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Characterizing Quality

Comprehensive fingerprinting of high-quality food products using GC×GC–TOF-MS

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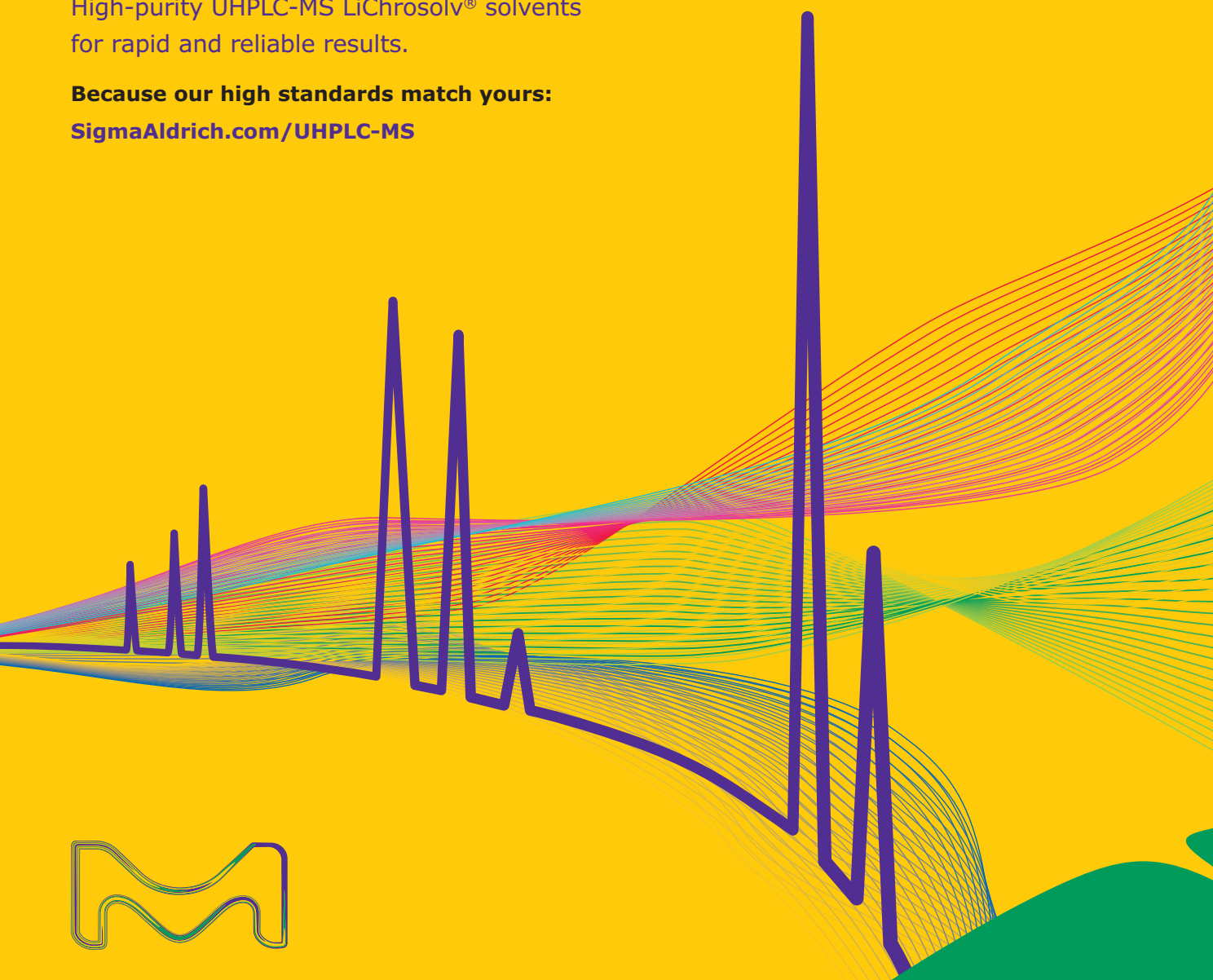

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INTERVIEW

Looking into Lipids

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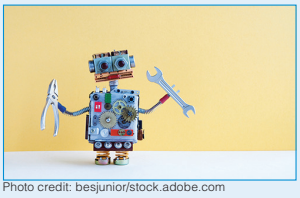
Lipidomics is one of the youngest branches of "omics" research. Maria Fedorova from Leipzig University, in Leipzig, Germany, discusses the latest trends and challenges in lipidomics research and highlights how innovative bioinformatics solutions are addressing data handling issues in this evolving field.

Read Here: http://bit.ly/2DHp2yy

INCOGNITO

The Rise of the Robots



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Incognito thinks that with respect to further enhancements in analytical automation and sample preparation robotics, we either evolve or die! What are your thoughts?

Read Here: http://bit.ly/2Whl8ni

LCGC BLOG

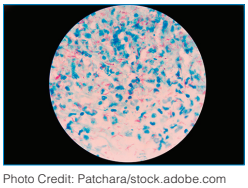
From Petrochem to Cannabis and Beyond: ISCC and GC×GC 2019 is Poised to Please

As we close in on ISCC and GC×GC 2019, my excitement burgeons. All of the groundwork has been laid to provide forums for presenting and discussing the latest advances in capillary and comprehensive separations science.

Read Here: http://bit.ly/2VcPPxF

NEWS

Investigating Mycobacteria FAME Profiles Using Alternative Ionization Energies in GC–MS



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Researchers have investigated alternative ionization energies for the analysis of volatile and semivolatile compounds using GC–EI-MS.

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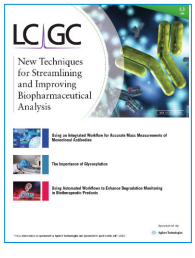
E-BOOK

New Techniques for Streamlining and Improving Biopharmaceutical Analysis



Biopharmaceutical analysis is fraught with numerous challenges, but integrated workflows that make use of automation and smart technology solutions might provide laboratories significant advantages in this area.

Read Here: http://bit.ly/2WeXRm8

WEBCASTS

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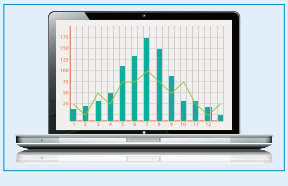

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GC×GC–TOF-MS and Comprehensive Fingerprinting of Volatiles in Food: **Capturing the Signature of Quality**

Federico Stilo[1], Erica Liberto[1], Carlo Bicchi[1], Stephen E. Reichenbach[2,3] and Chiara Cordero[1], [1]Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Turin, Italy, [2]Computer Science and Engineering Department, University of Nebraska, Lincoln, Nebraska, USA, [3]GC Image LLC, Lincoln, Nebraska, USA

Chemical fingerprinting can provide evidence for quality differences resulting from botanical and geographical origins of primary food ingredients, post-harvest practices, production processes (such as traditional versus industrial processes), and the shelf-life evolution of finished products. This article discusses the strategic role and potential of comprehensive two-dimensional gas chromatography (GC×GC) combined with time-of-flight mass spectrometry (TOF-MS) and pattern recognition using template matching for data processing to unravel the quality traits of high-quality food products. Practical examples dealing with high-quality cocoa and extra-virgin olive oil are described.

After the second industrial revolution the need for the standardization of quality for food production led to the introduction of periodic inspections, standardization of procedures, and quality controls (1,2). The International Organization for Standardization (ISO) 9000 is probably the best known modern international standard for quality management (3). Current European Union (EU) policy on food quality aims to protect products characterized by unique features, linked to their geographical origin as well as traditional expertise. With this policy, product names are granted by a geographical indication (GI), a protected geographical indication (PGI), or a protected designation of origin (PDO), if they have specificities strictly linked to the place where they are produced, including the compositional characteristics of raw materials, the climate, and the traditional processes of manufacturing and transformation (4).

An analytical platform or method capable of capturing the chemical traits of a food that are related to its perceived quality—mainly sensory quality, raw material authenticity, and processing impact—will contribute to the quality assessment process while providing a foundation for consumer-tailored strategies to improve a product's acceptance and loyalty.

Chemical fingerprinting can provide evidence for quality differences arising from botanical and geographical origins of primary ingredients, post-harvest practices, production processes (traditional versus industrial), and shelf-life evolution of finished products. Illustrative examples on how comprehensive chemical fingerprinting processes can be strategically valuable for characterizing the quality traits of food from our recent research are highlighted here. This article focuses on the potentials of comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOF-MS) to generate highly informative fingerprints of chemicals from complex fractions, including potent odourants responsible for the aroma signature, and technological markers that indicate the impact of the production process. When complex two-dimensional (2D) patterns of chemicals are explored by dedicated pattern recognition algorithms, a high level of information about sensory profile, product authenticity, and technological impact is revealed (5,6).

The first example deals with high-quality cocoa, a food commodity of global economic interest, and the second example deals with extra-virgin olive oil, an important local commodity in many Mediterranean countries, including Spain, Italy, Greece, and Tunisia. Both of

KEY POINTS

- Comprehensive chemical fingerprinting by GC×GC–TOF-MS can substantiate food quality.
- GC×GC–TOF-MS and advanced pattern recognition algorithms effectively exploit the chemical dimensions of food volatiles.
- Comprehensive two-dimensional gas chromatography applied to volatiles enables access to higher levels of information on food quality.
- GC×GC–TOF-MS unravels the chemical quality traits of high-quality food products.

these commodities have an intrinsic "added value" related to their flavour profile and perceived quality that are 80–90% a result of aroma-active compounds (7).

Experimental Procedures

Chemicals and Samples: The mixture of *n*-alkanes (*n*-C9 to *n*-C25) for calibrating linear retention indices (I^T) was from Sigma-Aldrich. The I^T solution was prepared in cyclohexane at a concentration of 100 mg/L.

Cocoa samples were from Gobino srl and were selected on the basis of their peculiar sensory profile from high-quality productions of different geographic origins. Processing was performed in three replicated batches using time and temperature protocols from 100–130 °C between 20–40 min. Processing was optimized for each origin and driven by a desirable flavour development. Hot-air roasting was conducted in a vertical roaster designed by Bühler AG.

Cocoa samples were frozen in liquid nitrogen immediately after each step of processing and then stored at -80 °C. Before headspace analysis, samples were ground in a laboratory mill up to approximately 300 µm (Grindomix GM200, Retsch); particle size homogeneity was verified by visual inspection.

Extra-virgin olive (EVO) oil samples were collected within the Italian "Violin" Project (Valorization of Italian OLive products through INnovative analytical tools – AGER Fondazioni in rete per la Ricerca Agroalimentare) and selected for their sensory profile by an expert panel. Examples cited in this paper refer to a commercial EVO oil with a PGI quality label (Azienda Agricola Mori Concetta, PGI *Toscano*, olives Mariolo cultivar, San Casciano in Val di Pesa, Firenze, Italy) and a PDO product (Azienda Agricola Leone Sabino, *Don Gioacchino Gran Cru*, DOP Terra di Bari Castel del Monte, 100% Coratina olives cultivar, Canosa di Puglia, Italy).

Headspace Solid-Phase Microextraction (SPME) Devices and Sampling Conditions: A divinylbenzene/carboxen/polydimethylsiloxane 1-cm SPME fibre from Supelco was used for HS-SPME sampling. The standard in-fibre procedure (8) was adopted to preload the IS (α-thujone) onto the fibre before sampling. A 5.0-µL solution of IS (α-thujone at 100 mg/L in diethyl phthalate) was placed into a 20-mL glass vial and subjected to HS-SPME at 50 °C for 5 min. After the IS loading step, the SPME device was exposed to 500 mg of cocoa in a headspace glass vial (20 mL) for 30 min at 50 °C or 100 mg of olive oil in a headspace glass vial (20 mL) for 60 min at 40 °C. Extracted analytes were recovered by thermal desorption of the fibre into the S/SL injection port of the GC system at 250 °C for 5 min.

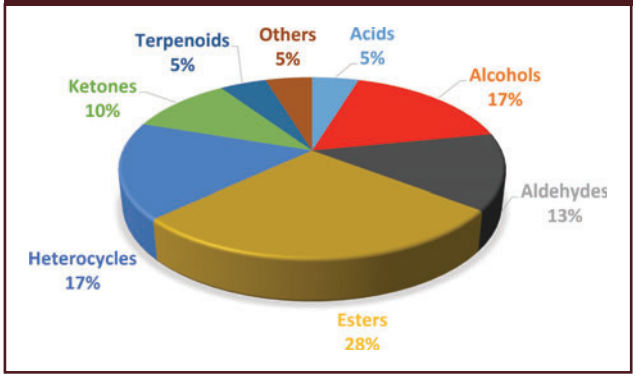
GC×GC–TOF-MS Conditions: GC×GC analyses were performed on an Agilent 7890B GC system coupled with a Bench TOF-Select system (Markes International) featuring Tandem Ionization that provides variable-energy electron ionization. The ion source and transfer line were set at 270 °C. The MS optimization option was set to operate with a mass range between 40–300 *m/z*; data acquisition frequency was 50 Hz for each channel; filament voltage was set at 1.60 V. Electron ionization energies explored were 70 and 12 eV.

The system was equipped with a two-stage KT 2004 loop thermal modulator (Zoex Corporation) cooled with liquid nitrogen controlled by Optimode V.2 (SRA Instruments). The hot jet pulse time was set at 250 ms, modulation period (P_M) was 4 s for cocoa and 3.5 s for olive oil, and cold-jet total flow was progressively reduced with a linear function from 40% of mass flow controller (MFC) at initial conditions to 8% at the end of the run.

GC×GC Columns and Settings: The column set was configured as follows: ^{1}D SolGel-Wax column (100% polyethylene glycol; 30 m × 0.25 mm d_c, 0.25-µm d_f) from SGE Analytical Science coupled with a ^{2}D OV1701 column (86% polydimethylsiloxane, 7% phenyl, 7% cyanopropyl; 2 m × 0.1 mm d_c, 0.10-µm d_f) from J&W (Agilent). SPME thermal desorption into the GC injector port was under the following conditions: split–splitless injector in split mode at 250 °C, split ratio 1:20. The carrier gas was helium at a constant flow of 1.3 mL/min. The oven temperature program was from 40 °C (2 min) to 240 °C at 3.5 °C/min (10 min).

The *n*-alkanes liquid sample solution for I^T_S determination was

Figure 1: Chemical classes distribution within the cocoa volatilome based on the list of targeted analytes reliably identified in the selected samples.



analyzed under the following conditions: split–splitless injector in split mode, split ratio: 1:50, injector temperature: 250 °C, and injection volume: 1 µL.

Raw Data Acquisition, 2D-Data Processing and Statistics: Data were acquired by TOF-DS software (Markes International) and processed using GC Image ver 2.8 (GC Image, LLC). Statistical analysis used XLStat (Addinsoft).

Results and Discussion

Cocoa Origin and its Distinctive Chemical Signature: Cocoa (*Theobroma Cacao* L. Malvaceae family) is the main raw ingredient for chocolate production (9). It is native to tropical forests of the South American continent, although recent statistics indicate that most of the production is concentrated in Africa with Cote d'Ivoire and Ghana covering about 56% of the global production, followed by Indonesia (12%), Nigeria (6%), and Cameroon (6%).

Cocoa and chocolate are considered comfort foods and are consumed worldwide for their pleasant sensory profile of unique and complex flavours. This complexity arises from multiple interconnected biochemical and chemical reactions occurring at post-harvest stage where pedoclimatic conditions and farming practices play a major role (10,11). Later in the processing chain, roasting, conching, and tempering develop the flavour profile and the distinctive sensory signature of chocolate (12–14).

Cocoa's complex aroma is modulated by a series of potent odourants (15–17), whose specific quali-quantitative distribution within the bulk of several hundreds of volatiles has been identified as a distinctive aroma signature also referred to as an *aroma blueprint*. From this perspective, GC×GC–MS would be the analytical technique of choice for an accurate and informative fingerprinting of such a complex fraction. Deeper insights on the quali-quantitative distribution of volatiles would help in delineating an origin-specific aroma blueprint, inform about the seasonal variations or the effect of climate changes, or help chocolate manufacturers in designing tailored blends evoking peculiar aroma notes.

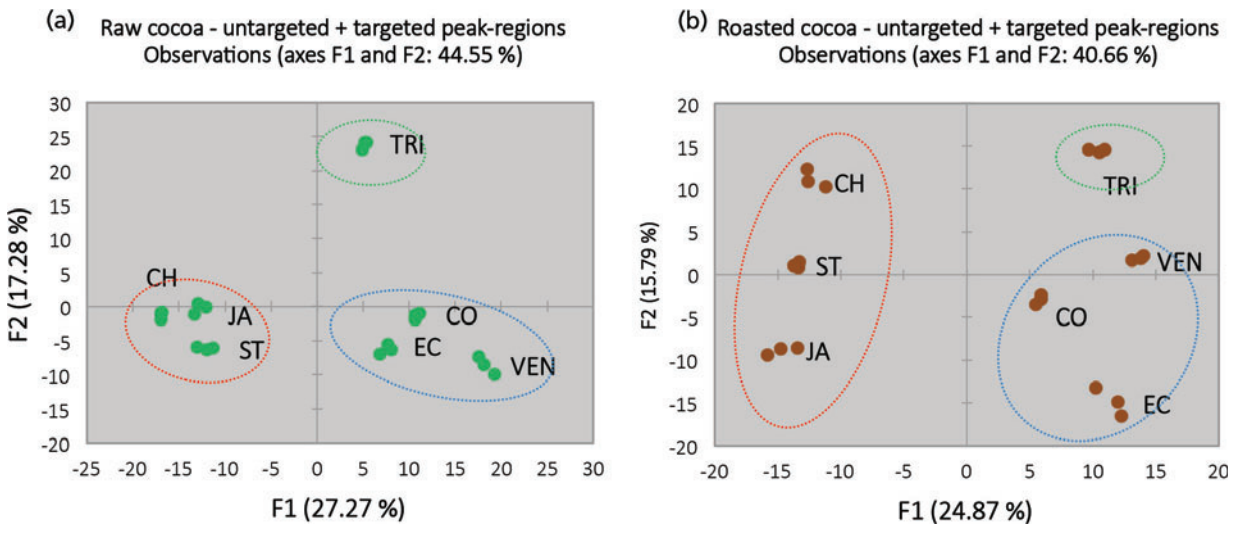
GC×GC–MS exploits the potential of two separation dimensions with the additional orthogonal information provided by MS. This results in: (i) increased separation power, (ii) meaningful 2D patterns with analytes structurally ordered in the chromatographic space, and (iii) enhanced sensitivity as a result of band focusing in space obtained by cryogenic modulation (18–20).

In a recent study, we investigated the unique signatures of volatiles from commercial, high-quality cocoa intermediates, with a pattern recognition strategy based on GC×GC–MS that extends the investigation potential to both untargeted and targeted analytes (21). The approach is based on the template matching principle and is named *untargeted/targeted (UT) fingerprinting* (22,23). Samples were from different geographical provenience (Mexico, Ecuador, Venezuela, Colombia, Java, Trinidad, and Sao Tomè), selected by experts for their unique aroma profiles and were studied along early steps of processing as raw, roasted, steamed, and grinded nibs. For some origins, cocoa liquor was also included to evaluate its aroma signature for chocolate products design.

The fraction of volatiles was extracted by automated headspace solid-phase microextraction (HS-SPME) and on-line analyzed by GC×GC–MS in a system equipped with a loop-type thermal modulator. Within 595 detectable analytes, delineated by unique 2D peak-regions and covering most of the chemical dimensions of cocoa volatilome, about 200 compounds were tentatively identified on the basis of ^{1}D Linear Retention Indexes (I^{T}) and MS spectral similarity with authentic standards or with spectra collected in commercial (Wiley 7n and NIST 2015) and in-house databases (23). Figure 1 shows chemical classes distribution within the cocoa volatilome. Esters dominate the volatile fraction and, together with alcohols and acids, bring information about fermentation and its impact on primary metabolites (mainly sugars and amino acids). Within the heterocycles, the subset of alkyl pyrazines is of great relevance because they provide information about the technological impact on some precursors present in raw cocoa, and for the most odour-active compounds, they bring the earthy/roasty notes to the global aroma. Alkyl pyrazines are formed from the early stages of processing during bean drying and later by roasting and steaming (9). Carbonyls (aldehydes and ketones) are formed mainly from fatty acids precursors by oxidative (chemical and enzymatic) reactions. The sub-group of Streker aldehydes (2- and 3-methylbutanal, methylpropanal, and phenylacetaldehyde) are fundamental for cocoa and chocolate flavour modulating the malty, buttery, and honey-like notes.

The quali-quantitative distribution of known and unknown volatiles, for example, the volatile metabolome fingerprint, is potentially informative, and helps in the discrimination and differentiation of geographical origin and manufacturing stage. As an example, unsupervised multivariate analysis, such as principal component analysis (PCA), can be applied to reveal the natural conformation (groups) of the analyzed samples and helps in localizing informative chemical features responsible for cocoa discrimination. Figures 2(a) and 2(b) show the scores plot resulting from the normalized response of 595 reliable peak-regions from raw (2[a]) and roasted (2[b]) cocoas of different origin (CH-Chontalpa/Mexico,

Figure 2: Scores plot resulting from the normalized response of 595 reliable peak-regions from (a) raw and (b) roasted cocoas of different origin (CH-Chontalpa/Mexico, VEN-Venezuela, CO-Colombia, EC-Ecuador, JA-Java, TRI-Trinidad, ST-Sao Tomè). Adapted with permission from reference 21.

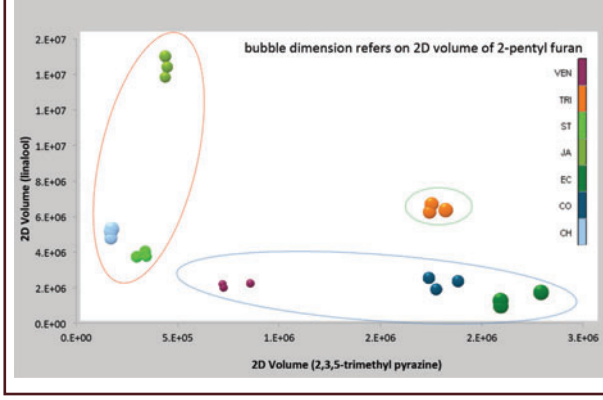


VEN-Venezuela, CO-Colombia, EC-Ecuador, JA-Java, TR-Trinidad, ST-Sao Tomè). Samples are grouped into three main clusters: Ecuador-Venezuela-Colombia (blue circles), Chontalpa/Mexico-Sao Tomè-Java (red circles), and Trinidad (green circles) (21). The total explained variability represented by the first two principal components (F1 and F2) is about 44% for raw and 41% for roasted beans.

Supervised approaches, at this stage, may help in defining or selecting highly discriminating variables. A classification model based on three variables, 2-pentylfuran, 2,3,5-trimethylpyrazine, and linalool, is capable of discriminating between cocoa nib origins. Figure 3 shows how samples could be discriminated in the Cartesian space of three variables: *x*-axis linalool, *y*-axis 2,3,5-trimethylpyrazine, and bubble-size 2-pentylfuran (21). This model confirms what unsupervised exploration by PCA showed (Figure 2[b]). Samples from Ecuador and Colombia are aligned along the *x*-axis (low linalool content) together with the Venezuela samples. Java is characterized by a low pyrazines signature (2,3,5-trimethylpyrazine is one of the most origin sensitive), but clustered together with Chontalpa/Mexico and Sao Tomè for their lower amount of linalool. Trinidad has an intermediate position between the two groups, but with a relatively high amount of 2-pentylfuran and trimethyl pyrazine.

Key aroma compound signatures (15–17) are of particular interest for perceived quality. These signatures are buried within the bulk of the cocoa volatilome, but their information is strategic for the confectionery industry and can be used in new origin selection and blending. Key aroma compounds include alkyl pyrazines (2,3,5-trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, and 3,5-diethyl-2-methylpyrazine), which are responsible for the earthy notes; and short-chain and branched fatty acids (acetic acid, butanoic acid, 2-methylpropanoic acid, and 3-methylbutanoic acid), whose presence at

Figure 3: Samples discrimination based on three variables: *x*-axis linalool, *y*-axis 2,3,5-trimethylpyrazine, and bubble-size 2-pentylfuran selected by regression tree analysis. Adapted with permission from reference 21.

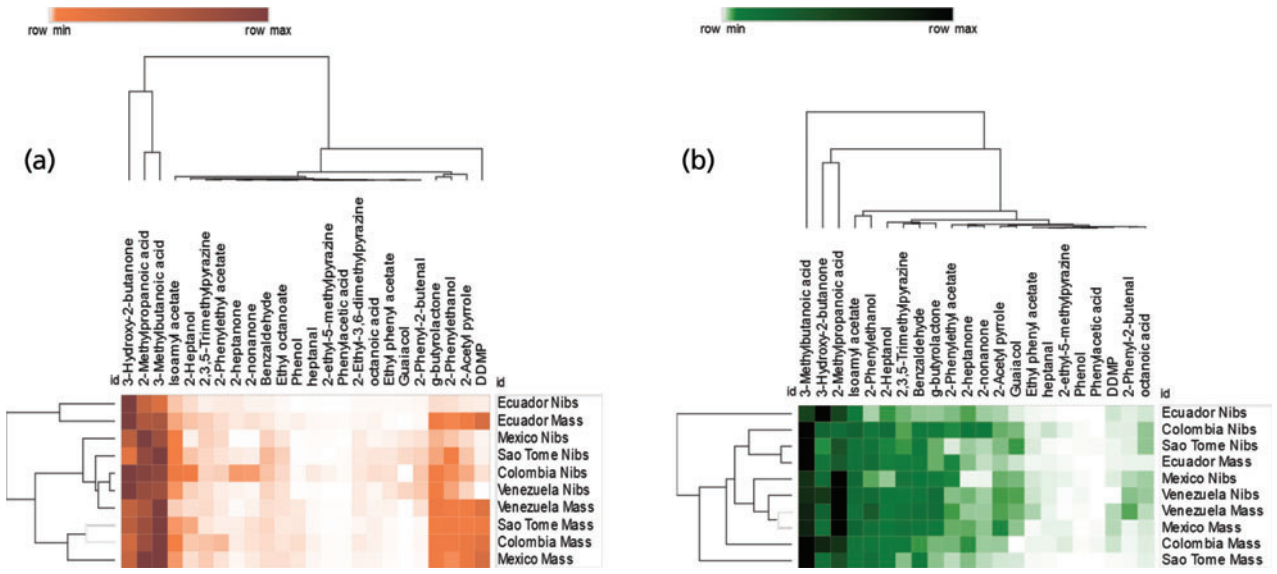


high concentrations can impart off-flavours as a result of their rancid, sour, and sweaty notes. Strecker aldehydes (2- and 3-methylbutanal), formed during fermentation and roasting, impress malty and buttery notes, and phenylacetaldehyde, derived from L-phenylalanine (L-Phe), imparts a pleasant honey-like note.

Other key-aromas are esters (ethyl-2-methylbutanoate: fruity; 2-phenylethyl acetate: flowery), linear alcohols (2-heptanol: citrusy), phenyl propanoids derivatives (2-phenylethanol: flowery), and sulphurous-derived compounds (dimethyl trisulfide).

Accurate quantitation of these analytes, performed by multiple headspace extraction SPME–GC–MS/flame ionization detection (FID) (24), are visualized as a heat map for a subset of origins in Figure 4. Odourants were quantified by external calibration and FID-predicted relative response factors. Amounts are reported for nibs

Figure 4: Heat map representing the quantitation results for a selection of key odourants. Data are expressed as (a) absolute concentration in the sample and (b) by odour activity value (OAV) computed as the coefficient of the concentration of an odourant (mg/kg) versus its odour threshold (mg/kg). Adapted with permission from reference 24.



and cocoa mass in Figure 4(a) and the odour activity value (OAV) is shown in Figure 4(b). OAV is computed as the coefficient of the concentration of an odourant (mg/kg) versus its odour threshold (mg/kg). OAV is a useful parameter for discriminating odourants from interfering components. Below an OAV of 1, which is generally used as a threshold value, it is assumed that an odourant does not play a role in eliciting its characteristic quality. However, several more parameters need to be considered to judge odour activity of volatiles. The capture of chemical complexity of volatile patterns by comprehensive approaches is currently one of the most effective strategies available (25).

Hierarchical clustering based on Euclidean distances helps in sample discrimination based on their aroma profiles. Key odourants, such as 2-methylpropanic acid, 3-methylbutanoic acid, acetoin, and 2-phenylethanol, show a homogeneous trend across all samples. A similar behaviour is seen for other odourants, such as isoamyl acetate, γ-butyrolactone, and 2-acetyl pyrrole, that cluster independently from the others. As expected, 2,3-dihydro-3,5-dihydroxy-6-methyl(4H)-pyran-4-one (DDMP) is an effective marker of processing (21): its concentration in cocoa mass is, on average, two orders of magnitude higher than in cocoa nibs.

To confirm the great flexibility of GC×GC in cocoa volatilome fingerprinting, it is interesting to explore the effect of steaming on roasted cocoa beans with simple and intuitive tools. Steaming is conducted on shelled cocoa beans after roasting. It is an on-line process conducted with overheated-steam that lowers the bacterial charge of the beans. However, steaming impacts cocoa aroma and its process parameters have to be carefully tuned to avoid off-flavour formation or the loss of key odourants. From this perspective, comparative visualization based on datapoint features (26) could be of great help. This is a pointwise approach where chromatograms are compared point-by-point or pixel-by-pixel. In a GC×GC–MS chromatogram, every datapoint corresponds to a detector event. With this approach, each datapoint is a feature and therefore datapoint features at the same retention times are implicitly matched. Figure 5 shows the 2D-patterns from Venezuela cocoa (Figure 5[a] roasted and Figure 5[b] steamed). Their comparative visualization is rendered as colourized fuzzy ratio (Figure 5[c]); within the zoomed area (orange rectangle), the pyrazines signature is visible (Figure 5[d]). This approach gives prompt information on analytes (relative) variations over the patterns and, by colour codes (green, red, and grey), informs about their higher or lower abundance in the samples. In this specific case, steaming has an impact on pyrazines quantitative distribution; those with short alkyl substituents are coloured by green pixels indicating that they were more abundant in the analyzed image (steamed beans). Conversely, red coloured pixels indicate analytes and chromatographic regions where the detector response was higher in the reference (roasted beans) image.

Italian Extra-Virgin Olive Oil: How to Capture Volatiles Chemical Complexity

Consumers appreciate high-quality olive oil, whether virgin (VO) or EVO oil, for its health benefits and pleasant and distinctive flavour. The objective quality and purity of this product is regulated by international organizations, including the EU, International Oil Council (IOC), and Codex Alimentarius. If from one side, EVO oil adulteration can be assessed by characterizing the nonvolatile fraction, for example, fatty acid methyl esters (FAMEs) profiles, sterol and triterpene dialcohol composition, wax content, and presence of conjugated dienes and trienes, the aroma quality can be evaluated by panel testing without any objective analytical protocol supporting the sensory assessment.

Figure 5: 2D-patterns from Venezuela cocoa ([a] roasted and [b] steamed) together with a comparative visualization rendered as colourized fuzzy ratio (c). The zoomed area (orange rectangle) highlights the pyrazines signature (d).

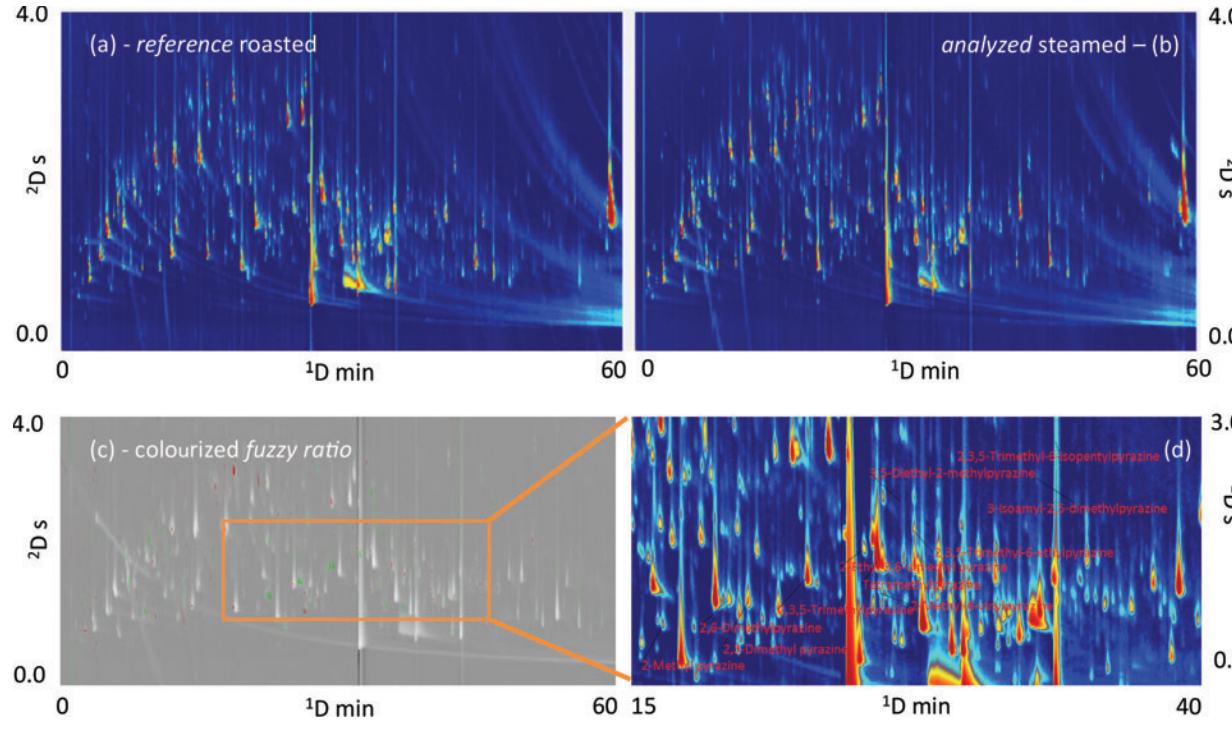
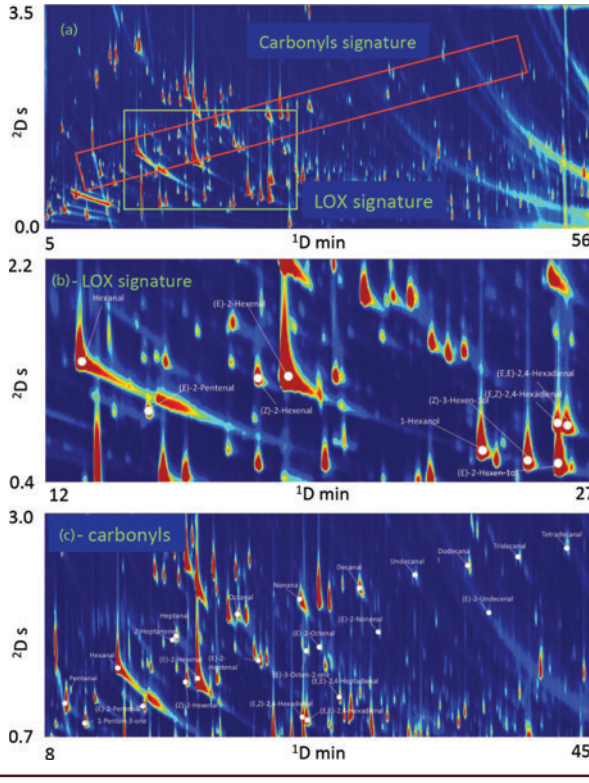


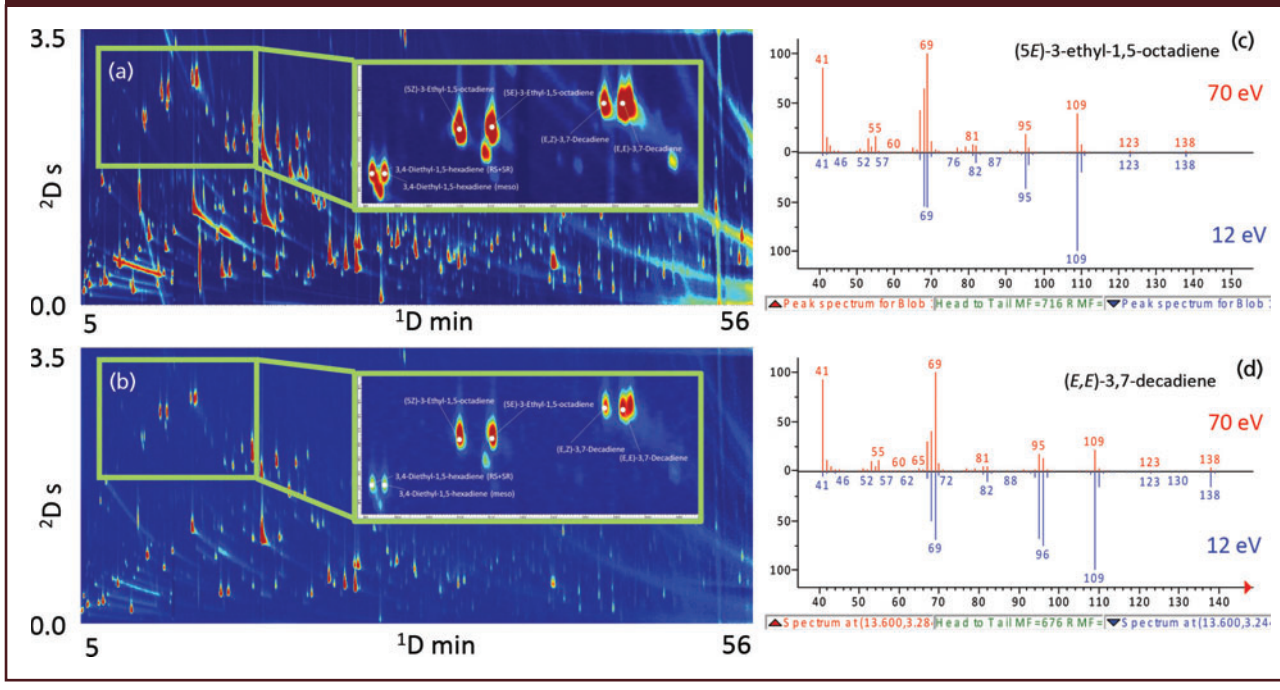
Figure 6: 2D chromatogram of (a) a PGI *Toscano* EVO oil, and in zoomed areas the lipoxygenase (LOX) signature (b), linear saturated and unsaturated aldehydes (c).



Olive oil production is located mainly in Europe with Spain, Italy, and Greece as the key players. Italian production, which is estimated at approximately 50% of Spanish production, is characterized by a relatively high number of PDO oils, with 46 registered products compared with 31 for Spain (European Commission – DOOR database http://ec.europa.eu/agriculture/quality/door/list.html). Quality labelling gives consumers a sense of tradition while improving trust and confidence for a high-quality product. Although these products must comply for higher quality standards compared to ordinary production, the EU Parliament, through the resolution of 14 January 2014 on "food crisis, fraud in the food chain and the control thereof", revealed that olive oil is, among others, one of the top foods subjected to adulteration. Consequently, the EU action plan focused on specific aspects related to quality, control, and promotion to counteract frauds and illicit operations.

Undoubtedly, quality control issues are partially related to the lack of powerful and informative analytical methods capable of supporting objective classification of olive oils, in particular for the distinction between VO and EVO oils based on their aroma signatures. Olive oil is currently the only food product with officially regulated sensory attributes; standardized protocols (27,28) for sensory assessment are established by law and highly trained panellists are involved in this task. Based on the presence or absence and the intensities of coded defects and, on the other side, of "fruity" perception, evaluated by smelling and tasting, virgin olive oil is then classified into three categories: EVO, VO, and lampante oil.

Figure 7: 2D chromatograms of a high quality PDO *Don Gioacchino Gran Cru* Terra di Bari EVO oil acquired at (a) 70 eV and (b) at 12 eV. Spectra for (*5E*)-3-ethyl-1,5-octadiene and (*E,E*)-3,7-decadiene at 70 and 12 eV are reported in (c) and (d).



In this scenario, GC×GC–MS represents an analytical tool with great potential to describe the chemical signature of sensory quality markers, including potent odourants related to oil-coded defects, that is fusty/muddy sediment, musty/humid/earthy, winey/vinegary, or rancid. Previous studies by our research team explored and confirmed the possibility of delineating meaningful patterns of potent odourants eliciting the coded defects in olive oils (5). At the same time, thanks to the separation power and enhanced resolution achievable with a comprehensive 2D-GC analysis, additional chemical information can be collected and rationalized before correlating it with oil sensory attributes, origin (29,30), or ripening status of olives (6).

The volatile fraction of olive oil is complex and connoted by high chemical dimensionality (31), a parameter defined by Giddings to describe the degree of order or disorder in multidimensional separations. The presence of several chemical functionalities, also represented by homologous series, generated by the multiple chemical and biochemical reactions occuring to olives primary metabolites, results in complex 2D patterns that require, for accurate fingerprinting, high-resolution separations and orthogonal detection by mass spectrometry. MS is fundamental here to extract the information on analyte fragmentation patterns for a reliable identification. Figure 6(a) shows the 2D chromatogram of a PGI *Toscano* EVO oil. The number of detectable 2D-peaks with a signal-to-noise ratio (*S/N*) threshold of 100 is about 750 and, for 180 of them, reliable identification was possible by matching $^1D\ I^T$ and MS spectrum with those collected in commercial and in-house databases (5,6). Reference compounds were also adopted in the case of key odourants related to positive attributes or coded defects.

Within the separated volatiles, the lipoxygenase (LOX) signature (Figure 6[b]) is fundamental to define fresh-green and fruity notes, the positive attributes. C6 unsaturated alcohols and aldehydes, (*Z*)-2-hexenal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexenol, (*E*)-2-hexenol, (*E,Z*)-2,4-hexadienal, and (*E,E*)-2,4-hexadienal, are formed from linoleic and linolenic acids oxidative cleavage. Figure 6(c) illustrates the linear saturated and unsaturated aldehydes together with a few ketones likely representing hydroperoxides (that is, primary products of lipid oxidation) cleavage products. This last group of analytes informs generally about shelf-life evolution, with increasing concentrations of potent odourants that bring rancid and fatty notes.

For an accurate and informative fingerprinting of EVO oil volatiles, an additional dimension at the detection level could be of help. Instruments capable of acquiring variable-energy electron ionization spectra by time-switching between two ionization energies across every single analytical run represent an interesting option to extend method dimensionality. Research on variable-energy electron ionization spectra acquisition also showed benefits for the identification of large isomeric species in unresolved complex mixtures (UCMs) of motor oil samples (32) and for light volatile organic compounds (VOCs) from human blood (33). The operation of the ion source at low energies (12–16 eV) allowed enhanced intensity for structure-indicating fragments, which can improve method specificity.

Recently, Freyre *et al.* (34) proposed a tile-based Fisher ratio analysis and a discovery-based investigation strategy to detect exogenous analytes (spiked at 50 ppm) in diesel fuel samples. Their strategy was based on data processing after tandem signals fusion. Our research group explored the cocoa complex volatilome by GC×GC–TOF-MS and variable-energy electron ionization proposing a novel approach based on the UT fingerprinting strategy but performed on fused data streams (hard and soft ionization

energies together) for effective cross-comparative analysis of samples (23).

Tandem data streams are complementary in terms of both spectral information/fragmentation pattern dissimilarity and absolute response. The first characteristic is of help when 70 eV spectra lack molecular ions and structurally informative fragments, while the differential response from the two channels opens new perspectives in terms of dynamic range and linearity. Although at lower energies (12–16 eV), the absolute number of ionized molecules is reduced, benefits are evident for background noise intensity and *S/N*. The latter benefit is accrued for analytes showing a reduced fragmentation at lower energies.

Figure 7 shows the volatile 2D pattern of a high-quality PDO *Don Gioacchino Gran Cru* Terra di Bari EVO oil acquired at 70 eV (Figure 7[a]) and at 12 eV (Figure 7[b]). The enlarged areas correspond to the elution region of unsaturated alkanes characteristic of early stages of olive ripening (35). They include: 3,4-diethyl-1,5-hexadiene (*RS* + *SR*), 3,4-diethyl-1,5-hexadiene (meso), (*5Z*) and (*5E*)-3-ethyl-1,5-octadiene, (*E,Z*)- and (*E,E*)-3,7-decadiene, and (*E*)-4,8-dimethyl-1,3,7-nonatriene (6,35). Spectra for (*5E*)-3-Ethyl-1,5-octadiene and (*E,E*)-3,7-decadiene at 70 and 12 eV are reported in Figures 7(c) and 7(d).

Conclusions

The concept of quality of food is complex and connoted by different meanings. Food compositional complexity offers the opportunity, through informative and reliable analytical protocols, to objectify the most relevant characteristics of quality. Multidimensional analytical platforms, and in particular those implementing comprehensive two-dimensional separations with mass spectrometry, have the intrinsic potential of delineating meaningful chemical fingerprints that can be explored for their targeted and untargeted feature distribution.

To achieve the higher level of information exploiting the quality concepts related to food origin, harvest practices, technological processes impact, and flavour profile, meaningful analyte patterns must be delineated. Data processing tools are therefore fundamental. “Flexibility” is a key characteristic for algorithms and workflows, and data patterns should be explored from different perspectives in relation to the objectives. If the full pattern—for example, the chemical signature—of untargeted and targeted analytes may help in describing chemical differences between samples of different origin, such as cocoa origins characterization, alternatively, datapoint features with visual comparisons may help in locating single analyte variations between sample pairs, such as cocoa processing.

Algorithm flexibility should be accompanied by adequate tools for data alignment, with effective transform functions (36) that support re-alignment of datasets acquired across wide time ranges, in analytical batches affected by random instrumental fluctuations, or

with multidimensional detectors, with variable-energy electron ionization TOF-MS, for example, for EVO oil volatile patterns.

In the scenario of linking meaningful chemical signatures to food quality concepts, the role of data processing software is central. Analysts should drive the exploration of complexity with simple and intuitive tools with an understanding of how data is pre-processed and treated along the steps of the data analysis workflow to fully understand and appropriately use results.

Interestingly, these research efforts have broken down barriers between academic and industrial research, indicating that these analytical tools and data mining concepts are not only academic exercises and speculations, but can also be concrete strategies to improve competitive advantages in a complex food market.

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Federico Stilo is a PhD student at the University of Turin, in Turin, Italy. Federico's project deals with Italian extra-virgin olive oil volatilome with particular emphasis on sensory active volatiles responsible for positive and negative attributes. The toolbox to achieve project objectives includes: comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry, automated headspace sampling, and dedicated data processing tools for effective chemical fingerprinting and profiling.

Erica Liberto is Associate Professor of food chemistry at the University of Turin. Her research interests cover food volatilome and sensometabolome (coffee, cocoa, olive oil, and vegetal matrices) and their chemical characterization by gas chromatography coupled to mass spectrometry, MS-nose platforms, and headspace sampling by miniaturized approaches and devices. Data elaboration by different chemometric approaches is also a field of active research.

Carlo Bicchi is Full Professor of pharmaceutical biology at the University of Turin. He has dedicated most of his research to the development of innovative analytical approaches and techniques, mainly based on gas chromatography, for the characterization of the complex volatile fraction of plants and food of vegetal origin. Research interests also cover the development of new GC stationary phases, and innovative approaches for headspace sampling based on solvent-free and fully automatic solutions.

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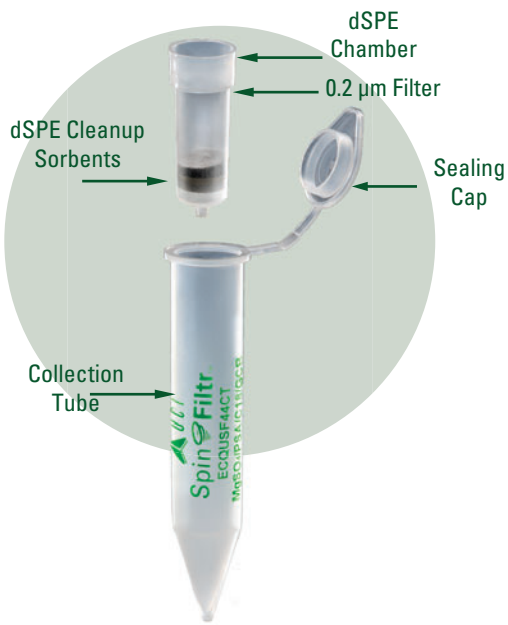

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Methods from Mars? Coping with Chromatographic Legacies

Dwight R.Stoll[1] and Tony Taylor[2], [1]LC Troubleshooting Editor, [2]Crawford Scientific, Strathaven, Scotland, UK

Sometimes, the conditions specified in legacy liquid chromatography (LC) methods seem like they come from a different planet. This month, we look at which conditions to keep, and which ones to let go of.

In method development for liquid chromatography (LC), instead of starting from scratch, there are a variety of possible starting points that we can use to save time. For example, we can look to column and instrument vendor application notes, the peer-reviewed literature, or perhaps methods developed for similar applications by co-workers within our organizations. One particularly common starting point is to use a method developed with objectives similar to our own, and that has been published by the United States Pharmacopeia (USP). *Given the long history of some of these methods, however, simply adopting older methods wholesale may involve the implementation of conditions that not only appear very strange to younger chromatographers, but have also been rendered practically obsolete, given advances in LC technologies over the past few decades. In some highly regulated environments, the user does not have much flexibility to make changes to the established method, even if it is obviously outdated. However, in recent years there has been an increase in flexibility afforded to method developers to adapt to changes in LC technology by widening the scope of allowable changes to existing methods. Making sound decisions about which elements of an older method should be carried forwards in the development of a new method requires a solid understanding of why particular conditions were used in the past, and whether or not they are still needed in light of new developments in LC column and instrument technologies. For this instalment of "LC Troubleshooting" I have asked Tony Taylor of Crawford Scientific to join me in discussing some of the chromatographic legacies he encounters in his day-to-day work in his laboratories and those of his clients.*

Dwight Stoll

Making sound decisions about which elements of an older method should be carried forwards in the development of a new method requires a solid understanding of why particular conditions were used in the past, and whether or not they are still needed.

Triethylamine as a Mobile Phase Additive: Take It or Leave It?

It is very common to find reversed-phase LC methods, most often designed for the analysis of amine-containing bases (for example, benzylamine), involving the use of triethylamine as a mobile-phase additive in the *USP* compendium, in vendor application notes, and in the peer-reviewed literature. For example, the *USP* methods for irbesartan, donepezil, and lamotrigine all call for triethylamine in the mobile phase (1). On the other hand, most users of LC–mass spectrometry (MS) instrumentation would not allow a bottle of triethylamine-containing mobile phase to come near their instruments (there are exceptions to this, but we'll leave that topic for another column). So, how do we reconcile these differences in perspective, and when we consider adapting a method that involves triethylamine, how do we decide whether to leave it in or take it out?

The short answer is that advances in column technology have largely rendered triethylamine obsolete for analyses where it was once considered essential.

A longer answer requires a good understanding of how the characteristics of silicas used for LC stationary phases have evolved over the past couple of decades. In the 1990s, methods were developed for producing porous, spherical silicas for use as stationary phase substrates that have much higher purities compared to previous materials, particularly with respect to metal content (for example, iron and aluminium). This change in the quality of the silica was so pronounced that materials produced by the earlier methods are referred to as "type A", and materials produced using the more modern methods are referred to as "type B" (2). Perhaps the most consequential impact of the metal impurities present in type A silicas is that they significantly lower the acid dissociation constants of

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Figure 1: Illustration of isolated silica surface silanol dissociation.

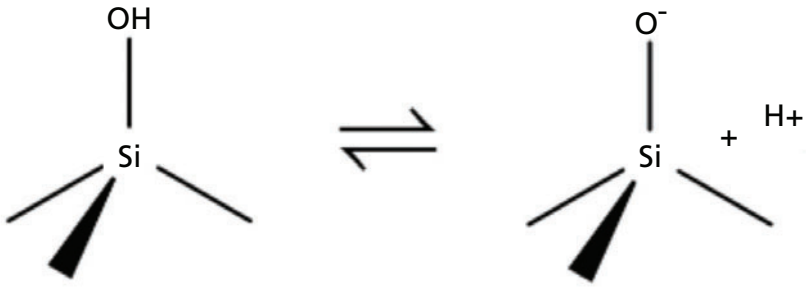
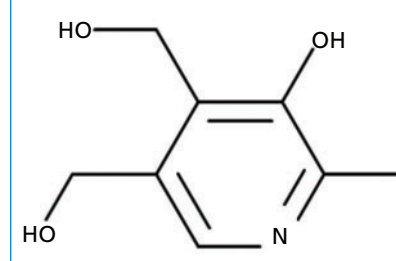


Figure 2: Structure of pyridoxine.



silanol groups on the surface of the silica particle. Figure 1 illustrates the loss of a proton (dissociation) from a surface Si-OH group yielding the negatively charged Si-O⁻ group. With type A silicas starting at around pH 3, the fraction of silanols in the Si-O⁻ form increases significantly (3). Below pH 3, the predominant form will be Si-OH, and above pH 3, the predominant form will be Si-O⁻. Whereas the Si-OH group can interact with analytes through hydrogen bonding interactions, the Si-O⁻ group can interact with positively charged analytes through much stronger electrostatic (charge–charge) interactions. These strong electrostatic interactions can lead to poor peak shape, particularly when there is a relatively small population of these sites on the silica surface, and this usually leads to serious peak tailing (4). In the late 1970s, researchers discovered that adding alkylamines such as triethylamine to reversed-phase LC eluents at concentrations on the order of 50 mM dramatically improved peak shapes for amine-containing analytes (5). Over his years writing this "LC Troubleshooting" column, John Dolan often wrote about the uses of and problems with triethylamine (6). It is understood that, under conditions where the triethylamine is protonated (below about pH 10), the triethylammonium ion interacts strongly with the surface Si-O-⁻ groups, effectively preventing analytes of interest from interacting with these sites and the expected poor peak shapes.

The reduction in metal impurities in type B silicas results in an increase in the pH at which a significant fraction of silanols becomes deprotonated and increases from about 3 to about 7 (3). This means that most surface silanols on type B silicas will be protonated and neutral in mobile phases buffered at pH 6 or lower (for example, even in 0.1% formic acid in water). At this pH, the detrimental electrostatic analyte–silanol interactions described above are avoided. Thus, with many type B reversed-phase columns, excellent separations of amine-containing bases can be obtained with simple acidic mobile phases (for example, phosphoric acid, or ammonium formate adjusted to pH 4) that do not involve triethylamine.

When considering whether or not to continue using triethylamine, we should consider not only whether or not it is actually needed, but also ways that it may create more problems than it solves. For example, triethylamine can act as an ion-pairing reagent for anionic analytes, potentially increasing their retention in surprising ways (see below for more on ion-pairing). In another example, equilibration of reversed-phase columns in mobile phases without triethylamine after the column has been used with triethylamine can be very slow, and lead to significant selecitivity changes over the course of days, or even weeks.

The bottom line is that if your environment requires continued use of a type A reversed-phase column, and the method was developed using triethylamine, then you are probably stuck with triethylamine for the life of the method. But, if you are adapting an older method that used triethylamine, but with a newer type B reversed-phase column, then it is worth taking the time to consider whether or not you really need triethylamine going forwards. If you can get the separation you need with good resolution and peak shape, but without triethylamine, let it go!

Historically, one of the weaknesses of reversed-phase separations has been low retention for hydrophilic compounds, particularly acidic and basic compounds.

Long Chain Ion-Pairing Reagents: Take Them or Leave Them?

Historically, one of the weaknesses of reversed-phase separations has been low retention for hydrophilic compounds, particularly acidic and basic compounds for which retention drops precipitously when the compound is protonated or depronated, and becomes positively or negatively charged. For example, with a C18 column and a mobile phase containing 10% acetonitrile and 90% phosphate buffer at pH 2.4, the retention factor of benzoic acid is about 35. Under these conditions, the acid is protonated and neutral, and not very soluble in the mostly aqueous mobile phase. However, when the pH is raised to 6, benzoic acid deprotonates and becomes negatively charged, increasing the water solubility dramatically. In this case, the retention factor is around 1, a decrease of a factor of 35 compared to the condition where the acid is protonated and neutral (7). In the case of benzoic acid,

there is still enough retention, even in the deprotonated state, to enable separation from other analytes, because the retention is influenced by the lipophilicity of the phenyl group. However, for other molecules where the rest of the molecule other than the ionogenic functional group is hydrophilic, it can be hard to get enough retention to develop a useful separation. Moreover, when analytes contain multiple cationic functional groups, interactions between these groups and the mobile phase may completely dominate retention, overwhelming any retention that might normally arise due to lipophilic parts of the molecule.

In the early days of liquid chromatography, this challenge of low retention for hydrophilic ionogenic compounds led to extensive use of what we refer to as *ion-pairing reagents*. These reagents are things like sulfonic acids (alkylsulfonates) and amines (alkylamines) with long (4 to 18 carbons) alkyl chains. The basic idea here is that the strong electrostatic interaction between the charged functional group of the ion-pairing reagent can form an ion pair with an analyte that has a functional group of the opposite charge. This ion pair has a lower water solubility than the unpaired analyte, which leads to higher retention under reversed-phase conditions. One can also find many examples of methods involving ion-pairing reagents as mobile-phases additives in vendor application notes, the peer-reviewed literature, and *USP* methods. For example, the *USP* method for pyridoxine (also known as *vitamin B6*, shown in Figure 2) calls for the use of hexanesulfonate in the mobile phase buffered at about pH 3 with acetic acid; here, the hexanesulfonate typically would be added to the solvent as a sodium salt. The pK_a associated with the form of pyridoxine with the aromatic nitrogen protonated is about 5.6. This means that below pH 5.6, most molecules in solution will be positively charged, and above pH 5.6 (at least up to about pH 9), most molecules in solution will be neutral.

There is no question that these ion-pairing reagents provide an effective means of getting a "handle" on molecules that would otherwise be hard to retain on most reversed-phase phases without them. However, there are several reasons to avoid long chain alkylsulfonates and alkylamines in the modern chromatography laboratory. First, these ion-pairing reagents tend to be highly retained by reversed-phase phases themselves, which means that they do not instantly "go away" if we change from a mobile phase containing the ion-pairing reagent to one that does not (8). One way of thinking about this is that the ion-pairing reagent partitions into the stationary phase and stays there unless we take specific steps to wash it out. We refer to this as *dynamic modification* of the stationary phase, and, in the case of using an alkylsulfonate, the stationary phase will acquire some degree of negative charge when the ion-pairing reagent partitions into the stationary phase. In other

words, the chemical characteristics of a reversed-phase column will be very different after it has been used with a long chain ion-pairing reagent, compared to when it was brand new. This kind of memory effect motivates many users to dedicate specific columns for use only with mobile phases containing long chain ion-pairing reagents. The second major reason to avoid these reagents in the modern LC laboratory is that they are not very compatible with MS, or at the very least are not MS-friendly. Alkylsulfonates are not volatile, and will quickly contaminate electrospray ionization sources. Long chain alkylamines are reasonably volatile, but are very difficult to remove from mass spectrometers after they have been introduced at the concentrations needed to have a useful effect on reversed-phase retention.

There are several viable alternatives to long chain ion-pairing reagents that can be used to increase retention of hydrophilic analytes.

The good news here is that there are several viable alternatives to long chain ion-pairing reagents that can be used to increase retention of hydrophilic analytes. As with triethylamine as discussed above, if your environment does not allow you to change column chemistries or separation modes, then you are probably stuck with these ion-pairing reagents for the life of the method. However, if you are allowed some flexibility, then it is worth taking the time to consider whether or not continued use of long chain ion-pairing reagents really is warranted.

Alternative #1: Fluorinated Short Chain Ion-Pairing Reagents: Whereas hydrophilic acid mobile phase modifiers, such as phosphoric acid and formic acid, do not ion pair strongly enough with positively charged analytes to significantly increase retention on reversed-phase columns, fluorinated short chain carboxylic acids such as trifluoroacetic (TFA) and heptafluorobutyric acid (HFBA) can increase retention enough to be useful in this regard (9). Trifluoroacetic in particular does not cause any serious memory effects with reversed-phase columns, and is volatile enough to be used with electrospray MS.

Alternative #2: Aqueous-Compatible Reversed-Phase Columns: Most major manufacturers of reversed-phase columns now sell specific chemistries that are advertised as "aqueous compatible" (10,11). Whereas with conventional C18-type stationary phases it is generally advised to avoid more than about 95% water in the mobile phase, these aqueous-compatible phases (commonly referred to as *AQ columns*) can be used in 100% aqueous mobile phases safely and effectively. The ability to go to a 100% aqueous mobile phase provides an avenue to increase retention of very hydrophilic molecules that would otherwise not be retained at all. For example, several small organic acids (such as acetic, tartaric, and succinic acids) can be readily separated using these phases (11). Finally, some phases designed for use with 100% aqueous mobile phases involve polar constituents (for example, polar endcapping ligands and polar embedded groups) that can interact with hydrophilic analytes through hydrogen bonding or electrostatic interactions.

Alternative #3: HILIC Separations: Although reversed-phase separations still dominate the LC separation landscape, users are becoming more comfortable with alternative separation modes, such as hydrophilic interaction liquid chromatography (HILIC) separations, as the community continues to build up an understanding about how and when HILIC works through fundamental research and new applications. HILIC and reversed-phase separations are complementary in many ways, not the least of which is retention for highly hydrophilic molecules. Although HILIC certainly will not replace all reversed-phase separations involving long chain ion-pairing reagents, it is certainly worth a try, particularly in situations where the long chain ion-pairing reagents are clearly undesirable for reasons such as those discussed above.

Tertiary Mobile Phase Mixtures: Take Them or Leave Them?

The final type of method we'll consider here is one that is focused on a very simple separation of one or two components, but involves a tertiary (or quaternary) mobile phase that specifies the use of two different organic solvents. Here again, numerous examples can be found in vendor application notes and *USP* methods. For example, the *USP* assay method for caffeine calls for a mobile phase containing 2.5% (*v/v*) acetonitrile and 2% (*v/v*) tetrahydrofuran (1). Now, it most definitely is the case that blending multiple organic solvents can be incredibly useful for adjusting selectivity in reversed-phase separations of multicomponent analyte mixtures (12). However, in assays that are focused on one or two constituents of the sample, the primary role of the organic solvent component of the mobile phase is retention control, which only requires one type of solvent. In the case of the caffeine method, the acetonitrile and tetrahydrofuran mixture could very easily be replaced with about 5% (*v/v*) acetonitrile, without affecting the assay results in any way. Moreover, this would simplify the method, make it more robust over the long term, and eliminate concerns around the use of tetrahydrofuran related to the potential for peroxide formation.

Summary

In this instalment, we have discussed a few conditions commonly encountered when working with reversed-phase liquid chromatography methods that were established one or more decades ago, including the use of triethylamine and long alkyl chain sulfonates or amines as mobile-phase additives, and the use of organic solvent mixtures as mobile-phase modifiers. Although these approaches were certainly warranted in the past, advances in LC column technology have

lessened the need for them. Understanding the origins of these approaches should help younger separation scientists in particular to decide which of these chromatographic legacies should be carried forwards, and which of them can be let go when adapting methods developed in the past to meet current analytical objectives.

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New Gas Chromatography Products for 2018–2019

John V. Hinshaw, GC Connections Editor

"GC Connections" presents the column's annual review of new developments in the field of gas chromatography (GC) seen at Pittcon and other venues in the past 12 months.

The 70th session of the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) was held 17–21 March 2019 at the Philadelphia Convention Center in Philadelphia, Pennsylvania, USA. As was the case last year, the conference and exhibition were a day shorter than previous meetings. The reduced time added some intensity to the experience, which I certainly felt as I hiked from exhibitions to presentations and back while trying to cover both sides of the conference. There were over 2000 technical presentations this year, given in more than two hundred sessions, including symposia, invited talks, oral sessions, posters, workshops, and award sessions. Attendance at this year's Pittcon was up about ten percent from Orlando last year, with a total of 12,541 from 94 countries, more than 900 of whom attended one or more of 75 Pittcon short courses. The exposition had 713 exhibitors—101 of them for the first time—from 27 countries, in a total of 1234 booths.

The conference celebrated its septuagennial (70th) anniversary with cake and cupcakes for all. I enjoyed some of the other activities in the Pittcon Park Demo Zone, particularly the portable planetarium show that benefitted the Parent Education and Advocacy Leadership (PEAL) Center, raising awareness for autism and other disabilities.

The 2019 *LCGC* Lifetime Achievement in Chromatography Award was presented to Milos Novotny (Indiana University, USA) (1), and the 2019 *LCGC* Emerging Leader in Chromatography Award went to Ken Broeckhoven (Vrije Universiteit Brussel, Belgium) (2).

The next Pittcon is set for a Chicago reunion 1–5 March 2020, at McCormick Place, as in past years in the Windy City. The conference will then circle around to New Orleans for the 2021 event.

The past year saw a significant number of new GC and GC-related product introductions.

This annual "GC Connections" instalment reviews gas chromatography (GC) instrumentation, columns, and accessories shown at this year's Pittcon, or introduced during the previous year. Table 1 shows a list of the companies that introduced new GC products since Pittcon 2018. For reviews of new products in other areas of chromatography, columns, and related accessories, please see the "Column Watch" and "Perspectives in Modern HPLC" columns in the April 2019 issue of *LCGC Europe* (3,4), and the "Sample Prep Perspectives" column in this issue (5).

The information presented here is based on manufacturers' replies to questionnaires, as well as on additional information from manufacturers' press releases, websites, and product literature about the past year's products, and not upon actual use or experience of the author. Every effort has been made to collect accurate information, but because of the preliminary nature of some of the material, *LCGC Europe* cannot be responsible for errors or omissions. This column instalment cannot be considered to be a complete record of all new GC products introduced in the past year, because not all manufacturers chose to respond to the questionnaire, nor is all of the submitted information necessarily included here, because of the limited available space, and the editors' judgment as to its suitability.

Table 1: Companies introducing new GC products for 2017–2018

Company Name
Agilent Technologies
DWK Life Sciences
JEOL
LECO
LNI Swissgas
Phenomenex
Proton OnSite
Shimadzu Scientific Instruments
Teledyne Tekmar
Thermo Fisher Scientific
VICI
Wasson ECE
Waters
YL Instruments

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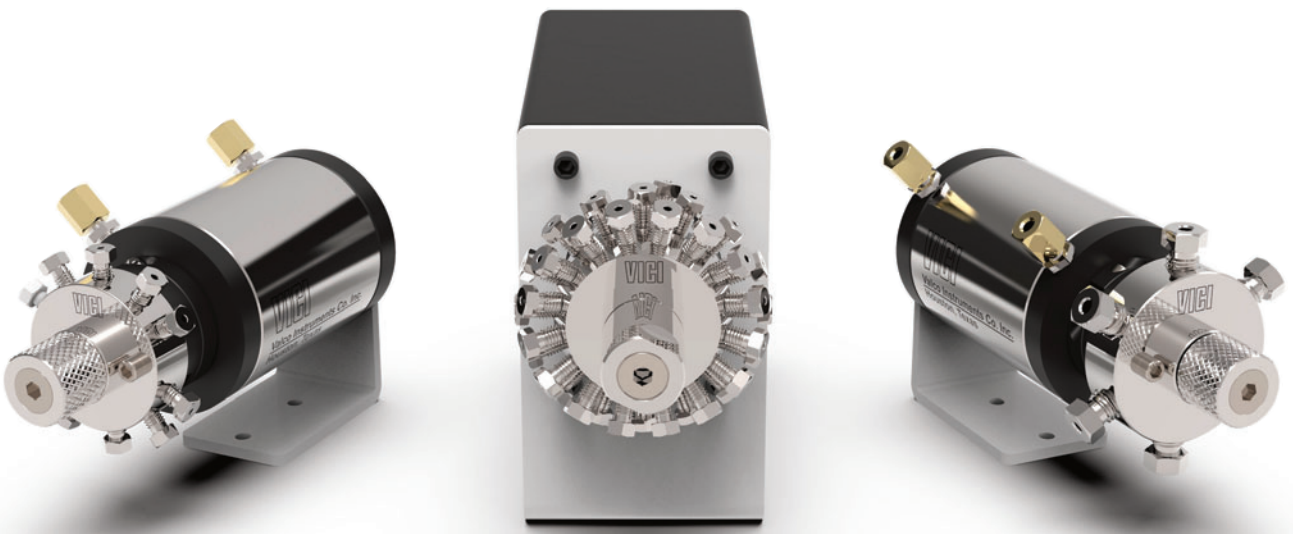

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- Manual, pneumatic, or electric actuation

Table 2: New GC instruments and systems

Company	Product	Description
Agilent Technologies	QuickProbe GC–MS system	Agilent's QuickProbe GC–MS system features a direct-insertion probe for quick determination of the composition of tablets, powders, and liquids, including the presence of controlled substances. It features rapid heating and separation with the QuickProbe inlet and short separation column; confident identification of compounds, including isomers, using electron ionization libraries such as NIST and Wiley; and near instantaneous determination of sample composition at a fraction of the cost compared to similar solutions. The QuickProbe is compatible with Agilent's 5977B GC–MSD (mass-selective detector) and 7000 Series GC–TQ (triple-quadruple mass spectrometer) with the 8890 GC or 7890B GC.
Agilent Technologies	8890 and 8860 GC systems	The Agilent 8860 system is designed to support many core routine applications, while the 8890 system is designed to support flexibility and expandability. Both are based on the technologies of Agilent's Intuvo 9000 GC, which was introduced in 2017. A browser interface providing remote connectivity enables monitoring of the GC system, checks system logs, and performs diagnostics tests, from inside, as well as outside, the laboratory. A full-function touchscreen gives a visual report of the system configuration, allowing method and sequence updates, maintenance routines, and instrument status checks. A built-in autonomous process monitors the health of the system, alerts the user of potential issues before they affect chromatographic performance, and offers step-by-step guides to resolve issues. A unique 6th-generation microchannel-based electronic pressure control (EPC) architecture provides significant improvements in reliability and longevity against gas contaminants, such as particulates, water, and oils, extending the life of the instrument consumables. Agilent's Capillary Flow Technology provides gas flow connections for multidimensional gas chromatography, including GC×GC or Deans switching, comprehensive two-dimensional gas chromatography (GC×GC) with flow modulation, and backflush at the beginning, middle, or end of an analytical column. The instruments support Agilent's single filament thermal conductivity detector (TCD), auto-ranging flame ionization detector (FID), and sulfur or nitrogen chemiluminescence detectors. Electronic pneumatic controllers, such as a helium conservation module, hydrogen sensors, and alternate carrier gas solutions, can significantly reduce the amount of helium used, and offer both flexibility and higher levels of safety in the laboratory.
JEOL	JMS-TQ4000GC triple-quadrupole GC–mass spectrometer	The JMS-TQ4000GC from JEOL is a GC–triple quadrupole mass spectrometer system that features the company's short collision cell technology. It enables high speed data acquisition, without crosstalk, at a selected reaction monitor (SRM) switching speed of up to 1000 channels/s while eliminating ion crosstalk interaction among SRM channels. The system also achieves high sensitivity with ion accumulation, and pulsed ion ejection at its short collision cell, which has both ion accumulation and pulsed ion ejection capabilities. The noise level of the signal can be reduced as much as possible by acquiring data only when an ion pulse arrives at the detector. The system has a mass range of 4 to 1022 Daltons. It is intended for routine analysis, such as residual pesticides analysis in agricultural materials, or monitoring of trace amount of chemicals regulated by tap water quality standards and environmental criteria.
Shimadzu Scientific Instruments	GCMS-QP2020 NX Single Quadrupole Gas Chromatograph-Mass Spectrometer	Shimadzu's GCMS-QP2020 NX single quadrupole gas chromatograph–mass spectrometer features a redesigned ion source for enhanced ionization and transport efficiency, and a large-capacity turbo molecular pump with enhanced exhaust efficiency. The spectrometer can easily switch between electron ionization (EI) and positive chemical ionization (PCI) methods using new Smart-CI source. It employs an advanced scanning speed protocol that minimizes sensitivity loss during high-speed scans. A new automatic flow controller with a central processing unit (CPU) uses various control methods to control carrier gas flow to a constant flow speed, flow rate, or pressure.
Shimadzu Scientific Instruments	GCMS-TQ8040 NX Triple Quadrupole Gas Chromatograph-Mass Spectrometer	The GCMS-TQ8040 NX has a uniform ion source box temperature with a shield that blocks out radiant heat generated from the filament that results in higher sensitivity. A high-accuracy mass filter with pre-rods minimizes quadrupole contamination and eliminates the need for quadrupole maintenance. OFF-AXIS Ion Optics, capable of bending the axis of the Q3 pre-rod, removes meta-stable and neutral ions without sacrificing sensitivity. High-speed multiple reaction monitoring (MRM) can operate at speeds up to 800 transitions per second, and a MRM optimization tool automatically optimizes MRM transitions for new compounds.
Shimadzu Scientific Instruments	GCMS-TQ8050 NX Triple Quadrupole Gas Chromatograph-Mass Spectrometer	The GCMS-TQ8050 NX from Shimadzu has a high-sensitivity detector that enables analysis of femtogram-level concentrations with fewer ions. An overdrive lens in front of the electron multiplier reduces random noise from helium or argon and improves signal-to-noise (*S/N*). The instrument has a uniform ion source box temperature with a shield that blocks out radiant heat generated from the filament and results in higher sensitivity. A high-efficiency collision cell minimizes crosstalk and enables trace analysis. The system uses a large-capacity differential vacuum system to maintain a high vacuum level even during MRM analysis with a collision gas (argon), enabling highly accurate trace analysis. Its high-accuracy mass filter with pre-rods minimizes quadrupole contamination and eliminates the need for quadrupole maintenance. The off-axis ion optics, capable of bending the axis of the Q3 pre-rod, remove meta-stable and neutral ions without sacrificing sensitivity. High-speed MRM can achieve speeds up to 800 transitions per second. Other MRM capabilities include automatic method creation and a MRM optimization tool that automatically optimizes MRM transitions for new compounds.

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Table 2: New GC instruments and systems (continued...)

Company	Product	Description
VICI	Chromatofast Fast GC components	VICI's Chromatofast high-speed capillary GC system incorporates the company's resistively heated fast temperature programmer module with their pulsed-discharge detector (PDD). The system on display separated C5 to C44 hydrocarbons in under a minute. Various fast GC capillary columns are incorporated into column bundles, and heated resistively under control of VICI's fast temperature program modules with a Windows user interface. The column module can be installed in an existing GC system, or built into a custom applied instrument.
Wasson ECE	Eclipse Process GC	Wasson-ECE Instrumentation's Eclipse process gas chromatograph is a high-resolution on-line capillary gas chromatograph that can utilize up to two MicroConvection ovens for programmed temperature control of capillary columns, or two isothermal ovens for packed columns, valving, and detectors. In addition, the system can incorporate one valve-inlet-detector (VID) oven for primary chromatographic valving, inlets, and detectors, heated bridges for controlled sample transfer, and sample panel options for complex sample handling and multiplexing. The Eclipse Process GC incorporates on-line mass-selective detection (MSD), TCD, FID, vacuum-ultraviolet detection (VUV), and pulsed-discharge helium ionization detection (PDHID) capabilities, full electronic pressure programming, and a local touchscreen interface for Wasson-ECE's new chromatography data system. The GC system is rated for hazardous locations, and it supports Modbus RTU, TCP, and ASCII communications.
Waters	Xevo TQ-GC mass spectrometry system	The Waters Xevo TQ-GC is a GC–MS/MS system that allows laboratories to consistently meet and exceed limits of detection when quantifying pesticides residues and other contaminants in food, using GC–MS/MS methods set forth by worldwide regulatory agencies and authorities. The system is designed as a workhorse system for non-MS experts for routine testing where rapid turnaround times are important. It allows laboratories to consistently meet and exceed regulatory detection limits when quantifying trace levels of pesticide residues and other contaminants in food, botanicals, and environmental samples. The Xevo TQ-GC uses a low noise, off axis, long life photomultiplier detector (PMT), and standard electrospray ionization (EI), with optional chemical ionization (CI) sources. The twist lock design of ion source simplifies routine maintenance and fast pump-down time, and enables the system to be returned to an operating state very rapidly. IntelliStart technology automatically performs source tuning, mass resolution, and calibration from a reference compound. The company's Quanpedia database stores and shares user-defined GC multiple reaction monitoring (MRM) acquisition and associated processing methods for the targeted quantification of named compounds. Xevo TQ-GC operates with MassLynx Mass Spectrometry Software and TargetLynx Applications Manager for automated data processing and data quality control checking and reporting.
YL Instruments	YL 6900 GC/MS	The YL 6900 GC/MS from YL Instruments features a scanning speed of up to 20,000 amu/s, a mass range up to 1200 amu, resolution of 0.7 amu, and a detection limit of less than 10 fg of octafluoronaphthalene (OFN). The quadrupole mass spectrometer's GC interface operates at up to 400 °C and has a 250 L/s vacuum pump. A direct-inlet probe option can introduce samples directly into the MS. The 6900 GC/MS incorporates the company's 6500 GC system.

GC: 2018–2019

The past year saw a significant number of new GC and GC-related product introductions. Continuing to be even more active than the previous year, the GC instrument realm gained at least 11 new GC and GC–mass spectrometry (MS) systems in 2018–2019.

Agilent updated its line with the 8860 and 8890 benchtop GC instruments, and added the Quick-Probe direct insertion probe to the 5977B GC/MSD system and the 7000-series GC/Triple-Quad MS system. JEOL introduced their JMS-TQ4000GC triple-quadrupole GC–mass spectrometer, while Shimadzu brought along three GC–MS products: the GCMS-QP2020 NX single quadrupole GC–MS, the GCMS-TQ8040 NX, and the GCMS-TQ8050 NX triple quadrupole GC–MS systems. Rounding out the GC–MS category was the Xevo TQ-GC mass spectrometry system from Waters, and the YL 6900 GC/MS from YL Instruments. In the fast GC realm, Wasson-ECE launched the Eclipse Process GC system with high-resolution capillary GC column capabilities, and VICI continued to expand and enhance its Chromatofast fast GC components with an in-booth demonstration of C_4–C_{44} hydrocarbon separation in under 1 min.

Still developing areas, such as fast capillary GC and GC×GC, continue to attain increased acceptance, while established techniques for regulated methods, such as GC–MS, headspace, and purge-and-trap, have received much attention towards making the methods more reliable, robust, and user-friendly in today's laboratory environments.

Multiple autosamplers and accessories for GC systems appeared this year, including the Teledyne-Tekmar AquaTek LVA water-sample purge-and-trap autosampler, and a new headspace

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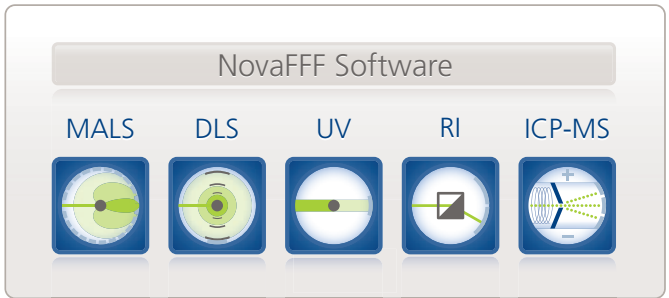

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Table 3: New GC accessories

Company	Product	Description
DWK Life Sciences	Wheaton MicroLiter BlueMAG screw cap	DWK Life Sciences introduced the Wheaton MicroLiter BlueMAG screw cap, a polymer-magnetic hybrid cap designed to address robustness issues with existing all-metal headspace vial caps, or caps with ferrous metal washers. The new screw caps feature a crimped surface feature that provides both a flat, squared surface with respect to the machine automation magnetic geometry, as well as extra ferrous mass, to ensure that even large headspace vials filled with dense sample can be securely transported through the sampling mechanism.
LECO	FLUX GC×GC Flow Modulator	LECO's FLUX flow modulator option for routine GC×GC analysis is a cost-effective option that does not require cryogens to carry out GC×GC. The design comprises a cross shape and a sideways tee fitting, connected together through a simple tube with a fixed gap between the columns. Method development is simplified because there are only two parameters for the user to manage; the flow programming is handled by software. The FLUX flow modulator is available for purchase with the company's Pegasus BT 4D, and also for existing Pegasus BT owners who wish to upgrade their system.
LNI Swissgas	Gas generators	LNI Swissgas laboratory gas generators include hydrogen and zero-air generators in various configurations. The HG Pro, HG ST Pro, HG Rack 2U Pro, and HG Kube are a series of hydrogen generators and combined hydrogen plus zero-air products that provide ultra-high purity proton-exchange membrane hydrogen at flows up to 1500 standard cubic centimetres per min, and air up to 2 litres per min (lpm). The ZA KFID, ZA Total Air, and Rack ZA FID Air generators produce zero air suitable for FID use at maximum flows from 3 to 30 lpm. The units feature an on-board CPU and touchscreen interface.
Proton OnSite	G4800/S20/S40 Hydrogen Lab Server	Proton OnSite's G4800/S20/S40 Hydrogen Lab Servers are large-volume high-purity hydrogen gas generators capable of supplying more than 40 GCs with carrier gas or over 200 GCs for FID alone. Flow rates are available from 4.8 to 18.8 litres per min, at output pressures up to 200 psig (13.8 barg).
Shimadzu Scientific Instruments	SCD-2030 Sulfur Chemiluminescence Detector	Shimadzu's SCD-2030 Sulfur Chemiluminescence Detector (SCD) utilizes a horizontally positioned redox cell. This design ensures an ample reaction time and reaction zone within the cell, which promotes the sample's redox reaction, and achieves long-term stability. The design also simplifies changing the consumable pyro-tube, and allows fast recovery of the instrument after maintenance. An ultrashort flow path results in high peak area sensitivity and signal-to-noise ratio, and new automation functions enable operators to finish preparations, such as gas control, temperature control, and conditioning.
Teledyne Tekmar	AQUATek LVA Waters-only Autosampler	The Teledyne Tekmar AQUATek LVA is a Purge and Trap (P&T) autosampler for samples such as drinking water and wastewater, including USEPA 5030 in conjunction with 502.1, 502.2, 524.2, 524.3, 524.4, 503.1, 601, 602, 603, 624, 8010, 8015, 8020, 8021, 8030, 8240, and 8260 ASTM and Standard Methods Massachusetts VPH and GRO Methods. It incorporates a manifold block design that provides fewer plumbing connections and results in fewer potential leaks. The sampler uses a high temperature deionized-water cleaning technique, with two internal reservoirs that heat blank water to 90 °C for rinsing the entire liquid pathway. It has a stackable configuration that minimizes footprint and saves laboratory bench space, and an 84-position fully insulated sample tray to ensure that samples remain cool.
Thermo Fisher Scientific	Thermo Scientific TriPlus 500 Gas Chromatography Headspace Autosampler	Powered by a completely new design, the Thermo Scientific TriPlus 500 Headspace Autosampler features a capacity for 12 heated vials, valve-and-loop technology, and integrated electronics pressure regulation. Optional sample trays and vial loader increase the capacity to 240 vials. The thermostatted vial oven operates from ambient + 5 °C to 300 °C, and the sample path is coated with SulfiNert. The system can handle hydrogen carrier gas as well as helium, nitrogen, or argon. The autosampler is integrated with the Thermo Scientific TRACE 1300 Series GC.

Table 4: New GC columns

Company	Product	Description
Phenomenex	Zebron™ ZB-614PLUS GC Column	Zebron™ ZB-624PLUS is intended for the analysis of volatile compounds for environmental, pharmaceutical, food, cannabis, and specialty chemicals. EPA methods (501.3, 502.2, 503.1, 524.2, 601, 602, 624, 8010, 8015, 8020, 8021, 8240, 8260), pharmaceuticals, residual solvents, and volatile organic compounds (VOCs). The "Plus" deactivation process enhances peak shape, improves signal-to-noise levels, and increases sensitivity for qualitative and quantitative analysis of active compounds. The columns have low bleed for GC–MS applications and a high temperature limit of 300/320 °C. The columns are available in a wide variety of sizes and film thicknesses, with various combinations from 20–60 m in length, 0.18–0.53-mm inner diameter, and 1–3 µm film thickness.

autosampler from Thermo Fisher Scientific, the TriPlus 500. From DWK Life Sciences, a new headspace vial cap promises to improve reliability with samplers that use magnetic vial transport. The new Flux flow modulator for comprehensive two-dimensional GC (GC×GC) from LECO replaces cryofocusing with fluidic flow switching for the critical sample hold-and-inject function at the column junction. Shimadzu's SCD-2030 sulfur chemiluminescence detector incorporates some innovative improvements, including a horizontal redox cell. In addition, the laboratory gas generator field gained a series of hydrogen generators from Proton OnSite that can serve an entire laboratory's needs, while LNI SwissGas introduced its hydrogen and zero-air generators in both benchtop and rack configurations, with some offerings that include both hydrogen and zero-air generation in the same unit. Finally, the Phenomenex Zebron ZB-614Plus GC column won a Pittcon Today Silver Excellence Award.

GC has seen significant innovations and developments in the past year. Still developing areas, such as fast capillary GC and GC×GC, continue to attain increased acceptance, while established techniques for regulated methods, such as GC–MS, headspace, and purge-and-trap, have received much attention towards making the methods more reliable, robust, and user-friendly in today's laboratory environments. I am looking forward to discovering the next year's worth of innovation and development in this field at the next Pittcon in Chicago.

Acknowledgements

I would like to thank the manufacturers and distributors that kindly furnished the requested information, which allowed a timely report on new product introductions over the past year. For those manufacturers who did not receive a "New Products" questionnaire this year and would like to receive one and be considered for early inclusion into the 2020 new GC and related product introductions review, as well as the other related review articles to be published in *LCGC*, please send the name of the primary company contact plus the mailing and e-mail addresses to Laura Bush, Editorial Director of *LCGC* and *Spectroscopy*, at LBush@MMHGroup.com. The questionnaire will be sent out later in 2019.

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New Sample Preparation **Products and Accessories**

Douglas E. Raynie, Sample Preparation Perspectives Editor

This yearly report on new products introduced since March 2018 covers sample preparation instrumentation, supplies, and accessories.

In late 2018, the *LCGC* editorial staff submitted a survey to vendors of sample preparation products. Responses to this survey are compiled in this review, as are other new product introductions observed during the past 12 months. Note that I, for only the second time since 1990, did not attend Pittcon this year, so the additional information usually gained during the exposition is lacking from this year's review.

New sample preparation technologies introduced in the past year were somewhat passive. Sorbents and accessories for solid-phase extraction (SPE) led the way, as did a multiplatform integration of sample preparation with gas chromatography (GC).

This review is presented in three sections. First, we discuss GC-specific sample preparation platforms and accessories. Next, new solid-phase sorbents and sorbent-based products are presented. Finally, we turn to other sample preparation accessories and supporting technologies. To assist the reader with some of the details behind these new products, each section presents a tabular summary of the associated products. In all cases, the new products we uncovered are presented in the annotated table, while the text highlights particularly noteworthy products.

Gas Chromatography–Specific Sample Preparation

A multiplatform sampling and concentration system for GC, Centri, was introduced by Markes International. A combination of robotics

Figure 1: Showing (a) high-split (50 mL/min) gas chromatographic analysis of off-flavours in wine using immersive adsorption with the HiSorb probe. In the (b) low-split (5 mL/min) bottom chromatogram, the recollected sample is injected with a low split flow to increase sensitivity. (Courtesy of Markes International).

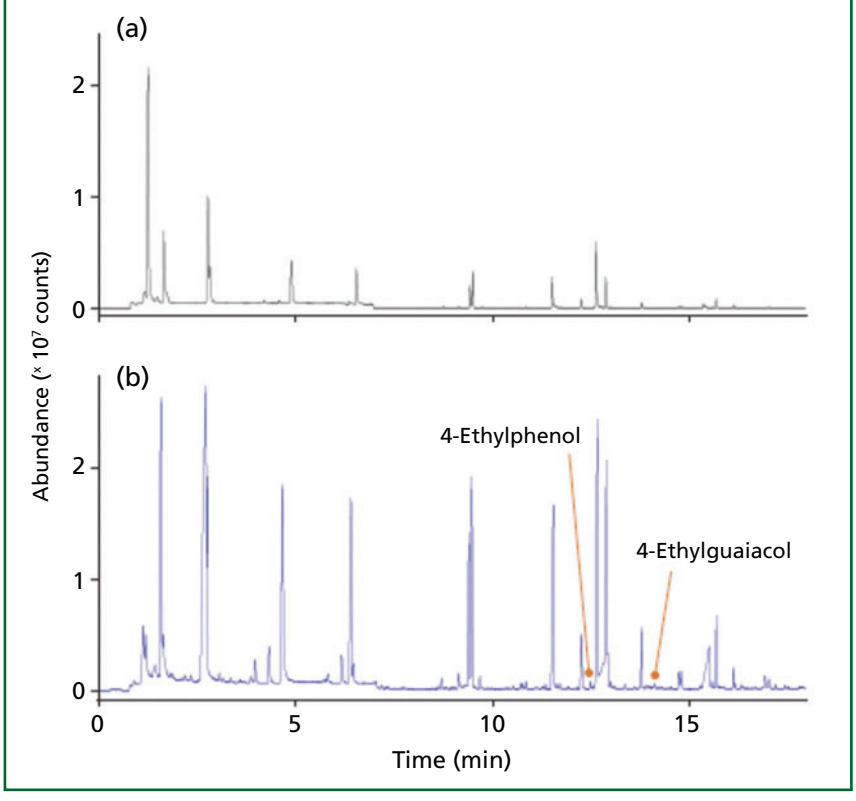


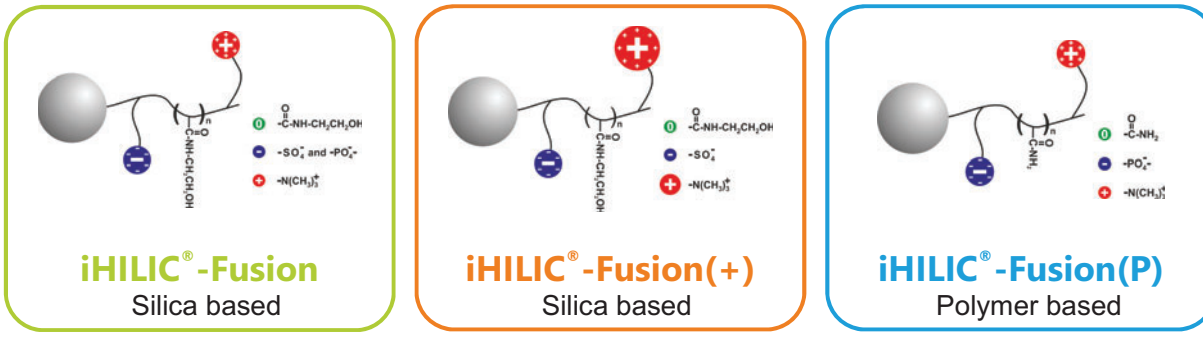
Table 1: Sample preparation instrumentation

Product Name	Supplier	Application	Main Use	Important Feature	Comments
Centri	Markes International www.markes.com	Multiple sampling modes for gas chromatography	GC sampling and sample injection	Recollection trap for enhanced sensitivity	Cryogen and solvent-free operation

HILICON

iHILIC®-Fusion

advances HILIC separations in UHPLC and HPLC

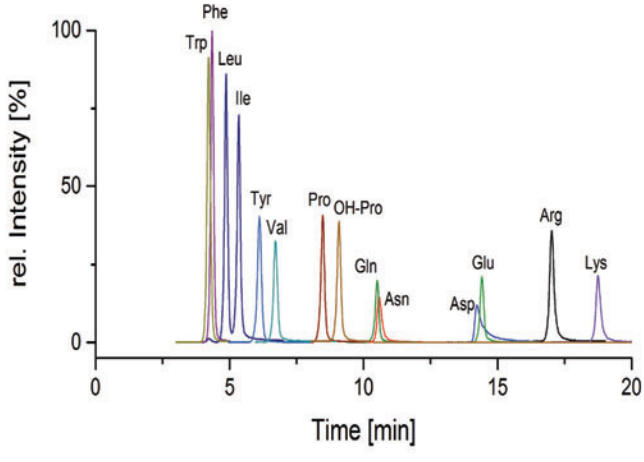


iHILIC®-Fusion
Silica based

iHILIC®-Fusion(+)
Silica based

iHILIC®-Fusion(P)
Polymer based

- Charge modulated hydroxyethyl amide/amide HILIC columns
- Three complementary selectivities for separation of polar compounds
- Excellent durability and ultra-low bleeding for LC-MS
- Versatile columns for "Omics" studies and other applications
- iHILIC®-Fusion and iHILIC®-Fusion(+): pH 2-8; 1.8, 3.5, and 5 µm
- iHILIC®-Fusion(P): pH 1-10; 5 µm



For more information:
Email: info@hilicon.com | Website: www.hilicon.com

Table 2: Solid-phase sorbent products

Product Name	Supplier	Product Type	Mode	Base Material	Functional Group	Dimensions	Comments
Strata-X Drug B Plus	Phenomenex	96-well plate	Strong cation	Polymer	Proprietary	96 wells with 10 mg or 30 mg per well	Sample clean-up of basic drugs of abuse from urine
SiliaPrep PAH	SiliCycle Inc.	SPE cartridge	Dual mode: reversed and ion exchange phases	Silica	Proprietary	Range from 30 mg/1 mL–100 g/276 mL	Extract polycyclic aromatic hydrocarbons from wastewater
bioZen N-Glycan Clean-up	Phenomenex	Microelution 96-well plate	Sample clean-up	Silica	Proprietary	96-well plate	Clean-up of labelled, released n-glycans before liquid chromatography
β-Gone Plus β-Glucuronidase Removal	Phenomenex	96-well plate	Enzymatic clean-up	Proprietary	Proprietary	96-well plate	Drugs of abuse from urine
SiliaFast FaPEx	SiliCycle Inc.	QuEChERS	Dispersive solid-phase extraction	Silica	Proprietary	7.0 × 1.5 cm	Pesticide residue analysis
Strata-X Peptide Screening Microelution 96-Well Plate	Phenomenex	Microelution 96-well plate	Mixed sorbent: weak cation exchange and strong anion exchange	Polymer	Ion exchange	2 mg/well	Small volume peptide extraction
BioPureSPN Graphite	Nest Group	Cartridges, 96-well plates	Adsorption	Graphite	Activated charcoal	20 mg, 50 mg, 100 mg spin columns and plates	Desalting glycans, phosphopeptide enrichment
BioPureSPN HIL-PSA	Nest Group	Cartridges, 96-well plates	HILIC, anion exchange, ERLIC	Silica	Amine	20 mg, 50 mg, 100 mg spin columns and plates	Proteomic fractionation
HisSep Kit	Nest Group	Spin column	Sample clean-up	Proprietary	Proprietary	10–50 mg and 200–500 mg capacities	Histidine removal from antibody formulations
BioPureSPN TARGA C18	Nest Group	Cartridges, 96-well plates	Water-wettable reversed phase	Silica	C18	20 mg, 50 mg, 100 mg spin columns and plates	Desalting
Phthalate-Free SPE Cartridges	Applied Separations	Cartridge SPE	Various	Various	Various	1, 3, 6, 12, 20, 35, and 60 mL	Trace analysis EPA Method 525.2

and analyte trapping is employed to accommodate a variety of sampling modes, including sorptive extraction, headspace sampling, solid-phase microextraction (SPME), and thermal desorption. The Centri's HiSorb sorbent extraction uses a large volume of polydimethylsiloxane adsorbent on a robust metal tip, for robustness and enhanced sensitivity in either the headspace or immersive modes, for the extraction and characterization of volatile and semivolatile organic compounds. Another feature of the Centri is a recollection trap. High split flows can be used in the initial GC separation of volatile samples, to minimize sample overload. The recollected sample may be injected with lower split ratios for high sensitivity, as illustrated in Figure 1. Thermal desorption with the Centri can be in the passive sampling, pumped sampling, dynamic headspace sampling, or direct desorption modes. Additional details of the Centri product are found in Table 1.

Solid-Phase Sorbents and Products

Although solid-phase extraction (SPE) has matured, developments continue in a variety of modalities to improve the ease of method development, application range, and other pending criticisms of the various formats of the technique. This year, the developments seem much more limited than in previous years, and are primarily oriented towards bioanalysis. Phenomenex, SiliCycle, and Nest each

Table 3: Solid-phase extraction and sample preparation accessories

Product Name	Supplier	Application Area	Product Type	Suggested Application	Comments
BioChromato Slit Seal Well Plate Seal	BioChromato	Bioanalysis	96-well plate cover	Used with automated handling of 96-well plates	PET and silicone construction to prevent solvent volatilization
Presston 1000 Positive Pressure Manifold	Phenomenex	SPE	SPE manifold	Processing of 96-well plates for SPE and similar extraction formats	Fully pneumatic positive-pressure manifold for consistent well-to-well sample processing
EZpress 144 EXP Positive Pressure Processor	Orochem Technologies	SPE	SPE manifold	Processing of 96-well plates for SPE and similar extraction formats	Can process up to 48, 96, or 144 cartridges in three separate zones
EZpress 96 EXP Positive Pressure Processor	Orochem Technologies	SPE	SPE manifold	Processing of 96-well plates for SPE and similar extraction formats	Can process up to 48 or 96 cartridges in three separate zones
EquaVap	Analytical Sales & Service	Bioanalysis	Well-plate evaporator	24-, 48-, 54-, and 96-well plates	Equal and consistent drying time among all wells
Smart Evaporator	BioChromato	Sample concentration	Solvent evaporation	Small volume solvent evaporation	Patented technology for use with high boiling point solvents
Pulverisette 11 Knife Mill	Fritsch Milling and Sizing	Particle-size reduction	Knife mill	Range of sample types: moist, oily, fatty, dry, and fibrous	Blending, homogenizing, and SOP capable
Vial Centrifuge	MicroSolv Technology	Chromatography samples	Centrifuge	Small volume centrifugation of GC, LC, and other samples	Used with 12 × 32 mm vials
Milli-Q IQ 7003/05/10/15	MilliporeSigma	Liquid chromatography	Water purification	Preparation of high-purity LC mobile phases	Compact design, mercury-free UV technology, more precise total organic carbon measurement
SolvFil 1000 Nylon Bottletop Solvent Filter	Chrom-Supply	Mobile phase filtration	Solvent filtration	Mobile phase preparation	Design prevents solvents from absorbing oxygen during decanting
MicroLiter Certified ULTRAPure Septa	Wheaton	Autosampler vial caps	11-mm crimp caps	Chromatography samples	Multiple syringe penetration. Minimal siloxane contamination

introduced a family of new sorbents. The range of new sorbent-based product introductions is given in Table 2.

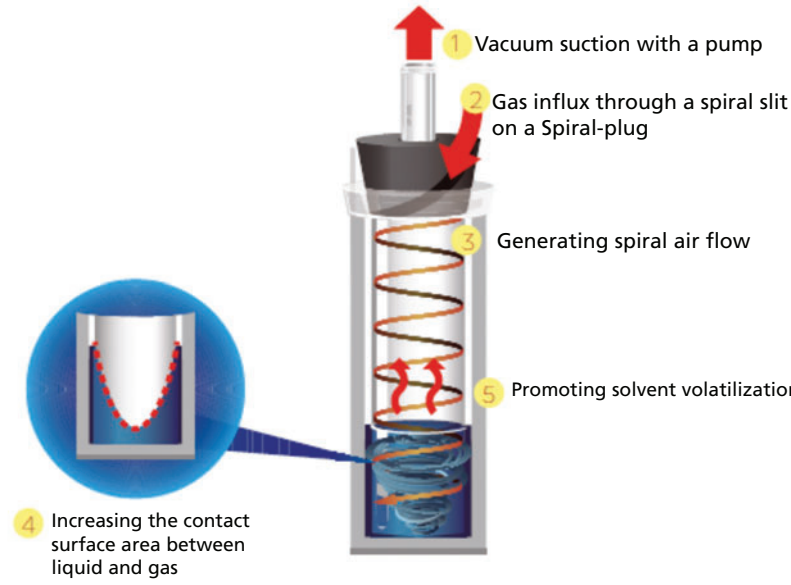
Phenomenex, primarily under the Strata-X family, developed the Strata-X Drug B Plus, bioZen N-Glycan Clean-Up, β-Gone Plus β-Glucuronidase Removal, and Strata-X peptide screening microelution 96-well plate products. The market for these products centres on analysis of drugs of abuse or enzyme hydrolysis. Each of the enzyme-hydrolysis products is in the microelution 96-well plate format, and allow in-well hydrolysis and filtrations. The extractions are cleaner than dilute-and-shoot samples, with low elution volumes, and shorter analysis times.

Meanwhile, the SiliCycle suite of products was developed for the environmental market. The SiliaFast FaPEx is an enhanced QuEChERS-type cartridge for determination of a wide spectrum of pesticide residues in a faster method than more traditional approaches. The SiliaPrep PAH uses a proprietary endcapping with irregular

silica of 40–63 µm, 60 Å, 500 m^2/g 13% C, and a 2.0–10.0 pH range. This exact silica is available in bulk, SPE cartridge, and flash cartridge formats.

Products from the Nest Group are available in either µ-spin cartridge or 96-well plate formats. Desalting is a feature of these products, and the BioPureSPN HIL-PSA product used for proteomic fractionation provides a salt-free first dimension for two-dimensional analysis, and can capture polyphosphorylated peptides. The HisSep Kit is designed for histidine removal from antibody formulations prior to polyacrylamide gel electrophoresis, and features 20–50 or 75–150 µL capacities. A water-wettable reversed-phase offering (BioPureSPN TARGA C18) is capable of loading 100% water to desalt glycans and phosphopeptides. Although novel sorbent phases or chemistries were not developed, Applied Separations launched a phthalate-free SPE cartridge available in a range of sizes that can be packed with any of their sorbents. For those using 96-well plates, BioChromato presented the BioChromato Slit Seal well plate seal, which features a three-layer polyethylene terephthalate and silicone construction for easy syringe penetration and prevention of solvent evaporation.

Figure 2: Diagram of patented vacuum-assisted vortex concentration technology used with BioChromato Smart Evaporator (Courtesy of BioChromato).



Constant, positive-pressure manifolds for SPE processing was the theme of products from Phenomenex and Orochem. Use of positive pressure, instead of vacuum manifolds, allows constant flow across each position in the manifold. The Presston 1000 manifold from Phenomenex has both high- and low-pressure gauges for use at a broad range of pressures. The Orochem EZpress is developed as two products to accommodate up to 96 or 144 one-millilitre cartridges, processed in two or three zones.

Sample Preparation Accessories and Related Products

Sample preparation accessories, including those previously discussed, are summarized in Table 3. Two new solvent evaporation systems were introduced in the past year. The EquaVap series from Analytical Sales & Services provides solvent evaporation from plates of 24, 48, 54, and 96 wells. The blowdown evaporators have an internal flow equalizer for equal output across all needle ports. The BioChromato Smart Evaporator accommodates single sample or up to 10 vials. A patented Spiral Plug technology allows evaporation of dimethylsulfoxide, dimethylformamide, and other high boiling solvents by placing solvents in a vacuum vortex under atmospheric pressure to eliminate bumping. This technology is diagrammed in Figure 2.

Other sample preparation accessories spanned a number of areas. Fritsch introduced the Pulverisette 11 knife mill for use with a range of sample types, from moist, oily, and fatty samples to dry and fibrous samples. The Vial centrifuge from MicroSolv Technology is utilized for small-volume samples associated with chromatography. The next generation Milli-Q system (IQ 7003/05/1015) from MilliporeSigma includes removal of colloids, particles, ions, and free chlorine, reverse osmosis, UV bacteria inactivation, and UV oxidation in a system with a smaller footprint than previous ultra-high purity water purifiers. When preparing mobile phases, a large membrane solvent filtration SolvFil 1000 Nylon Bottletop Solvent Filter is available from Chrom-Supply. A next generation of Wheaton vial caps (Microliter certified UltraPure septa) allows multiple syringe penetration, with minimal siloxane carryover to retard sample evaporation.

Conclusion and Future Directions

The trend, if any, emerging the past year seemed oriented towards bioanalysis, particularly using SPE technology. Whether the trend in coming years is aimed at tool-building or problem-solving for food, environmental, and related analysis, is the question.

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A Clinical **Approach**

Isabelle Kohler from Leiden University, in Leiden, The Netherlands, spoke to *LCGC Europe* about the latest trends in clinical metabolomics using chromatography and how the field is likely to evolve in the future.

Interview by Alasdair Matheson, Editor-in-Chief, *LCGC Europe*

Q. What is the definition of clinical metabolomics and why is it important?

A: Metabolomics can be defined as the comprehensive analysis of all metabolites, which are intermediates and end-point products of the metabolism with a mass lower than 1 kDa, present within a biological system. In clinical metabolomics, the aim is to identify—and ideally quantify—all small molecules present in patient-derived samples. Metabolomics, together with other "-omics" techniques, such as genomics and proteomics, plays a key role in the implementation of personalized medicine in patient healthcare. Indeed, by providing a metabolite "snapshot" (phenotype) at a certain time, the information gathered at the metabolite level may contribute to a better understanding of biomolecular mechanisms involved in (patho)physiological conditions, the possibility for earlier diagnosis of diseases, or the implementation of individualized treatment therapies.

Q. What are the main challenges facing chromatographers involved in this field and how are they being addressed?

A: Even more than in other "-omics" techniques, metabolomics strongly relies on the quality of the obtained data. Indeed, the analytical variability should be lowered to its minimum to extract the relevant biological or clinical differences in the metabolome between the studied groups. It is therefore essential to opt for state-of-the-art and reliable analytical platforms for the analysis of the samples, control the storage and sample handling conditions, and implement multiple quality controls throughout the whole workflow. Many excellent papers have been published to raise the awareness of the community on the importance of high-quality data while also providing some useful tips, including a white paper from the Metabolomics Society Initiative (1) and a recent review from Beger *et al.* showing the importance of quality assurance and quality control in untargeted metabolomics studies (2).

The information gathered at the metabolite level may contribute to a better understanding of biomolecular mechanisms involved in (patho) physiological conditions, the possibility for earlier diagnosis of diseases, or the implementation of individualized treatment therapies.

From an analytical perspective, the two challenges that chromatographers have to face are the need for (i) high-throughput and (ii) high-resolution techniques. In metabolomics, a strong experimental design relies on the number of subjects for each group studied, which has to be high enough to ensure a sufficient statistical power of the study. Large-scale clinical studies therefore involve the analysis of thousands of samples—not including all the additional quality controls—justifying the need for development of high-throughput techniques. There is also a strong trend towards being capable of analyzing more and more metabolites to potentially enable the discovery of novel biomarkers.

The latest release of the Human Metabolome Database (HMDB 4.0), considered the standard metabolomics resource for human metabolic studies, has reported more than 110,000 fully annotated metabolites (3)! This is an incredible number of metabolites to be exploited in the clinic. This also highlights the need to use the latest analytical developments in the field of metabolomics to expand the coverage of the metabolome, and have better access to these potential metabolite biomarker candidates.

Q. Can you tell us more about the role of liquid chromatography–mass spectrometry (LC–MS) and gas chromatography mass spectrometry (GC–MS) in clinical metabolomics?

A: GC–MS was the first separation technique used in metabolomics and still remains very popular because of the excellent separation efficiency that can be obtained. However, it presents some relevant drawbacks, for example, possible loss of thermolabile analytes, cumbersome and time-consuming sample preparation, limited metabolome coverage, and higher variability compared to LC–MS.

The innovative technological developments performed in LC over the last 15 years, are, notably, the advent of sub-2-µm porous particles for ultrahigh-pressure

Photo credit: Original materials: traffic_analyzer/Getty Images

liquid chromatography (UHPLC) and superficially porous particles (core–shell technology) to achieve high-throughput and high-resolution analysis.

LC–MS, mostly UHPLC–MS, is now considered the gold standard in metabolomics. Chromatographers who are not familiar with fast LC analysis should refer to the paper of Fekete *et al.* (4), which also highlights the essential role played by the instrumentation.

Most of the metabolomics applications performed in the last decade were performed using reversed-phase LC, typically using a stationary phase based on C18 chemistry combined with an aqueous-organic mobile phase composed of methanol or acetonitrile with 0.1% formic acid. A very simple and versatile setup, well-adapted for a lot of simple applications but suffering from two major drawbacks, namely (i) a possible ion suppression caused by the presence of coeluting phospholipids (plasma or serum analysis), and (ii) a poor retention of polar compounds. Moreover, metabolites with very similar physicochemical properties (for example, isomers) are not well separated using reversed-phase LC. Improving the metabolome coverage will therefore rely on the use of other approaches more powerful for the analysis of the polar metabolome or the discrimination between closely related compounds, such as supercritical fluid chromatography (SFC), capillary electrophoresis (CE), or two-dimensional (2D-)LC.

Q. Can you discuss in more detail the role that CE, SFC, and comprehensive 2D-LC may play in clinical metabolomics?

A: CE, SFC, and 2D-LC are increasingly considered in clinical metabolomics as complementary approaches to conventional reversed-phase LC–MS. CE–MS, for example, is very interesting because it allows the highly efficient separation of polar and ionized compounds—without the need for large sample volumes. Indeed, only a few nanolitres are required for a CE injection and 2–10 µL of sample in the vial for reproducible injection. CE–MS is therefore particularly well-suited for the analysis of limited sample amounts, for example microfluidics three-dimensional (3D)-cell culture models (limited number of cells), microdialysates, or other samples (plasma, cerebrospinal fluid, urine) collected on small animal models (5). However, only a few applications of CE–MS in large-scale studies have been reported.

SFC has shown a spectacular comeback in the last decade in the field of pharmaceutical analysis, mostly as a result of the impressive technological developments performed in this technique. The advent of the latest generation of SFC instruments has also fostered the development of columns packed with sub-2-µm fully porous (ultrahigh-performance SFC, [UHPSFC]) and sub-3-µm superficially porous particles specially designed for SFC analysis, as well as new interface designs for hyphenating SFC with MS. This metamorphosis of SFC–MS into a powerful analytical technique is also highly beneficial for metabolomics applications. SFC–MS is not only well suited for the analysis of lipids, where it enables the separation of

isomers that are difficult to analyze using other LC-based techniques, but also for polar compounds. Indeed, the state-of-the art instruments allow for a large flexibility in tuning the experimental conditions (for example, addition of acids, bases, salts, or water to the mobile phase; use of different stationary phases). Moreover, excellent kinetic performance can be obtained with (UHP)SFC, similar to (UHP)LC, but at higher mobile phase velocity and with a lower pressure drop compared to (UHP)LC (6). SFC remains little used in metabolomics, but on the basis of recent work carried out in this field by the group of Holčapek and co-workers (7) or Guillarme and colleagues (8), I believe that this will significantly change in the near future.

Last but not least, 2D-LC has also seen a significant breakthrough in the last couple of years that will also benefit metabolomics. Indeed, adding another separation dimension on-line is an excellent strategy to improve the metabolome coverage. Comprehensive 2D-LC (LC×LC), where all the peaks are captured in the first dimension into the second dimension, is very interesting in untargeted approaches to significantly increase the number of features detected. On the other hand, multiple heart-cutting 2D-LC (LC–LC), where one or few fractions are collected in the first dimension and sent to a high-resolution second dimension, is promising to increase the separation between closely related compounds such as isomers. Since a large diversity of chromatographic modes can be combined in 2D-LC, this opens up new perspectives in the field of metabolomics to further expand the metabolome coverage. However, method development for this technique is perceived as complicated for inexperienced users, so this technique is also in its infancy in clinical metabolomics. New users are strongly encouraged to refer to guidelines and tutorials published by experts in the fields, including Pirok and Schoenmakers (9,10), as well as Stoll and co-workers (11,12).

Q. There has also been some interesting work using hydrophilic interaction liquid chromatography (HILIC). Why is HILIC useful in clinical metabolomics?

A: If there is one field where HILIC can prove its usefulness, it is in metabolomics. Indeed, a large variety of polar or ionizable metabolites, such as small organic acids, amino acids, nucleosides, or phosphate derivatives, are very important in multiple (patho) physiological processes, but are not easily analyzed using reversed-phase LC because of poor retention. HILIC is based on a multimodal separation mechanism involving hydrophilic partitioning, dipole-dipole interaction, hydrogen bonds, and electrostatic interaction, which makes it very well-suited for the analysis of polar compounds.

There is also a strong trend towards being capable of analyzing more and more metabolites to potentially enable the discovery of novel biomarkers.

However, HILIC is not as straightforward as reversed-phase LC; reproducible and high-quality data can only be obtained with adequate procedures. Notably, it is well-known that the sample injection diluent and injected volume, the composition of the mobile phase (especially the organic solvent chosen and the composition of the buffer), and the column equilibration time have to be carefully controlled to ensure the reproducibility of the analysis. Inexperienced users are referred to the reviews published by Kohler *et al.* (13,14) and McCalley (15) for discussion of these important parameters and some useful practical recommendations.

Q. It has been reported that chirality can affect the results obtained in clinical metabolomics. Why is that and how is this issue being addressed?

A: Since the thalidomide crisis in the 1960s, we know that two enantiomers from a pharmaceutical racemic mixture may have different pharmaceutical activity and potency. This is also the case for chiral metabolites, which often show different biological activities as a result of, for instance, different receptor affinities. Among the reported examples, we can cite the importance of chirality in the interaction between D-serine (and not the L-form) and the NMDA receptor to modulate synaptic plasticity, playing an important role in depression and neurological diseases; the detection of trace levels of D-amino acids in blood from patients with kidney diseases; or the oncometabolite D-2-hydroxyglutarate, which is produced upon mutations of the enzyme isocitrate dehydrogenase and causes malignant transformation—while L-2-hydroxyglutarate blood levels are not affected. The latter example has been well discussed by Struys (16).

In my opinion, chirality still remains overlooked in current metabolomics applications, but this is probably about to change along with the advent of the innovative technologies mentioned earlier. Indeed, CE, SFC, 2D-LC, and especially ion mobility mass spectrometry have an important role to play in this field because they enable the discrimination between enantiomers (17). I believe that these techniques will be the core of the analytical toolbox used in modern bioanalysis—not only in clinical metabolomics, but also in forensic toxicology, environmental toxicology, and plant metabolomics.

Q. Is clinical metabolomics applied in a routine setting?

A: A lot of small molecule biomarkers are routinely used in the clinic, such as glucose, total cholesterol, lactate, acylcarnitines, urea, or creatinine. However, none of them has been discovered using metabolomics-based approaches. As discussed by Goodacre and colleagues recently, many metabolite biomarkers have been reported in the literature while almost zero have made it to the clinic (18). Metabolomics is now well-established, but the literature is saturated with small-scale preliminary-type studies. The potential biomarker candidates discovered are rarely confirmed in replication studies or validated for clinical use.

Clinical metabolomics is extremely promising in personalized medicine, but it is only by performing large cohort multi-centre studies with adequate experimental design

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www.chromatographyonline.com/LCGCSocialMedia

that possible metabolite biomarker candidates will succeed in being translated into point-of-care or rapid diagnostics. Goodacre and co-workers also mentioned that a large number of publications "claim" to have discovered a biomarker using metabolomics, despite the fact that most of this research failed to acquire sufficient statistical power because of limited sample size (<100 subjects in total) (18). Additionally, the replication of the results using independent cohorts is crucial to increase confidence in the validity of the findings and the clinical utility of the biomarkers discovered.

Q. Your research is often focused on clinical metabolomics in the brain. What are the main analytical challenges in this research, how have you overcome them, and what have been your main findings so far?

A: Studying neurological diseases, such as Alzheimer's disease, Parkinson's disease, or addiction, is very exciting and can have a strong impact, but it comes with a lot of challenges. First, it is difficult to study the core of the disease in affected patients, or only in post-mortem material, which does not provide any information on prodromal phases of such diseases. Animal models are an alternative solution, but many of them are actually not fully representative of the (patho)physiological processes happening in humans. Second, analyzing brain tissues is very challenging, because lipids account for a large proportion of the total metabolite composition. The sample preparation step is therefore essential to extract the metabolites of interest, which makes the method development rather time-consuming. The analysis of cerebrospinal fluid (CSF) seems to be an interesting alternative because it has a direct connection with the brain. However, despite being a relatively simple matrix (compared to plasma–serum), a large number of metabolites of interest are present at very low concentrations, which requires extensive method development and the latest generations of MS instruments to achieve sufficient sensitivity.

We are currently very interested in the role that lipid-based signalling molecules play in neurological disorders, mostly in Alzheimer's disease. We have therefore implemented a method that enables the simultaneous analysis of oxylipins, lysophospholipids, isoprostanes, free fatty acids, bile acids, and endocannabinoids in plasma and tissue samples. We are also currently measuring plasma samples from a large cohort of patients with dementia, mild-cognitive impairment, and Alzheimer's diseases (N > 5000 subjects) with this method. I am very curious about the results.

Clinical metabolomics is extremely promising in personalized medicine, but it is only by performing large cohort multi-centre studies with adequate experimental design that possible metabolite biomarker candidates will succeed in being translated into point-of-care or rapid diagnostics.

Q. What else is your group focusing on at the moment?

A: Our metabolomics facility is currently measuring multiple samples obtained from large-scale studies, in which we also have access to relevant biological information on the genome, transcriptome, and proteome, which gives us the opportunity to integrate this information with metabolomics data. We are also focusing on developing novel approaches to speed up the analysis time, improve the metabolite coverage, adapt current methods to the analysis of samples from microfluidics 3D-cell cultures from on-chip format and other biomass- and volume-limited samples, and improve the separation of exosomes and lipoproteins. All our projects linked to method development actually aim to tackle the two challenges I mentioned earlier, namely, the need for high-throughput and high-resolution analytical techniques.

Q. How will clinical metabolomics evolve in the future?

A: The value of clinical metabolomics in routine practice has not been fully demonstrated yet, but the community believes that the translation of metabolomics to clinics relies on the development of novel point-of-care tests, such as dipstick approaches, breath measurements for volatiles, and electrochemical detection for analytes such as glucose and cholesterol.

Indeed, state-of-the-art LC–MS techniques are very powerful for the discovery of new biomarker candidates, but they are too laborious to be used for large-scale screening of very large populations, or at the general practitioner's. As an aficionada of biohacking techniques, I believe that biosensors used in wearable technologies (for example, smartphones, smart watches, health bands, contact lenses) together with the use of machine learning and artificial intelligence represent the next generation of metabolomics towards real personalized medicine.

Q. What steps need to be taken for clinical metabolomic to evolve faster?

A: Many of the techniques I mentioned earlier, such as CE, HILIC, SFC, and 2D-LC, still remain underexploited in metabolomics—and more generally in bioanalysis. The reluctance in using these techniques is, in my opinion, mostly explained by the lack of basic practical knowledge by inexperienced users. These techniques are indeed not as straightforward as reversed-phase LC, but it is possible to reach similar performance and data quality if the operator is aware of the potential analytical pitfalls, and knows how to prevent or solve them. Therefore, and this is particularly important for me because I am involved in a lot of teaching activities, I would like to encourage all senior scientists or leading experts in such fields to helps inexperienced users by giving short courses and tutorials at conferences, publish guidelines and background review papers, give access to helpful on-line tools, and teach analytical students the state-of-the-art techniques, ideally

alongside practical experience. Today's undergraduate students are our future colleagues; the quality of their knowledge relies on their education.

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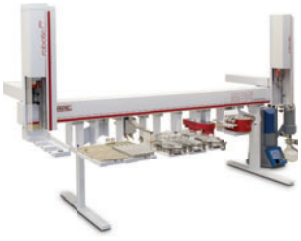
Isabelle Kohler studied pharmacy at the University of Geneva, in Geneva, Switzerland. She carried out her Ph.D. at the School of Pharmaceutical Sciences at the University of Geneva, and obtained her Ph.D. in pharmaceutical sciences in 2013, focusing on the use of capillary electrophoresis hyphenated to mass spectrometry in clinical and forensic toxicology. She moved to the Netherlands for a postdoctoral fellowship at the Leiden University Medical Center, where she investigated the biomolecular mechanisms of familial hemiplegic migraine in a transgenic mouse model using untargeted and targeted metabolomics approaches. She is currently working as Assistant Professor in the group of Analytical Biosciences and Metabolomics at the Leiden Academic Center for Drug Research. Her research interests include clinical metabolomics, bioanalysis, brain metabolism, and neurological diseases, as well as capillary electrophoresis, and hydrophilic interaction chromatography coupled to mass spectrometry.

SFC columns

Available with different selectivities (diol, PFP, C18, hybrid-silica), particle sizes (1.9-, 3-, 5-µm), and dimensions, YMC-Triart columns cover the full range of applications from analytical to (semi)preparative scale. Full SFC compatibility and safety has been officially certified by an independent institute. The well-known YMC reproducibility also comes with its renowned outstanding stability and reliability, according to the company.

https://ymc.de/sfc-columns.html
YMC Europe GmbH, Dinslaken, Germany.

3-MCPD analysis system



An automated GC–MS-based system that determines 3-MCPD, 2-MCPD, and glycidyl fatty acid esters in edible oil, meeting the requirements of standard ISO, AOCS, and DGF methods. Samples are automatically prepared and analyzed, including analyte derivatization and evaporation of excess reagent and solvent for best limits of determination and system stability.

www.gerstel.com
Gerstel GmbH & Co. KG, Mülheim an de Ruhr, Germany.

Flow modulator

LECO has introduced a new Flux flow modulator option for routine GC×GC analysis. While LECO's traditional thermal modulation alternative is still available and provides high sensitivity, the Flux flow modulator is a cost-effective option that makes GC×GC more accessible and easy to use, according to the company. Another advantage of the Flux is that it does not require cryogens to carry out GC×GC, saving the user time and resources in the laboratory.

https://info.leco.com/flux-lcgc
LECO Corporation, Saint Joseph, Michigan, USA.

Dextran GPC/SEC calibration kit

ReadyCals allows for the rapid preparation of reproducible GPC/SEC calibration curves without the inconvenience of individually weighing the standards, according to the company. New in the PSS ReadyCal family are the Dextran ReadyCals: reference material: dextran, nine different standards with Mp 180–298,000 Da; application: calibration of aqueous GPC/SEC systems; set contains 3 × 5 vials 1.5-mL for five calibrations.

www.pss-shop.com
PSS GmbH, Mainz, Germany.

UHPLC system

Shimadzu has released the Nexera Ultra High-Performance Liquid Chromatograph series, incorporating artificial intelligence as Analytical Intelligence, allowing systems to detect and resolve issues automatically. According to the company, the Nexera series makes laboratory management simple by integrating IoT and device networking, enabling users to review instrument status, and optimize resource allocation.

www.shimadzu.eu
Shimadzu Europa GmbH, Duisburg, Germany.

HILIC columns

Hilicon offers a broad range of hydrophilic interaction liquid chromatography (HILIC) products to separate polar compounds. Three column chemistries in UHPLC and HPLC, iHILIC-Fusion, iHILIC-Fusion(+), and iHILIC-Fusion(P), provide customized and complementary selectivity, excellent durability, and very low column bleeding, according to the company. The columns are suitable for the LC–MS analysis of polar compounds in "omics" research, food and beverage analysis, pharmaceutical discovery, and clinical diagnostics.

www.hilicon.com
Hilicon AB, Umeå, Sweden.

Dioxin analyzer

The Thermo Scientific Dioxin Analyzer is a new gas chromatography–mass spectrometry-based workflow, designed to address the high cost and complexity of testing food and animal feeds for dioxins and polychlorinated biphenyls. The validated workflow provides a full confirmatory method that complies with European Union regulations aimed at controlling the maximum levels of these contaminants in the food chain.
www.thermofisher.com/dioxin-analyzer
Thermo Fisher Scientific, Sunnyvale, California, USA.

MALS detector

Introducing the next generation DAWN multi-angle static light scattering (MALS) detector for absolute characterization of the molar mass and size of macromolecules and nanoparticles in solution. DAWN offers high sensitivity, a wide range of molecular weight, size, and concentration, and a large selection of configurations and optional modules for enhanced capabilities.
www.wyatt.com/dawn
Wyatt Technology, Santa Barbara, California, USA.

Microchip column

µPAC is PharmaFluidics' chip-based chromatography column for nano-liquid chromatography. According to the company, perfect order in the separation bed is achieved by etching a regular pattern of pillars into a silicon wafer using micromachining technology. The column allows high-resolution separation of tiny, complex biological samples, with an unprecedented robustness. µPAC is suitable for lipidomic, metabolomic, and peptide profiling.
www.pharmafluidics.com
PharmaFluidics, Ghent, Belgium.

FID gas station

The VICI FID gas station combines the reliability of the VICI DBS hydrogen and zero-air generators into one compact and convenient package, according to the company. Available in high and ultrahigh purity for all GC detector and carrier gas applications. The generator is available in two styles: flat for placement under a GC, or the Tower. Available in H_2 flow ranges up to 1 L/min and 10.5 bar.
www.vicidbs.com
VICI AG International, Schenkon, Switzerland.

LC accessories

Restek has expanded the company's line of liquid chromatography accessories for chromatographers. High-quality couplers, fittings, unions, tees, and crosses; PEEK and stainless steel tubing; mobile phase maintenance and safety products, including bottle tops, valves, filters, and spargers are now available.
www.restek.com/LCacc
Restek Corporation, Bellefonte, Pennsylvania, USA.

QuEChERS mixes

Within a few years of its development by Anastassiades *et al.*, the "QuEChERS" method has gained a leading position for the determination of pesticide residues in food samples by GC–MS or LC–MS, allowing "quick, easy, cheap, effective, rugged and safe" clean-up of strongly matrix-contaminated samples. Macherey-Nagel offers a selection of pre-weighed Chromabond QuEChERS mixes according to EN 15662 as well as to AOAC 2007.01 method.
www.mn-net.com
Macherey-Nagel GmbH & Co. KG, Düren, Germany.

LC columns

Based on a sulfonated, cross-linked styrene-divinylbenzene copolymer, Eurokat columns are available in several ionic forms. One advantage of this particular cation exchanger is the application of organic, solvent-free methods. According to the company, the columns are also eco-friendly, as well as cost-efficient during application, and very long-lasting. They are suitable for the analysis of sugars, organic acids, and alcohols.
www.knauer.net/columns
Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany.

Nitrogen generator

Genius XE Nitrogen is a cutting-edge nitrogen generator combining advanced technology with refined and robust engineering, according to the company. Two model options are available: XE 35 with up to 35 L/min and XE 70 with up to 70 L/min. The generator reportedly provides a premium standalone nitrogen solution for high performance LC–MS and other mission-critical laboratory applications where performance and reliability are paramount.
www.peakscientific.com/genius
Peak Scientific, Scotland, UK.

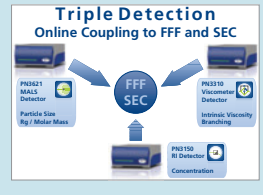
Electrochemical detector

The Decade Elite from Antec Scientific is designed as an easy-to-use electrochemical detector that can integrate with any LC system on the market, according to the company. The system can reportedly handle fast eluting peaks in (U)HPLC and deliver fast stabilization from dedicated flow cells. When used with the SenCell, the system is a highly sensitive electrochemical detector.
www.AntecScientific.com
Antec Scientific, Zoeterwoude, The Netherlands.

Crimping and decapping

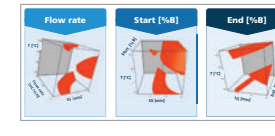
The CR-1000 is a crimping and decapping machine for all types and sizes of vials and caps. The force is adjustable according to the type of vials, caps, and septum. According to the company, the machine is simple to use, offers reproducible crimping, and is suitable in any type of room and for any power supply socket. The CR-1000 reportedly provides an average rate of 400 vials/h. The company are looking for partnership in Europe .
www.sertir.fr
Action Europe, Sausheim, France.

Triple detection



Postnova has introduced the Triple Detection for thermal field-flow fractionation (FFF) and GPC/SEC. Triple Detection is the combination of multi-angle light scattering (MALS), viscosity detection, refractive index detection, and UV detection. In a single separation experiment, Triple Detection provides molar mass distribution, molecular size distribution, and molecular structure (branching, composition) of polymers, biopolymers, polysaccharides, proteins, and antibodies.
www.postnova.com
Postnova Analytics GmbH, Landberg, Germany.

Method modelling software



Molnár-Institute's DryLab software has a 35-year history in scientific method modelling. Using a DoE of 12 input runs, the software integrates the theory of solvophobic interactions and linear solvent strength (LSS) to predict the movements of peaks, selectivity changes, and retention times of any multidimensional design space. The software's automation module creates method sets in the most economic and ecologic order, executes runs, and acquires results from the CDS. Mass and other integrated data are retrieved and ambiguity in peak tracking is reduced to a minimum.
www.molnar-institute.com
Molnár-Institute, Berlin, Germany.

Volumetric dispenser

A one volumetric precision dispenser for aggressive liquids and solvents, the dispenser has a programmable volume from 10–1100 mL and can be repeated to pump several litres in portions or repeatedly fill vessels. The process is operated using a touch panel. No computer is required, the user can type in the desired volume and it will withdraw the volume.

www.biotechfluidics.com/products/pumps/volumetric-precision-dispenser/
Biotech AB, Onsala, Sweden.

Chromatography software

DataApex has launched a new version of Clarity Chromatography software. Clarity version 8 comes with a graphically enhanced user interface, improvements in MS and GLP options, and new control modules. According to the company, Clarity brings easy operation, user support, and optional extensions for various applications, such as PDA, MS, GPC, NGA, and many more. A free demo is available from DataApex's website.

www.dataapex.com
DataApex, Prague, Czech Republic.

Sample automation

Markes' new Centri multitechnique platform is an advance in sample automation and concentration for GC–MS, according to the company, and offers four sampling modes: HiSorb high-capacity sorptive extraction, headspace, SPME, and thermal desorption. The company reports analyte focusing allows increased sensitivity in all modes, state-of the-art robotics increases sample throughput, and sample re-collection allows repeat analysis without having to repeat lengthy sample extraction procedures.

http://chem.markes.com/Centri
Markes International Ltd., Llantrisant, UK.

Metabolomics 2019

Metabolomics 2019, the Jubilee 15th International Conference of the Metabolomics Society, will be held in **The Hague, The Netherlands**, from **23–27 June 2019**. The conference will be held at the **World Forum Conference Centre**, located in the Hague, and an hour from Schiphol Airport and Rotterdam The Hague Airport. The Netherlands has a strong academic and industrial tradition in metabolomics. To ensure this year's conference is a major success, the Metabolomics Society will work with other European metabolomics associations to highlight the applications of metabolomics in industry and biological research.

The main conference days will include four parallel tracks, one exclusively on **biomedical applications**, one exclusively on **plant, food, environmental, and microbial applications**, and one on **technology**. In the technology track, new analytical methods and technologies will be discussed; one hot topic this year is metabolite identification. The fourth track is a new addition and will focus on **new frontiers**. This track will include presentations highlighting new developments that may impact the field of metabolomics, such as regulatory aspects, novel technologies, including artificial intelligence, deep learning, and single-cell metabolomics, and novel applications, such as stem cells, organoids, and organ-on-chips. In addition, attendees can expect to hear about many exciting applications of metabolomics in the field of biomedical and clinical applications, including the gut microbiome, and nutrition and plant research.

There will be 18 pre-conference workshops on Sunday afternoon and Monday before the conference starts, covering all aspects and application areas of metabolomic research, which will be of great educational value for early career scientists and students.

Another novel segment of this year's conference is two day-long workshops on data acquisition (Sunday) and data (pre-)processing and biostatistics (Monday) for newcomers to the field.

Sunday evening will also feature another new element: career night. This is the ideal opportunity for early career researchers to prepare for the next career step by meeting research groups, research organizations, and companies with job openings. In addition, round table discussions on career-related topics ranging from networking to work-life balance will be organized.

Before the conference two (independent) satellite meetings will be organized: on Saturday 22 June "Metabolomics and Epidemiology" and on Sunday morning "Metabolomics Enabling Tools for Large Studies and Biobank Initiatives".

The conference is the official meeting of the Metabolomics Society, and the largest metabolomics meeting worldwide. The Metabolomics Society expects this event to attract metabolomics researchers from around the world, with an anticipated 1000–1200 visitors, and over 800 registrations already received. As an added bonus to registrants, the conference dinner, which is being held at a beach club, is included in the registration fee.

The conference will also have exceptionally good exhibitor opportunities with an "all on one floor" concept featured in the conference layout; all meeting rooms and exhibitor space are on one floor, thereby providing optimal flow of visitors to the exhibitor booths.

The organizers look forward to welcoming you to The Hague this June.

E-mail: info@metabolomics2019.org **Website:** www.metabolomics2019.org

Image credit: Photocreo Bednarek/stock.adobe.com/

23–23 May 2019
3rd International Conference and Exhibition on Petroleum, Refining, and Environmental Technologies (PEFTEC 2019)
Rotterdam, The Netherlands
E-mail: info@ilmexhibitions.com
Website: www.ilmexhibitions.com/peftec/

11–12 June 2019
5th International Workshop on Electrochemistry–Mass Spectrometry (ELCHEMS^5 2019)
University of Münster, Münster, Germany.
E-mail: ElCheMS2019@wwu.de
Website: www.uni-muenster.de/Chemie.ac/en/karst/workshops/elchems.html

16–20 June 2019
48th International Symposium of High Performance Liquid Phase Separations and Related Techniques (HPLC 2019)
Milano-Bicocca University, Milan, Italy
E-mail: hplc2019@effetti.it
Website: www.hplc2019-milan.org

17–18 June 2019
Analytical Quality by Design
Naarden-Bussum, The Netherlands
E-mail: info@kantisto.nl
Website: www.kantisto.nl/index.php/agenda/30-agenda-items/38-analytical-quality-by-design

18–20 June 2019
LABWorld China 2019
SNIEC, Shanghai, China
E-mail: salesoperations@ubm.com
Website: www.pmecchina.com/labworld/en

7–10 July 2019
International Symposium on Preparative and Process Chromatography (PREP 2019)
Baltimore, USA
E-mail: janet@barrconferences.com
Website: www.prepsymposium.org

15–18 September 2019
The 30th International Symposium on Pharmaceutical and Biomedical Analysis (PBA 2019)
Tel Aviv, Israel
E-mail: bioforum@bioforum.co.il
Website: www.pba2019.org

Please send any upcoming event information to Lewis Botcherby at lbotcherby@mmhgroup.com

Modern Mobile Phase Design for **HPLC**

In this excerpt from *LCGC Europe*'s e-learning platform, CHROMacademy.com, mobile phase design for high performance liquid chromatography (HPLC) is discussed.

In recent years, there has been a paradigm change in the way we approach mobile phase design for high performance liquid chromatography (HPLC). The trends are towards more simple linear binary gradients, with fewer additives (buffers, sacrificial bases, exotic pH adjusting reagents). This is possible due to improvements in column stationary phase design, the requirement for volatile buffers to be compatible with atmospheric-pressure ionization mass spectrometry (API-MS) detection, and a desire for improved robustness of analytical methods.

Perhaps the best examples of this new approach are popular eluent systems employing 0.1% w/w trifluoroacetic acid, 0.1% w/w formic acid, or 10 mM ammonia. These simpler systems work on the premise that analytes will be either fully ionized or fully nonionized in solution, well away from the pK_a of the analytes of interest, improving robustness by removing any susceptibility to changes in selectivity as a result of small variations in eluent pH. Of course, this predicates that the pK_a values of our analytes are known or can be reasonably accurately estimated, to ensure that there are no analytes with particularly high or particularly low pK_a values.

Reversed-phase retention of ionized analytes can be attained by using polar endcapped- or polar embedded-type stationary phases, which have only been available recently. Furthermore, trifluoroacetic acid and formic acid are simple ion-pairing reagents, which may also improve retention of ionized basic analytes under acidic conditions. Although using ion-pairing reagents is straightforward, it does somewhat restrict method development choices to altering stationary phase chemistry, the organic modifier used (acetonitrile or methanol), and the gradient slope. It is also important to realize that these additives are relatively volatile, and steps should be taken to avoid their evaporative loss from the eluent reservoir during extended campaigns of analyses. Also, ion-pairing reagents, such as trifluoroacetic acid, may strongly bind to the stationary phase, and should be carefully washed from the column post analysis or used only with columns dedicated to analyses that use these additives. A further note of caution is that the specification of additive concentrations needs attention; 0.1% w/w trifluoroacetic acid produces a significantly different pH from 0.1% w/v of trifluoroacetic acid.

For certain separations, the ionic strength of the mobile phase can alter the selectivity obtained, and this will not only depend on the analyte chemistry, but also on the nature of the bonded phase and silica substrate. Retention and resolution variability can occur as a result of secondary ionic strength gradients (higher to lower ionic strength) that occur when the "buffer" is added to the aqueous component (mobile phase A) only. The magnitude of these selectivity changes will depend on the nature of the interactions between the analyte and the bonded phase ligand or stationary phase surface. However, these effects can be mitigated via the use of a ternary pumping system in which the "buffer" is added at a constant concentration. For example, if 10 mM ammonium acetate is required, a third pump can deliver a constant 5% of a 200 mM solution of ammonium acetate, in order to deliver the constant ionic strength required during the whole gradient analysis, the organic and aqueous portions being proportioned accordingly. A similar approach can be taken with trifluoroacetic acid or formic acid, if required. There are secondary benefits to this approach, which include more robust and repeatable electrospray LC–MS results, and a reduction in baseline "slope" during the method, which is particularly true when using UV detection at wavelengths below 220 nm.

We have also noted that modern HPLC users strive for the fewest numbers of operations in eluent preparation, to improve repeatability and robustness. Users tend to select higher grade solvents, which do not require filtration, and contribute less to the background signal. Additionally, more and more often we see eluents being prepared using accurate volumetric or gravimetric techniques, negating the requirement for pH adjustment using a pH electrode, which again helps to improve accuracy and removes potential cross-contamination from the electrode itself.

The use of nonvolatile buffers continues to decline, perhaps in line with the rise in popularity of API-MS detectors. We have seen a moderate increase in more volatile "true" ion-pairing reagents, such as heptafluorobutyric acid, which produces satisfactory retention for ionized analytes in reversed-phase LC, while retaining good analyte sensitivity with MS detection.

We see a large upturn in the use of acetonitrile in favour of methanol as the first-choice organic modifier in many application areas, perhaps as a result of perceived superior selectivity, efficiency, and sensitivity with UV detection. Although this may be true in some instances, there will be applications in which methanol produces the best selectivity, and when using phenyl stationary phases, when it should be the *de facto* choice to achieve optimum selectivity. We would urge method developers to screen separations using both modifiers to make more informed choices.

More Online:

Get the full tutorial at
www.CHROMacademy.com/Essentials
(free until 20 June).

Analysis of Bisphenol A in Food by Solid-Phase Microextraction Using an Overcoated Fibre

Katherine Stenerson, MilliporeSigma

Solid-phase microextraction (SPME) has not been widely used for the analysis of suspected endocrine disruptor bisphenol A (BPA) in food matrices due to sensitivity and fibre ruggedness issues associated with exposure to matrix components. The purpose of this work was to revisit the use of SPME to develop an easy and sensitive method for analysis of BPA in a variety of food products. The issues mentioned previously related to food matrices and SPME were addressed through the use of an overcoated (OC) DVB fibre. The overcoating, which consists of polydimethylsiloxane (PDMS), protects the DVB layer from contamination, and increases the physical robustness of the fibre.

Experimental

The final, optimized SPME method using the OC fibre is described in Table 1. After extraction, the fibre was desorbed in the inlet of a 7890/7000C GC–MS/MS system, and analysis of BPA (underivatized) proceeded by MS/MS. Samples (spiked and unspiked) were quantitated against matrix-matched calibration curves.

Results and Discussion

The SPME method described in Table 1 was optimized to enable a single set of extraction parameters to be used for multiple sample types. Four matrices were studied, and BPA and the internal standard, BPA-d_{16}, could be detected free of interferences from all of them. In Figure 1, an example is shown for the heaviest matrix, canned pumpkin. As is shown for 10 ppb spiked replicates in Table 2, good accuracy and reproducibility were obtained using the SPME method.

Table 1: Optimized SPME procedure for extraction of BPA from food samples

Sample and Matrix:	10 mL vial containing 0.5 g of sample and 6.5 mL of water at pH 4 containing 25% sodium chloride, spiked with 10 ppb of BPA-d_{16} internal standard
SPME Fibre:	Overcoated PDMS-DVB, 23 gauge
Incubation:	10 min, 50 °C, 400 rpm
Extraction:	Immersion, 50 min, 50 °C, 250 rpm, vial penetration 34 mm
Wash:	0.5 min, 250 rpm, vial penetration 34 mm
Desorption:	3 min, 260 °C
Post Bake:	6 min, 270 °C

In comparison to a standard PDMS/DVB fibre, the OC fibre was expected to show greater durability when exposed directly to these matrices. This was confirmed by doing multiple extractions of canned pumpkin spiked with BPA-d_{16}. The OC fibre outperformed the standard fibre for physical ruggedness and response repeatability.

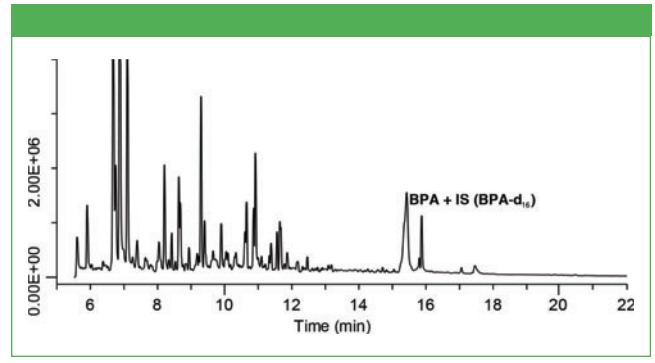


Figure 1: Detection of BPE, and BPA-d_{16} in canned pumpkin international standard (IS).

Table 2: Accuracy and reproducibility for SPME method applied to food samples

Sample	Container Type	BPA level measured in unspiked*	Spike level	Avg. amount measured	RSD
Fruit flavoured energy drink	can	0.8 ng/mL	10 ng/mL	11.5 ng/mL	1% (*n = 3*)
Baby food, carrots	glass jar with metal lid	0.65 ng/g	10 ng/g	11.7 ng/g	2%**
Cream of chicken soup (condensed)	can	12.7 ng/g	10 ng/g	8.2 ng/g	9%**
Pumpkin	can	1.6 ng/g	10 ng/g	11.0 ng/g	13% (*n = 6*)

*determined by standard addition; **%RPD, *n = 2*

Summary and Conclusions

An immersion SPME method using an overcoated PDMS/DVB fibre was developed for the low level analysis of BPA from various food products. Method accuracy and reproducibility at a 10 ppb spiking level was 80–110%, with RSD/RPD values of <15%. Durability testing showed the OC fibre to be more physically robust, with more consistent response, compared to a standard fibre. The SPME method had only a few steps and was easy to automate. In addition, it was highly sensitive, and when combined with GC–MS/MS, provided the selectivity necessary to be used with different matrices.

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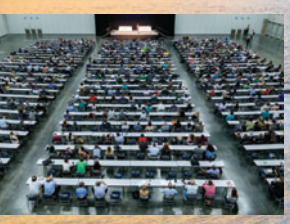



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