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NEXT-GEN PTR-MS TASTES CHOCOLATE

The chocolate benchmark: benefits for complex aroma real-time analysis with new high-performance IONICON PTR-TOF instruments

One of the main fields of application of proton transfer reaction–mass spectrometry (PTR-MS) is food and flavor science. Here, we present a recent study on one of the world's most popular foods: chocolate. We demonstrate how IONICON's Next-Gen PTR-TOFMS take chocolate analysis to the next level.

Direct sample injection without the need for any sample preparation, real-time quantification, extremely high sensitivity, and detection limits in the low pptv region within less than one second are just the perfect prerequisites for in-vivo and in-vitro foodstuff analysis. PTR-MS unites all of these benefits. Thus it is not surprising that over the past 25 years, countless PTR-MS studies have been published on samples ranging from various drinks, dairy products, fruits and vegetables, meat, snacks, and, of course, chocolate.

IONICON's Next-Gen PTR-TOFMS: chocolate analysis with unmatched insights

Real-time nosespace analysis during the consumption of chocolate is very challenging for analytical instrumentation because the nosespace gas not only consists of chocolate compounds, but also of chemicals present in room air and substances originating from human metabolism. Many of these compounds are isobars with only small differences in exact m/z but huge differences in intensity.

IONICON's PTR-TOF 10k, which offers a mass resolution of 10,000 to 15,000 m/Δm, is ideally placed to meet this challenge. We find that above 10,000 m/Δm, up to nine peaks per nominal m/z can be identified in chocolate nosespace. With such an excellent mass resolution, we can quantify the release of trimethylpyrazine – one of the key aroma compounds of chocolate – into the test subject's nosespace, while the close isobar C_9H_{14} is present in room air at high concentration. The PTR-TOF 10k perfectly separates relevant isobars, and thus takes selectivity to the next level. It also enables aroma monitoring with sub-second time resolution in the pptv region.

The FUSION PTR-TOF 10k is equipped with a revolutionary reaction chamber and outperforms common PTR-MS devices with a stunning sensitivity of up to 80,000 cps/ppbv. In food and flavor research, such an extreme sensitivity can be crucial when the available time per measurement is strongly limited but high-quality data is required. In a proof-of-concept test, we sampled the headspace above differently flavored chocolate pieces at room temperature. After only 1s of measurement time, the relative errors of the relevant aroma compounds were already below 1 percent. This means that even in the 100 ms region, excellent data quality can still be expected.

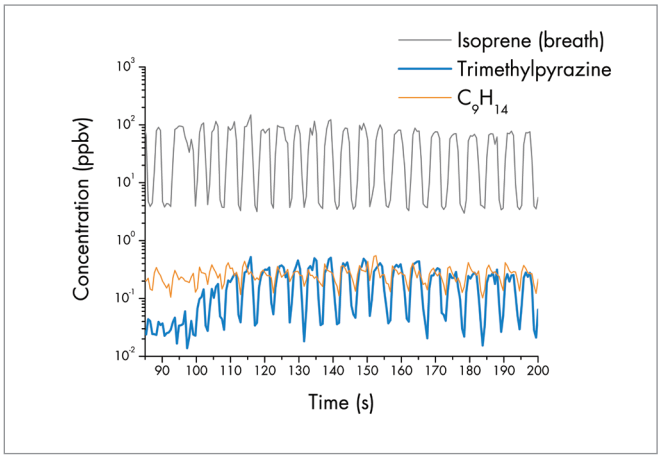


Figure 1. Real-time quantification of trimethylpyrazine in nosespace during the consumption of chocolate.

WORKFLOW FOR THE ASSESSMENT OF KEY AROMA COMPOUNDS OF PUMPERNICKEL BREAD VARIATIONS

An analytical workflow combining GCxGC-TOFMS analysis with automated and statistical data processing

By Lena Dubois and M. A. Majcher

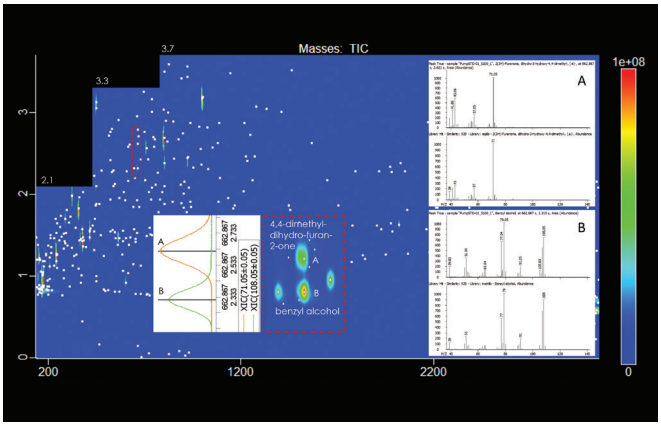
GCxGC-TOFMS and ChromaTOF® Tile Software combine for an efficient, supervised statistical analysis. See how subtle variations in recipe can change the aroma profile of pumpernickel bread.

Subtle variations in a recipe can have vastly different results in the final product. This can be especially true for baked goods, where a different oven may use a different heat cycle that changes the final flavor profile, or the origin of ingredients might sway the results. With an enormous global industry, the production of baked goods such as pumpernickel bread is subject to much analytical scrutiny. Anything one producer can do to give themselves a leg up on the competition is a huge advantage for what may be a subtle change.

Pumpernickel bread comes with a complex and challenging matrix. While 1D GC-MS is a typical first step, here it can be more efficient to jump straight to GCxGC-TOFMS analysis. The added dimension dramatically improves separation as analytes that coelute in the first dimension can be easily differentiated when run through a column of differing polarity.

GCxGC-TOFMS provides such rich and detailed chromatographic data that the sheer volume of information may become nearly unmanageable after just a few samples. Comparing these sets of data aren't easy. Small variations in run times could misalign the chromatograms, making it impossible for basic statistical analysis to produce coherent automated information.

ChromaTOF Tile software, however, is far more than a basic statistical analysis package. Seamlessly running alongside ChromaTOF, ChromaTOF Tile creates Fisher-Tile ratio calculations for each set of data and compares those values. Rather than going individually, point by point, these tile rations enable the software to accommodate for misalignment, efficiently and effectively sorting truly statistically significant finds to the top of a list for your scientists to study. Even when it comes to a sample as challenging as pumpernickel bread, ChromaTOF Tile, combined with GCxGC-TOFMS data, can cut hours, days, weeks, and even months off your analysis times.



WORKFLOW FOR CHEMICAL CHARACTERIZATION AND DATABASE CURATION FOR LC/MS AND GC/MS DATA

Confident identification of known and unknown components to create a curated centralized database

By Cindy Roberson, Joseph Buchman, and Baljit Bains

Learn how scientists at Medtronic use the structure verification and knowledge management capabilities in ACD/MS Structure ID Suite to create a data-handling workflow for chemical characterization and database creation.

Medical device manufacturers are required to evaluate the biocompatibility of medical devices to manage biological risk. The ISO 10993 series provides the framework for these evaluations. Medtronic conducts extractable and leachable (E&L) testing in accordance with ISO 10993 Part 18, to create a profile for every medical device they supply, generating an immense amount of data that must be appropriately managed and stored.

Medtronic uses MS Structure ID Suite for their data-handling workflow to strategically curate relevant spectral databases for LC/MS and GC/MS data from reference standards and reference materials that it can search against. The software is used to standardize, integrate, and store analytical data with its scientific context (structure, metadata, retention time, etc.), allowing Medtronic to elucidate each component's structure confidently.

Data-handling workflow

The Intelligent Component Recognition (IXCR) tool is used to screen GC/MS and LC/MS mass spectra samples, search databases to compile structure hits (hit quality index, HQI%), and conduct spectral comparisons via mirrored plots.

Depending on the HQI%, further verification may be required using the AutoAssignment tool. It interrogates LC/MS data and top structure candidates to determine an assignment score that shows structure and match factor for the compounds.

For truly unknown components, ACD/Labs' Molecular Formula Generator tool can propose potential molecular formulae and display expected isotopic abundance. The software searches for a target component's accurate parent mass and predicted molecular formula to generate a ranked structure hit list. The AutoAssignment tool is used to determine the best structural matches. An additional check is performed with the ChromGenius tool to predict retention time.

Once an accurate structure verification is achieved, the analytical and metadata are stored in a centralized database. The workflow complies with regulatory requirements, including 21 CFR Part 11 (i.e., traceability, versioning, etc.), and information necessary for regulatory documentation is easily accessed. Scientists can access live, curated data remotely, allowing them to work and make decisions more efficiently. With MS Structure ID Suite, Medtronic consolidates its analytical and metadata in a centralized database, ensuring analytical knowledge generated today is retained and leveraged for the future.

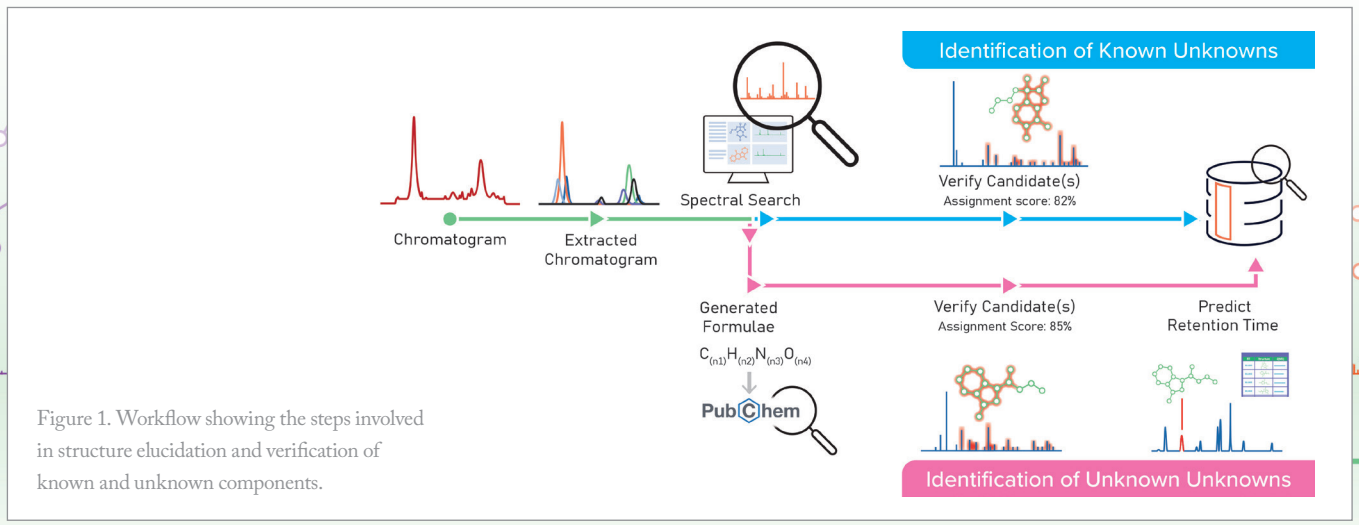


Figure 1. Workflow showing the steps involved in structure elucidation and verification of known and unknown components.

SYFT TRACER™: REVOLUTIONARY PRODUCTIVITY FOR VOLATILE IMPURITIES ANALYSIS

By Vaughan S. Langford

This application note summarizes a scenario relevant to contract research organization (CRO) and contract drug manufacturing organization (CDMO) laboratories where multiple volatile impurity methods need to be conducted in short runs. Five analyses are considered that can be handled by one Syft Tracer but require multiple legacy, chromatography instruments. For these analyses, SIFT-MS reports the first quantitative results 2- to 12-fold faster, and has sample throughputs 3- to 17-fold higher than the conventional procedures.

Headspace analysis of volatile impurities using conventional chromatographic approaches usually have long set-up times, are slow to report quantitative results due to frequent calibration, and have low daily sample throughputs. Syft Tracer™, the latest innovation in SIFT-MS, addresses these challenges through a single, stable instrument configuration that requires very little set-up and infrequent calibration, while simplifying sample analysis. A single Syft Tracer instrument revolutionizes multiple workflows and redefines productivity – allowing for five dissimilar analyses to be conducted in one day.

Chromatographic methods suffer from long run times, the requirement of expertise, significant sample prep time, high system maintenance, and the need for a configuration change (and hence re-calibration) when switching from one method to another.

Syft Tracer addresses these challenges in a unique way by simplifying multiple workflows and delivering revolutionary productivity enhancements via reduced calibration demand and higher throughput. These benefits are realized because Syft Tracer provides flexible sample analysis from a single configuration together with very stable, reproducible analysis provided by direct sample analysis using ultra-soft chemical ionization. Furthermore, sample derivatization is not required, and Syft Tracer is easily operated by non-specialist personnel.

This application note summarizes a scenario (see http://bit.ly/3ZU3SEJ for full details and references) relevant to contract research organization (CRO) and contract drug manufacturing organization (CDMO) laboratories where multiple volatile impurity methods need to be conducted in short runs. Five analyses are considered that can be handled by one Syft Tracer (comparison of legacy instruments required in parentheses): (a) residual solvents (three GC-FID or GC/MS systems), (b) volatile nitrosamines from tablets (one GC or LC system), (c) ethylene oxide in Polysorbate 80 (one dedicated GC-FID), (d) formaldehyde in polyethylene glycol excipient (one GC or LC system), and (e) benzene in an emulsion (run on third residual solvents system). For these analyses, SIFT-MS reports the first quantitative results 2- to 12-fold faster, and has sample throughputs 3- to 17-fold higher than the conventional procedures.

Figures 1(a) to 1(d) demonstrate that running just 70 test samples across five methods is a major undertaking for conventional instrumentation. In contrast (Figure 1(e)), one Syft Tracer instrument comfortably accomplishes the equivalent analytical tasks with 13 hr available for additional analyses. Syft Tracer revolutionizes workflows and maximizes productivity for volatile impurities analysis.

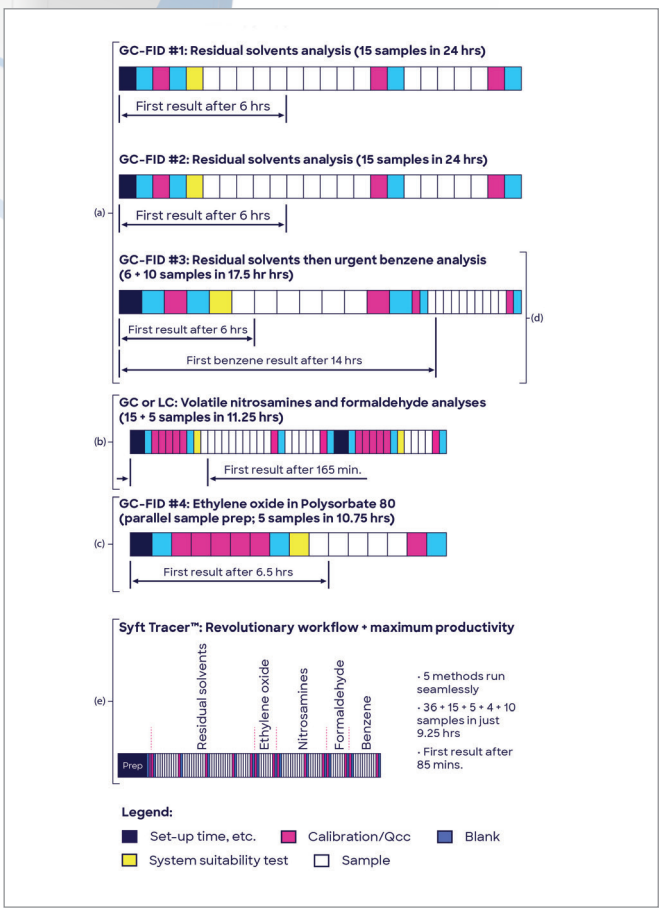
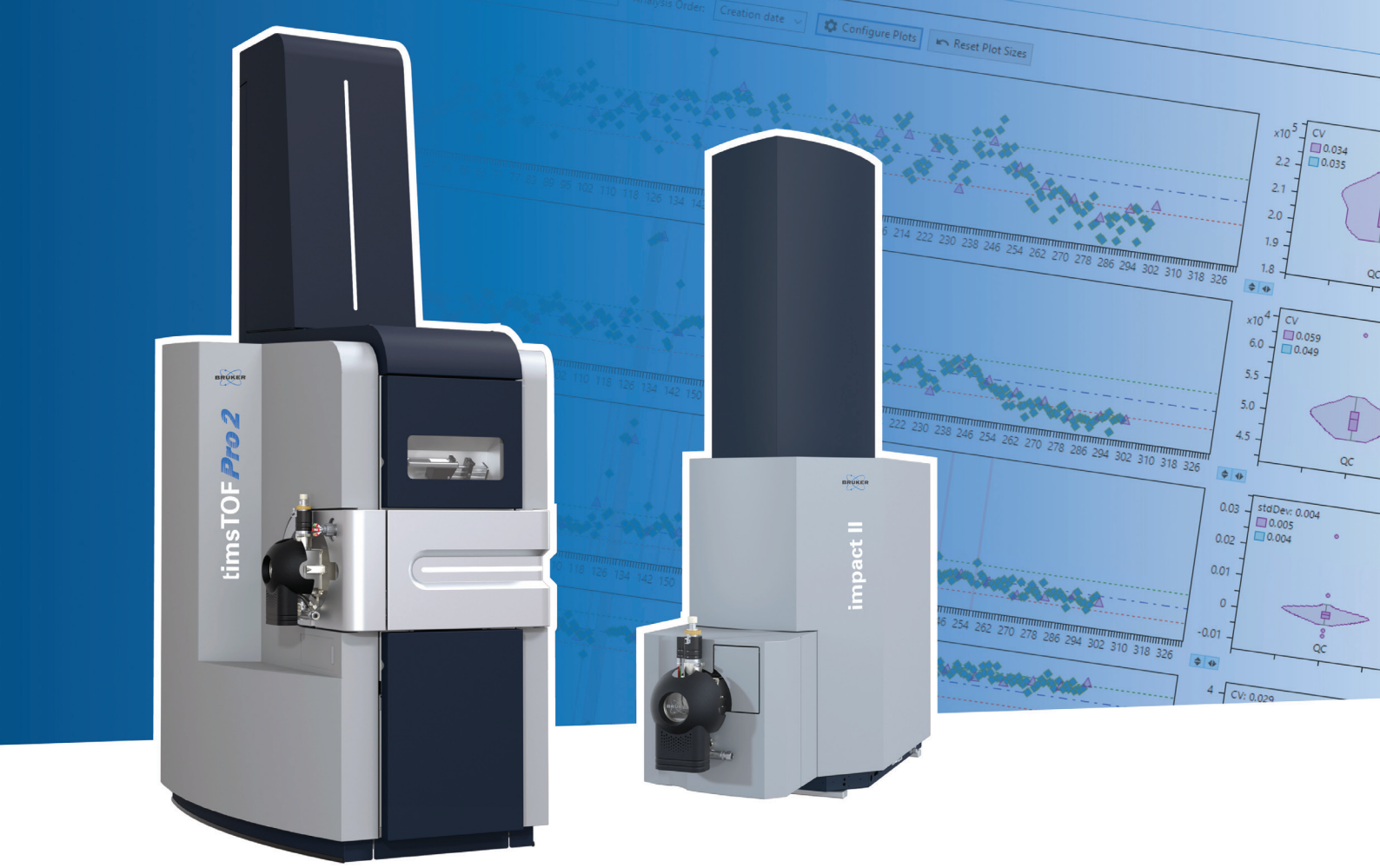


Figure 1. Five conventional chromatography instruments are required to handle 70 samples across five methods, whereas with one automated Syft Tracer instrument, they are handled with ease. See the text below for descriptions of labels (a) to (e).



REAL-TIME PRECISION ASSESSMENT FOR ENHANCED QUALITY CONTROL IN UNTARGETED METABOLOMICS AND LIPIDOMICS

Flying blind with quality control? Bruker RealTimeQC offers analysts a transformative upgrade.

By Patrick Groos, Ilmari Krebs, and Matthew R. Lewis

BRUKER

Bruker RealTimeQC allows the quality of LC-MS and LC-TIMS-MS measurements to be clearly seen during data acquisition for informed decision making and improved data quality outcomes.

Review of data quality during liquid chromatography mass spectrometry (LC-MS) data acquisition in untargeted metabolomics and lipidomics studies is commonly performed in a superficial manner by overlaying chromatogram traces from repeated injections of pooled quality control (QC) samples and looking for gross changes in retention and signal intensity. Such an approach lacks the necessary depth and detail required to assure analysts that the data they are producing will be fit for purpose in downstream data analysis, because assessment of retention time and peak intensity drift are typically qualitative, and deviations in mass accuracy, collisional cross section measurement, and isotopic fidelity may go entirely unnoticed.

Bruker RealTimeQC software provides analysts with better control of their data quality by providing quantitative and run-order-based assessment of critical measurements in user-defined QC analytes. Clear visualization of trends in measurement drift can aid users in the early detection of deviations from expected performance where it matters – in the data itself – allowing for quick corrective action to be taken to avoid compromised results. The software facilitates informed decision-making in real-time, allowing users to suspend or terminate an analysis and spare sample material from consumption under non-ideal performance conditions. Bruker RealTimeQC software allows users to monitor and verify that analytical instruments are performing accurately and consistently, ensuring that data quality remains within intended limits of control and reducing the incidence of measurement errors in datasets that would otherwise only be discovered after completion of the analysis. Together, these benefits help increase confidence in the data and support better decision making in untargeted metabolomics and lipidomics studies.

NEGATIVE MODE ANALYSIS OF SYNTHETIC OLIGONUCLEOTIDES USING THE MALDI-8030

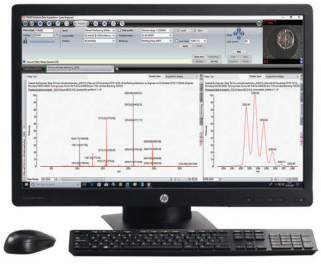
Simple analysis of oligonucleotides in negative ion mode to reduce adducts, on an affordable benchtop MALDI-TOF

By Simona Salivo

This app note uses a MALDI-TOF with dual polarity switching for the analysis of synthetic oligonucleotides – and demonstrates the benefits of the negative ion mode detection for eliminating the desalting sample clean up step in the analysis of oligonucleotides, while still producing good signal sensitivity.

Synthetic oligonucleotides are short DNA or RNA sequences with various applications in molecular biology, such as primers used in DNA sequencing and amplification by the polymerase chain reaction (PCR) (Figure 1). Recently, synthetic oligonucleotides have also been explored for therapeutic and diagnostic purposes, such as DNA-based diagnostic test kits, in several conditions. Cystic fibrosis is an example of a condition that develops at the DNA level. It is the most common autosomal recessive disorder among Caucasians. Besides its clinical relevance, cystic fibrosis can be useful for demonstrating genotyping workflows in teaching laboratories because of the multiple mutations (>1500) identified, which may involve different molecular assays. These are typically PCR related assays followed by gel electrophoresis. But this involves the use of harmful reagents, making detection using MALDI-TOF a safer option.

Here, we present the dual polarity MALDI-8030 benchtop linear mass spectrometer for genotyping, using cystic fibrosis disease (Phe508del mutation) as an example (figure 1). A scenario where oligonucleotides had been generated following PCR was simulated – based on cystic fibrosis alleles. Samples of synthetic oligonucleotides, corresponding to the sequence of the CFTR gene where the mutation causing cystic fibrosis occurs, were purchased from Merck Life Science. Sample desalting was carried out for the analyses in positive ion-mode. Dowex cation exchange resin works through exchange of hydrogens for sodium and other salts. No desalting was performed for the negative mode analyses. Samples were pre-mixed with matrix (1:2) prior to spotting onto the MALDI target. MALDI analyses were conducted on the MALDI-8030 in positive and negative ion modes using the desalted and non-desalted samples, respectively.



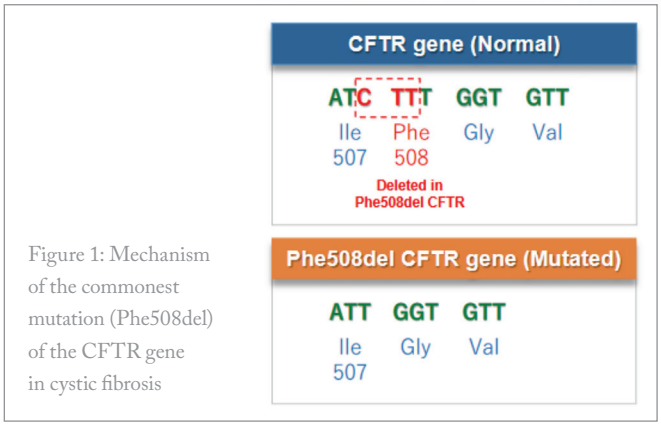


Figure 1: Mechanism of the commonest mutation (Phe508del) of the CFTR gene in cystic fibrosis

The results demonstrated that analysis in negative mode provides an advantage over the positive mode because it simplifies sample preparation without compromising spectral quality. The overall analysis workflow is simple and faster than when performed via gel electrophoresis. The technique could therefore be useful in training students in teaching laboratory sessions following PCR amplification, and also in more routine laboratories, for genotyping.

AN EFFICIENT OLIGONUCLEOTIDE IMPURITY ANALYSIS WORKFLOW USING LCMS Q-TOF

Unique algorithms for precise calculation of percentage impurity by MS1 and accurate sequence assignment by MS2

By Kosuke Uchiyama, Noriko Kato, and Atsuhiko Toyama

This app note demonstrates the impurity analysis of oligonucleotide samples using a LCMS Q-TOF. The benefit of data acquisition speed and high mass accuracy for impurity identification and sequencing is described.

In recent years, oligonucleotide therapeutics are attracting much attention as a new drug discovery modality, shown by a rapid increase in the development of new nucleic acid medicines. However, an analytical strategy for comprehensive detection and identification of impurities is yet to be established for oligonucleotides, due to difficulties resolving small changes in the medium-sized structures by column chemistry alone. Moreover, incomplete chromatographic separation necessitates the use of mass spectrometry signals for quantitative assessment of impurity levels, rather than the classical photometric detectors.

Herein, we briefly report the results of impurity analysis using a time-of-flight mass spectrometer LCMS-9050. With a mass resolving power of 45,000 FWHM, fine MS1 spectra were obtained for charge deconvolution and determination of component masses at low-ppm accuracies. Additionally, MS/MS spectra were obtained by DDA and were associated with each MS1 assigned component. Figure 1 shows the TIC chromatogram of a crude, freshly synthesized 20mer oligonucleotide, with the averaged zero-charge spectrum of the chromatographic peak for the full-length product (inset "MS1") and with the sequence assignment diagram generated from the MS/MS spectrum (inset "MS/MS Sequencing"). A comprehensive set of accurate-mass MS1 and MS/MS sequencing were show to be useful for screening the predicted impurities and understanding how the synthesis progressed. The simplicity allows a quick turnaround of trial-and-errors needed for finding the optimum synthetic condition.

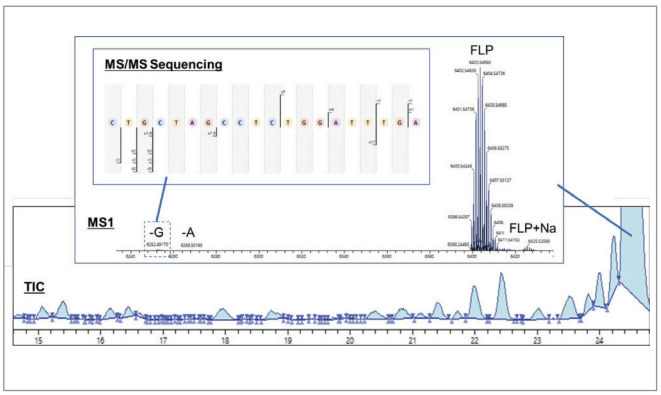


Figure 1. Analysis of a crude oligonucleotide sample, showing the MS1 TIC, MS1 spectrum and sequence assignment by MS/MS.

INCREASING SENSITIVITY AND SAVING SOLVENT WITH ADVANCED LC COLUMN TECHNOLOGY

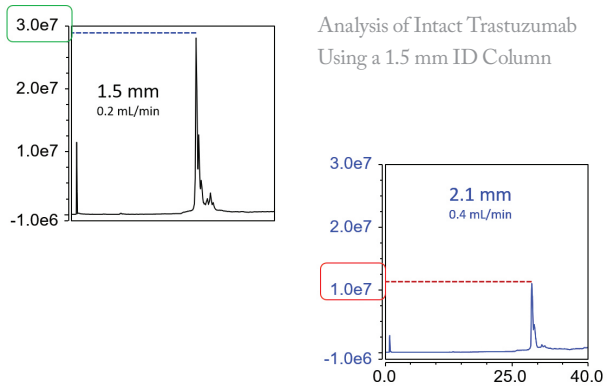
For all of the reasons you moved your LC-MS methods to 2.1 mm ID columns, the time has come to adopt the new 1.5 mm ID

By Stephanie Schuster

We demonstrate that employing the new HALO® 1.5 mm ID Diphenyl column provides a threefold gain in MS sensitivity for intact mAb analysis. Using a 1.5 mm ID column format results in a 50 percent reduction in solvent usage compared to 2.1 mm ID columns. Finally, Fused-Core® particle technology accommodates faster analysis times with higher efficiencies compared to fully porous column packings.

Advanced Materials Technology introduces its new line of LC and LC-MS columns in a 1.5 mm ID column format. Bridging the gap between traditional 2.1 mm ID and micro 1 mm ID columns, the HALO® 1.5 mm ID provides a new dimension in LC and LC-MS separations for increased sensitivity and decreased solvent usage with a robust format for user-friendly operation. As UHPLC systems have become more commonplace in the laboratory, more analysts are benefiting from their design advantages, including reduced diameter tubing and flow cells configured for less diffusion. Given that newer UHPLC systems are designed for operation at elevated back pressures and the ability to use smaller particle size packings, they can inherently benefit from smaller ID column hardware. Moving down from a 3 mm ID or 2.1 mm ID column to the new 1.5 mm ID column provides a demonstratable signal intensity boost and significant solvent savings (50 percent less than what is used for separations using a 2.1 mm ID column). For the analyst, this makes it possible to interrogate smaller peaks for more accurate quantitative and qualitative analysis and monetary savings in the lab – using the capital equipment they have already invested in, versus the acquisition of micro or nano flow technology.

Implementation of the HALO® 1.5 mm ID column is also straightforward. Unlike capillary columns that must be handled with care and involve sensitive instrument connections, the HALO® 1.5 mm ID column physically looks and handles like a 2.1 mm ID column, so installation is simple and the hardware is rugged. Using zero dead volume connection tubing also leads to greater chromatography efficiency gains.



Analysis of Intact Trastuzumab Using a 1.5 mm ID Column

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msFINEANALYSIS AI: NOVEL QUALITATIVE ANALYSIS SOFTWARE

Exclusively available with the JEOL AccuTOF GC-Alpha

By Ayumi Kubo

We discuss how we used the NIST20 library to train the AI for predicting EI mass spectra from structural formulas and then used it to evaluate prediction accuracy. From the accuracy evaluation results, we confirmed that the AI generated database is useful for determining the structural formulas of unknown compounds. We introduce features of msFineAnalysis AI and provide our evaluation results.

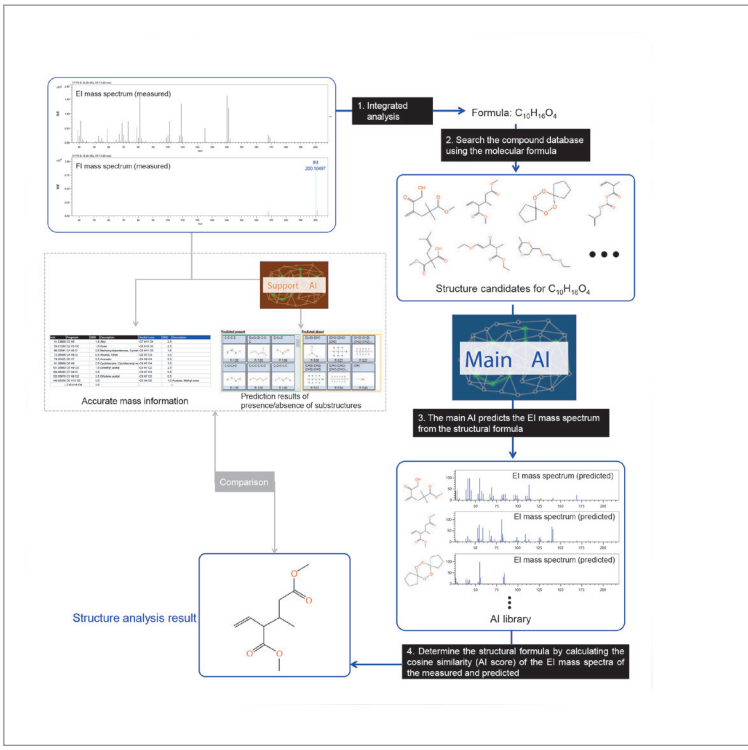
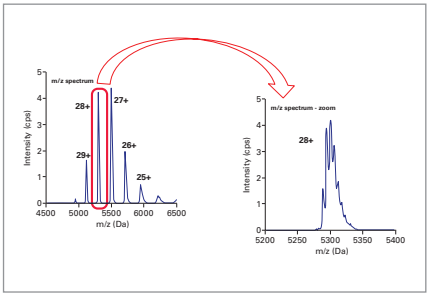


Figure 1. Overview of AI structural analysis.

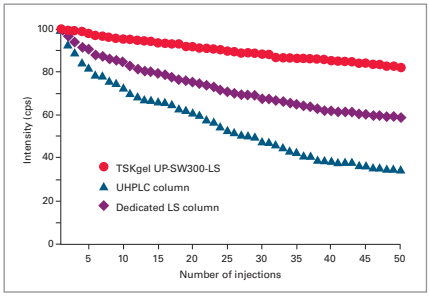
msFineAnalysis AI is equipped with a structural analysis method using artificial intelligence (AI), called "AI structural analysis." This AI structural analysis incorporates two different structure analysis tools: an in-silico database consisting of AI-generated electron ionization (EI) mass spectra for the +100 million structures in the PubChem database, and a support AI that predicts the presence and absence of common substructures. These advanced AI technologies allow msFineAnalysis AI to provide a unique automatic structure analysis capability that was not previously available for GC-MS qualitative analysis and can even provide structural analysis results for compounds that are not registered in the available EI database libraries (unknown compounds).

First, the integrated qualitative analysis capabilities available with previous versions of msFineAnalysis are used to identify the molecular formula of an unknown compound. Next, the software uses this molecular formula information to search the PubChem database for candidate compounds and then compares the AI predicted EI mass spectra for these candidates to the measured mass spectrum. Additionally, the support AI predicts the presence and absence of common substructures. The final results are then ranked accordingly using all of this information, with the top candidate identified as the most likely molecular structure.

Selecting the Optimal Column for Native SEC-MS of Monoclonal Antibodies



A Mass spectrum of mAb sample



B Ionization efficiency

Characterization of monoclonal antibodies (mAbs) is essential for product safety and efficacy. Size exclusion chromatography (SEC) coupled with mass spectrometry (MS) is increasingly used to identify the accurate molecular mass of mAbs and their impurities. However, traditional SEC generates high particle shedding, which decreases ionization efficiency over time. To avoid shedding for MS and multi-angle light scattering (MALS) applications, Tosoh Bioscience developed TSKgel® UP-SW3000-LS U/HPLC size-exclusion columns. In this application note, the column was coupled with an MS instrument for the analysis of a mAb standard. Data demonstrate that the TSKgel UP-SW3000-LS column surpasses competitive UHPLC columns and a dedicated low shedding column for SEC of proteins in terms of particle shedding observed by MS. Moreover, the column helps maintain ionization efficiency in the electrospray ionization (ESI) source >90% compared to the initial injection over >50 injections, thus increasing data quality and reducing ion source cleanings.

Read the full application note here: bit.ly/SEC-MS-of-mabs

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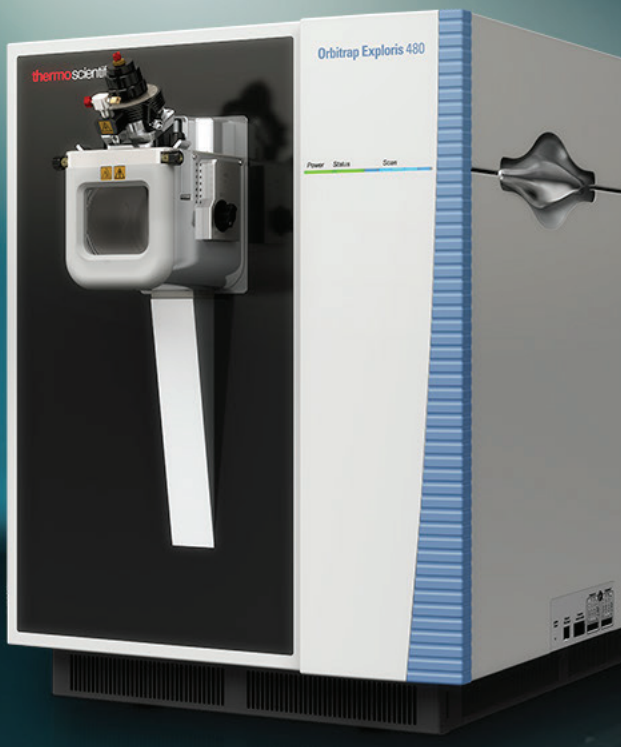

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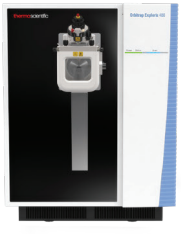

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