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The Column

the interactive e-publication for the global separation science industry

Instrumental Innovations 2019

Selected highlights of innovative chromatography products

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Selected highlights of innovative chromatography products

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Gesa J. Schad, Shimadzu Europa GmbH
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31 **The Basics of HPLC Peptide Analysis**
To fully characterize a protein biopharmaceutical, it must be broken down into smaller segments (peptides). Several HPLC techniques can be used to provide a wealth of information on everything from post-translational modifications to glycol profile to information on similarity when characterizing biosimilars.

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"Green" GPC/SEC and Solvent Alternatives
Daniela Held and Jasmin Preis, PSS Polymer Standards Service GmbH
The "greenest solution" is certainly using no solvent but GPC/SEC as a LC technique requires the use of a mobile phase. The growing awareness of the need for more sustainable (greener) solutions has focused attention on environmentally and health friendly solvents and solutions.

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Instrumental Innovations 2019

Julien Eichinger/stock.adobe.com

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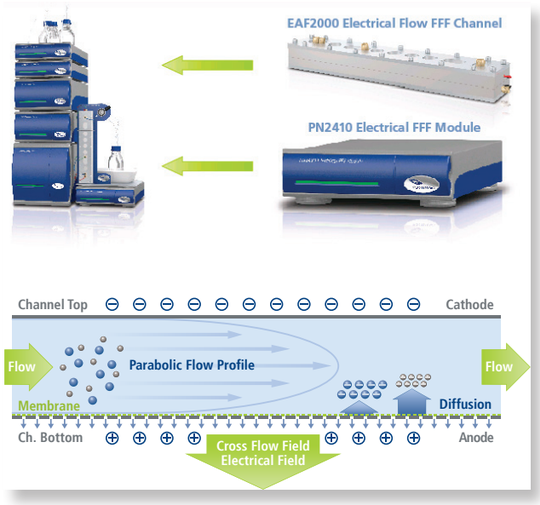
FFF Products

Electrical/Asymmetrical Flow Field-Flow Fractionation



Traditional separation technologies for biopharmaceutical and nanoparticle applications provide particle size or molar mass distributions as the final result. However, it is clear that particle and molecular charge play a primary role in many applications such as protein aggregation, polymer flocculation, particle agglomeration, and in pharmaceutical formulations in general. The Postnova EAF2000 instrument using electrical asymmetrical flow field-flow fractionation (EAF4) technology allows the particle size or molar mass distributions to be further differentiated and transformed into charge distributions. This identifies charge heterogeneities present within the different size and molar mass fractions and will help to aid research or establish more efficient product development processes.

The instrument works by combining the principle of electrical and AF4 in one system. According to the company, this instrument is a key tool, particularly for protein research, because existing techniques for zeta-potential are limited by concentration and are simple batch techniques giving just an average value for all components in the solution. The EAF2000 can determine the zeta-potential of each individually separated component, such as protein monomer and dimer (or higher aggregates) or antibody monomer and fragments–aggregates.

www.postnova.com/overview_759.html

GC Products

Ultrafast GC

The latest breakthrough is the launch of ultrafast gas chromatography (UFGC), which typically reduces output times in runs measured in minutes to seconds. Designed for rapid and repeated analysis, UFGC can reduce analysis cycle times by typically five- to 10-fold when compared to traditional chromatography. Applications include environmental, petrochemical, and pesticide analysis, as well as the analysis of waste streams in manufacturing, particularly in cleanroom technology.

By directly heating only the column rather than an entire oven, temperature ramps can be much faster than conventional GC. This rapid temperature programming can be used to drive compounds through the column much faster creating sharper peak shapes and better resolution on shorter columns. As only the column needs to cool rather than an entire oven, cool down is significantly faster. This means that less energy is used for heating so the energy used for each analysis is tiny when compared to a conventional GC system.

Ellutia's 500 Series GC integrates conventional, fast, and UFGC functionality in one space-saving, easy-to-use, and easy-to-maintain instrument, delivering an ultrafast typical analysis cycle time of five minutes with minimal system downtime, according to the company.

As UFGC moves into common use, the next phase will see faster multichannel detectors that improve capabilities, including time-of-flight GC–mass spectrometry (MS), infrared absorption spectroscopy, atomic emission detection, and far-UV absorption spectroscopy.

www.ellutia.com/our-instruments/gas-chromatographs/500-series-gc/

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Olfactory Detection Port

The newly developed Olfactory Detection Port (ODP 4) from Gerstel enables simultaneous detection of compounds with a mass spectrometer and the human nose, a combined approach frequently used in the analysis of food, flavour, and fragrances. The ODP 4 is highly inert and uniformly heated, resulting in good recovery and sensitive olfactory detection, even for high-boiling and polar compounds. Make-up gas with or without humidification can be added to the GC column effluent as required. The multi-swivel holder is firmly held in place with a single turn of the fixation knob for easy positioning and optimized ergonomics. Marker pins ensure accurate and reproducible nasal positioning and best possible results, even at low flows. Sorbent tubes can be inserted for trapping and concentration of interesting fractions for further analysis, for example, when unknown off-flavours must be identified in a product.

The ODP 4 is delivered with the Olfactory Data Interpreter (ODI) software, which enables time-aligned sensory evaluation of compounds eluting from the GC–MS system complete with intensity levels and qualifiers displayed in a combined olfactogram. Compounds can be searched in the NIST database and their identity added and reported. According to the company, the ODI software provides the analyst with helpful software tools that are also relevant for evaluation of sensory panel data such as: cumulative olfactogram presentation, aroma extract dilution analysis (AEDA), flavour dilution (FD), as well as multivariate data analysis (PCA).

www.gerstel.com

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Light Scattering Products

Hydrogen Generator for GC–FID

This year, Peak Scientific unveiled the smallest laboratory-grade hydrogen generator for gas chromatography–flame ionization detection (GC–FID): Precision Hydrogen SL. Designed primarily to supply hydrogen to flame detectors for GC, Precision Hydrogen SL is compact, with a total footprint measuring less than 20% of the size of its predecessor.

Available in both 100 cc and 200 cc, Precision Hydrogen SL produces hydrogen gas at 99.9995% purity and can be purchased in black or white. It has been developed to enhance its usability with one button for both start-up and shutdown, and only simple user maintenance, requiring the replacement of a sealed-capsule desiccant system and de-ionizer cartridge, both of which can be changed by the user.

With minimal gas storage and fail-safe auto-shutdown features, Precision Hydrogen SL is reportedly a safer hydrogen gas solution for GC–FID, particularly when compared with gas cylinders. Removing concerns of running out of gas and eliminating costs of repeat cylinder deliveries and rental charges, it offers a cost-effective and reliable gas solution for GC detector flame, according to the company.

www.peakscientific.com/precisionsl

Multi-Angle Light Scattering

Over the past four decades, low-angle and multi-angle light scattering (LALS and MALS) instruments have been commercially available, each with its own benefits and drawbacks, forcing scientists to choose one between them. Tosoh Bioscience's recent introduction of a new light scattering technology combines the benefits from each type of detector.

The new LenS3 MALS detector features a novel, elongated flow path geometry that maximizes scattered light collection, to increase the detector's response, while the non-refractive material of the chamber eliminates noise from stray light. The advanced optical design also provides signal-to-noise at the crucial extreme angles (10° and 170°) for molecular weight and size measurements. In addition, a new angular dissymmetry plot has introduced a simpler and more direct approach to size determination.

The resulting enhanced capabilities and sensitivity of the new LenS3 MALS detector provide a direct and accurate measurement of molecular weight, even for oligomers and low *dn/dc* samples. Moreover, the angular dependence can be reliably detected to a considerably lower level, thus extending the range of radius of gyration (Rg) determination by light scattering down to a few nanometres for the very first time, according to the company.

http://bit.ly/LenS3-Detector

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Next-Generation SEC-MALS Detectors

Wyatt's NEON generation of instruments for macromolecular characterization includes:

- DAWN, miniDAWN, and microDAWN: multi-angle light scattering (MALS) instruments for absolute molar mass and size
- Optilab and microOptilab: differential refractometer for universal concentration measurement
- ViscoStar and microViscoStar: differential viscometers for polymer intrinsic viscosity.

The "micro" instruments are for use with ultrahigh-pressure liquid chromatography (UHPLC), while the others are for use with high performance liquid chromatography (HPLC) and fast protein liquid chromatography (FPLC) systems.

According to the company, each new instrument maintains industry-leading performance in terms of sensitivity and range. The NEON models incorporate new features to enhance usability and maintainability, including: i) State-of-the-art, multi-touch front-panel display to control and monitor the instrument and signals; ii) System-ready monitor and health indicators—a display of chromatography and detector status that lets users know if the system is ready to acquire high-quality data, and if not what needs to be remediated; iii) Built-in diagnostics and modular design, enabling full on-site repair, including a field-replaceable MALS flow cell.

Each MALS instrument incorporates a COMET ultrasonic device to minimize flow cell cleaning cycles, and a Forward Monitor for accurate analysis of laser-absorbing samples. These features offer maximum productivity and confidence when analyzing proteins and polymers.

www.wyatt.com/next-gen

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LC Products

High Flow Rate Degasser

Efficient degassing of liquids at high flow rates is a tough challenge. Gases dissolved in liquids often cause problems in fluidic systems. The gas molecules can form bubbles when temperature or pressure changes, and this will affect the precision, accuracy, and performance of the equipment. Online degassing efficiently removes dissolved gases from the fluid stream and prevents bubble formation, reduces noise, improves baseline stability, reduces start-up times, and ensures more consistent results.

Regardless if you work with process chromatography, have sensitive manufacturing processes in biotechnology, perform critical dissolution testing, or design sophisticated dispensing equipment, the new Biotech Degasi high flow 1000 eliminates problems with dissolved gases, according to the company. Equipped with a vacuum pump that ensures ultrahigh degasification efficiency, while operating with a minimum of vibrations and being almost completely silent, the Degasi high flow 1000 can be used in most environments. With this device, bubbles and dissolved gases can be removed from water-based solutions up to 1000 mL/min.

Learn more about the new benchmark for high-throughput degasification applications at: **www.biotechfluidics.com/products/degassing-debubbling/degasi/degasi-high-flow-1000**

Polymeric Achiral Phases

Daicel has created three new polymeric phases for use in achiral separations. These phases combine polymeric robustness with unique chemical composition to ensure new and reliable separations.

Daicel DCpak P4VP is a polymeric ethyl pyridine phase that has increased robustness for the separation of achiral compounds in supercritical fluid chromatography (SFC) and high performance liquid chromatography (HPLC). Testing shows that this selector has a longer lifetime, which is critical for users that are currently replacing 2-ethyl pyridine (2EP) columns regularly.

Daicel DCpak PBT is a unique polymeric selector based on butyl terephthalate and has selectivities for polar compounds that cannot be found with other currently available columns for SFC and HPLC, according to the company. Innovative separations include glucosylceramides.

A third column has just been introduced. Daicel DCpak PTZ is a polymeric tetrazole selector that has been designed to provide a polar polymer that creates a larger water layer around the silica for use in novel HILIC separations. It has been shown to provide excellent separations for complex compounds such as uridines, lipids, and vitamins. **https://chiraltech.com/whats-new/**

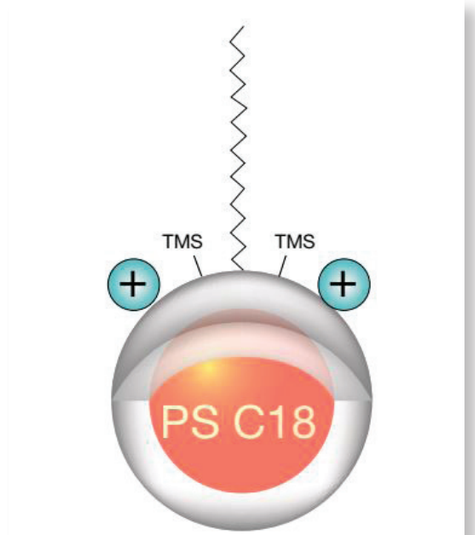
Microbore HILIC Columns

Hilicon is a leading company in developing and manufacturing hydrophilic interaction liquid chromatography (HILIC) products for the separation of polar and hydrophilic compounds. It has recently started to expand its iHILIC-Fusion and iHILIC-Fusion(+) product lines into a 1 mm microbore column format. The initial products use a 3.5 µm particle for HPLC and will soon use 1.8 µm for UHPLC. The columns utilize a PEEK-lined stainless-steel column hardware that minimizes the undesirable surface interactions between samples and steel and achieve the best chromatography performance. They are versatile for the LC–MS-based analysis of polar compounds in "omics" research, for example, metabolomics, proteomics, glycomics, and lipidomics, in the fields of clinical diagnostics and pharmaceutical discovery.

The packed iHILIC stationary phases are charge-modulated amide silica particles that are covalently bonded with various neutral, positively charged, and negatively charged hydrophilic functional groups, following the company's innovative and unique surface-bonding technologies. iHILIC-Fusion is neutral and slightly negatively charged at pH 2–8, while iHILIC-Fusion(+) is constantly slightly positively charged. According to the company, the columns provide customized and complementary selectivity, ultimate separation efficiency, and ultra-low column bleeding, and are particularly suitable for LC–MS applications. Furthermore, they also simplify method development and improve the productivity and data quality in chemical and biological analysis.

www.hilicon.com

Core–Shell Family



Phenomenex Inc. has introduced the Kinetex PS C18—the 11th selectivity in the Kinetex core–shell high performance liquid chromatography/ultrahigh-pressure liquid chromatography (HPLC/UHPLC) family. According to the company, the Kinetex core–shell (superficially porous) particle technology delivers higher efficiency (N) and performance compared to columns packed with fully porous particles. In addition, the manufacturing process allows for greater control of the overall average particle diameter promoting improved column-to-column consistency and chromatographic performance. Combining the Kinetex core–shell 2.6 µm particle with the unique PS C18 selectivity affords scientists an HPLC/UHPLC column solution well suited for the analysis of different classes of acid, bases, and natural compounds under typical reversed-phase conditions. Additionally, the Kinetex PS C18 selectivity is particularly applicable for the analysis of polar basic compounds that contain basic functionality and typically exhibit poor peak performance on traditional C18 phases. While traditional alkyl C18 phases are prone to peak tailing issues for basic compounds as a result of uncontrolled secondary interactions occurring at the silica surface, the surface of the Kinetex PS C18 contains a bonded positive charge that repels basic species and delivers sharper peak shape for basic compounds.

www.phenomenex.com/Kinetex

Polysaccharide-Based Chiral Columns

Regis Technologies has been developing innovative chiral stationary phases since 1972. They were the first manufacturer to bring immobilized chiral phases to the market and are focused on improving the tools available to separations scientists. Using nearly 50 years of experience, they launched their Reflect line of immobilized and coated polysaccharide chiral columns earlier this year.

Reflect chiral columns are rugged polysaccharide phases suitable for a wide range of chiral compounds, scalable from analytical to preparative separations. According to the company, they boast a unique, proprietary phase coverage that provides excellent peak shape and resolution. High resolution greatly improves preparative loading, leading to greater productivity and higher purity separations. These rugged columns are suitable for a wide range of chiral compounds and built for durability.

Columns are available in analytical to preparative sizes in six phases: immobilized amylose A; immobilized cellulose B, C, and J; coated amylose A; and coated cellulose B.

Comparative data are available as well as a free chiral screening service to try the columns on compounds of interest with no obligation to purchase.

www.chiral.com

UHPLC System

Shimadzu's new Nexera UHPLC series LC-40 is setting new benchmarks in intelligence, efficiency, and design, according to the company. By incorporating new sensor technology and IoT functions (Internet of Things) it defines a new standard in "Analytical Intelligence".

As overall efficiency depends not only on the performance of one instrument but also on the management of all devices within a laboratory, device networking enables users to review instrument status and optimize resource allocation.

According to the company, the systems maximize reliability and uptime with new "Analytical Intelligence" features. Fully unattended workflows span startup to shutdown, and new auto-diagnostics and auto-recovery capabilities allow issues to be detected and resolved, such as pressure fluctuations, automatically.

With a fast injection speed and capability of connecting several plate changers, automated high-speed analysis of thousands of samples is offered.

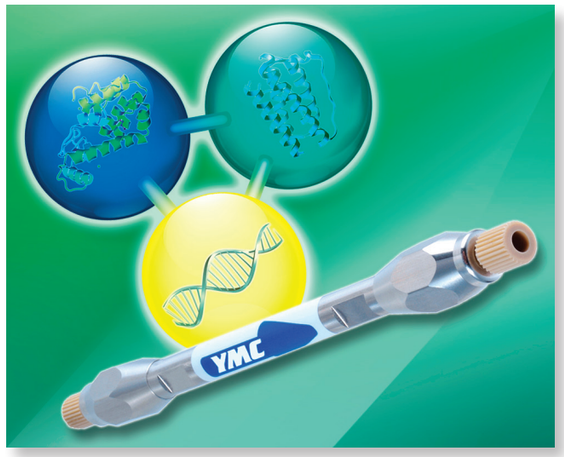
The compact design saves bench space compared to other models. It also supports an environmentally friendly laboratory via an energy-saving standby mode.

The i-PDeA II function (intelligent Peak Deconvolution Analysis) enables extraction of a single peak from coeluting compounds, by utilizing differences in the analyte's spectral data. This unique processing method eliminates discussion of integration methods for coeluting components and helps to detect underlying impurities in a target peak.

www.shimadzu.eu

Mass Spectrometry Products

UHPLC/BioLC



New ultrahigh-performance liquid chromatography/high performance liquid chromatography (UHPLC/HPLC) phases for biomolecules, such as peptides, proteins, antibodies, or oligonucleotides, have to meet numerous challenging criteria including high temperature, pH stability, high resolution, and mass spectrometry (MS)-compatibility and inertness. Properties such as highly robust phases and particularly excellent lot-to-lot reproducibility for quality control (QC) purposes have become major priorities. YMC's main focus for satisfying these needs for BioLC users has been the production and supply of reliable products together with the introduction of new, innovative products.

YMC-Triart Bio C4 and the all new YMC-Triart Bio C18 are widepore phases for reversed-phase (U)HPLC. According to the company, they offer the perfect solution for outstanding selectivity for peptide and protein analyses as well as antibody separations with their 300 Å pore size. Method flexibility is enhanced as a result of high temperature (up to 90 °C) and pH stability (Bio C18: pH 1–12; Bio C4: 1–10). Their lot-to-lot reproducibility offers reliable BioLC QC data. These phases are available in 1.9 µm for UHPLC as well as 3 µm and 5 µm for HPLC separations, together with bioinert and semipreparative YMC-Actus columns for improved separations and purifications.

https://ymc.de/rp-bioseparation.html

Mass Selective Detector

The InfinityLab LC/MSD iQ is the newest mass selective detector on the market today. According to the company, the compact LC/MSD iQ is the perfect partner for laboratories performing small molecule analysis in pharmaceutical drug discovery, development, quality assurance/quality control (QA/QC), as well as academia, food, and chemical laboratories that need mass spectral data for making data-driven decisions.

The InfinityLab LC/MSD iQ is specifically designed for chemists, chromatographers, and new users looking for more certainty in their LC-based results. The Auto Acquire mode sets up the acquisition thereby eliminating the learning curve. The detector incorporates intelligent instrument health tracking to ensure robust, reliable, and routine operation with embedded sensors. In conjunction with this, an overall assessment of the entire liquid chromatography–mass spectrometry (LC–MS) system is done with a system suitability check. With a focus on overall laboratory productivity, early maintenance feedback features help laboratory managers plan routine maintenance.

The InfinityLab LC/MSD iQ resides beneath the Agilent's InfinityLab HPLC stack, saving valuable space. To accommodate changing space and layout requirements, the new Agilent InfinityLab Flex Bench MS enables mobility, modular mounting of all system components, and easy access to all system areas, in addition to providing an integrated solution for waste management, as well as system noise reduction.

https://www.agilent.com/en/products/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-instruments/single-quadrupole-lc-ms/lc-msd-iq

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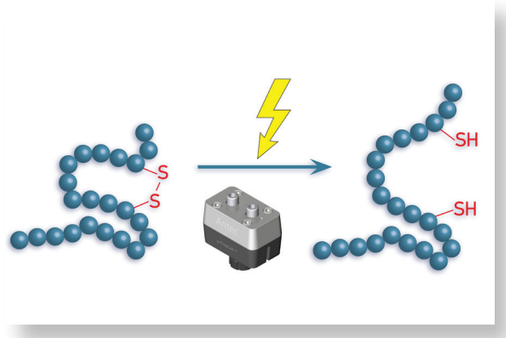
Protein Characterization

With Antec Scientific's new µ-PrepCell-SS, electrochemical reduction of disulfide bonds in peptides, proteins, monoclonal antibodies (mAbs), and other biopharmaceuticals becomes possible routinely in every liquid chromatography–mass spectrometry (LC–MS) workflow. Typical features include:



- Fast and efficient reduction of S-S bonds
- Robust on-line reduction in top-down and bottom-up proteomics
- Reagent free, no interfering DTT or TCEP, ideal for HDX-MS
- Superior peptide sequencing and S-S bond assignment

The new cell allows for continuous operation over several days without any loss in reduction efficiency. It can be used in pre- or post-column high performance liquid chromatography (HPLC) configurations and is suited for the reduction of highly disulphide bond-stabilized proteins.

Online reduction occurs within several seconds as compared to conventional offline chemical methods, which can take hours or longer to achieve similar results. Full reduction of inter- and intra-molecular SS bonds in mAbs can be achieved with complete sequence coverage at the hinge region.

For cystine knot peptides (CKPs), 100% sequence coverage was observed, unlike with CID, ETD, or HCD, which resulted in zero sequence coverage.

The cell has a pressure rating of 350 bar making it ideal for use in HDX-MS, according to the company. Overall much higher sequence coverage has been found in MS proteomics when compared to TCEP or DTT reduction and new cysteine peptides could be identified for the first time.

www.AntecScientific.com

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New-Generation Benchtop MS

The Thermo Scientific Orbitrap Exploris 480 mass spectrometer (MS) is a new-generation benchtop instrument designed to address the increasing need for quantitative proteomics across high-throughput biopharmaceutical, translational, and academic research applications. Built on the guiding principle of ease-of-use and reliable hardware, the Orbitrap Exploris 480 MS combines proven technology, advanced capabilities, and intelligence-driven data acquisition strategies for rigorous, high-throughput protein identification, quantitation, and structural characterization of biotherapeutics and translational biomarkers, according to the company. The intelligent data acquisition methods ensure any analyst, regardless of their experience, can obtain confident insights from the analysis of a wide range of compounds: from small molecules to peptides, intact proteins and their complexes across a mass range of 40–6000 *m/z*. The coverage of the proteome offered by the Orbitrap Exploris 480 MS can be increased by 20% when combined with the Thermo Scientific FAIMS Pro interface. The system also provides enhanced quantitative performance across label-free and tandem mass tag (TMT) experiments, as well as access to new Thermo Scientific SureQuant methods for ultra-sensitive targeted protein assays within complex biological matrices. Within a smaller footprint than previous generations, the Orbitrap Exploris 480 MS reportedly maximizes laboratory bench space, while maintaining high resolution, mass accuracy, and spectral quality through a robust design that supports a range of new features intended to extend uptime and improve serviceability for researchers in high-throughput laboratory environments.

https://www.thermofisher.com/order/catalog/product/BRE725532

Mass Spectrometer

The Waters Synapt XS Mass Spectrometer is a new, highly flexible, high-resolution mass spectrometer for R&D laboratories focused on discovery applications requiring the greatest variety of analytical strategies to tackle inherently challenging questions. By providing high levels of flexibility through inlets and acquisition modes, the Synapt XS delivers greater freedom of analytical choice to support scientific creativity and technical success for any application, according to the company.

The newest iteration in the Synapt family of research-grade mass spectrometers, the Synapt family is known for both its flexibility and its unique T-Wave ion mobility mass spectrometry (IMS) configuration, which significantly extends the power of high-resolution analysis. IMS capability on the Synapt increases the extent and confidence with which a scientist can profile complex mixtures and characterize complex molecules, and dramatically enhances sample definition.

According to the company, the inherent power of Synapt is enhanced in the new Synapt XS with new technology building blocks that provide increased sensitivity for challenging compounds while further improving the levels of analytical robustness at far superior mass resolution than previous models. In addition, complementary modes of operation that increase analytical peak capacity providing "clean and clear" fragmentation data provide an investigative toolbox for the interrogation of complex mixtures.

www.waters.com/synaptxs

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Sample Preparation Products

Solid-Phase Extraction Module

In August 2019, PerkinElmer released a new component to its QSight series of triple quadrupole liquid chromatography tandem mass spectrometry (LC–MS/MS) instruments, expanding its sample handling capabilities, and enabling high-throughput mass spectrometry workflows. The QSight SP50 online solid-phase extraction (SPE) system, controlled via Simplicity 3Q software, facilitates sample clean-up, enrichment, and concentration, obviating the need for elaborate and time-consuming sample preparation procedures. The QSight SP50's Precision Sampling Module features two six-port valves on the autosampler that facilitate software-controlled switching between traditional direct injection ultrahigh-pressure liquid chromatography (UHPLC) analyses, and online SPE and preconcentration. Further, QSight SP50's High Pressure Dispenser delivers conditioning, washing, and loading solvents at controlled flow rates at pressures of up to 300 bar. Users can choose up to nine different solvents, providing the customization and versatility required in high-throughput environmental and food testing laboratories.

According to the company, pairing the QSight SP50 system with the QSight triple quad LC–MS/MS provides the increased sensitivity needed to achieve increasingly lower detection limits for traditional and emerging contaminants, such as pharmaceuticals and personal care products (PPCPs), estrogens, and mycotoxins. Automation of extraction and concentration processes reduces the risks associated with variability and analyst error, improving overall data quality and reproducibility.

https://www.perkinelmer.com/Product/qsight-sp50-high-press-dispenser-n2994500

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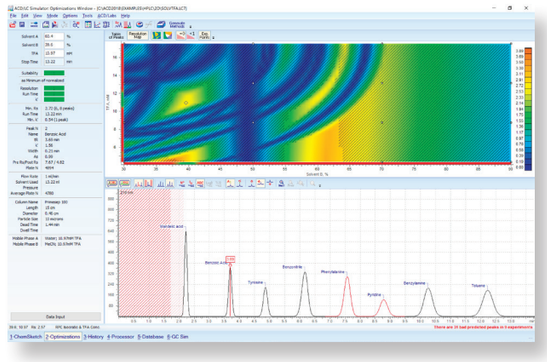
Software Products

Method Development Software



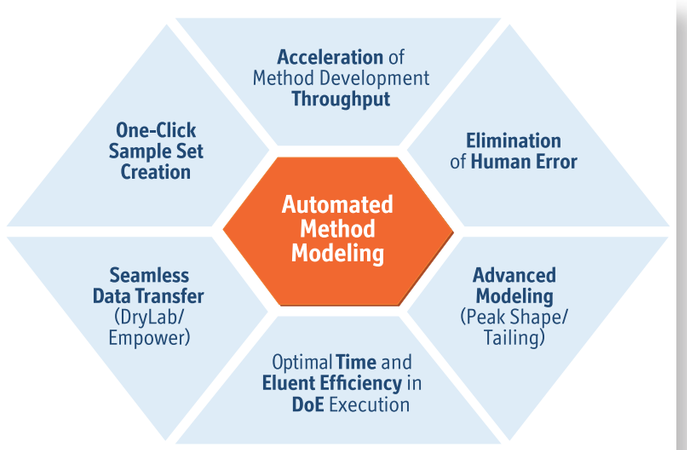
ACD/Labs continues to implement updates to its primary method development software solutions, ACD/Method Selection Suite and ACD/AutoChrom. The v2019.1 releases of Method Selection Suite and AutoChrom expand method-screening, optimization, and reporting options for reversed-phase separations, particularly increasing onboard solvent screening possibilities. For example, two-dimensional optimization of gradient and solvent ratio is now available, allowing users to adjust these two parameters simultaneously.

According to the company, the 2019.1 versions of Method Selection Suite and AutoChrom improve support for several major instrument vendor data formats, including the ability to import chromatographic rider peaks directly from chromatography data systems. AutoChrom can now be used to design mixed mode (ion exchange/reversed phase) and hydrophilic interaction chromatography (HILIC) separations. Specifically, alternative solvent roles are defined for mixed mode and HILIC methods, compared to the traditional solvent roles of reversed-phase chromatography.

Finally, ACD/Labs' complete software architecture has been upgraded to 64-bit, eliminating any technological limitations scientists may face, such as the import and export of large liquid chromatography–mass spectrometry (LC–MS) datasets. With Method Selection Suite and AutoChrom v2019.1, users are able to design robust methods that align with quality by design (QbD) principles.

www.acdlabs.com/methdev

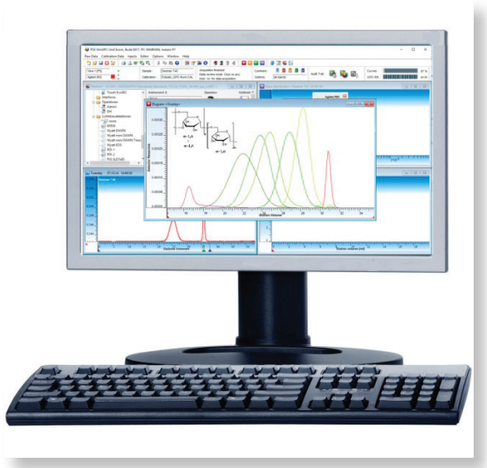
Automation Software



DryLab is an industry-standard chromatographic method modelling software package for (U)HPLC method development and optimization. Based on fundamental scientific theories such as the solvophobic interactions and linear solvent strength (LSS) model, DryLab requires only the minimum number of input experiments to pinpoint selectivity and resolution changes. The visualized multidimensional design space—corresponding to over a million virtual chromatograms—allows for method optimization choices and subsequent *in-silico* robustness assessment. Recent development in integrating Empower CDS includes three-level automated steps in the software's AQbD-workflow by creating, performing, and retrieving input runs, model-verification, and robustness verification experiments. Generated method sets are performed in the most economic and ecologic order, also taking into account subsequent equilibration steps. Following data-stream allows for additional peak-information regarding peak shapes (T_f, $w_{0.5}$), and peak names to be accessed, which can be directly used for model-adjustment. According to the company, this provides an easy modelling platform for making science-based flexible method development choices, mitigates risk, improves analytical throughput, and ultimately delivers method development success.

www.molnar-institute.com

Dextran Analysis Solutions

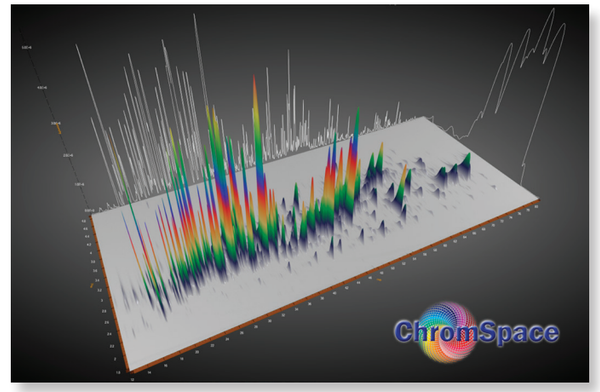


Size-exclusion chromatography (SEC) is the standard technique in pharmaceutical testing of Dextran 40, 60, and 70. National and international pharmacopoeias describe the determination of their molecular weights and molecular weight distributions (MWDs). USP and Ph. Eur. require the use of a very specific calibration routine. A dedicated e-workflow has been added to PSS WinGPC MCDS to allow for characterization in agreement with these standards. The workflow comprises data capture, specific dextran calibration, data analysis, and compliant reporting. Regulated laboratories can rely on the WinGPC UniChrom Compliance Pack, which enables compliance with FDA 21CFR11 by providing a data safe, comprehensive audit trails, and electronic signatures.

Dextrans are also used as standard calibrants in aqueous GPC/SEC. Calibration with narrow standards is a time-consuming process because it requires the accurate weighing of a larger number of different molar mass reference materials for every single calibration. PSS ReadyCals allows for the rapid preparation of reproducible calibration curves without the inconvenience of individually weighing the standards. New in the PSS ReadyCal family are the Dextran ReadyCals offering colour-coded vials filled with a pre-weighed mixture of high quality reference materials with precise concentrations and molar masses.

https://www.pss-polymer.com/support/librarypss-publications/full-featured-application-notes.html

Software Platform



ChromSpace is a software platform for multidimensional GC that reduces the workload for analysts in busy laboratories. According to the company, analysts previously put off by the complex data processing associated with two-dimensional gas chromatography (GC×GC) will benefit from ChromSpace's quick-to-learn and easy-to-use interface. Following feedback from experts in industry and academia, ChromSpace was designed to meet the needs of those working in high-throughput laboratories.

ChromSpace enables analysts to enjoy full instrument control and the flexibility to explore multiple GC×GC data files in a single window. According to the company, data navigation can begin while a sample is still running, saving time and streamlining the process. An instant display of library matches also helps analysts to make a quick identification.

Multiple file formats are supported in ChromSpace, enabling files from mass spectrometry (MS) and single-channel detectors from most major manufacturers to be read, taking the hassle out of the process and avoiding the expense of purchasing multiple software packages.

ChromSpace uses unique algorithms to ensure that trace or masked peaks are not overlooked or incorrectly merged, a deconvolution tool to identify coeluting peaks, classification "stencils" for simple group-type reporting, and a scripting tool to filter complex data and identify target compounds or chemical classes.

http://chem.sepsolve.com/LCGC/Chromspace

News

Joel M. Harris Receives EAS Award

The Eastern Analytical Symposium (EAS) Award for Outstanding Achievements in the Fields of Analytical Chemistry was presented to Joel M. Harris at EAS 2019 on Wednesday 20 November. Harris presented a plenary lecture, "Spectroscopy through the Microscope: Chemical Analysis at Liquid/Solid Interfaces" following the award.

Harris, a distinguished professor of chemistry at the University of Utah (Salt Lake City, Utah, USA), also holds an adjunct appointment in the Department of Bioengineering. He earned his Ph.D. from Purdue University (West Lafayette, Indiana, USA) and then joined the faculty of the University of Utah. His research has focused on analytical chemistry and spectroscopic studies of low concentrations of molecules in liquids and at liquid–solid interfaces. Along with his students, he has advanced new concepts in photothermal spectroscopy, methods to analyze multidimensional spectroscopic data, Raman spectroscopy of transient species and interfaces, and quantitative analysis of interfacial molecular populations by imaging and counting individual fluorescent molecules. He and his students have applied these methods to investigate the kinetics and energetics of excited-states and reactive-intermediates, and molecular transport, adsorption, and binding kinetics that govern separations and analysis at liquid–solid interfaces.

Investigating Primate Fertility Cues Using GC–MS

Researchers from the University of Leipzig have used gas chromatography–mass spectrometry (GC–MS) to study the fertility cues of non-human primates (1).

The use of odour cues can be seen throughout the animal kingdom and are used by animals to deliver strong messages to those around them, including the marking of territory, defensive posturing, or even to attract prey. These odour cues exist even in primates and a number of species have well developed olfactory systems. However, large knowledge gaps remain in our understanding of the chemical underpinnings of primate odour cues.

A common odour cue in primates revolves around reproduction and in particular odour-based messages that detail the fertility state of females and their reproductive quality. For example, in common marmosets (*Callithrix jacchus*) both males and females show scent-marking behaviour from two specialized glands and some non-specialized glands. This makes them ideal targets for olfactory studies in primates as they have a lot of complex chemical signals to investigate. A number of studies have shown that female marmosets lack visual cues to indicate reproductive readiness and yet males can detect when females are fertile, with studies determining odour cues as the source for the males (2–4). However, detailed analysis of the chemical compounds responsible has yet to be carried out.

To address this knowledge gap, researchers used behavioural bioassays and GC–MS to analyze marmoset secretion odour particles, attempting to identify fertility-related substances as well as factors that may alter or impact them.

Their findings noted that menstrual cycle states, age, and the experience of the female in offspring birthing affected the chemical profiles of marmosets, confirming previous evidence of odour cues being linked to fertility. Males would likely use these cues to optimize their mating efforts and therefore the potential for offspring. In the case of experience, or *parity*, as the papers terms, this refers to a high likelihood for first time marmoset mothers to lose their first litter, and thus the male preference for more experienced females. The overall chemical profile of an individual marmoset consists of a few hundred substances offering a wealth of information, but more studies are required to fully understand their roles and find their metabolic pathways.—L.B.

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2. L. Digby, *Folia Primatol.* **70**, 136–145 (1999).
3. T.E. Smith and D.H. Abbott, *Am. J. Primatol.* **46**, 265–284 (1998).
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Peter de Boves Harrington Receives EAS Award

The Eastern Analytical Symposium (EAS) Award for Outstanding Achievements in Chemometrics was presented to Peter de Boves Harrington on Tuesday 19 November. Harrington went on to present his talk "Chemometrics for the Masses: How to Painlessly Improve Your Science." Harrington received his Ph.D. from the University of North Carolina-Chapel Hill in 1988. From 1987 to 1989 he created the DOS-based software platforms Resolve and Presager for identifying bacteria from their pyrolysis-mass spectra, while working for Kent Voorhees at the Colorado School of Mines. He joined the faculty of Ohio University (Athens, Ohio, USA) in 1989. In 1992, he founded the Center for Intelligent Chemical Instrumentation. He is the Director of the Ohio University Center for Intelligent Chemical Instrumentation and is a Fellow of the American Academy of Forensic Sciences and the North American Academy of Sciences. Harrington's current research focuses on the development and coupling of artificial intelligence to chemotyping by spectrometric measurements of botanical medicines and foods.

The 2020 *LCGC* Awards Winners are...

LCGC is proud to announce that Daniel W. Armstrong and Szabolcs Fekete are the winners of the 13th annual *LCGC* Lifetime Achievement and Emerging Leader in Chromatography Awards, respectively. Armstrong and Fekete will be honoured in a symposium as part of the technical programme at the Pittcon 2020 conference in Chicago, USA, on 3 March 2020.

The Lifetime Achievement Award

The Lifetime Achievement in Chromatography Award honours an outstanding professional for a lifetime of contributions to the advancement of chromatographic techniques and applications. Armstrong, the 2020 winner, is the R.A. Welch Distinguished Professor of Chemistry and Biochemistry at the University of Texas at Arlington. He has worked on an extremely broad range of separation techniques including HPLC; GC; SFC; micellar liquid chromatography; thin-layer chromatography; countercurrent chromatography; CE; and FFF, among others. He developed the theory and mechanistic background behind many of the practical advances in these techniques. Further, he advanced the use of separations techniques as a means to obtain important physico-chemical data. His most recent work in ultrafast separations and signal processing is driving fundamental changes in the field. Among Armstrong's greatest contributions is his work in the field of enantiomeric separations. He published a seminal paper in *Science* in 1986 that described in detail the mechanism of chiral recognition by cyclodextrins in aqueous and hydro-organic solvents. This was also the first example of small-molecule molecular modelling. This study indicated the necessity of chromatographic enantiomeric separations for purity assessment and pharmacokinetic and pharmacodynamic studies, and provided impetus that led the U.S. Food and Drug Administration to issue new guidelines in 1992 for the development of stereoisomeric drugs. This changed the way that the pharmaceutical industry operated worldwide and these changes still reverberate today.

The Emerging Leader Award

The Emerging Leader in Chromatography Award recognizes the achievements and aspirations of a talented young separation scientist who has made strides early in his or her career towards the advancement of chromatographic techniques and applications.

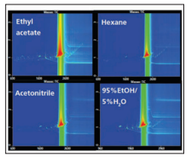
Fekete, the 2020 winner, earned his Ph.D. degree in 2011 from Technical University of Budapest, Hungary, and is currently a scientific collaborator at the University of Geneva, Switzerland. Fekete was the first to evaluate the impact of operating pressure on proteins retention and selectivity in reversed-phase LC, and has also published valuable work on the retention modelling of large proteins (monoclonal antibodies) using computer simulation, suggesting a generic method development approach and platform methods that are very important for pharmaceutical industrial laboratories. He has also carried out fundamental studies in which the effect of longitudinal temperature gradient on retention, caused by frictional heating, was experimentally dissociated from the combined effect of pressure and frictional heating. Through this work, the specific contributions of these effects to the overall retention were determined for both small and large solutes.

Visit the *LCGC* website or information about past *LCGC* award winners or for information on how to nominate a candidate for a future award.

Peaks of the Month

- **The *LCGC* Blog: HPLC Diagnostic Skills II – Tailing Peaks**—In HPLC Diagnostics Skills Part I we looked at baseline issues, and we continue here with HPLC peaks and in particular the skills required to identify tailing peaks, the causes of peak tailing, and most importantly, how to fix the issues that give rise to this peak deformation. **Read Here>>**

- **Sizing Up Size-Exclusion Chromatography (Part 2)**—In the second part of this interview on size-exclusion chromatography (SEC), André Striegel discusses the benefits of the technique to analyze nanoparticles and quantum dots, the complementary role of hydrodynamic chromatography (HDC), and the future of SEC in two-dimensional liquid chromatography (2D-LC) separations. **Read Here>>**



- **Suspect Screening of Chemicals in Food Packaging Plastic Film by Comprehensive Two-Dimensional Gas Chromatography Coupled to Time-of-Flight Mass Spectrometry**—The occurrence of additives, such as plasticizers in plastic food packaging, has been documented, but information on their potential migration into foods is limited. In this pilot study, 2D GC–MS was used to identify chemicals extracted from a common stretch plastic film with a series of organic solvents. **Read Here>>**

- **Advances in Food and Beverage Analysis**—This *LCGC Europe* supplement features the most recent methodological advances in food and beverage analysis, including: an update on IM-MS, plastic food packaging assessments, untargeted GC–MS analysis, and much more. **Read Here>>**

- **Making Nontargeted LC–HRMS Screening Quantitative**—Anneli Kruve discusses the challenges of developing quantitative nontargeted liquid chromatography high resolution mass spectrometry (LC–HRMS) methods. **Read Here>>**

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News In Brief

Thermo Fisher Announce Owlstone Medical Collaboration

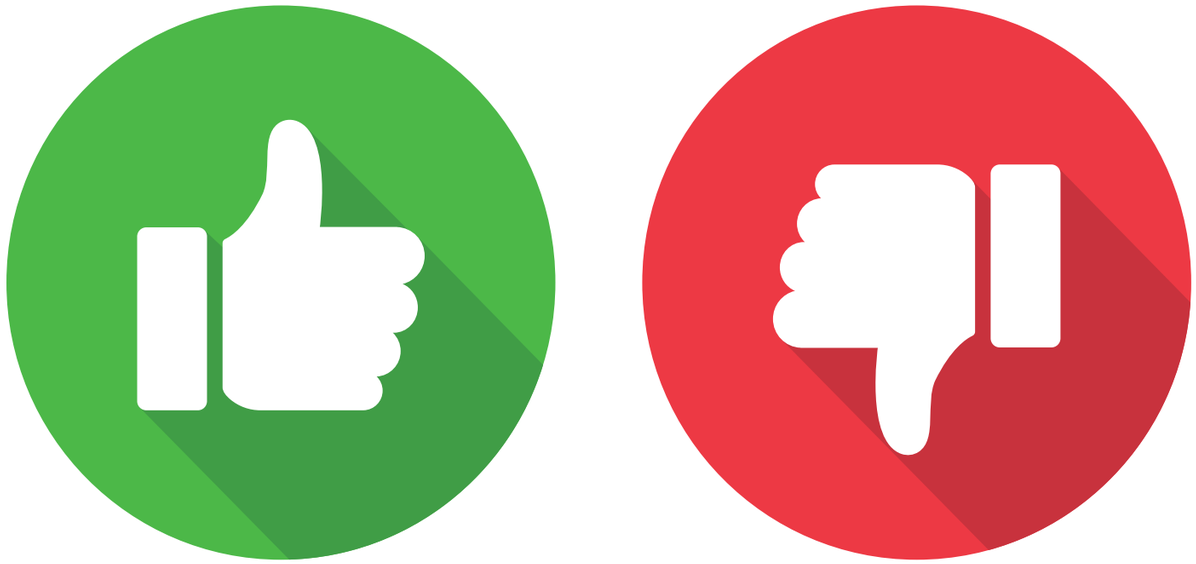
Thermo Fisher Scientific has announced a collaboration with Owlstone Medical to advance the early diagnosis of cancer and other diseases utilizing novel biomarkers and non-invasive breath sampling.

"There is a growing need for non-invasive diagnostic solutions to support early disease detection, patient treatment and increase remission rates," said Morten Bern, director of marketing, gas chromatography, Thermo Fisher Scientific.

The collaboration will see Orbitrap gas chromatography–mass spectrometry (GC–MS) instrumentation integrated with Owlstone Medical's Breath Biopsy platform allowing the detection of new biomarkers via a validated discovery and routine analysis project.

"With an installed base of GC Orbitrap systems, our collaboration with Thermo Fisher Scientific represents an exciting opportunity for cross-promotion of the platform and technique, by which the benefits of breath biopsy can be broadly realized," said Billy Boyle, co-founder and chief executive officer at Owlstone Medical.

For more information, please visit: **www.thermofisher.com**



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How Does Your Laboratory Measure Up On Glassdoor?

What does a best-in-class laboratory workplace look like?

There won't be many of you who hasn't heard of Glassdoor (www.glassdoor.com), a website dedicated to recruitment and company reviews. The site allows companies to find new employees and job seekers to research companies, find new positions, and compare salaries. It's a growing force both inside and outside of the workplace, and can tell employers a lot about their businesses, including the types of things that rarely get mentioned in exit interviews. It's fair to say that the company review sections can often make difficult reading for businesses, and laboratories or companies with laboratories are no exception.

As part of a recent project, I've been reviewing the reviews posted by analytical laboratory workers on Glassdoor and there are some very clear themes that emerge, not only from the disaffected but also as part of more balanced feedback. Consistent themes include:

- Boring repetitive work with no challenge
- No support from management when things go wrong
- Poor management skills
- High workload, low pay
- No visibility of the wider business (laboratory operates as a silo)
- Few or no opportunities for progression
- Little or no training
- High staff turnover affects morale and puts pressure on staff with more time served.

I guess this could read true for just about any workplace (it does, I checked); however, that doesn't mean we should ignore it out of hand. I think most people working in an analytical laboratory would agree with at least some of the points above, and I have seen these things to be

true when working with other laboratories, and dare I say it, even in my own labs.

There will always be the minority of folks who will complain about their working conditions, I fully accept that. However, regular readers will cast their minds back to a previous Incognito (1) in which I explained the attitude of the millennial and post-millennial workers, who work "with" employers rather than "for" them and who are much more "employer mobile" given their very valuable modern education and skills. Layer on top of this the connectedness of this generation, with sites such as Glassdoor helping them to evaluate current and prospective employers, and I'd venture that the scourge of the modern laboratory, high staff turnover, isn't going to improve for employers who don't have their act together.

So, what can be done? Well, my recent project involved trying to answer the question "What does a best-in-class laboratory workplace look like?". I'm sharing some of the results in case it might help you improve your Glassdoor ratings, assuming of course you have the time, inclination, or resources (people and money) to act upon it!

Eliminate Repetitive, Dead-End Jobs: It's tempting to design a "body shop" model when pressure to produce results is high, but project-oriented roles, rather than task-oriented roles, tend to develop laboratory staff much more quickly and comprehensively. Work will be much more varied and, whilst this may be a bold approach, try to develop staff across all the project disciplines and stages as quickly and efficiently as possible. Staff may still be stratified in each discipline or stage, such as subject matter expert and instrument (technique) champion and operator, and tasks at each level may be assigned accordingly. A matrix of project discipline against these levels of expertise can form an excellent grid for measuring staff progression.

Don't Let Your Quality Systems Limit Your Ambitions for Staff: Fear of staff "doing something wrong" or "creating a non-compliance" should not dictate whether they can operate new (to them) or more advanced equipment or become involved with new analytical methods. A properly designed process for staff development should help to alleviate these fears, even if some equipment must be earmarked for non-regulatory work, or they have to be mentored through the activities. Allow more junior staff to be involved in project meetings and to contribute to project decision-making.

Build Tools to Identify Tensions Within the Workflow: Are you able to quickly and effectively identify which departments are overloaded with work? Are there departments or techniques where knowledge is limited? Are there techniques or departments with particularly high failure rates or low productivity? Can your business quickly respond to these tensions and resolve them efficiently? By building project-based models and ensuring good cross-training is in place, you will build in the ability to move resources with the workload.

Challenge Even the Most Junior Staff: Are you aware of your staff skills? I'm not referring to which methods, techniques, or operating procedures they have been "signed off" on. I'm talking about their inherent knowledge and aptitudes because strengths are developed from a combination of interests and abilities. Work hard to challenge staff to develop their interests and to provide development activities for them to improve their abilities. Don't allow good people to stagnate, just because you are too busy with yet another report for management or fighting yet another fire! Why hasn't Bob used the ultrahigh-pressure liquid chromatography–mass spectrometry (UHPLC–MS) instrument yet? Is he interested? What does Mary know about the theory of gas chromatography (GC)–MS? How can this be measured and tested? Communication is often key to better understanding the abilities of your staff, and once you have identified their strengths and interests, ensure that they are challenged to grow and develop in these areas. Most importantly of all, if they struggle or fail, support them and have the safety net of good mentors to limit the impact of these failures.

Create a Mobile Workforce: A workforce who can respond to workload demands will be much more effective, but this is only possible with good training and mentoring. Your training matrix should contain critical skills such as troubleshooting, statistical approaches, instrument maintenance, regulatory knowledge, general chemistry, and product knowledge. Include cross-training objectives into personal development plans and your people development review programme.

Protect Your Business-Critical Knowledge: Plan to help retain knowledge by implementing a risk matrix of criticality of knowledge (generally known to be irreplaceable) and the time taken to replace it (2–6 years). In this way one could quickly identify the risks associated with knowledge concentration

(that is, critical information being held by only a few staff) or knowledge loss. Again cross-training and mentoring will help alleviate knowledge concentration or loss and this topic should form part of your laboratory operations risk register. Some companies plan for events such as phased retirement or early succession hiring.

Create a Mentoring Culture: Ensuring that the knowledge of more experienced staff is effectively communicated down and across the laboratory organization is good for everyone involved, and for the business. This can be achieved through several different methods including:

- War stories—Allow more senior staff the time to explain why things are done as they are, what has happened in the past to shape the way analyses are performed, and the nature of your work.
- Lessons learned—A military strategy often employed in project debriefs where the intended outcomes are compared to the actual outcomes and learnings distilled into working practice.
- Procedures and documentation—This only works with explicit knowledge, but a well-organized and maintained information repository can be very effective at disseminating knowledge of this type.
- Formal mentoring or work shadowing networks—A crucial part of a healthy laboratory environment, this approach allows more junior staff to develop whilst feeling supported and valued. Of course, it's easy to allow the mentoring programme to slip when workloads and time pressures are high, but protect your mentoring programme as fiercely as you are able—it's the key to your future success.
- Instrument champion presentations—One should strive for time in work for more senior staff to present on their work with analytical instruments or techniques. It's a highly effective way to disseminate knowledge.

Create Regular Communications Platforms: This will help to engage and develop more junior staff and identify where individual staff members strengths and interests lie. It's also a great way to quickly identify those who are engaged and keen to develop from those who are not. Forums such as chemistry club, departmental presentations, poster presentations from work presented at conferences, project review meetings, new equipment or procedures train-out, and sessions on the wider business operations (where do samples come from, who uses your data, and how they use it) all help to create context and share knowledge. These wider communication initiatives are critical and should form a part of the regular cadence of laboratory operations.

Person Centric Training: People development should not concentrate merely on who is "trained" to carry out which procedures or methods on what equipment, but should take a more holistic approach to professional development. The wider training curriculum can and should include topics such as business operations, regulatory understanding, method development, method validation, and theory of analytical techniques, all topics which are required to properly develop the "whole person" and should be available to everyone within the laboratory operation.

Optimize Recruitment: Recruit for talent, aptitude, and interest and not pairs of hands. Manage poor performance quickly and manage perennially poor performers out of the business quickly. If you are giving staff every chance to perform and develop, then there is no time or resource available in the modern laboratory to allow this to continue. Focus on staff who are performing adequately or well, and supercharge their performance.

Does all of the above seem like nirvana, or even naïve, amongst the maelstrom of your sample backlog, the reports that need to be written, the capital expenditure submissions you need to develop, the recruitment activities that call upon your time, the instrument vendor who isn't providing you with the servicing levels they promised, and the staff discipline issues that you need to speak with HR about? Well, I can guarantee you that by not doing any of the above, your high staff turnover will cost you more time and effort than implementing the above programmes and cultures.

By the way, if you are in middle or upper management and are frustrated by how little support you have in getting any of the above done in your workplace, go look on Glassdoor, you may just find an organization who is fit for the future, and who are not afraid to read their company reviews. I can assure you, your employees will certainly be doing so.

Reference

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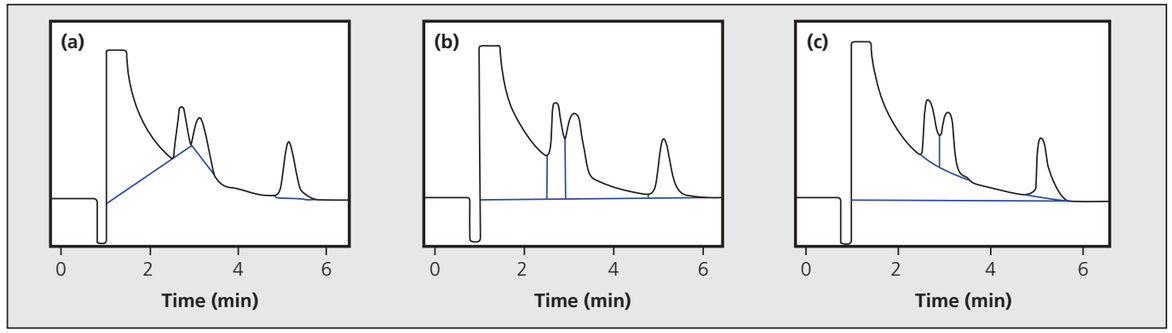
Advanced Peak Processing to Reduce Efforts in Method Optimization

Gesa J. Schad, Shimadzu Europa GmbH, Duisburg, Germany

Partial coelution of chromatographic peaks is an often-encountered issue in high performance liquid chromatography (HPLC) analysis, despite best efforts in method development and optimization. Even though there are several ways of integration of overlapping signals, accurate quantification of single compounds using conventional photodiode array (PDA) detection is almost impossible without baseline separation. While extensive signal processing is well established in spectroscopic analyses such as infrared (IR) or nuclear magnetic resonance (NMR), it has not yet been commonly adapted to improve chromatographic data evaluation. This article introduces the theory and application of a novel data analysis technique for PDA detection to accurately determine and quantify single compounds, even from overlapping peaks, without the need for mass spectrometry (MS) detection.

Despite advances in separation technologies, chromatographic method development, and optimization tools, coelution of compound peaks is still a common issue. Achieving baseline separation can be a challenge, particularly in complex samples with a large number of analytes or with isomers that can't be distinguished by different mass and exhibit similar elution behaviour. There are a number of integration options for

Figure 1: Integration example of overlapping peaks using (a) valley-to-valley integration, (b) perpendicular drop, or (c) peak skim method.



overlapping peaks, such as valley-to-valley integration, perpendicular drop, or peak skim method as visualized in Figure 1, but none of them offer truly accurate quantification of the single compounds. To quote John Dolan's "LC Troubleshooting" advice: "Integration of poorly resolved peaks is only an estimate of the more accurate results you would get when the peaks are baseline resolved" (1).

However, despite best efforts, baseline separation is sometimes more easily said than done, and achieving that last extra bit of resolution considerably increases the time and effort, and hence cost, of method development.

An alternative is the use of intelligent peak deconvolution analysis (i-PDeA), where multivariate curve resolution-alternating least squares (MCR-ALS) is applied to photodiode array (PDA) detector data, so that single analytes can be extracted from coeluting peaks (2). This novel approach enables visualization and detection of a minor single impurity even when it is coeluting with a main component. It therefore facilitates the individual, accurate quantification of hard-to-separate peaks through computer processing and deconvolution of spectral information, thereby reducing the effort required to further optimize separation parameters.

Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS)

MCR-ALS is a powerful tool that allows underlying elution and spectral profiles for a chromatogram to be estimated, even in the case of complete overlap of peaks. As the PDA detector collects full spectral data, it allows the construction of

Figure 2: Data matrix (D) showing peak profile (C) and spectral data (S) from a three-component mixture (2).

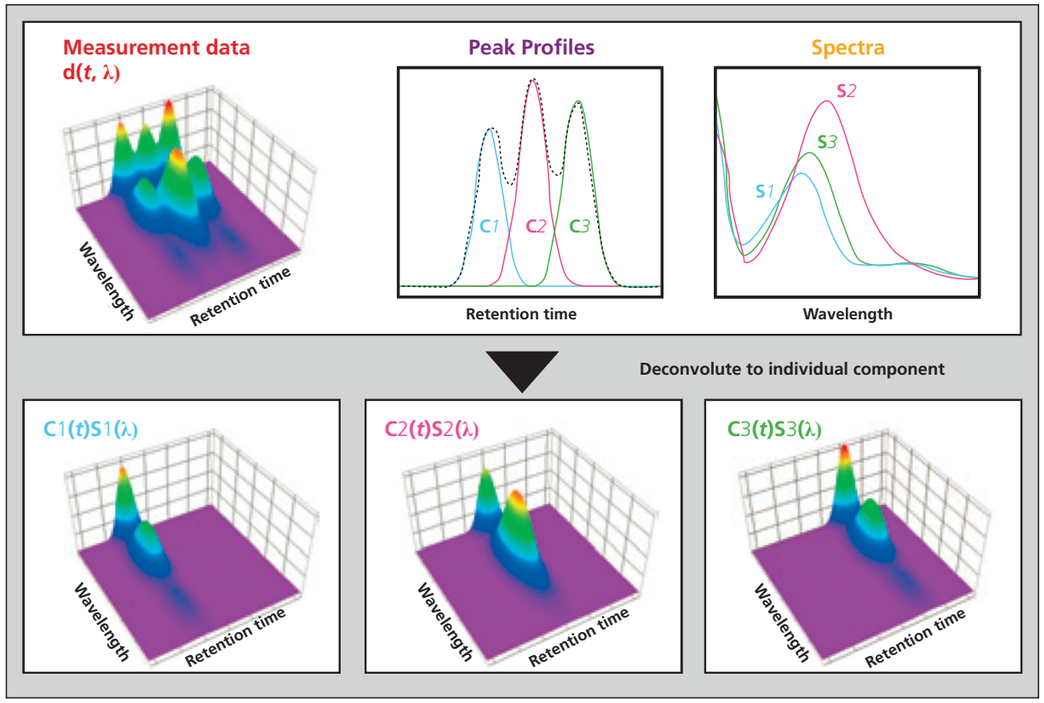


Figure 3: Chemical structures of three antioxidants added to polystyrene.

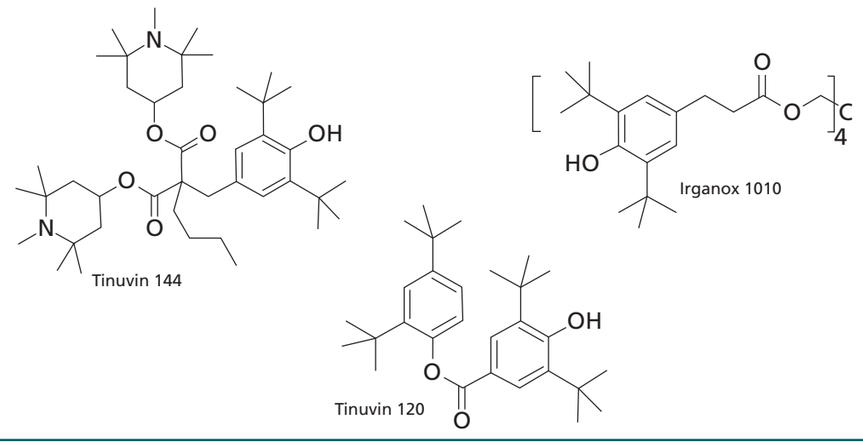
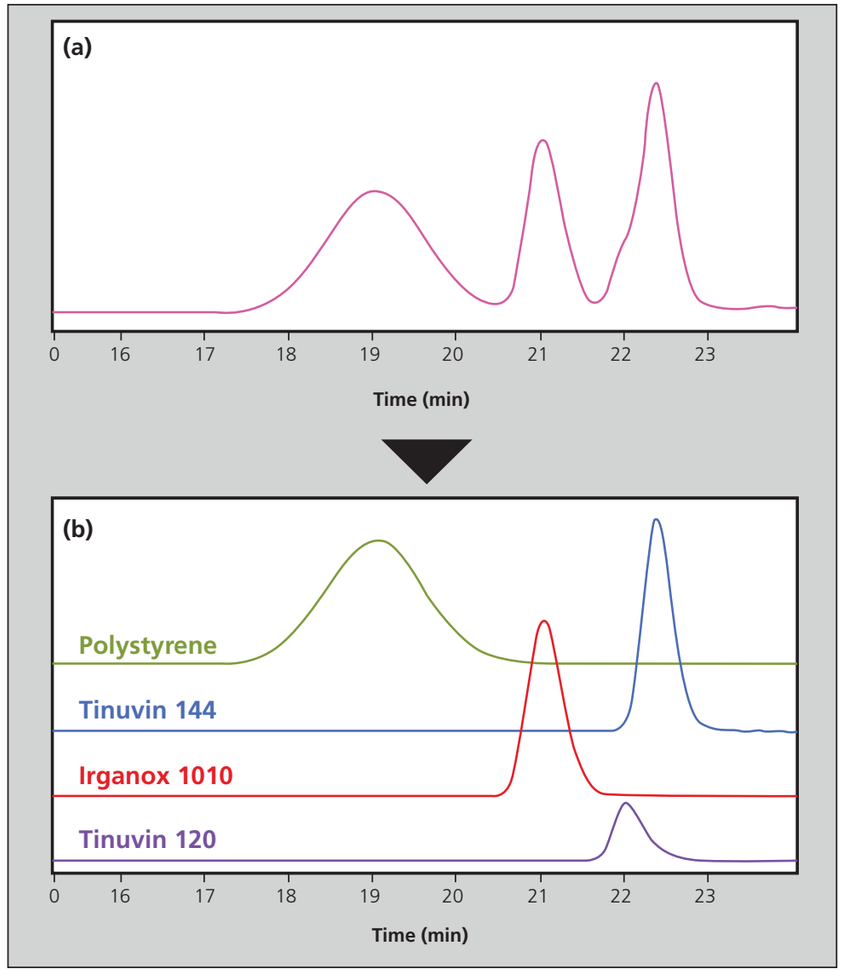


Figure 4: (a) Chromatogram at 240 nm and (b) i-PDeA extracted traces of the simultaneous analysis of polystyrene and three additives (PDA).



a multidimensional data matrix. MCR-ALS is used to decompose the observed data matrix (D) of a chromatogram into elution (C) and pure spectral profiles (S^T) that optimally fit the data matrix. Considering measurement error, noise, and unpredictable factors, and given a remainder, R, the measurement data can be modelled as $D = CS^T + R$ (2,3). This relational expression is valid for any number of components (Figure 2).

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Table 1: Linearity of the calibration curve and quantitative results

	Irganox 1010	Tinuvin 144	Tinuvin 120
Linearity (R^2)	0.999	0.995	0.998
Content (mg/g)	49.2	23.1	27.4
% RSD	1.28	1.93	1.47

Peak Deconvolution for Incomplete Separation of Polymer Additives

The merit of individual, accurate quantification of the three antioxidants in a gel permeation chromatography (GPC) run that doesn't offer baseline separation is described in the following paragraph. It saves the time and effort associated with setting up a second, separate method for analysis and quantification of the additives. **Method:** column: 300 × 8 mm shim-pack GPC 805 + 801 (Shimadzu); mobile phase: THF; flow rate: 0.8 mL/min; oven temperature: 40 °C; injection volume 10 µL; sample 0.5% polystyrene containing three additives.

In the simultaneous determination of molecular weight distribution of polymer compounds by GPC using a refractive index detector and polymer additives in an additional PDA detector, separation of these additives is almost impossible on any GPC column, due to very small differences in molecular weight of these antioxidants.

Figure 4(a) shows the PDA chromatogram of the analysis of polystyrene in the presence of three antioxidants at 240 nm, with only two peaks in addition to the polystyrene signal. In Figure 4(b) the extracted traces of each single compound after peak deconvolution using i-PDeA can be seen. The obtained peak areas were used for accurate quantification of the amount of each additive in the polystyrene sample.

Calibration curves for the three compounds were created in the range 0.01 to 0.1% (w/v). Linearity of this calibration curve and quantitative results of each antioxidant from six consecutive injections are summarized in Table 1.

Conclusion

While digital peak processing to improve data quality is widely accepted in the field of spectroscopy, separation scientists have so far been hesitant to adapt mathematical manipulations to enhance peak resolution and evaluation. A peak deconvolution algorithm, embedded in the chromatographic data system, has been shown to aid in the interpretation of data from overlapping peaks and to enable accurate quantification of single components without the need for time-consuming method optimization to achieve baseline resolution. In an application example, this technology was used for simultaneous determination of polystyrene and antioxidant additives in a single GPC run.

This approach can also be applied as a cost-effective alternative to mass spectrometry (MS), especially for analysis of isomers with similar retention behaviour and identical mass-to-charge ratio (*m/z*) values.

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Tips & Tricks GPC/SEC: “Green” GPC/SEC and Solvent Alternatives

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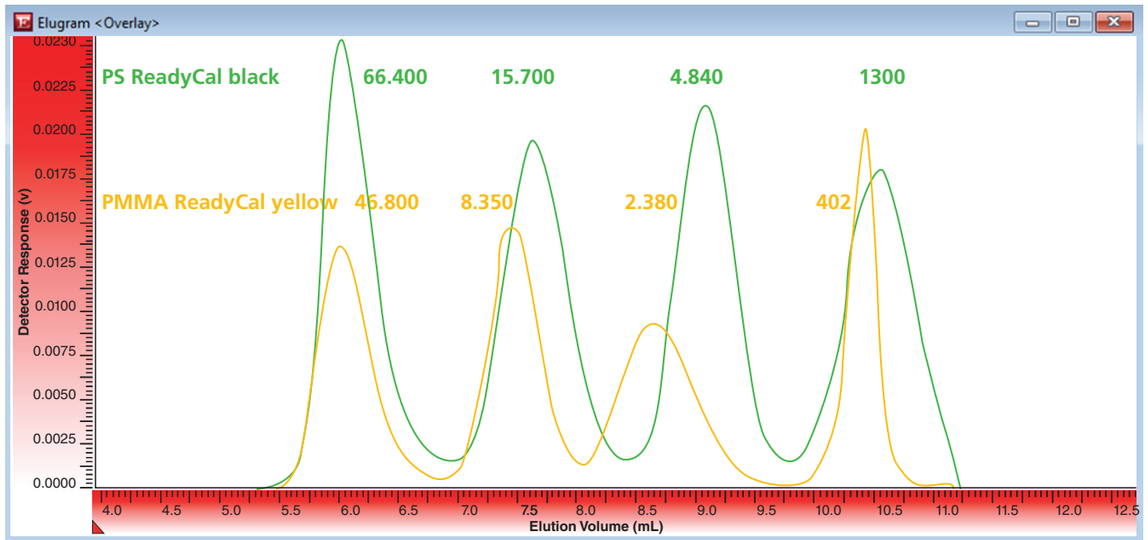
The “greenest solution” is certainly using no solvent but gel permeation chromatography/size-exclusion chromatography (GPC/SEC) as a liquid chromatography (LC) technique requires the use of a mobile phase. The growing awareness of the need for more sustainable (greener) solutions has focused attention on environmentally- and health-friendly solvents and solutions.

Traditionally gel permeation chromatography/size-exclusion chromatography (GPC/SEC) is a “slow” method that uses long columns with large diameters. Water-soluble macromolecules can, by their very nature, be analyzed under relatively “green” conditions, but for other macromolecules specific, environmentally challenging organic solvents are often required.

In contrast to high performance liquid chromatography (HPLC), where separations can be optimized by varying the solvent gradient or the temperature, GPC/SEC separations rely solely on the accessible pore volume. A minimum amount of material or pores is required to achieve sufficient resolution and therefore, compared to HPLC, longer columns with a larger diameter have to be used. To increase the pore volume (and thus the resolution), often several columns are combined to create column combinations or column banks (1).

A drawback of this approach is that analysis time and eluent consumption increases with the number of columns. Despite these very challenging experimental conditions, strategies have been implemented to reduce the solvent

Figure 1: A PS (green) and a PMMA (orange) mixture of four different molar mass standards analyzed in ethyl lactate on a styrene-divinylbenzene phase using RI detection.



consumption (2). This includes software solutions such as overlaid injection and, if applicable, a reduction of particle size and the use of small particles in columns with smaller dimensions in optimized hardware.

Besides approaches for solvent reduction the chemical industry is also investigating complete substitution of the solvent. In some areas solvents from renewable resources or less harmful alternatives have already been successfully applied. For example, ethyl lactate, an ester of lactic acid, is a "green" solvent derived from processing corn. As a result of its high solvating power, it has replaced solvents such as toluene, acetone, or xylene in the polyurethane industry as a cleaner alternative, resulting in safer workplaces. Other greener solvent alternatives for traditional organic mobile phases are summarized in Table 1.

However, the availability of a greener alternative is not the only parameter to be considered when researching more sustainable GPC/SEC solvents. Prerequisites for the suitability of a GPC/SEC mobile phase are also that there is:

- a matching stationary phase
- conditions that allow for interaction-free size separation
- soluble calibration standards that allow for the creation of a calibration curve
- sufficient detection options.

Medium Polar Organic Mobile Phase

A relatively straightforward approach is available for all materials that are analyzed in medium polar solvents, such as dimethylformamide (DMF). Medium polar stationary phase materials for interaction-free GPC/SEC have been developed for the polarity range of these solvents and have proven their applicability in the last few years.

With only a very few exceptions, samples that are soluble in DMF are also soluble in dimethylacetamide (DMAc). As DMAc is less harmful compared to DMF, it is preferred for method development.

Another potential alternative is dimethyl sulfoxide (DMSO), a solvent for which no H and P warnings (hazard and precautionary warnings) are reported. However, it is a penetration enhancer, so skin contact should be avoided. When working with

Table 1: Typical organic GPC/SEC solvents and potential "greener" alternatives

Mobile Phase	Potential Less Harmful Alternative	Comment
Pentane/Hexane	Heptane	Ready to use
DMF/DMAc	Dimethylsulfoxide (DMSO)	DMAc less harmful than DMF, DMSO no H/P warnings but penetration enhancer, ready to use
THF	2-Methyl THF	Derived from renewable raw materials such as corncobs and bagasse
THF	2-Methoxy-2-Methylpropane (MTBE)	Lower miscibility with water and less peroxide formation
THF	Cyclopentylmethyl Ether (CPME)	Less peroxide formation
THF/ Dichloromethane	Ethyl Lactate	No significant health risks, not teratogenic
THF/ Dichloromethane	Ethyl Acetate	Lower health risks

DMSO in GPC/SEC, higher temperatures (60–80 °C) are required as a result of the high viscosity of this mobile phase. Suitable calibration standards are polymethyl methacrylate (PMMA) and (depending on the stationary phase chemistry) polystyrene (PS).

Nonpolar Organic Mobile Phases

Tetrahydrofuran (THF) is still one of the most commonly used nonpolar organic mobile phases in GPC/SEC. Potential replacements for THF are ethyl lactate and 2-methyl THF.

Ethyl lactate is a colourless liquid with a discreet scent. Both PMMA and PS standards are soluble in ethyl lactate. To investigate the potential of this solvent as a THF substitute, a typical styrene-divinylbenzene phase was transferred from THF to ethyl lactate. The transfer was straightforward and fast, and the plate count test confirmed the successful solvent exchange. Mixtures of PMMA and PS standards were then analyzed using a GPC/SEC system equipped with a refractive index (RI) detector. Figure 1 shows an overlay of a PS and a PMMA mixture, each comprising four different molar mass standards.

Figure 2: Overlay of the PMMA (orange) and PS (green) calibration curves on a styrene-divinylbenzene phase in ethyl lactate. PS oligomers elute with or after the system peaks, most probably because of interaction of the low molar mass PS with the stationary phase.

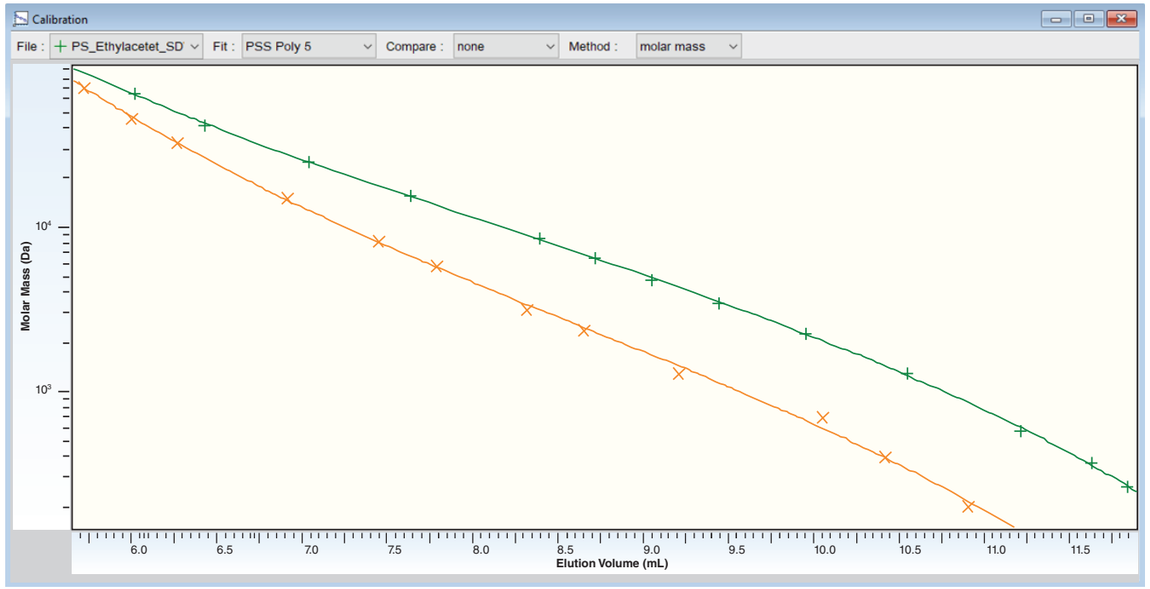
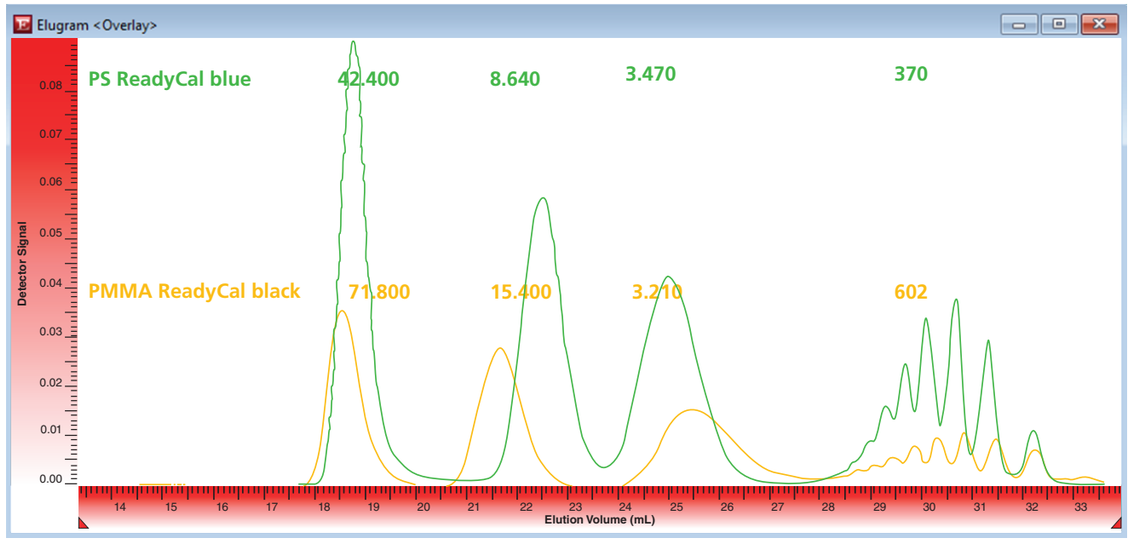


Figure 3: A PSS (green) and a PMMA (orange) mixture of four different molar mass standards analyzed in 2-methyl THF on a styrene-divinylbenzene phase, detection: RI. The oligomers could be resolved into single peaks.



Although at first glance the chromatograms of PS and PMMA look promising, the elution of the PS standard of 1300 Da close to the RI system peak is a clue that a very thorough investigation is required. Lower molar masses of PS (oligomers) elute even with or after the typical RI system peaks. The overlay of the calibration curves (compare Figure 2) shows that the PMMA and the PS calibration curves differ strongly in the low molar mass region. Interaction of the PS oligomers in this mobile phase–stationary phase combination is the potential root cause for this observation.

Thus, only PMMA is a suitable calibration candidate when working with ethyl lactate on styrene-divinyl benzene materials, because PS most probably interacts with the stationary phase material.

2-Methyl THF was the second alternative to be tested. It is also a colourless liquid with a discreet scent. Again the two most common calibrants, PS and PMMA, were soluble and could therefore be investigated further. The first chromatograms looked promising for both standard types, and additional tests were performed on a column combination with the highest resolution in the low molar mass area.

Figure 3 shows an overlay of the results for a mixture of four different molar mass standards of PS (green) and PMMA (orange). As a result of the very high resolution of the chosen column set in the low molar mass region, the lower (last eluting) PMMA and PS standards could be resolved into single oligomers resulting in multiple peaks.

PMMA and PS molar masses do not match 100%, which was expected because GPC/SEC separates based on hydrodynamic volume and not on molar mass. However, all peaks clearly elute before the total permeation limit and thus both substances are applicable for calibration.

As in the case of ethyl lactate, RI detection was used for the separations in 2-methyl THF. The difference in signal intensity for a comparable injected mass shows that PS has a higher *dn/dc* value and therefore larger response in 2-methyl THF compared to PMMA. This was less pronounced in ethyl lactate. However, in 2-methyl THF, detection with a UV detector

is also possible for wavelengths above 230 nm.

Conclusions

- There are a few solvents that could be considered as "greener" organic solvents because they are produced from renewable resources or have less health risks.
- DMSO or DMAc are preferred over DMF for samples that can be analyzed in medium polar solvents.
- Alternatives for THF are currently being investigated. Stationary phases are suitable for the alternatives 2-methyl THF and ethyl lactate.
- Potential calibrants for 2-methyl THF are PS and PMMA. For ethyl lactate, only PMMA calibrations should be used when working on styrene-divinylbenzene stationary phases.

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The Basics of HPLC
Peptide Analysis

To fully characterize a protein biopharmaceutical, it must be broken down into smaller segments (peptides). Several high performance liquid chromatography (HPLC) techniques can be used to provide a wealth of information on everything from post-translational modifications (PTMs) to the glycoprofile to information on similarity when characterizing biosimilars.

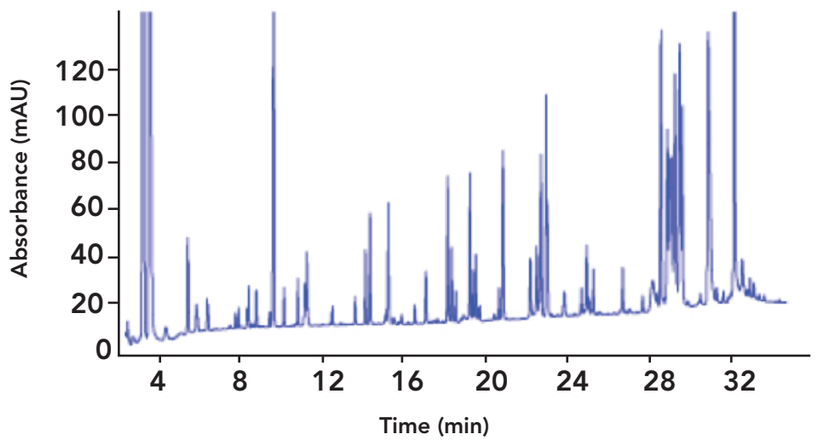
Much information is available when biomolecules are analyzed at the protein level, such as molecular weight, structural integrity, charge variants, aggregation, and post-translational modifications (PTMs). However, identification of PTM modification sites, as well as other critical quality attributes such as the glycoprofile, requires digesting the protein into representative peptides using a suitable proteolytic digestion enzyme.

The digested peptide-containing solution is then chromatographed, commonly using a generic reversed-phase liquid chromatography (LC) methodology that consists of an acidic mobile phase, a steeper gradient over a wider range, and a longer alkyl chain stationary phase (such as C18, for example) as compared to the method employed to analyze an intact protein.

A typical peptide map of a digested monoclonal antibody (mAb) is shown in Figure 1. It is considerably more complex than those generated for intact proteins because of the number of peptides liberated and the artifacts that arise from the digestion process, such as residual reagents and missed cleavages.

Great care and consideration are required during the digestion process, as the proteolytic enzymes used and the conditions employed (pH, temperature, even storage time) not only affect the overall number of peptides liberated but also the stability of associated PTMs, and can even introduce protein modifications of their own.

Figure 1: Typical reversed-phase chromatogram of a mAb peptide map.



Broadly speaking, the digestion process can be broken down into three separate steps: reduction, alkylation, and digestion.

The first stage in the reduction step is to denature the mAb. This is commonly accomplished with an acid-labile surfactant that removes the higher order structure of the protein and exposes many otherwise internal disulfide bonds. These disulfide bonds are then ready for reduction, which is achieved using dithiothreitol. The pH is maintained at physiological levels throughout the process using buffers. To prevent reformation of disulfide bridges across the thiol groups of the cysteine (C) residues, the protein is then incubated with an alkylating agent such as 2-iodoacetamide, once again at physiological pH. The final stage is the addition of a proteolytic agent capable of site-specific protein digestion. Table 1 details these enzymes and highlights their specific cleavage sites. Typically, fewer cleavage sites leads to larger and, therefore, fewer resulting peptides, and vice versa.

Table 1: Common proteolytic digestion enzymes and their specific cleavage sites

Enzyme	Site of Cleavage
Trypsin	Lys, Arg (C)
Chymotrypsin	Phe, Trp, Tyr (C)
Asp-N protease	Asp, Glu (C)
Pepsin	Leu, Phe, Trp, Tyr (N)
Elastase	Ala, Gly, Ser (C)
Cyanogen bromide	Met (C)
Endoproteinase Lys C	Lys (C)

Due to the precise and predictable nature of the hydrophobic retention of reversed-phase LC, estimates as to where the modified peptide will elute in relation to the native, unmodified variant can be made (Table 2). This can be a helpful tool when trying to identify and assign unexpected peaks. Asparagine deamidation can produce both pre- and post-peaks, due to deamidation occurring via the succinimide intermediate, iso-Asp (pre-peak), and Asp (post-peak) in a 3/4:1 ratio.

Table 2: Peptide PTM reversed-phase LC peak prediction relative to the unmodified parent peptide

Modification	Reversed-Phase LC Peptide
Aspartate isomerization	Pre-peak
Asparagine deamidation	Post-peak + pre-peak
Oxidation	Pre-peak
PyroGlu from Glu (-H_2O)	Post-peak
PyroGlu from Gln (-NH_3)	Post-peak
Succinimide	Post-peak
Sugar	Pre-peak
C-terminal lysine	Pre-peak
Fragmentation	Variable

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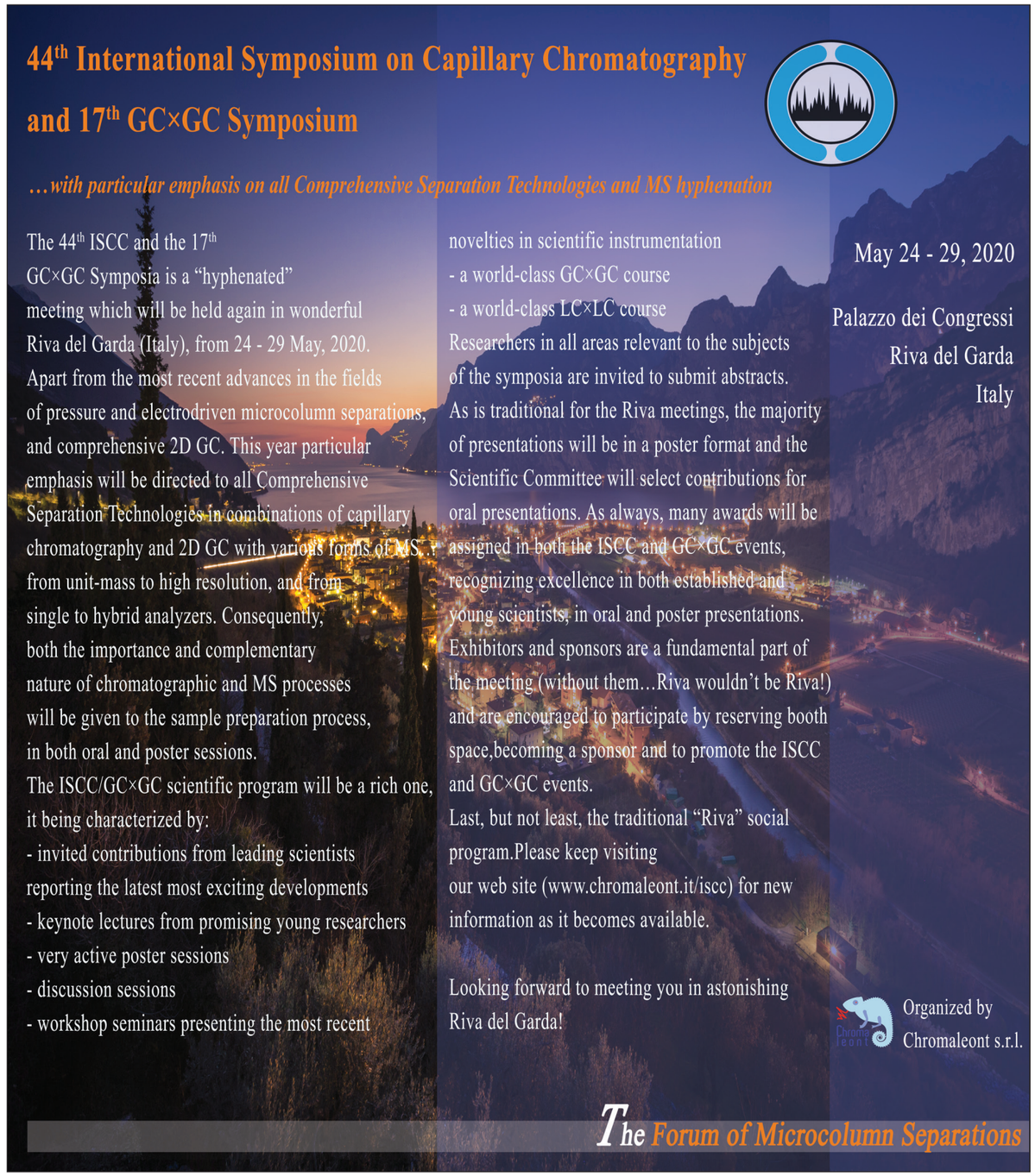

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