Mass spectrometry is transforming how we diagnose and treat disease, protect our environment, while also improving industry efficiencies – thanks to instrument innovators who continue to push the boundaries. From lipid characterization, to PFAS, to synthetic aviation fuels, this supplement showcases a selection of impactful mass spectrometry applications from leading companies.

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This study investigates the efficacy of the Thermo Scientific™ Orbitrap Exploris™ GC 240 mass spectrometer coupled with Compound Discoverer™ software for analyzing soil samples. By detecting a range of contaminants such as PCBs, PCDDs, PAHs, BFRs, and pesticides, the method demonstrates its capability for comprehensive differential analysis. The results reveal significant statistical differences between soil samples from various locations, highlighting the potential of this approach for environmental monitoring and assessment.

Environmental samples, (e.g., soil, sediments, or surface water) can contain a broad spectrum of volatile or semi-volatile contaminants, including polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polyaromatic hydrocarbons (PAHs), brominated flame retardants (BFRs), and pesticides. The combination of gas chromatography (GC) with quadrupole-based mass spectrometers is a common analytical setup for detection of these contaminants. GC-MS quadrupole-based instruments are well known for their robustness and ease of use. However, they have some important limitations for this application. Single quadrupole instruments can perform full scan analysis, but their sensitivity and selectivity in this mode are limited. Triple quadrupole (GC-MS/MS) systems have greater sensitivity and selectivity; however, their advantages are limited to targeted acquisition within a specified compound list. Creation of targeted methods requires time-consuming optimization and use of analytical standards, which can be very expensive and, on occasion, unavailable. However, the biggest drawback is that only targeted compounds included in the method can be detected, whereas other contaminants present will be overlooked.

High-resolution accurate mass (HRAM) mass spectrometry provides a very sensitive and selective non-targeted application and surpasses quadrupole instruments in all non-targeted applications. Orbitrap™ MS-based instruments offer unmatched resolving power (up to 240 000 at m/z 200), mass accuracy greater than 1 ppm, wide dynamic range, and high sensitivity. However, to fully realize the benefits of a HRAM system, powerful software is essential to convert high quality data into scientific discovery. Thermo Scientific™ Compound Discoverer™ software is designed to process large non-targeted data sets acquired using high-resolution mass spectrometry instruments, like the Orbitrap® Exploris GC 240 mass spectrometer (www.thermofisher.com/OrbitrapExplorisGC240). The software contains a wide range of tools for unknown compound identification and statistical analysis.

In this study, GC-Orbitrap technology and Compound Discoverer software were used to assess the chemical profile of soil sample extracts taken from three locations near Bremen, Germany. Data were acquired in full-scan with electron ionization (EI) mode. Positive chemical ionization (PCI) and negative chemical ionization (NCI) were used to confirm the elemental composition of the molecular ions using accurate mass information, isotopic match (measured versus theoretical), and presence of specific adducts.
DETERMINING OPTIMAL BUFFER CONCENTRATION FOR HIGH-THROUGHPUT INTACT PROTEIN ANALYSIS

By Jacob W. McCabe, Aaron Stella, and Anuja Bhalkarikar

This application note demonstrates a rapid method to determine the optimal buffer conditions for the intact protein analysis of myoglobin. Choosing the correct buffer is crucial to maintaining the activity and folding of a protein. To preserve the desired activity, scientists must prepare their protein in traditional, biochemically relevant buffers (Good's buffers), regardless of the endpoint analysis.

This application note demonstrates a rapid analysis of myoglobin in 16 different buffers at 9 different buffer concentrations, performed on the SCIEX Echo® MS+ system with ZenoTOF 7600 system (Figure 1). Rapid method development was performed using standard peak mode (1 second per sample) with an additional delay of 3 seconds per sample to account for changes in buffers and buffer concentrations. This added delay between ejections is not required for general analytical analysis. In approximately 34 minutes, the optimal buffer and concentration were determined for apo- and holo-myoglobin in triplicate, for increased confidence. This method is not limited to myoglobin and can be applied to other analytes of interest.

Optimal buffer concentration

The optimal buffer concentration for protein mass spectrometry experiments can be challenging since many buffers are available for such studies. Furthermore, choosing the optimal buffer at the optimal concentration adds complexity to the experiment. Method development for selecting the appropriate buffer and correct concentration is time-intensive and can delay final assay experimentation without a high-throughput solution.

The Echo® MS+ system uses acoustic ejection mass spectrometry (AEMS) technology. AEMS is a label-free sampling technique that operates at rates of up to 1 sample per second. The high-throughput nature of AEMS enables the rapid determination of optimal buffer conditions for 384 samples in approximately 10 minutes, compared to the 32 hours that would be required for a conventional method utilizing LC-MS at a rate of 5 minutes per sample. The Echo® MS+ system with ZenoTOF 7600 system offers a high-throughput solution for determining the optimal protein buffer concentration. In this study, apo- and holo-myoglobin in varying buffer concentrations were acoustically ejected using an Echo® MS+ system with ZenoTOF 7600 system. The data were then reconstructed using the mass reconstruction workflow in SCIEX OS software to monitor the intact mass of apo- and holo-myoglobin. Average peak areas of the reconstructed proteins were calculated and plotted to visually determine the optimal buffer concentration for a particular protein in a particular buffer.

Sample preparation

An array of 16 traditional, biologically relevant buffers were selected and plated onto a 384-well microtiter plate. The buffers were 2x serially diluted horizontally across the plate until row T. All 10µM myoglobin was added to each well to monitor the response of the apo- (blue trace) and holo- (pink trace) myoglobin under the different buffer conditions. The PBD structure is shown from 1MBN. 2.

Key benefits of high-throughput intact protein analysis:

- Determine optimal buffer concentration at a rate of 4 seconds per sample
- Platform sample preparation: easily apply the sample preparation method to small and large molecules
- Increased data confidence: perform customized replicate analysis using technical or analytical replicates (n=3) of 100 nL per ejection

Conclusion

- The optimal concentrations were determined across buffer conditions for apo- and holo-myoglobin
- Sixteen buffers were screened at 10 concentrations in approximately 11 minutes
- Low sample consumption of 100 nL per ejection allowed for technical replicate analysis

Figure 2: Average reconstructed area values for apo- and holo-myoglobin in buffers with varying TRIS, MgCl2 and TCEP concentrations. Average area response is shown in light blue for apo-myoglobin and dark blue for holo-myoglobin. Error bars represent 1 standard error of the mean.
**OXYGEN ATTACHMENT DISSOCIATION (OAD) MS/MS FOR THE STRUCTURAL CHARACTERIZATION OF LIPIDS**

Innovative fragmentation technology enables the identification of carbon-carbon double bond positions in lipids that relate to their biological functions

By Emily G. Armitage and Neil J. Loftus

Oxygen Attachment Dissociation (OAD) is an innovative fragmentation technology, providing additional structural information in Collision Induced Dissociation (CID) on the LCMS-9050 QTOF Mass Spectrometer. Irradiating oxygen radicals are used to specifically oxidize/dissociate double bonds between carbons, which enables structural characterization of organic compounds such as lipids.

Structural classification of lipids requires the identification of head group, length of carbon side chains and the number and position of double bonds within them. Collision Induced Dissociation (CID) is useful for the identification of lipid class and length of carbon side chains, however the identification of carbon-carbon double bond position(s) in the fatty acid moieties presents a major challenge in unsaturated lipid characterization. Revealing double bond position(s) in lipids is vital to understanding their biological roles, since minor structural differences between positional isomers can drastically alter the biochemical function of a lipid.

OAD-MS/MS and CID-MS/MS spectra acquired simultaneously in positive or negative ion mode generate sufficient information to identify organic compounds such as lipids to the structural level from a minimal amount of sample. The enhanced level of structural identification provided by OAD-MS/MS allows the potential to improve our understanding of the biological roles of lipids and other organic compounds. The use of this new technology aims to enhance life science research by providing further clarification of biological processes and a better understanding of the roles of lipids as potential markers of health and disease states. Moreover, expanding the application of OAD to bioprocessing may bring about the development of higher quality and more functional products in the future.
proteomics are accessible with the MALDI HiPLEX-IHC workflow, which allows for highplexing of antibodies via an IHC methodology. Combining protein information with small molecules, including drug targets, increases the contextualization of biological systems.

The basic workflow using technology from AmberGen, Inc. includes the following steps: i) section tissue on conductive IntelliSlides®, ii) stain with photocleavable mass-tagged antibodies (AmberGen, Inc.) using a MALDI-friendly IHC staining process, iii) photocleave mass-tagged antibodies prior to matrix deposition using UV light, iv) apply MALDI matrix for analysis, v) analyze with a Bruker timsTOF fleX or rapifleX®, vi) visualize data in SCiLS Lab software from Bruker. The total workflow preparation time is around 2 hours, with whole slide profiling at 50 µm in under an hour.

Herein, a multiomic experiment was conducted, for targeted lipids and a drug molecule. On the same tissue section, target spatial proteomics using the MALDI HiPLEX-IHC workflow. Typical lipid signals for distinct regions of the brain were observed, for example the hypothalamus and Purkinje cells. Co-localization of lipids was observed with targeted proteins as well. Additionally, lipids co-localized to the drug target, JQ1.

By utilizing the MALDI HiPLEX-IHC workflow, targeted whole protein localization was linked to the distribution of a target drug molecule and lipids. Specifically, due to the low intensity of the drug JQ1, colocalization was used to suggest possible distributions of the drug, with examples showing unique distributions throughout the rest of the tissue. Two separate imaging acquisitions were used to acquire images for small molecules, as well as targeted protein localizations utilizing the mass tags from the MALDI HiPLEX-IHC process. Drug interactions with proteins of interest, as well as additional small molecule signals, are all accessible with MALDI Imaging workflows. The MALDI HiPLEX-IHC workflow combines MALDI Imaging with IHC to generate highly multiplexed images that can be combined with other imaging techniques for multimodal data, or subsequent MALDI imaging runs for multiomic data.

The interdisciplinary field of spatial biology continues to connect omics research areas with the goal of understanding the spatial distribution of biomolecules that influence biological processes and functions. Advanced imaging techniques continue to emerge on the market, but matrix-assisted laser desorption ionization (MALDI) Imaging, a mass spectrometry-based technique, is a widely accepted methodology for determining spatial localization of analytes on tissue and has been around for more than 20 years.

MALDI Imaging offers the only label-free spatial analysis technique for metabolites and lipids, and additional workflows make released glycans and intact proteins accessible for multiomic connections. New applications in targeted spatial proteomics are accessible with the MALDI HiPLEX-IHC workflow, which allows for high plexing of antibodies via an IHC methodology. Combining protein information with small molecules, including drug targets, increases the contextualization of biological systems.

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MALDI Imaging offers the only label-free spatial analysis technique for metabolites and lipids, and additional workflows make released glycans and intact proteins accessible for multiomic connections. New applications in targeted spatial
Performing at its best where others fail: real-time nosespace analysis with IONICON’s FUSION PTR-TOF 10k

Proton-transfer-reaction mass spectrometry (PTR-MS) is one of the only technologies that brilliantly masters the analysis and quantification of in-vivo volatile flavor release from different foods in real-time. IONICON’s latest PTR-MS advancements easily eliminate problems existing devices may have with highly complex matrices.

Due to the fact that the flavor of food is strongly determined by the compounds entering the nose during mastication and swallowing, nosespace analysis is of particular importance for food and flavor scientists. Thus, PTR-MS coupled to a nosespace-sampler has become an indispensable tool for food industry and research.

Several years ago, we attempted a nosespace study during the consumption of different instant soups utilizing a (back then) state-of-the-art PTR-MS instrument with about 5,000 m/Δm mass resolution. To our surprise, we could not find reasonable correlations between flavor-relevant compounds in the soup and quantified in real-time, with the measured concentrations decreasing with each subsequent exhalation. Because of the extremely high sensitivity of the PTR-MS instrument, the compound can still be detected at a slightly elevated level after 20 exhalations, which is when a second sip was ingested.

In summary, the study confirms that the FUSION PTR-TOF is indeed the "next generation" of PTR-MS, opening up analytical worlds that were previously inaccessible.

The synthetic aviation fuels (SAF) market has seen recent growth that is expected to continue thanks to regulations like the European Union’s “ReFuel EU” proposal and the United States Sustainable Aviation Fuel Grand Challenge. SAFs derived from sustainable and renewable resources are less impactful on air quality than traditional fossil-based aviation fuels due to their processing pathways reducing the total aromatics and sulfur content. However, a certain amount of aromatic content is still necessary to maintain the proper freeze points, viscosity, and polymeric sealing properties in a jet fueling system. Thus, it is of high importance to know not only the physical properties of a new fuel, but also the chemical makeup. With a combination of comprehensive two-dimensional gas chromatography (GCxGC) and time-of-flight mass spectrometry (TOFMS), the high-quality information necessary for a deeper understanding of the composition of synthetic aviation fuels can be produced and utilized to expedite the certification process and gain approval for use.

Figure 1. (upper panel) Two mass spectra of chicken soup nosespace analysis. After 10 "blank" exhalations through the nose (sampled directly into the FUSION PTR-TOF 10k, without the need for any preparation) to establish a baseline, the participant takes a sip of chicken soup. C,H,O is detected and quantified in real-time, with the measured concentrations decreasing with each subsequent exhalation. Because of the extremely high sensitivity of the PTR-MS instrument, the compound can still be detected at a slightly elevated level after 20 exhalations, which is when a second sip was ingested.

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STREAMLINING HYPerHENED MS DATA PROCESSING ACROSS DATA FORMATS

Easier accessibility to MS data with browser-based data processing, analysis, and storage

By Baljit Rains

Browser-based applications are accessible from any device and offer easier accessibility and flexibility to decrease the barrier to data handling. The Spectrus JS family of applications offers vendor-neutral, browser-based hyphenated MS data processing, analysis, databasing, and reporting of low and high-resolution GC/MS, LC/MS, and LC/UV/MS (xC/UV/MS) data.

Browser-based component analysis of hyphenated MS data and cloud-based storage

The demand for more efficient analysis of complex samples and the need for flexible access due to the new norm of remote/hybrid working environments are key driving factors in the evolution of technology to analyze mass spectrometry data. Browser-based applications are accessible from any web browser and computers offer the most flexibility.

Vendor-neutral hyphenated data processing

With labs commonly using multiple instruments and vendors, instrument diversity adds to the complexity of deciding which processing tool to use. In addition to being browser-based, the Spectrus JS family consists of applications that offer vendor-neutral hyphenated xC/UV/MS data processing, further decreasing the barrier to data handling.

These applications allow low and high-resolution xC/UV/MS data (including DDA (MSn) and DIA (AIF)) to be processed, stored, and reported in the same easily accessible interface, so that scientists are no longer tied to instrument software, or the lab/office computer.

Component analysis of xC/UV/MS data

The component identification process involves the extraction of ion chromatograms (XICs), peak integration, and grouping spectral features to generate a component mass spectrum within the chosen web browser. The advanced component analysis algorithm coming soon to the Spectrus JS family of applications, runs datasets for untargeted extraction of chemical features through peak detection, ion grouping, and component interpretation. It can also be used in a targeted manner when supplied with structure(s) and/or mass(es). Results from the component extraction algorithm include extracted components (including those that co-elute), pure component spectra, and component MS2 spectra, along with spectral interpretation (molecular, confirmatory, and fragment ions). The algorithm reduces interpretation time and allows the identification of small components that may be overlooked in manual assessments.

Cloud-based storage of results

Spectrus JS applications allow scientists to store results in centralized cloud-based databases, preserving chemical context (chemical structure, user interpretations, metadata, etc.) and data integrity with an audit trail. Databases are easily searchable by chromatographic, and mass spectral features and data can be retrieved for seamless review or re-analysis through the web-based application. Reports can be created and viewed instantly.

The Spectrus JS family enables more accessible and efficient processing, analysis, and storage of hyphenated MS data.
MEASUREMENT OF PFAS IN COMPLEX CONSUMER PRODUCT MATRICES USING NEGATIVE REAGENT IONS

By Leslie P. Silva, Stefan Swift, Patrik Španěl, and Vaughan S. Langford

Selected ion flow tube mass spectrometry (SIFT-MS) is a direct MS technique that utilizes eight reagent ions simultaneously to provide real-time analysis of compounds in whole air. This application note demonstrates the unique selectivity and high-sensitivity of SIFT-MS for quantitating volatile PFAS compounds. It was used to characterize two fluorinated species in consumer products such as shaving cream, fabric softener, deodorant, and dishwasher rinse aid.

Characterizing the presence of PFAS compounds in consumer products is important due to their persistence in the environment and potential toxicity to humans and animals. Testing for low-concentration PFAS impurities using direct measurement techniques can be challenging because many consumer products contain high concentrations of volatiles, resulting in reagent ion depletion and saturation effects (1). This leads to a loss of sensitivity for the PFAS contaminants.

Selected ion flow tube mass spectrometry (SIFT-MS) is a direct MS technique that utilizes eight reagent ions simultaneously to analyze whole air. This is advantageous in complex mixtures like personal care and household products, as each reagent ion offers unique selectivity through orthogonal ionization properties, potentially mitigating matrix effects caused by the more abundant volatile species present in these products. While PFAS compounds react with the eight reagent ions available in SIFT-MS, three reagent ions are insensitive to the more abundant components in these products: O$_2^-$, NO$_2^-$, and NO$_3^-$. This application note demonstrates selective, high-sensitivity quantification of two fluorinated species in consumer products such as shaving cream, fabric softener, deodorant, and dishwasher rinse aid.

PFPA and HFBA were measured in four consumer products using the five negative reagent ions (OH$^-$, O$_2^-$, NO$_2^-$, NO$_3^-$, O$^-$). Figure 1(a) displays the reagent ion signals for the negative reagent ions in a blank and fabric softener sample. Saturation was observed for O$^-$ and OH$^-$ (Figure 1(a) – light blue and pink traces) and they were excluded from the concentration calculations. Reagent ion signals for O$_2^-$, NO$_2^-$, and NO$_3^-$ (red, navy and purple) remain high in these complex matrices, enabling the remaining product ions for PFPA and HFBA to be averaged for determination of the headspace concentration. The concentrations obtained for the products are shown in Figure 1(b) (experimental details in full documents).

SIFT-MS successfully quantified PFPA and HFBA in consumer products at low levels, even when the overall matrix VOC concentration was high. The eight reagent ions in Syft Tracer™ provide enhanced selectivity, because three negative reagent ions can measure low concentrations of PFAS without any ion suppression effects. This prevents the need for sample dilution or pre-concentration, facilitating rapid sample screening.

Reference

Figure 1. (a) Comparison of reagent ion levels in a blank (filled circles) vs fabric softener (patterned circle). (b) Measured levels of PFPA and HFBA in four consumer products.
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