the Analytical Scientist

The Application Book 2023

Recent application notes across a range of exciting topics

- An Ultra-High Sensitivity Analysis of PFAS Compounds in Multiple Water Sources
- Enhancing VOC Analysis with Vocus CI-IMS-TOF: Isomer Separation Unraveled
- Accurate and Reproducible Quantitation of xC/UV/MS Data from Any Instrument
- A Novel Approach to Assess Encapsulation Efficiency of mRNA-Lipid Nanoparticles
- Multi-Technique, Vendor Neutral Analytical Data Handling for Chemists
- Low-pg/mL Quantification of TL 13-112, a Proteolysis Targeting Chimera (PROTAC) in Rat Plasma
- Food Analysis with Confocal Raman Microscopy
- Achieve new heights in PTM analysis with in-depth peptide mapping solutions for complex therapeutics
- Expand Your PFAS Analysis
- DAR Measurement of Intact Antibody Drug Conjugates Using an icIEF-UV/MS Workflow
- Sensitive Quantification of AAVs and Their Impurities
- Accelerating Residual Solvents Analysis in 21 CFR Part 11 Compliant Settings
- Targeted and Non-targeted Analysis of Fentanyl Analogs and Their Potential Metabolites Using LC-QTOF
- Sensory Directed Analysis of Tuna and Plant-Based Tuna

Please click the circles to navigate

Targeted and Non-targeted Analysis of Fentanyl Analogs and Their Potential Metabolites Using LC-QTOF

Using the SCIEX X500B QTOF system and SCIEX OS software

By Matthew Standland, Aaron Stella, Mike Shaw, Holly Lee, Olin Jackson, and Glen Taylor

This technical note describes a high-throughput method that enabled the simultaneous quantitation and both targeted and non-targeted screening of fentanyl and its analogs in a single injection of urine samples. Using the X500B QTOF system, SWATH data-independent acquisition (DIA) collect MS/MS data for spectral matching against the SCIEX NIST 2017 MS/MS library and metabolite identification using the Molecule Profiler module of SCIEX OS software.

The Centers for Disease Control and Prevention (CDC) estimates that approximately 150 overdose deaths occur daily due to synthetic opiates, such as fentanyl. The structural diversity of fentanyl complicates this public health crisis because as many as 1,400 analogs are known to date. Forensic toxicology laboratories typically perform immunoassay tests to screen for different drug classes in urine. However, their lack of specificity often requires LC-MS/MS analysis to quantify and identify specific compounds. This combined approach is lengthy and laborious, and it often excludes new designer analogs that are illicitly produced. These drugs are often produced with sub-optimal synthesis and stored in poor conditions, which often lead to the final product containing many analogs and impurites that necessitate a non- targeted approach to capture as much information as possible for confident identification. SWATH DIA is a novel LC-MS acquisition technique that produces accurate mass spectra for all ions and all fragments in a single sample injection. The resulting spectra can be retrospectively mined for further identification.

Key features of SWATH DIA using the X500B QTOF system and SCIEX OS software:

- High-throughput workflow combining quantitation based on TOF MS peak areas and both targeted and non-targeted screening based on TOF MS/MS spectra in a single injection.
- SCIEX OS software offers a single platform for data acquisition, processing and rapid review of complex data.
- Molecule Profiler module delivers an intuitive workflow for identifying fentanyl and its analogs in urine samples.

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vMethod Application
Expand your NPS screening capabilities using the vMethod application for forensic toxicology screening

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Forensic Testing & Analysis
Overview

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Sensory Directed Analysis of Tuna and Plant-Based Tuna

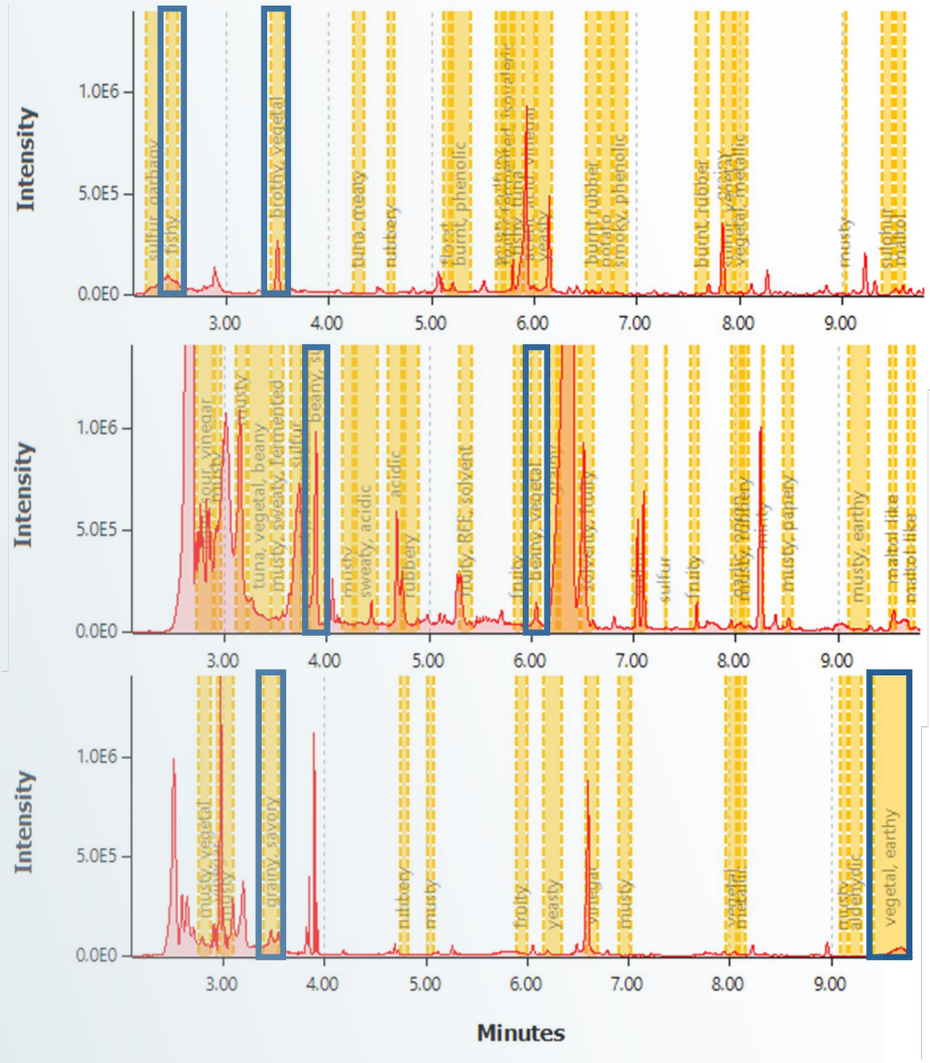
Only the nose knows: sensory directed analysis of key flavors in tuna and plant-based tuna using GC-MS with olfactory detection

By Nicole C. Kfoury, Jaqueline A. Whitecavage, Megan C. Harper, and John R. Stuff

Plant-based protein sources produce aromas that are not generally associated with animal- based foods. In this work, Sensory Directed Analysis with GC-O/MS were used to determine, identify, and compare key sensory-active flavor compounds in plant-based tuna with those in animal tuna to facilitate plant-based food product development.

The plant-based food market is projected to grow significantly as more and more consumers reduce their intake of animal-based foods. Fish replacement products have emerged in recent years and manufacturers face the challenge of replicating fish flavor and texture for consumers. Sensory directed analysis (SDA) is a process that utilizes gas chromatography in combination with human olfactory detection and mass spectrometry to identify sensory-active (flavor) compounds. Parallel olfactory and MS detection enables simultaneous determination of sensory-active regions of the chromatogram and mass spectral identification of the associated flavor compounds. As a result, SDA can be used to solve sensory-related challenges by determining the compounds responsible for producing desirable and undesirable flavors in food products. In this study, dynamic headspace (DHS) was used as an automated, solventless means of extracting analytes. Selectable 1D/2D-GC-O/MS or "heart-cutting" GC was used to resolve the complex compound mixture. Two GC columns with dissimilar column phases and a valveless, software-controlled column switching device were used for easy implementation of the 2D GC separation. This combination of techniques, software, and the SDA approach enabled the identification of key flavor compounds in real and plant-based tuna fish products.

View the full application note online



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On Demand Webinar
Sensory Directed Analysis
A Method for Identifying Off-Odors

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An Ultra-High Sensitivity Analysis of PFAS Compounds in Multiple Water Sources

Using the SCIEX 7500 system

By Bertram Nieland, Jack Steed, Rens-Jan Jaspers, Kay Hup, Abdessamad Chahboun, and Jianru Stahl-Zeng

In this method, sub-parts per trillion (ppt) levels of detection for 26 PFAS compounds was achieved with LOD values of 0.2 ng/L in diluent and calculated method detection limits in drinking, ground, and surface water ranging between 0.06 ng/L and 1.12 ng/L.

PFAS in our environment are becoming increasingly prevalent due to decades of overuse and their persistent nature, which means that they will be of concern for decades to come. It is, therefore, imperative to employ rigorous and sensitive analytical testing in order to develop regulations for these compounds and try to limit their possible effects on human health. In December 2020, the European Parliament and Council of the European Union released a new directive that sets the limit of PFAS in drinking water to 0.5 µg/L for the sum of all PFAS compounds identified, and 0.1 µg/L for a subset of PFAS compounds that are particularly concerning for humans. The 0.1 µg/L limit applies to the compounds included in this list, which contain a perfluoroalkyl moiety with three or more carbons (i.e., $-CnF_2n-$, n ≥ 3) or a perfluoroalkylether moiety with two or more carbons (i.e., $-CnF_2nOCmF_2m-$, n and m ≥ 1). The 0.5 µg/L limit applies to all PFAS compounds in total. The method presented here is demonstrated for drinking water, surface water and groundwater, and thus can help to ensure that these water sources are not contaminated and that drinking water is not affected.

The key benefits of PFAS analysis using the SCIEX 7500 system are:

- Ultra-high levels of sensitivity with LOQ values between 0.2 ng/L and 2.0 ng/L – see Figures 1 and 2 which highlight the sensitivity achieved
- Calculated method detection limits between 0.06 ng/L and 1.12 ng/L in three different water matrices
- Twenty-six relevant PFAS compounds analyzed in LC-MS grade, drinking, ground and surface water
- The mitigation of equipment, which can cause PFAS contamination to decrease blank contamination and interference at the analyte retention time

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Per- and perfluoroalkyl (PFAS) solutions for water analysis using the SCIEX 7500 system

On Demand
PFAS summit: a scientific series

Explore
SCIEX 7500 system

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Enhancing VOC Analysis with Vocus CI-IMS-TOF: Isomer Separation Unraveled

Revolutionizing mass spectrometry for unambiguous isomeric identification

By Michael Kamrath and Felipe Lopez-Hilfiker

This application note explores the breakthrough capabilities of the Vocus CI-IMS-TOF in resolving isomeric compounds, addressing challenges in atmospheric VOC analysis. By combining chemical ionization with ion mobility spectrometry, this instrument provides a two-dimensional view for precise identification and quantification, surpassing conventional methods. Real-time monitoring further elevates its utility, making it indispensable in dynamic atmospheric chemistry environments.

Chemical ionization mass spectrometers reliably analyze VOCs in a variety of environments. However, when a compound is masked by either an isomeric or isobaric interference, this presents ambiguity in the analysis. Interferences introduce uncertainty in mass spectrum peak identification because there is no definitive measurement of the structures contributing to a mass spectrum peak. As an example, this is a common issue in flavor and fragrance chemistry where a single molecular formula (ion) may be present in several isomeric forms. Each ion is of importance since they uniquely contribute to the perceived flavor or fragrance. Figure 1 presents two vanillin isomers – methyl salicylate and methyl paraben. To separate and differentiate between these isomers, mass spectrometry must be supplemented with another dimension of separation. When distinct isomers need to be resolved, ion mobility spectrometry (IMS) is a technique that can be used in conjunction with mass spectrometry. IMS discriminates between ions that adopt structures which differ in their rotationally averaged collision cross sections (i.e., average molecular size). With Vocus IMS-MS, analytes are ionized by chemical ionization and the resulting ions are separated by their collision cross sections before mass analysis. This provides two-dimensional information: the mass-to-charge ratio and cross section-related IMS. This deconvolutes multiple isomeric or isobaric components present in a single mass spectrum peak.

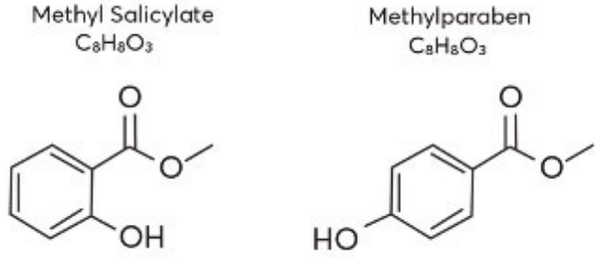

Figure 1. The isomeric structures of methyl salicylate and methylparaben.

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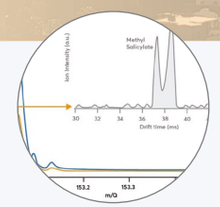
Webinar
Unlocking the Power of Isomer Separation with the Vocus CI-IMS-TOF



Isomer Separation
with Vocus Ion Mobility Spectrometry

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Accurate and Reproducible Quantitation of xC/UV/MS Data from Any Instrument

Process and quantify all xC/UV/MS data in a single software interface with software compatible with major vendor formats

By Baljit Bains and Anne Marie Smith

Process and quantify all xC/UV/MS data in a single software interface from any instrument. Learn how to consistently achieve reliable and accurate results using the quantitation workflow in ACD/Labs MS Workbook Suite software.

Quantitative analysis addresses scientific challenges across various industries, such as food, environment, pharmaceutical, and more. It is crucial to ensure the quality of a product by answering "How much?". This question is answered using essential quantitation xC/UV/MS workflows. Using MS in tandem with gas chromatography (GC) or liquid chromatography (LC) detects and quantitates analytes of interest in highly complex mixtures, and allows for an efficient, selective, and sensitive separation.

In present-day multi-vendor laboratories, there are challenges in learning and navigating multiple applications with different interfaces and difficulty in standardizing data. Using software compatible with most leading vendor data formats allows for consistency and standardization of quantitative analysis.

MS Workbook Suite software offers a fully integrated quantitative workflow where all xC/UV/MS data can be processed and quantified in a single interface – ensuring consistently reliable and accurate results.

The quantitation workflow is used to determine the amount of analyte present and consists of:

SET UP
Define sample information including sample type, type of trace, and the compound(s) for quantitation.

PROCESS
Define the processing parameters and identify the peaks for quantitation via retention time. Apply peak detection and integration parameters to create a calibration curve and calculate the linear regression, standard deviation, and r^2. Determine the suitability and validity of the statistical regression model using the analysis of residuals.

REVIEW, STORE & REPORT
Automatically save the full set of data (raw and processed) to a database where it is possible to visualize data with its chemical context and results. View and/or modify the results and track changes made in the processing interface for data integrity purposes. The final step is to create and share customized reports.

The quantitation workflow within MS Workbook Suite ensures connectivity between data and numerical results for routine delivery of reproducible and accurate quantitative data analysis.

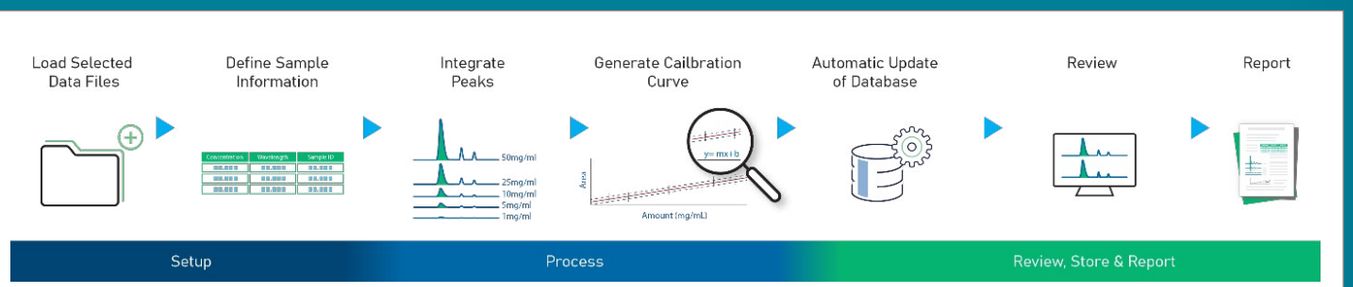

Figure 1: The intuitive calibration and quantitation workflow in MS Workbook Suite.

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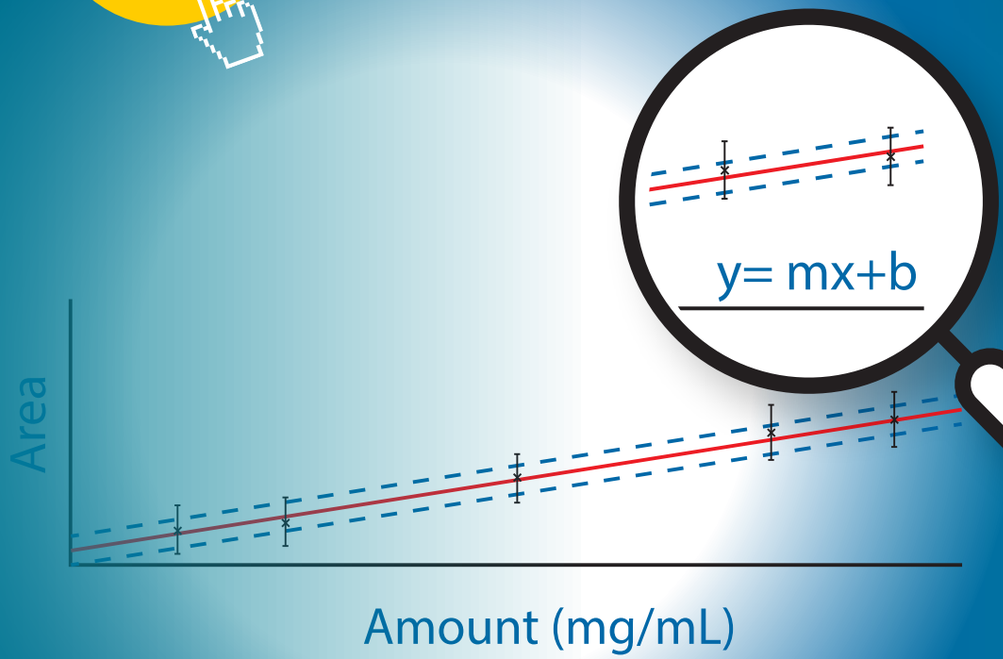

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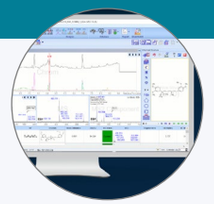
Resource
Accurate and Reproducible Quantitation of xC/UV/MS Data from Any Instrument



MS Workbook Suite
Mass Spectrometry Software for Spectral Interpretation and Component Characterization

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A Novel Approach to Assess Encapsulation Efficiency of mRNA-Lipid Nanoparticles

Determination of mRNA integrity, purity and encapsulation efficiency of mRNA-lipid nanoparticles (mRNA-LNPs) using the BioPhase 8800 system and the RNA 9000 Purity & Integrity kit

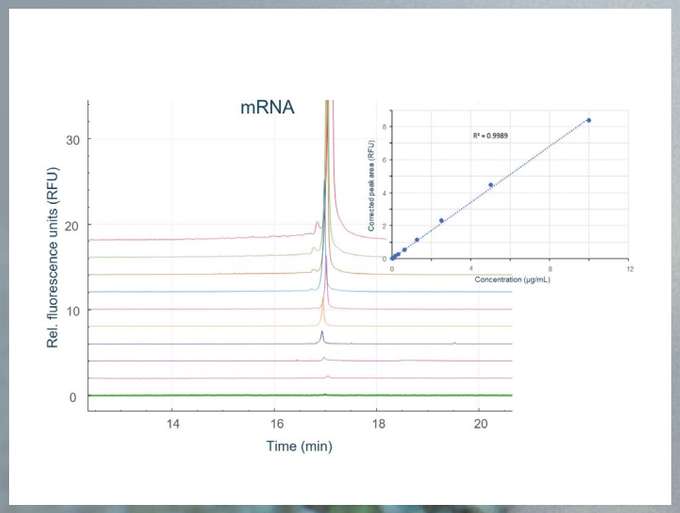
By Jane Luo, Zhichang Yang, Sahana Mollah, Adam Kowalczyk, Razvan Cojocaru, and Jon Le Hurayg

In this technical note, a new method for mRNA-LNP encapsulation efficiency determination by capillary gel electrophoresis coupled to laser-induced fluorescence detection (CGE-LIF) is showcased. The high-resolution separation of the intact mRNA from impurities and other species provide insights into the mRNA quality, adding another dimension for improved encapsulation efficiency determination.

mRNA-LNPs are becoming increasingly significant in pharmaceutical applications as prophylactic vaccines and next-generation therapeutics. In addition to mRNA integrity and purity, the efficiency of encapsulating mRNA in the nanoparticle is a quality attribute of mRNA-LNPs. Commonly, fluorescent dye assays are used to determine encapsulation efficiency. While these tests are simple, they cannot differentiate between full-length mRNA and degraded species. Additionally, the use of the detergents during sample preparation can introduce background noise and excipients in certain LNP formulations, interfering with the binding of the dye to mRNA.

Encapsulation determination with CGE-LIF include capabilities to:

- Leverage high sensitivity to enable accurate encapsulation efficiency determination with a 2.7 log linear dynamic range
- Achieve simultaneous mRNA integrity determination to understand your mRNA quality
- Break through boundaries of separating impurities for mRNA purity assessment with high-resolution power
- Minimize user-errors and simplify operation with the ready-to-use RNA 9000 Purity & Integrity kit



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mRNA analysis

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Messenger RNA analysis

On Demand
Better mRNA-LNPs

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Multi-Technique, Vendor Neutral Analytical Data Handling for Chemists

Accelerate analytical data interpretation and decision-making processes with a single, easy-to-use software application

By Sanji Bhal

Handling all your analytical data in one application allows you to free-up valuable instrument time, assemble all related analytical and chemical information together for ease of interpretation, and create publication-ready reports. This application note explores the how, in addition to these benefits, Spectrus Processor's structure integration and chemically intelligent tools further simplify routine analytical data processing.

Scientists use a diverse range of analytical techniques to confirm, identify, and characterize chemical structures. Instruments used to collect data are rarely from a single vendor, and each comes with its own software to process that data. The primary goal of this data is to help answer questions such as: "Did I make what I think I made?", "How much is there?", and "how clean/pure is it?".

This plethora of software is not only a training burden; valuable instrument time is often "tied up" by scientists processing their data, and frustratingly, all the analytical information gathered to support a stop/go decision cannot easily be brought together and reviewed in one place. Furthermore, today more than ever, the primary responsibility for data interpretation often falls to non-specialists in analytical chemistry.

Wouldn't it be great if you could use a single piece of software to answer these questions, no matter the analytical technique or instrument vendor?

Spectrus Processor allows you to view, process, interpret and report data from all major vendor formats. Whether you use NMR, chromatography, mass spectrometry, FT/IR, Raman or other analytical techniques, this single software platform can process and interpret all your spectrometric and spectroscopic data.

This application note addresses how Spectrus Processor provides immediate feedback to the chemist who can confirm the expected structure or question the outcome of a reaction quickly, allowing them to make faster, more informed decisions about the relationship between a chemical structure and analytical data.

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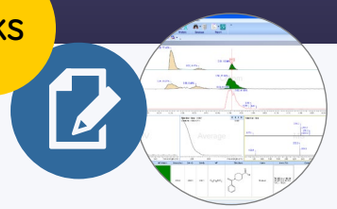

Spectrus Processor
Process, Analyze, and Report All Your Analytical Data in One Interface

Blog
5 things you need to STOP doing in your lab

Case Study
Takeda Streamline Analytical Handling with Spectrus Processor

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Low-pg/mL Quantification of TL 13-112, a Proteolysis Targeting Chimera (PROTAC) in Rat Plasma

Excellent quantitative performance for PROTACs in matrix using the SCIEX 7500 system

By Ebru Selen, Rahul Baghla and Eshani Nandita

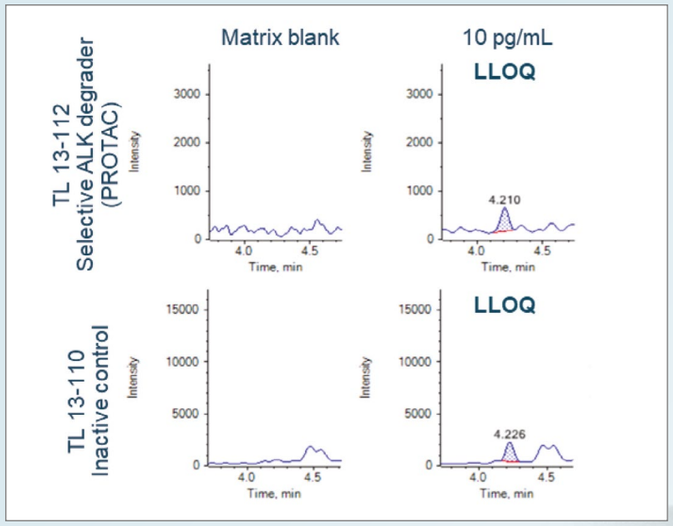
This technote demonstrates a highly sensitive, robust, and rapid quantification workflow for TL 13-112, a selective ALK degrader, and its inactive control, TL 13-110, in rat plasma on a high-end triple quadrupole mass spectrometer. A lower limit of quantification (LLOQ) of 10 pg/mL was achieved for both TL 13-112 and TL 13-110 using a fast and simple protein precipitation method with a 10-minute LC-MS/MS analysis.

The interest in targeted protein degradation has shifted from academia to industry after the therapeutic potential of a PROTAC was documented in 2001. PROTACs have emerged as a therapeutic modality and several candidates have moved into clinical trials. The potential of PROTACs is coded in its structure. A linker connects a protein of interest (POI) binding moiety to a ubiquitin E3 ligase recognition moiety (Figure 2A). The heterobifunctional structure enables PROTACs to bring the POI and E3 ligase closer in proximity. This induces the ubiquitination of the POI, which is then targeted by the disposal machinery of the cell.

One of the many attractive hallmarks of PROTACs is their high potency in nanomolar drug concentrations. Although their potential is well-documented, challenges remain for the analysis of PROTACs. Sensitive and selective assays for high-confidence detection and quantification of PROTACs are needed to ensure the safety and efficacy in the drug development pipeline.

Key features of the quantification of PROTACs using the SCIEX 7500 system are:

- New levels of quantification of low-dose, high-potency drug modalities: achieve low-pg/mL level LLOQs for the quantification of PROTACs in rat plasma on the SCIEX 7500 system equipped with an innovative front-end design
- Robust analytical performance: reach exceptional quantitative performance with strong linearity and excellent accuracy and precision for low-level quantification
- Streamlined data management: employ fast, intuitive and integrated data acquisition and processing using SCIEX OS software



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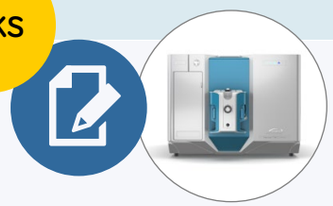
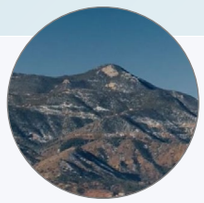


Sciex 7500 system



Additional Resources
Biopharma / pharma research

Additional Resources
Pathways to a new frontier of bioanalysis

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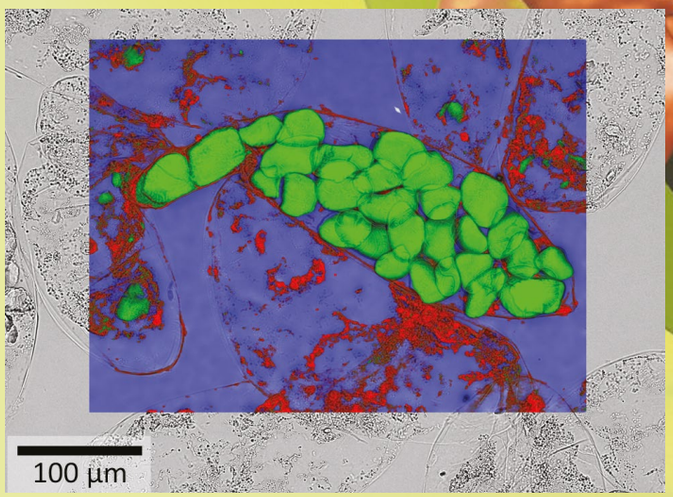
Food Analysis with Confocal Raman Microscopy

High-resolution chemical characterization reveals how the distribution and structure of ingredients and additives affect food properties

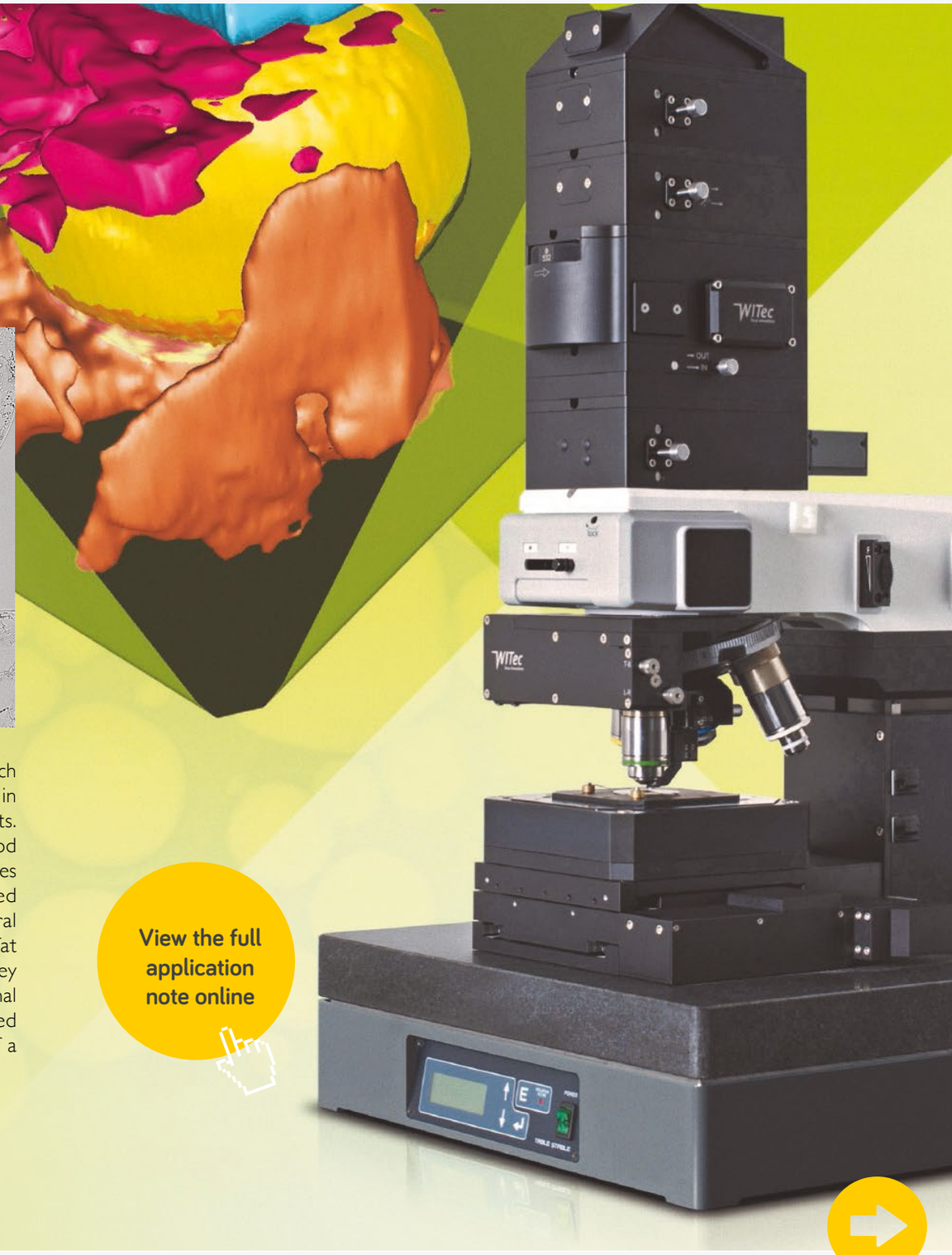
This application note shows how confocal Raman imaging, topographic Raman imaging and Raman-based particle analysis can characterize fat spreads, conventional and spreadable butter, white chocolate, honey, sugar, banana pulp, particulate baking ingredients and more.

In the food industry, various ingredients and additives such as emulsifiers, stabilizers or thickeners are commonly used to optimize the texture or flavor of food. Their distribution and microstructure strongly influence the properties of the final product. Therefore, research and development, as well as quality control, require powerful analytical methods for studying the distribution of compounds in food.

Confocal Raman microscopy is a versatile tool for analyzing the chemical composition of samples on the sub-micrometer scale that is well suited to analyses in food science. The physical phenomenon that underlies Raman microscopy, known as the Raman effect, is the slight wavelength shift in light that has been inelastically scattered by molecules of gaseous, liquid or solid materials. The incident photons from a monochromatic excitation source cause vibrations of the molecule's chemical bonds, leading to a specific change in energy that is visible in a Raman spectrum. Every chemical compound produces a unique Raman spectrum when excited and can be quickly identified by it. Raman imaging microscopy acquires a Raman spectrum at each image pixel over a sample area. This information is then compiled in an image that visualizes the distribution of its chemical components.



This survey shows how Raman imaging can characterize food samples to help understand the products and production processes with confocal measurements, scans guided by an integrated profilometer, and investigations that employ a Raman spectral database. It describes experiments on white chocolate, fat spreads, a sugar bar, a squashed banana pulp sample and a honey pollen grain. It also features 3D Raman imaging of conventional and spreadable butter, topographic Raman imaging of frosted gingerbread, and Raman-based automated particle analysis of a mixture of baking ingredients.



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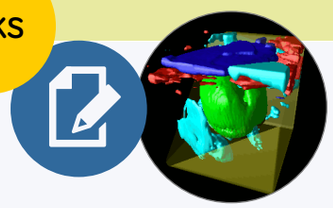

Application
Food Technology

Product
alpha300 R –
Raman Imaging Microscope

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Achieve New Heights in PTM Analysis with In-Depth Peptide Mapping Solutions for Complex Therapeutics

Gain confident sequence confirmation of an ADC and accurate localization of the payload

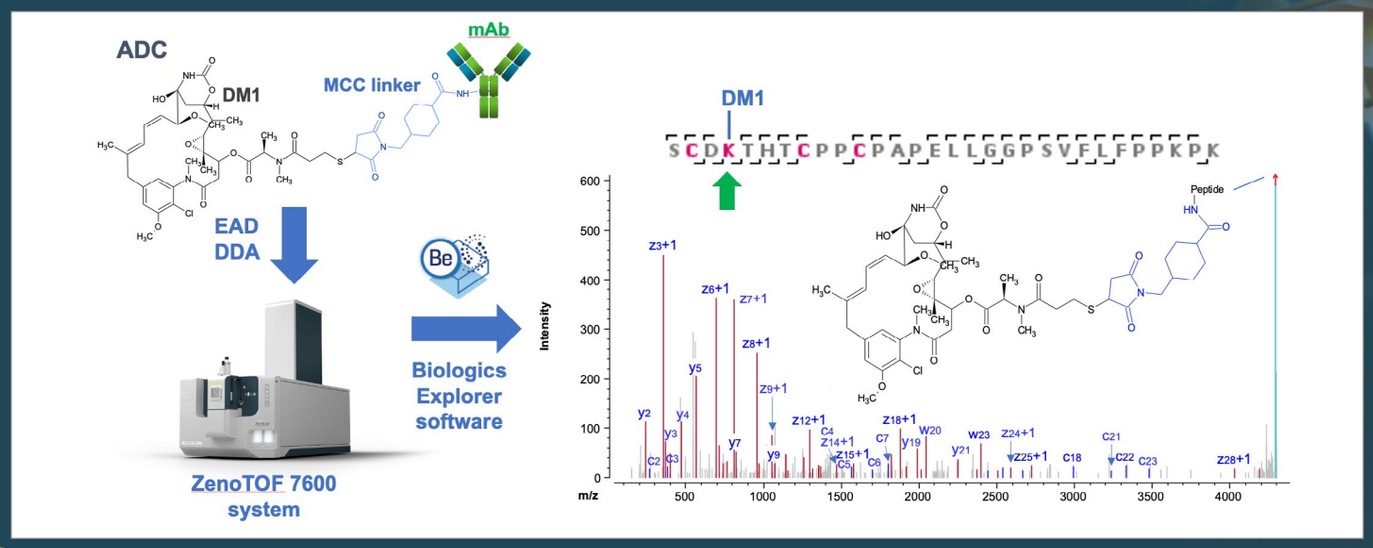
By Zoe Zhang, Haichuan Liu, Takashi Baba, Pavel Ryumin, Bill Loyd, Jason Causon and Kerstin Pohl

- Enable comprehensive analysis of mAbs and ADCs in a single injection
- Confident payload localization and detailed structural characterization
- Simplify method implementation with automated interpretation of EAD DDA data

Antibodies and ADCs have become an important class of biotherapeutics. ADCs are typically composed of a monoclonal antibody (mAb) covalently coupled with a cytotoxic payload through synthetic linkers. It is critical to fully characterize ADCs during their development to ensure drug quality, safety and efficacy.

Comprehensive characterization of ADCs typically involves confirmation of the mAb sequence, identification and localization of post-translational modifications (PTMs) on the mAb, measurement of the drug-to-antibody ratio (DAR) and determination of payload conjugation sites. Intact mass analysis is commonly utilized to determine the DAR, while sequence confirmation and localization of the payload are typically achieved using a bottom-up approach.

This technical note discusses a bottom-up, peptide mapping workflow enabling simultaneous sequence confirmation and accurate payload localization in a single injection.



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Up the game for ADC analysis

Content Pack
Deep dive into the characterization of an ADC

Boost your biotherapeutic
Software to get exactly what you want

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Produced by the Analytical Scientist

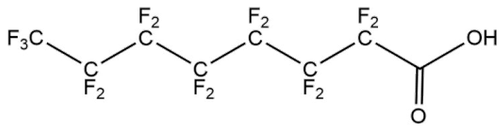
Expand Your PFAS Analysis

Screen for targets and identify unknowns simultaneously

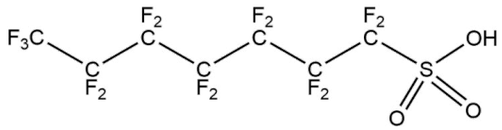
By Nick Jones and David Alonso

This application snapshot focuses on the rapidly expanding area of PFAS analysis and highlights how screening for known PFAS targets, as well as discovering and identifying unknown PFAS chemicals, can be performed using high-performance GCTOFMS.

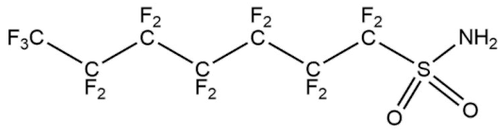
Per- and polyfluoroalkyl substances (PFAS) chemicals- also known as "forever chemicals" have been a topic of research recently due to their high stability in our environment and food chain. Regulatory control of these species in our environment continues to accelerate. Analysis of PFAS can be challenging due to the large number and variety of types of PFAS chemicals. To achieve the best results of PFAS analysis, it is recommended that Gas Chromatography (GC) with Time-of-Flight Mass Spectrometry (TOFMS) methods are used.



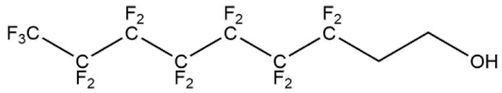
Perfluorooctanoic acid (PFOA)



Perfluorooctanesulfonic acid (PFOS)



Perfluorooctanesulfoamide acid (PFOSA)



Octylfluorotelomer ethanol (8:2 FTOH)

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DAR Measurement of Intact Antibody Drug Conjugates Using an icIEF-UV/MS Workflow

Assess and monitor product quality during ADC development and manufacturing with the Intabio ZT system

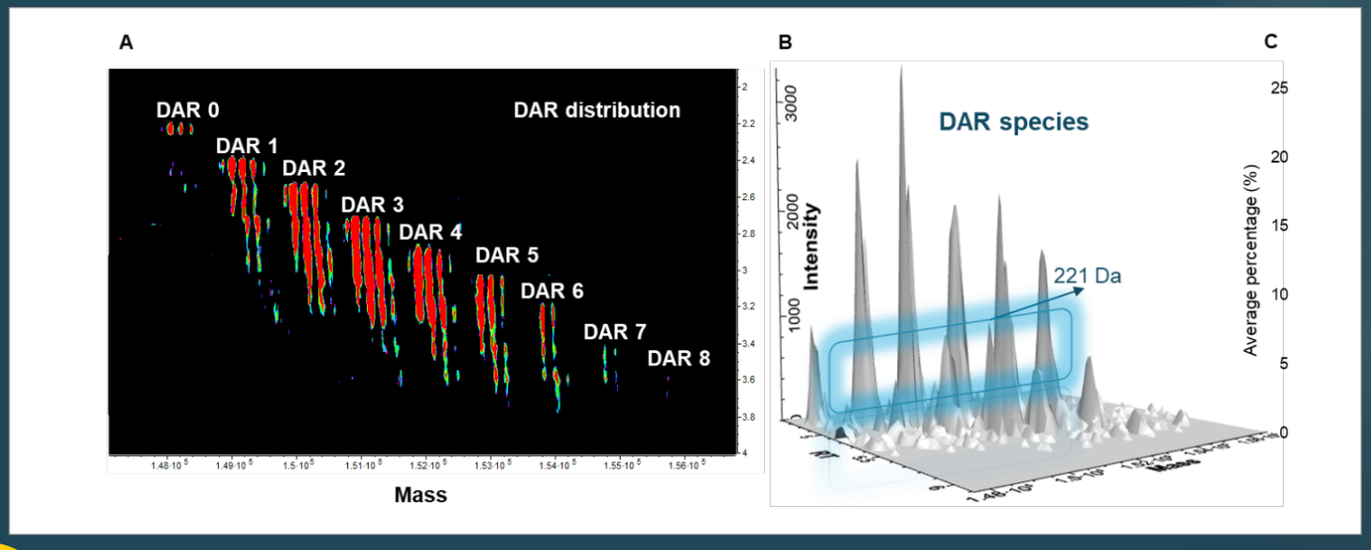
By Rashmi Madda, Jingwen Ding, Haichuan Liu, Scott Mack, Maggie Ostrowski and Zoe Zhang

- *Single-platform analysis of intact ADCs: Charge-based separation using icIEF, confident charge-variant identification with high-resolution MS and accurate DAR measurement in a single injection*
- *Accurate DAR measurement: automated protein deconvolution, confident peak annotation and DAR calculation*
- *Routine ADC assessment: Incorporate into standard procedures to assess and monitor product quality during ADC development and manufacturing*

ADCs are cutting-edge biotherapeutics that selectively target specific cells and are constructed by linking potent cytotoxic drugs to monoclonal antibodies (mAbs). Reliable determination of drug-to-antibody ratios (DARs) is important to assess and monitor product quality. Variations in the number of drug molecules attached to the backbone mAb result in a complex mass spectrum, and the types of linker chemistry used along with post-translational modifications can significantly contribute to the charge heterogeneity of an ADC product.

Performing charge-based separation prior to intact MS analysis simplifies the complexity of the spectrum, enables the confident identification of charge variants and accurate DAR measurement.

In this technical note, a streamlined icIEF-UV/MS workflow was leveraged to obtain a high-resolution charge variant separation of T-DM1 and accurate DAR measurement.



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LINKS

Resources
Up the game for ADC analysis

Content Pack
Deep dive into the characterization of an ADC

Product
Comprehensive charge variant analysis made simple

Sponsored by SCIEX The Power of Precision

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Sensitive Quantification of AAVs and Their Impurities

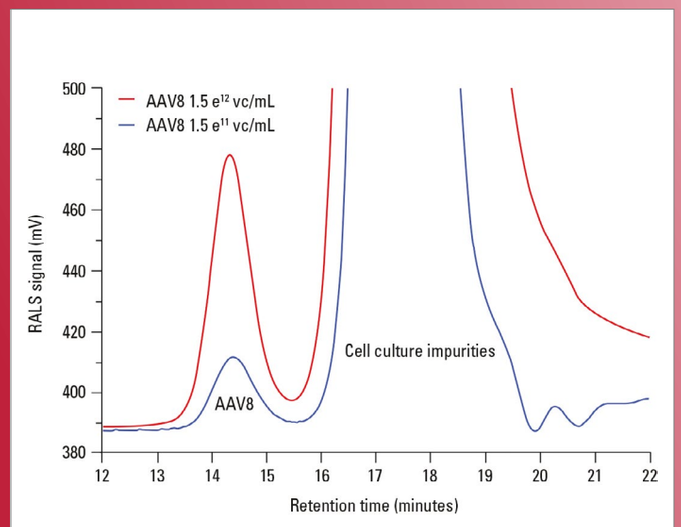
SEC-MALS sensitively quantifies adeno-associated viruses and detects impurities

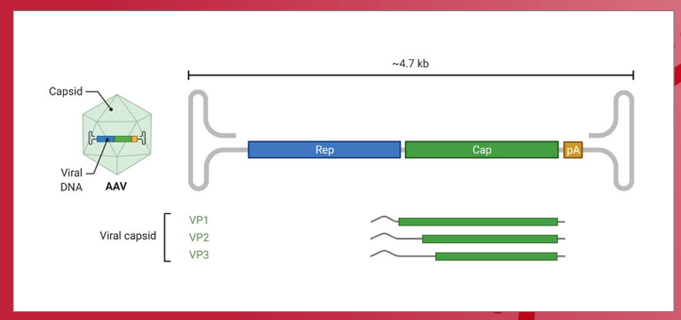
A rapid and reproducible analytical methodology is crucial for the timely development of high-quality tools for cell and gene therapy applications. This application note describes a powerful analytical SEC-MALS technique for the sensitive detection of AAVs using the LenS3 MALS detector and the TSKgel GMPWXL column.

Cell and gene therapy in medicine relies on efficient and specific delivery of therapeutic genetic material to target cells. These delivery devices are called vectors, and many have been developed from viruses. The parvovirus family of viruses called adeno-associated viruses (AAVs), have emerged as the vector of choice in therapeutic gene delivery due to their optimized tissue and cell specificity and their ability to edit the genome precisely. AAVs are non-enveloped viruses with an icosahedral structure belonging to the parvovirus family and containing single-stranded DNA (ssDNA) (~4.7 kb in length) as their genome.

Development of clinically desirable AAV capsids with optimal genome design requires rapid, accurate, and robust analytical methods to assess AAV purity, capsid titer, DNA content, and structure-activity relationships. Size exclusion chromatography (SEC), when coupled with multi-angle light scattering (MALS), offers a powerful analytical method for AAV characterization. In contrast to the traditional method of AAV analysis using the UV 260/280 nm absorbance ratio, the advantage of MALS detection is the high sensitivity for AAVs due to their considerable capsid mass (~3.7 MDa), inherently providing a strong light scattering response for analytical characterization.

A rapid and reproducible analytical methodology is crucial for the timely development of high-quality tools for cell and gene therapy applications. This application note describes a powerful analytical SEC-MALS technique for the sensitive detection of AAVs using the LenS3 MALS detector and the TSKgel GMPWXL column. The limit of detection (LOD) for the method was determined to be as low as 7.0 x 109 vc/mL (2.8 x 108 vc loaded in 40 µL). The high sensitivity of SEC-MALS detection allows the injection of low-concentration AAV samples which is a highly sought attribute in the early development stages with often limited availability of material. In addition, AAV separation directly from HEK cell culture supernatant was possible enabling direct MALS detection without prior AAV purification. In summary, the SEC-MALS methodology described here provides a sensitive, powerful, and robust tool for the detection of AAVs during product development, viral vector production, and throughout quality control.





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LINKS

Webinar
AAV characterization by SEC-MALS

Product
LenS3 Multi-Angle Light Scattering Detector

Product
GMPWXL

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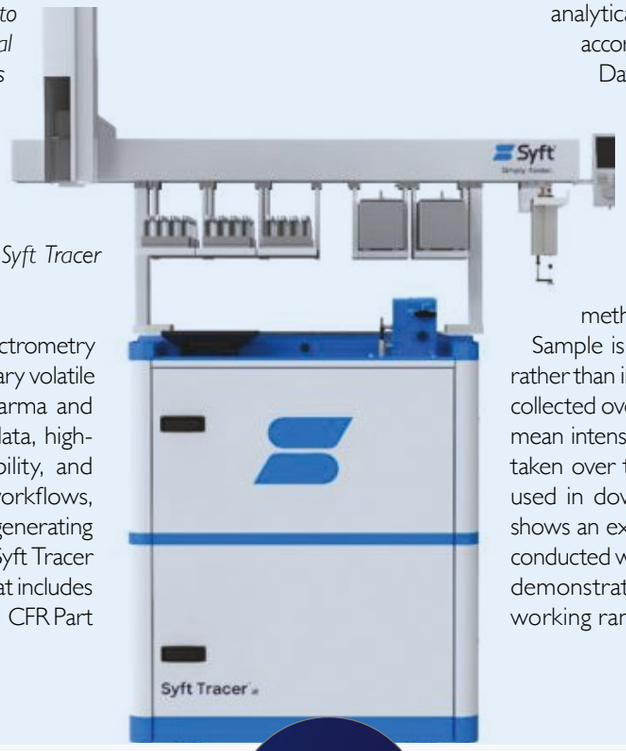
Accelerating Residual Solvents Analysis in 21 CFR Part 11 Compliant Settings

Using real-time mass spectrometry to streamline high throughput CDMO and Pharma applications through faster time to data and analytical versatility

By Dr. Sophia Whitlock, Dr. Daniel Milligan, and Paul Johnson

SIFT-MS is a direct, real-time mass spectrometry (MS) technique which offers revolutionary volatile compound analysis capabilities to Pharma and CDMO labs due to its fast time to data, high-throughput capacity, analytical flexibility, and ease of use. It expedites analytical workflows, such as residual solvents analysis, by generating faster results than traditional methods. Volatile impurities such as nitrosamines, ethylene oxide, and residual solvents can be characterized in real-time using the Syft Tracer Pharm11 automated solution.



SIFT-MS is a direct, real-time mass spectrometry (MS) technique which offers revolutionary volatile compound analysis capabilities to pharma and CDMO labs due to its fast time to data, high-throughput capacity, analytical flexibility, and ease of use. It expedites analytical workflows, such as residual solvents analysis, by generating faster results than traditional methods. Syft Tracer Pharm11 is a SIFT-MS-based solution that includes SyftAuditTracer software designed for 21 CFR Part 11 compliant environments. Volatile impurities can be characterized in real-time including nitrosamines, ethylene oxide, and residual solvents.

Syft Tracer Pharm11 provides unmatched ease of use while increasing workflow efficiency through minimal sample preparation requirements, high sample capacity, and analytical flexibility. System or column changeover between analytical methods is not required, saving both analyst and instrument time. SIFT-MS eliminates the need for chromatographic separation and achieves selectivity via rapidly switchable reagent ions. Analytes undergo different reactions with each reagent ion, generating product ions via independent ion chemistries that are diagnostic of species identity.

SyftAuditTracer software is designed to maximize the speed and ease of residual solvents analysis while supporting 21 CFR Part 11 compliant processes. It allows users to easily acquire, process, and report on data while maintaining a traceable account of all user actions, records, and system access within the audit trail.

SIFT-MS is well-suited to residual solvents analysis (USP<467>), with analytical performance previously validated according to USP<1467> (Biba et al. (2021)). Data are generated in real-time, with each SIFT-MS sample analysis completing in several minutes (≤3 minutes). Sample throughput of >200 per day is achievable with this automated solution. Additional batches can be queued to run immediately after a sequence starts, even if different methods are used.

Sample is injected over a long, slow injection, rather than in a tight, fast band. The raw signal data collected over the injection is averaged to obtain a mean intensity (see Figure 1 showing an injection taken over the course of 100 seconds), which is used in downstream data processing. Figure 2 shows an example calibration curve from a study conducted with the Class 2 solvent chlorobenzene, demonstrating excellent linearity across the working range (10-750 ppb in solution).

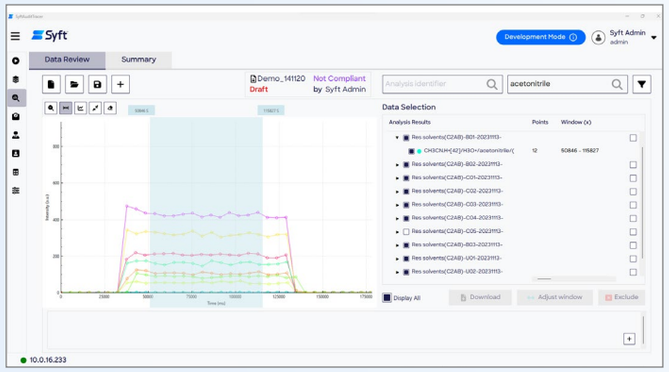


Figure 1. The first step of data processing involves computing a mean intensity across the sample injection profile. The plot illustrates the long, slow injections required for real-time SIFT-MS analysis. In this example, data is collected over the course of 100,000 ms (100 seconds).

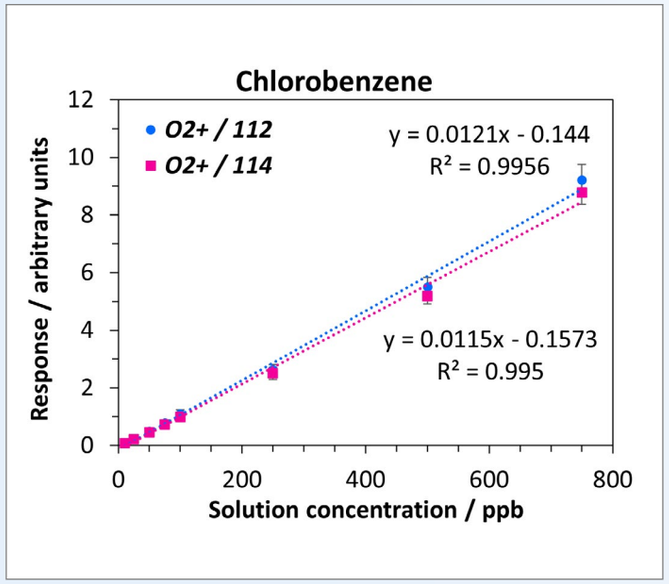


Figure 2. An example calibration curve for the Class 2 residual solvent chlorobenzene, exhibiting excellent linearity across the chosen range.

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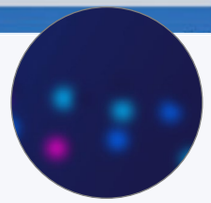
LINKS

Product Selection
SIFT-MS



Webinar
Rapid Analysis of Residual Solvents and Volatile Impurities for High-Throughput CDMO Workflows

Brochure
Syft Tracer Pharm11

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