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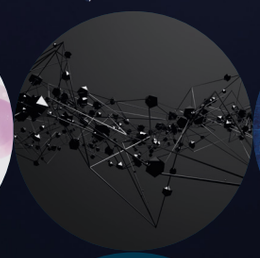
Mass Spectrometry at the Cutting Edge

From testing for toxins, to revealing the inner workings of the cell, the potential for mass spectrometry is limitless. Here, we've gathered a selection of the latest innovations and applications in this ever-evolving field.

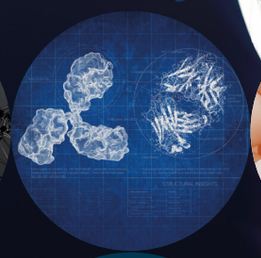
Click the circles to navigate

Be a better spy with LRI!

Peptide sequencing using Bayspec's field deployable mass spectrometer

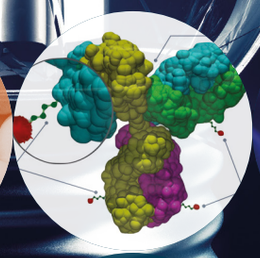


Improve the performance of your proteomics LC-MS facility with QuiC

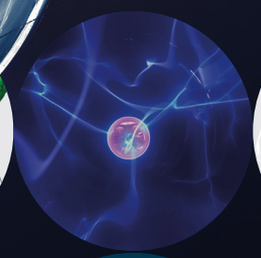


Full characterization of heterogeneous antibody samples under denaturing and native conditions

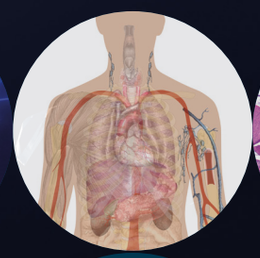
Rapid analysis of fipronil and fipronil sulfone in eggs by LC-MS.



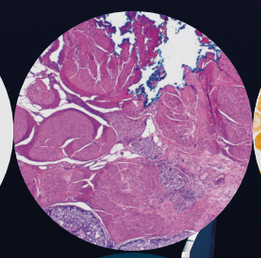
Rapid screening of intact antibody and antibody-drug conjugates



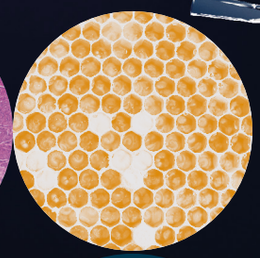
Quantitative analysis of estradiol and testosterone in plasma



Discovery to targeted metabolomics on a single HRAM Orbitrap LC-MS system



Applied metabolomics delivered



IONICON presents CHARON – the revolutionary direct aerosol inlet for PTR-TOFMS analyzers

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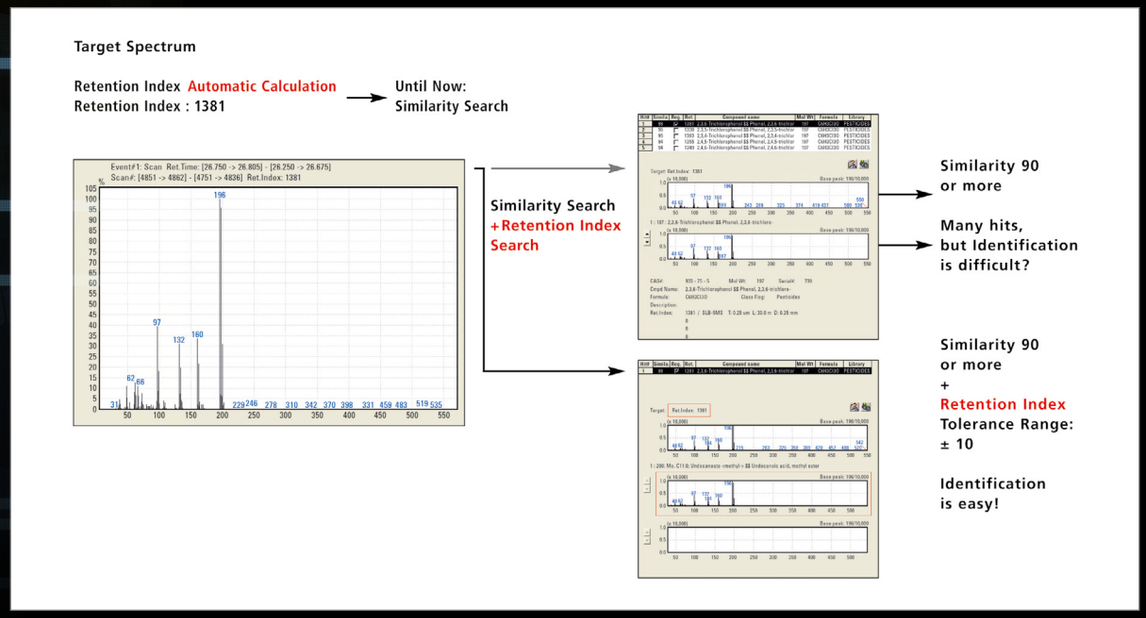
BE A BETTER SPY WITH LRI!

Linear Retention Indices (LRI) for better compound identification



Untargeted GC-MS analysis relies completely on the possibility to identify measured compounds based on library spectra for comparison. Even targeted analysis can benefit greatly from additional scan information, decreasing false positive results due to matrix or component interference. Therefore, independent of the application field, the reliability of this identification process is a key factor for routine analysts.

Particularly difficult are co-elution cases, which are of growing importance due to the ubiquitous increase of target components to be monitored, often accompanied by the need for higher sample throughput while reducing analysis times. Even more challenging are applications which involve compounds with very similar electron impact spectra. Unique identification is difficult as long as the spectral comparison is not considered.

LRI significantly improves identification reliability

Shimadzu applies the well-known linear retention indices (LRI) as an additional filter during library search. Instead of spending valuable time in manual post-processing, retention index information improves automatized identification reliability.

Isomers are difficult to assign reliably using only library spectra – aside from possible false positive results, their spectra are nearly identical. In these cases, the use of LRI as additional criteria improves identification reliability significantly and reduces manual post-processing.

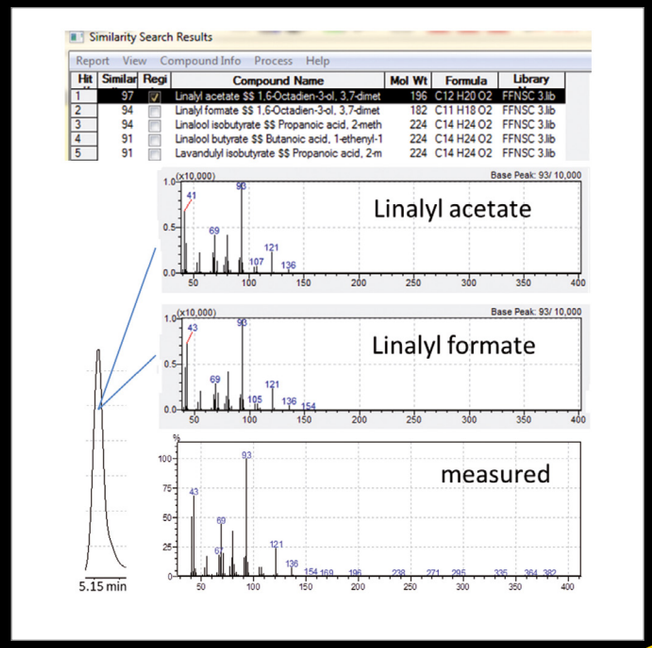
The example above shows the assignment of 2,3,6-Trichlorophenol to the measured scan spectrum on the left. The possible different isomers can only be distinguished based on their corresponding LRI included in the library information. By filtering via retention index allowance during the library search the correct isomer is obtained directly in a single hit.

LRI facilitate compound identification for various compound classes in diverse application fields.

GET MORE INFORMATION

Shimadzu provides retention index information (see below) in its dedicated libraries for flavor and fragrance, pesticides and lipids analysis.

www.shimadzu.eu



LINKS

FFNSC 3.0

Registered Retention Indices: SLB-5MS, Supelcowax 10, Equity-1

LIPIDS LIBRARY

Registered Retention Indices: SLB-5MS, Supelcowax-10, Equity-1

PESTICIDES LIBRARY

Registered Retention Indices: SLB-5MS, Equity-1

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PEPTIDE SEQUENCING USING BAYSPEC'S FIELD DEPLOYABLE MASS SPECTROMETER

Mass spectrometry can be now deployed for onsite peptide sequencing and identification in real time

Peptide identification and sequencing strategies by mass spectrometry have been very well-developed during the last 25 years following the introduction of soft ionization techniques. When sequencing peptides with tandem mass spectrometry (MS^n), peptides are cleaved at various locations off their backbones to generate fragment ions of different masses based on their amino acid sequences. The most common product ions are the b-, y-, and a-ions generated from the cleavage of amide bond (CO-NH) and the subsequent loss of CO from the b-ions to form a-ions. The resulting MS^n spectra can be matched with a database or computed with an algorithm to get the original sequence of known or unknown peptides.

Here we present an example of peptide sequencing with BaySpec's highly-portable (22 kg) and battery operated Continuity™-series portable mass spectrometer. It features a linear ion trap with collisionally induced dissociation (CID) for MS^n analysis, similar to the Portability™ series mass spectrometers. In addition, Continuity™ is equipped with a continuous atmospheric pressure sampling inlet with differential pumping, allowing the detection of a larger mass range (110 – 950 amu).

BaySpec's Continuity™ mass spectrometer is ideal for on-site peptide sequencing.

In this application note, we show an example of peptide sequencing in a standard mixture (H2016, Sigma-Aldrich) with Continuity™ mass spectrometer. The mixture was dissolved in HPLC grade water, and then further diluted with an electrospray solution (50:50 methanol:water with 0.5 percent acetic acid). The diluted solution is directly infused into the API inlet of the Continuity™ mass spectrometer without any further treatment or separation.

BaySpec's Miniature Mass Spectrometer

GET MORE INFORMATION

Figure 1 shows the full scan (MS^1) spectrum of the peptide mixture. Each peptide was isolated with stored waveform inverse Fourier transform (SWIFT), before fragmentation by CID. The resulting MS^2 spectrum consists of the sequencing a-, b- and y-ions. Shown in Figure 2 and Figure 3 are the SWIFT isolation and MS^2 spectra, respectively, of the largest and doubly-charged peptide, DRVYIHPF. The fragment ions in the MS^2 spectrum were successfully assigned based on the sequence.

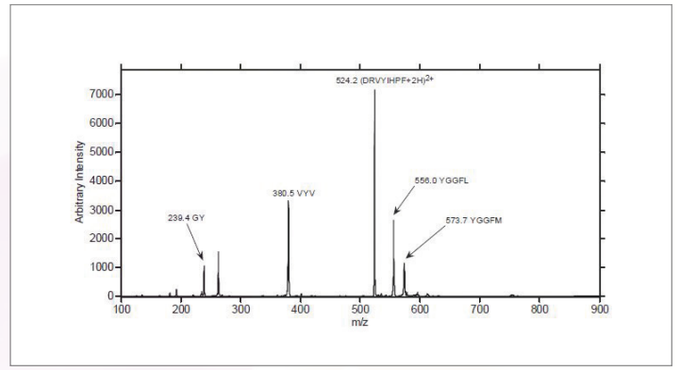

Figure 1. Full scan (MS^1) spectrum of the peptide mixture H2016 with Continuity™.

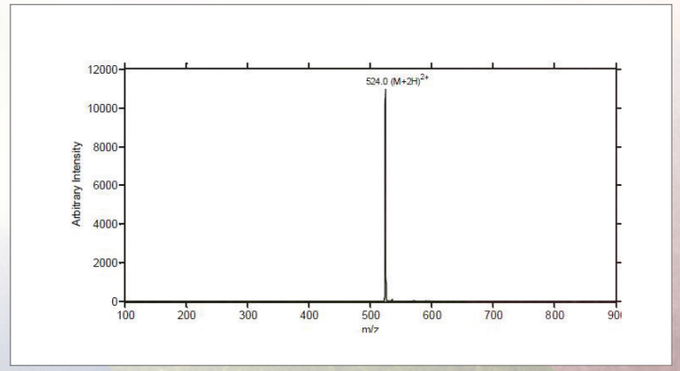

Figure 2. Swift isolation of the doubly charge peptide DRVYIHPF

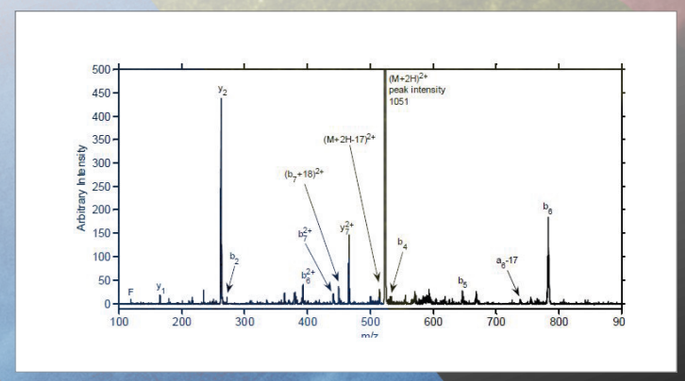

Figure 3. MS^2 spectrum of the peptide DRVYIHPF, and the assignment of the fragment a-, b- and y- ions.

LINKS

WEBSITE
More information on the Portability™ transportable mass spectrometer.

ARTICLE
Detection of residual pesticides on fruits and vegetables using Portability™

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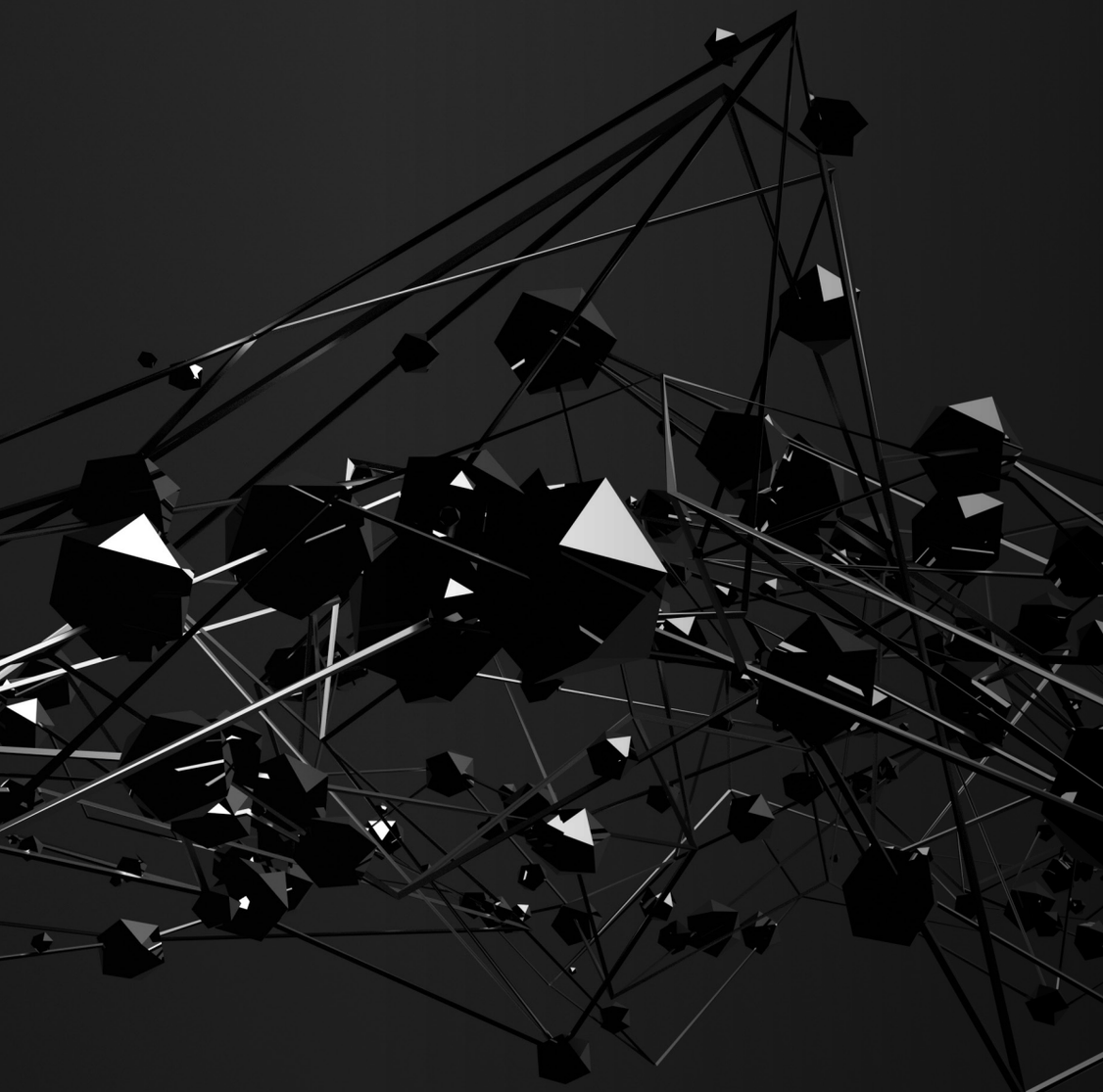


IMPROVE THE PERFORMANCE OF YOUR PROTEOMICS LC-MS FACILITY WITH QUIC

Easy to use QC monitor for main proteomics workflows

LC-MS based proteomics has become the method of choice for the comprehensive identification and quantification of proteins over large sample sets. However, as a highly complex analytical technique, mass spectrometry based proteomics can be subject to a large variability between runs. This can influence the accuracy and reproducibility of your results. Therefore, robust and accurate quality controls (QC) during data acquisition and in between instrument maintenance are essential to ensure that high quality data with low systematic errors is collected. This enables stable performance of your instruments and more predictable maintenance, consequently unlocking the full potential of your LC-MS facility.

To have a comprehensive overview of the performance of your LC-MS facility try QuiC, a simple and easy to use LC-MS QC tool developed by Biognosys. QuiC enables you to monitor the QC of all your proteomics workflows across different vendors in near real time. This is a tremendous help for the managers and operators of LC-MS facilities to improve data quality. It generates QC readouts from raw files of Thermo Scientific™ and SCIEX mass spectrometers operating in MRM, PRM, DIA and DDA mode.

QuiC tool provides:

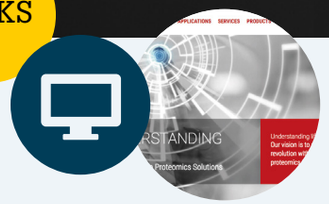
- an active queue of per-run QC analyses that supports real-time folder monitoring
- a multitude of readouts for main proteomics workflows based on iRT peptides
- for discovery proteomics workflows, DIA and DDA a background library can be specified and additionally targeted to better QC the samples and sample processing
- proper handling of corrupted files and duplicate run checking
- intuitive visualization and customizable reporting for documentation

QuiC enables users to judge data quality and intervene as needed to prevent sample loss, LC-MS issues and low data quality. It allows you to keep track of and monitor interventions and maintenance in your LC-MS facility and have something tangible at hand when negotiating with MS vendor engineers.

GET MORE INFORMATION

QuiC is available for download at www.biognosys.com/shop/quic.

LINKS



WEBSITE
https://www.biognosys.com/

PRODUCT OVERVIEW
Easy to use QC monitor for main proteomics workflows.

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NEXT GENERATION PROTEOMICS

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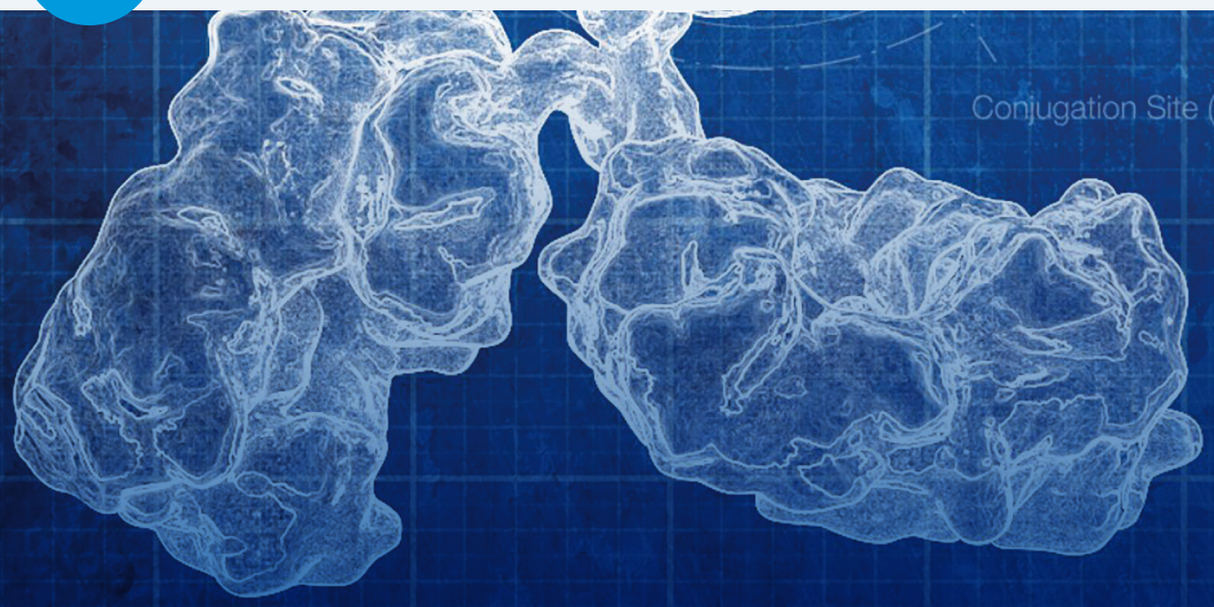

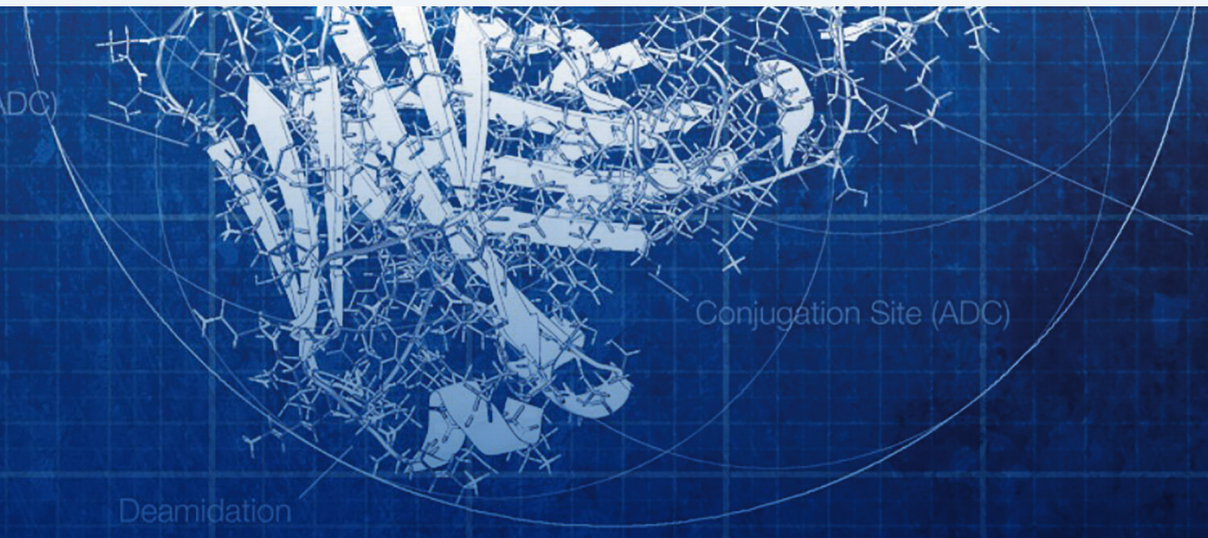

FULL CHARACTERIZATION OF HETEROGENEOUS ANTIBODY SAMPLES UNDER DENATURING AND NATIVE CONDITIONS

Showcasing the capabilities of the Thermo Scientific™ Q Exactive™ BioPharma mass spectrometry platform

MS analysis of antibodies at the protein and peptide levels is critical during development and production of biopharmaceuticals. The compositions of current generation therapeutic proteins are often complex due to the heterogeneity caused by various modifications that are relevant for their efficacy. Intact proteins analyzed by ESI-MS are detected in higher charge states that also provide more complexity in mass spectra.

Analysis of proteins in native or native-like conditions with zero or minimal organic solvents and neutral or weakly acidic pH can allow proteins to preserve non-covalent interactions and retain high degrees of folding. This effect has analytical benefits: greater protein folding leads to reduced charge states, increased mass separation, and increased signal at higher m/z. This strategy has been utilized for the analysis of antibodies and antibody–drug conjugates present in highly complex mixtures of different antibody/drug combinations. Requirements for performing native MS on antibody samples include scanning towards 8,000 m/z and increased transmission optimization for large compounds. This feature has so far only been available on the Thermo Scientific™ Exactive™ Plus EMR mass spectrometer. After successful implementation of the HMR Mode as part of the BioPharma Option, the Thermo Scientific™ Q Exactive™ Plus and Q Exactive HF™ mass spectrometers add the capability to perform native MS analysis with mass detection up to 8,000 m/z without compromising performance of normal operation modes. These enhanced capabilities are necessary for the analysis of antibody samples on the intact level under native conditions requiring the detection of masses beyond the standard mass range of up to 6,000 m/z. The BioPharma Option adds superior denatured and native MS intact mass analysis and subunit top/middle-down analysis capabilities to the most powerful benchtop peptide mapping instruments available.

The BioPharma Option offers distinct operational modes that have been optimized for the top three protein characterization workflows:

- Intact mass analysis under native and denaturing conditions with the new High Mass Range Mode
- Subunit and top/middle-down analysis with Protein Mode
- Peptide mapping with Standard Mode

For the Q Exactive Plus mass spectrometer, the BioPharma Option includes: Standard Mode, Protein Mode, Enhanced Resolution Mode with resolution up to 280,000 @ m/z 200, and the High Mass Range Mode with extended mass range up to m/z 8,000. For the Q Exactive HF mass spectrometer, the BioPharma Option includes: Standard Mode, Protein Mode, and High Mass Range Mode with extended mass range up to m/z 8,000.

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RAPID ANALYSIS OF FIPRONIL AND FIPRONIL SULFONE IN EGGS BY LC-MS

Quick, simple method for the determination of fipronil and fipronil sulfone in eggs using a modified QuEChERS acetonitrile extraction protocol.

Extraction

* Weigh 5 g homogenized eggs into 50 mL polyporpylene tube and add 5 mL of water

* Add 10 mL acetonitrile, shake for 2 min

* Add 4 g $MgSO_4$ and 1 g NaCl (60105-340 HyperSep Mylar pouch 4000 mg Magnesium Sulfate and 1000 mg NaCl)

* Shake for 2 min and centrifuge for 5 min at 4000 rpm

dSPE cleanup

* Transfer 4 mL of acetonitrile phase into empty 15 mL tube; avoid pipetting the hexane upper layer

* Add dSPE cleanup sorbent consisting of 600mg anhydrous $MgSO_4$ and 500 mg C18 (ACROS Organic, 413485000; C18 dSPE pouches – P/N 60105-367-SP)

* Shake for 2 min and centrifuge for 5 min at 4000 rpm

LC-MS/MS

* Transfer the supernatant into LC-MS vial

* Inject 1 µL into LC-MS system

Millions of eggs contaminated with the insecticide fipronil were distributed to more than 17 countries, and in some cases, the pesticide fipronil was mixed with another formulation and sprayed on chickens against ticks, fleas and lice. This created a demand for quick and efficient methods for the determination of both substances in egg matrix and potentially in chicken meat.

To address this need, a quick and simple method for the determination of fipronil and fipronil sulfone in eggs was developed using an in-house modified QuEChERS acetonitrile extraction protocol, a Thermo Scientific™ UltiMate3000™ RSLC system, a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer and Thermo Scientific™ TraceFinder™ software for data analysis.

The results demonstrate the suitability and robustness of the UltiMate 3000 RSLC system and TSQ Quantis MS for the analysis of fipronil and fipronil sulfone in egg extracts. The limit of quantification (LOD) of 0.5 ng/g was determined as the lowest calibration level with repeatability of <20 percent relative standard deviation (RSD), which is 5× below the EU statutory MRL for sum of fipronil and fipronil sulfone, and are in full compliance with the SANTE11945/2015 analytical quality control guidelines for pesticides, and even after 100 egg samples were analyzed, there was no need for system maintenance.

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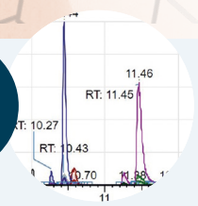
LINKS

PRODUCT
Get confident quantitation with the TSQ Quantis MS



DOWNLOAD
Download a free trial of TraceFinder software

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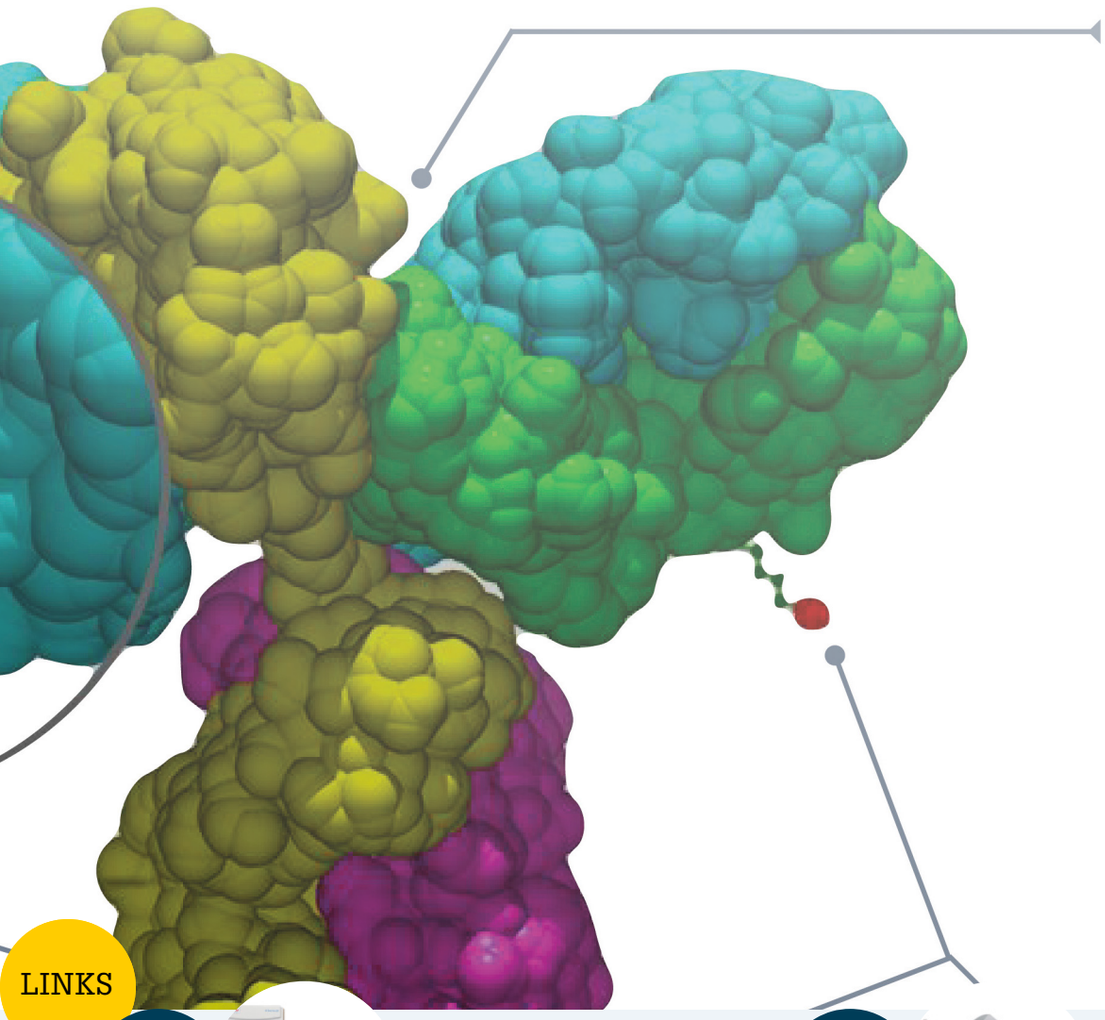
RAPID SCREENING OF INTACT ANTIBODY AND ANTIBODY–DRUG CONJUGATES

An integrated microfluidic capillary electrophoresis (CE) and mass spectrometry (MS) workflow



Monoclonal antibodies (mAb) and antibody–drug conjugates (ADC) constitute two of the most important biopharmaceuticals within the class of biotherapeutic drugs. During drug development and manufacturing, undesired mutations and in vitro modifications may introduce sample heterogeneity, causing changes to the protein structure that may lead to the loss of drug efficacy. The availability of a quick screening method at the intact protein level to detect and assess any variability that might occur during drug development is attractive.

For this study, NIST mAb and NIST mAb ADC were analyzed with the ZipChip™ (908 Devices) system, a Thermo Scientific™ Q Exactive™ Plus hybrid quadrupole-Orbitrap™ MS with the BioPharma option and Thermo Scientific™ BioPharma Finder™ software, utilizing the same experimental settings. With this CE-MS workflow, the fast analysis of the heterogeneity of the intact NIST mAb and ADC samples was achieved within 3 minutes, which can significantly improve lab productivity. This approach is even more amenable to quick screening and characterization of mAbs and ADCs during various phases of drug development and production as it requires minimal sample preparation and offers online desalting capabilities.

Compared to infusion-based MS methods, the separation of charge variants by capillary electrophoresis reduces sample complexity for MS detection and allows a more complete sample profiling and was able to identify and quantify all known variants with a high dynamic range in a few minutes.

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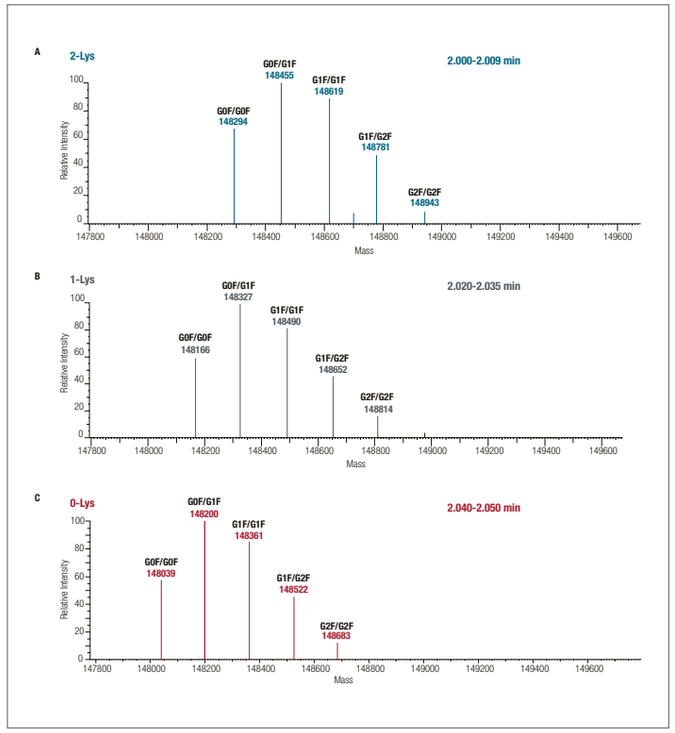

Figure 1. Three deconvoluted mass spectra from different migration time intervals, which correspond to different lysine variants.

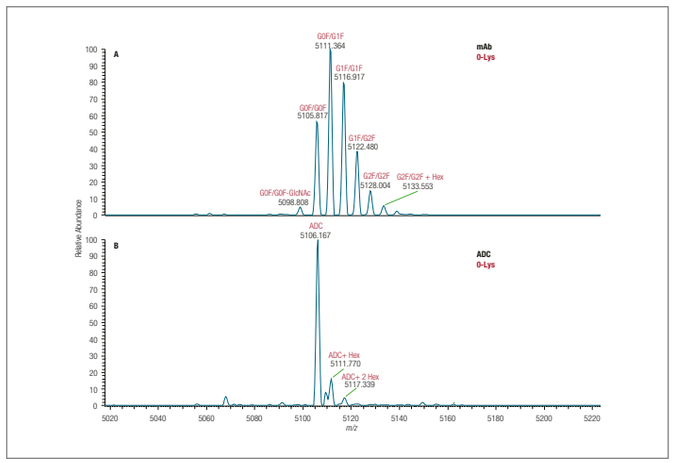

Figure 2. Identification of peaks from raw mass spectra acquired by CE-MS from A) NIST mAb and B) NIST mAb ADC.

LINKS

PRODUCT
ZipChip System for Mass Spectrometry

PRODUCT
Orbitrap LC-MS for the highest accuracy and precision

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QUANTITATIVE ANALYSIS OF ESTRADIOL AND TESTOSTERONE IN PLASMA

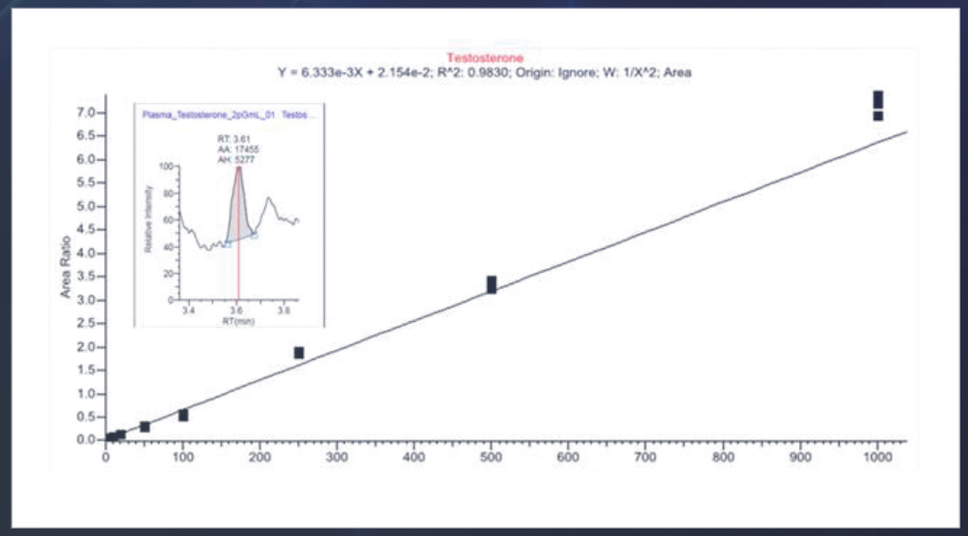
For clinical research using the TSQ Altis triple quadrupole mass spectrometer

Analysis of estradiol and testosterone in plasma samples for clinical research requires a sensitive analytical method. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been widely adopted as an analytically sensitive and selective technique for estradiol and testosterone analysis in complex matrices such as human serum or plasma.

To develop a sensitive LC-MS/MS method for quantitative analysis of estradiol and testosterone in plasma for clinical research, an LC-MS workflow was employed utilizing a Thermo Scientific™ Vanquish™ Flex Binary HPLC system, a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer and Thermo Scientific™ TraceFinder™ software.

The results for the lower limits of quantitation (LLOQ) of testosterone in plasma was 2 pg/mL - 20 fg on column (linearity range: 2–1000 pg/mL). For estradiol in plasma, the LLOQ was 20 pg/mL - 200 fg on column (linearity range: 20–10,000 pg/mL). The precisions were less than 8 percent and 7 percent for testosterone and estradiol, respectively, for all replicates at all concentrations.

The TSQ Altis triple quadrupole mass spectrometer provides the superior sensitivity required for the analysis of estradiol and testosterone in plasma for clinical research.



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LINKS

PRODUCT
Separate your science from the status quo with a Vanquish UHPLC system

PRODUCT
TSQ Altis MS: Sensitivity and robustness without compromise

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DISCOVERY TO TARGETED METABOLOMICS ON A SINGLE HRAM ORBITRAP LC-MS SYSTEM

Integration of three mainstream approaches – discovery, targeted screening and quantitation for a comprehensive integrated approach to metabolomics

Mass spectrometry-based strategies for metabolomics provide both qualitative and quantitative information. This not only enables identification of the metabolite structures in a biological system but also allows the monitoring of changes that occur within a system. Traditionally, MS-based metabolomics studies can be classified into two primary strategies – a discovery-driven untargeted profiling approach using high-resolution, accurate mass (HRAM) mass spectrometry followed by a hypothesis-driven targeted approach using a triple quadrupole mass spectrometer

A paradigm shift in integrated metabolomics research is presented, performed all on one MS platform. This provides a holistic approach to confidently identify metabolites that are of biological significance, in an untargeted or targeted fashion, followed by validation or verification of these metabolites by targeted quantitative means – all of which can be carried out on a single MS system, bypassing the tedious process of method transfer between different instrument platforms.

The HRAM capability of the Thermo Scientific™ Q Exactive™ MS system provides high specificity and uncompromised sensitivity, which are essential success factors for a comprehensive metabolomics workflow. The high resolution and stable mass accuracy of the Orbitrap analyzer provides greater reliability in peak detection critical for untargeted profiling experiments, while the outstanding MS/MS spectral quality contributes to a more robust targeted screening workflow. The software tools used are highly interactive and offer complementary solutions for unbiased data interpretation to demonstrate statistical significance based on the observations made from the data.

To showcase this capability, the metabolic profiles of the human pancreatic cancer cell line Panc-1 were investigated and the steps involved are outlined in the full application note.

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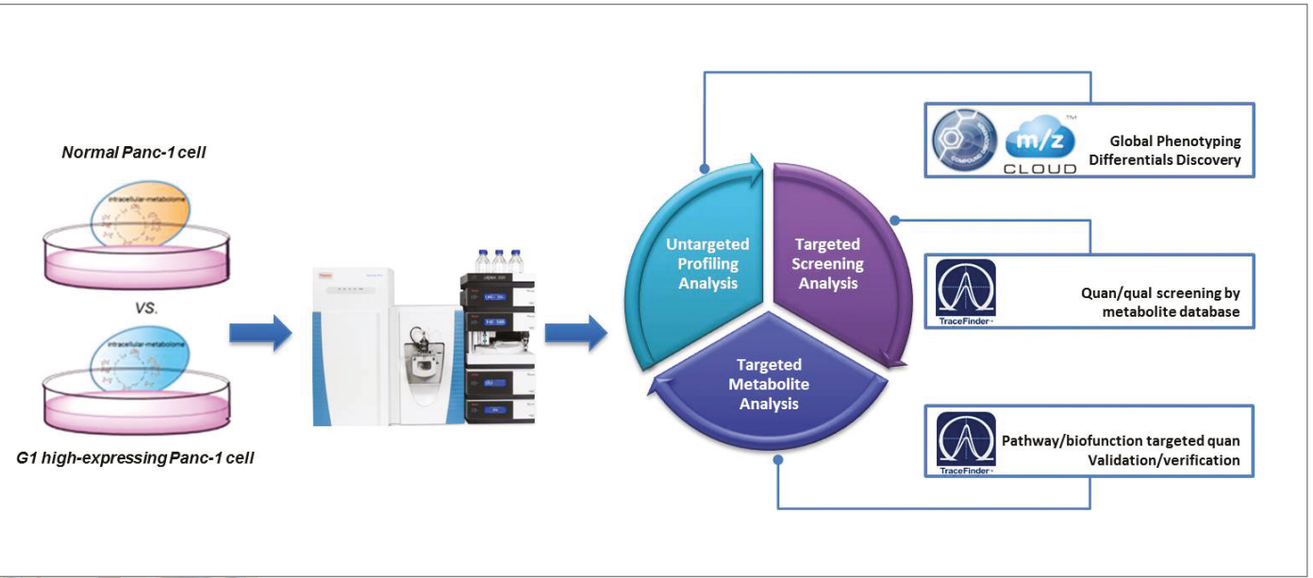

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APPLIED METABOLOMICS DELIVERED

Metabolomics differentiates known disease classifications of prostate cancer

Metabolomics focuses on the chemical processes central to cellular metabolism, however the structural and chemical diversity of metabolites that make up these metabolic processes means that their analysis on a global scale can be challenging. A robust mass spectrometry solution for screening metabolites is of increased interest, allowing for a more integrated and routine analysis. A new QTOF System was developed for routine, robust workflows which require minimal MS expertise. The system integrates all data acquisition, processing and review in a single software. A prostate cancer study was used to determine whether the untargeted metabolomics workflow using the X500R System could find key differences between the samples.

Using untargeted metabolomics one can differentiate sample groupings based on their metabolic profiles. In this study, samples with known disease classifications as well as QC samples (spiked with mix of known standard compounds) and control urine (matrix QC) were analyzed by MarkerView Software. In this data set, samples from the same group cluster together well, highlighting good reproducibility across the data collection. Removing the control urine and QC samples and repeating the PCA, a clear differentiation between the groups of samples is seen, highlighting that the metabolic profile is different between healthy and disease samples.

At this stage these are still features that need identification and confirmation. The m/z - RT ion pairs of interest are copied directly into SCIEX OS Software for identification (library search), peak integration, quantitation and any other statistics which may need to be reviewed for confidence in assignment. The Formula Finder algorithm then generates a set of possible formulas based on the parent mass, mass error and isotope distribution pattern, which can then be searched in databases for possible structures.

In this study, samples from a pilot prostate cancer study were analyzed and a clear difference between healthy and disease urine samples was detected using this untargeted metabolomics approach, confirming the original disease classifications. Most changes were in the small molecule amino acids. This pilot study provided confidence in the approach, and the next phase of the study analyzing a much larger set of samples is underway.

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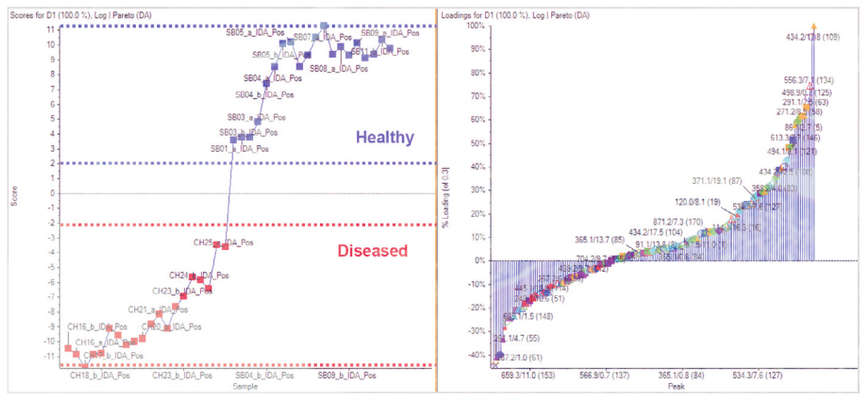

LINKS

APP NOTE
Targeted metabolite screening on the X500R

BLOG
Why we love metabolomics (and you should too!)

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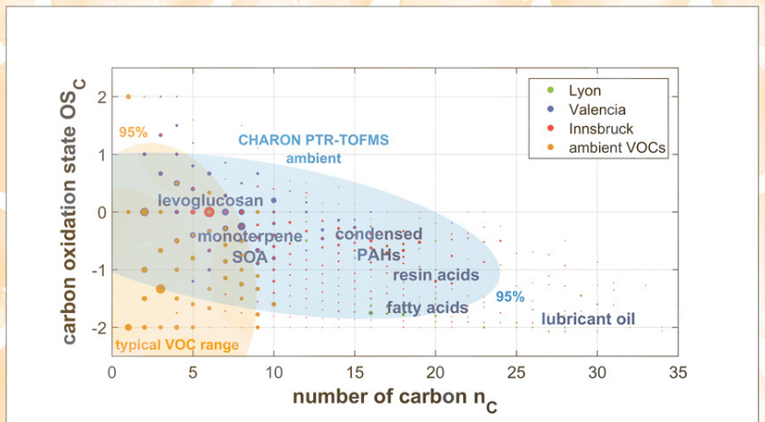

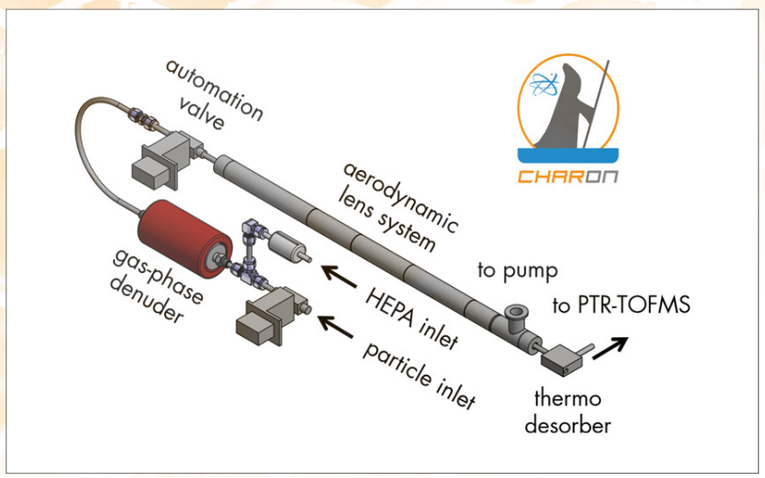

IONICON PRESENTS CHARON – THE REVOLUTIONARY DIRECT AEROSOL INLET FOR PTR-TOFMS ANALYZERS

All-in-one: a single instrument for real-time gas-phase and particulate organics monitoring. CHARON-PTR-TOFMS detects almost the full range of atmospheric organic carbon.

CHARON-PTR-TOFMS is a novel on-line analytical technique that combines the real-time ultra-sensitive analysis of volatile organic compounds with the possibility to characterize the molecular composition of non-refractory sub-µm organic particles on a chemical composition level at one-minute time-resolution. The CHARON aerosol inlet aboard an IONICON PTR-TOFMS offers the most abundant range of atmospheric organic carbon with a single instrument. Unlike other options on the market, CHARON is a direct inlet system that does not sacrifice one of the main benefits of PTR-MS: the real-time monitoring capability. IONICON customers can now use their real-time instrument to monitor VOCs and condensed intermediate to low volatile organics.

This revolutionary new inlet enables IONICON PTR-TOFMS series instruments to measure aerosols directly with the most versatile, reliable and proven technology for VOC analysis available on the market: PTR-MS. IONICON is the market-leader in PTR-MS instruments based on time-of-flight (TOF) mass spectrometry. PTR-TOFMS real-time analyzers are known for sub-pptv detection limits, high mass resolving power and the speed enabling VOC analysis in a fraction of a second.

The CHARON particle inlet consists of a honeycomb activated charcoal denuder that efficiently adsorbs organic gases and transmits particles, a high-pressure aerodynamic lens system that collimates and extracts sub-µm particles, and a thermo-desorber that evaporates non-refractory organic particulate matter at moderate temperatures of 100-160°C and reduced pressures of a few mbar.

These organics are subsequently analyzed as gas-phase analytes with one of IONICON's high-resolution PTR-TOFMS instruments. By coupling the CHARON inlet to a PTR-TOFMS, the VOC inlet remains fully operational. An automated valve system allows for scheduled switching between gas- and particle-phase measurements as well as zeroing of the particle inlet.

With its high temporal resolution and the high degree of conserved chemical composition information, CHARON PTR-TOFMS is thus the perfect analytical technique to identify and quantitatively follow atmospheric particulate tracer compounds like levoglucosan and polycyclic aromatic hydrocarbons.

The CHARON particle inlet is available as an exclusive add-on for selected IONICON PTR-TOFMS series instruments and best combined with the new PTR-TOF 6000 X2, for an ultimate performance experience, high mass resolving power and utmost detection sensitivity.

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