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The Application Book

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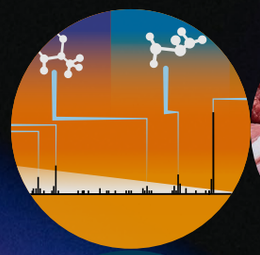
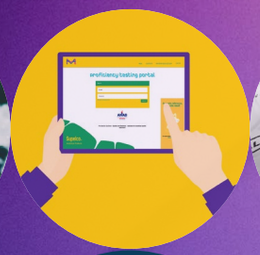


Tracking Down the Structure of an Unknown LC/MS Component

Veterinary Drugs in the Meat we Eat

An LC/MS Solution to Study Water Pollution



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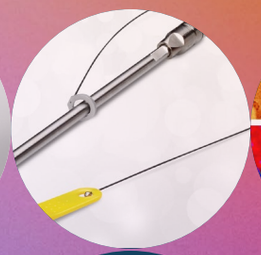
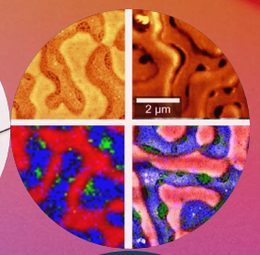


Increasing Chromatographic Performance of Acidic Peptides in RPLC-MS-Based Assays with Acquity Premier



Correlative Raman Imaging of Polymeric Materials

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TRACKING DOWN THE STRUCTURE OF AN UNKNOWN LC/MS COMPONENT

Find the best structural candidate among thousands of compounds by piecing together multiple clues

Charis Lam, Richard Lee and Joe DiMartino

The best structural candidate for an unknown LC/MS component is found by assembling several pieces of evidence. Using accurate mass, isotopic pattern, substructures, and predicted fragments, a database of 92 million compounds is narrowed first to 5,752 matches, and finally to one candidate.

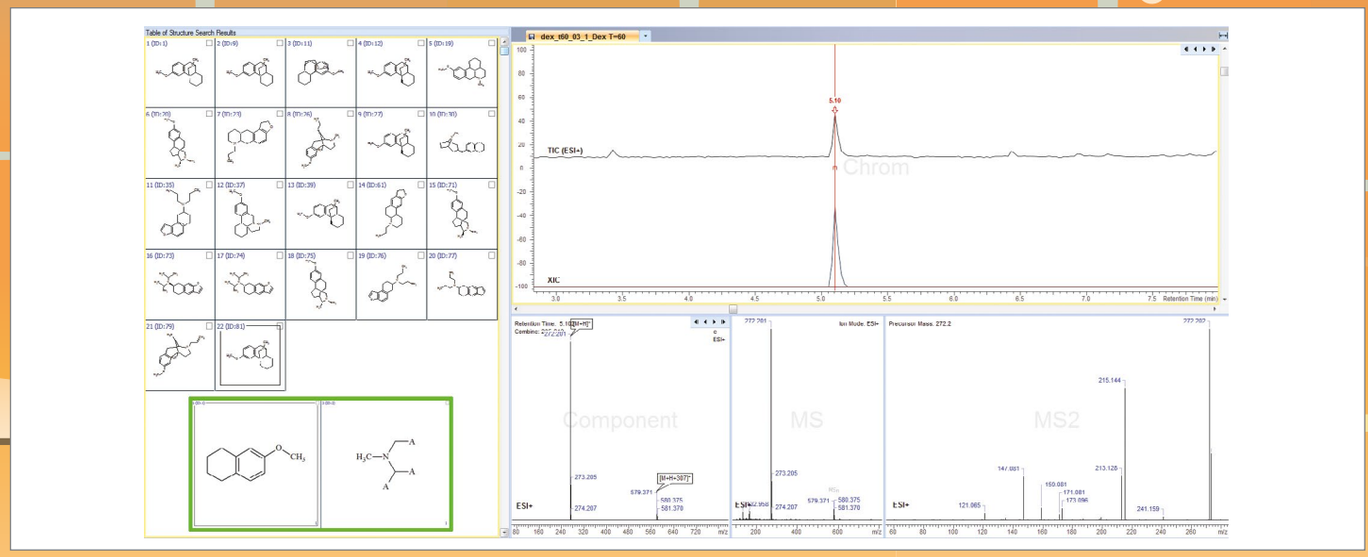

Figure 1. The list of structural candidates was narrowed from 5,752 to 22 after known substructures (inset, green border) were included as a search filter.

What is the structure of an unknown LC/MS component? It's hard to answer. Even with advances in LC/MS, each clue provides only partial information. High resolution narrows down the elemental composition, but doesn't distinguish between structural isomers. Fragment analysis highlights functional groups, but doesn't show how they piece together.

Each bit of evidence paints a partial picture, which means we can only reconstruct the whole image by using them all. Here we present a software workflow for that purpose: assembling known analytical information piece by piece, to narrow a list of structural candidates from thousands of compounds to only a few – or even one.

We used the software package ACD/MS Structure ID on HPLC-FTMS data from a pharmaceutical sample. After chromatographic deconvolution was performed on the TIC (+ve), one component (RT of 5.102 minutes, $[M+H]^+$ signal at *m/z* 272.2017) was chosen as an example. From the isotopic pattern, this compound was expected to contain only C, H, N, and/or O atoms. The software's Formula Generator used this information and the accurate mass to suggest the formula $C_{18}H_{25}NO$.

PubChem and ChemSpider databases were searched for this mass and formula. From a combined database size of 96 million compounds, the search returned 5,752 matches. To further filter the list, structure fragments were identified by examining the MS2 spectrum and other analytical data. The identified substructures were found in only 22 candidates.

Finally, mass fragments were predicted for these 22 compounds. Theoretical fragment ions were generated for each structure and matched against the experimental MS2 spectrum. Only two candidates had a match score above 0.80 (out of 1). More predicted fragments were generated for these two, and the match was run again – identifying the top candidate.

From a database of 96 million to a candidate list of 5,752 to a single compound – one workflow guided the progressive filtering. With software designed to help assemble analytical evidence, it's easy to find the one correct structure among millions.

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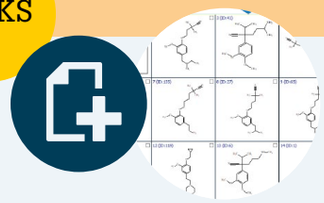

ACD/MS STRUCTURE ID SUITE

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VETERINARY DRUGS IN THE MEAT WE EAT

An end-to-end workflow for quantitative screening of multiclass, multiresidue veterinary drugs in meat using the Agilent 6470 Triple Quadrupole LC/MS

Siji Joseph, Aimei Zou, Chee Sian Gan, Limian Zhao and Patrick Batoon

This application note describes the development of a comprehensive LC/MS/MS workflow to quantify 210 veterinary drug residues in meat. Performance was evaluated across chicken, pork and beef using two different Agilent triple quadrupole LC/MS models (an Agilent 6470 and a 6495C triple quadrupole LC/MS). The aim: to accelerate and simplify routine testing.

A comprehensive LC/MS/MS workflow was developed for targeted screening or quantitation of 210 veterinary drug residues in animal muscle prepared for human consumption, with the intention to accelerate and simplify routine laboratory testing. The workflow ranged from sample preparation through chromatographic separation, MS detection, data processing and analysis, and report generation. The workflow performance was evaluated using three muscle matrices – chicken, pork and beef – and was assessed on two different Agilent triple quadrupole LC/MS models (an Agilent 6470 and a 6495C triple quadrupole LC/MS). A simple sample preparation protocol using Agilent Captiva EMR–Lipid cartridges provided efficient extraction and matrix cleanup. A single chromatographic method using Agilent InfinityLab Poroshell 120 EC-C18 columns with a 13-minute method delivered acceptable separation and retention time distribution across the elution window for reliable triple quadrupole detection and data analysis.

Workflow performance was evaluated based on evaluation of limit of detection (LOD), limit of quantitation (LOQ), calibration curve linearity, accuracy, precision, and recovery, using matrix-matched spike samples for a range from 0.1 to 100 µg/L. Calibration curves were plotted from LOQ to 100 µg/L, where all analytes demonstrated linearity R2 >0.99. Instrument method accuracy values were within 73 to 113 percent. Target analytes response and retention time %RSD values were ≤19 percent and ≤0.28 percent respectively. Analyte recovery and reproducibility at three levels of fortified quality control (QC) samples – 1, 10, and 25 µg/kg in meat – were used to validate the method applicability for confident routine screening of veterinary drugs. The recovery repeatability (intrabatch technical replicates) and recovery reproducibility (interbatch technical replicates) were calculated using QC samples, and the results were within acceptable limits of 20 and 32 percent, respectively (1). The workflow method performance results across the chicken, beef, and pork muscle matrices showed excellent overlap, and confirm the method applicability for routine multiresidue screening in various animal origin matrices.

Reference

1. *Guidelines for Standard Method Performance Requirements, AOAC Official Methods of Analysis (2016) Appendix F.*

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AN LC/MS SOLUTION TO STUDY WATER POLLUTION

Analysis of >50 legacy and emerging PFAS in water using the Agilent 6495B Triple Quadrupole LC/MS

Timothy L. Coggan, Jeff Shimeta, Bradley O. Clarke, Tarun Anumol and James Pyke

Per- and polyfluoroalkyl pollution is a serious concern that must be addressed by analytical techniques at the trace level. This application note describes a sensitive and reliable method for simultaneous quantitation of 53 legacy and emerging PFAS compounds using an Agilent 1290 Infinity II LC coupled to an Agilent 6495B triple quadrupole LC/MS.

The contamination of the environment with per- and polyfluoroalkyl substances (PFAS) is a serious concern to regulators, scientists and the public worldwide, due to their ubiquitous presence, persistence and toxicity (1–3). Robust analytical techniques that can accurately and precisely quantify these pollutants at trace levels are necessary for understanding their environmental fate, ecological impacts and impacts on public health. Appropriate analytical techniques and the fundamental data they generate allow scientists and regulators to make informed assessments of PFAS use in modern society. This application note describes a sensitive and reliable method for the simultaneous quantitation of 53 legacy and emerging PFAS from 14 compounds classes. The method uses isotope dilution on an Agilent 1290 Infinity II LC coupled to an Agilent 6495B triple quadrupole LC/MS (4).

References

1. *M Houde et al., "Biomagnification of perfluoroalkyl compounds in the bottlenose dolphin (Tursiops truncatus) food web," Environ Sci Technol, 40, 4138 (2006).*
2. *L Ahrens and M Bundschuh, "Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review," Environ Toxicol Chem, 33, 1921 (2014).*
3. *JP Giesy and K Kannan, "Global distribution of perfluorooctane sulfonate in wildlife," Environ Sci Technol, 35, 1339 (2001).*
4. *TL Coggan et al., "A single analytical method for the determination of 53 legacy and emerging per- and polyfluoroalkyl substances (PFAS) in aqueous matrices," Anal Bioanal Chem, 411, 3507 (2019).*

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PFAS PRESENTATION
from Marcus Chadha

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In the fast-paced world of routine lab testing, increasing pressure is placed on chemists and technicians to analyze more samples in shorter timeframes, and often with fewer personnel. Analytical processes and methods therefore need to be streamlined to improve efficiency.

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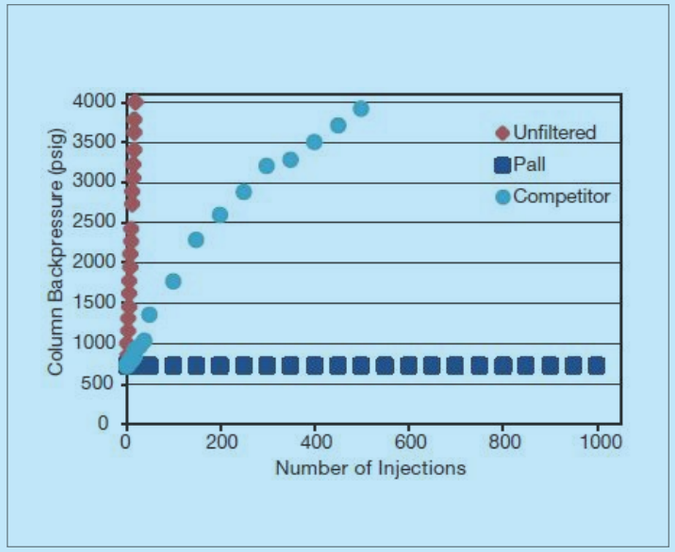
ACRODISC® SYRINGE FILTERS FOR ANALYTICAL SAMPLE PREPARATION; INCLUDING HPLC AND DISSOLUTION TESTING

An analytical technical guide for choosing a syringe filter to extend HPLC column life and limit extractables and drug binding

Thomas Valorose



This technical guide contains performance studies and useful selection criteria to help you choose the right syringe filter for your application. This guide is divided into sections, containing information on why filtration is necessary, main considerations for your application, minimizing the occurrence of extractables, Pall's product quality assurance and certifications, and more.

Sample and mobile phase filtration are simple, economical practices that serve to extend the life of consumable HPLC parts. In 1974, Pall Laboratory revolutionized sample preparation for analytical chemists with the development of the Acrodisc syringe filter. Today Pall produces high-quality filters for sample preparation and dissolution testing that meet the unique requirements of every lab we serve. Pall's microporous materials and filtration devices are manufactured under precise, highly controlled conditions. Pall's device manufacturing facilities utilize the most advanced sealing technologies, vision systems, and robotics platforms to ensure optimum lot-to-lot consistency.

Sample and mobile phase filtration are simple, economical practices that serve to extend the life of consumable HPLC parts, decrease system wear and tear, and preserve the integrity of the HPLC system. The adverse effects of improper filtration practices that occur to each component of the HPLC system are systematically and thoroughly explored in this guide. By reviewing these consequences, the analyst can become familiar with the early warning signs of filtration-related problems and avoid the expense and downtime related to lengthy maintenance repairs and replacement costs.

Choosing the proper filter requires knowledge of filter/solvent compatibility and the chemical/physical characteristics of the filter. These characteristics include pore size, pore distribution, filter thickness, extractables, hydrophobic/hydrophilic character, binding properties, pyrogenicity, gas and liquid flow rate, burst strength, autoclavability, pore size, and nominal particulate retention. Download the technical guide today for more detailed information, and to find out about the benefits of filtration for sample preparation.

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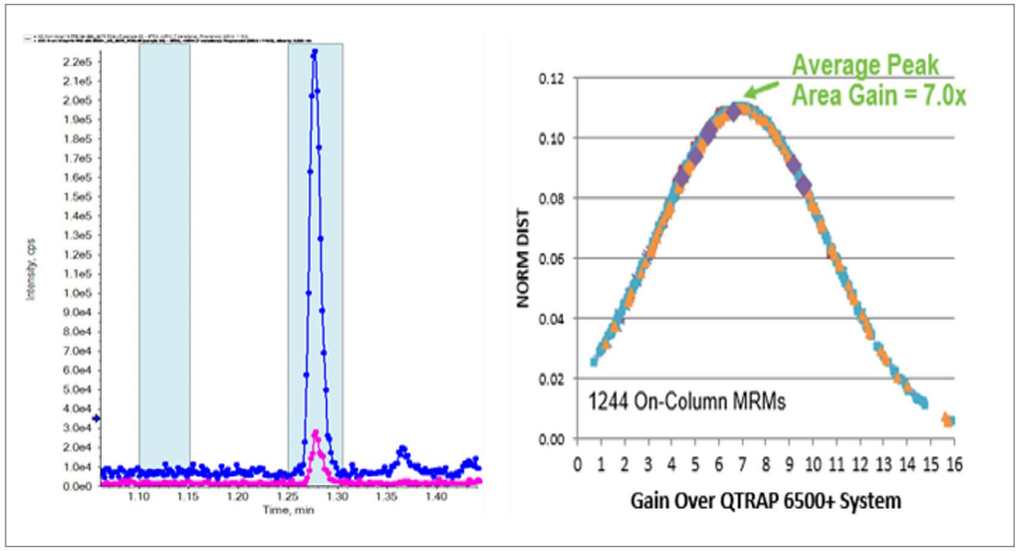
Ian Moore



Sensitivity gains for SCIEX 7500 System over QTRAP 6500+ System. Large numbers of analytes were run in various matrices on both systems and the peak areas and S/N gains were measured. Left: example data from propranolol in rat plasma, area gain of 9x with S/N gain of 3x. Right: summary of comparison of 1244 MRMs in positive and negative mode across 10 studies (pesticides, drugs, peptides), average peak area gain across the compounds was 7x.

The SCIEX 7500 System is enabling new levels of quantification across a large suite of sample types and workflows. The greater sensitivity is achieved through the introduction of hardware features that enable significant gains in the generation, capture and transmission of ions.

With the same ruggedness and robustness of previous generations, the Triple Quad™ 7500 LC-MS/MS System – QTRAP® with D Jet™ Ion Guide and integrated E Lens™ Technology combine to deliver significant sensitivity gains over previous generation instruments; average peak area gains of 7x and signal to noise gains of 2.5 to 3x.

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INCREASING CHROMATOGRAPHIC PERFORMANCE OF ACIDIC PEPTIDES IN RPLC-MS-BASED ASSAYS WITH ACQUITY PREMIER

Learn how MaxPeak HPS Technology can be applied in the development and manufacturing of therapeutic drug products

Robert E. Birdsall, Jacob Kellet, Samantha Ippoliti, Nilini Ranbaduge, Henry Shion and Ying Qing Yu

Analyte/surface adsorption in liquid chromatography as a contributing factor to poor peak shape, tailing, and diminished recovery can lead to increased assay variability, reduced assay sensitivity and misinterpretation of results for analytes susceptible to surface interactions. ACQUITY PREMIER with MaxPeak HPS Technology is Waters' solution to these challenges. Waters' ACQUITY PREMIER Columns are designed to deliver exceptional chromatographic performance while minimizing analyte/surface interactions of sensitive compounds.

Metal-ion mediated adsorption of analytes as a contributing factor in poor peak shape, tailing, and diminished recovery of compounds in LC-based techniques can negatively impact data quality and assay robustness. Analytes exhibiting phosphate groups, uncharged amines, and deprotonated carboxylic acids are particularly susceptible to these phenomena and are commonly encountered in the development and manufacturing of protein-based therapeutics. Current methods for addressing analyte/surface adsorption include ion-pairing additives, hardware passivation, and high-ionic strength mobile phase. These strategies, while proven effective, can be challenging to deploy in terms of instrument/technique compatibility in the case of MS-based methods as well ensuring they can be implemented in a safe and efficient manner with respect to lengthy passivation procedures that can involve corrosive reagents. The newly introduced ACQUITY PREMIER brand columns with MaxPeak HPS Technology is Waters' solution to these challenges.

In this study, the performance gain of ACQUITY PREMIER Columns with MaxPeak HPS Technology is demonstrated with increased recovery, reproducibility, and robustness of RPLC-MS-based peptide mapping assays using the Waters' NIST mAb tryptic digest standard. Collectively, this study shows that MaxPeak HPS Technology can be broadly applied in the development and manufacturing of therapeutic drug products to deliver the chromatographic performance expected from Waters' technologies while increasing reproducibility, peak shape, and recovery of analytes prone to surface interactions.

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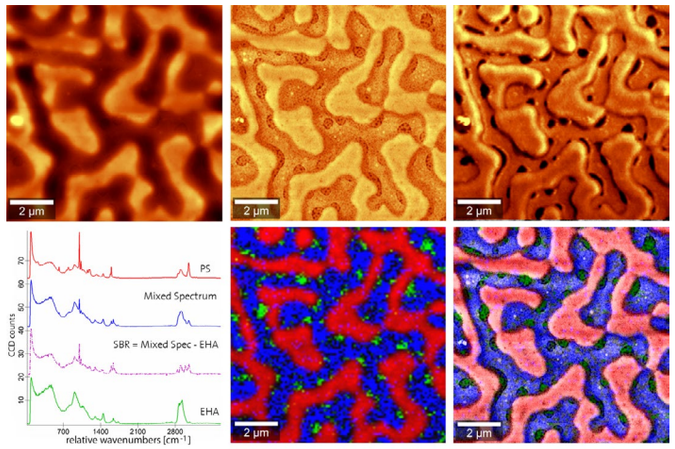
CORRELATIVE RAMAN IMAGING OF POLYMERIC MATERIALS

Raman microscopy combined with AFM and SNOM delivers more comprehensive sample characterization



This study shows a series of correlated Raman imaging, atomic force microscopy and scanning near-field optical microscopy measurements that characterize and depth-profile polymer samples. The substances investigated include spin-coated PS-SBR-EHA and PMMA-SBR blends, an orange juice container inner coating, adhesive layers on paper and bioinspired nanofibers.

Polymers have widely varying chemical, mechanical and optical properties. Knowledge of their morphology and composition is crucial to advancing their development and monitoring their production. Raman imaging correlated with atomic force microscopy and scanning near-field optical microscopy can facilitate the investigation of polymer sample properties that are difficult to measure with conventional methods.

Confocal Raman microscopy is a spectroscopic technique that is able to characterize a sample nondestructively and without staining or other specialized sample preparation, and due to its beam path geometry, three-dimensional measurements such as depth profiles can be performed. The chemical sensitivity of high-quality confocal Raman microscopes also allow data acquisition from extremely small sample volumes and concentrations. AFM measurements reveal structural information at the sub-micron level and SNOM can evaluate a specimen optically with resolution below the diffraction limit. Cantilever-based SNOM sensors can be used with the same beam-deflection optics and control electronics as an AFM and are also more robust than traditional SNOM tips. A truly correlative system can use all three methods in concert on the same sample area and the data recorded provides a more thorough understanding than possible with the individual approaches in isolation.

In this study a thin-film mixture of PMMA-SBR is investigated and its components are differentiated with Raman-AFM, a Raman-AFM-SNOM measurement of a PS-SBR-EHA mix is presented, depth-profiles of a polymer coating and an adhesive layer are shown, and the topography and chemical composition of bioinspired nanofibers are characterized with Raman-AFM. The Raman spectra of the constituent substances are displayed along with color-coded images that show their distributions over a sample area in x-y scans or the positions and thicknesses of their layers in scans along the z-axis.

The instrument used for all measurements was a WITec alpha300 RAS correlative Raman-AFM-SNOM microscope. This system fully integrates all three techniques in a modular architecture with a common software environment and beam path. The speed, sensitivity and resolution of the alpha300 series, along with its intuitive user interface and configurability, enables researchers to carry out challenging experiments quickly and routinely.

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