From biopharmaceutical discovery to decontamination following a chemical warfare agent attack, mass spectrometry is constantly breaking new ground. Here, we showcase just some of the ever-expanding list of applications, alongside recent advances in Tandem Ionization and automated deformatiation workflows.
Are you ready for a step-change in data independent acquisition?

Introducing

Zeno SWATH

data independent acquisition (DIA)

exclusively on the ZenoTOF 7600 system

Enhance speed, sensitivity and accuracy of your workflows using Zeno SWATH data independent acquisition (DIA) for MS-based proteomics.

Harnessing the power of the Zeno trap for Zeno SWATH DIA allows you to:

- Confidentlydiscover and translate significant biomarkers
- Identify and quantify up to twice as many proteins as with traditional SWATH DIA approaches
- Shortened run times with minimal compromise in proteome coverage

Seeing is believing at:

SCIEX.com/zenoswathdia
04 Improved Purification Workflow in Drug Discovery

06 Chemical Warfare Agents and Decontamination measurements with PTR-TOF

07 Designed for Speed: Fast GC

08 Tandem Ionisation® – Revolutionary Soft Ionisation to Enhance Confidence in Identification

10 Automated Deformation of LC/MS and GC/MS Data

12 High-Throughput Biotherapeutic Glycosylation Profiling Using High-Resolution Ion Mobility-Mass Spectrometry (HRIM-MS)

13 Speed Up Multiresidue Pesticides Analysis in Food with Low-Pressure GC-MS
This application note introduces the automated target screening and preparative purification by LC/MS using a dedicated open-access software to assist the entire drug discovery workflow.

Preparative LC is widely known as a means of purifying target compounds from mixtures. In drug discovery, synthesis, screening, and purification of new active pharmaceutical ingredients are performed following a conventional workflow (Figure 1). Synthesized products are screened by LC-MS, followed by analytical method development and scale-up for target purification by preparative HPLC.

These steps can be tedious and time-consuming when carried out manually, so workflow automation offers significant improvement with regards to efficiency and sample throughput.

This article introduces application of automated target screening and preparative purification by LC/MS using a dedicated open-access software – Open Solution – to assist the entire drug discovery workflow. Separation, co-elution or missing targets are identified in an initial LCMS screening run, using target m/z information; results are then color-coded for simple visual evaluation; before auto scale-up from an analytical to a preparative focused gradient method is performed using the ASAPrep™ algorithm. Separated analytes can then be chosen for collection.

This workflow automation can be used to increase productivity, reduce cost and the risk of human error.
Figure 1: Conventional workflow of target screening and purification

<table>
<thead>
<tr>
<th>Screening Chromatogram</th>
<th>ASAPprep Algorithm</th>
<th>Purification Chromatogram</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="image1">Image of chromatogram</a></td>
<td>Target mass 254.28, Retention time 2.85 min, Focused gradient Int. B Conc. 36.9 %</td>
<td><a href="image2">Image of chromatogram</a></td>
</tr>
<tr>
<td><a href="image3">Image of chromatogram</a></td>
<td>Target mass 330.24, Retention time 2.53 min, Focused gradient Int. B Conc. 36.9 %</td>
<td><a href="image4">Image of chromatogram</a></td>
</tr>
<tr>
<td><a href="image5">Image of chromatogram</a></td>
<td>Target mass 392.29, Retention time 3.11 min, Focused gradient Int. B Conc. 46.3 %</td>
<td><a href="image6">Image of chromatogram</a></td>
</tr>
</tbody>
</table>

Figure 2: Results for LCMS screening and preparative purification for three pharmaceuticals in the presence of related impurities.
There's a need to check, if surfaces are "clean and safe" after decontamination procedures following CWA attacks and no solution is available on the market. PTR-TOF works in real-time without sample preparation and is extremely sensitive, thus meets all the requirements for CWA decontamination control. Here, we present results from two recent measurement campaigns at certified facilities.

The threat of Chemical Warfare Agents (CWAs) is very real as has been shown in the recent past by the assassinations at KL airport (2017) and in Salisbury (2018). Unfortunately, due to the current armed conflicts in Europe, it could remain topical in the near future. From an analytical point of view, the detection of high CWA concentrations in air during and immediately after an attack is relatively simple even with handheld devices. However, verifying that large surfaces (e.g. the floor of an airport) are indeed clean and safe following standardized decontamination procedures is currently not possible because of the lack of suitable analytical instrumentation.

The IONICON Proton-Transfer-Reaction–Time-Of-Flight (PTR-TOF) technology is a real-time direct injection trace gas analysis method with extremely high sensitivity, selectivity and excellent Limits-of-Detection (LoDs). In combination with the proprietary Automated Measurement and data Evaluation (AME) software no trained scientist is necessary to operate the PTR-TOF and to interpret the obtained data. AME “translates” the scientific data into straight-forward graphical compound concentration readings. Therefore, IONICON PTR-TOF is the ideal tool for real-time trace gas monitoring and control.

Results of CWA measurement campaigns
In most scientific literature on CWA measurements, analogues have been used because of the restricted access to live agents even for analytical purposes. We therefore took our compact PTR-TOF devices to measurement campaigns at certified facilities in the Czech Republic and in Austria.

At the first campaign we found that our patented (EP3503161) NH₄⁺ reagent ion production method is particularly beneficial for sensitive CWA detection. Even with the most compact PTR-TOF QB model (two stackable and separable 19” cubes) we determined 1s LoDs between 10 and 30 pptv for the CWAs nitrogen mustard, soman, sarin, cyclosarin, tabun and VX.

For the second campaign we utilized a high-mass-resolution PTR-TOF 6000 to investigate residual CWA gas phase concentrations above various surfaces (from metal and glass to pig skin) before and after decontamination with different common decontamination agents. We found that only after the treatment with some of these agents and only for absolutely smooth surfaces no residual contamination could be detected anymore.

These results confirm that IONICON PTR-TOF is indeed perfectly suited for the real-time verification of the absence of highly toxic compounds, such as CWAs.
The shortage of helium and its increased costs are encouraging laboratories to find suitable alternatives. Learn how LECO achieved results using Hydrogen as a carrier gas.

Even the best-trained experts can only move as fast as their equipment will allow, and as helium prices increase and supplies drop, the fast spectral acquisition speeds of GC-TOFMS instruments stand ready to reap the benefits of fast GC separations afforded by utilizing hydrogen carrier gas.

While the advantages of helium as a carrier gas for mass spectrometers have long been known, it is a limited natural resource. On the other hand, hydrogen gas generators can be installed in any lab for a constant supply and, conveniently, increased chromatographic speeds.

Not every instrument is capable of benefiting from using hydrogen as a carrier gas. The reduced viscosity of hydrogen can cause issues with the vacuum pump, and the higher speeds can confuse a detector that can’t collect data fast enough to deconvolute and quantify the narrower peaks. However, LECO’s Pegasus® BT was designed to be able to use hydrogen instead of helium as a carrier gas.

With no mechanical issues when switching from helium to hydrogen, the next question is: What is the quality of the data produced?

LECO’s experts have been running samples through the Pegasus BT with both helium and hydrogen carrier gases and comparing the results. Our results have been extensively documented through on-demand webinars, poster presentations, and application notes on our Fast GC Forum. In a recent app note, LECO tested tea tree oil.

The quality of essential oils such as tea tree oil can be impacted by various processing or storage conditions, and it can degrade or change over time. Fast and reliable analytical screening is therefore critical for quality control of these products. A typical analysis of tea tree oil using a Pegasus BT and helium carrier gas can take around 16 minutes, with fantastic chromatographic resolution.

By switching the carrier gas and optimizing the method for hydrogen using an online method translator, that time dropped to 3.45 minutes.

By transferring to hydrogen, it was possible to dramatically increase throughput without significantly impacting resolution. Mass spectral fragmentation was highly similar to that obtained with helium, while the overall sensitivity actually increased by about 40 percent.

Figure 1: Chromatograms of each method translation step. As reference point for analysis speed, the elution time of globulol is indicated in every chromatogram.
TANDEM IONISATION®
– REVOLUTIONARY
SOFT IONISATION TO
ENHANCE CONFIDENCE
IN IDENTIFICATION

Tandem Ionisation for BenchTOF2™ mass spectrometers acquire hard (70 eV) and soft (10–20 eV) electron ionisation in a single analysis
Tandem Ionisation is an exclusive development in ion-source technology for BenchTOF2 time-of-flight mass spectrometers. By simultaneously providing reference-quality 70 eV spectra and complementary soft EI spectra, Tandem Ionisation has allowed analysts, for the first time, to fully benefit from the inherent advantages of soft ionisation for gas chromatography–mass spectrometry — without any of its historic disadvantages. This white paper describes the theoretical background to the technique, the benefits that stem from soft EI and how Tandem Ionisation breaks new ground by providing sample characterisation in a single, streamlined workflow.

Electron ionisation (EI) is the production of ions by direct bombardment of analyte molecules by electrons. Conventionally, EI is used with an accelerating potential difference of 70 V, because at approximately 70 eV, the efficiency of energy transfer from electrons to most organic molecules is at a maximum and varies little with electron energy. This results in a relatively high degree of ionisation (and thus good sensitivity) and consistent mass spectra — the two principal reasons 70 eV EI is standard practice for gas chromatography–mass spectrometry analysis. Indeed, some large commercial libraries, such as NIST and Wiley, have been developed almost exclusively on the basis of EI at 70 eV.

Nevertheless, such extensive fragmentation is not always desirable, and there are a number of gentler “soft ionisation” techniques. These ultimately result in analyte molecules becoming ionised without giving them excess energy. The consequence of this is a limited degree of analyte fragmentation, meaning that a higher proportion of the original ionised analyte molecules reach the detector intact. This ability to provide information about the unfragmented molecule makes soft ionisation of great value to analysts.

Tandem Ionisation provides fast switching (multiplexing) between two ionisation energies, enabling two sets of spectra to be acquired at the same time — reference-quality 70 eV spectra and “soft” ionisation spectra with stronger molecular ions and less fragmentation, for improved confidence in identification. The multiplexing speed can be extended to 200 Hz, enabling compatibility with two-dimensional gas chromatography data acquisition.

Tandem Ionisation allows complex samples containing structurally similar compounds to be comprehensively characterized. For example, the technique can be used to improve isomer speciation. In cases where analytes exhibit weak molecular ions and/or extensive fragmentation at 70 eV, analysts have previously resigned themselves to a low-confidence multi-analyte assignment (“This is probably A but could be B, C or D”) — or indeed failure to identify the component at all. In such situations, Tandem Ionisation can be used to generate complementary low-energy spectra that aid correct and confident identification, as illustrated in figure 1 for the isomers neral and geranial.

![Figure 1. Comparison of the 70 eV and 12 eV spectra obtained for the isomers neral and geranial, showing the enhanced differences in ion ratios obtained when using soft EI, for improved isomer speciation.](image-url)
AUTOMATED DEFORMULATION OF LC/MS AND GC/MS DATA

Identify components faster! Select from an unbiased list of structures, molecular formulae, or component names that fit your data.

Anne Marie Smith, Richard Lee, Artsiom Piatrouski, Andrey Paramonov, and Vitaly Lashinu

IXCR is an automated workflow for deconvolution of LC/MS or GC/MS datasets and database query for efficient and confident component identification of unknowns. Read how you can use this software to generate an extensive, unbiased, and relevant list of structures or component names to accelerate deformulation of complex MS samples.

Accelerate the dereplication of MS data with an automated workflow that includes chromatographic deconvolution and spectral search.

With ACD/MS Workbook Suite you can initiate automated chromatographic feature-finding and component identification for entire GC/MS and LC/MS datasets using the intelligent compound recognition workflow (IXCR). Use the Wiley/NIST library and user-created mass spectral databases, in combination, to help with component identification.
Step 1 – Import and process GC/MS or LC/MS data from all major instrument vendors
Native support for all major instrument vendor formats means you can apply the same processing and analysis, no matter what instrument the data is collected on.

Step 2 – Automated isolation of all the components in your chromatogram
The feature-finding IntelliXtract algorithm performs chromatographic deconvolution to isolate components. A mass spectrum is generated for each component – MS1, and MS2 when available.

Step 3 – Automated database search to identify components
Setting your component identification preferences at the outset helps refine the database search for LC/MS data. Search MS1 or MS2, single or averaged scan spectra and set up a variety of thresholds to narrow your search. You can also apply the NIST MS Search algorithm.

Convenient component identification
Experimental and search results are displayed as mirrored spectra to make visual comparison quick and easy. Click through each component in the table to see the labelled component spectrum, a mirror plot of the experimental versus database spectra, and values to help you evaluate spectral match (HQI% or MF when using NIST MS Search).

Spectra are automatically annotated with potential fragment and adduct ions when possible (full and partial [Markush] structures are supported). Depending on the complexity of the data and the database(s) used, you can further filter search results by collision energy or retention time.

Finally, store the processed data file, create a report, or store the results in a database for use in future searches.

Figure 1. The IXCR workflow in MS Workbook Suite generates a list of components. Each component is linked with its experimental spectrum. See search results mirrored with experimental spectra for convenient comparison.
HIGH-THROUGHPUT BIOThERAPEUTIC GLYCOSYLATION PROFILING USING HIGH-RESOLUTION ION MOBILITY-MASS SPECTROMETRY (HRIM-MS)

A complete analytical and bioinformatics solution with MOBIE and HRIM-Byos

Steven Broome, Rose Lawler, Dmitry Avtonomov, Andrew Nichols, and Anhishek Roushan

This note discusses the use of MOBIE for High-Resolution Ion Mobility Separation and profiling of released glycans. Demonstrating that the addition of HRIM to a traditional LC method provides a solution for rapid fingerprinting of glycosylation profiles in biotherapeutics.

In the pharmaceutical manufacturing space, protein glycosylation is a critical quality attribute (CQA) for biotherapeutics that is regularly characterized and monitored due to glycosylation’s direct impact on product safety and efficacy. The non-template driven nature of glycan biosynthesis results in high structural heterogeneity, with even minor changes to the manufacturing process potentially causing significant alteration in glycosylation profiles.

Glycoform characterization typically involves liquid chromatography (LC) separations followed by detection with either laser-induced fluorescence of fluorescently labeled glycan species or mass spectrometry (MS). However, liquid-phase separations alone are limited in capacity for resolving glycan isomers, and efforts at separating isomeric species can require methods with extended separation times.

By incorporating High-Resolution Ion Mobility-MS into a traditional LC workflow for comprehensive characterization of N-linked glycan species released from a protein biotherapeutic, we present a solution for high-throughput glycan profiling for biotherapeutics.

LC methods of varying run times were implemented to assess the ability of HRIM to resolve glycans in the gas phase while LC separation times were gradually reduced. The data collected were then analyzed using a novel, automated workflow designed to enable seamless processing and reporting of LC-HRIM-MS released glycan data using Protein Metrics HRIM-Byos Released Glycan module.

LC-HRIM-MS analysis of released N-glycans from Aranesp® across multiple LC gradients ranging from 60- to 10-minutes provided consistent and reproducible relative quantitation results. With the addition of HRIM, separation of potential glycan isomers was achieved. Given the data-rich results provided by LC-HRIM-MS, the tedious nature of high-dimensional data analysis for this technique can be a major challenge facing the larger adoption of high-resolution ion mobility separations. HRIM-Byos workflows alleviate this bottleneck and enable the complex 4D data to be processed rapidly and efficiently with automatically generated reports.

The MOBIE platform combined with automated data analysis can provide a solution for rapid fingerprinting of glycosylation profiles for biotherapeutics.

Figure 1. Consistent ion mobility separations were achieved for all LC gradients assessed. A) Example total ion current chromatograms for the 60- (blue), 30- (green), and 10-minute (red) HILIC gradients used to analyze the Aranesp® released N-glycan samples. Highlighted areas represent the elution regions corresponding to the extracted HRIM arrival time distributions in (B). B) Overlay of extracted ion mobiligrams (XIM) for Aranesp® glycan (HexNAc)5(NeuAc)3(Fuc)1(Hex)6 (m/z =1113.09, +3 charge state) observed with 60-, 30-, and 10-minute HILIC gradients. Four replicate XIMs plotted of the LC-HRIM-MS runs collected for each HILIC gradient for the same released N-glycan signal. “Extracted ion mobiligram” represents the ion mobility arrival time trace for a single m/z.
Multiresidue pesticides analysis is a cornerstone of food safety testing, and labs are generally under pressure to manage both a high volume of samples and rapid turnaround time requirements. This creates demand for faster GC-MS and GC-MS/MS methods, but typical approaches involve expensive instrumentation or “fast GC” techniques that have capacity issues (narrow-bore columns) or MS-compatibility concerns (hydrogen carrier gas). Low-pressure GC-MS (LPGC-MS) is an option that can provide significant speed gains without these drawbacks, but, historically, the challenging setup has been a barrier to implementation.

As shown in this LPGC-MS pesticide analysis of 209 compounds in strawberry, all analytes elute quickly with deltamethrin eluting last at 8.33 minutes. This time is three-fold faster than our analysis of the same extract on a conventional 30 m, 0.25 mm ID, 0.25 µm 5-type column where deltamethrin was again the final compound and eluted at 26.34 minutes. This LPGC-MS pesticides analysis utilizes a unique low-pressure GC column kit that is comprised of a narrow restrictor column (5 m x 0.18 mm ID) that is factory coupled to a wider Rtx-5ms analytical column (15 m, 0.53 mm ID, 1 µm plus 1 m integrated transfer line on the outlet end). The manufactured connection is tested to ensure leak-free performance and is more robust than manual connections.

Using this kit allows the speed gains of LPGC-MS to be obtained by making a simple column change and updating the instrument method with the new column dimensions, oven ramp, and flow rates. Note that for this particular LPGC-MS pesticides analysis, the GC oven must be capable of a 35 °C/min ramp rate at oven temperatures in excess of 300 °C. For 120V ovens, an oven insert kit will be necessary to achieve this rate. While this LPGC-MS setup provides a significant speed gain; tall, narrow peaks that may improve sensitivity; and high capacity from the thick film analytical column, the overall plate count will be somewhat lower than on conventional columns. Although peak resolution will be lower with LPGC-MS, the mass spectrometer can offset this effect by spectrally distinguishing most target analytes. However, it is important to note that isobaric compounds must be chromatographically separated because the MS cannot resolve them. For example, in this analysis, isobars 4-4'-DDD and 2,4'-DDT are not fully resolved, so if their separation is critical, further method development would be needed. The other highlighted separations are compounds that either have both shared and unique ion transitions (trifluralin/benfluralin and cyhalothrin/cypermethrin); are adequately separated (cis- and trans-permethrin); or are commonly reported as a group (cyfluthrin and cypermethrin isomer clusters) and thus are still quantifiable even though they may not be completely chromatographically resolved.

For busy food labs needing faster methods, the speed gains of LPGC-MS pesticides analysis are an effective way to increase sample throughput. This once-challenging setup is now much simpler to implement using a low-pressure GC column kit from Restek.

Find the full article here: tas.txp.to/restek-ms
Compact Mass Spectrometers: Controlled Substance Detection

The landscape of controlled substance analysis is changing with compact mass spectrometers. BaySpec’s Portability™ and Continuity™ mass spectrometer series offers a new way to approach controlled substance analysis. No longer do samples need to go to the lab, but now the lab can go to the samples. With the growing risk of exposure to fentanyl and its analogues, there is a need in law enforcement and decontamination services for fast and dependable analysis that requires little to no training.

Portability™ and Continuity™ offer real-time analysis at the site. These devices offer high sensitivity based on BaySpec’s proprietary custom vacuum design and cutting-edge linear ion trap.

The revolutionary technology of the linear ion trap mass analyzer allows for the compact size of the instrumentation while still maintaining laboratory quality performance, which is made possible by its high tolerance for low vacuum.

Don’t Wait for Answers

Get them with BaySpec’s Portable Mass Spectrometers

Features:
- Fully field portable with rapid deployment
- High sensitivity and can be used with wide range of applications
- Miniature linear ion trap with MS/MS capability
- Compatible with any atmospheric ionization source including ESI, APCI, TD-ESI, TD-APCI and DBDI

sales@bayspec.com

© 2022 BaySpec, Inc. All rights reserved. All BaySpec products are made in the USA
SWITCH TO **GAS GENERATORS**!
Make your own gas.

The most advanced **LC-MS CALYPSO gas generator** on the market

Our **Nitrogen gas generators** are quieter. With more control functionality than any other gas generator on the market, this gas generator offers an enhanced user experience. We can also offer both PSA and Membrane technology depending on the application and end user preference.

Thanks to our close collaborations with the various manufacturers of LC-MS instrumentation, we have received multiple feedbacks for our CALYPSO where F-DGSi has currently the largest range of Nitrogen generators for LC-MS application. **Upgrade your workflow with F-DGSi gas generators!**

---

**F-DGSi**
Innovative Gas System Company

*Your local gas generation partner*

---

**FIND OUT MORE**

Call: +33 1 64 98 21 00
Email: info@f-dgs.com

[www.f-dgs.com](http://www.f-dgs.com)