The Column
4 December 2013 Volume 9 Issue 22
the interactive e-publication for the global separation science industry

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A selective review of companies who launched products in 2013.

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26 **Q&A: Miniaturizing Military Detectors**
Scientists at Sandia National Laboratories are thinking smaller in the development

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ADVANSTAR

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26 Q&A: Miniaturizing Military Detectors
Scientists at Sandia National Laboratories are thinking smaller in the development of detectors for military use — from the detection of explosives and chemical weapons to humans. Scientist Ron Manginell from the laboratories spoke to Bethany Degg of *The Column* about the on-going research in this area.

28 Diisobutyl Columns Reduce Solvent Consumption and Offer Rapid HPLC–MS Analysis of Plasma Oxycodone and its Metabolites
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Shoulders in gel permeation/size-exclusion chromatograms (GPC/SEC) can be a result of sample characteristics or down to the wrong choice of columns or column combinations. Proper selection helps to measure true results.

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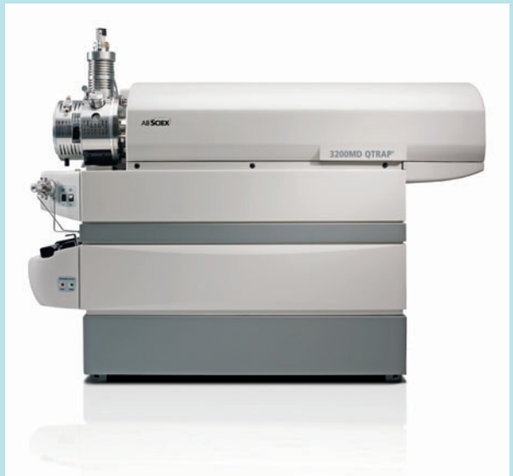
Instrumental Innovations

A selective review of companies who launched products in 2013.

Mass spectrometer for in vitro use

AB Sciex introduced its first series of CE-marked mass spectrometers and kits designed specifically for in vitro diagnostic use*. The AB Sciex API 3200MD and 3200MD QTRAP CE-IVD LC–MS–MS systems have been developed for clinicians and researchers in hospital laboratories performing analyses of trace level multiple compounds in human samples for diagnostic purposes. The company has also recently announced a series of CE-marked reagent kits for diagnostic testing. Mass spectrometry (MS) has the potential to improve the sensitivity and accuracy of testing in clinical diagnostics, while also reducing costs compared to other technologies. These instruments are reported to bring the power, reliability, and speed of MS in a robust, off-the-shelf, and easy-to-use format. The SCIEX IVD-MS kits, coupled with the 3200MD CE-IVD instrumentation, are reported to offer complete application-specific solutions to European hospitals and clinical laboratories to improve diagnostics.



*** Only available in some European countries.**

www.absciex.com

Encapsulated bonding technology

ACT has developed "encapsulated bonding technology", which is reported to increase the ligand coverage of silica surfaces to eliminate the effect of unbonded silanol groups in liquid chromatography (LC) separations. The higher ligand coverage is reported to improve inertness, chromatographic performance, and stability. The resultant ACE SuperC18 UHPLC/HPLC columns are inert and stable across an extended pH range (pH 1.5 to 11.5), allowing exploitation of beneficial selectivity changes at low, intermediate, and high pH. The columns can be used with both methanol and acetonitrile mobile phases and can be rapidly equilibrated without memory effects. Designed for use with LC–MS compatible buffers, they reportedly give ultra-low bleed for improved LC–MS compatibility. Columns of 2-, 3-, and 5-µm particle sizes are supplied with dual compatible UHPLC/HPLC Excel hardware and are stable up to pressures of 1000 bar or 15,000 psi. A 10-µm particle size is also available. The wide range of preparative column dimensions allows reproducible scale-up from analytical dimensions, allowing increased loading capacity at elevated pH. The columns are reportedly ideal for method development.

www.ace-hplc.com

Flow path inertness products

As regulatory agencies drive detection limits lower for increasingly active and more complex samples, chemists cannot afford adsorption caused by flow path activity. This is particularly critical for food, environmental, and forensic sample matrices. To address this need, Agilent laid the groundwork for flow path inertness in 2008 by introducing Agilent J&W Ultra Inert GC columns — reported to deliver consistent inertness and exceptionally low column bleed.

Since then Agilent has expanded the range of Ultra Inert liners and added inert fittings, ferrules, and supplies. By minimizing activity along every step of the GC and GC–MS flow path, the flow path solutions are reported to improve system performance, give better results, and allow the processing of more samples without unplanned maintenance and recalibration. The complete Inert Flow Path includes: Ultra Inert liners, Inert Flow Path split/splitless inlet, Ultra Inert gold seals, Inert MS source, Inert CFT devices, UltiMetal plus Flexible Metal ferrules, Gas Clean purifier, and Agilent J&W Ultra Inert GC column.

www.agilent.com

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Triple quadrupole liquid chromatography mass spectrometer

Winner of three awards in summer 2013, the EVOQ triple quadrupole liquid chromatography mass spectrometer (LC–MS TQ) from Bruker reportedly offers a comprehensive solution for routine laboratories, enabling them to reliably quantify thousands of samples in the fastest sample to report time possible. The company has also added the EVOQ Elite ER to the range in 2013, providing high sensitivity and specificity needed for proteomics quantitation. The models are used in laboratories in a wide range of application areas, including food safety, environmental testing, petrochemicals, and drug testing.

The instruments feature ultrahigh pressure liquid chromatography (UHPLC) pump technology that gives short run-to-run cycle times and reproducible separations; as well as several innovations in the ion source to maximize sensitivity and robustness. Vacuum-Insulated Probe Heated Electrospray (VIP-HESI) technology preserves and ionizes thermally fragile molecules, and "Active Exhaust" entrains sprayed gases in the source for high sensitivity, reducing chemical noise and cleaning. In addition, the EVOQ Elite ER is reported to offer an extended mass range of 2,000 *m/z*.

Novel PACER software delivers exception-based data review, reportedly increasing efficiency and accelerating sample-to-report times without compromising on data quality. Dash Reporting software gives customized reporting to all users, with an intuitive system which allows control of all report elements. The innovations behind the EVOQ and the new EVOQ Elite ER reportedly deliver a complete platform for routine laboratory testing.

www.bruker.com

HPLC system

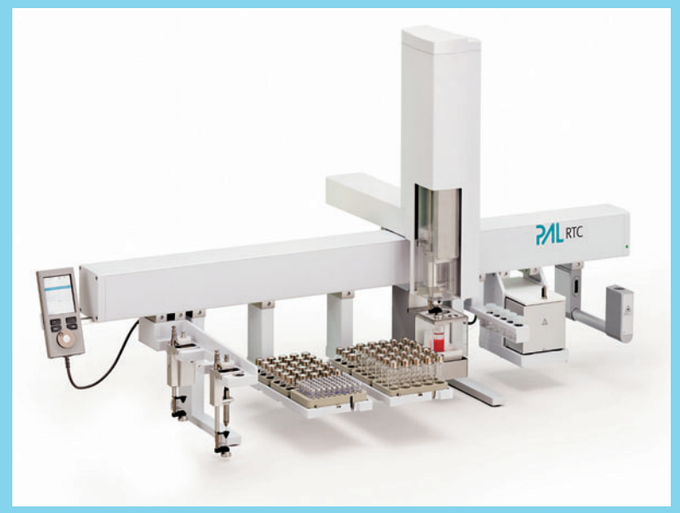
Many scientists performing high performance liquid chromatography (HPLC) require the most innovative instrumentation to accommodate a wide range of current and future applications. Many HPLC instruments have a vast array of functions to meet these requirements; however, with this comes additional pricing costs and system complexity. Furthermore, in some applications, technological developments are not required and can be detrimental to use. According to Cecil Instruments, Merit HPLC systems are low-cost and easy to use, ideal for applications such as multi-compound screening, initial method development, production situations, process development, and teaching. The systems are available in isocratic and binary modular configurations. The company report that in initial method development an ultra-fast scanning UV–vis WaveQuest detector, instead of a diode array detector, may be used to check that all known components of the injected solution are eluted with appropriate baseline resolution. Each system offers ultra-low drift and durable modules, with full PC control. This control is provided by software on-board the instrument modules, so there is virtually no software to learn to master.

www.cecilinstruments.com/merit-hplc.html

Robotic platform

With the PAL RTC (Robotic Tool Change) robotic platform from CTC Analytics, sample preparation is no longer the bottleneck. In seconds the RTC changes automatically between different syringes (1 µL to 10 mL), liquid injection, headspace, and solid-phase microextraction (SPME) methods without the need for manual intervention. This boosts productivity and expands the application range. The optional vortex mixer, incubator, and different syringes can automate most sample preparation steps. Liquid-liquid extraction, derivatization, standard addition, and dilution reportedly become smooth and traceable.

PAL Sample Control software controls the RTC. With a few clicks you can import or generate sample lists and start the data acquisition. Or you can quickly set up a new workflow to eliminate tedious manual operations. PAL Sample Control interfaces with most chromatographic systems.



www.palsystem.com

Syringe filters

Whatman disposable syringe filters from GE Healthcare can be categorized into different families. Each family is optimized for specific applications or filtration requirements. For example, Roby can be utilized for automated filtration systems and Whatman GD/X for hard-to-filter samples. Within each syringe filter family there are options for different diameters, pore sizes, membrane types, and sterility. Whatman disposable syringe filters are designed to provide fast and efficient filtration of aqueous and organic solutions. The syringe filters are sealed without glue to maintain the purity of the filtered sample. The filters can be used in numerous pharmaceutical, environmental, biotechnology, food and beverage, and agricultural applications.

www.gelifesciences.com/LaboratoryFiltration

GC–MS headspace sensitivity

The enrichment technique hot injection and trapping (HIT) for gas chromatography coupled to mass spectrometry (GC–MS) combines analyte trapping from several headspace injections for each run, thereby offering extra concentrating power. The technique can be performed with a MultiPurpose Sampler (MPS) instrument and Thermal Desorption Unit (TDU) combined with a Cooled Injection System (CIS) PTV-type inlet, all available from Gerstel. The system is reportedly easily switched between standard and HIT operation by specifying the number of injections in the Maestro software. HIT can be performed under integrated control with the GC–MS system or independently using Maestro software. The performance of the HIT technique has been demonstrated for flavour and off-flavour compounds in drinking water and beverages, resulting in good linearity and repeatability and very low limits of detection (LOD).

www.gerstel.com

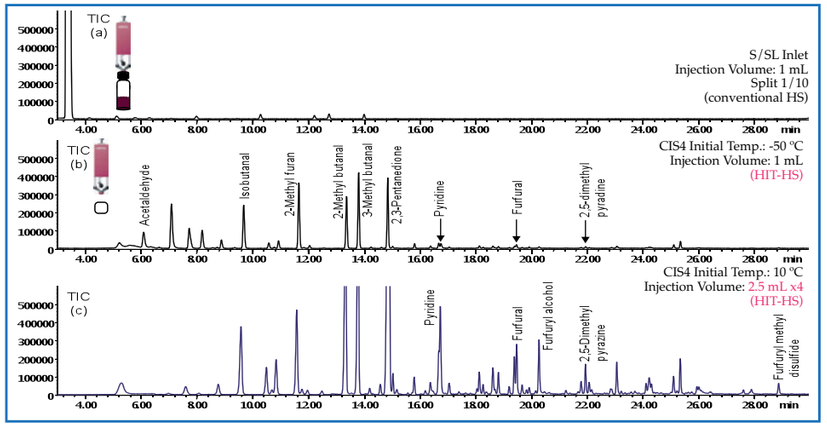
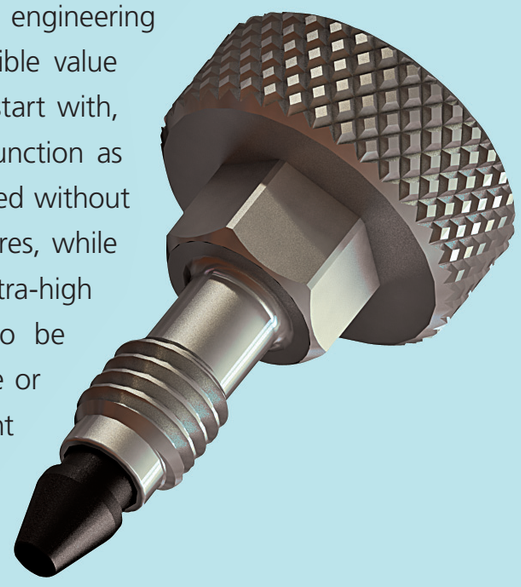


Figure 1: Total ion chromatograms of canned coffee by SHS analysis.
(a) Conventional SHS with S/SL inlet (injection volume 1 mL, Split 1/10);
(b) HIT-HS with CIS 4 Initial Temp. -50 °C (injection volume 1 mL, Split 1/1);
(c) HIT-HS with CIS 4 Initial Temp. 10 °C (injection volume 2.5 mL ×4, Split 1/1).

Fingertight UHPLC fittings

With the increasingly popular transition to UHPLC technologies, there remains a demand for fittings that can be "finger-tightened" into place. This year, IDEX Health & Science has expanded its growing line of reusable fittings for UHPLC, adding the new VHP-3200 fitting to its portfolio. Senior technical specialist, John Batts, shared his thoughts on this new fitting: "One of the major benefits of UHPLC is speed. There is a greater demand for convenience without sacrificing quality. To that end, we developed the VHP-3200. We focused on engineering features into the fitting that brought tangible value to those using them in the laboratory. To start with, the fitting's patented design allows it to function as a 'one-piece' fitting. It can be hand-tightened without tools and still hold to typical UHPLC pressures, while allowing the use of a wrench for those extra-high pressure applications. This fitting can also be reused multiple times – with the same tube or with different tubes in the same or different receiving ports, even in higher-temperature applications and aggressive chemical environments." He added: "These features help facilitate more rapid column change-overs, less system down-time, and therefore higher productivity per system."



www.idex-hs.com

Light scattering detector

Multi-angle light scattering (MALS) detectors are widely used in gel permeation/size-exclusion chromatography (GPC/SEC) within biopharmaceutical research. The Viscotek SEC–MALS 20 detector from Malvern Instruments measures absolute molecular weight across a range of macromolecular applications and provides good radius of gyration (Rg) measurements for molecules larger than 10–15 nm. The system reportedly gives a good data fit for molecular weight and size extrapolation, improving accuracy even at high molecular weights. Its vertical flow cell with radial optics can increase sensitivity and accuracy for low angle detection and allows the use of different mobile phases without the need to alter the optical properties of the detector.

www.malvern.com/secmalslaunch

Thermal desorper

In 2013 Markes International launched the next generation of the TT24-7 thermal desorber for continuous monitoring of trace-level volatile and semi-volatile organic vapours in air or gas by gas chromatography (GC) or direct read-out instruments. According to Matt Bates, thermal desorption product manager at Markes, the TT24-7 Series 2 desorber uses a reciprocating twin-trap system to capture airborne volatiles to avoid breaks in sampling.

He said: "The inert, uniformly-heated flow path makes it compatible with compounds ranging in volatility from C_2 through to C_{40}, including reactive species." He added that the design of the instrument and the cold traps allows sampling flows up to 800 mL/min with quantitative trapping, which he says provides outstanding levels of sensitivity. The same flexibility, he points out, also makes it possible to achieve cycle times as low as 3–5 min, for situations where a rapid response to raised levels of toxic airborne compounds is needed.

These and other innovations, Bates says, make the TT24-7 Series 2 suitable for a range of challenging air monitoring applications, including atmospheric chemistry research, continuous monitoring of controlled industrial environments, counter-terrorism (NRT detection of chemical warfare agents), and continuous monitoring of changing odour profiles.

www.markes.com

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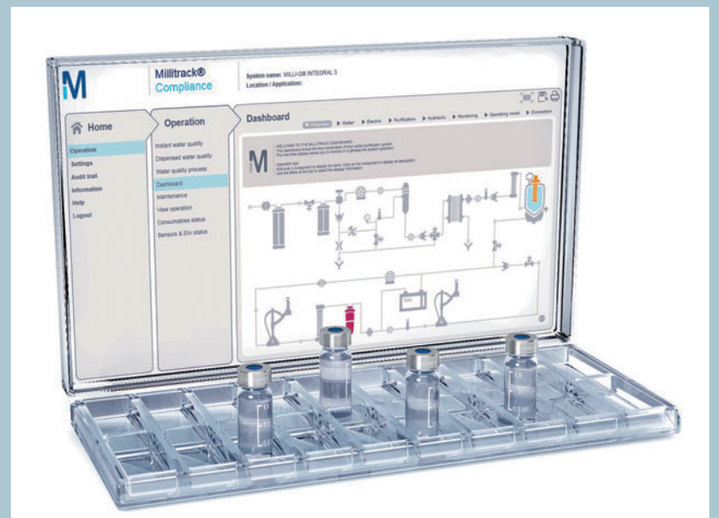
Purification software

Millitrack Compliance from Merck Millipore is a novel e-solution designed to allow regulatory compliance and optimization for Milli-Q Advantage and Milli-Q Integral laboratory water purification system users. Jean Mahooti, responsible for Merck Millipore Lab Water Compliance services said: "This e-solution enables laboratories to achieve good archiving practices and ultimately offers full compliance for users' water systems." He added: "The built-in Millitrack Compliance e-solution is the optimal solution for companies who want to move towards a zero-paper environment. In addition, Millitrack Compliance was designed with remote monitoring and advanced diagnostic capabilities to facilitate work in the laboratory and boost lab productivity, allowing laboratories to save time and increase productivity while minimizing downtime."



www.merckmillipore.com/millitrackcompliance

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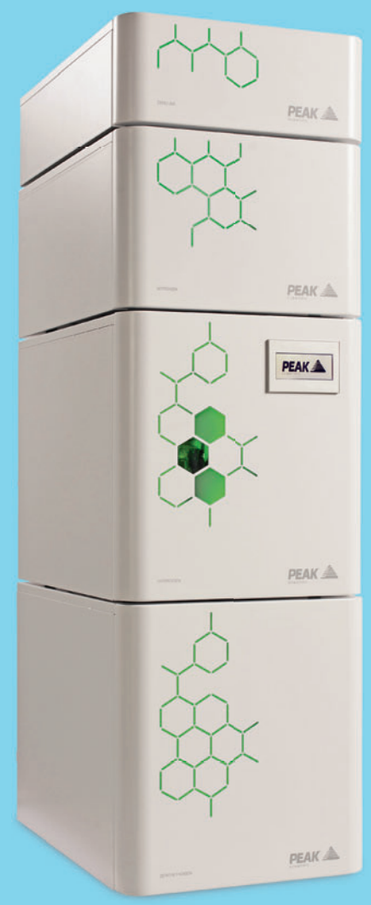
Ion chromatography system

Metrohm has recently improved the operation of the Professional IC Vario Series. A liquid handling station enables a number of sample preparation techniques such as dilution, partial loop injection, pick-up techniques, and preconcentration. Although each instrument consists of different units, they are very compact with short connections and visible flow paths. The new suppressor set-up is reportedly more environmentally friendly; all suppressor types are compatible with the same holder and can be easily exchanged. The suppressor is said to be robust and does not require any exchange of spare parts. The module for eluent production handles up to four solutions. A water purification system from ELGA can be connected for ultrapure water supply. This contamination-free, stable system runs automatically and offers flexibility combined with user-friendly operation and reliability.

www.metrohm.com

Modular gas generators

Peak Scientific has released the Peak Precision Series, a group of compact modular gas generator systems that allow the separation and analysis of multiple gas types and complex mixtures with high accuracy. The laboratory is a unique environment demanding precision, accuracy, reliability, and design. The Precision Series is reported to maintain gas flow with accuracy and reliability. The systems incorporate a space-saving stackable design and can fit neatly into confined spaces. Looking to the future as natural resources run low and costs rise, the system is able to accommodate helium to hydrogen conversion. The modular design reportedly allows for different combinations to suit single gas chromatography (GC) and multiple GC applications. The system eliminates the inconvenience of gas cylinders — no more changing over, no more supply issues, and no safety concerns. As a result, the range is very low maintenance and provides long-term cost stability.



www.peakscientific.com/peak-precision

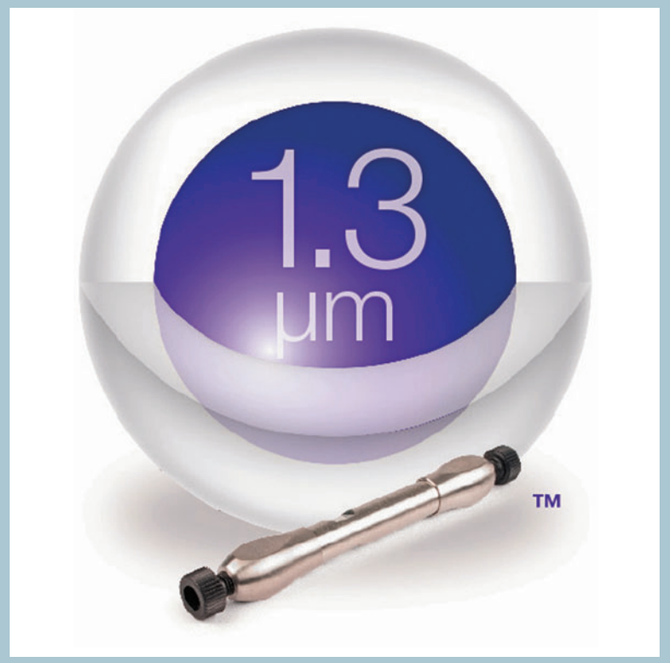
GC–MS–MS system

PerkinElmer has introduced the AxION iQT GC–MS–MS system which has the identification capabilities of a QTOF and the quantitation capabilities of a triple quadrupole in one instrument, according to the company. It has minimal setup requirements and is reported to be able to measure up to 500 compounds per second. The system delivers automated method development tools to users, includes advanced software, and is reportedly easy to use and maintain. The company state that it is compatible with a wide array of gas chromatography (GC) instruments, enabling rapid and efficient ion transmission and providing the quantitative dynamic range and low level sensitivity required for most complex matrices. The system offers a broad array of user-interchangeable ion sources (dual-filament electron ionization [EI], chemical ionization [CI], or cold EI). It can also characterize and quantify compounds for a variety of applications, including the analysis of pesticides in food samples to ensure food safety and authenticity, quantitation of non-derivatized steroids, cholesterol and therapeutic drug monitoring in research applications, analysis of drugs in forensic toxicology, and thorough analysis of chemical compositions.

www.perkinelmer.com/outsidethebox

Core–shell particle columns

In 2013, Phenomenex continued to expand the line of sealable high performance liquid chromatography/ultrahigh pressure liquid chromatography (HPLC/UHPLC) Kinetex core–shell particle columns, the company's most important innovation in recent years. A new addition to the line is a high-powered 1.3-µm particle, the smallest diameter in the Kinetex family. With high manufacturing quality and a tight specification range, Kinetex 1.3-µm columns are reported to give a performance approximately 50% greater than fully porous 1.7-µm particles, according to the company. This efficiency allows greater chromatographic resolution, performance, and productivity. The Kinetex core–shell family provides separation solutions for a wide range chromatographic applications and offers easy method transferability from UHPLC to HPLC to preparative-scale LC.



www.phenomenex.com

Automated soluble fraction system

Polymer Char recently introduced the CRYSTEX QC instrument for soluble fraction determination in quality control laboratories at polypropylene manufacturing plants on to the market. Traditionally, this test is performed through gravimetric and wet chemistry methods which are time-consuming and have safety implications for the operator because they require manual solvent handling at high temperature. The system fully automates temperature rising elution fractionation to obtain the soluble fraction in resins that is reported to be safer and simpler to use over traditional methods. Samples do not have to be weighed and all solvent handling is automatic throughout the analysis. High repeatibility is obtained as the instrument allows the use of up to 4 g of polymer. Furthermore, it is reported that precise quantification is delivered through a built-in infrared detector that also measures ethylene content. An integrated viscometer provides intrinsic viscosity in the same analytical process that lasts in total approximately 2 h.

www.polymerchar.com

MALS technology

Postnova has introduced the MaxMALS Multi-Angle Light Scattering (MALS) technology for the absolute molar mass, particle size, and structural characterization of proteins, biopolymers, macromolecules, and particles, using a maximum number of 21 detection angles. This technology is incorporated into the company's new line of multi-angle light scattering detector systems and reportedly overcomes the traditional limits of currently available multi-angle light scattering set-ups, such as insufficient number of detection angles and a — more or less — complete lack of low detection angles. This has been a significant problem for correct and reproducible characterization of large biomolecules, polymers, and particles, as the information for molar mass and size is directly derived from these low angles. The total number of angles and the availability of low angles are absolutely crucial for precise molar mass and particle size measurement. Up to now only instruments with a smaller number of angles, such as 3°, 7°, 8°, or 18° in the best case, have been available. Typically, the lowest angles available in MALS are located at 35° for most commercial instruments and around 20° for others. MaxMALS provides a maximum number of 21 detection angles and a complete series of low angles located at 7°, 12°, 20°, 28°, and 36°. MaxMALS reportedly offers the highest number of total and lowest detection angles available on the market and the most precise MALS instrument for molar mass and size determination.

www.postnova.com

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Deep well plates

Porvair Sciences has developed and introduced 96-well round deep well plates that deliver a full 2.00 mL liquid capacity for each well in an automation compatible SLAS/ANSI footprint, according to the company. The plates measure 45 mm in height and are compatible with microplate stackers, washers, and automated equipment. The plate design incorporates features that prevent "locking" when stacked and enable easy heat sealing. Manufactured under class 10,000 conditions from ultra-pure grade polypropylene, the plates are certified as RNase/DNase free, and do not contain measurable contaminants, according to the company. Plates are also available irradiated for sterile biological applications. A matching thermoplastic elastomer "cap mat" is reported to provide easy friction sealing for transport, shipping, and short-term storage. For longer periods, the plate can be heat sealed to provide peelable, pierceable, and re-sealable storage options.

www.porvair-sciences.com

Light scattering detector

The SLD7000B from PSS is a seven-angle multi-angle light scattering detector that can be operated with any gel permeation/size-exclusion chromatography (GPC/SEC) system or as a stand-alone instrument in batch-mode to measure absolute molar masses and sizes of macromolecules in solution. A metal-free version has recently been released that is reported to enable operation for applications at extreme pH values and with high salt concentrations. It combines the cell design of the SLD7000 with the robustness against corrosion of the PSS BioSECcurity SEC line. The detector measures the scattering intensity directly in the cell, therefore avoiding problematic interfaces and the need for angular correction with the solvent. The detector can be used in the pH range 1–13 and is ideal for protein molar mass, purity, and aggregation analysis in combination with a concentration detector (UV, RI). The wetted parts are PEEK (cell, tubing, frits).

www.pss-polymer.com

GC filter status indicator

The Super Clean Gas Filter Electronic Indicator from SGT displays real-time filter status, rather than traditional last-minute colour change indication. It has an audio-visual early warning system, and can be linked to a virtual indicator platform, or be configured to a preferred replacement schedule. It has three operating modes: Factory, manual, and virtual indicator. The factory mode is based on default replacement advice of 12 months and no configuration. The manual mode is reportedly useful for users with a replacement schedule in place that deviates from the standard manufacturer's replacement advice and want to use the device for updates on status. The virtual indicator mode is an advanced operation mode that calculates replacement-advice tailored to specific gas configuration and instrument usage.

www.virtualindicator.com

LC–MS instrument

Shimadzu has released the LCMS–8050 triple quadrupole LC–MS–MS system, which is reported to feature extremely fast data acquisition rates. The system is reported to provide attogram-level (10^{-18} g) sensitivity as the result of two features: The UFsweeper III collision cell which enhances collision-induced dissociation (CID) efficiency by optimizing the collision cell pressure; and a newly designed heated electrospray ionization (ESI) source which improves desolvation and enhances ionization efficiency by adding a heated gas combined with the nebulizer gas. The instrument's newly engineered high voltage power supply enables a maximum scan rate of 30,000 u/sec and a 5 msec polarity switching time. It is reported that 1000 events with up to 32 channels can be included for a maximum of 32,000 multiple reaction monitoring (MRMs) for each analysis. The "plug-in & play" ion source is reported to enable an easy transition from ESI to atmospheric-pressure chemical ionization (APCI) or to dual ionization mode (DUIS). The LabSolutions LCMS Version 5.60 software integrates high performance liquid chromatography (HPLC) and mass spectrometry (MS) control. An automated MRM optimization routine is reported to provide unattended optimization of multiple compounds.

www.shimadzu.eu

Complex biological sample analysis

The Thermo Scientific Orbitrap Fusion Tribrid LC–MS instrument was introduced in June 2013 for the analysis of complex biological samples. The instrument is reported to improve the results of tandem mass tag (TMT) experiments by increasing the depth and quality of data compared to previous tools. This can solve many throughput challenges by enabling mass spectrometer users to determine relative quantification of proteins in multiple samples simultaneously. The instrument combines three mass analyzers — quadrupole, Orbitrap, and linear ion trap — into a unique "tribrid" architecture. The three different mass analyzers are reported to work together to improve analytical performance and enable new experimental methods. The quadrupole mass analyzer selects precursors at isolation widths down to 0.4 amu for excellent sensitivity and selectivity. The ultrahigh-field Orbitrap offers resolution greater than 450,000 and scan rates up to 15 Hz. The ion routing multipole followed by dual-pressure linear ion trap is reported to provide MSn higher energy collision dissociation (HCD), collision-induced dissociation (CID), and electron-transfer dissociation (ETD) fragmentation, as well as fast, sensitive mass analysis with scan rates up to 20 Hz.

www.thermofisher.com

Size-exclusion chromatography

In 2013, Tosoh Bioscience presented a new high temperature gel permeation/size-exclusion chromatography (GPC/SEC) system to their SEC portfolio, the HLC-8321GPC/HT (HT EcoSEC System). It is reported to provide stable thermostatization up to 220 °C for up to eight columns, autosampler, pumps, and an extremely stable refractive index detector with an independent temperature control. The optional DF-8321 sample processing unit is able to process up to 24 samples at temperature programmes ranging from 40–220 °C. In addition, the TSKgel SW series of aqueous HP-SEC columns was expanded by three new ultrahigh-pressure liquid chromatography (UHPLC) columns tailored to different aspects of antibody analysis: According to the company the TSKgel SuperSW mAb HTP enables easy transfer of high performance liquid chromatography (HPLC) methods to fast UHPLC analysis; the TSKgel SuperSW mAb HR provides high resolution for the analysis of fragments, monomers, and aggregates in one run; and the TSKgel UltraSW Aggregate covers a range of higher molecular weights, ideal for separating antibody dimers and higher aggregates.

www.tosohbioscience.de

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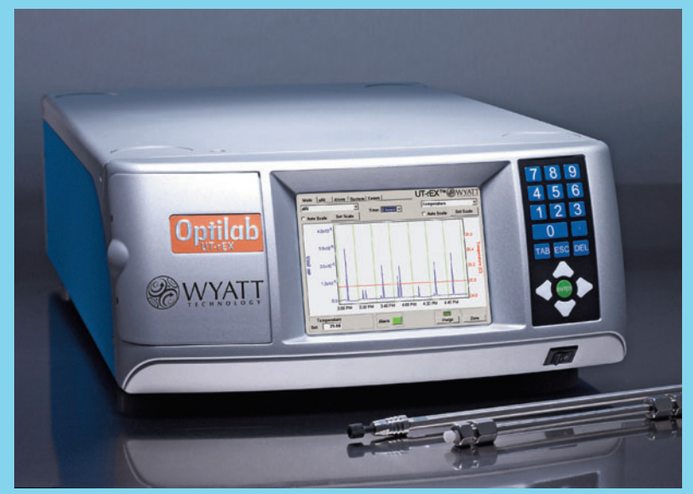
Mass detector for chromatography

This year, Waters introduced the Acquity QDa detector, which is reported to bring high-quality, mass spectral data to chromatographic separations. A synergistic component of a chromatographic system, it is a small and easy-to-use mass detector that brings mass spectrometry to chromatography in a way that gives analytical scientists access to mass spectral data regardless of their previous experience with mass spectrometry. The detector fully automates sample analysis and eliminates sample-specific adjustments for certainty in sample results. Robust and reliable enough for routine use, the detector generates the mass spectral data expected of a single quadrupole mass spectrometer in a mass detector no bigger than a familiar photodiode array (PDA) detector. By simplifying laboratory workflows, the detector is reported to increase productivity by eliminating the need to run additional assays or turn to time-consuming alternative techniques to establish the levels and identity of specific sample compounds.

www.waters.com/separate

UHPLC RI detector

The Optilab UT-rEX from Wyatt is a refractive index (RI) detector specifically designed for use with ultrahigh-pressure liquid chromatography (UHPLC) systems using columns packed with small beads. Using a combination of miniaturized components, semiconductor photodiode technology, and proprietary computer algorithms, the detector is reported to improve sensitivity, stability, and temperature range. As a universal detector, it can be operated either stand-alone — as the sole on-line detector downstream of a UHPLC column — or in combination with another detector, such as UV–vis absorption.



The temperature regulation capabilities of the detector are reported to allow control of the flow path temperature above and below ambient. The absolute refractive index measurement of the solution is reported to improve resolution, reduce sample and solvent usage, and save time. In addition, the detector is linear over a wide range and is sensitive to samples that are not UV-active, while the dead volume for the instrument is <1.5 µL. The small flow cell and proprietary temperature regulation enable stable RI baselines and RIS signals, further enhancing the detector's sensitivity.

www.wyatt.com

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PHARMACEUTICAL/DRUG DISCOVERY

Sophisticated Antibody Analysis by GPC/SEC with RALS

Monoclonal antibodies (mABs) are increasingly growing in importance for the diagnosis and therapy of various diseases, including cancer and autoimmune and inflammatory disorders. One essential parameter to define their quality is the content of aggregates (dimers, trimers, and higher aggregates). These aggregates can be formed during processing and purification or are the result of long-term storage. As a result of aggregation, antibodies lose their pharmaceutical efficacy and can facilitate an immunology response.

Antibody fragments which lack the Fc region can be used for the treatment of diseases. They can also be the result of degradation of full length antibodies. Therefore, a GPC method, which offers the opportunity to analyse antibodies and their aggregates, as well as antibody fragments simultaneously, with superior resolution and high sensitivity is invaluable.

Experimental

GPC/SEC analysis was performed on a PSS SECcurity GPC system, equipped with a PSS SECcurity SLD1000 light scattering detector, using the following conditions:

Columns:	PSS PROTEEMA, 5 µm, 2 × 300 Å (8 × 300 mm each) + precolumn
Solvent:	100 mM sodium phosphate pH 6.7 + 0.25 M NaCl
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	Refractive index (RI), ultraviolet (UV) at λ = 214 nm, PSS SLD1000 (right-angle light scattering [RALS]) at λ = 488 nm
Calibration:	Light scattering
Injected mass:	60–80 µg
Data acquisition, calibration, and evaluation:	PSS WinGPC UniChrom 8.1

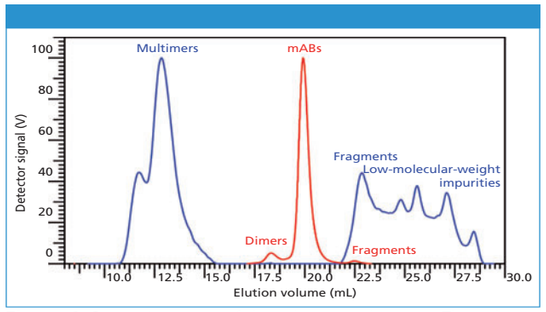

Figure 1: Separation range of the column combination. The red curve shows the UV signal of a full length antibody and its dimers plotted against the elution volume. The blue curve is the elugram of antibody fragments and their high level aggregates.

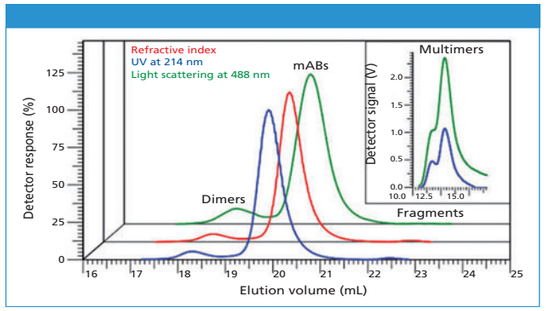

Figure 2: Sensitive analysis of antibody aggregates. The light scattering signal for the dimer is relatively high compared to that of the mABs because of molar mass dependency and provides improved sensitivity for the detection of high aggregates (inset).

Results

Figure 1 shows an overlay of elugrams obtained for a full length antibody and antibody fragments analysed on a single set of columns.

All three detector signals for the analysis of a monoclonal antibody are shown in Figure 2. The light scattering signal shows improved sensitivity for high aggregates compared to the other signals.

Conclusion

The GPC/SEC method including UV, RI, and RALS can be used for the simultaneous determination of aggregate content of monoclonal antibodies as well as antibody fragments. The column combination covers the separation range for all three types and provides a high resolution for the determination of the dimer content. Because of its molecular weight dependency, the PSS SLD1000 RALS detector offers high sensitivity for very small quantities of high aggregates and also allows the determination of the absolute molecular weight of the antibodies. In addition, it has a unique feature for a light scattering detector as the wavelength can be altered to increase the sensitivity.

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News

Designer Cannabinoids

The variance in the toxic effects of designer cannabis drugs marketed throughout Europe and the USA as "K2" or "Spice" could be the result of stereoselective metabolism of enantiomers by lung and liver enzymes, according to a study published in *Analytical Chemistry*. Chiral liquid chromatography–tandem mass spectrometry (LC–MS–MS) was applied to the analysis of JWH–018 and AM2201 metabolites in human urine. JWH–018 and AM2201 are metabolized by lung and liver enzymes to generate three primary chiral metabolites — (ω)-carboxyl, (ω)-monohydroxyl, and (ω-1)-monohydroxyl metabolites that have a high affinity for the human cannabinoid type-1 receptor (CB1R). Solid-phase extraction with chiral LC–MS–MS determined specific excretion patterns associated with both JWH–018 and AM2201 compound metabolism. The authors suggest the method could be used in future clinical studies. — A.L. Patton et al., *Anal. Chem.* **85**, 9390–9399 (2013).

Mummified Snacks

The ancient Egyptians embalmed meat mummies as an afterlife snack in the same way as their dead, according to the findings of a gas chromatography–mass spectrometry (GC–MS) analysis. The team from the University of Bristol (Bristol, UK) and the American University in Cairo (Cairo, Egypt) collaborated to analyze the chemical compositions of samples and tissues from meat mummies excavated by the Cairo Museum and the British Museum. Of particular interest, the team found evidence of the use of *Pistacia* resin on a beef rib mummy, a substance rarely even seen in the embalming of humans. — K.A. Clark, S. Ikram, and R.P. Evershed, *PNAS Early Edition,* DOI: 10.1073/pnas.1315160110 (2013).

Hijacking the Immune System

Staphylococcus aureus (*S. aureus*) evades clearance by the immune system by hijacking the very traps it produces, transforming these same traps into a toxic compound according to a paper published in *Science*.[1] Scientists from the University of Chicago (Chicago, USA) used high performance liquid chromatography coupled to mass spectrometry (HPLC–MS) to identify the toxin as 2'-deoxyadenosine (dAdo).

Bacterial invasion of tissue triggers an array of processes as part of the immune response, in an attempt to first contain and then destroy an infection. *S. aureus* can permanently colonize the human body without causing harmful effects, usually on the skin and within the nose causing boils or abscesses. However, if it enters the blood stream it can result in blood poisoning, endocarditis, and meningitis among other conditions.

S. aureus tissue infections are characterized by abscesses formed by immune cells cornering off the infection for clearance by a specific type of cell known as a macrophage — so-called "big eaters" that roam the body clearing away infections. The traps are released by neutrophils (neutrophil extraceullar traps [NETs]) in an attempt to contain the infection until the arrival of the macrophage. In the case of *S. aureus* however, there is no macrophage activity and the infection remains uncleared.

To investigate this mystery, *S. aureus* was co-cultured alongside immune cells, including neutrophils and macrophages. When NET production was activated, the macrophage began to die suggesting the production of a toxin by the bacterium. The team performed HPLC–MS to identify this toxin as 2'-deoxyadenosine (dAdo). Combining these results with data from genetic experiments using mutant strains of *S. aureus*, the team deduced that the bacterium converted NETs to dAdo that is toxic to macrophages.

Olaf Schneewind, senior author of the paper, said: "These bacteria have endowed themselves with weapons to not only anticipate every immune defense, but turn these immune defenses against the host as well." He added: "Sooner or later almost every human gets some form of *S. aureus* infection. Our work describes for the first time the mechanism that these bacteria use to exclude macrophages from infected sites."

So what does this mean for drug development? Theoretically, scientists could target this mechanism of evasion using therapeutic proteins to block bacterial enzymes involved in the transformation of NETs by *S. aureus*. This is promising but requires a lot more work. — B.D.

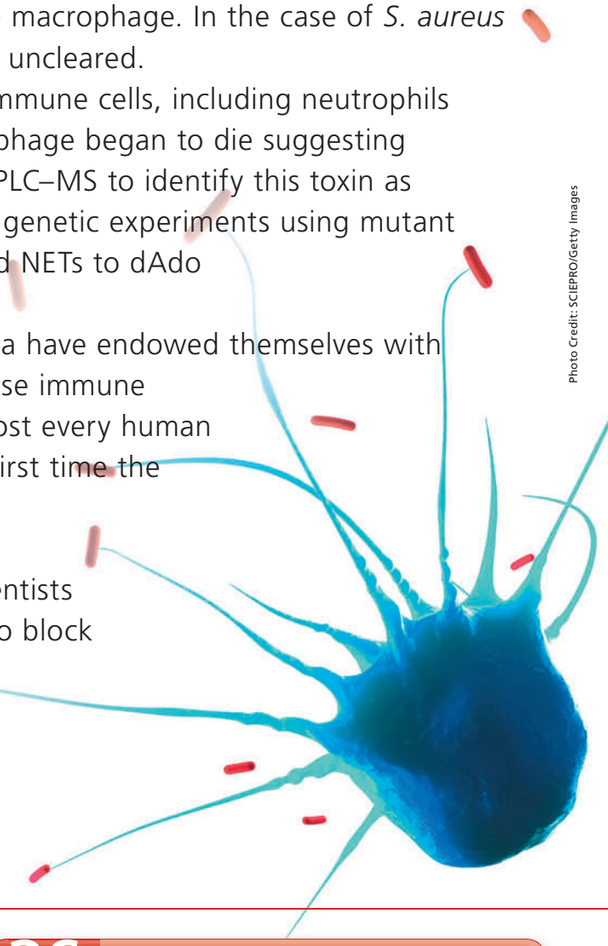

Photo Credit: SCIEPRO/Getty Images

Reference

1. V. Thammavongsa et al., *Science* **342**, 863–866 (2013).

Gulf of Mexico Research Initiative Grant Competition

The Gulf of Mexico Research Initiative (GoMRI) has announced plans to invest $105 million between 2015 and 2017 to support research consortia investigating the effect and impacts of hydrocarbon release into the environment. The funding will be awarded to four or more institutions over two years, under the programme known as RFP–IV.

gulfresearchinitiative.org

Biosimilar Characterization Laboratory Opens in Austria

The Christian Doppler Laboratory for Biosimilar Characterization has opened at the Paris-Lodron University of Salzburg (Salzburg, Austria). The laboratory was established to develop and transfer to practice novel and more efficient methods to characterize the active ingredients in protein-based medicines. Scientists from the University of Salzburg, Sandoz Pharmaceuticals, and Thermo Fisher Scientific are collaborating in these efforts.

www.thermoscientific.com

PerkinElmer Opens Center for Innovation in Massachusetts, USA

PerkinElmer has opened a new Center for Innovation in Hopkinton, Massachusetts, USA. The facility features laboratory space where engineers, scientists, and customers can meet and discuss developments to benefit the life sciences market.

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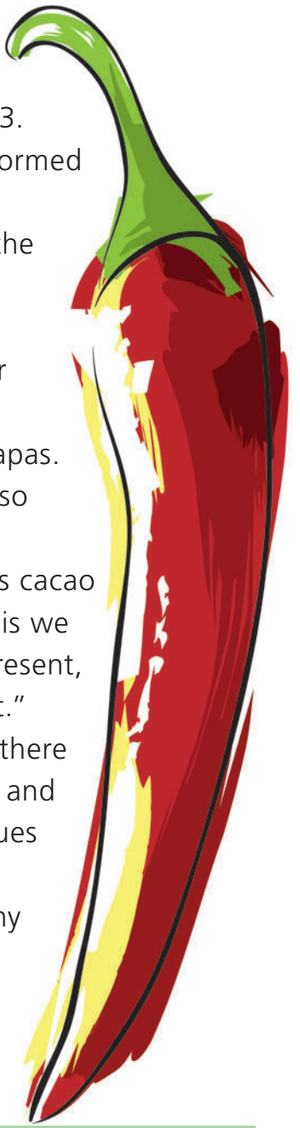
Early Use of Chili in Mexico



Residues of chili pepper (*Capsicum*) have been found on pottery dating back 2000 years in southern Mexico, according to a study published in *PLOS ONE*.[1] The New World Archeological Foundation (NWAF) first excavated the pottery (thought to date from 400 BCE–300 CE) from Chiapa de Corzo between 1955 and 1963.

Ultrahigh pressure liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS–MS) was performed on residues collected from the pottery. Extracts were collected by lightly scraping the interior of 13 pieces of pottery with sandpaper, which were subsequently analyzed. *Capsicum* residues were identified in five out of the 13 pieces.

There has long been a connection between the fields of analytical chemistry and archeology leading to the development of molecular archeology, specifically when investigating the diet of ancient peoples. Lead author Terry Powis told *The Column*: "I was asked by a Mexican colleague, Emiliano Gallaga, one of my co-authors, about two years ago to look for cacao in pottery vessels from the site of Chiapa de Corzo in the State of Chiapas. Although our focus was primarily to look for cacao in 2000-year-old pottery vessels from the site, we were also interested in looking for any additives or flavourings that may have been included in these particular drinks."

The discovery of chili residues was an unexpected one, according to Powis. The vessels had been selected as cacao residues had been identified in similar artefacts in other areas. He said: "During the mass spectroscopy analysis we were completely surprised at the fact that no cacao was present in any of the pots tested. In fact, chili was present, although we have no way of knowing at present whether the amount is strong or weak, just that it is present."

As an archeologist, the primary goal of Powis was to identify the exact species of chili pepper used but, as there are five different species, they were not able to determine this. He told *The Column* that this is a future path, and that they are attempting to conduct DNA analysis of *Theobroma cacoa* on archeological samples to link residues to different modern species now in existence in different areas of the New World.

When discussing the importance of the work, Powis said: "Because of our findings we can now pursue many different cultural questions about how the Mixe-Zoque people used chili peppers in their daily social lives. We will also be able to move beyond its culinary use (but we still have a way to go) to include how they used it in pharmaceutical and ritual contexts as well." — B.D.

Reference

1. T.G. Powis, E.M. Gallaga, R. Lesure, R. Lopez Bravo, L. Grivetti, et al, *PLoS ONE* **8**(11), e79013 (2013).

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Photo Credit: jkir/Getty Images

Peaks of the Week

We have a rich range of informative and practical content across our print and digital portfolio that we tweet, share, and open for discussion. Get on-line today and join the discussions! Some of the highlights:

- **What's the Problem with the LLOQ? — A Case Study:** Liked and shared by Twitter and Facebook followers. bit.ly/1b5PjUc
- **John Dolan: Dr. Troubleshooting:** "John Dolan column in *LCGC* is a read reference for all users of liquid chromatography. John is a true master in this regard. In this 30th anniversary of this column I hope he can continue to contribute for many years to the resolution of LC troubleshooting." — bit.ly/17DPZKp
- **Professor Barry L. Karger: Scientist, Mentor, and Innovator: In graduate school, do you think that your thesis advisor should also be your mentor?** "No. If you know why you are going to graduate school, you can focus on your studies & your research..." "While I have not been in graduate school since the previous millennium I think lack of mentorship is the biggest failing for US post-graduate education. But what faculty member is qualified to mentor advanced degree chemists for anything other than academic positions? Most graduates move into industrial positions and a career academic has little of first-hand practical utility to share. — Comments from LinkedIn. bit.ly/Ic7c7k

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Editors' Series: Fundamentals, Optimization, and Practical Aspects of UHPLC: Series 1: Fundamentals, Optimization, and Practical Aspects of UHPLC
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- **An Insight into Analytical Quality by Design in the Pharmaceutical Industry:** bit.ly/15Dp8fa

Interviews

News In Brief

Studies of Autism
Chromatographic methods are used in studies of autism to identify novel diagnostic biomarkers. Zurawicz and colleagues have reviewed the methods available in the journal *Biomedical Chromatography*, presenting a theoretical introduction as well as example applications. **DOI: 10.1002/bmc.2911**

Graphene Oxide Adsorbent
Graphene oxide (GO) has been proposed as an effective adsorbent of aflotoxins prior to HPLC analysis. The different parameters affecting extraction were investigated, and the method was applied to the determination of aflotoxin levels in peanuts. **DOI:1016/j.chroma.2013.10.006**

Plastic Pollution
A study published in the journal *Scientific Reports* suggests that chemicals from plastic debris could impact fish health. Microscopic plastic debris found worldwide can be ingested and bioaccumulated by fish resulting in liver toxicity. **DOI: 10.1038/srep03263**

Capillary LC and Nano LC in Food
Luigi Mondello and colleagues have reviewed and compared the general principles of two miniaturized techniques, capillary LC and nano LC, to conventional LC in relation to food analysis. **DOI: 10.1016/j.trac.2013.05.021**

Tips & Tricks GPC/SEC: Select the Right Columns for Your Molar Mass Range

Daniela Held, PSS Polymer Standards Service GmbH, Mainz, Germany.

Shoulders in gel permeation/size-exclusion chromatograms (GPC/SEC) can be a result of sample characteristics or down to the wrong choice of columns or column combinations. Proper selection helps to measure true results.

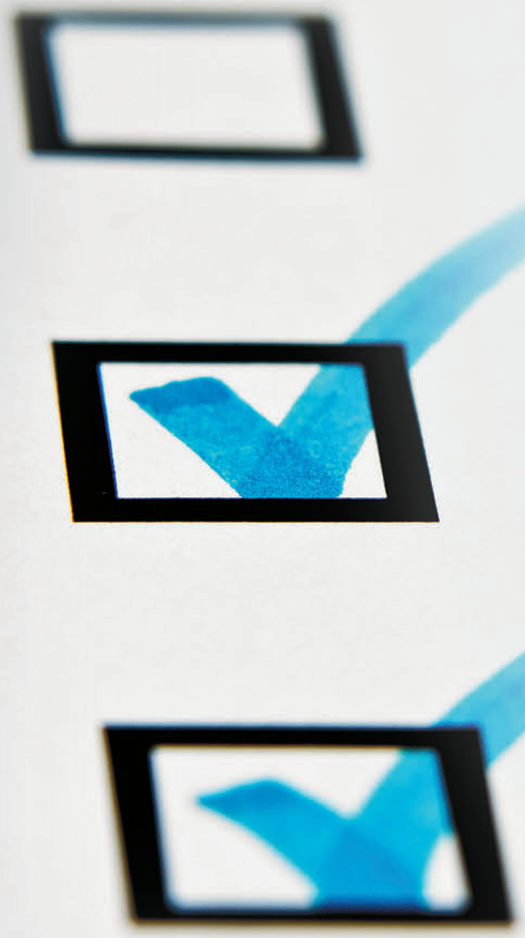


Photo Credit: Daniel Kulinski/Getty Images

Resolution, molar mass separation range, solvent consumption, and analysis time are key factors that have to be balanced when performing gel permeation chromatography/size-exclusion chromatography (GPC/SEC). Depending on the application and environment, whether routine quality control (QC) or research and development (R&D), one of these parameters can be dominant and will affect GPC/SEC column selection.

The concept of specific resolution has been developed for GPC/SEC to understand and compare resolution and to determine the influence of the molar mass accuracy.[1] Inspection of the calibration curve of a GPC/SEC column can give a good indication of resolution. The slope of the calibration curve directly indicates resolution; the flatter the slope, the higher the resolution.[2]

Good resolution (a flat slope) can only be achieved for a limited molar mass separation range when one column is used, which is acceptable for protein (or antibody) and oligomer analysis. However, when analyzing synthetic polymers or natural macromolecules with a broad molar mass distribution, an efficient separation over a large molar mass separation range is required. For this, columns of the same dimensions and particle size (but different porosity) are often coupled in series. This is a very successful approach but has some potential pitfalls such as mismatch, which can lead to artifacts in chromatograms and the resulting molar mass distributions.

Column Types

In general, linear, mixed-bed, or multipore columns have a broad pore size distribution and cover a wide molar mass range with a constant resolution. Single porosity columns with a narrow pore size distribution cover a smaller molar mass separation range but with a high resolution. Resolution can be increased for all types of columns by adding a column of the same type and porosity. The separation range of single porosity columns can be expanded by adding a column of the same type with different, correctly matched porosity. The differences between linear, mixed-bed, multipore columns, and single porosity columns was covered in detail in a past instalment of Tips & Tricks.[3]

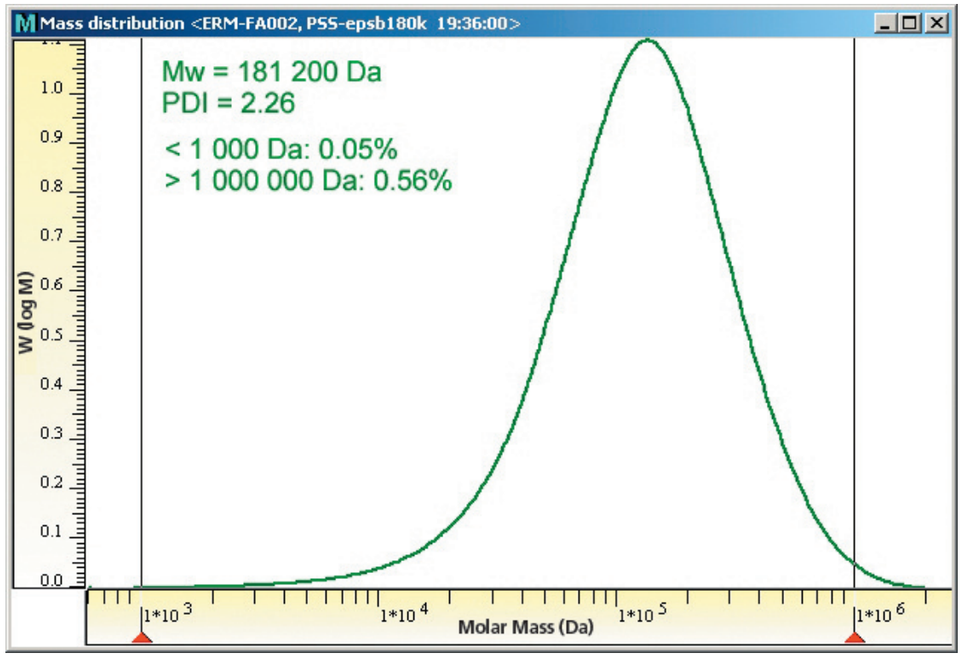
Selecting the Upper Molar Mass Separation Limit

When selecting a column or combination of columns for GPC/SEC, users often only know the approximate average molar mass (average weight molecular [M_W]). However, wrong column choices can be made if the breadth of the molar mass distribution (related to the polydispersity index [PDI]) of the sample is not considered.

For example: If a typical sample PDI value for synthetic polymers, produced by free-radical polymerization, is two, the molar mass range covered can be very broad. In this case, users should select an upper molar mass limit that is 10× higher than the M_W of the highest samples to be analyzed. Figure 1 shows the molar mass distribution for a European reference material, a poly(styrene) of M_W = 181,200 Da and PDI = 2.26. Although the average molar mass is relatively low for this polymer, molar masses up to 2 million Dalton are present.

Figure 2 shows an example of when a fraction of the sample molar masses is higher than the exclusion limit (the upper molar mass separation limit). It shows the refractive index (RI) detector signal (green) overlaid with the corresponding

Figure 1: Many synthetic and natural macromolecules have a broad molar mass distribution and therefore a higher polydispersity index (PDI). This example shows the molar mass range covered for an poly(styrene) European reference material with a PDI around two and a medium-molecular-weight.



calibration curve (red). The two red dots mark the highest and lowest molar mass of the calibration standards.

An artificial shoulder appears in the chromatogram at approximately 6 mL elution volume when the exclusion limit has been reached. This is not related to sample characteristics, but is the result of the resolution change indicated by the change of the slope of the calibration curve.

This problem can be solved by adding a second, correctly matched column with a higher exclusion limit than the first column.

Selecting the Right Combination

It is not possible to combine linear or mixed bed columns and single porosity columns in all combinations — if the porosities and the calibration curve slopes do not match for each of the columns combined, chromatographic artifacts (such as shoulders) can arise giving erroneous results.

Figure 2: An upper molar mass separation limit that is too low can lead to artificial shoulders in the chromatogram. Choosing the proper separation range helps to solve this problem.

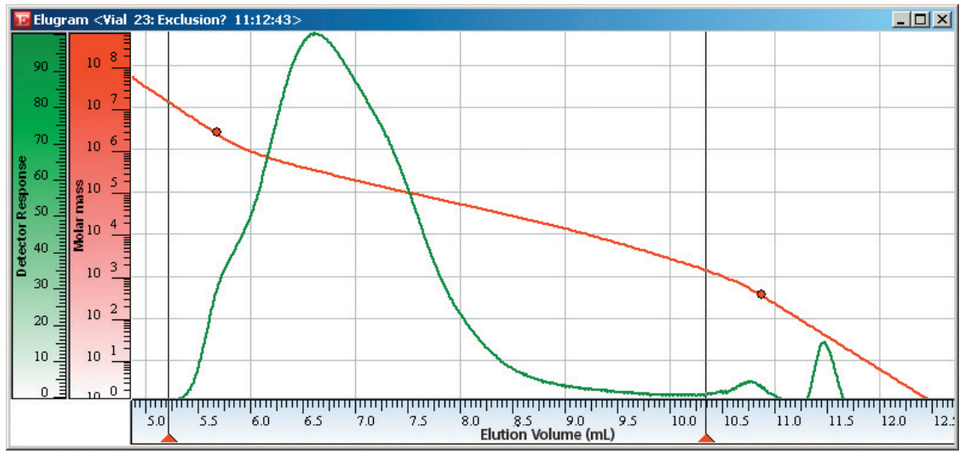


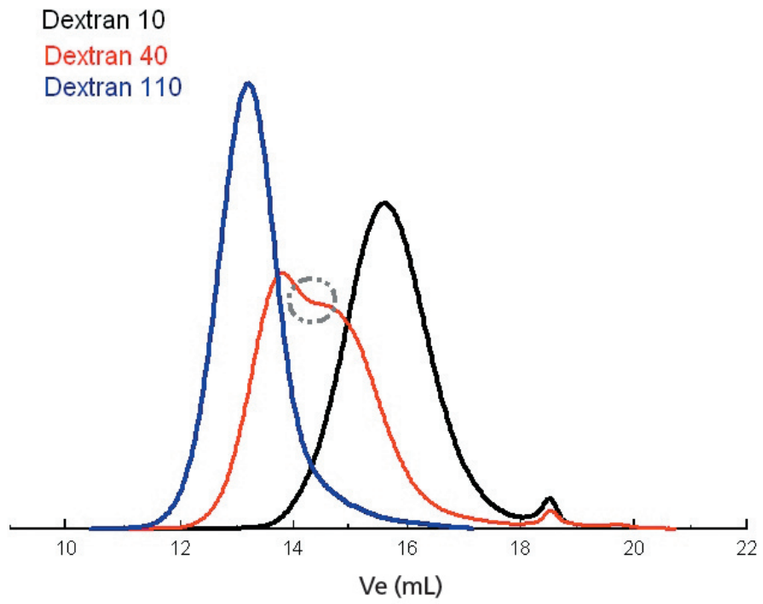
Figure 3 shows an analysis of three different Dextran samples on two analytical GPC/SEC columns with different porosities. One of the samples, Dextran 40 (red), seems to be bimodal as a shoulder is present. However, this shoulder is not part of the sample and cannot be seen on a single column. This shoulder is a result of a mismatch of the porosities.

Subsequent calculation of the raw data erroneously creates a shoulder in the molar mass distribution of this sample. Furthermore, small changes in the column manufacturing process can shift or eliminate the shoulder making it appear to be a real property of the sample. Long-term reproducible results based on such a setup are therefore extremely hard to achieve.

Porosity mismatch is not always readily visible in the calibration curve. Figure 4 shows a calibration curve where a very small porosity and a very large porosity column have been combined. The resulting calibration curve has two regions with very different slopes and an inflection point can be identified. This combination would definitely produce chromatographic artifacts.

On the other hand, the calibration curve can look as would be expected but there could still be a mismatch problem. Such a mismatch can only be detected by probing the region of the inflection point using reference materials with a broad molar mass distribution.[3]

Figure 3: Chromatograms of three Dextrans with broad molar mass distributions and different molar mass averages. The red trace seems to be bimodal, but this is a chromatographic artifact resultant of porosity mismatch of the two combined columns.



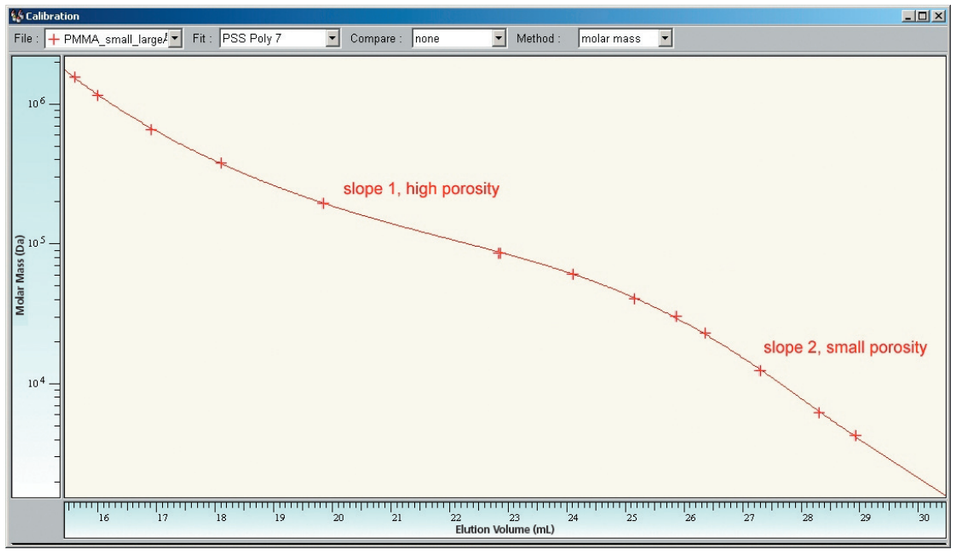
Up to now, it is not possible to overcome these problems with advanced calibration data fitting even if absolute detection, such as on-line laser light scattering, is used. Molar mass distributions can still show artificial shoulders if the porosities do not match. A better solution is to optimize the separation by adding correctly matched columns.

Summary

There are some general rules that are more or less true for all columns, independent of the manufacturer:

- Ensure that the upper molar mass separation limit is sufficient taking the PDI of your samples in account. Verify that all parts of the sample elute in a region with sufficient separation by overlaying the calibration curve with the sample.
- Avoid combining linear columns of different types; linear columns with single porosity columns (for example, linear + 100 Å); and single porosity columns with non-matching porosities — or only do so following the manufacturer's recommendation or by doing careful mismatch tests.

Figure 4: Calibration curve of two columns with non-matching porosities. The curve has two different slopes resulting in erroneously chromatograms with shoulders.



- Investigate unexpected shoulders carefully. Review the calibration curve with respect to changes in slope and use reference materials with a broad molar mass distribution to detect hidden porosity mismatch. Please note that porosity mismatch can even occur within one column, for example, if the manufacturer blends materials in a linear or mixed column to cover a wider molar mass range.

In conclusion, applying these three basic rules will help to establish GPC/SEC methods that can be used over a long time period with high reproducibility.

References

1. W.W. Yau, J.J. Kirkland, D.D. Bly, and H.J. Stoklosa, *Journal of Chromotography A* **125**(1), 219–230 (1976).
2. D. Held, *The Column* **7**(2), 14–16 (2011).
3. T. Hofe, *The Column* **4**(4), 20–23 (2008).

Daniela Held studied polymer chemistry in Mainz (Germany) and is working in the PSS software and instrument department. She is also responsible for education and customer training.

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Website: **www.pss-polymer.com**

Q&A: Miniaturizing Military Detectors

Scientists at Sandia National Laboratories are thinking smaller in the development of detectors for military use — from the detection of explosives and chemical weapons to humans. Scientist Ron Manginell from the laboratories spoke to Bethany Degg of *The Column* about the on-going research in this area.

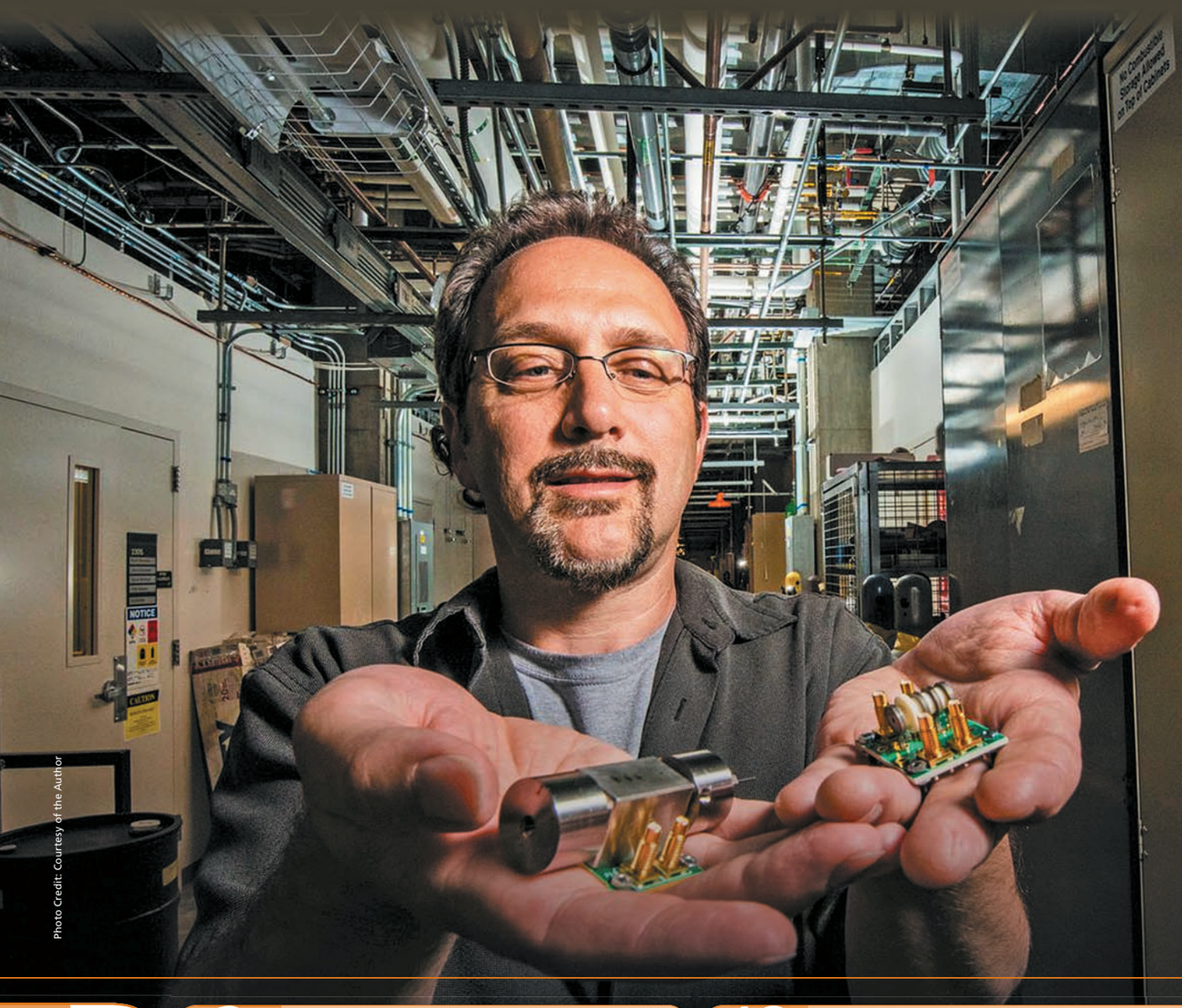

Photo Credit: Courtesy of the Author

Q. You work for the Microsystems-Enabled Detection Department at Sandia National Laboratories, part of a network of US National Security Laboratories — what is the purpose of this laboratory?

A: Sandia's primary missions are in nuclear weapons and national security.

Q. What are the main research interests of your department?

A: My department is primarily concerned with developing miniature, portable detection systems for chemical, biological, and physical detection. Our main thrust for many years has been developing and "fielding" handheld chemical detection systems for chemical agents and industrial uses.

Q. How did you become an inventor working for Sandia?

A: I began working at Sandia as a graduate student intern, then as a postdoctoral appointee, and finally as a full-time employee. The work in our area at Sandia has always been centred on research and development, with a strong emphasis on new inventions.

Q. Sandia released details of MicroChem Lab — a detector for the military — in the late 1990s. What was unique about the technology that you developed?

A: The MicroChemLab system was very unique in its system approach. While many groups have developed interesting detectors, the MicroChemLab miniaturized laboratory system approaches to chemical detection. This includes inventing the world's first microfabricated preconcentrators, implementing the first miniature gas chromatographs, and sensitive microdetectors. Each of these elements had chemical selectivity built in, allowing sensitive field analysis to take place in minutes, rather than hours, and usability by lay persons.

Q. What are the applications of the detector now?

A: The system is still used for detecting chemical agents, but other targets have been added over time, including toxic industrial chemicals, pharmaceutical solvents, natural gas, and so on. Recently, our development of a miniature pulsed-discharge ionization detector (mini-PDID) has allowed us to detect a much broader range of compounds, including unique volatiles emitted by bacteria.

Q. Why is gas chromatography (GC) your method of choice when developing sensors for the detection of VOCs?

A: Even the best detectors, such as the gold-standard laboratory-grade mass spectrometers, utilize GC. GC separates out a mixture of chemicals so that they are presented to a detector, like the mass spectrometer, in a time series. This simplifies the job of the detector, as it only has to detect one compound at a time if the GC separation is good. Our systems use GC for the same reason, to simplify the detector's job. In addition, the time at which compounds exit a GC can be used in their identification. Many groups claim to have the latest and greatest detectors. But like gold-standard MS, if they do not have a separation column in front of them, they are very likely to be confused in the real world.

Q. Your team is currently working on the development of a miniature pulsed-discharge ionization detector (mini-PDID). What are the key features of this detector?

A: This detector is small, and has the ability to detect organic compounds at parts-per-billion levels or lower. It generates intense ultraviolet (UV) light internally that can ionize any chemical except neon and helium. This opens up a very broad range of chemical analyses. The ionization energy can also be tuned downwards if desired to create chemical selectivity based on chemical ionization potential, or the willingness for a chemical to give up an electron and be ionized. Detection is electronic, meaning it is fast and sensitive.

Q. What are the advantages of your prototype technology compared with current "e-nose" technology?

A: The bare response of our detectors are at least a factor of 10 better than typical e-nose systems, but probably a factor of 100 in many cases. Further, we can detect not only patterns of response, as e-noses do, but also specific chemicals, thereby imparting greater chemical specificity.

Q. What are the current applications that you have tested? Could it be used in the field to test for the use of chemical weapons?

A: Absolutely. That was the original intent and the chemical target for which most of our test data was obtained.

Q. What further developments are required in order for it to be put into action? Where do you see your future research taking you?

A: Our research now is focused on implementing our miniature GC-based systems with detectors like the mini-PDID and miniature MS. We are looking at these for a variety of chemicals, including those produced by bacteria in their metabolism.

Dr Ron Manginell is a principal member of the technical staff in the MicroSystems-Enabled Detection Department at Sandia National Laboratories. He has worked at Sandia since 1995 on microsensors, microfabrication, and microanalytical systems and has over 20 patents. In the last 15 years he has focused on microanalytical systems for the detection of chemical agents, explosives, toxic chemicals, and biological compounds. Ron has also developed microfluidics and control systems for biological applications, including cell, antibody, DNA and protein capture, and sensing systems. In 1997 Ron received a PhD in physics with honours from The University of New Mexico (Albuquerque, New Mexico, USA) for research performed at Sandia National Laboratories; he received a B.S. degree in physics with honours from the Rochester Institute of Technology (Rochester, New York, USA) in 1991.

To reach Ron Manginell contact Sue Holmes, Sandia Media and Communications, at sholmes@sandia.gov

Diisobutyl Columns Reduce Solvent Consumption and Offer Rapid HPLC–MS Analysis of Plasma Oxycodone and its Metabolites

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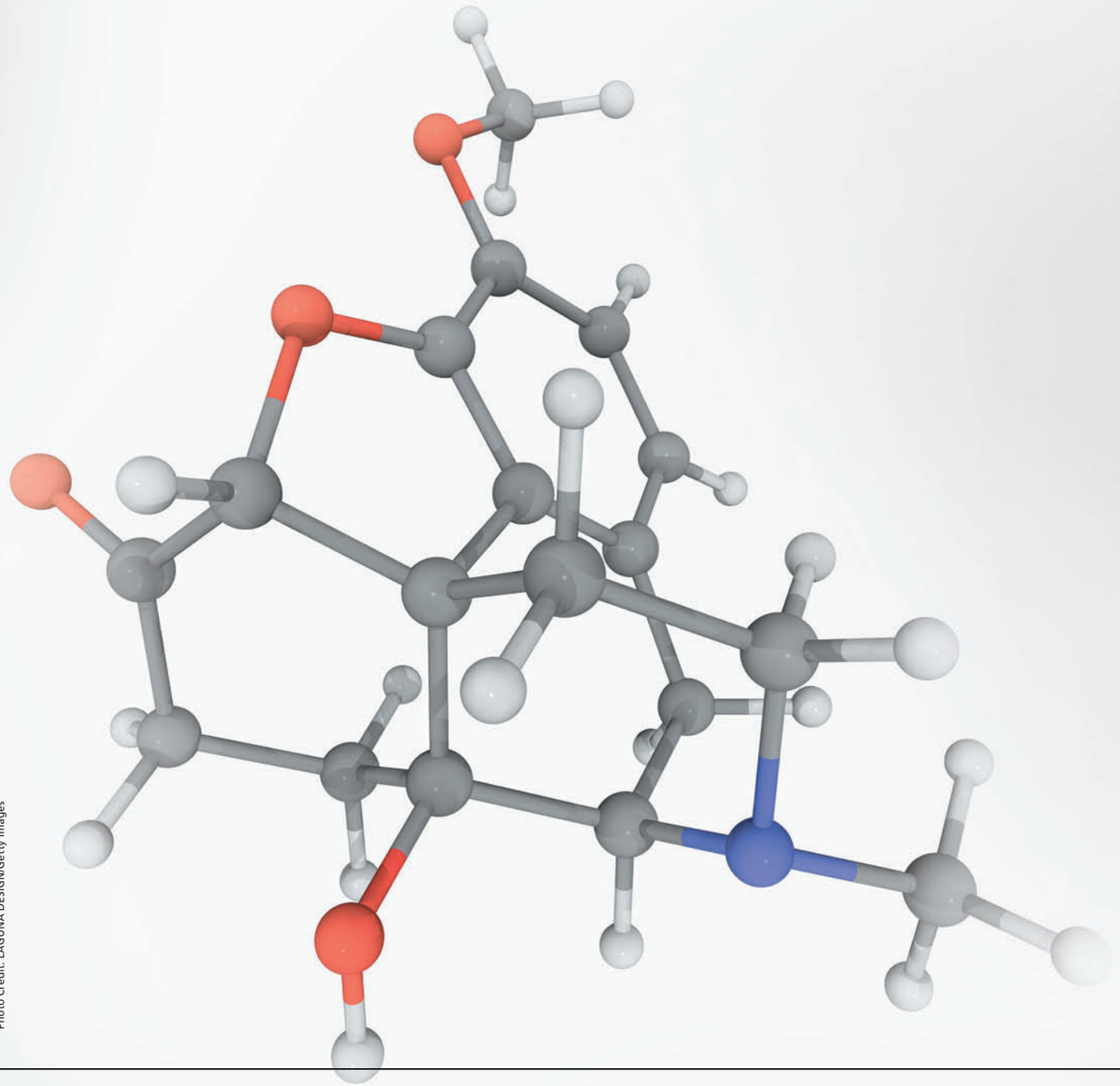

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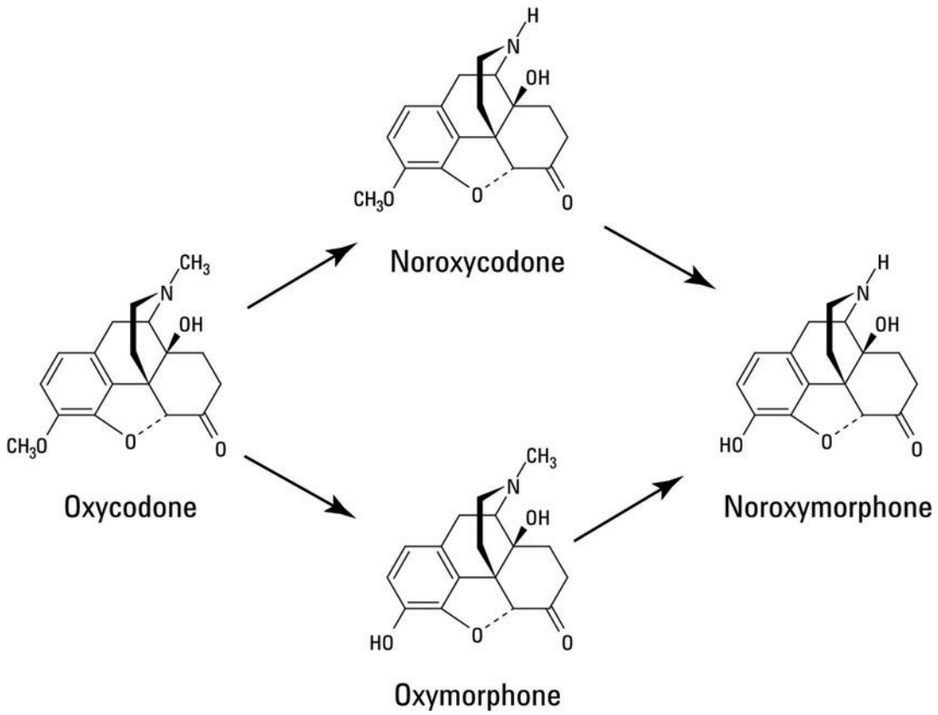
Oxycodone and its metabolites (noroxycodone, oxymorphone, and noroxymorphone) were analyzed by high performance liquid chromatography–mass spectrometry (HPLC–MS) using a diisobutyl stable bond column. Opioid compounds were isolated from human plasma samples using solid-phase extraction (SPE) prior to HPLC–MS. The proposed method is highly linear (R^2 >0.9900) and reproducible (<10% difference between duplicates) for all compounds; and also reduces analytical turnaround time and reduces solvent consumption.

Oxycodone is a semi-synthetic opioid synthesized from poppy-derived thebaine. It was developed in Germany in 1916 to replace the only narcotic analgesic available at the time: Diacetylmorphine (heroin).[1] Today, oxycodone is a schedule II drug in the USA[2] and a class A drug in the UK[3] indicated for the relief of moderate to severe pain. Although it has proven medical uses, the high abuse potential of oxycodone led to the reformulation of extended release formulations in an attempt to reduce abuse through non-oral routes.[4]

Oxycodone is metabolized by the cytochrome P450 enzyme system (CYP2D6 and CYP3A4 isoforms) to noroxycodone, oxymorphone, and noroxymorphone (a secondary metabolite).[5] Figure 1 shows the metabolic scheme of oxycodone. Oxymorphone is approved for use as an analgesic in its own right, but like oxycodone, it was also reformulated in 2011 owing to high rates of abuse.[6,7] Substance abuse of drugs such as oxycodone and oxymorphone makes accurate identification and quantification of these compounds and their multiple metabolites a clinical priority.

In recent years, high performance liquid chromatography–mass spectrometry (HPLC–MS) (or LC–MS–MS) has become increasingly important in the field of clinical and forensic toxicology. It has enabled the analysis of aqueous matrices, as well as hydrophilic, thermolabile, and non-volatile analytes that were not sufficiently covered by the established gold standard technique

Figure 1: Oxycodone and its metabolic products.

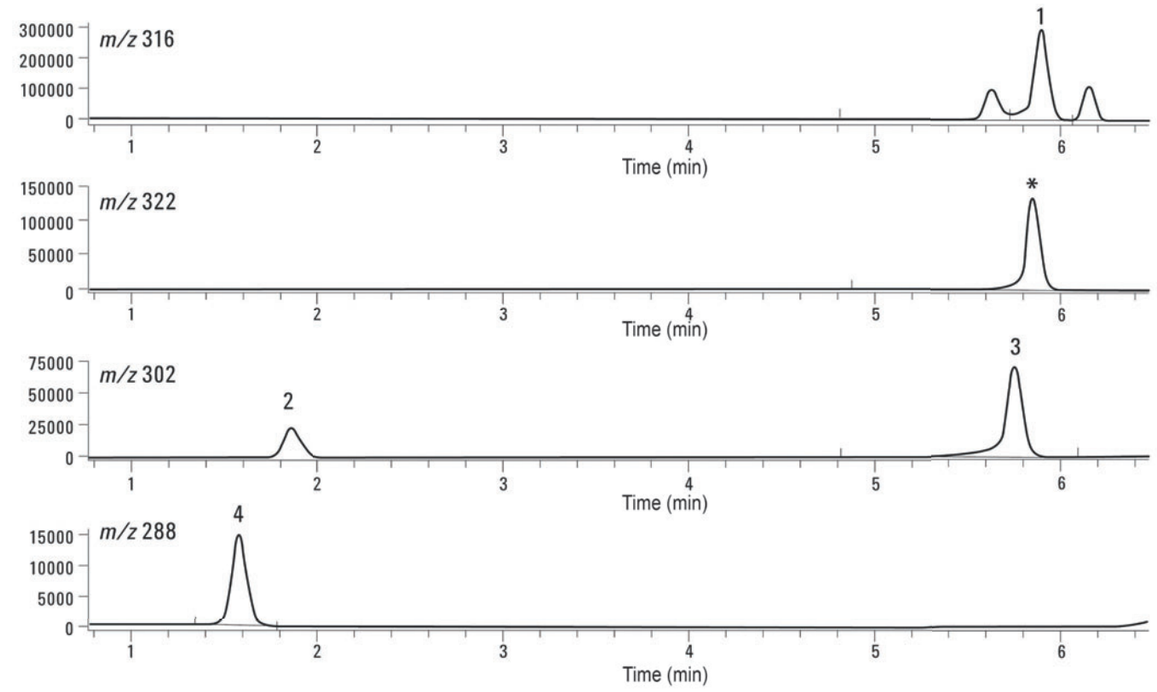


gas chromatography–mass spectrometry (GC–MS).[8]

Multi-analyte procedures that allow simultaneous analysis of multiple drugs from a drug class, or several closely related drug classes, are often used in clinical and forensic toxicology. These procedures help to save time, limit the number of methods that need to be established for a broad spectrum of analytes to be covered, and reduce the resources required during method development.[8] However, complex samples such as human plasma usually require preparation prior to injection onto an HPLC column. In spite of clean-up with techniques such as SPE, matrix effects can either reduce analyte response or enhance it. Both can compromise quantification accuracy and ion suppression can lead to false negative results.[8]

Chromatographic column and stationary phase selection can help to overcome the challenges of multi-residue analysis in complex matrices. For example, HPLC using stationary phases with sub-2 µm particle sizes can enhance separation power and reduce run

Figure 2: Extracted ion chromatogram of a human plasma sample spiked with oxycodone, noroxycodone, d6-oxycodone.



time; mixed mode column sorbents may offer a balance between extraction of as many analytes as possible while also minimizing sample matrix interference.[8] This study describes the successful application of a reversed phase HPLC using a stable bond column for separation of oxycodone and its metabolites from human plasma extracts prior to MS identification.

Methods

Reagents: Acetonitrile, ammonium acetate, methanol, methylene chloride, isopropanol, and ammonium hydroxide were purchased (Fisher Scientific) and Boric acid (JT Baker). Standard solutions of oxycodone, noroxycodone, oxymorphone (all 1 mg/mL), and noroxymorphone (0.1 mg/mL) in methanol (Cerilliant) were used.

A pooled internal standard was prepared by mixing 25 µL aliquots of oxycodone, noroxycodone, and noroxymorphone with 2.5 µL of oxymorphone and 25 mL methanol.

Sample Preparation: Matrix samples were prepared by spiking 1 mL of clean human

plasma with different concentrations of the pooled internal standard.

Mixed-mode SPE extraction of metabolites is summarized as follows:

- Sorbent material: Octyl (C8) and benzenesulphonic acid (SCX).
- Pre-condition: 2 mL methanol, then 2 mL deionized (DI) water.
- Load: 1 mL spiked plasma sample + 1.5 mL borate buffer (pH 8.9).
- Wash: 2 mL DI, 1 mL 10 mM ammonium acetate (pH 4), 2 mL methanol.
- Elute: 3 mL methylene chloride/isopropanol/ammonium hydroxide (80:20:2).
- Samples were then air-dried at 60 °C and the remaining pellets reconstituted in 60 µL 10 mM ammonium acetate (pH 4)/acetonitrile (95:5).

HPLC–MS Analysis: HPLC separation was performed on an 1100 series HPLC system (Agilent); Chromatographic column: 2.1 mm × 50 mm, 1.8-µm Zorbax Narrow Bore RRHT StableBond SB-C18 (Agilent); Mobile phase A: 20mM ammonium acetate, pH 4.0; mobile phase B: Acetonitrile. Mobile phase flow rate: 0.300mL/min; Gradient: Hold 5% B: 2.33 min. Increase B from 5–20%: 2.33–4.33 min. Stop: 6 min. Post time: 4 min; Column compartment temperature: 30 °C; MS identification: Electrospray ionization; Polarity: Positive; Spray chamber gas temperature and flow rate: 350 °C at 12 L/min.

Results and Discussion

This study describes the successful chromatographic separation of oxycodone and its metabolites in human plasma using reversed phase HPLC on a stable bond column, followed by MS identification. Rapid, multi-compound analysis within a single analytical run is highly valuable in the forensic determination of opioid compounds in human plasma. Optimal chromatographic separation of compounds in a complex sample matrix such as human plasma will depend on column and stationary phase selection. Compared with end-capped stationary phases, non-end capped stationary phase allow chromatographic separation of a mixture of compounds with varying polarity because of additional interactions with exposed silanol groups. These interactions can be optimized by altering mobile phase conditions. The small 1.8-µm particle size allowed for superior resolution and efficiency over 3.5-µm or 5-µm particles. In addition, use of a short column length allowed a lowered analytical turnaround time, while the small internal diameter reduced solvent consumption.

Figure 2 shows the extracted ion chromatograms (EIC) of a human plasma sample spiked with 50 ng/mL each of oxycodone and noroxycodone, 5 ng/mL noroxymoprhone and 40 ng/mL d6-oxycodone (an internal standard) and then extracted by SPE. Despite plasma being a complex sample matrix, chromatograms were well resolved for each of the five compounds.

This method employed demonstrated high linearity, sensitivity, and reproducibility. Calibration curve coefficients of determination (r^2) were ≥0.99000 over the concentration range of 2–50 ng/mL for oxycodone and noroxycodone, and 0.2–5 ng/mL for oxymorphone and noroxymorphone. Limits of detection were: 0.5 ng/mL for oxycodone, 1 ng/nL for noroxycodone, and 0.2 ng/mL for both oxymorphone and noroxymorphone. Inter-sample variability of the method was low, with less than 10% difference between duplicate samples over the aforementioned concentration range for limits of detection.

Conclusion

Oxycodone and its metabolites were successfully extracted from human plasma, then separated using reversed-phase HPLC prior to MS identification over a linear concentration range. The separation technique employed allowed for rapid analytical turnaround, clear chromatographic peak resolution, and reduced solvent consumption This method demonstrated good linearity, sensitivity, and reproducibility. These advantages may make the method useful for routine multi-compound forensic analysis of opioids in human plasma.

In addition, such systems may aid development of optimized drug formulations and help to monitor compliance with pain medication where urine analysis is not possible.

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A Mixed-Mode Stationary Phase for the Structural Analysis of Labelled *N*-Glycans Using LC–MS–MS

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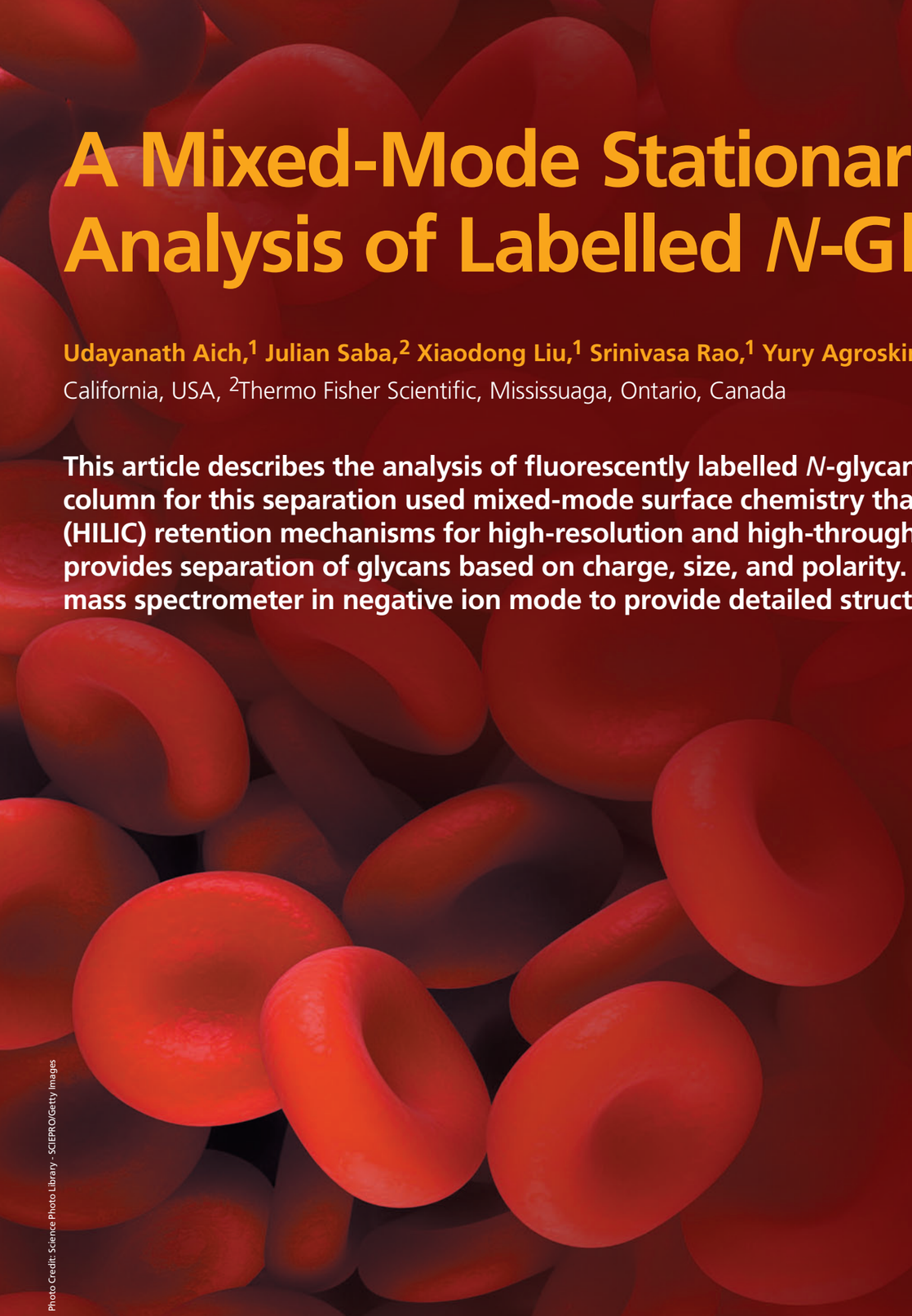
This article describes the analysis of fluorescently labelled *N*-glycans released from proteins by liquid chromatography–mass spectrometry (LC–MS). The column for this separation used mixed-mode surface chemistry that combines weak anion-exchange and hydrophilic interaction liquid chromatography (HILIC) retention mechanisms for high-resolution and high-throughput analysis of glycans. This combination of modes offers unique selectivity that provides separation of glycans based on charge, size, and polarity. MS and MS–MS analyses were performed using a hybrid quadrupole-orbitrap-based mass spectrometer in negative ion mode to provide detailed structural information of *N*-glycans released from proteins.



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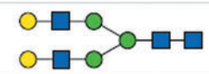
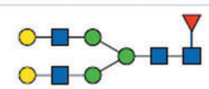
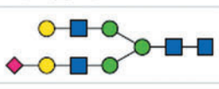
Glycans are involved in a wide range of biological and physiological processes, including recognition and regulatory functions, cellular communication, gene expression, cellular immunity, growth, and development.[1] They are commonly investigated in therapeutic protein drug development because there is strong evidence that bioactivity and efficacy are affected by glycosylation.[2] Both the structure and types of glycans attached to the proteins are usually examined. For these reasons, understanding, measuring, and controlling glycosylation in glycoprotein-based drugs, glycan content of glycoprotein products, and thorough characterization of biosimilars has become increasingly important.

The structures of glycans are highly diverse, complex, and heterogeneous because of post-translational modifications. This makes it challenging to comprehensively characterize glycan profiles and to determine their structures.[3] To understand the detailed structure of glycans using liquid chromatography tandem mass spectrometry (LC–MS–MS), it is essential to separate all isomeric, charge, and branching glycan variations.

Liquid chromatography (LC) coupled to mass spectrometry (MS) has emerged as one of the most powerful tools for the structural characterization of glycans. Various modes of high performance liquid chromatography

Table 1: Structural characterization of glycans present in each peak by the separation of 2AB-labelled *N*-glycans from bovine fetuin.

Peak	Compound Stucture (2AB labeling are not shown)
1	
2	
4	
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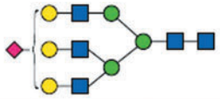
6	
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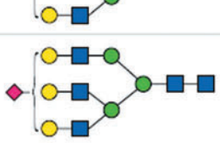
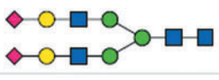
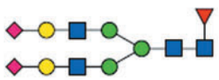
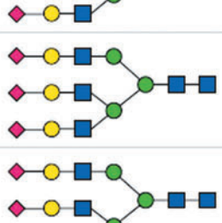
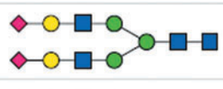
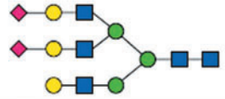
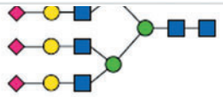
8	
9	
10	
11a	
11b	
11c	
12a	

Peak	Compound Stucture (2AB labeling are not shown)
16	
17	
18	
19	
20	
21	
22	
23	
24	

Peak	Compound Stucture (2AB labeling are not shown)
12a	
12b	
13	
14	
15	

Peak	Compound Stucture (2AB labeling are not shown)
24	
25	
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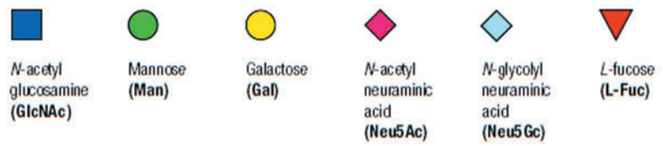
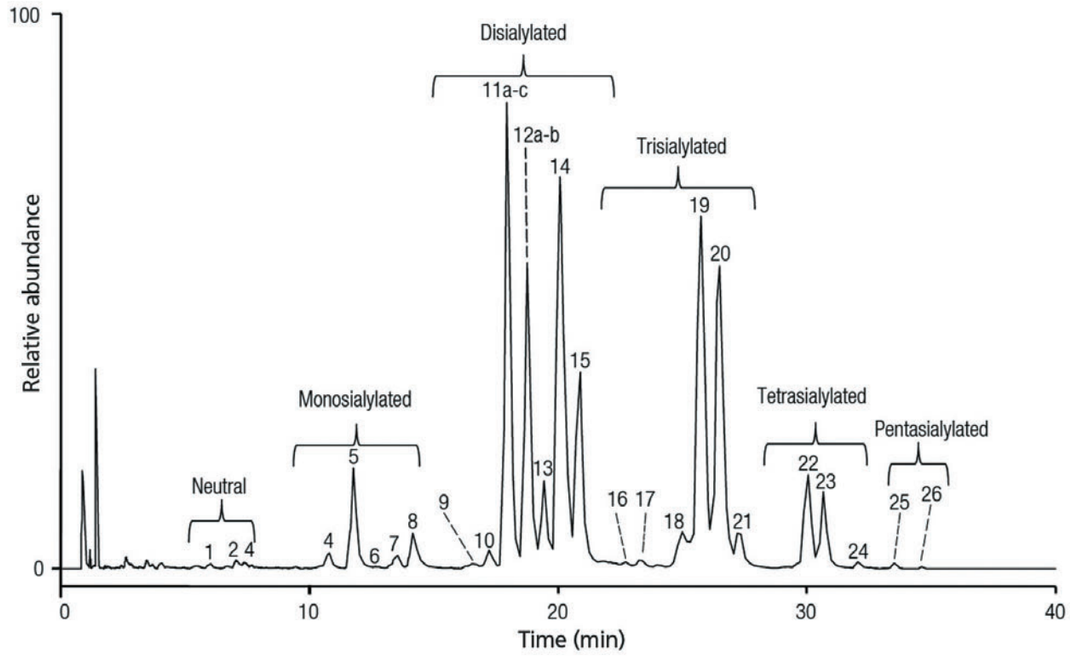


(HPLC) separation have been developed for the analysis of glycans, including normal phase (NP) or hydrophilic interaction (HILIC),[4] ion-exchange (IEX), and reversed-phase.[5] Porous graphitized carbon LC columns that work based on a reverse phase mechanism have also been used for the LC–MS analysis of native unlabelled *N*- and O-glycans.[6] Because glycans are highly hydrophilic and polar substances, HILIC amide columns are extensively used for glycan analysis as compared to reverse phase chromatography.[7] The separation columns are typically amide, amine, or zwitterionic-based packing materials. The HILIC amide columns[8] separate glycans by hydrogen bonding, resulting in a size and composition-based separation. HILIC amide columns are particularly useful for the separation of 2AB or 2AA labelled *N*-glycans released from antibodies, for example MAb, in which the majority of glycans are neutral (0 charge). However, HILIC amide columns don't provide good separation of highly charged state (≥-2) sialylated *N*-glycans because glycans of different charge states are intermingled in the separation envelope. Highly sialylated glycans also retain strongly in HILIC columns and that leads to unsatisfactory separation. In addition, the inherited strong ionic interactions between multiply charged

Figure 1: LC–MS analysis of 2AB-labelled *N*-glycans from bovine fetuin.



glycans and the stationary phase often requires a high eluent buffer concentration for HILIC amide column, adversely affecting MS sensitivity.

This article will describe the use of a high-performance HPLC/ultrahigh-pressure liquid chromatography (UHPLC) column specifically designed for structural analysis of glycans, either labelled or native, by LC-fluorescence or LC–MS methods. The column is based on innovative mixed-mode surface chemistry combining both weak anion-exchange (WAX) and HILIC retention mechanisms. The WAX functionality provides retention and selectivity for negatively charged glycans while the HILIC mode facilitates the separation of glycans according to their hydrophilicity and size. As a result, the column provides unparalleled separation capabilities for highly charge native and 2AB or 2AA labelled *N*-glycans. Furthermore, analyzing unlabelled glycans eliminates the extra reaction step and time-consuming cleanup methods during labelling. Characterization of native glycans also retains the original glycan profile without the changes to the glycan profiles that labelling reaction can introduce. Standardized MS data acquisition method and data analysis software are essential to identify known and unknown *N*-glycan structures present in proteins. LC–MS–MS analysis of glycans requires the processing of large set of data. The incorporation of appropriate software alleviates this issue, which enables the development of a true high-throughput workflow.

In this article, we highlight the structural analysis of 2-aminobenzamide (2AB) labelled *N*-glycans from bovine fetuin using LC–MS. An example of a step-by-step method for the release, labelling, separation, and structural elucidation of native and labelled *N*-glycans from proteins by LC–MS–MS technology is also presented.

Experimental Details

Buffer Preparation: Ammonium formate (80 mM, pH 4.4): Dissolve 5.08 ± 0.05 g of ammonium formate (crystal) and 0.60 g of formic acid in 999.6 g of deionized water. Sonicate the resulting solution for 5 min.

Sample Preparation:

1. Native *N*-glycans were released from glycoproteins with PNGase F enzyme (New England Biolabs) and purified with a 6 mL Hypercarb cartridge (Thermo Scientific). with the help of a 24-port solid-phase extraction (SPE) vacuum manifold (Thermo Scientific) under vacuum. The released glycans were conjugated with a 2-amino benzamide (2AB) (Fisher Scientific) label group.[9]
2. 2AB labelled *N*-glycans from fetuin (5,000 pmol) were dissolved in deionized water (25 µL) in an autosampler vial (Thermo Fisher Scientific) (volume 250 µL).
3. Acetonitrile (75 µL) was added to the same vial and mixed until uniform.

Separation Conditions: Column: 2.1 mm × 150 mm, 1.9 µm GlycanPac AXH-1 (Thermo Scientific); Mobile phase A: Acetonitrile–water (80:20) (v/v). Mobile phase B: Ammonium formate (80 mM, pH 4.4). Column temperature: 30 °C. Sample volume: 1 µL.

Gradient:

Time (min)	%A	%B	Flow Rate (mL/min)	Curve
-10	97.5	2.5	0.4	5
0	97.5	2.5	0.4	5
30	87.5	12.5	0.4	5
35	75.0	25.0	0.4	5
40	62.5	37.5	0.4	5

MS Conditions: MS instrument: Q Exactive hybrid quadrupole-Orbitrap MS; Ionization mode: Negative ion mode; MS scan range: 380–2000 *m/z*; Resolution:

70,000; AGC target: 1 × 106; Max IT: 60 ms; dd-MS2 resolution: 17,500; MS–MS AGC target: 2 × 105; MS–MS max IT: 1000 ms; Isolation window: 2 *m/z*; Dynamic exclusion: 90 s.

Data Processing and Software: Chromatographic software: ChromQuest Chromatography Data System version 5.0 (Thermo Fisher Scientific); MS data acquisition: Thermo Scientific Xcalibur software version 2.2 SP1.48; MS–MS data analysis: SimGlycan software (Premier Biosoft).

Results

Figure 1 shows the separation of neutral and acidic 2AB labelled *N*-glycans from bovine fetuin. The glycan elution profile consists of a series of peaks grouped into several clusters. The neutral glycans elute first, followed by monosialylated, disialylated, trisialylated, tetrasialylated, and, finally, pentasialylated species. Analytes in each cluster represent glycans of the same charge. Within each cluster, glycans with the same charge are further separated according to their size and polarity by HILIC interaction.

The 2AB labelled *N*-glycans from bovine fetuin were separated based on the separation conditions using a two eluent system and analysed. The total ion chromatogram (TIC) is shown in Figure 1. For structural elucidation, data dependant MS–MS spectra were acquired on all precursor ions (z ≤ 2) and software was used for data analysis.[10,11] The detailed structural information obtained from the MS–MS data (Table 1) further validated the ability of the stationary phase to separate glycans based on charge, size, isomers, and polarity. These results also confirmed that the column would be ideal for MS use.

Conclusion

The mixed-mode surface chemistry of the column used here, combining weak anion-exchange and HILIC retention mechanisms, is a high-performance, silica-based HPLC column for simultaneous separation of glycans by charge, size, and polarity. This mixed-mode approach offered unique selectivity and excellent resolution of glycans released from bovine fetuin.

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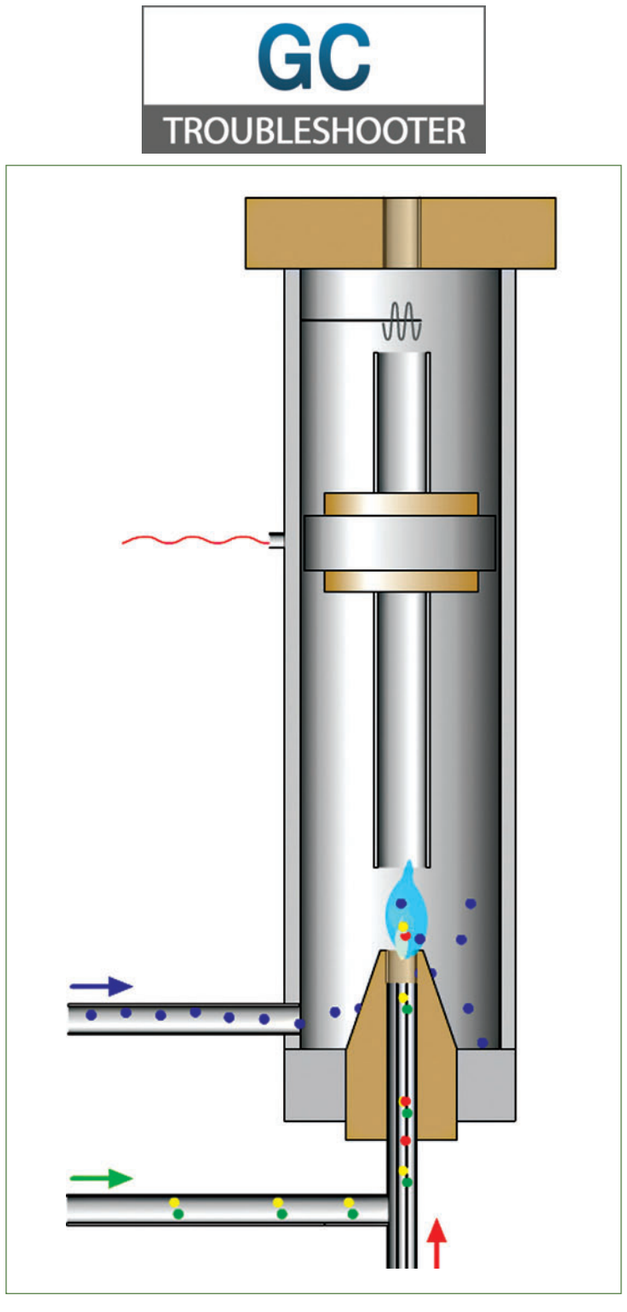

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20–22 January 2014
The Open University, Milton Keynes, UK
Website: http://anthias.co.uk/training-courses/PE-GC

GC Troubleshooting and Maintenance
12 March 2014
Hilton Reading, Drake Way, Reading, UK
Website: http://www.crawfordscientific.com/training-online-calendar.asp

Gas Chromatography: Fundamentals, Troubleshooting, and Method Development
17–20 June 2014
Axion Analytical Laboratories, Chicago, Illinois, USA
Website: http://proed.acs.org/course-catalog/courses/GCPC/

The Basics of Gas Chromatography Online Short Course
TBA
Website: http://proed.acs.org/course-catalog/courses/GCWEB/

HPLC/LC–MS

Essentials of Modern HPLC/

UHPLC 1: Fundamentals and Applications
27 January 2014
Sheraton La Jolla Hotel, La Jolla, California, USA
Website: http://proed.acs.org/course-catalog/courses/EMHU/

HPLC Troubleshooting and Maintenance
5 February 2014
Hilton Glasgow Grosvenor, Great Western Road, Glasgow, UK
Website: http://www.crawfordscientific.com/training-online-calendar.asp

LC–MS for the Chromatographer
18 March 2014
Hilton Glasgow Grosvenor, Great Western Road, Glasgow, Scotland
Website: http://www.crawfordscientific.com/training-online-calendar.asp

The Theory of HPLC
On-line training from CHROMacademy
Website: http://www.chromacademy.com/lc-hplc-overview.asp

Basics of Preparative HPLC
On-line training from CHROMacademy
Website: http://www.chromacademy.com/Preparative_HPLC_Essential_Guide.asp?tpm=1_2

Fundamental LC–MS
On-line training from CHROMacademy
Website: http://www.chromacademy.com/mass_spec-overview.asp

HPLC Troubleshooter
On-line training from CHROMacademy
Website: http://www.chromacademy.com/hplc_troubleshooting.html

METHOD VALIDATION

Introduction to Analytical Method Validation
29 April 2014
Hilton Reading, Drake Way, Reading, UK
Website: http://www.crawfordscientific.com/training-online-calendar.asp

SAMPLE PREPARATION

Solid-Phase Extraction
On-line training from CHROMacademy
Website: http://www.chromacademy.com/sample-prep-training.html

GPC

GPC Academy
11–13 May 2014
PolyRMC Facilities at Tulane University, New Orleans, Louisiana, USA
Website: http://tulane.edu/sse/polyRMC/polyrmc-gpc-academy.cfm

MISCELLANEOUS

Light Scattering Training
10–12 December 2013
Santa Barbara, California, USA
Website: http://www.wyatt.com/training/training/light-scattering-training.html

Rapid and Rational Development of Stable Lyophilized Protein Formulations
11–14 March 2014
Warminster, Pennsylvania, USA
Website: http://www.spscientific.com/ProteinHandsOn/

Please send your event and training course information to Kate Mosford kmosford@advanstar.com

Event News

28–31 January 2014
HTC-13/HTSP-3
Old Saint John Conference Centre, Bruges, Belgium
Organizers: KVCV (BE)/RSC (UK)
Tel: +32 (0) 9 264 9606
E-mail: info@htc-conference.org
Website: www.htc-conference.org

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27 April–1 May 2014
30th International Symposium on MicroScale Bioseparations
Kodály Center, Pécs, Hungary
Organizers: University of Pécs and the Hungarian Society of Separation Sciences
Tel: +36 72510497
E-mail: info@msb2014.org
Website: www.msb2014.org

6–9 July 2014
8th International Conference on Breath Research & Cancer Diagnosis
Torun, Poland
Organizers: Nicolaus Copernicius University, Polish Chemical Society, and the Committee of Analytical Chemistry PAS
Tel: +48 56 6114753
E-mail: conference@breath2014.pl
Website: www.breath2014.pl

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31 August–5 September 2014
Dioxin 2014: 34th International Symposium on Halogenated Persistent Organic Pollutants
Hotel Meliá Castilla, Madrid, Spain
Organizers: IQOG-CSIC, Madrid, Spain
Tel: +34 91 400 93 84
E-mail: dioxin2014@mci-group.com
Website: www.dioxin2014.org

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Mission Statement
The Column (ISSN 2050-280X) is the analytical chemist's companion within the dynamic world of chromatography. Interactive and accessible, it provides a broad understanding of technical applications and products while engaging, stimulating and challenging the global community with thought-provoking commentary that connects its members to each other and the industries they serve.
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