THE BRITISH MASS SPECTROMETRY SOCIETY

AI & EFA SIG 1-DAY MEETING

GUILDFORD ✪ 27 APRIL 2022

E-ABSTRACT BOOK

In partnership with

UNIVERSITY OF SURREY

www.bmss.org.uk

BMSS

THE BRITISH MASS SPECTROMETRY SOCIETY

SPONSORS

www.bmss.org.uk

THE BRITISH MASS SPECTROMETRY SOCIETY

CONTENTS

www.bmss.org.uk

THE BRITISH MASS SPECTROMETRY SOCIETY

The 42nd BMSS Annual Meeting

MANCHESTER ✪ 13-15 SEP 2022

REGISTRATION NOW OPEN

The BMSS are happy to announce the Royal Northern College of Music as the venue for the 42nd BMSS Annual Meeting. A hybrid event with asynchronous on-line outreach

Engage with your Society!

RNCM

www • bmss • org • uk

BMSS
BRITISH MASS SPECTROMETRY SOCIETY

THE BRITISH MASS SPECTROMETRY SOCIETY

PROGRAMME

9.00 – 10.00am	Registration and coffee
10.00– 10.05	Introduction – Prof Paul Townsend, University of Surrey Lecture theatre D, Stag Hill Campus
	Chair: Michael Wilde, University of Plymouth
10.05 – 10.45	Simon Hird, Waters. Using Direct MS to verify product authenticity in the food and beverage sector
10.45 – 11.10	Mark Perkins, Anatune. Automated Headspace Analysis of Selective Fragrance Binding and Release from Cucurbiturils using Selected Ion Flow Tube Mass Spectrometry.
11.10 – 11.35	Yuying Du, University of Birmingham. Native LESA mass spectrometry of Intact Proteins and Protein Complexes Directly from Living Bacterial Colonies
11.35 – 12.00	Posters Introduction
12.00 – 1.30	Exhibition, posters and lunch
	Chair: Oli Hale, University of Birmingham
1.30 – 1.55	Jan-Christoph Wolf, Plasmion. Advanced applications of the SICRIT ion source: From fast classification and fraud detection to detailed aroma profiling and emission monitoring
1.55 – 2.20	Alice Flint, Queen's University Belfast. The Development of Rapid Evaporative Ionisation Mass Spectrometry for the Detection and Diagnosis of Plant Parasitic Nematode Infections in Crops
2.20 – 2.55	Andrew Hambly, University of Bristol. Identification of β-Carotene Oxidation Products Produced by Bleaching Clay Using UPLC-ESI-MS/MS
2.55 – 3.10	Coffee
	Chair: Andrew Ray
3.10 – 3.35	Henriette Krenkel, University of Reading. LAP-MALDI MS for high-speed sample screening: from 5 to 40 samples/s
3.35 – 4.00	Valerio Converso, LGC. Meat Speciation Via Deployable ASAP-QDa
4.00 – 4.25	Nick Molden, Oxford Indices. According to chemistry, whisky is a solution
4.25 – 4.30	Closing Remarks, Pete Ryan Award
4.30 – 7.30	Networking event – Waites House

www.bmss.org.uk

THE BRITISH MASS SPECTROMETRY SOCIETY

ORAL ABSTRACTS

www.bmss.org.uk

Direct Mass Spectrometry for High Throughput Food Analysis

Simon Hird – Waters

Aims: Mass spectrometry, using ambient ionisation techniques, combined with multivariate statistical analysis, is a technique for rapid characterization of food and animal tissues, either with no requirement for sample preparation or just simple dilution/extraction. Here we focus on two of those techniques; Rapid Evaporative Ionization Mass Spectrometry (REIMS) and Atmospheric Solids Analysis Probe (ASAP) mass spectrometry.

Results: Due to their high market value, meat and fish are often targeted for fraud. REIMS has been successfully used as a rapid profiling technique to tackle issues associated with species substitution, adulteration and mislabelling (e.g. geographical origin or type of production). It has also been successfully used for characterization of production metrics such as aging method, aging time and for prediction of quality attributes such as carcass type, production background, breed type, muscle tenderness and palatability. Lastly, the utility of REIMS has also been explored for the direct analysis of meat samples from livestock treated with illegal growth promoters.

Although REIMS is rapid, requires no sample preparation and is simple to use, the technology is coupled to a high-resolution mass spectrometer (HRMS), which may prove prohibitive for most point of control testing. One innovative solution being explored is the potential of other ambient ionisation techniques fitted to a compact, easy to use, nominal mass detector, which has greater potential for deployment away from the research laboratory environment. The RADIAN ASAP is a novel, dedicated, direct analysis system specifically designed for direct MS, which has been used for the investigation of authenticity and adulteration issues. It comprises of a redesigned Atmospheric Solid Analysis Probe (ASAP) fitted on an ACQUITY QDa mass detector and LiveID informatics package.

Conclusions: We will explore a series of case studies demonstrating that Direct MS, using untargeted, metabolomics workflows, multivariate statistics, and real time recognition software, can be considered as a high-throughput and accurate strategy for real-time

Automated Headspace Analysis of Selective Fragrance Binding and Release from Cucurbiturils using Selected Ion Flow Tube Mass Spectrometry.

Mark Perkins - Anatune, Ben Cheesman - Aqdot Ltd, Jessica Lenderyou - Aqdot Ltd

Aims: Cucurbiturils are barrel-shaped molecules that can selectively bind and hold fragrance (or other) molecules. The bound molecules can then be released in response to triggers such as humidity, heat, pH, or displacement by other molecules such as malodours. Analysis of the headspace above aqueous mixtures of fragrance can yield important information regarding differential binding of compound classes. However, this requires the analytical technique to be both fast and largely immune to humidity. Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) is a soft ionisation mass spectrometric technique that offers real-time, targeted measurement of volatile and semi-volatile compounds without the need for chromatographic separation, or the removal of residual water. By integrating SIFT-MS with autosampler technology it is possible to analyse dynamic headspace conditions over long periods of time.

Results: A number of fragrance/cucurbituril mixtures were analysed over approximately 18 hours using repeated headspace sampling, followed by vial flushing, to mimic fragrance loss over time. Additionally, malodours were added to stimulate fragrance release. Analysis of the headspace concentrations clearly demonstrated the effect of differing fragrance/cucurbituril ratios as well as fragrance functional group effects.

Conclusions: Automated Headspace SIFT-MS allowed for multiple headspace measurement to be made, directly from complex aqueous mixtures, that confirmed the binding and release capabilities of cucurbiturils on different functional fragrances. The high-throughput nature of the technique allowed for the released profiles to be generated, in real-time, and with minimal preparation or manipulation of the samples.

Native LESA mass spectrometry of Intact Proteins and Protein Complexes Directly from Living Bacterial Colonies

Yuying Du, Helen Cooper, Emma Sisley, Oliver Hale, Robin May - University of Birmingham

Aims: We have previously demonstrated liquid extraction surface analysis mass spectrometry (LESA- MS) for the analysis of denatured intact proteins directly from living colonies of bacteria. In order to breach the peptidoglycan cell wall and outer membrane of bacteria, denaturing solvents have been used which cause the loss of structural information. Native mass spectrometry enables the analysis of tertiary and quaternary protein structure in the gas phase. By using LESA-MS which is particularly suitable for the analysis of intact proteins, proteins can be sampled under near native-like conditions from a range of biological substrates and structural information can be obtained. By coupling LESA MS with electroporation, which can rupture the thick cell wall and form micropores in the cell membrane with minimal heating, it is possible to analyse intact proteins directly from bacteria. Here, we have combined electroporation with LESA MS using native-like solvents to perform native MS of proteins directly from bacteria.

Results: Colonies were grown on lysogeny broth (LB) agar and subjected to electroporation using a home-built device. LESA was performed using a TriVersa NanoMate with a solvent comprising 200 mM ammonium acetate. Mass spectrometry experiments were performed using an Orbitrap Eclipse. Data were acquired in the 900-4000 m/z range at a resolution of 120000 at m/z 200. MS/MS spectra were searched using the Prosight software and putative protein assignments confirmed by manual analysis.
Native-like solvents 200mM ammonium acetate were used for the detection of E.coli K12 proteins by LESA MS. Proteins were observed both with and without electroporation; however, a much higher mass spectral reproducibility was obtained with electroporation. The success rate of 77% for detection of proteins with electroporation was significantly higher than the 14% without electroporation. Peaks corresponding to 25 proteins were observed following native LESA MS, of which thirteen proteins were identified by top-down mass spectrometry. Acyl carrier protein was detected with a range of different post-translational modification. Seven proteins were previously undetected including Csp E, YibT, HPr, PSiF, YgiW, Antigen 43 alpha chain and superoxide dismutase. Protein antigen 43 alpha chain (~49.8 kDa) is the highest molecular weight protein detected following LESA of bacterial colonies to date. Three multimeric proteins were identified – the HDE A, HDE B and superoxide dismutase homodimers. The superoxide dismutase dimer was observed with 2 Mn^{2+} ions bound.Conclusions: Our results confirm that electroporation followed by LESA MS using ammonium acetate is a suitable approach for direct native mass spectrometry of bacteria. This work is the first demonstration of native LESA MS of intact proteins and protein complexes in living bacterial colonies. Bacterial proteins were analyzed directly from native-like conditions without complicated pretreatment steps. Several proteins from cytoplasm was obtained by using electroporation technology. We demonstrate detection of intact protein assemblies, as well as higher molecular weight proteins .

Advanced applications of the SICRIT ion source: From fast classification and fraud detection to detailed aroma profiling and emission monitoring

Jan-Christoph Wolf, Markus Weber - Plasmion GmbH

Aims: Soft Ionisation by Chemical Reaction in Transfer (SICRIT) is an ambient ionisation source that enables various new applications of mass spectrometry. Due to its “flow through” operation principle multiple sampling and enrichment techniques can be easily interfaced. Since its introduction 2018 many different applications have evolved around this technology. In this presentation we want to share some insights on recent and advanced applications of this technology in the area of food and environmental analysis. Possible applications of SICRIT range from direct headspace measurements for fast and easy fraud detection or origin classification to solidphase extraction – gas chromatography high resolution mass spectrometry (SPME-GC-HRMS) for highly sensitive and selective identification and quantification of individual trace components.

Results: In a recent study SICRIT was applied for the direct headspace measurement of honey samples. The combination of a PAL autosampler, SICRIT ionization source and a single quadrupole MS presents a fast and cost-effective way to classify honey based on floral origin. Various types of honey like manuka, linden, rape, chestnut and pine could be distinguished and altered samples were detected with a measurement time of only 3 minutes per sample. For a more detailed analysis the SICRIT ionization source was coupled with gas chromatography and an orbitrap MS to conduct detailed aroma profiling and record terpene profiles of different honey samples. Similar studies were performed for coffee and olive oil.

If the goal is ultimate sensitivity, SICRIT enables easy combination with sample enrichment techniques like SPME. This allows to achieve limits of detections matching legal regulations of pesticides in surface and drinking water. SPME was also used for aroma profiling of trace components in the headspace of various types of beer.

Conclusions: The third way to use SICRIT for environmental and food analysis are direct measurements. Due to its flow through geometry and the wide range of ionizable compounds SICRIT allows for real time measurement of dynamic processes in ambient air. This can be applied to food processing steps like roasting of coffee beans. The development of different aroma compounds was observed in real time depending on roasting temperature and time. In the field of environmental analysis exhaust gas measurements directly at a vehicle test bench were conducted to observe the release of pollutants during a real world engine test cycle.

The Development of Rapid Evaporative Ionisation Mass Spectrometry for the Detection and Diagnosis of Plant Parasitic Nematode Infections in Crops

Alice Flint, Simon Cameron, Ryan Weir - Queen's University Belfast, Jonathan Dalzell - Agri-Food and Biosciences Institute

Aims: Plant parasitic nematodes (PPNs) such as root-knot and cyst nematodes are major agricultural pests which exhibit broad host ranges, including members of the Fabaceae, Poaceae and Solanaceae families. PPNs cause wilting, stunting and chlorosis, with resultant yield losses up to 25% costing an estimated £100 billion annually. However, these above-ground PPN symptoms resemble other environmental stresses resulting from root dysfunction and can display slow onset. Current PPN diagnosis requires root and soil analysis; a low throughput, destructive process necessitating specialist expertise. Therefore, the development of non-destructive assay would enable early detection, improving management and monitoring.

Plant responses to pathogen challenge include the production of defence hormones and phytoalexins, structural reinforcement, nutrient remobilisation and the generation of reactive oxygen and nitrogen species, requiring the rewiring of primary and secondary metabolism. Thus, metabolomics has proven an effective tool for the diagnosis and study of plant-pathogen interactions. However, current chromatography-based mass spectrometry metabolomic analyses require time-consuming processing, extraction and separation, are restricted to laboratory settings and again necessitate specialist expertise.

Rapid evaporative ionisation mass spectrometry (REIMS) is an ambient ionisation technique developed for the clinical detection of cancerous tissue boundaries, which utilises sample heating to generate an analyte-rich vapour. Ionisation and analysis can be performed within 5 seconds, compared to typical LC-MS runtimes of 10 to 20 minutes. Furthermore, samples require minimal preparation, vastly increasing throughput and usability. Subsequently, REIMS has been widely employed for microbiological diagnostics and food authenticity analyses but has only recently been applied to plant tissues, with analysis of Capsicum and Kigelia fruit demonstrating detection of amino, organic and fatty acids, lipids and phenolics. Therefore, REIMS analysis of above-ground vegetative tissue has great potential for the simple and rapid detection of PPN-induced host metabolic changes, to enable diagnosis and further understanding of plant-nematode interactions.

The Development of Rapid Evaporative Ionisation Mass Spectrometry for the Detection and Diagnosis of Plant Parasitic Nematode Infections in Crops

Existing REIMS methods heat samples via surgical electrical diathermy handpieces or infrared CO2 lasers. However, electrical diathermy is an unsuitable method as plant foliage has poor conductivity, whereas surgical CO2 lasers are costly, preventing widespread uptake of this technology. Therefore, in addition to the optimisation of laser-assisted REIMS in vegetative plant tissue with existing technology, this work aims to apply low-cost heating methods to develop a viable solution for the in-field diagnosis of PPN infections. A soldering iron and 450 nm blue light laser were selected for trial, each priced below £200.

Results: Tomato was chosen as a model crop for the development of a REIMS-based PPN diagnostic tool in Solanaceae. Four PPN species have been selected for this study to determine whether distinction at the genus and species level is possible: the root-knot nematodes Meloidogyne incognita and M. javanica and the cyst nematodes Globodera pallida and G. rostochiensis. PPN infected leaves will be subject to REIMS analysis via CO2 and 450 nm laser orsoldering iron sample heating throughout an infection time course over four weeks.

Each heating modality was optimised for greatest signal intensity. Comparison of the modalities at optimised conditions demonstrated the soldering iron generates the best signal intensity but is less reproducible than laser heating. Nevertheless, the 450 nm laser provided improved signal and reproducibility over the CO2 laser, suggesting this technology is a viable alternative to expensive infrared lasers. However, the 450 nm laser produced the most distinct spectra indicating heating and ionisation of different components.

Early results from REIMS analysis of leaves 10 days post-M. incognita infection demonstrate metabolomic separation of control and infected plants by OPLSDA following heating with the 450 nm laser or soldering iron. Changes in 28 metabolites were detected via 450 nm laser heating, whereas 15 differential metabolites were isolated for the soldering iron. Only one spectral bin, putatively identified as the C18 fatty acid linoleic acid was shared between modalities, suggesting potential changes to jasmonate synthesis and signaling upon M. incognita infection.

Conclusions: This work has optimised laser-assisted REIMS for analysis of plant vegetative tissue and developed low-cost sample heating technologies to enable greater application and uptake of this technology. Early results suggest REIMS can detect PPN-associated metabolic changes and could present a simple and economical solution for the diagnosis of PPN infections, enabling improved management and treatment, securing future crop yields. Moreover, REIMS has broad potential for applications within agricultural research, including investigation of abiotic and biotic stress responses, seed analysis and high-throughput screening.

Identification of β-Carotene Oxidation Products Produced by Bleaching Clay Using UPLC-ESI-MS/MS

Andrew Hambly, Dr Paul J Gates, Dr Jeroen S Van Duijneveldt - University of Bristol

Aims: Vegetable oils often undergo some degree of refining before being made into products for the food and pharmaceutical industries. One of the refining steps is known as 'bleaching' or decolourising. Here the oil is mixed with an acid-activated clay (AAC) adsorbent and contaminants which are detrimental to the final product quality are removed. The contaminants include lipid oxidation products and plant pigments chlorophyll and β-carotene. While it is known that β-carotene adsorbs readily to AACs, it is not understood whether this removal process is purely physical or involves chemical degradation of the pigment as well.
In this work we aim to provide extra mechanistic insight into the interaction between β-carotene and AAC adsorbents by studying the extracts of the spent AAC using UPLC-ESI-MS/MS.

Results: AAC particles were exposed to β-carotene at a concentration that coated them with a monolayer. These particles were then isolated and extracted. Extracts were first analysed using direct infusion MS. This revealed a mass spectrum which indicated no β-carotene was present but a mixture of compounds of higher m/z and a mass defect characteristic of carotenoids. This suggested that a chemical process was taking place.

A UPLC-ESI-MS/MS method was developed which was capable of separating the components of the carotenoid extract by retention time or m/z. The extracts were then analysed using this method. The results showed that the extracts contained over 30 carotenoid oxidation products (COPs). Most abundant were COPs with higher m/z and one or two oxygens. Co-injections and comparison of retention time and MS/MS spectra revealed that one of these COPs was the xanthophyll canthaxanthin. Another COP was identified as 3,4-didehydro-caroten-4'-one by MS/MS spectra interpretation. Interpretation of molecular formulae, retention times and comparison with literature allowed tentative assignments of other COPs, including common oxidation product β-apo-8-carotenal.
To provide further mechanistic insight and reveal what the source of the oxygen was, AAC which is usually a few percent water by weight was prepared with 18-O isotopically labelled water instead. The extracts were prepared and analysed by the same method. Accurate mass measurements showed that the COPs produced in this instance contained 18-O instead of 16-O, showing that water present on the clay surface is involved.

Identification of β-Carotene Oxidation Products Produced by Bleaching Clay Using UPLC-ESI-MS/MS

Finally, extracts were also prepared similarly but with other clay adsorbents which had not been acid-activated. These were an untreated clay and a clay which had its structure modified with the addition of alumina 'pillars' ('alumina-pillared clay', APC). The extracts were then analysed with the same UPLC-ESI-MS/MS method. Python scripts were written which then summed the total intensity for all ions of each molecular formulae detected. These intensities were compared across samples prepared from the AAC, the untreated clay and the APC. This showed that AACs are unique in their ability to oxidise β-carotene to a greater extent than either of the other clays. Physicochemical characterisation of the clays showed that this process is likely catalysed by Brønsted acid sites and accelerated by higher surface area and porosity.

Conclusions: From the results above we draw the following conclusions:

Firstly, the process by which vegetable oil contaminant β-carotene interacts with acid-activated clays (AACs) involves a chemical adsorption process.

Secondly, UPLC-ESI-MS/MS analyses show this process oxidises β-carotene to a variety of carotenoid oxidation products (COPs). These include higher m/z COPs such as canthaxanthin and 3,4-didehydro-caroten-4'-one, and lower m/z scission products such as β-apo-8-carotenal.

Thirdly, accurate mass measurements on samples after exposure of β-carotene to 18-O labelled clay showed that water which is normally present on the clay surface is involved in the oxidation.

Lastly, extracts prepared with AAC, untreated clay and alumina-pillared clay were analysed again using UPLC-ESI-MS/MS, and their compositions were compared. This showed that AACs are unique in their ability to oxidise β-carotene. They produced more COPs and COPs of a higher oxidation state relative to β-carotene. This, combined with physicochemical characterisation of the clays showed that the clay's Brønsted acid sites catalyse this process and it is accelerated by AACs higher surface area and porosity.

LAP-MALDI MS for high-speed sample screening: from 5 to 40 samples/s

Henriette Krenkel, Rainer Cramer - University of Reading, Jeffery Brown, Keith Richardson, Emmy Hoyes, Michael Morris - Waters Corporation

Aims: Fast analyses are in general desirable for obvious cost and convenience reasons, but particularly large-scale experiments require high sample throughput. Mass spectrometry offers a label-free alternative for photometric readouts of enzymatic assays used in clinical screening experiments and diagnostics. Although providing less false-positive readouts, the throughput of MS techniques is still not matching traditional methods. Liquid atmospheric pressure matrix-assisted laser desorption/ionisation (LAP-MALDI) tries to close this gap by providing contact-free laser-based desorption for fast sample exchange and a good compatibility with reagents commonly used in biological assays.

Results: A commercial QToF instrument was fitted with a heated stainless-steel inlet tube as described before1. Several hard- and software adaptations were necessary to increase sample throughput beyond the 5 samples/s shown previously1.

Analyte ion signal intensity scales nearly linearly with the number of laser shots encountered in a given time frame. Thus, a laser with a higher pulse repetition rate allows shorter residency time on each sample and hence a significantly increased throughput. However, the reported “self-healing” effects of the liquid sample is impeded at very high pulse repetition rates and hence sets an upper limit on the residency time.

Furthermore, the standard MS software on the commercial QToF instrument employed is not capable of providing enough temporal resolution to differentiate between samples at high speed. To break one summed ToF scan into several spectra, the software was adapted to store the spectral information in 200 individual bins similar to the commercial SONAR mode. The minimal scan times could be reduced from 16 ms to 0.93 ms. Additionally, the occurrence of the interscan time (10 - 15 ms), normally after every scan, could be reduced to a scan-to-interscan ratio of 200:1.

Different sample raster patterns and sample layouts were also investigated. Changing from 384-well microtiter plate (MTP) format to 1536-well layouts, higher analysis speeds could be achieved. Sample batches were analysed as one data file and sliced into individual samples post-acquisition using a custom script.

LAP-MALDI MS for high-speed sample screening: from 5 to 40 samples/s

As a proof-of-principle, an enzyme assay using Angiotensin Converting Enzyme (ACE) as an example was carried out and analysed without prior purification. The natural substrate Angiotensin I as well N-Hippuryl-His-Leu, a substrate commonly used in photometric readouts, were incubated with enzyme and 'buffer-only' as a control. After mixing with a liquid support matrix (LSM), samples were irradiated with a 343-nm laser (2000 Hz) using an extraction potential of ~3kV between sample and inlet. The LSM consisting of α-CHCA (5 mg/mL in 50:50 H2O/MeCN, v/v) and propylene glycol (+ 60% v/v) were mixed with the assay sample 1:1.

Substrates as well as products (Angiotensin II and His-Leu) were observed and monitored in the enzyme-treated samples and untreated samples.

1) Krenkel et al, Analytical Chemistry, 2020, 92, 2931-2936.

Conclusions: By changing the hard- and software at our LAP-MALDI MS setup we were able to overcome several bottlenecks and reach analysis speeds of up to 40 samples/s for small molecules. Multiply charged peptides were analysed at slightly lower speeds in the presence of buffers and without any sample clean-up.

In LAP-MALDI only a small fraction of the sample is consumed during analysis, so an additional decrease in sample volume and tighter sample spotting has the potential to further increase throughput without sacrificing any analytical performance.

The capability of LAP-MALDI to generate multiply charged analytes combined with the low sample consumption provides the possibility of re-analysis of interesting samples flagged in screening experiments and perform fragmentation experiments.

Meat Speciation Via Deployable ASAP-QDa

Valerio Converso - LGC, Bryan McCullough - Waters Corporation, Daniel Burns - LGC

Aims: The aim of this study is to explore the potential use of ambient mass spectrometry in the food industry. In particular, a deployable ASAP-QDa has been used for meat speciation analyses in a non-laboratory environment. The developed process from sample receipt to results is quick and user friendly, not requiring scientifically trained personnel performing it and interpreting the data.

Results: The target selected to test the technique is meat, in particular beef, pork and chicken (based on the most common ones in the food industry). Speciation of adulterated food products is usually performed with a variety of techniques (depending on availability and needs). The ones using mass spectrometry traditionally use time consuming chloroform extractions. The aim of this study is to drastically reduce preparation time and complexity, with a simple, relatively safe extraction medium, and process. ASAP-QDa (Waters AQUITY) was used to generate characteristic mass spectra of samples, which can be used as “fingerprints” to differentiate between sample types. The data generated were analysed via the Waters LiveID software, that uses a variety of statistical modelling approaches to de-convolute and reduce dimensionality of mass spectra, producing models which can then be applied to unknown samples for identification. Different statistical models were applied to a variety of unadulterated meats and adulterated meats at different percentages and the most appropriate was found to build a model capable of correctly categorise “unknown” samples as pure or adulterated. The analytical results are obtained in less than 1 minute and the sample preparation step currently sits at around 15 minutes, but further work is aiming at shorten the sample preparation time even more. Moreover, different sampling methods are being tested to lower the detectable adulteration levels (<5%).

Conclusions: Deployable ASAP-QDa gave promising results for the speciation of meats and live detection of adulterated samples. Further experiments could be planned to try and simplify the sample preparation more and more and to expand the range of meat types and adulterated samples that can be added to the method. The intrinsic day-to-day variability of the ambient techniques also needs to be considered and the method robustness needs to be assessed over time. Overall, deployable ambient mass spectrometry showed potential as a technique for on-site rapid testing of food adulteration.

Meat Speciation Via Deployable ASAP-QDa

Valerio Converso - LGC, Bryan McCullough - Waters Corporation, Daniel Burns - LGC

Aims: The aim of this study is to explore the potential use of ambient mass spectrometry in the food industry. In particular, a deployable ASAP-QDa has been used for meat speciation analyses in a non-laboratory environment. The developed process from sample receipt to results is quick and user friendly, not requiring scientifically trained personnel performing it and interpreting the data.

Results: The target selected to test the technique is meat, in particular beef, pork and chicken (based on the most common ones in the food industry). Speciation of adulterated food products is usually performed with a variety of techniques (depending on availability and needs). The ones using mass spectrometry traditionally use time consuming chloroform extractions. The aim of this study is to drastically reduce preparation time and complexity, with a simple, relatively safe extraction medium, and process. ASAP-QDa (Waters AQUITY) was used to generate characteristic mass spectra of samples, which can be used as “fingerprints” to differentiate between sample types. The data generated were analysed via the Waters LiveID software, that uses a variety of statistical modelling approaches to de-convolute and reduce dimensionality of mass spectra, producing models which can then be applied to unknown samples for identification. Different statistical models were applied to a variety of unadulterated meats and adulterated meats at different percentages and the most appropriate was found to build a model capable of correctly categorise “unknown” samples as pure or adulterated. The analytical results are obtained in less than 1 minute and the sample preparation step currently sits at around 15 minutes, but further work is aiming at shorten the sample preparation time even more. Moreover, different sampling methods are being tested to lower the detectable adulteration levels (<5%).

Conclusions: Deployable ASAP-QDa gave promising results for the speciation of meats and live detection of adulterated samples. Further experiments could be planned to try and simplify the sample preparation more and more and to expand the range of meat types and adulterated samples that can be added to the method. The intrinsic day-to-day variability of the ambient techniques also needs to be considered and the method robustness needs to be assessed over time. Overall, deployable ambient mass spectrometry showed potential as a technique for on-site rapid testing of food adulteration.

According to chemistry, whisky is a solution

Nick Molden, James Hobday - Oxford Indices

Aims: Whisky comes with much history, folklore and personal tastes, but it is also big business that thrives as a premium product made with high quality methods. Liquid sunshine is also complex chemistry. The manufacturing process is deep science with a dash of art, which requires high skill to create perfectly flavours and odours, but also sets up the incentive and possibility for fraud and adulteration
The objective of this presentation is to demonstrate the greater potential for VOC identification and discovery enabled by two dimensional gas chromatography time-of-flight mass spectrometry.

Results: As each whisky has its own unique combination of compounds, we tested 50 different products, covering 36 different single malts and 14 blends, drawn from around the world. Each was sampled from recently opened bottles stored in controlled conditions, and then analysed in our laboratory using liquid injection into a two-dimensional gas chromatography and time-of-flight mass spectrometry system from Markes International and SepSolve Analytical.
The benefit of this analytical approach is that the two dimensions of the chromatography allow for near-complete separation of all the organic compounds, in a way not possible with standard one-dimensional separation, which is limited by coeluting peaks on the chromatogram. The time-of-flight mass spectrometry allows for powerful identification of the separated compounds even using standard spectral libraries. With a sensitivity down to parts-per-billion, trace amounts of compounds can be discovered effectively by this approach.
A corollary of being able to identify compounds is that we can then compare and contrast different whiskies. Principal Component Analysis (PCA) is used to reduce the complexity of making comparisons. With dozens of compounds in whiskies, the comparative analysis would be impossible to understand. The PCA use a best-fit approach to create axes that a synthesis of multiple compounds.

Conclusions: The next stage in this project is to expand the dataset further, to hundreds of whiskies, to reveal patterns and correlations reflecting geographies, maturation process, ageing and other factors. In early 2022, Oxford Indices will launch a research database that will show the individual compounds detected and their relative concentrations. Characterising the market of whiskies to this level detail presents many opportunities, from engineering new products to protecting the value in an important and high-value sector.

THE BRITISH MASS SPECTROMETRY SOCIETY

POSTER ABSTRACTS

www.bmss.org.uk

A liquid chromatography-vacuum differential mobility-mass spectrometry method for the analysis of isomeric pyrrolizidine alkaloids in tea

Patrick Knight - Shimadzu Research Laboratory (Europe),Maria Cifuentes Girard - University of Geneva Life Sciences Mass Spectrometry, Gerard Hopfgartner - University of Geneva Life Sciences Mass Spectrometry

Aims: Pyrrolizidine Alkaloids (PAs) are found in many flowering plants. Several hundred PAs have been identified to date and are linked to acute and chronic toxicity. As a result, PA content in food stuffs and herbal products is regulated in many jurisdictions. The analysis of PA compounds is complicated by the existence of several isomeric species which can lead to interference in LC-MS based methods. Ion mobility is one method for resolving co-eluting isomeric species. Here we present a method for the analysis of isomeric PA compounds in tea by means of LC-vacuum differential mobility (vDMS)-MS.

Results: 19 tea samples were purchased from supermarkets. Four sets of isomeric PA compounds (14 compounds and 2 internal standards in total) were analysed. The results of the LC-vDMS-MS analysis were compared with a published LC-MRM method (repeated in this work). Several of the isomeric sets co-elute by LC and have no selective fragments in MS/MS.
The LC-vDMS-MS analysis was carried out on a modified Shimadzu LCMS8060 with a prototype vDMS cell situated between the desolvation line and the first quadrupole. The LC-vDMS-MS system was operated in SIM mode. LC was carried out on a Shimadzu Mikros microflow LC system.
The LC and vDMS conditions were optimised using laboratory standards prior to analysing the tea samples. The LC solvent and flow rate have a strong effect on the vDMS separation. The final conditions were: the LC was operated in trap/elute mode with isocratic elution in 15% MetOH/0.1% FA at 100 µL/min with a 50 mm C18 analytical column while the vDMS was operated at 198 Td. After optimisation, the analysis was performed with fixed EdN/EcN pairs for each compound – thus the vDMS separation does not compromise the analysis time.
The LC-vDMS-SIM method presented here facilitates the analysis of 10 out of 14 isomeric PA compounds from tea in 5 minutes analysis time and with very simple sample preparation. In comparison, the conventional LC-MRM method can separate 9 of the 14 compounds in 12 minutes and requires more extensive sample clean up prior to analysis.

Conclusions: LC-vDMS-MS is a novel method for the analysis of PA compounds. LC and vDMS provide orthogonal separations. The benefits of vDMS are seen in throughput, selectivity and noise reduction. The total analysis time (sample preparation, analysis and data reporting) is much reduced which will provide a significant advantage for routine laboratory use.

The Use of Thermal Desorption Gas Chromatography Mass Spectrometry (TD-GC-MS) to Characterise Microplastics

David Jones - Manchester Metropolitan University, Laura Miles - Markes International Ltd, Massimo Santoro - Markes International Ltd, Michele Edge - Manchester Metropolitan University, David Megson - Manchester Metropolitan University

Aims: Microplastics are ubiquitous within our world today. With a size fraction of 1 µm to 5 mm, these polymer particles can arise from many sources, both in urban and non-urban environments.
Previously, a standard method for the sampling and characterisation of microplastics within the air had not been an area of focused research. Instead, the focus has been on microplastics in marine environments. Moreover, techniques like pyrolysis, although common, tend to be very destructive to polymers.

Results: In collaboration with Markes International – specialists in thermal desorption technology – and Eurofins IPROMA, a methodology was developed for characterisation of polymers using thermal desorption gas chromatography mass spectrometry (TD-GC-MS).
A normalised dataset of fifty fragments from TD-GC-MS analysis of six different polymers was used. The polymers analysed were low-density polyethylene (LDPE), nylon-6 (NY6), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC).
Principal component analysis identified fragment ion clusters that correlated with each polymer, and Agilent's MassHunter software was then used to refine these assignments. The parent ion from the total ion chromatogram for each compound was used to create new extracted ion chromatograms, the peaks of which were then integrated and subsequently a signal-to-noise ratio was calculated.
A calibration graph of peak area against sample mass was then constructed for each of these identified potential marker compounds, to check correlation and confirm marker assignment.

Conclusions: Through this, several potential marker compounds were successfully identified, suggesting that quantitative analysis of microplastics is indeed possible with TD-GC-MS.

Rapid Screening of Submicroliter Urine Samples for Drugs of Abuse utilizing Pulsed Gas Ambient Ionization

John Moncur, Scott Campbell - SpectralWorks Limited, Brian D Musselman, Celia Peterson - IonSense, Inc., Saugus, MA, Christopher Esposito, Francois Espourteille - Comprehensive LCMS Solutions Inc.

Introduction. Rapid detection of trace drugs-of-abuse in urine is demonstrated using a pulsed gas direct analysis in real time ambient ionization equipped time-of-flight mass spectrometer. Submicroliter sampling combined with analysis at multiple temperatures serve to minimize the matrix effects normally encountered in direct analysis of urine without pre-processing to remove salts, proteins, metabolic products including creatinine and urea. Accurate control of the temperature of ions exiting the DART source and limiting thermal desorption time to one second per sample serve to permit rapid sensitive detection of drugs-of-abuse. Addition of isotopically labelled reference standards and their ionization serve to provide semi-quantitative determination for those drugs detected making the method suitable for high throughput screening of raw urine.

Results: Method.

Analysis of urine samples presented in 96-well SBS format plates were completed by sampling using a 12-pin replicator (VP Scientific) with the 45-micron diameter pins serving as both sample collector and desorption support. The replicator was mounted on the linear rail presentation system of a JumpShot DART-MS to permit automated presentation of each sample. The release of a one second pulse of heated ionizing gas from the DART source was coordinated with the introduction of each sample into the desorption ionization region. Ion detection was completed using a high-resolution time-of-flight MS. Both positive ion and negative ion analysis were completed for each sample at low (200 C) and high temperature (350 C). Results.

Sample sets were obtained from a commercial testing lab. Row A of the 96-well plates contained calibrator samples for performance testing of the DART AccuTOF (JEOL Ltd.) the remaining 8 rows of 12 samples each were analyzed in sequence. An array of 12 pins 45 micron in diameter (replicator tool, VP Scientific, San Diego) was used to simultaneously sample liquid direct from all samples in each row. The sample array was transferred to the automated sampling arm to permit programmed sample introduction into the ionization region. The pulsed gas JumpShot DART was programmed to release ionizing gas for 1 second only when sample was present. A 3 second delay between sample analysis permitted movement of the subsequent sample into position and time for ion production from the previous sample to cease. While the ionization gas was present for only one second detection of ions was up to 2 seconds was observed to be consistent at all desorption gas temperatures. Determination of presence of a collection of 24 drugs-of-abuse and 9 isotopically labelled standards drugs at concentrations above the LC/MS/MS method cutoff was completed in order to compare the methods.

Conclusions: Conclusions.

Analysis using low temperature (250 C) ionizing gas proved valuable for detection of more volatile drugs such as methamphetamine. This low gas temperature permitted the determination of the relative concentration of creatinine as the signal for that compound did not saturate the MS detector. Analysis of the majority of the drugs targeted was completed using the higher (350 C) temperature gas. As expected, acids such as THC-acid were detected in negative ion mode. Results of these analysis vs those collected using the LC/MS/MS confirmation method will be reported for those compounds where the limited 10000 resolving power of the TOF proved sufficient to isolate the drug signal from background.

Rapid microbial identification for pharmaceutical microbiology using Laser-Assisted Rapid Evaporative Ionisation Mass Spectrometry (LA-REIMS)

Toma Ramonaite, Yuchen Xiang, Zoltan Takats - Department of Metabolism, Digestion and Reproduction, Division of Systems Medicine, Imperial College London, - Department of Metabolism, Digestion and Reproduction, Division of Systems Medicine, Imperial College London, Andrew Ray, Miriam Guest - New Modality Product Development, Pharmaceutical Technology & Development, Operations, AstraZeneca, Macclesfield, UK.

Aims: Development of rapid, sensitive, and precise technologies for identifying various microorganisms has gotten a lot of interest in the pharmaceutical industry in recent years. Introduction of MALDI-ToF has reduced identification times, although it still requires the use of a matrix to aid ionisation. Furthermore, additional extraction processes are required for some microbial groups, such as yeasts, in order to get precise species level identifications.
LA-REIMS (Laser-Assisted Rapid Evaporative Ionization Mass Spectrometry - first automated sample-preparation-free mass spectrometry platform) is rapid microbial detection technology that can provide high-accuracy results in as little as a few minutes after initial incubation. Using single MS technique, a wide range of bacteria, yeast, and fungus can be identified at the species, genus, and family levels.
Results: LA-REIMS works by applying a CO2 laser beam to the microorganism, which causes rapid heating of microbial biomass, resulting in the formation of aerosol containing gas phase ions of metabolites and structural lipids, which are aspirated into to a mass spectrometer (Xevo G2-XS QToF) for MS analysis. Aerosol is co-aspirated, at a flow rate of 0.2 mL/s, with 2-propanol (HPLC Chromasolv grade, Sigma-Aldrich), containing Leuenkephalin at a concentration of 10 ng/µL. Data was acquired in negative mode from three repeats per sample and using repeat laser pulsing with super pulse and 2 W power.
The automated LA-REIMS platform has a wide range of industrial applications and designed to analyse samples from any size culture plates, as well as 96 well plates, 24 well plates or tubes with no preparative steps. Quality control, critical production process testing, screening raw materials or final product release testing are just a few of the LA-REIMS platform's application possibilities in industry.
Here we present work on, environmental monitoring, water analysis, and absence and presence of microbial contaminants. Environmental and absence-presence samples were directly analysed from the culture plates, and water samples were directly analysed from the membrane placed on the culture plates. Speciation accuracy was tested using initial incubation plates as well as refrigerated plates for 24 hours, 48 hours, and 72 hours at 4C fridge before being analysed.
The trained model, including 25 isolates per class, was developed by combining 15 of the most common pharmaceutical bacteria and yeast species identified in water and environmental testing, as well as required species in pharmacopoeia for absence and presence tests. In-house peak picking platform with biomarker detection setup was used to generate a data matrix with class labels for post-processing, with 748 peaks identified across the spectral range and 141 ultimately selected as unique features for random forest model building. The accuracy of the model was first tested using leave-one-out cross validation (CV), which yielded an accuracy of 98.93%. Secondly, a validation set was produced using independent data by the trained model and contained the same 15 species and 10 isolates for each class. The accuracy obtained at species level for 150 isolates was 98.66%, although most incorrectly identified isolates were still placed in the right Genus group. And finally, after 24 hours in the 4C fridge, classification accuracy was 98.00%, 100% after 48 hours, and 98.00% after 72 hours.

Conclusions: REIMS analysis could be performed directly from a culture plate, with no requirement for sample pre-treatment or extraction, and thus allows for a substantially higher throughput than other platforms. Thus, in contrast with existing methods and procedures in pharmaceutical companies, REIMS technology could potentially be a technique with great savings for the business (e.g. saving time and money, increasing sustainability, integrity and robustness, reducing delays for batch and stock release).

Ambient ionisation for the trace detection of explosives

Simone Mathias - University of Surrey, Carol Crean - University of Surrey, David Douce - Waters Corporation, Patrick Sears - University of Surrey

Aims: Rapid screening for trace levels of explosives is necessary for security purposes within the aviation industry. Typically, this is carried out using ion-mobility spectrometry, however these instruments are often low resolution which can give out false positive results. Using mass spectrometry can provide specificity through the generation of multiple characteristic ions whilst also providing acceptable levels of sensitivity. When coupled with an ambient ionisation technique results can also be produced rapidly with minimal sample preparation required. Here we investigate the sensitivity for 5 different explosives: HMTD, TNT, RDX, tetryl and PETN using two ambient ionisation techniques, atmospheric solids analysis probe (ASAP) and thermal desorption corona discharge (TDCD).

Results: Five explosives were selected to reflect the most common classes of high explosive; HMTD (peroxide), TNT (nitro aromatic), RDX (nitroamine), tetryl (nitromine/nitro aromatic), and PETN (nitrate ester). Explosive certified reference standards were diluted in methanol to produce calibration curves (n=5) and a set of infinite dilutions to determine the limit of detection. Thermal desorption experiments were carried out on the Waters Acquity QDa mass spectrometer with fibre glass swabs for sample introduction and ASAP experiments were carried out on the Waters RADIAN mass spectrometer using glass rods for sample introduction. The identity of the ion species produced from each explosive have been identified and the ion chemistries elucidated. The linearity and repeatability were in line with what would be expected for an ambient ionisation method for all the explosives analysed. The limits of detection show nanogram sensitivity on both ambient techniques, with picogram sensitivity observed for RDX, TNT and tetryl using ASAP.

Conclusions: Both ambient ionisation techniques provide limits of detection that may be suitable for the trace detection of explosives within a security setting. Whilst ASAP appears to be more sensitive as a result of lower background signals (due to the glass rod sample introduction), the fibre glass swabs used with thermal desorption provide a better sampling device in a real-world setting (effectively providing sample pre-concentration by sampling from a wide area).

Simplifying the detection of explosives in a walk-up environment

John H. Moncur - SpectralWorks Limited, Peter Luke - Mass Spec Analytical, Richard Sleeman - Mass Spec Analytical, Charles Liddell - Mass Spec Analytical, Scott J. Campbell - SpectralWorks Limited

Aims: Rapid screening and detection of explosives is a critical capability for global security. Here we present an ambient ionisation interface to a triple quadrupole mass spectrometer instrument which allows the comprehensive MS data to be reduced to a simple Yes/No result for non-MS users such as security personnel. The touch screen software interface is scalable from single instrument implementation through to an extensive enterprise or cloud-based solution. The instrument agnostic software and ambient ionization hardware allows for the application to be run on any MS vendors' instrument from quadrupole to time of flight. As well as instant feedback to the user, results can be reviewed by expert users, either onsite or remotely, as well as archived and retrieved for data mining purposes and trend analysis.

Results: The following hardware and software are used in this workflow:
Thermal Extraction Ion Source (TEIS) – Mass Spec Analytical, Bristol, UK.
SCIEX Triple Quadrupole 3500 LC-MS System – SCIEX, Concord, Toronto, Canada.
RemoteAnalyzer Open Access Software – SpectralWorks, Runcorn, UK.
Scentinel (powered by RemoteAnalyzer) Software – Mass Spec Analytical/SpectralWorks.
The analytical workflow is as follows:

1. QC Check using explosives mixture or simulant

Prior to sampling the validity of the instrument needs to be verified by inserting a check solution into the desorber and obtaining a result within predetermined parameters. The results are shown as a Red or Green indication.

2. Blank checks of gloves and swab

A clean swab is sampled to ensure it won't trigger a positive result. The swab is then used to check the user's gloves and/or working area. Each sample is passed through the desorber in turn and if any positive results should occur the swab and gloves are replaced, work area cleaned if required, and retested. The process is repeated until a clean indication is obtained

Simplifying the detection of explosives in a walk-up environment

John H. Moncur - SpectralWorks Limited, Peter Luke - Mass Spec Analytical, Richard Sleeman - Mass Spec Analytical, Charles Liddell - Mass Spec Analytical, Scott J. Campbell - SpectralWorks Limited

3. Sample Collection

A Whatman No2 filter paper has been selected as our swab. It is rubbed back and forth by hand across any surface of interest approximately 4 or 5 times to allow transfer from the surface to the swab.

4. Sample Acquisition

Touch screen selection or a few mouse clicks is all that is needed to select the sample type and method before initiating the start sample. On-screen directions and audio, if selected, instruct the User on when and how long to insert the swab.

5. Results

The analytical run takes 8-10 seconds. The screen updates with a list of all the compounds looked for and positive results, if any, are indicated in red.

6. Confirmation of positive results (optional)

Positive indications can be repeated by selecting a confirmation file from a drop-down menu. The confirmation file is more specific and only includes the compound of interest. In the case of the Sciex MSMS instrument used we have included more ion pairs in the method to increase specificity.
Replace the swab with a clean one, re-test the gloves and swab. Re-swab the area where the positive result was obtained and submit the sample again. As before, the sample result will be shown with either a red or green indicator to show a positive or negative result.

Conclusions: A simple workflow for the detection of explosives has been shown. Each swab takes aproximately 8-10 seconds to analyse and indicate near instantanious results easily understood by a non technical/scientific User.
Where traceability is required automated data storage along with reporting and E-mail notification dependant on results can also be selected in the functionality.
This approach can be extended to other analytes of interest and has been demonstrated with drugs of abuse and pesticides.The number of analytes simultainiously detected and displayed on screen is only limited by the instrument selected. A simple scroll function or a float to the top feature can be utilised to bring results to the users attention in instances where the number of compounds exceed what will physically fit on screen.

ASAP mass spectrometry provides rapid answers to synthesis problems

Patrick Sears - University of Surrey, Ian Riddlestone - University of Surrey, Ashley Sage - Waters Corporation, David Douce - Waters Corporation, Ethan Bexley - University of Surrey

Aims: The aim of this work was to establish whether a synthetic process (producing mesityl amidinate ligand from bromomesitlyene, mesitylene and carbodiimide) had been successfully completed.

Results: 1H-NMR was attempted but due to the complexity of the spectra (including cyclohexyl groups and a significant delocalisation) it was not possible to assign all the peaks such that the product could not be identified. RADIAN ASAP was attempted instead. The MS data clearly showed the desired product together with traces of the reactants.

Conclusions: ASAP mass spectrometry can be used to rapidly identify organic synthesis products. The low level of fragmentation generated by this ambient ionisation technique made interpretation of the signals from mass spectrometer easy, even for an inexperienced user!

The low cost and ease of use of the mass spectrometer used for this study makes it suitable for use by undergraduate researchers.

Direct Probe ionisation Mass Spectrometry for rapid and accurate screening of environmental samples

Patrick Sears - University of Surrey, Tim Sidnell - University of Surrey, Madeleine Bussmaker - University of Surrey, Dave Megson - Manchester Metropolitan University

Aims: Analysis of environmental contaminants such as per- and polyfluoroalkyl substances (PFAS) and pesticides is typically achieved using chromatographic techniques, such as high-performance liquid chromatography, combined with mass spectrometry. These techniques can be complex, expensive, time consuming and can lead to inaccurate quantification due to sample container sorption and contamination by polytetrafluoroethylene (PTFE) components.

Results: Here, we demonstrate the novel application of Direct Probe ionisation Mass Spectrometry (DPiMS) to quantify a range of environmentally relevant compounds. We show how rapid quantification of different PFAS molecules can achieved over a wide concentration range with limits of detection in parts per billion.

Conclusions: Direct probe ionisation mass spectrometry (DPiMS) is a highly effective tool to add to the existing range of technologies for the quantification of environmental contaminants at concentrations seen in many real-world samples.

Direct analysis of synthetic cannabinoids

Patrick Sears - University of Surrey, David Douce - Waters Corporation, Ashley - Waters Corporation, Steph Brown - University of Surrey

Aims: A range of synthetic cannabinoids (SCs) have come onto the market in recent years and are widely mis-used in prisons across the country. The detection and identification of these materials poses many challenges for staff within prisons with few established methods suitable to detect materials being smuggled or even directly sent via mail. This work aims to use a simple ambient ionisation technique, atmospheric solids analysis probe (ASAP) to detect contamination on the outside of envelopes (such as transferred by unwitting criminals by means of Locard's principle).

Results: Samples of 7 different SCs have been analysed using ASAP. Optimisation of the analytical method has been completed and method parameters including desorption temperature, cone voltage, linearity and limits of detection have been assessed. The final method was used to detect 50 ng of SC which had been transferred to the outside of an envelope.

Conclusions: ASAP-MS is a sensitive method which can be used to detect and identify SCs with sub ppm limits of detection. Contamination could be detected on real world samples with no sample preparation necessary and with rapid clear-down times and good sensitivity.

Analysis of Aromatic Components in Tomato Juice

John Moncur - SpectralWorks Limited,Y. Kawakita - Shimadzu Corporation, Y. Sakamoto - Shimadzu Corporation, Scott J. Campbell - SpectralWorks Limited

Aims: It is known that taste, texture, and flavour are important elements of the "deliciousness" of food. In the recent food and beverage markets, differentiation from other products has been promoted through product development with the aims of higher quality and higher functionality.
Breeding and selection of food materials is one method for achieving differentiation. For example, it is known that the genetic differences between farm products influence differences in their aromatic components. Vegetable and fruit drinks are also marketed as foods with health claims which do not contain flavour additives, heightening the need for evaluation of differences in the materials (products) themselves. A multivariate analysis of the metabolites in three types of tomato juice was carried out, and the differences in the components contributing to taste and functional components between the products were introduced.
In this article, an analysis of aromatic components by the headspace (HS) method was conducted using the three commercially-available tomato juices, and deconvolution and multivariate analysis were conducted using the AnalyzerPro XD software (SpectralWorks Limited).

Results: Three types of commercially available tomato juice were used as samples. These products were selected because the raw materials used in production were stated to be limited to only tomatoes or tomatoes and common salt (table salt). A Shimadzu headspace (HS) gas sampler was used to capture the aromatic components. The trap model of the HS-20 headspace gas sampler series features a built-in electronic cooling trap and enables high sensitivity analysis by concentrating aromatic components. Samples containing moisture can also be analysed by concentrating low boiling point compounds to high boiling point compounds. Samples were prepared by taking 5 mL of the tomato juice in a 20 mL headspace vial and adding 10 µL of a 10 µg/mL solution of p-bromofluorobenzene (methanol) as an internal standard.

Analysis of Aromatic Components in Tomato Juice

John Moncur - SpectralWorks Limited,Y. Kawakita - Shimadzu Corporation,Y. Sakamoto - Shimadzu Corporation,Scott J. Campbell - SpectralWorks Limited

AnalyzerPro XD is a software program that can carry out the entire series of processes from direct reading of the data acquired by a Shimadzu GC-MS, deconvolution, peak detection, library search, alignment and comparison of the differences between samples. In multivariate analyses, it can conduct principal component analysis (PCA) and volcano plots for two-group comparisons. Data were acquired 3 times for each sample, and the intensities of the detected compounds were normalized by using p-bromofluorobenzene as an internal standard.

Principal component analysis (PCA) was conducted using the results obtained from the three tomato juices.

Conclusions: The distinctive components between tomato juice products could be searched by capture of aromatic components with a headspace sampler and an non-targeted multivariate analysis by AnalyzerPro XD. An objective evaluation of the influence of differences in the types of raw materials/production process on aromatic components is possible by this approach

Direct Analysis of Drugs in Saliva and Plasma

Toni Jackson - University of Surrey, Dr Patrick Sears - University of Surrey, Dr Valerio Converso - LGC, Chris Hopley - LGC

Aims: The screening of drugs in biological matrices is a frequent practice, for which it is important to have rapid and reliable analyses. Driving under the influence of drugs (DUID) has attracted attention in the last decade, which has seen the UK government introduce legislation specifying threshold limits, in whole blood, for a suite of drugs known to cause impairment. Subsequently, this has pressed for the development of effective, yet efficient, methods to provide on-site screening of individuals suspected of driving whilst impaired, as the current preliminary tests present limitations.

The emergence of ambient ionisation techniques and their coupling to low-cost miniaturised mass spectrometers have demonstrated promise for routine in-situ analyses beyond traditional laboratory settings. Preliminary experiments evaluated the use of atmospheric solids analysis probe (ASAP-) and thermal desorption (TD-) techniques in conjunction with a Waters QDa; a small footprint, inexpensive single quadrupole mass spectrometer (QMS). These techniques were assessed for their applicability to identify a small number of medicinal drugs in synthetic saliva and plasma by way of selectivity and sensitivity, with the intention to support DUID incidents.

Results: Calibration standards (1000 - 10000 ng/mL) were prepared in methanol, synthetic saliva and crashed plasma. Methanol and synthetic saliva were spiked directly with the drug, whilst crashed plasma was dried under nitrogen before being reconstituted with the corresponding concentration of drug in methanol. Samples were introduced into the ASAP-QDa system by a sealed glass capillary that had been dipped into the solution, whereas samples were introduced into the TD-QDa system by a PTFE-fibreglass coated sample trap onto which the drug had been deposited. Where required, a series of infinite dilutions (<1000 ng/mL) were analysed in each matrix to estimate the limit of detection (LOD) for each drug.

Direct Analysis of Drugs in Saliva and Plasma

Toni Jackson - University of Surrey, Dr Patrick Sears - University of Surrey, Dr Valerio Converso - LGC, Chris Hopley - LGC

The parameters to have exhibited the greatest influence on the detection of the drugs were desorption temperature and the cone voltage. An optimum desorption temperature that yielded the strongest signal intensity across the selection of analytes was chosen. Variable sample cone voltages generated analyte-specific fragment ions and introduced a degree of specificity not normally associated with QMS. Both methods demonstrated high selectivity when a multi-analyte solution was employed for analysis.
Greater sensitivity was demonstrated by the ASAP-QDa system (LODs <1000 ng mL-1) compared to the TD-QDa system (LODs ≥ 1000 ng mL-1). An unmatched internal standard (IS) improved the linearity and standard error at each concentration of the calibration curve, as a matched IS shared fragment ions with two of the drugs present in the multi-analyte solution.

Conclusions: Drugs were detected in synthetic saliva and plasma by both ASAP- and TD-QDa, and suitable specificity and selectivity required for a screening method were observed. However, the sensitivity was greater for ASAP-QDa as the LODs were closer to the legislative threshold limits than TD-QDa. As expected, the LODs were greater in synthetic saliva and plasma than in methanol as a result of matrix effects. The linearity and standard error were improved by the introduction of an internal standard, but plasma on the ASAP-QDa system yielded the greatest results at the tested concentration range.

The preliminary work for both ASAP-QDa has demonstrated greater promise for use a screening method to support on-site analyses of DUID incidents. However, sensitivity for the TD-QDa could be improved by altering the sample substrate material and/or introducing a pre-treatment procedure to reduce interferences from any impurities that may be present on the surface of the sampling material.

Ambient ionization of intact soluble and membrane protein assemblies.

Oliver J. Hale - University of Birmingham, Helen J. Cooper - University of Birmingham

Aims: Ambient ionization techniques have generally focused on the analysis of low molecular weight analytes, e.g., metabolites and lipids, and have found use in determining the spatial distribution of these analytes in samples including thin tissue sections. Analysis of higher mass analytes, such as proteins, has generally been limited to molecular weights up to 20 kDa and the use of denaturing conditions resulting in the loss of structural information. Protein function within organisms often relies on the formation of non-covalently bound assemblies with metal ions, small molecule ligands, or other proteins. We recently modified nanospray-desorption electrospray ionization (nano-DESI) for intact protein analysis under non-denaturing “native” conditions i.e. quaternary structure remains intact. These conditions have enabled the analysis of analytes exceeding 100 kDa in molecular weight without tissue preparation or protein purification. These protein assemblies present in their endogenous ‘native’ stoichiometry and are often found with endogenously-associated ligands and/or metal ions. Operated in a mass spectrometry imaging (MSI) mode, nano-DESI can map the spatial distribution of protein assemblies within tissue. Furthermore, unknown assemblies and their constituents can be identified through accurate mass measurements and top-down analysis. Examples of the capabilities of nano-DESI as a platform for native protein analysis are presented.

Results: Fresh frozen tissue (rat kidney, mouse and rat brain, sheep eye lens) was sectioned to 10-20 um thickness and thaw mounted onto glass slides. Tissue was not further washed or prepared for analysis.

All experiments were performed using a home-built nano-DESI ion source attached to an Orbitrap Eclipse mass spectrometer operated in positive or negative ion modes as required. The solvent system was 200 mM aqueous ammonium acetate + C8E4 detergent added at 0.5 – 2x its critical micelle concentration. Solvent flow rate was 2 uL/min and spray voltage optimized between 0.8 and 1.3 kV.

Ambient ionization of intact soluble and membrane protein assemblies.

Oliver J. Hale - University of Birmingham, Helen J. Cooper - University of Birmingham

MSI was enabled by scanning the nano-DESI probe across tissue sections at 10-20 um/s and assembling the line scans into a single imzML file post-analysis.
Top-down analysis was also performed directly from tissue. Proton transfer charge reduction (PTCR) and high-resolution mass spectrometry was used for intact mass determination. Top-down analysis of membrane and soluble protein assemblies was performed using collisional dissociation (HCD or resonant CID) and high-resolution mass spectrometry.
Intact soluble protein assemblies were analysed directly from rat brain and kidney. For example, the homodimer of malate dehydrogenase 2 (66 kDa) was homogenously distributed in the kidney but exhibited enhanced distribution in grey matter of the brain, whereas zinc-bound carbonic anhydrase (29 kDa) was found most abundantly in brain white matter. The homodimer of alpha-enolase (94 kDa) binds four magnesium cations and was observed distributed within kidney cortex tissue.
The membrane protein Aqp0 was analysed from eye lens tissue by solubilisation in detergent micelles. Aqp0 is a tetrameric assembly with intact mass approx. 113.1 kDa. Aqp0 ions were released from detergent micelles by application of collision voltage in the source region of the mass spectrometer. The tetrameric stoichiometry was confirmed by dissociation of the assembly to monomer subunits (28.3 kDa). Sequence information was obtained by HCD of the monomer, resulting in a sequence coverage of approx. 11%. Homotetrameric soluble proteins of molecular weight approx. 94 kDa were co-analysed and identified.Conclusions: Intact protein assemblies were sampled and analysed directly from tissue by nano-DESI with non-denaturing solvent systems. Furthermore, addition of detergent micelles to the solvent allowed the analysis of intact membrane protein assemblies exceeding 100 kDa in molecular weight. Nano-DESI also enabled mass spectrometry imaging of protein assemblies, by which their spatial distribution was mapped. Dissociation of the protein assemblies to their constituents enabled determination of assembly stoichiometry. Moreover, sequence ions determined from proteins and fragments from small molecule ligands enabled confident identification of the constituents. The work shows nano-DESI coupled to a high performance mass spectrometer to be a comprehensive platform for in situ analysis of protein assemblies.

THE BRITISH MASS SPECTROMETRY SOCIETY

AI & EFA SIG 1-DAY MEETING

GUILDFORD ✪ 27 APRIL 2022

E-ABSTRACT BOOK

In partnership with

UNIVERSITY OF SURREY

www.bmss.org.uk

BMSS