the Analytical Scientist

SPICING UP MASS SPECTROMETRY

From picking the perfect pepper to finding new biomarkers, applications of mass spectrometry are all around us. Here, we present five application notes that highlight the diversity of MS technology.

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Contents

SUPERCRITICAL FLUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY FOR THE ANALYSIS OF PESTICIDE RESIDUES IN DRIED SPICES

By Víctor Cutillas, María Murcia-Morales, María del Mar Gómez-Ramos, Sherif M. Taha, and Amadeo R. Fernández-Alba

Spices are complex matrices containing large amounts of essential oils, plant nutrients and secondary metabolites such as flavonoids, terpenes and alkaloids. These interfering matrix components produce ion enhancement or suppression which can be very strong and depends on the origin of the sample. Supercritical fluid chromatography is a separation technique with unique capabilities and advantages. It is considered a technique with "liquid-like solvation power and gas-like viscosity" and "tunable solvent" capability, also being a "green alternative" to conventional chromatography using organic solvents.

The technique has great potential for this purpose. Matrix effects – the main difficulty in the analysis of spices – are usually low in supercritical fluid chromatography for a wide variety of samples. In the present work, development and validation of a multiresidue method for the analysis of 162 pesticides in spices was carried out by SFC-MS/MS (Nexera UC, LCMS-8060, Shimadzu). These matrices, which show clear differences in their composition and appearance, were used in order to obtain representative data of spices. The validation study was performed in terms of recovery, linearity, matrix effect, intra-day and inter-day precision.

Different clean-up sorbents were tested for both matrices: PSA (primary secondary amine), Z-Sep (zirconium dioxide) and EMR (enhanced matrix removal). A matrix fingerprinting study was carried out in order to identify the extract with a lower number of coextracted matrix compounds. EMR sorbent provided the best results in both matrices. Samples of blank matrices (black pepper and cayenne) were spiked with a mixture of 162 pesticides at 50 and 200 μg kg^{-1} for the study of recoveries of the extraction method. Recoveries for the majority of compounds were in the 70-120 percent range recommended by DG-SANTE guidelines. At the level of 200 μg kg^{-1}, 152 compounds (corresponding to 94 percent) in black pepper and 146 compounds (91 percent) in cayenne showed recoveries within that range. Similar results were obtained at 50 μg kg^{-1}, with 149 and 144 compounds showing recoveries between 70 and 120 percent in black pepper and cayenne, respectively.

According to DG-SANTE, a practical default range of 60–140 percent may be used for individual recoveries in routine analysis if they are consistent (RSD ≤ 20 percent). Applying these criteria, at least 95 percent of pesticides meet the recovery range in all matrices at both concentration levels. Furthermore, 85 percent of the pesticides in black pepper and 91 percent in cayenne presented RSD values below 10 percent at the lowest recovery level (50 μg kg^{-1}). Linearity of the method was evaluated by employing matrix-matched standards corresponding to the 5-500 μg kg^{-1} range in the samples.

Most of the pesticides studied met the requirement to be identified at the lowest concentration level of 5 μg kg^{-1} in both matrices. It is remarkable that in most cases (93 percent of compounds in black pepper and 99 percent in cayenne), coefficients of determination of higher than 0.999 were achieved. Out of the 162 pesticides studied, 132 (corresponding to 82 percent) showed weak matrix effect in cayenne and 91 (56 percent) in black pepper. Strong matrix effect, on the other hand, was only found in 10 (6 percent) pesticides in cayenne and 27 (17 percent) in black pepper. These results compared favourably to those obtained in previous studies using LC-MS/MS to analyze pesticides in spices. The lower matrix effect can be explained on the basis of (1) lower flow rate in the source, resulting in a reduced size of the microdroplets and (2) replacement of water in the mobile phase with methanol, which has lower polarity and surface tension. These factors improve ionization efficiency of the analytes, resulting in lower signal suppression.

Furthermore, it is usual to inject more than 1 mg of sample using liquid chromatography coupled to mass spectrometry, producing important matrix interferences in the analysis of complex matrices. However, using this SFC-MS/MS method, the total amount of sample injected corresponds to 0.2 mg. To demonstrate the effectiveness of the method in routine analysis of spices, 47 real samples from different origins (Spain, Egypt, Portugal, India and Brazil) were analyzed. Within these 47 real

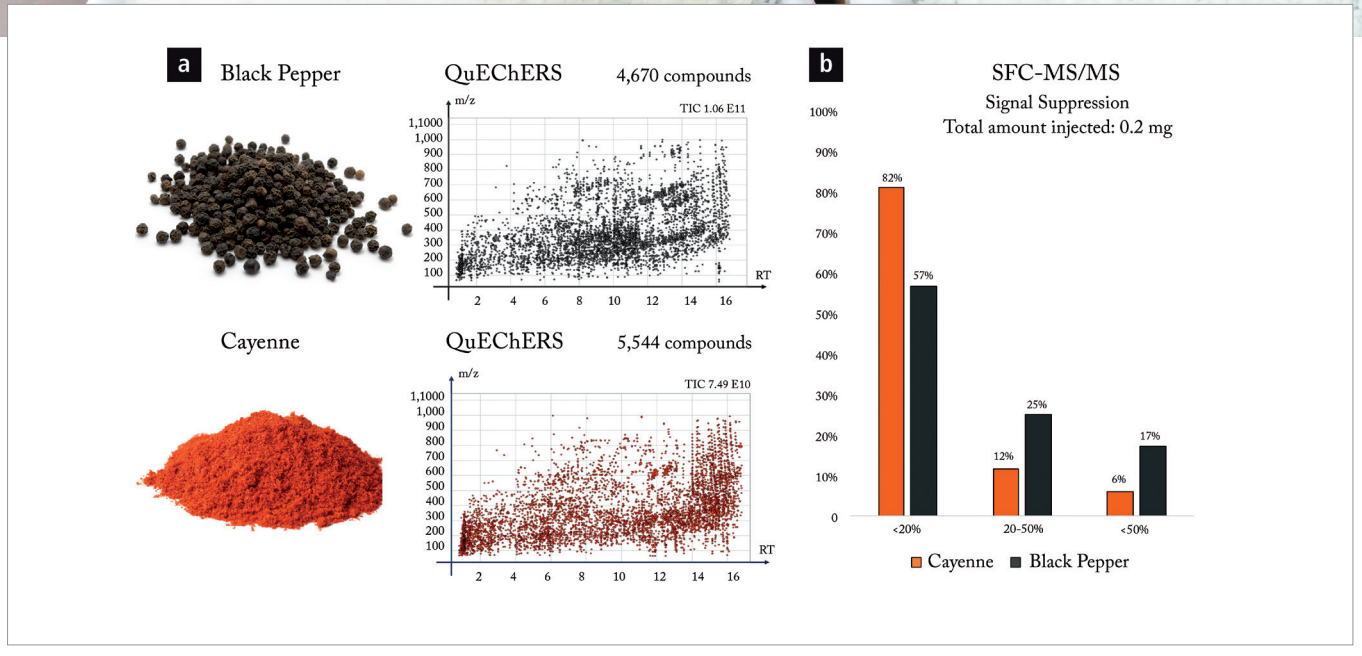


Figure 1. a) Coextracted matrix compounds in black pepper and cayenne. b) Signal suppression obtained analyzing both matrices by supercritical fluid chromatography coupled to mass spectrometry.

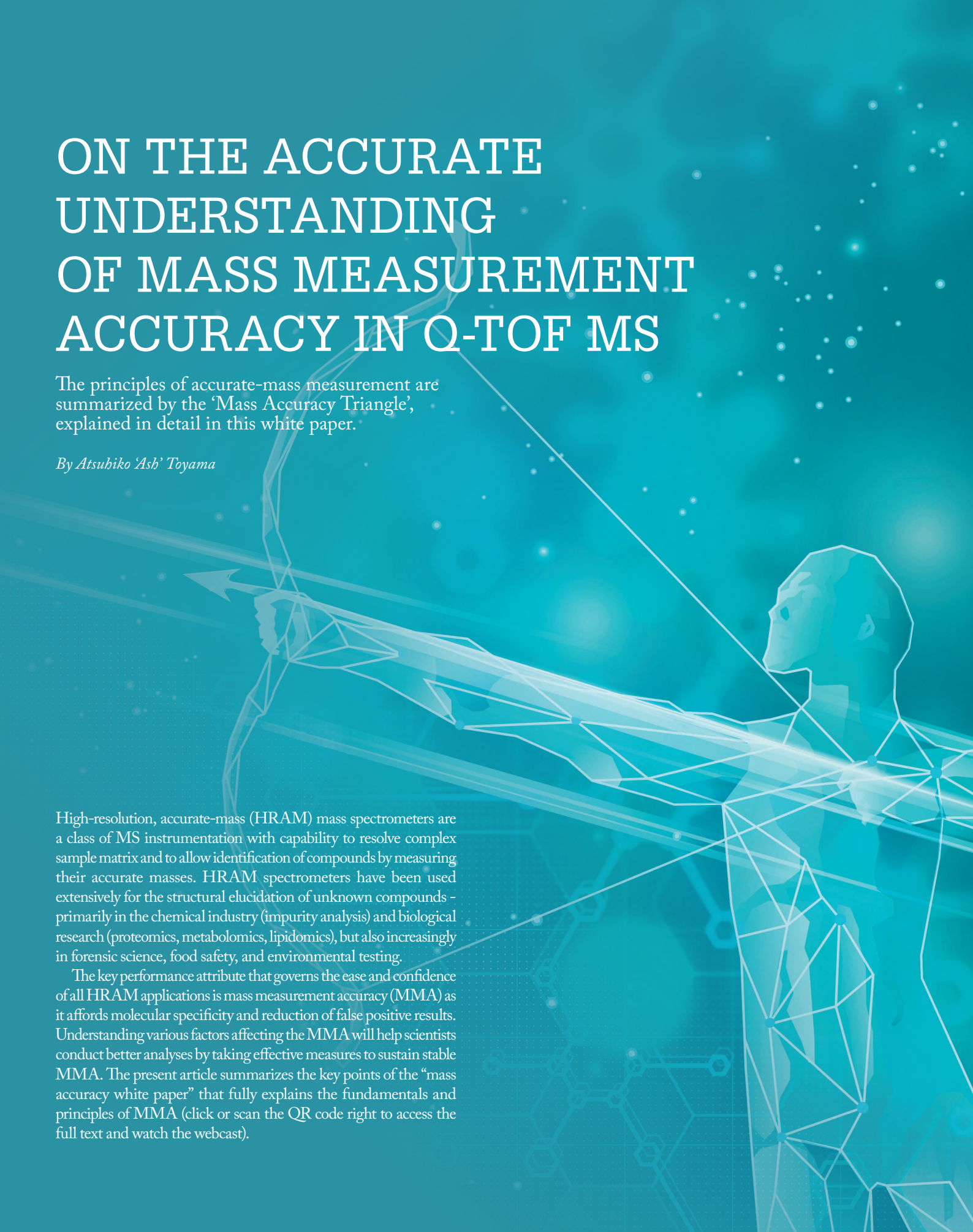
samples, 8 corresponded to black pepper and 12 to cayenne. The remaining 27 samples corresponded to paprika, cinnamon, dill, mint, thyme, oregano, parsley, tarragon, rosemary, basil, cumin, turmeric, ginger, curry, cardamom, anise, mate, clove, sesame and nutmeg. Of the 162 pesticides validated in the method, 46 were detected in the real samples analyzed. Pesticides were not found in 9 samples; therefore, in 81 percent of the samples, one or more pesticides were detected in a concentration exceeding the LOD. Fifteen samples (32 percent) contained 5 or more pesticides. The samples with the highest number of pesticides correspond to four cayennes and one paprika with 22, 20, 18, 14 and 10 pesticides respectively. In total, 198 positive results were found in the samples.

The most frequently found pesticides were carbendazim (40 percent of the samples), imidacloprid (34 percent), acetamiprid (28 percent), azoxystrobin (26 percent), metalaxyl (26 percent) and tebuconazole (21 percent). The rest of the pesticides were found in less than 20 percent of the samples. Twelve of the pesticides detected are not approved by the European Commission. Ten of these 12 pesticides were present in less than 10 percent of the samples analyzed but it is remarkable that the most detected pesticide (carbendazim) is one of these non-approved pesticides. Supercritical fluid chromatography coupled to mass spectrometry has shown itself to be a good technique for the analysis of pesticides in difficult matrices. The use of SFC-MS/MS provided a reduction of the ion suppression in most of the 162 pesticides studied in cayenne and black pepper. Despite the complexity of the matrix, the results obtained in the validation showed that the method was effective for the analysis of pesticides in spices.

ON THE ACCURATE UNDERSTANDING OF MASS MEASUREMENT ACCURACY IN Q-TOF MS

The principles of accurate-mass measurement are summarized by the 'Mass Accuracy Triangle', explained in detail in this white paper.

By Atsuhiko 'Ash' Toyama



High-resolution, accurate-mass (HRAM) mass spectrometers are a class of MS instrumentation with capability to resolve complex sample matrix and to allow identification of compounds by measuring their accurate masses. HRAM spectrometers have been used extensively for the structural elucidation of unknown compounds - primarily in the chemical industry (impurity analysis) and biological research (proteomics, metabolomics, lipidomics), but also increasingly in forensic science, food safety, and environmental testing.

The key performance attribute that governs the ease and confidence of all HRAM applications is mass measurement accuracy (MMA) as it affords molecular specificity and reduction of false positive results. Understanding various factors affecting the MMA will help scientists conduct better analyses by taking effective measures to sustain stable MMA. The present article summarizes the key points of the "mass accuracy white paper" that fully explains the fundamentals and principles of MMA (click or scan the QR code right to access the full text and watch the webcast).

Ion statistics – resolution and sensitivity

MMA, expressed as standard deviation of mass errors in ppm, can be predicted by dividing 425,000 by the product of mass-resolving power (in FWHM) and the number of ions being measured. This relationship explains why it is generally difficult to keep improving the MMA with increasing mass resolution, due to the inherent compromise of ion abundance in achieving exceptional mass resolution.

Tips on mass calibration

The process through which detector recordings are converted into *m/z* is called calibration. The white paper describes the rationale behind the 'rule of thumb' in mass calibration and the impact of reference compound selection on MMA, demonstrated with data acquired with Shimadzu's quadrupole time-of-flight (Q-TOF) MS, the LCMS-9030.

Stability of mass calibration

Calibration needs to be performed routinely to offset the changes occurring to MS hardware. In Q-TOF MS, two principal factors that cause 'mass drift' are temperature changes and electronic instability. To better understand the nature of mass drift, the LCMS-9030 was subjected to rigorous temperature changes within a 24-hour period, during which analyses were performed to evaluate the MMA. Three key findings given by the results (Figure 1) evidently support the conclusion that LCMS-9030 has overcome the temperature issue altogether. First, the pattern of mass drift closely synchronized with the room temperature change in agreement with the operating principle. Second, the mass errors returned to the original level at the end of the experiment when the room temperature returned to 24°C, demonstrating that the environmental interference on the instrument is reversible. Finally, the amplitude of mass drift was as small as ±1.5 ppm after experiencing ±3°C change in room temperature. This data has the potential to totally refashion the Q-TOF user experience, supporting long calibration intervals without requiring additional corrections.

Stability of mass calibration was further evaluated under a constant laboratory temperature to demonstrate the electronic robustness resisting mass drift. This time, all measurements resulted in less than 1 ppm mass error throughout the 60-hour duration of the experiment without any additional mass calibration. The mean of the errors over the 60 hours converged to near-zero for all compounds without a sign of mass drift.

Lock-mass correction

Given that external calibration is now reliable, it is recommended that users consider benefits and precautions on the usage of lock-mass correction and select the most appropriate approach.

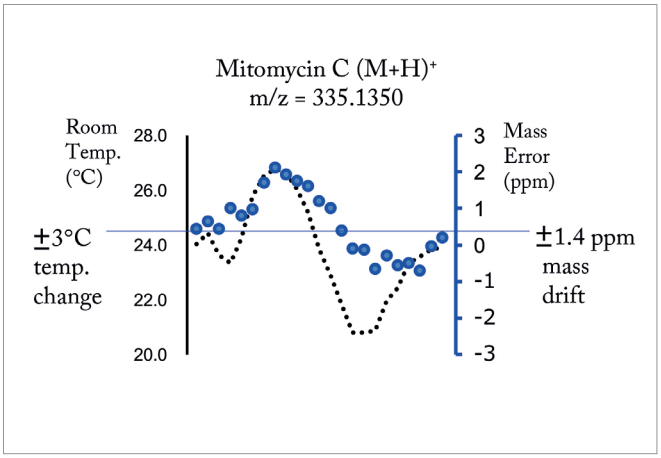


Figure 1. Hourly acquisitions were repeated for 24 hours and mass errors recorded (closed circles), while the ambient temperature was shifted by ±3°C.

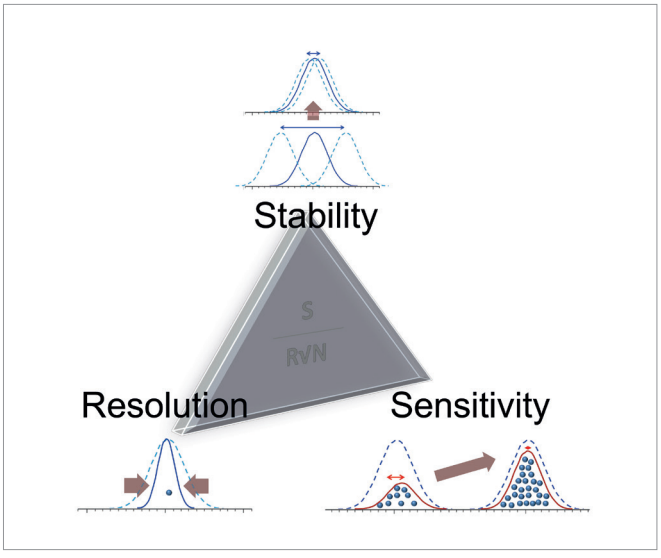


Figure 2. The 'Mass Accuracy Triangle' summarizes the important elements for attaining good mass measurement accuracy.

Conclusion

Understanding the 'Mass Accuracy Triangle' (Figure 2) clarifies that stability is the critical factor affecting the MMA in practice. Hence, the benefit of LCMS-9030 lies in its outstanding stability of MMA, giving users real confidence in compound identification and quantification with exceptional ease of use.

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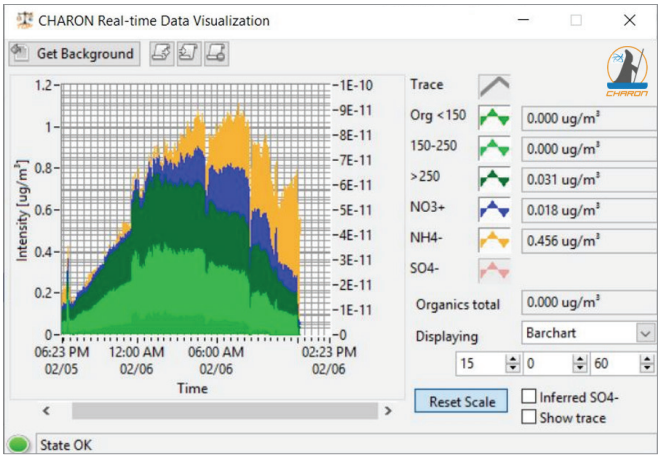
The revolutionary set-up enables IONICON PTR-TOFMS series instruments to measure aerosols directly with the most versatile, reliable and proven method for VOC analysis available on the market: PTR-MS. Now, one single IONICON instrument covers VOCs and also allows the molecular-level characterization of sub-µm particulate organic matter in real time. Low limits of detection enable laboratory-based studies as well as ambient measurement campaigns.



Real-time data visualization of sub-µm particulate matter captured by CHARON PTR-TOFMS at a conference in February 2019.

Watch the CHARON Webinar and learn more:
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PLASMA PROTEOMICS GOES HIGH THROUGHPUT

The timsTOF Pro with PASEF and the Evosep One for biomarker discovery in large sample cohorts of human blood plasma – 500 plasma proteins in 11.5 min.

By Thomas Kosinski [1], Raphael Heilig[2], Dalila Bensaddek[1], Nicolai Bache[3], Ole Bjeld Hørning3, Roman Fischer[2], and Heiner Koch[1]

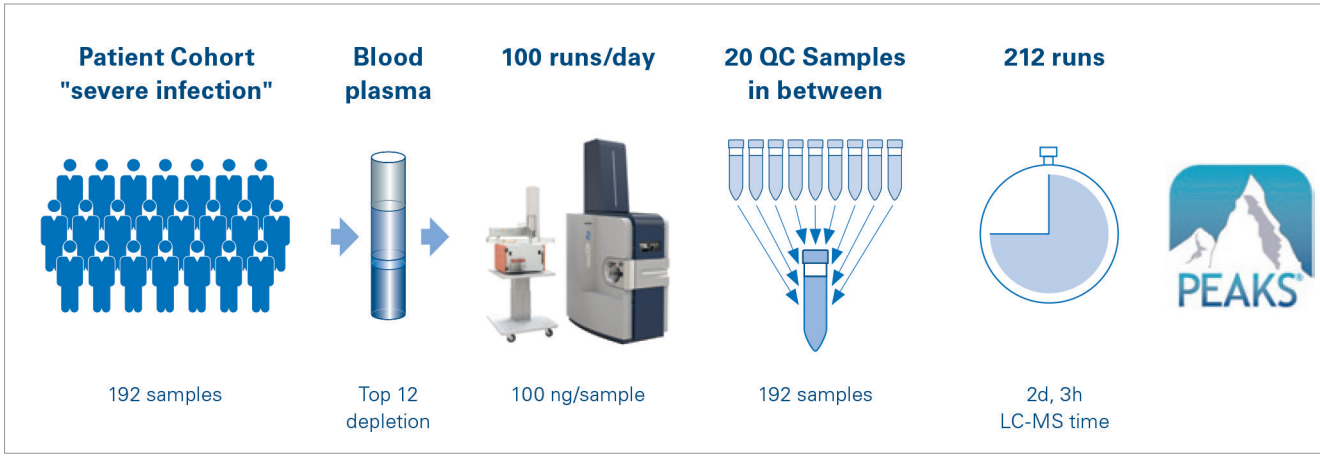
1 Bruker Daltonik GmbH, Bremen, Germany

2 Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom.

3 Evosep Biosystems, Odense, Denmark

Blood analysis is one of the most commonly performed procedures in medicine, where clinical parameters are used for diagnosis and for decisions on treatment options. Currently, biomarkers are typically derived from enzymatic or immunoassays and lack comprehensiveness. LC-MS/MS-based proteomics has long been a powerful research tool but has not provided the robustness and throughput to decipher new biomarkers in large cohort studies of blood plasma. Here, we have combined the robustness and speed of the timsTOF Pro with PASEF and the high-throughput LC separation provided by the Evosep One to analyze 100 ng samples of blood plasma from 192 severe infection patients at a sampling rate of 100 runs per day (11.5 min. gradient). After every 10th, a QC sample is included (20 total) to monitor the LC-MS/MS performance. High quantitative reproducibility was found across the study (R2 > 0.97, median CV 9.3 percent) and no systematic drift in peptide and protein identification. PEAKS X software (Bioinformatics Solutions) was utilized, which aligns features in four dimensions – retention time, intensity, m/z and ion mobility – to transfer identifications in a "match between run" design. Using this alignment approach, the number of quantified proteins was dramatically imroved from 188 to 500 protein groups on average per run. This depth of quantification is often not even achieved on LC-MS/MS runs of several hours in length. In the sample set, several proteins of intermediate or lower abundance (CRP, PSA, IFN-γ) were found, demonstrating that this workflow can facilitate biomarker discovery even at medium to low abundance analyte levels. Taken together, this provides a robust and high-throughput LC-MS/MS solution with sufficient depth for unbiased discovery of new biomarkers in samples of blood plasma relevant to clinical applications.

To download the full application note please see https://bit.ly/2Y9fJiV



Study design for plasma proteomics on the timsTOF Pro and Evosep One. Plasma samples from severe infection patients were collected and depleted for the top 12 most abundant proteins. Tryptic peptides (100 ng per sample) were separated on the 100 runs per day LC method of the Evosep One for subsequent delivery to the timsTOF Pro with PASEF. A pooled sample of plasma peptide digest (QC sample) was injected after each ten runs to monitor LC-MS/MS performance over time, resulting in a total analysis time of 2 days and 3 hours for 212 LC-MS/MS runs. Raw data were submitted post acquisition to data analysis in PEAKS studio.

AN UPDATE FOR PHARMACEUTICAL STRESS TESTING ENABLED BY MODERN INFORMATICS TECHNOLOGIES

How evolving analytical and automation tools are positively impacting forced degradation studies in pharmaceutical development.

Stress testing or forced degradation represents a fundamental part of the drug development process, specifically related to purity through control of stability. Quantitative assessments of pharmaceutical stability require "stability-indicating" methods (SIMs), and the development and validation of such methods is built on the foundation of well-designed and conducted stress testing studies. Their primary goal is to comprehensively induce pharmaceutically-relevant degradation pathways, such that all realistic degradation products are formed and can be analytically detected through validated SIMs.

The field of pharmaceutical stress testing has matured greatly in the past 20 years, and this whitepaper aims to summarize the growing impact of enabling technologies on the subject, and share how applying these new technologies can help alleviate the burden that stress testing places on process analytical teams. These advancements represent novel analytical and automation tools to support the stress testing process, broadly assisting with all relevant steps: guiding protocol design through theoretical predictions, designing and optimizing chromatographic separation and detection, elucidating degradation product structures, tracking degradation product peaks, and preparing meaningful and retrievable reports that can facilitate decision-making and collaboration.

Screenshot of ACD/Labs' Luminata displaying how forced degradation processes can be visually represented, where process map structures are directly associated with corresponding experimental conditions, LC/UV/MS data, and kinetic information.

For example, ACD/Labs' Luminata™ represents a novel tool designed to provide comprehensive analytical data management and decision support for stress testing, chemical process development, formulation development, impurity investigations, and associated control strategies. Luminata enables systematic capture, review, query, visualization, storage, and reporting of many data types, including LC/UV/MS datafiles from most major instrument vendors. It also allows for intuitive representation of forced degradation schemes (by condition), including color-coded structural process maps (with both observed and theoretical degradants), associated analyses, kinetic plots, spectroscopic characterization data, and interpreted reports. By connecting forced degradation reaction schemes with associated LC/UV/MS datasets, Luminata provides scientists with an automated means to assess whether known or theoretical degradation products were formed under any specific stress condition, through user-defined thresholds/levels.

Ultimately, pharmaceutical stress testing will continue to progress as degradation chemistry knowledge grows and associated analytical, spectroscopic, automation, and informatics tools are invented, developed, and implemented. Such informatics tools will hopefully be positioned to facilitate the transformation of data to information to knowledge, leading to informed, wise decisions.

Learn more at: https://www.acdlabs.com/StressTestingWhitepaper

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