O_7: Applications of Two New Ambient Ionization Technologies for Direct Sample Analysis: PaperSpray and Flowprobe

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Paper spray mass spectrometry (PS-MS) and liquid microjunction surface sampling probe (LMJ-SSP) are two relatively new ambient ionization technologies that enable rapid and direct analysis of samples with little to no sample preparation and no chromatographic separation. PS-MS is well suited for quantitative analysis of xenobiotics in biological fluids, while LMJ-SSP facilitates rapid qualitative analysis of samples using a liquid microjunction with a sample surface. We have developed a number of quantitative bioanalytical methods using PS-MS, including quantitation of multiple NSAID drugs in equine and porcine plasma, and quantitation of an antifungal drug in equine tears and plasma. For NSAIDs in equine plasma, PS-MS quantitative results were compared with those generated using an established, validated, LC-MS method and shown to be equivalent. Each PS-MS method was validated with respect to selectivity, linearity, accuracy, precision, matrix effect, and carryover. Method validation results and figures of merit will be presented as will results from analysis of authentic unknown samples. We have also developed a method using LMJ-SSP for rapid, untargeted screening for veterinary drug residues and xenobiotics in food and companion animal tissues. Examples of applications of this method include identification of the veterinary anesthetic pentobarbital and the anti-seizure compound phenytoin simultaneously in canine liver, identification of the growth and leanness promoting drug ractopamine in porcine liver, and identification of the NSAID drug flunixin in a porcine muscle homogenate smear. Considerations regarding parameter selection and optimization when implementing the LMJ-SSP as part of an untargeted screening workflow will be reviewed. For PS-MS methods, a Velox 360 automated paper spray source (Prosolia, Inc., Indianapolis, IN, USA) was interfaced with a Q Exactive Focus quadrupole-orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). For the LMJ-SSP work, a flowprobe (Prosolia, Inc, Indianapolis, IN, USA) was interfaced with a Q Exactive Focus mass spectrometer.

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O_8: Efficient Total RNA Analysis Using Ionic Liquid-based In Situ Dispersive Liquid-liquid Microextraction

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The extraction and purification of total ribonucleic acid (total RNA) from complex biological sample matrices is essential for numerous downstream applications in molecular biology such as quantitative reverse-transcription polymerase chain reaction (RT-qPCR), electrophoresis, protein expression, sequencing, transfection and gene therapy. Traditionally, RNA extraction protocols use organic solvents, which pose environmental hazards, and are often time-consuming as they can contain multiple steps. Commercial kits have been developed to address these issues. However, they are usually very expensive and may require specialized equipment to use. In this study, a simple, environmentally-friendly and rapid extraction technique has been developed for the effective determination of total RNA from aqueous solutions. Six ionic liquids (ILs) were tested as possible extraction solvents in an in situ ionic liquid-based dispersive liquid-liquid microextraction (IL-DLLME) technique involving aqueous solutions spiked with total RNA, ionic liquid, and lithium bis[(trifluoromethyl)sulfonyl]imide (LiNTf2) as the anion-exchange reagent. Total RNA is preconcentrated into the IL extraction phase and indirect analysis on the amount of total RNA extracted is performed by injecting an aliquot of the upper aqueous phase after extraction to high-performance liquid chromatography with diode array detection following a new ion-pair reversed-phase method (IP-RP-HPLC).

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O_9: Advances in Pre-Analytical Sample Preparation: Unclogging the Bottleneck from Sample to Answer

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Successful detection of pathogens in foods involves the seamless integration of three interdependent steps: 1) statistically validated sampling, 2) pre-analytical sample preparation and 3) detection. Weak links in any of these three steps will propagate through the system and degrade the end result. In a worst-case scenario, this could lead to false-negative results. "Upstream" methods for sampling have long been established, and the past decade has seen a revolution in development of the hardware and reagents needed for truly rapid detection of pathogens. However, even the most sophisticated detection methods cannot reach their full potential without next-level advances in pre-analytical sample preparation, which is still the key bottleneck in getting from sample to answer when detecting pathogens in foods. Further, sample preparation is not only of critical importance for detection of pathogens in foods, but also for detection of any analyte (chemical, biochemical, cellular, etc.) distributed in any medium. Where appropriate, this talk will address sample preparation-related motivations, problems - and potentially, solutions - that are common to even very different disciplines such as chemistry and food science.

O_10 : Exploiting Magnetic Ionic Liquids to Develop a One-step Method for Plant DNA Isolation

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Nowadays, there is an increasing demand for fast sustainable, and reliable plant biomolecular analyses. Plant DNA can be used for several fast screening applications including, for instance, DNA barcoding for food traceability [1]. In general, DNA isolation requires multiple and time-consuming steps to remove cellular interferences with the consequence that sample preparation is the main bottleneck in a bioanalytical workflow. New perspectives have been opened by the use of Magnetic Ionic Liquids (MILs), proposed in 2015 by Anderson's group, as new materials for selective extraction and purification of nucleic acids [2]. Hydrophobic MILs can be used in magnet-assisted Dispersive Liquid-Liquid Microextraction (maDLLME) by dispersing the MILs into very small microdroplets in a water solution containing the nucleic acids and by recovering the MILs with the application of a magnetic field. This approach results in reduced extraction time and increased extraction efficiency toward nucleic acids. So far, it has been applied to DNA (purified or in complex solutions containing proteins) and to bacterial cell lysate, but, to date, no applications on plants have been reported [3]. In the present study, three low-viscosity hydrophobic MILs, containing Co, Mn and Ni [4], were tested for the isolation of plant DNA. Preliminary tests were carried out on purified DNA from Arabidopsis thaliana (L.) Heynh. while subsequent experiments were performed directly on the plant material. The isolation of the DNA was performed by maDLLME after lysis of the plant cells and the DNA was then recovered by back extraction. The isolated DNA was subjected to qualitative and quantitative analyses, with interesting results in terms of MILs-based method extraction performance. In the field of plant molecular biology, the application of maDLLME with MILs could represent a powerful tool for fast, sustainable and potentially automatable screening methods involving DNA. *[1] Galimberti A, De Mattia F, Losa A, Bruni I, Federici S, Casiraghi M, Martellos S, Labra M, DNA barcoding as a new tool for food traceability. Food Res Int. 2013; 50 (1): 55-63 [2] Clark KD, Nacham O, Yu HL, Li TH, Yamsek MM, Ronning DR, Anderson JL. Extraction of DNA by Magnetic Ionic Liquids: Tunable Solvents for Rapid and Selective DNA Analysis. Anal Chem. 2015;87(3):1552-9 [3] Clark KD, Trujillo-Rodríguez MJ, Anderson JL. Advances in the analysis of biological samples using ionic liquids. Anal Bioanal Chem. 2018, doi: 10.1007/s00216-018-0898-9 [4] Pierson SA, Nacham O, Clark KD, Nan H, Mudryk Y, Anderson JL. Synthesis and characterization of low viscosity hexafluoroacetylacetonate-based hydrophobic magnetic ionic liquids. New J Chem. 2017;41(13):5498-505*.