyzed by both GC-MS and HPLC-FLD. The dual-layer SPE cartridge was evaluated with olive oil samples spiked with light and heavy PAHs, and found to yield recoveries of >70% and % RSD values.

P.44

THE CHARACTERIZATION OF VOLATILE COMPONENTS IN BEER AND ITS INGREDIENTS

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Aroma plays a very important part in the flavor in many of the things we ingest – including beer. Most brewers use skilled and experienced beer tasters to monitor the organoleptic character of beer and so help to ensure the high quality of their product. While this approach is usually effective, such tasting is highly subjective and would be greatly complemented by the availability of objective analytical data.

A gas chromatographic system has been developed to assist in the objective characterization of beer aroma. This system can be used for the quality control of hops and adjuncts prior to brewing and to troubleshoot beer after production. A wide variety of hops types have been examined using this system including American West Coast strains, English strains and Noble strains. A range of beers has also been examined.

The system can also be used to detect and monitor the presence of flavor defects such as dimethyl sulfide (vegetative), diacetyl (butterscotch), acetaldehyde (green apple), mercaptans (skunky) and *t*-2-nonenal (stale cardboard).

The first component of this system is an equilibrium headspace sampler with an integral adsorbent trap. A hop or beer sample is placed in a sealed vial and maintained at an elevated temperature for a fixed period of time. During this time, volatile organic compounds (VOCs) from the sample migrate into the vapor (headspace) phase inside the vial. This vapor is then vented into a cooled adsorbent trap to focus and concentrate the VOCs. The VOCs in the trap are thermally desorbed and delivered to a gas chromatograph (GC) for component separation. The use of such a trap enables more VOCs to be collected and be delivered to the gas chromatograph thus increasing detection limits significantly. The chromatographic column used for the separation is a 60m x 0.32mm x 0.5µm Carbowax column (same stationary phase as used in ASBS Method Hops-17). The effluent from the chromatographic column is split between a mass spectrometer (MS) and an olfactory port (OP). The splitting device is fabricated using chemically deactivated laser-etched micro-channel wafer technology to ensure minimum dispersion and adsorption of compounds eluting from the GC column. The MS system enables the detection, identification and quantification of each VOC component. The MS used in this work is a new single quadrupole designed specifically for GC use and has an enhanced sensitivity to enable spectral identification of VOCs at very low levels. The olfactory port is a new design that enables the operator to smell each component as it elutes from the GC column in relative comfort. In this way, the chemical profiles generated by the MS may be correlated against the subjective organoleptic information obtained from the olfactory port. This poster will describe the design and application of this system.

P.45

HEADSPACE SOLID PHASE MICROEXTRACTION TANDEM WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS AS USEFUL STRATEGY TO ESTABLISH THE VOLATOMIC PROFILE OF BLACKBERRY FRUIT

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Blackberry (*Rubus fruticosus, Rosaceae*) is a fruit-bearing widely distributed throughout Madeira Island. This fruit contains high levels of anthocyanins and other phenolic compounds, mainly flavonols and ellagitannins, which contribute to its high antioxidant capacity and biological activities. Several studies showed their potential to fight oxidative stress, cardiovascular disease, and its protective effects on certain cancers and age-related neurodegenerative diseases. To gain insights on the volatile metabolomic pattern of blackberry, a useful strategy involving headspace-solid phase microextraction (HS-SPME) and gas chromatography with mass spectrometry (GC–qMS) was developed and optimized. An experimental univariate design was used to determine the effect of SPME variables, namely, SPME fibers, adsorption temperature, extraction time, amount of salt, sample amount and sample concentration on the extraction efficiency of volatile organic metabolites (VOMs) and therefore to find out the most appropriate conditions for the extraction of blackberry volatiles. DVB/CAR/PDMS at 40 °C for 40 min and 30% (w/w) of NaCl under stirring mode resulted in the highest extraction efficiency of VOMs. Fifty two compounds were identified by GC–qMS when using the optimized extraction conditions. Blackberry was dominated by norisoprenoids, ethyl esteres, aldehydes and acids. The major VOMs identified in blackberries were: *p*-cimene, terpinolene, 2-heptanol, terpinen-4-ol, myrtenol, limonene and 2-hexenal, accounting for 62% of total volatile composition of investigated blackberries.

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P.46

DYNAMIC HEADSPACE ANALYSIS OF KOREAN TRADITIONAL SPIRITS

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Distilled spirit, also called distilled liquor, distilled spirit: fermentation and distillation process for producing high alcoholic beverage (such as brandy, whisky, rum, or arrack) that is obtained by distillation from wine or other fermented fruit or plant juice or from a starchy material (such as various grains) that has first been brewed. In Korea, the major crop has historically been rice, and thus most Korean traditional alcoholic beverages have been made from rice, of both the glutinous and non-glutinous variety, which are fermented with the aid of yeast and nuruk, a wheat-based source of the enzyme amylase. The distillation procedure influences the occurrence and concentration of volatile flavor compounds in the distillate. Particularly in the manufacture of strong spirits, it is customary to improve the flavor of the distillate by stripping it of low-boiling and high-boiling compounds to a greater or lesser degree. In this study, the volatile flavoring components profiles of the more than five different Korean traditional spirits (such as Ikangju, 10 years aged soju, Andong soju, Hwanyo, Ilfumjinro etc) were studied. The volatiles were sampled by dynamic headspace method (such as SPME and thermal desorber) and analyzed by gas chromatograph coupled to mass spectrometer (GC-MS) and GC-FID (Retention index system). Most of the identified flavoring components are ester compounds such as ethyl acetate, 2-methylbutyl acetate, ethyl hexanoate, ethyl heptanoate, diethyl butanoate, etc. The characteristic flavoring profiles of Korean Traditional Spirits depending on the materials and aging method are presented.

P.47

MULTICLASS MYCOTOXINS DETERMINATION IN COOKIES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Mycotoxins are a group of naturally occurring toxic compounds produced by the secondary metabolism of many filamentous fungi (mainly Penicillium, Fusarium and Aspergillus genera) [1]. Whenever several physical, chemical, and biological conditions take place, mycotoxin contamination may occur, and most mycotoxins are moderately stable in most food processing systems thus they can still be found even in finished products [2]. A sensitive, simple and rapid method for the simultaneous determination of 19 mycotoxins in biscuits (a dry matrix containing cereals and egg) has been developed using high performance liquid chromatography coupled to tandem mass spectrometry with electrospray source working in both positive and negative mode.

Due to the matrix complexity and the high amount of contaminants, a solid phase extraction method using graphitized carbon black was optimized for an effective clean-up step. Accuracy was carried out in the selected matrix using blank samples spiked at three analyte concentrations. Recoveries between 63 and 107% and relative standard deviations lower than 12% were obtained. For all considered mycotoxin classes, i.e. thricotecenes A and B, zearalenone and its metabolites, fumonisins, ochratoxin A, enniatins and their structurally related beauvericin, the method was validated in terms of linearity, recovery, matrix effect, precision, limit of detection and limit of quantification. Matrix-matched calibration was used for quantification purposes, in order to compensate for matrix effect. The coefficients of determination obtained were in the range of 0.9927-1. The limits of quantification, ranging from 0.04 µg kg-1 for enniatin B1 to 80.2 µg kg-1 for nivalenol, were always lower than maximum permitted levels for every regulated mycotoxin by the current European legislation.

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P.48

GOUDA AND CHEDDAR CHEESE ANALYSIS BY SPME-GC-MS

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Cheese flavour is for a large part due to the flavour compounds formed by the microorganisms living in the cheese. The breakdown of protein and fat by the microorganisms results in flavours that are characteristic for the individual type of cheese.
Chr.Hansen A/S is producing bacteria for cheese making and we are in a process of improving our flavour analysis capabilities.
Automated SPME-GC-MS has been implemented and relevant parameters are investigated in order to obtain maximum sensitivity and selectivity. These results will be presented.

P.49

DETERMINATION OF POLYPHENOLS IN CAPSICUM ANNUM L. BY LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY DETECTION AND EVALUATION OF THE BIOLOGICAL ACTIVITY

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In recent years phenolic compounds have attracted the interest of researchers because they show promise of exerting beneficial effects toward human health, as related e.g. to the antioxidant and antimicrobial activities. Polyphenols are a group of secondary metabolites, which are synthesized by plants as a result of adaptation to biotic and abiotic stress conditions; since they cannot be produced by the human body, they must be taken in mainly through the daily diet. Knowledge about the nutritional and therapeutic role of dietary phenolic antioxidants is essential for the development of functional foods, which refers to the improvement of conventional foods with added health benefits. On the other hand, detailed chemical composition of foods considered to be functional is needed, and the main goal of the chemistry of natural compounds is screening for promising biologically active substances of plant origin.
This research was aimed to achieve the full characterization and quantification of flavonoids and other phenolic components extracted from Capsicum annuum pepper fruits, to correlate to the antimicrobial activity tested. An RPLC-DAD-MS system was optimized, employing a partially porous octadecylsilica column as stationary phase. Determination was carried out on the basis of the complementary information obtained from their migration times, diode array spectra, MS ions and MS/MS fragments.

P.50

METABOLITE PROFILING ON COFFEE VOLATILE COMPOSITION BASED ON SOLID PHASE MICROEXTRACTION AND GAS-CHROMATOGRAPHY QUADRUPOLE MASS SPECTROMETRY

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Aroma is one of the most contributory factors for the high acceptability of coffee by consumers. Its complex composition, involving more than 800 volatile compounds, depends on the botanical variety of coffee, processing, grinding, and packaging and especially the roasting process and extraction method. Maillard or non-enzymatic browning reaction between nitrogen containing substances, amino acids, proteins, strecker degradation; degradation of sugar and degradation of individual amino acids as well as carbohydrates, hydroxy-acids and phenols, are the main chemical processes that influence the development of volatile compounds in coffee.
In this study a dynamic headspace solid-phase microextraction (HS-SPME) followed by thermal desorption gas chromatography-quadrupole mass spectrometry analysis (GC-qMS), was developed for the acquisition of metabolite profiles of coffee volatiles. As a first step, an experimental design was applied to find out the most appropriate conditions for the extraction of coffee volatile metabolites by SPME. The selected SPME method was applied in profiling of three different coffee varieties by GC–qMS.
Furan compounds are found to be the most predominant group of volatile metabolites amongst the coffee aromatics. They typically have caramel-like odors since they result from the pyrolysis of sugars. The pyrazines are the second most abundant class of volatile metabolites and contribute to the roasted, walnut, or toast-like flavors in coffee. Along with thiazoles, the pyrazines have the lowest odor threshold and therefore can significantly contribute to the coffee aroma. The pyrroles are responsible for some of the sweet, caramel-like, and mushroom-like aromas in coffee. Conversely, the thiophens, produced from Maillard reactions between sulfur containing amino acids and sugars, are known to have a meaty aroma. Thiazoles, formed via sugar degradation, have an even smaller presence in the overall volatile profile.

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P.51

THE COMPOSITION OF HUNGARIAN SCHNAPPS (PALINKA) RESULTED BY THE AFTER DISTILLATION TREATMENTS

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Palinka is a “Hungaricum” and according to the legal definition it is made from fruits without adding any other source of sugar. The distilled hard drink is very often treated to modify the taste and make it more delightful. This treatment might be storage in barrels – fresh or used for wine fermentation. Palinka has typically strong smell and taste, therefore only fresh barrel treatment is applied. Another post-distillation treatment is the storage and maturation on (dried) fruits – usually the same kind then the palinka is made from. In all cases the composition of components responsible for aroma and taste is modified, namely the volatile components of the barrique barrel enrich the palinka and the non-volatile components (poly-phenols, tannins) add acrid-bitter taste to the fruity base.

Using gas chromatograph-mass spectrometer we could identify some volatile components typical for barrique treatment. The non-volatile components were extracted by selective solid-phase extraction using reversed phase tubes. The component identification was performed via HPLC-MS and it was found that the appearances of several components are dependent on the treatment and the characteristic components for different treatments are listed on the poster. Using the database the origin of the palinka can be determined, and the wood of the storage barrel of the palinka can be identified.

The complex composition of the volatile components required more separation power. Preliminary results of GC x GC experiments, the resolution of overlapping peaks gave the possibility to identify those components, too. Components of some palinkas (for example cherry) may contain nitrogen or sulphur, they can be detected by specific detector.

Additional study was made to determine effect of the adulteration on the composition of palinka. During this process the palinka becomes “smooth”, the chemical composition changes.

P.52

MEDICINAL DRUG COMPONENTS IN HONEY

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Honey is a well known sweetener collected and prepared by bees. The herbal medicines have very often intolerable taste and smell, the intake of them is unpleasant. Apiarists let prepare honey products containing medicinal drugs by bees including the leaves and berries. Our aim was to prove that the active ingredients originated from the herbs are also present in the honey products. We analyzed the volatile components of honey consisting of medicinal plants. The characteristic compounds of plant leaves, berries, and flowers were found in honey; therefore it was obvious that the components responsible for the medicinal effects (if they are not identical with the volatile components) were also transferred to honey. The components from the syrupy and honey samples obtained by SPME were analyzed by GC and GC-MS. The components appearing in almost all of the samples were used as references in the fingerprints while the specific components prove the presence of the active components in the honey product. For fingerprinting capillary GC analyses were used, the component identification was performed by mass spectrometry.

The syrupy samples were prepared from peppermint (*Mentha x piperita*) leaves, stinging nettle (*Urtica dioica*) leaves, wild rose (also known as dog rose) (*Rosa canina*) berries, black elderberry (*Sambucus nigra*) berries, scented mayweed or chamomile (*Matricaria recutita*) sprout, smallflower hairy willowherb (*Epilobium parviflorum*) leaves, common medlar (*Mespilus germanica*) fruits, and garden sage (*Salvia officinalis*) leaves.

Those honey products are commercially available and they are labeled as „TÖBBMINTMÉZ" (MORETHANHONEY).

P.53

MONITORING KEY TEA METABOLITES TO ASSESS CLIMATE VARIABILITY EFFECTS ON TEA QUALITY

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We received funding by the National Science Foundation to study the impact of climate variability on tea management systems, including both natural and human components. Over the last 50 years the East Asian monsoons are arriving earlier and lasting longer in Yunnan, China, with farmers believing these changes have adversely impacted tea quality. With this in mind, we are investigating if changes in rainfall are affecting tea chemistry and if the secondary metabolites are associated with perceived changes in taste and aroma. Initial LC analyses of tea harvested before and after the monsoon onset suggest statistically lower concentrations of catechins (bitter, astringent phenols) are produced by the plant in only five days after the rains begin whereas total phenolic content increased. Automated sequential GC-GC/MS produced libraries of 400 and 1000 small molecule, secondary metabolites from unprocessed (microwaved to stop oxidation) and processed (commercial) tea, respectively. For unprocessed teas, GC-GC/MS analyses were made for samples collected 5-days before and 10-days after the onset of the monsoon. One leaf from five plants per plot was ground and homogenized, replicate samples from two additional plots were also analyzed. Once the libraries were made, spectral deconvolution of GC/MS data showed some metabolites decreased in concentration, others increased in concentration, and some compounds exhibited no statistical change in concentration. Changes in secondary metabolite chemistry occurred in as little as five-days once rains began, which was consistent with LC results and with farmers' perception of time and taste. Findings are from the 2012 harvest; 2013 samples are being analyzed, with 2014 pre-monsoon samples scheduled for the spring. Two sets of pre- and post-monsoon samples must be analyzed: the first, to measure metabolite chemistry as a function of changing environmental conditions and the second, to assess farmer and consumer taste perceptions. Simultaneous distillation-extraction of unprocessed tea leaves was used in the first analysis and for the second, twister extraction from water of brewed tea. The rationale and methodology for making small molecule metabolite libraries will be explained. Illustrative examples of how newly developed spectral deconvolution data analysis software automates the library-building, high throughput screening analysis of target compounds and unknowns in complex mixtures such as tea will also be presented.

EVALUATION OF FILM THICKNESS OF STATIONARY PHASE AND LOADING CAPACITY OF MICROFABRICATED COLUMNS FOR GAS CHROMATOGRAPHY

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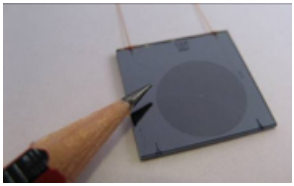
Columns are basics and heart of gas chromatograph. Development of microchip columns is the first steep of micro-GC advances, but the control of fabrication requires an evaluation of physical and chemical properties of micro-columns and presents a real challenge for micro-systems.
For example functionalization protocol (deposit of stationary phase in the micro-channel for micro-system) must be controlled, especially for the sample capacity of the column and for film thickness of stationary phase. Film thickness is well-known for its impact on chromatographic separation and can be evaluated through different ways: by chromatographic tests or by direct way (Scanning Electron Microscopy observations). Standardized chromatographic tests, like Grob test, have been developed for classical capillary column (e.g. columns with cylindrical section, longer and larger than the most classical columns on chip) and we evaluate them for column on chip. Furthermore, evaluation of saturation phase level for some compounds can be used as dedicated chromatographic test for sample capacity evaluation.



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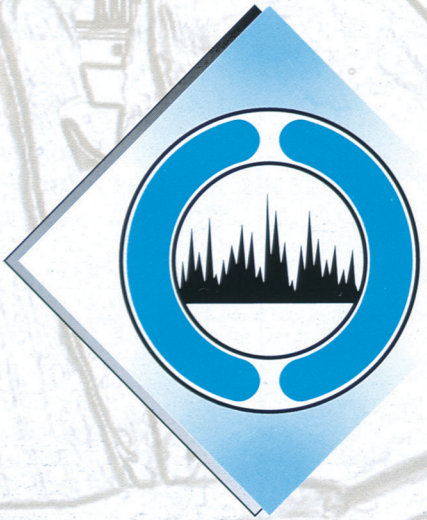

TOGETHER.