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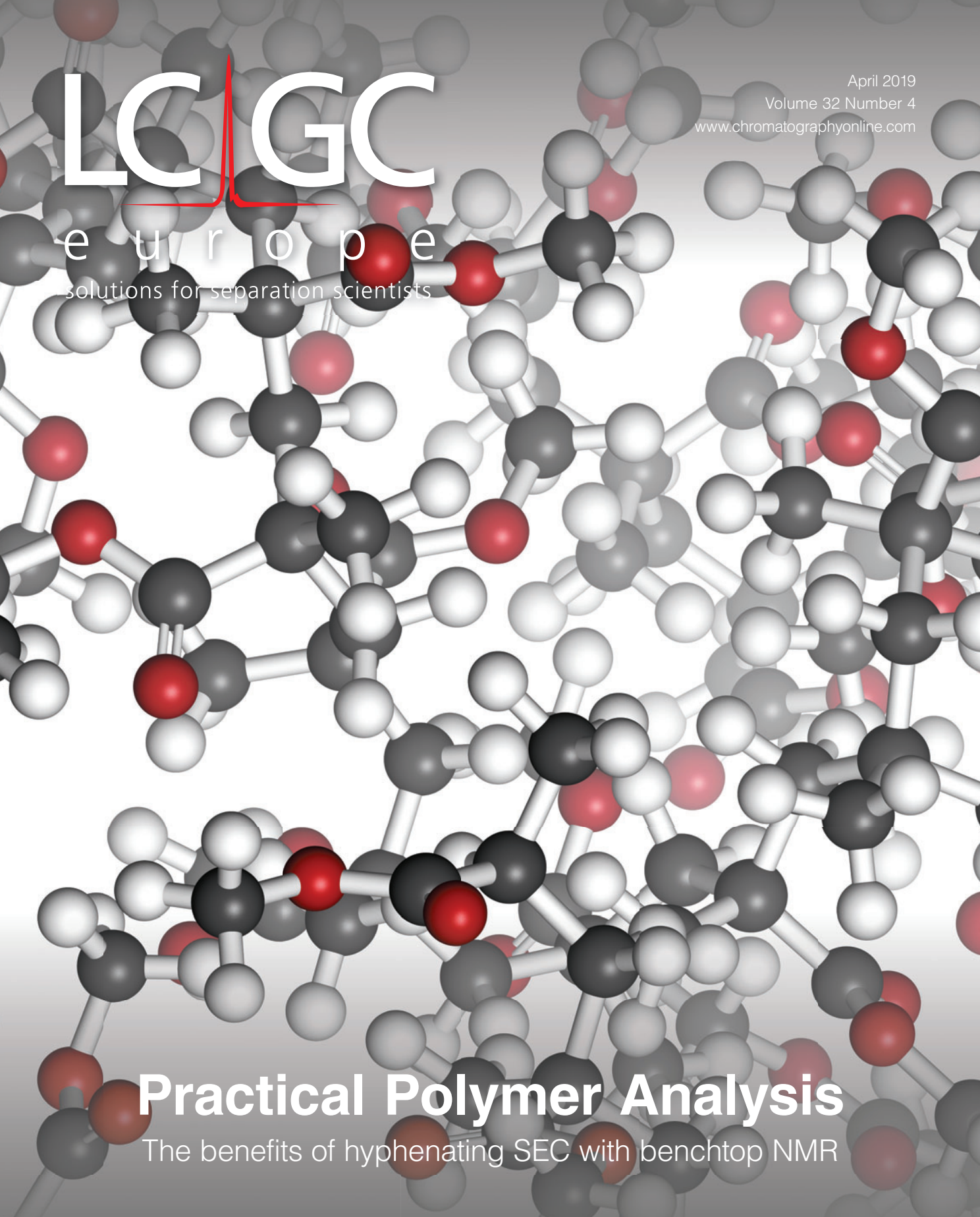

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The benefits of hyphenating SEC with benchtop NMR

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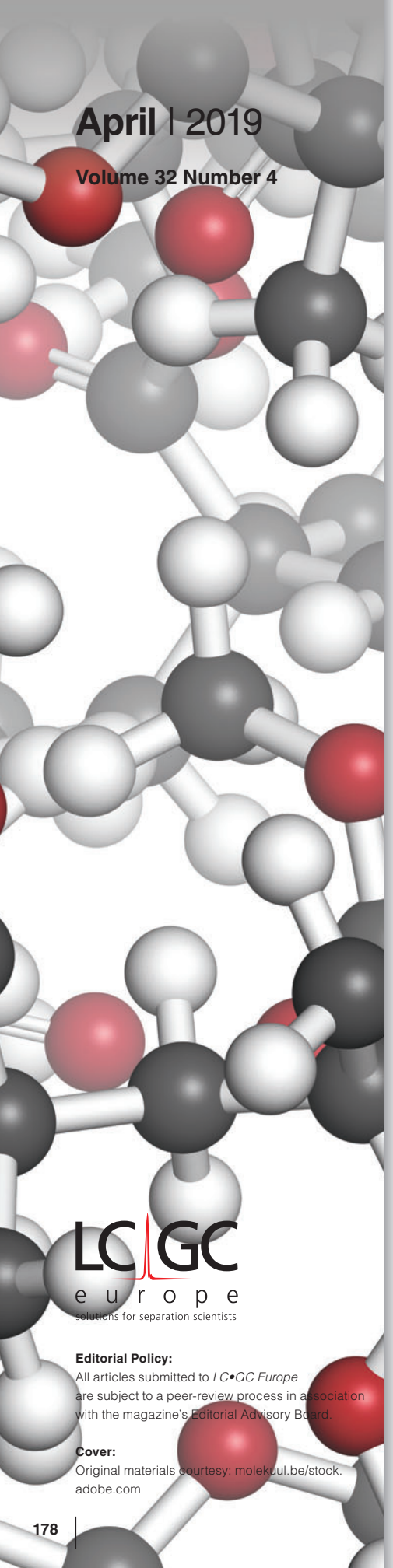

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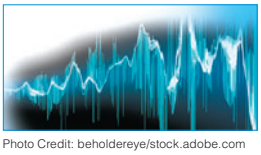

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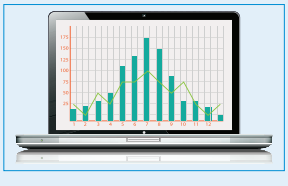

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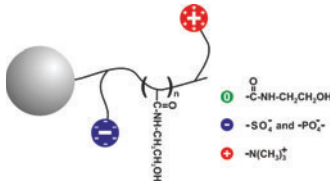

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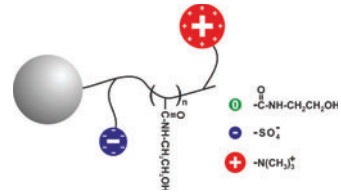

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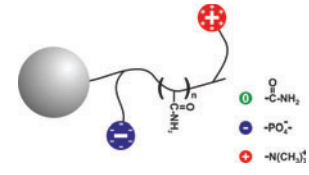

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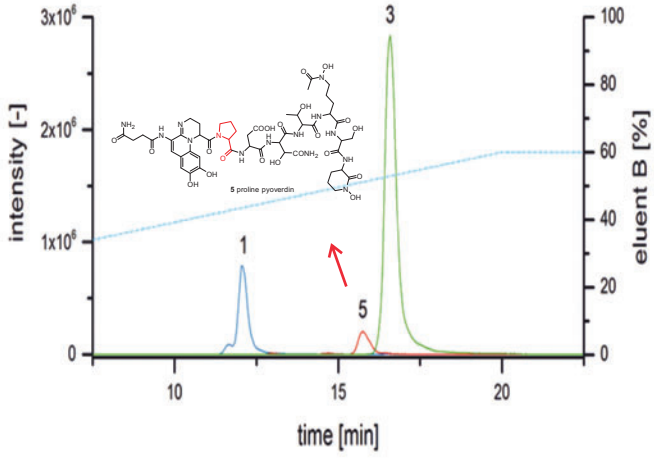

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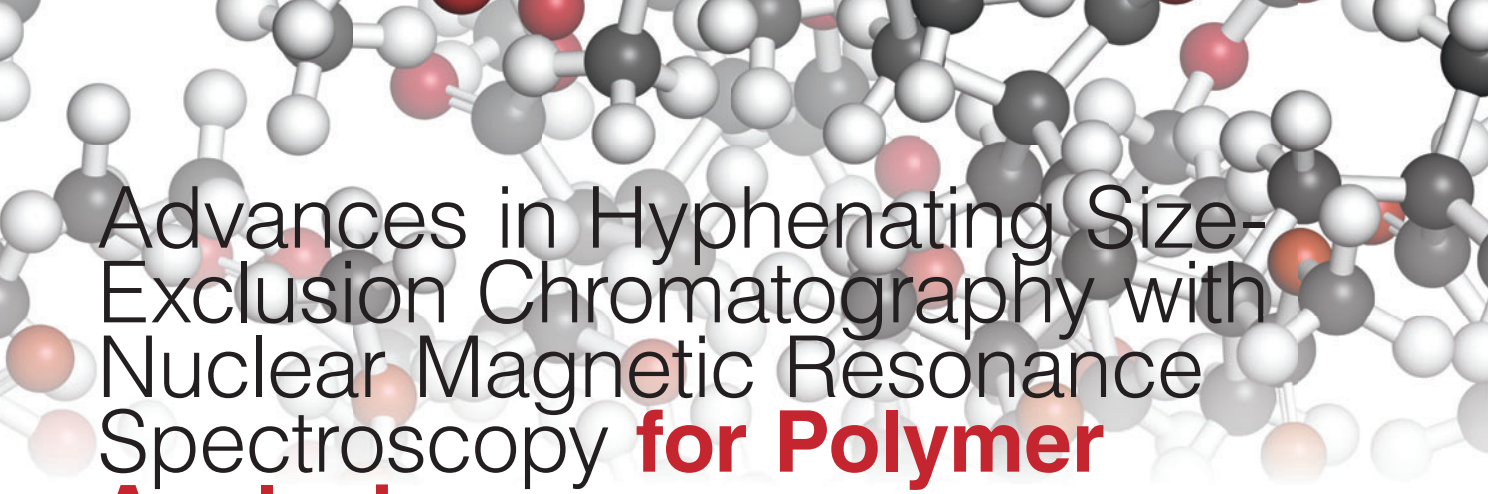

Advances in Hyphenating Size-Exclusion Chromatography with Nuclear Magnetic Resonance Spectroscopy for Polymer Analysis

Paolo Sabatino[1], Marcel van Engelen[1], Hamed Eghbali[1], Alex Konig[1], Matthias Pursch[2], Robert Zeigler[3], Klas Meyer[4], Jürgen Kolz[4], and Andreas Schweizer-Theobaldt[1], [1]Dow Benelux B.V., Core R&D, Analytical Science, Terneuzen, Netherlands, [2]Dow Stade Produkt. GmbH&Co OHG, Core R&D, Analytical Science, Stade, Germany, [3]The Dow Chemical Company, Core R&D, Analytical Science, Freeport, Texas, USA, [4]Magritek GmbH, Aachen, Germany

Nuclear magnetic resonance (NMR) spectroscopy provides unique structural information on organic molecules as well as quantitation without standards, but is often limited by the ability to differentiate components in mixtures. A hyphenated technique, such as liquid chromatography (LC)–NMR, is therefore desirable because it simplifies the ^{1}H–NMR spectrum by separating different components in the chromatographic column prior to analysis and permits the direct monitoring of compositional changes during the separation process (on-flow) because the eluents are sampled in "real-time". However, despite the progress in this field, LC–NMR is not currently used routinely, mainly because of practical operation issues. This article demonstrates that size-exclusion chromatography (SEC) coupled to a benchtop NMR instrument allows shifts of bulk polymer composition to be monitored directly by on-flow analysis and allows reliable semiquantitative analysis to be performed. The applicability of SEC–NMR is demonstrated by characterizing several samples, such as acrylate blends, gradient co-monomer acrylate, as well as surfactants blends.

Nuclear magnetic resonance (NMR) spectroscopy is one of the most important and most powerful technologies for structure elucidation and characterization of chemical species because it provides unique molecular information on organic molecules as well as the possibility to quantify species without the need for standards.

However, NMR is often limited by its ability to differentiate components in mixtures. The use of more elaborate two-dimensional (2D)-NMR techniques can overcome this limitation, but in many cases a separation process before structural characterization by NMR is required. Diffusion-ordered spectroscopy (DOSY) (1,2) experiments can be very useful in these circumstances because they offer the possibility of separating components according to their diffusion coefficients, directly inside the NMR tube. The methods that rely on diffusion coefficient differences to resolve mixtures are often referred to as NMR chromatography (3). These methods, however, have limited applicability for higher molecular weight polymer characterization where strong magnetic field gradients would be needed to follow the spatial displacement of slowly diffusing species.

Under these conditions, a physical separation method is needed and a hyphenated technique such as liquid chromatography (LC)–NMR is very desirable. This would allow the simplification of the ^{1}H–NMR spectrum by separating different components in the chromatographic column and the direct monitoring of compositional changes during the separation process (on-flow) as the eluents are sampled in "real-time" while flowing through the NMR detection coil. The latter would result in a pseudo three-dimensional (3D)-plot (intensity versus ppm versus retention time) (4–6).

KEY POINTS

- Hyphenation of SEC with benchtop NMR was established to monitor change in chemical composition as a function of molecular weight distribution.
- Experiments demonstrated that small shifts in bulk polymer composition can be detected and quantified by direct on-flow analysis of the eluted material.
- By installing a switch valve, stop-flow mode NMR analysis could be used to overcome sensitivity limitations by measuring different chromatogram segments for longer periods.

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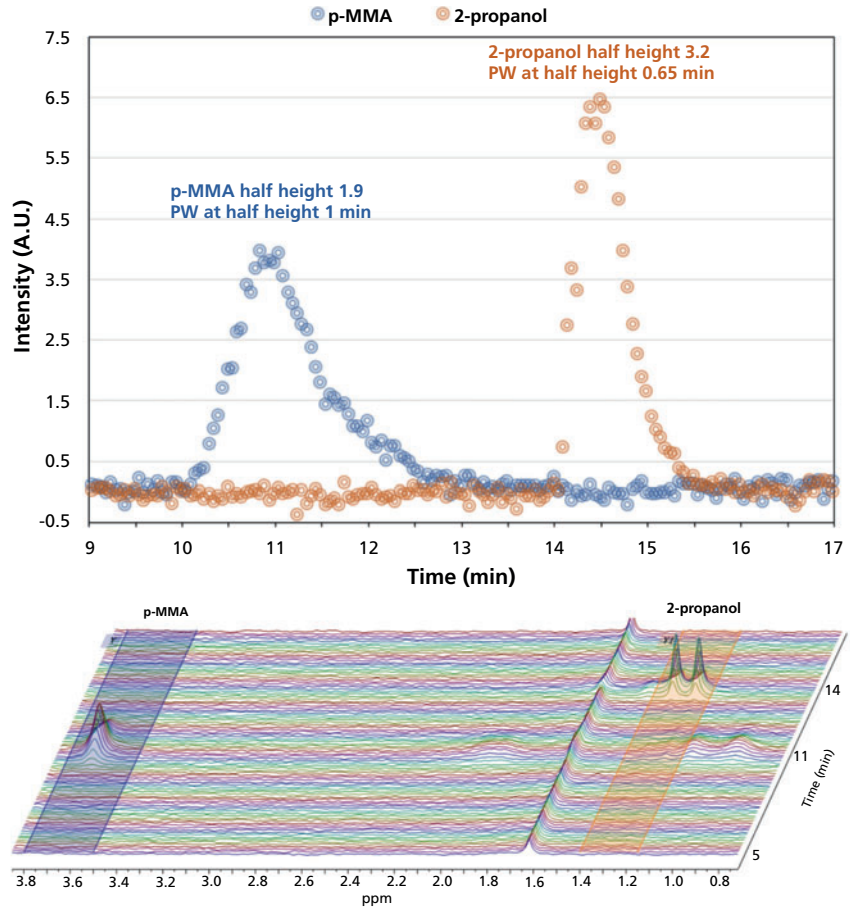
Figure 1: NMR stacked plot and SEC–NMR chromatogram of the p-MMA peak (blue crosses) and 2-propanol signals (orange circles).



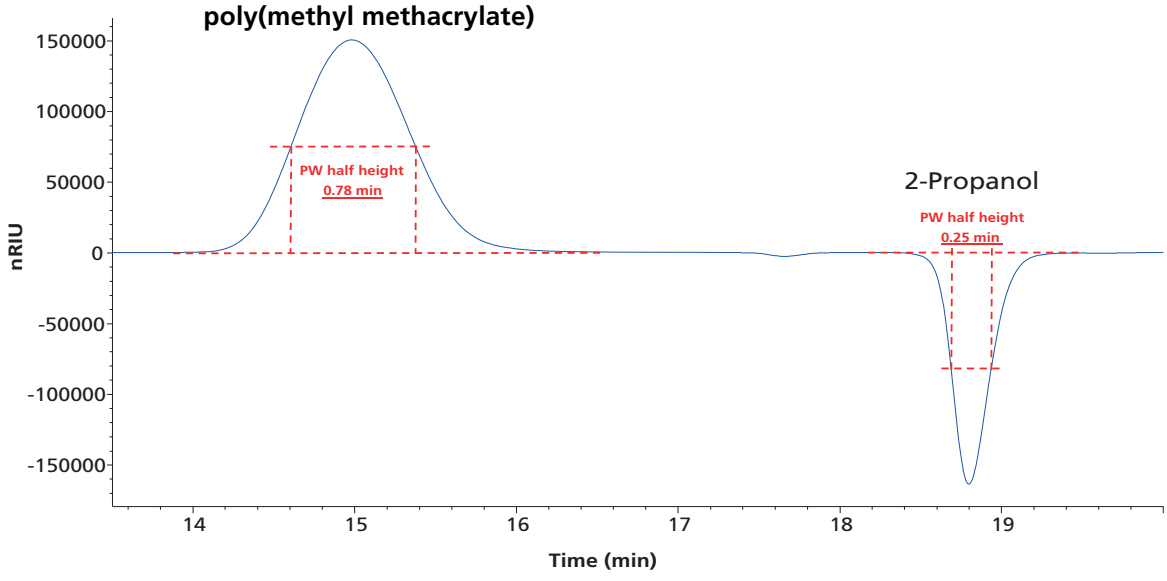
The first attempt at LC–NMR hyphenation dates back to the seminal work of Watanabe and Niki in 1978 (7). However, the poor sensitivity of NMR as the detector and the sample preparation effort involved have strongly limited the application range of this technique. NMR technology has advanced only recently with the development of stronger superconducting magnets and cryogenic probes, which established LC–NMR as an analytical technique (8–10).

The current main limitation of LC–NMR is the high cost associated with the instrumentation. While the LC system is relatively inexpensive, high-field NMR equipped with cryoprobes are prohibitive costs. Therefore, the possibility of hyphenating an LC system with benchtop NMR systems has been investigated. The performance of benchtop NMR technology has developed to the point that a much broader use of NMR technology is now feasible (11,12). The implementation of solvent suppression techniques and effective flow cells allows the hyphenation of this type of NMR instrument with liquid chromatography.

Size-exclusion chromatography (SEC) was the first choice for coupling LC with benchtop–NMR for various reasons. First, the characterization of polymers was a worthwhile target to pursue because structural characterization by NMR in coupled mode can overcome the molecular weight limitations of MS techniques, where multiple charging and limited sensitivity inhibits insight into higher molecular weight materials. Initial work on SEC with a benchtop NMR system has been published very recently, with a focus on NMR itself (13). NMR can also provide reliable quantitative information not easily attainable by MS. This article describes considerations from the separation point of view and presents several applications.

This article aims to show that the current challenges associated with benchtop NMR can be addressed by hyphenating with this chromatographic technique. Low sensitivity can be compensated for by injecting large amounts of sample. Peak band broadening from the large volume of the NMR flow-cell does not severely impact the analytical information because the volumetric band variance contribution in conventional SEC is significant due to the large column volumes and large particle sizes. Furthermore, the rather low peak capacity of SEC allows time and cost-efficient detailed analysis of major parts of a chromatogram or even a full comprehensive characterization by stopped-flow mode with a reasonable number of SEC runs because the entire SEC trace can be cut into a reasonable low number of fractions for NMR analysis.

Figure 2: SEC–DRI trace of poly(methyl methacrylate) and 2-propanol. A 2.5-mg measure of each is injected.



It will be demonstrated that LC–NMR using a benchtop system can be applied to copolymer analysis and has the potential to become widely implemented in analytical laboratories.

Experimental Procedures

Materials: Poly(methyl methacrylate) (p-MMA) analytical standard with average M_w of ~15000 Da was purchased from MilliporeSigma (#200336). Poly(butyl acrylate) (p-nBA) solution 25 wt. % in toluene with average M_w of 60000 Da and M_w/M_n = 3.0 was purchased from MilliporeSigma (#181412). Gradient random copolymer poly(styrene-co-methyl methacrylate) with MMA concentration varying from 60–90%, M_n = 68000 Da and M_w/M_n = 4.2, was obtained from Polymer Source Inc. Canada (product id P9160–SMMAgra). Poly(methyl methacrylate) with average M_w of 2500 Da and M_w/M_n = 1.1 was purchased from PSS Polymer Standards Service GmbH (product id PSS–mm2.5k). 2-Propanol anhydrous, 99.5% purity, was obtained from MilliporeSigma (#278475). Chloroform, purity ≥ 99.5%, containing 100–200 ppm amylenes as stabilizer and dichloromethane for liquid chromatography LiChrosolv, was purchased from MilliporeSigma (#C2432 and #106044). Ethoxylated and propoxylated secondary alcohols surfactants $C_{12}H_{25}OEO_{4.5}PO_{5.5}$ (M_w = 702 Da) and $C_{10}H_{23}OEO_9PO_{12}$ (M_w = 1249 Da) were obtained from Dow Chemical.

NMR: NMR experiments were performed on a benchtop Ultra Spinsolve NMR System (Magritek Ltd) operating at ^1H frequency of 62.08 MHz. A standard single pulse sequence was used for acquiring the experiments. The experiments were acquired with 5000 Hz spectral width and 32k points spectral size. The number of transients and the overall pulse repetition time were set depending on the type of sample investigated and are mentioned later in this article. The NMR datasets were processed using the Reaction Monitoring plugin within NMR software Mestrenova (Mestrelab Research S.L.), which allowed the construction of SEC–NMR plots in which the signal intensity is plotted as a function of time.

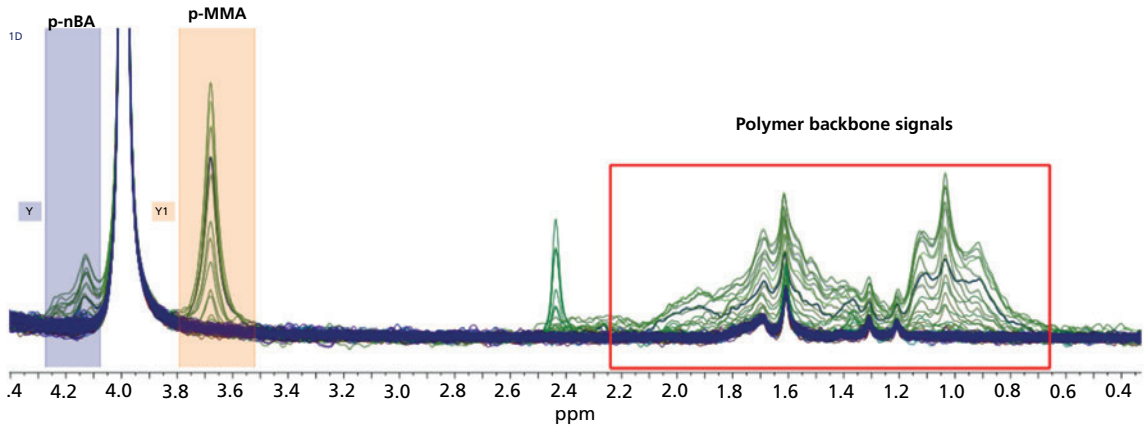
Size-Exclusion Chromatography (SEC)–NMR: *Experimental Conditions:* Column: two 300 × 7.5 mm, 5-µm PLgel MIXED–D PL1110–6504 columns (Agilent) in series; injection volume: up to 100 µL; column oven: 28 °C; eluent: CH_2Cl_2 or $CHCl_3$ stabilized with 2-methyl-2-butene; flow: 1.0 mL/min; UV detection: 230 nm, 3.43 Hz acquisition rate; DRI detection: Agilent 1100 Series G1362A RID connected to LC system in place of the NMR spectrometer to record reference data, 2.28 Hz acquisition rate.

A 1100 LC system (Agilent) with UV detection consisting of a G1310A isocratic pump, G1313A autosampler, G1322A degasser, G1314A variable wavelength detector, and G1316A column oven equipped with a switching valve was coupled with the benchtop NMR using the standard monitoring flow cell (Magritek Ltd). The switching valve in the column oven was mounted in the eluent flow after the UV detector and allowed the eluent to be switched to the NMR system or directly to the waste bin. This design allowed the NMR system to be bypassed in case no measurements were performed, or stopped the flow in the NMR flow cell to analyze a particular fraction of the size-exclusion chromatogram (stopped-flow analysis). Some analyses were performed coupling a G1362A RID detector (Agilent) with the LC system instead of the NMR spectrometer.

Results and Discussions

Extra-Column Band Broadening: The extra-column band broadening by the NMR flow cell was investigated by comparing the peak width at half height of a 10% solution of narrow p-MMA (2500 Da, polydispersity 1.1) and 2-propanol separated by SEC and detected by a refractive index

Figure 3: Superimposed ^{1}H–NMR plot of the p-MMA + p-nBA sample acquired on flow mode. The peak assignment is displayed on the figure. The blue and orange bands define the spectral region integrated over time to construct the plot in Figure 4(a).



detector and by the NMR spectrometer. A 25-µL measure of the solution was injected. A total of 340 NMR spectra were recorded collecting one scan per experiment with 2 s repetition time. Figure 1 shows the NMR stacked plot and the SEC–NMR plot for both p-MMA and 2-propanol along with the determination of the peak width at half height. This parameter was evaluated again after replacing the NMR by the DRI detector (Figure 2).

The peak width at half height of the p-MMA increased from 0.78 min (SEC–DRI) to 1.0 min (SEC–NMR), while that of 2-propanol increased from 0.25 min (SEC–DRI) to 0.65 min (SEC–NMR). The data indicated that the used NMR flow cell leads to considerable extra-column band broadening. This extra-column broadening would be too large for an LC with a benchtop NMR coupling of smaller internal diameter (i.d.) LC columns, such as conventional 4.6-mm-i.d. types, and this prohibits LC analysis by adsorption chromatography or SEC using these column dimensions. In particular, for smaller internal diameter columns (2.1–3 mm) packed with very small particles (< 3 µm), an extra-column broadening of 5–10 µL will already have a significant negative impact on peak broadening (14). With 4.6-mm i.d-columns and 5-µm particle size packing, the acceptable extra-column broadening is much larger—in the order of 20–60 µL. The extra-column volume of the NMR flow cell is one order of magnitude higher and would prohibit LC analysis of small molecules or polymers with common columns. Although the observed band broadening is too large for accurate molecular weight separation, it can be considered acceptable for the qualitative analysis of chemical structures of sample components separated by their size at a given chromatographic speed and resolution. A possible solution to the band broadening effect would rely on the use of smaller NMR flow cells. This approach, however, would lead to lower sensitivity of the NMR spectrometer because of smaller sample volumes in the measurement zone and therefore would not lead to the desired result. A more viable method was explored and recently published by Höpfner *et al.* (13) using 20-mm i.d. semipreparative SEC columns with a similar LC and benchtop NMR system setup. Compared to the conventional 7.5-mm i.d. columns used in this study, increased loadability of the preparative columns should allow sharper peaks to be eluted when injecting the same amounts of sample.

Polymer Analysis:

p-MMA + p-nBA Blend: A sample containing 7.5 mg of p-MMA with weight-average molecular weight of 15000 Da and 6.0 mg of p-nBA with weight-average molecular weight 60000 Da was dissolved in 1 mL dichloromethane. For the analysis of this sample the eluent flow rate was set to 1 mL/min and a total of 80 NMR spectra were recorded by acquiring 4 scans/spectrum with a repetition time of 7 s/scan. Figure 3 shows the superimposed plots of the ^{1}H–NMR experiments acquired in on-flow mode, emphasizing the signals' intensity build-up of the eluting species.

By following the evolution of the p-MMA and p-nBA integral values as a function of the elution, it was possible to create the plot displayed in Figure 4(a). As expected, it is clearly visible that the p-nBA is the first component to elute (higher molecular weight), followed by the p-MMA. For comparison, Figure 4(b) shows the SEC trace recorded by replacing the NMR spectrometer with a DRI detector in the LC system. By injecting the two homopolymers separately, the elution profiles of the two materials were investigated. Accordingly, the elution times of the two homopolymers in the mixture are marked by curved brackets in the figure. Considering the overall separation pattern, it can be noted that there is a significant time shift (> 2 min) between the NMR and DRI chromatogram. This can be explained by the fact that both separations were obtained with two completely different instruments with different internal volumes. The lift-off of p-nBA signal begins at 9.3 min, while the elution of the p-MMA starts 2.2 min later and overlaps with the p-nBA signal. The total polymer elution time is approximately 7 min (from 9 to 16 min). Similar findings were made by analyzing the SEC–NMR chromatogram: the p-nBA signals are first observed at 6.7 min, while the p-MMA is only visible from 8.5 min. This means that p-MMA starts eluting approximately 1.5 min after the p-nBA, which is in agreement with SEC–DRI results. Moreover, the last spectrum containing polymer signals is recorded after 12.1 min, providing a total elution time of 5.1 min. At the permeation volume, toluene coeluted with other low-molecular-weight impurities of the

Figure 4: Chromatogram of the polymer elution as detected by (a) NMR and (b) DRI. The blue circles in the NMR plot selectively detect poly(butyl acrylate), while the orange squares represent the poly(methyl methacrylate).

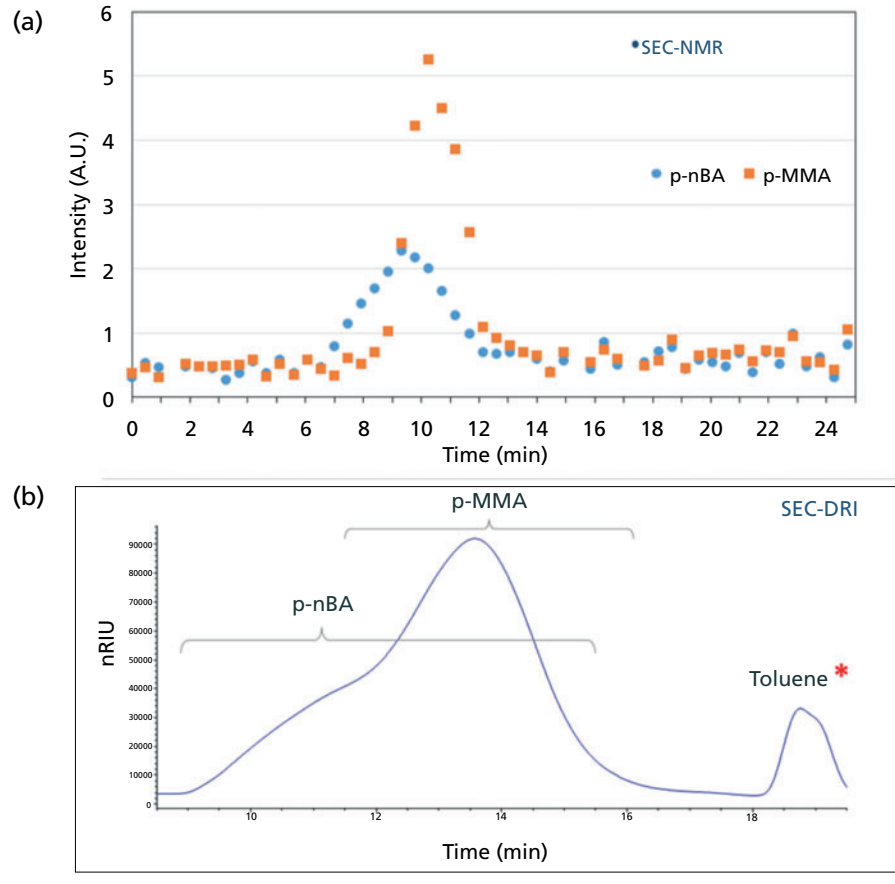
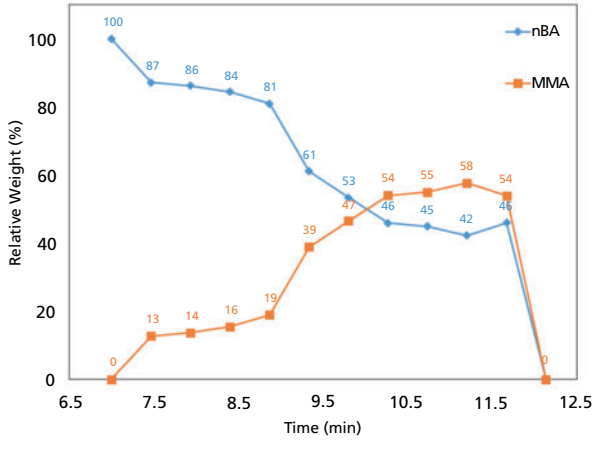


Figure 5: Quantitative compositional analysis of the blend p-nBA–p-MMA sample indicates the change of relative amounts of the two species during the elution.



sample solution in a broad signal. By comparing the SEC–DRI and SEC–NMR traces, sharper signals are indicated in the latter one. This is in contradiction with the significant extra-column band broadening effect of the NMR flow cell reported above. This observation can be explained by the intrinsic insensitivity of the NMR as a detector. This leads to late detection of peak lift-off and early detection of peak-end and to a falsely narrower SEC–NMR elution profile, masking the brand broadening effect and complicating quantification. Adjusting the conditions of the SEC–NMR experiments (bigger columns, higher number of scans) could partially solve the sensitivity and band broadening issues, paving the way to reliable quantitative analysis.

For the p-MMA/p-nBA blend it is possible, theoretically, to quantitate the relative amounts of polymer at any given time during the elution by integrating the nBA and MMA resonances in the NMR spectra. The information can be used to construct the plot in Figure 5. Considering that the p-MMA and p-nBA peaks used count for three ($O\text{-}CH_3$) and two ($O\text{-}CH_2$-) protons respectively, the relative weight fractions of the two monomers can be calculated using equations 1 and 2:

$$W_{p-nBA}(t)\% = \frac{\left(\frac{Int_{p-nBA}(t)}{2} * MW_{nBA}\right)}{\left(\frac{Int_{p-nBA}(t)}{2} * MW_{nBA} + \frac{Int_{p-MMA}(t)}{3} * MW_{MMA}\right)} * 100 \quad (1)$$

$$W_{p-MMA}(t)\% = 100 - W_{p-nBA}(t)\% \quad (2)$$

Where $W_{p\text{-nBA}}$ (*t*)% and $W_{p\text{-MMA}}$ (*t*)% are the relative weight fractions of the two monomers at time, *t*, $Int_{p\text{-nBA}}$ (*t*) and $Int_{p\text{-MMA}}$ (*t*) are the integrals value of p-nBA and p-MMA signals at *t* and MW_{nBA} and MW_{MMA} are the molar masses of the nBA and MMA monomers.

Analogously to Figure 4, the plot in Figure 5 indicates that at the beginning of the elution only p-nBA is present and the amount of p-MMA progressively increases as a function of the elution time. Figure 5 captures the strength of LC–NMR hyphenation, where the separation capability of the SEC enables the NMR compositional quantification of the species present in the sample. This example demonstrates that the sensitivity for detecting composition drifts allows very useful, semiquantitative information to be gained.

p-Styrene–p-MMA Gradient Copolymer: In order to investigate the sensitivity of the benchtop NMR spectrometer in on-flow mode for detecting copolymer composition shifts, a gradient random copolymer of poly(styrene-co-methyl methacrylate) MMA 60 –> 90%, M_n = 68000 Da, was analyzed. The material was a random copolymer prepared by atom transfer radical polymerization of styrene and methyl methacrylate with the monomer feeding process repeated and controlled to adjust the gradient composition. As expected, the analysis by SEC coupled with UV and DRI detection revealed a minor shift of the styrene to methyl methacrylate ratio with molecular weight. Higher intensity of the UV—compared to the DRI—detected SEC trace in Figure 6(a) indicated increased styrene content in the lower molecular weight fractions of the material. The abnormal shape of the SEC trace clearly indicates exclusion and column overload of high-molecular-weight material at and above about 400000 Da. The composition shift was detected by the NMR as well. A 1D NMR plot over all spectra was recorded with 15 mg of sample injected. A total of 72 NMR spectra were recorded collecting four scans per experiment with 7 s repetition time. Figure 6(b) indicates that there is a shift of the MMA–styrene ratio over the molecular weight distribution. The styrene level reaches its maximum after the maximum for MMA, indicating that the low-molecular-weight fraction is richer in styrene content. The small intensity difference between the normalized UV–254 nm and DRI–detected SEC traces in Figure 6(a) indicates that the drift in the styrene–MMA ratio over the molecular weight span is quite small. This test highlights that this method is capable of sensitively following composition variation by direct on-flow analysis.

Surfactant Blends Analysis: Besides the experiments on acrylate model systems mentioned above, the performance of SEC–NMR was also tested by the analysis of ethoxylated and propoxylated alcohol surfactants. The constituents in blended materials were characterized to explore the applicability of the hyphenated system for current analytical needs. The test sample

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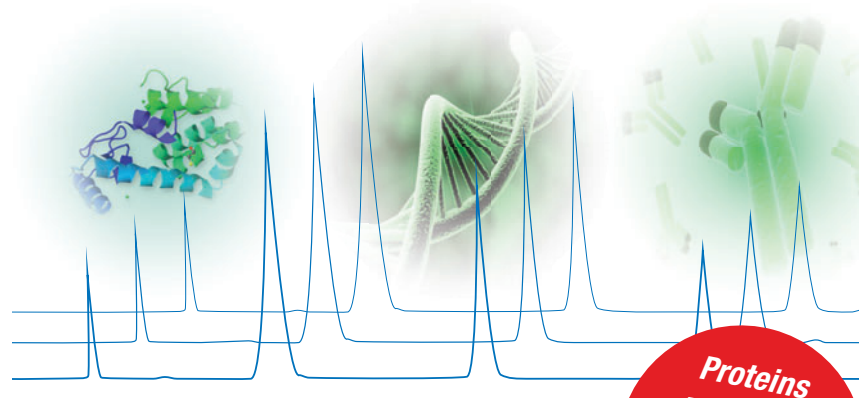

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Figure 6: SEC–UV–DRI and SEC–NMR comparison. The shift of the styrene curve in the SEC–NMR plot (b) indicates a higher content of styrene in the low-molecular-weight fraction in line with the higher UV response visible in the normalized SEC–UV-RI plot (a).

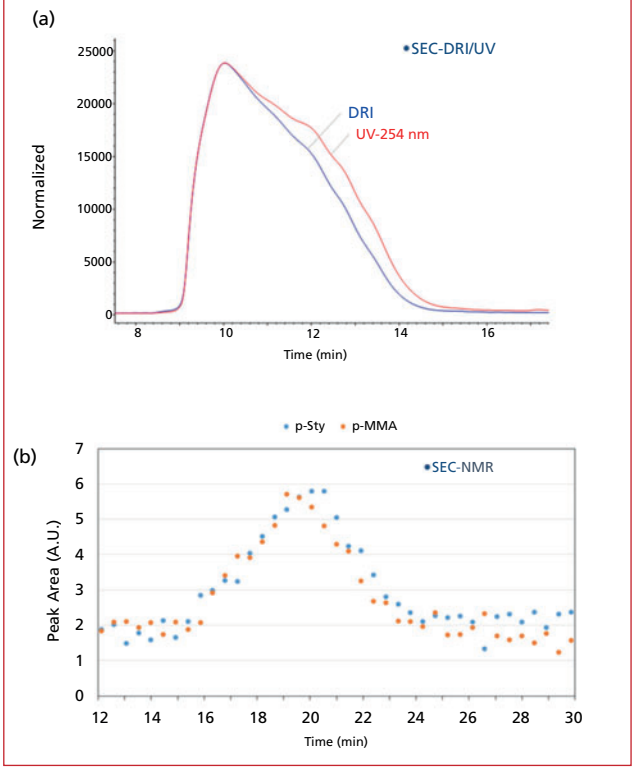
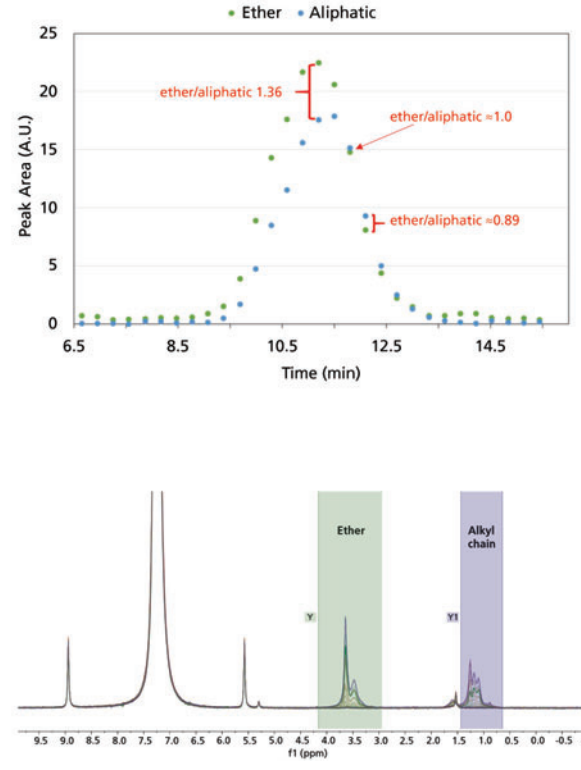


Figure 7: Superimposed NMR plot and SEC–NMR chromatogram obtained by integration of ether and aliphatic signals.



consisted of $C_{12}H_{25}O(EO)_{4.5}(PO)_{5.5}$ (M_w = 702 Da) and $C_{10}H_{21}O(EO)_9(PO)_{12}$ (M_w = 1249 Da) surfactants in equal concentration. The two products are characterized by different molecular weights and by different chemical compositions, and hence, different aliphatic proton ratios. The ether to aliphatic proton ratios in the two surfactants were 1.35 and 0.92 for the higher and lower molecular weight species, respectively. The main target for this analysis was to check whether a sensitive differentiation between similar polymers is possible by monitoring the change in ether and aliphatic protons signal intensity.

A blend of 6.5 mg of each component was injected setting the flow rate to 1 mL/min. In total, 73 NMR experiments were recorded acquiring 4 scans per spectrum at repetition rate of 4 s/scan.

Figure 7 shows the superimposed NMR spectra. At the chromatogram lift-off (~ 9 min), the ratio of ether to aliphatic protons is >1—as expected for the higher molecular weight surfactant eluting faster. At the apex of the chromatogram (11.2 min), the ratio of ether to aliphatic protons is 1.36, suggesting that mainly the higher molecular weight surfactant is eluting. This ratio progressively decreases and reaches equality at 11.8 min, meaning that the two surfactants are coeluting at the same concentration. At the peak end a ratio of <1 (0.89 at 12.1 min) can be observed when only lower molecular weight surfactant is eluting. The detection of the composition shift in on-flow analysis confirmed the availability of this approach.

Conclusions

The opportunities and limitations of current low-field benchtop NMR spectroscopy for hyphenation with liquid separation techniques were investigated by coupling with SEC. Bearing in mind the limited sensitivity of the benchtop instrument and the large volume of the NMR flow cell, this chromatographic technique was selected because it allows, with some limitations, a high sample load without fundamentally deteriorating the separation.

The analysis of homopolymer blends, copolymers, and gradient copolymers by SEC using a benchtop NMR spectrometer was used to explore current limitations regarding composition characterization of separated materials in an on-flow mode. The studies demonstrated that small shifts in chemical composition can be detected and roughly quantified in the eluted material. Although very high sample load of the LC columns and a quite large NMR flow cell is needed to obtain sufficient sensitivity, the qualitative features of the chromatograms are maintained at an extent that allows reasonable composition analysis. It should be noted that the analytical sensitivity achieved so far by on-flow analysis may be considerably improved by using larger diameter columns or by analyzing chromatogram segments in stop-flow mode.

The performance of benchtop NMR spectrometers has reached a level where coupling to LC is starting to become a valuable option for material characterization. As a result of sensitivity limitations, reasonable data

can currently be obtained by SEC operated at a very high sample load only, and with technological advances SEC with benchtop NMR spectroscopy has the potential to become a routine, highly informative LC detection technique.

Acknowledgements

The authors thank Dr. David Meunier for the fruitful discussions.

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Reversed-Phase Liquid Chromatography and Water, Part 2: **Re-equilibration of the Stationary Phase Following Gradient Elution**

Dwight R. Stoll, LC Troubleshooting Editor

How long does it take to re-equilibrate reversed-phase stationary phases following gradient elution, especially when starting with a highly aqueous eluent?

A little more than 15 years ago, Adam Schellinger and I started what turned into an extensive series of experiments aimed at better understanding reversed-phase column re-equilibration following solvent gradient elution. We were both graduate students at the time, studying with Professor Peter Carr at the University of Minnesota, USA. Adam was focused on fundamental aspects of gradient elution, including optimization and method transfer, and I was focused on improving the speed of two-dimensional liquid chromatography (2D-LC) separations. I can still recall the place in the laboratory where I asked Adam why re-equilibration of reversed-phase columns required so much time. Neither of us could come up with a clear answer based on our reading and understanding about how columns worked, so we decided to do some simple experiments and find out for ourselves. The prevailing thought at that time was that reversed-phase columns should be re-equilibrated with about 10 column volumes of the initial eluent used in the gradient following one separation, and before injecting the next sample. A typical 150 mm × 4.6 mm i.d. column has a dead volume of about 1.5 mL. Even using a flow rate of 2 mL/min, this translates into a re-equilibration time of about 8 min; these days, many separations are entirely completed in a fraction of that time.

The essence of what we learned was that, under most circumstances, reliable chromatographic results can be obtained with much shorter re-equilibration times corresponding to re-equilibration with just one to two column volumes of the initial eluent used in the gradient.

The essence of what we learned was that, under most circumstances, reliable chromatographic results can be obtained with much shorter re-equilibration times corresponding to re-equilibration with just one to two column volumes of the initial eluent used in the gradient. For conventional LC separations, this can result in tremendous time savings and improve throughput of gradient elution methods. In 2D-LC, this was a transformative finding, because we realized that this would enable high quality 2D separations on the timescale of an hour or less (1).

In Part 1 of this "LC Troubleshooting" series earlier this year, I discussed the use of reversed-phase stationary phases designed for use in highly aqueous eluents, and how "dewetting" of traditional reversed-phase stationary phases can occur under these conditions (2). One question I alluded to in that article but did not address in detail was how long does it take for an aqueous-compatible reversed-phase stationary phase to equilibrate when switching from an eluent containing some organic solvent to a completely aqueous

eluent? This is a question of practical significance, both for isocratic separations involving a completely aqueous eluent, and gradient elution separations that involve an initial eluent that is completely aqueous.

Essential Concepts for Re-equilibration Following Gradient Elution

The results of our initial studies of column re-equilibration several years ago were summarized in a series of journal articles. One was focused primarily on eluents containing acetonitrile and water, and nonionogenic solutes (3). The other two papers dealt with more complex situations involving buffered eluents and ionogenic solutes (4,5). Readers interested in the effects of variables on re-equilibration, such as flow rate, temperature, solute retention, and eluent additives, are encouraged to read these articles. It is also worthwhile noting here that McCalley has recently published two papers that address questions about the rate of column re-equilibration

Figure 1: Solvent program used in gradient elution (solid line), and the eluent composition observed at the column inlet (dashed line). The change in composition is offset in time due to the delay time (t_d) that results from the time it takes for a change in composition to travel from the mixing point to the column inlet. Particularly problematic for fast gradient separations is the exponential flush-out of the strong solvent observed at the end of the programmed gradient.

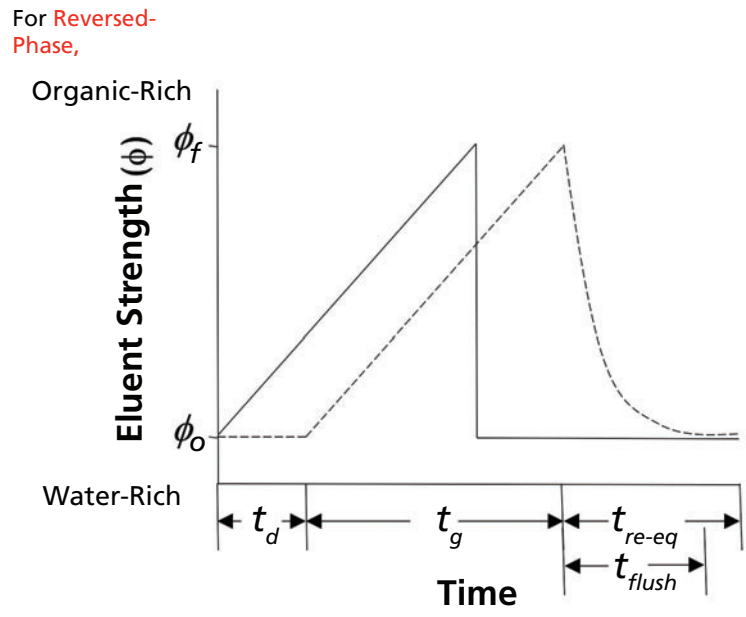

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Figure 2: Chromatogram for the separation of six organic acids on an AQ-C18 column. Chromatographic conditions: olumn, HALO AQ-C18, 50 mm × 2.1 mm, 2.7-µm superficially porous particles; eluent A, 10 mM phosphoric acid in water; eluent B, acetonitrile; gradient elution from 0–75–75–0–0% B from 0–1.5–2.0–2.01–7.0 min; flow rate, 0.50 mL/min; temperature, 40 °C; injection volume, 1 µL; solutes: 1 – tartaric acid, 2 – succinic acid, 3 – propionic acid, 4 – butyric acid, 5 – phenylacetic acid, 6 – 4-butylbenzoic acid. The retention factor of tartaric acid is about 0.5. The pressure at the column inlet at the beginning of the separation was about 160 bar. HALO is a trademark of Advanced Materials Technology, Inc.

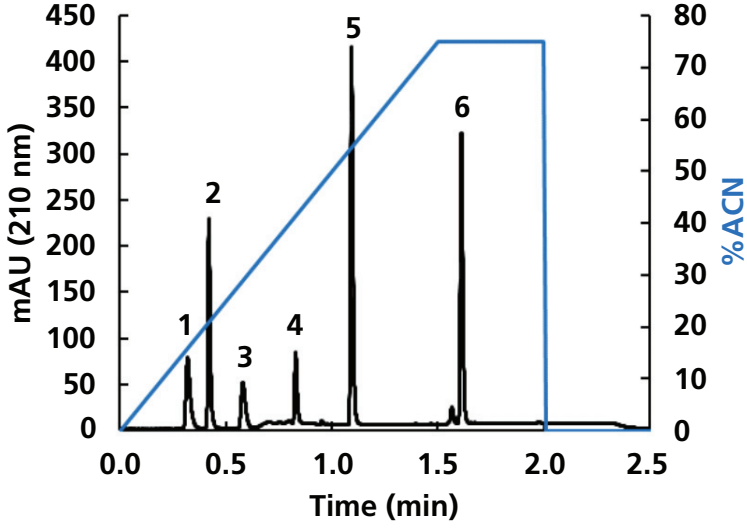
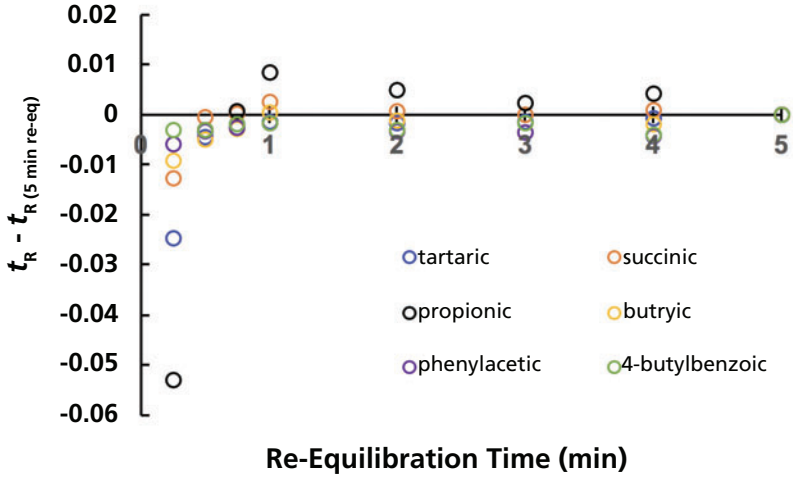


Figure 3: Difference between retention at a given re-equilibration time and the retention time with a re-equilibration time of 5 min for the AQ-C18 column. Conditions are the same as those described in Figure 2.



under hydrophilic interaction liquid chromatography (HILIC) conditions (6,7). The two most impactful outcomes from our own work on reversed-phase separations were:

- Learning that we had to distinguish between two very different "states" of column re-equilibration following gradient elution: i) a state in which retention was highly repeatable as long as the re-equilibration time between separations was fixed and precisely controlled; and ii) a state in which retention was independent of re-equilibration time between separations; we refer to this as a state of *full re-equilibration*.
- Learning that in many situations the time it takes to flush the "strong solvent" (for example, acetonitrile in the case of reversed-phase gradient elution) from the pumping system at the end of a gradient is a big contribution to the apparent required re-equilibration time. This time is a property of the instrument, and has nothing to do with the column itself.

Figure 1 illustrates the exponential flushing out of the strong solvent from the pumping system and connections to the column (in many cases, flushing this strong solvent from the autosampler takes significant time too) that is observed at the end of the solvent gradient program. For practical purposes, in my laboratory we estimate the flush-out time (t_{flush}) as two times the delay time (t_d). In many cases, actual re-equilibration of the column requires flushing with just one to two column volumes of initial eluent beyond t_{flush}. This is why it is important to have a sense for this time, especially when using columns with small volumes (that is, diameters of 2.1 mm or less).

Quantifying the Rate of Re-equilibration in Aqueous Eluents

To quantify the rate of rate-equilibration of an AQ-C18 column in a completely aqueous eluent following a solvent gradient, I varied the re-equilibration time, and tracked the retention times of six probe compounds ranging from the hydrophilic tartaric acid to the more hydrophobic 4-butylbenzoic acid. A representative chromatogram for this mixture is shown in Figure 2, where the solvent gradient starts with completely aqueous eluent and ends with 75% acetonitrile.

Figure 3 shows the difference between average retention time for a given solute from four replicate separations at a given re-equilibration time, and the retention time for that solute with a re-equilibration time of 5 min (which corresponds to about 25 column volumes of re-equilibriation). We observe that retention of the probe solutes is nominally independent of re-equilibration time all the way down to a re-equilibration time of 0.5 min.

The retention of propionic acid appears to vary slightly; however, the retention of this probe is significantly less repeatable than the others, as is indicated by the standard deviations shown in Table 1.

One of the most impactful outcomes from our own work on reversed-phase separations was learning that in many situations the time it takes to flush the "strong solvent" from the pumping system at the end of a gradient is a big contribution to the apparent required re-equilibration time.

These experiments were performed using an instrument optimized to reduce the gradient delay volume to about 70 µL. At a flow rate of 0.5 mL/min, the gradient delay time is about 10 s, and the flush-out time is 20 s. Under these conditions the dead time of the column is about 12 s. Given that the retention times of the probes are already stabilized at a re-equilibration time of 30 s, this means that the column is effectively fully equilibrated after flushing with just one column volume of completely aqueous eluent. Although the retention times of the probes are clearly different with a re-equilibration time of 15 s compared to 30 s, the separations are still highly repeatable as shown by the excellent precision of retention time in the last row of Table 1, so long as the re-equilibration step is also precise.

What About Re-equilibration of a Conventional C18 Column in Highly Aqueous Eluents?

Given how fast the AQ-C18 column equilibrates with the completely aqueous eluent as shown above, it is reasonable to ask if a conventional C18 phase behaves differently under these conditions. In the first work Adam and I did on this topic many years ago, the lowest percentage of starting organic solvent we used was 1% acetonitrile. Figure 4 shows

Figure 4: Difference between retention at a given re-equilibration time and the retention time with a re-equilibration time of 5 min. Conditions are exactly the same as those described in Figure 2 except that a HALO C18 column was used. HALO is a registered trademark of Advanced Materials Technology, Inc.

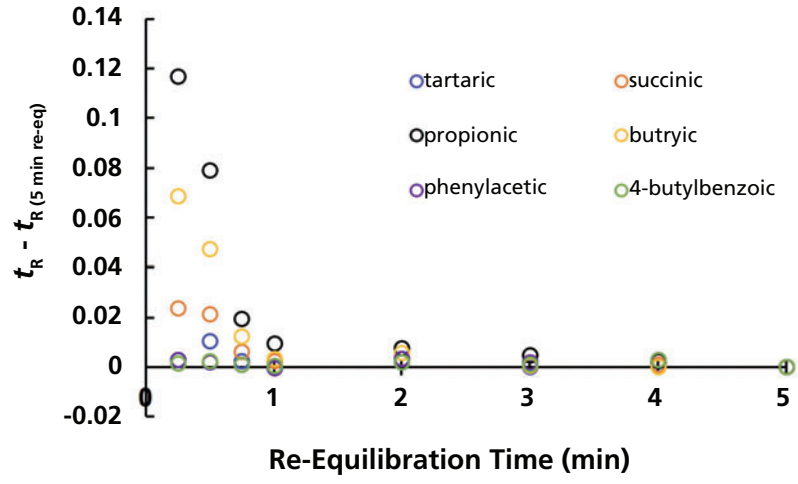


Figure 5: Comparison of chromatograms obtained with the C18 column and a re-equilibration time of 1 min, (a) before, and (b) after turning the flow off for 30 min. The slight splitting of the second peak is due to the partial separation of acetic and succinic acid. Acetic acid was also in the mixture used with the AQ-C18 column, but was not resolved from succinic acid, as shown in Figure 2. Conditions are the same as those described in Figure 4.

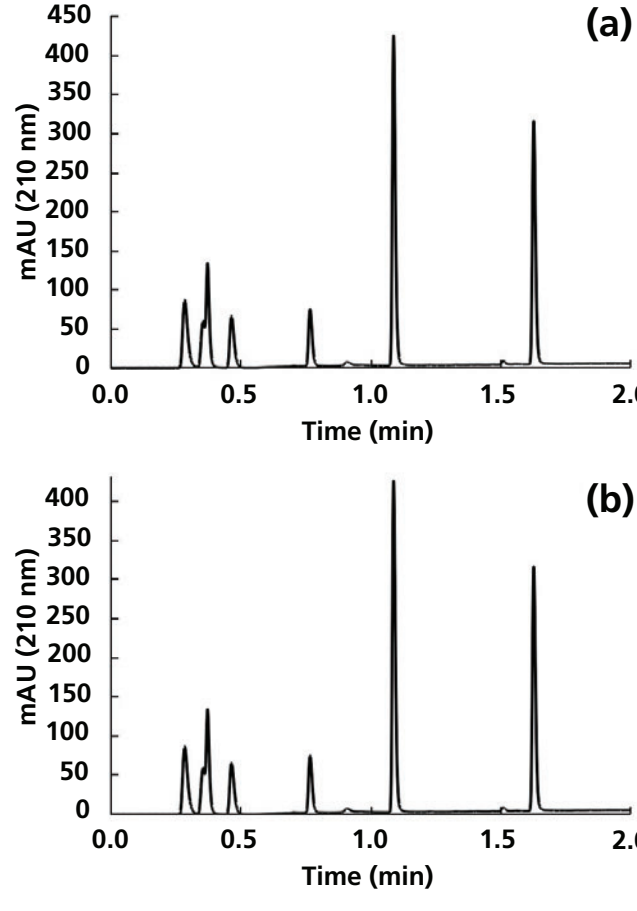


Table 1: Repeatability of retention times of organic acid probe solutes on the AQ-C18 column at different re-equilibration times*

$t_{re\text{-}eq}$ (min)	tartaric	succinic	propionic	butyric	phenylacetic	4-butylbenzoic
5	0.0011	0.0023	0.0072	0.0037	0.0023	0.0014
4	0.0009	0.0018	0.0077	0.0021	0.0027	0.0025
3	0.0009	0.0018	0.0024	0.0036	0.0016	0.0021
2	0.0002	0.0009	0.0031	0.0014	0.0024	0.0024
1	0.0003	0.0008	0.0027	0.0009	0.0016	0.0011
0.75	0.0011	0.0022	0.0059	0.0047	0.0013	0.0029
0.50	0.0013	0.0029	0.0074	0.0045	0.0009	0.0016
0.25	0.0004	0.0003	0.0009	0.0019	0.0007	0.0023

Source: *Reported values are the standard deviations (in minutes) of retention times obtained from four replicate separations.

the results from the same type of experiment described above, where the initial eluent used in the gradient was 100% aqueous, but with a conventional C18 column. These results are similar to those shown in Figure 2 in that retention times are nominally independent of re-equilibration time down to re-equilibration times as short as 1 min. In this case, however, retention times increase as the re-equilibration time is decreased. It is not immediately obvious to me how we might rationalize this result. Nevertheless, we see again that these separations are highly repeatable, even at the shortest re-equilibration time of 15 s. The conclusion here, then, is that the conventional C18 column requires a bit more time to fully equilibrate with the completely aqueous eluent (that is, four column volumes rather than one), but not dramatically more time.

Keeping in mind the result shown in Part 1 of this series that we only observed dewetting of the C18 phase when the eluent flow through the column was stopped for 10 min (2), I did one final experiment to check for this effect under these gradient elution conditions. Figure 5 shows chromatograms for the C18 column before and after turning off the flow for 10 min, both with a re-equilibration time of 1 min, and each after a first "warmup" gradient. We observe that there is no statistically significant difference between the retention times observed in these two cases. It seems that, even if the C18 phase does dewet when the flow is turned off, it is re-wetted quickly during the first gradient such that the separations observed thereafter are indistinguishable from those obtained prior to turning off the flow.

It seems that, even if the C18 phase does dewet when the flow is turned off, it is re-wetted quickly during the first gradient such that the separations observed thereafter are indistinguishable from those obtained prior to turning off the flow.

Summary

In this instalment of "LC Troubleshooting", I have discussed the results of simple experiments aimed at understanding how quickly reversed-phase stationary phases equilibrate with highly aqueous eluents when they are used as the initial eluent in solvent gradient elution. We observe that an aqueous-compatible AQ-C18 is effectively fully equilibrated with a completely aqueous eluent after flushing with just one column volume of initial eluent beyond the flush-out time of the instrument, at least for the solutes studied here. It is likely that other solutes that may be more sensitive to the chemical state of the stationary phase might require longer re-equilibration periods. Finally, similar experiments with a conventional C18 column showed that this phase required slightly, though not dramatically, longer times to fully equilibrate with a completely aqueous initial eluent.

Acknowledgements

I'd like to thank Tom Waeghe for our discussion of highly aqueous eluents that eventually led to the experiments described in this article.

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New HPLC Systems and Related Products Introduced in 2018–2019: **A Brief Review**

Michael W. Dong, Perspectives in Modern HPLC Editor

This instalment describes high performance liquid chromatography (HPLC), mass spectrometry (MS), and related products introduced at Pittcon 2019 and during the year prior. It reviews new HPLC and MS systems, modules, chromatography data systems (CDS), and other related software and summarizes their significant features and user benefits. A brief description of instrumentation trends and the current market is also included.

This instalment marks my 7th anniversary as a columnist for *LCGC Europe*'s "Perspectives in Modern HPLC" column. My first contribution covered new high performance liquid chromatography (HPLC) product introductions in 2013. And though even then our coverage of new products reached well beyond Pittcon, the event remains a major event in analytical chemistry and one where many new products continue to be launched. And for that reason, we produce this review after Pittcon each year, and include in our coverage the sense of the field that we get from attending.

At the Pittcon 2013 meeting, I recall the country was still feeling the aftermath of the 2008 financial crisis and the host city, Philadelphia, was not immune to its effects. Much has changed in the years since. The economy is in much better shape, and Philadelphia is thriving, undergoing an urban renewal with many new buildings, hotels, and upscale restaurants springing up in Center City.

Philadelphia is an appropriate conference site for conferences in the Northeast. The city has many industrial and pharmaceutical research centres, as well as plenty of notable universities such as the University of Pennsylvania, Drexel University, and Temple University. Philadelphia's proximity to major cities also helps to make it an excellent site; it is within a two- or three-hour drive of both New York City and Washington D.C. The Pennsylvania Convention Center is a few blocks from Independence Hall, the famous site where the U.S. founding fathers signed the Declaration of Independence in 1776 and later the Constitution of the United States in 1787. Besides the historical richness of the location, the convention centre is across the street from the Reading Terminal Market and Philadelphia's Chinatown, where one finds many ethnic eateries offering great food at reasonable prices.

Pittcon 2019 boasted ~13,000 attendees from industry, academia, and government agencies representing over 90 countries. There were 200 plus technical sessions, including plenary lectures, invited, contributed, and award symposia, workshops, posters, and networking sessions, as well as ~90 short courses, and a huge three-day exposition with more than 700 vendors.

Appropriate to the historical significance of the city, Dr. Fenella G. France, the Chief of the Preservation Research and Testing Division at the Library of Congress, gave the plenary lecture on "Preserving and Revealing History—Challenges of a Cultural Heritage Scientist". In the Wallace H. Coulter lecture, Nobel Laureate Professor Fraser Stoddart discussed a "New World of Wonders on Materials Beyond Cyclodextrins". Other presentations by award winners in separation sciences were delivered by Weihong Tan of the University of Florida, by the founders of Supelco, Walter Supina and Nicholas Pelick, and by Peter Schoenmakers of the University of Amsterdam, Milos Novotny of Indiana University, and Ken Broeckhoven of Vrije Universiteit of Brussel.

Trends in HPLC and Mass Spectrometry (MS) Products and the Current Market

Before describing any new products introduced over the last year, I will start with a brief discussion of modern trends in HPLC and MS instrumentation, and the current market for them. The market for HPLC and MS instruments was measured at ~$10 billion in 2018. This market size estimate appears surprisingly low, especially when considering the impact that these instruments have in driving scientific discovery (1–3).

Current Market for HPLC Systems

Four major HPLC manufacturers, Waters, Agilent, Thermo Fisher Scientific, and Shimadzu, have been consistently responsible for more than 80% of the global market in recent years.

Waters Corporation has been the HPLC market leader since the 1970s. They were the first to commercialize ultrahigh-pressure liquid chromatography (UHPLC) technology in 2004 with their Acquity

An entire chromatographic system in a small 6x6 inch footprint.

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Figure 1: Amino acid analysis of Roswell Park Memorial Institute (RPMI) as (a) 1650 cell culture media, and (b) an amino acid standard solution using the Agilent AdvanceBio amino acid analysis (AAA) column (2.7 µm, superficially porous particles). Mobile phase A: 10 mM disodium phosphate (Na_2HPO_4), and 10 mM sodium borate ($Na_2B_4O_7$) pH 8.2; Mobile phase B: 45:45:10 (*v/v/v*) acetonitrile–methanol–water. The system is capable of quantitating both primary and secondary amino acids (prolines and hydroxyproline). Details are available from reference 8.

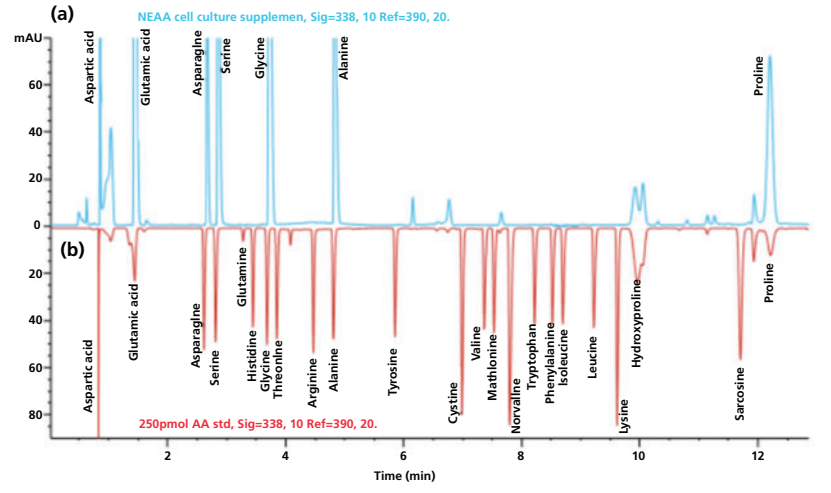
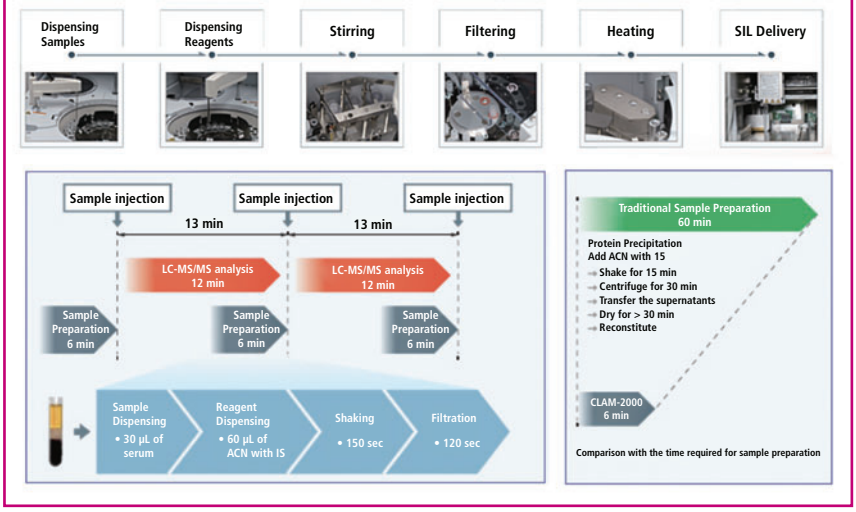


Figure 2: Automation workflow schematics for the Shimadzu CLAM-2030 for analysis of serum with some of the supported functionalities compared to those of a traditional manual workflow Details are available from reference 10. ACN = acetonitrile.



UPLC instrument (4–6). Their success has persisted with their newer UHPLC systems such as the Acquity H-Class, I-Class, M-Class, and the Acquity Arc systems. Moreover, their Empower CDS has enjoyed widespread acceptance by regulators as demonstrated by its ubiquity in pharmaceutical laboratories.

Agilent's HPLC systems are popular in research laboratories thanks to their modular 1100, 1200, 1260, and 1290 series HPLC product lines. Agilent's current product line consists of the second-generation UHPLC Infinity II series, which includes the 1290 binary and quaternary, the 1260, and 1220 series. Recently revamped versions of Agilent's OpenLab CDS greatly improved data handling and included regulatory compliance features that have enhanced its competitiveness in quality control laboratories.

Thermo Fisher Scientific, already well known for its innovative MS products, became a serious competitor in the chromatography market following the acquisition of Dionex in 2011. The acquisition added ion chromatography, the Chromeleon CDS, and the Ultimate 3000 UHPLC systems to its portfolio. The introduction of the Vanquish UHPLC in 2014 further bolstered Thermo's presence in chromatography.

Shimadzu offers the Prominence and Nexera Series, two well-developed, integrated, and modular HPLC and UHPLC product lines covering microflow through preparative purification applications. These products are supplemented by preconfigured systems for specific applications (such as the Cannabis Analyzer) and automated sample preparation modules (such as the Clinical Laboratory Automation Module, or CLAM-2030, for LC–MS). The recent introductions of its supercritical fluid chromatography (SFC) system that includes preparative SFC and a supercritical fluid extraction system, and a more complete MS product line, add to Shimadzu's presence in the food, environmental, pharmaceutical, quality control, and industrial markets.

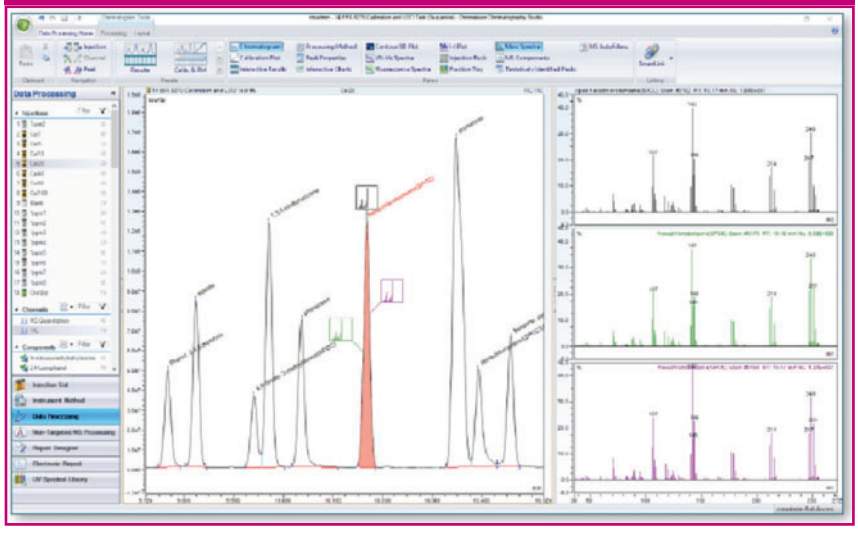
Other HPLC Companies: Other global providers of HPLC instruments include Danaher (Sciex, Eksigent), Jasco, Knauer, Hitachi, and PerkinElmer, as well as producers of HPLC modules such as Metrohm, Scientific Systems (Teledyne Isco), Bischoff, LEAP Technologies, Showa Denko, Sonntek, Spark Holland, Tosoh Bioscience, and Wyatt Technologies. Additionally, a few new HPLC manufacturers have seen some localized success in recent years, particularly in academia. New entries in the HPLC market find it difficult to compete with the major brands but can penetrate the market with niche instruments, such as the portable LC system (Focus) launched by Axcend in 2018.

Mass Spectrometry (MS): Mass spectrometers separate analytes by their mass-to-charge ratio (*m/z*) in a high vacuum and offer unprecedented analytical sensitivity and selectivity for ionizable

Table 1: Summary of new HPLC, MS, and CDS product introductions from 2018 through Pittcon 2019

Exhibitor/Vendor	Product Name	Description
ACD/Labs	ACD/Method Selection Suite	Software that streamlines chromatographic method development by combining physicochemical property predictions with method optimization tools
Applied Separations	Zephyr High-Pressure Pump	A high-pressure preparative pump module that supports mass flow control
Bruker	timsTOF	A high-resolution trapped ion mobility MS system for analysis of isomeric compounds
Buchi Labortechnik AG	Pure C-850 FlashPrep System	An all-in-one dual-use flash or preparative HPLC system for purification of organic compounds
Agilent	InfinityLab Workflow Solutions	Provides system configurations that support bioinert analysis, gel permeation chromatography, and amino acid analysis
	OpenLAB 2.3 CDS and ChemStation Edition	Updated OpenLAB version with an improved user interface, data handling, and regulatory compliance feature to support mainstream CDS needs for UV and MS detection. The ChemStation Edition is used to provide instrument control of Agilent's other instruments such as GC, CE, and 2D systems.
Leman Instruments	Gas Generators	Provide high-purity nitrogen and hydrogen at a flow rate up to 500 mL/min
Merck KGaA (MilliporeSigma)	Solvents for LC–MS	A new line of ultrapure HPLC–MS-grade solvents
Postnova	EAF2000 Electrical Flow FFF Series	A separation system based on field-flow fractionation for separation of particles, polymers, and proteins
PerkinElmer	QSight Series 400	Triple-quadrupole MS instrument for high-throughput analysis now offered with QSight LX-50 UHPLC
S-Matrix	New enhancements to Fusion QbD Software	"PeakTracker" using UV and MS data to enhance HPLC method development
Shimadzu Scientific Instruments	Nexera Series UHPLC	New compact UHPLC Series with enhanced productivity and automation features
	Nexera Bio UHPLC	A biocompatible 9000 psi or 600 bar UHPLC
	Prep SFC	A preparative-scale supercritical fluid chromatography system
	LCMS 9030 Q-TOF	The LCMS-9030 delivers high resolution and accurate mass with patented technologies
	CLAM 2030 sample preparation module for LC–MS	An automated sample preparation module for blood, urine, serum, and plasma samples using the Nexera X2 UHPLC and triple-quadrupole MS systems
	Hemp Analyzer	A dedicated HPLC system for cannabinoids in hemp
Thermo Fisher Scientific	Orbitrap ID-X Tribrid MS	A tribrid MS (Q-orbitrap-ion trap) system, designed for small-molecule identification and structure elucidation
	Q-Exactive UHMR	An orbitrap hybrid system with a mass range of 350–80,000 *m/z* and resolution up to 200,000 and mass accuracy of 3 ppm
	ISQ EM SQ MS	A single-quadrupole MS system with heated ESI or APCI source with control by Chromeleon CDS
	Chromeleon 7.2 CDS	Updated CDS to support instrument control and data processing for Thermo Scientific MS systems including SQ, TQ, and Exactive Orbitraps. Chromeleon XPS-Open Access is a simplified user interface for walk-up and multiuser access by non-chromatographers.
Tosoh Bioscience	$Lens_3$ MALS Detector	A new multi-angle laser light scattering detector for absolute measurements of molecular weights of polymers
Waters	Acquity Class Plus, H-Class Plus, H-Class Plus Bio	Updated quaternary and binary UHPLC systems with reduced system dispersion and dwell volumes
	BioAccord System	An integrated LC–MS system for biopharmaceutical analysis based on Acquity I-Class Plus, RDa TOF, and Unifi
	Renata DX Screening System	An in-vitro diagnostic device designed for dried blood analysis based on flow-injection triple-quadrupole MS
	Dart QDa System	An automated sampling system for the Waters compact QDa MS for direct introduction of solid samples
	Empower 3 CDS	Improved version with enhanced regulatory compliance, easier peak integration, and a new toolset for administrators
Wyatt Technology	DAWN, microDAWN, MiniDAWN	Multi-angle light scattering instrument with optional embedded dynamic light scattering detector for determination of absolute molar mass, size, conformation, and conjugation of macromolecules and nanoparticles
	Optilab, microOptilab	Differential refractive index detector for HPLC and UHPLC
	ViscoStar, microViscoStar	Differential viscometer for HPLC and UHPLC

Figure 3: A screenshot of the Chromeleon 7.2 chromatography data system (CDS) displaying the total ion chromatograms (TIC) and mass spectral plots. Chromeleon 7.2 contains the necessary MS-specific data views, data processing, and reporting capabilities to streamline both chromatography and MS quantitation workflows in a single application.



compounds. HPLC–MS is arguably the most powerful analytical technique in scientific discovery, particularly in biosciences (3–4). Major types of MS include the following:

- Magnetic sector: the oldest type of MS system, using a permanent magnet; primarily used in gas analyzers.
- Single quadrupole: the most common type of MS instrument, with unit mass resolution useful for peak identification and confirmation.
- Triple quadrupole or tandem MS: With two single quadrupoles in series with a middle radio frequency-only quadrupole for collision-induced fragmentation, triple quadrupole or tandem MS instruments use multiple reaction monitoring as the gold standard for trace quantitation of complex samples in bioanalytical and multiresidue assays.
- Ion trap: a compact type of MS system useful for structure elucidation by trapping analyte ions and performing sequential fragmentation.
- Time-of-flight (TOF): a high-resolution type of MS system using a long flight tube that differentiates ions by measuring their times of flight. A reflectron is often used to extend the flight path (and to reduce the overall instrument footprint).
- Fourier-transform ion cyclotron resonance (FT-ICR): a type of MS offering very high resolution and mass accuracy based on the cyclotron frequency of the ions in a fixed magnetic field cooled by liquid helium and nitrogen.
- Orbital ion trap: an elliptical ion trap instrument that utilizes a Fourier transform algorithm to yield very high mass resolution for qualitative and quantitative analysis. This type of instrument is more compact than FT-ICR and is a proprietary product marketed solely by Thermo Scientific.
- Hybrid and tribrid: MS instruments combining two or more types of MS such as QTOF or Q-orbital trap-ion trap are particularly useful for structure elucidation and the analysis of complex samples (proteomics) and biomolecules.

It is not surprising that the top four HPLC manufacturers are also successful providers of MS instruments. Waters entered the MS market via their acquisition of Micromass in 1997 and continues to offer a competitive line of MS instruments. Agilent (formerly Hewlett-Packard) was an early manufacturer of single-quadrupole MS instruments for gas chromatography (GC). They offer a wide choice of single-quadrupole, triple-quadrupole, TOF, and QTOF-MS instruments. Finnigan Instruments, acquired by Thermo Scientific in 1990, was the first maker of single-quadrupole and ion-trap instruments. Thermo Scientific is currently a leading manufacturer of diversified MS equipment including single-quadrupole, ion-trap, orbital-ion trap, FT-ICR, and various hybrid and tribrid systems. Shimadzu has recently expanded its MS offerings of single-quadrupole and triple-quadrupole systems to include QTOF equipment.

Sciex (a subsidiary of Danaher) was the first company to introduce triple-quadrupole systems and continues to dominate the market for bioanalytical analysis, with instruments like the 6500+. Bruker, the leader in nuclear magnetic resonance (NMR) instruments, also supplies FT-MS, ion-trap, triple-quadrupole, TOF, QTOF, and ion mobility MS systems. Other MS manufacturers include Advion, Hitachi, Jeol, LECO, and PerkinElmer. Additionally, there have been several recent entries of compact and transportable MS instruments from 1st Detect, 908 Devices, and Microsaic Systems (3).

Emerging Trends for HPLC and MS Systems: The most important development in HPLC was the introduction of UHPLC instruments. Compared to HPLC, UHPLC is capable of higher operating pressures and lower system dispersion (5–7) used in conjunction with sub-2-µm particle columns. The debut of the first commercialized UHPLC system in 2004 spurred on waves of UHPLC instrument introductions by other major manufacturers. Current HPLC systems available include UHPLC (>15,000 psi or 1000 bar), conventional HPLC (<6000 psi or 400 bar), intermediary (9000–12000 psi or 600–900 bar), dual-path systems (Acquity Arc, Thermo Vanquish Flex, and Duo), and systems preconfigured for specific workflows or applications such as method development, two-dimensional LC (2D-LC), and cannabis analysis).

MS is currently undergoing a boom in development fueled by the increasing demand from the pharmaceutical, biotechnology,

industrial, environmental, food, and clinical diagnostics industries. New instruments are trending towards more compact laboratory systems such as the Waters Acquity QDa, Advion expression CMS, and Agilent Ultivo. Recent trends include highly portable point-of-use instruments, such as those from 1st Detect and 908 Devices, and high-resolution hybrids or tribrids for accurate mass analysis of complex mixtures, as afforded by Thermo's Orbitrap, or QTOFs by many manufacturers.

New HPLC, MS, and CDS Products Introduced in 2018–2019

Although new introductions of HPLC systems appear to be slowing down, manufacturers are turning their attention to tailored applications and sample preparation systems, particularly for LC–MS.

Table 1 lists new HPLC, MS, and CDS products, in alphabetical order by supplier name, introduced at Pittcon 2019 or in the prior year, followed by descriptions of and commentaries about each product.

New HPLC and UHPLC Systems and Line Extensions

New UHPLC systems introductions have slowed while manufacturers appear to be focusing on LC–MS line extensions for specific applications, such as clinical diagnostics, and sample preparation.

Agilent is offering several new Infinity-Lab Workflow Solutions to support bioinert analysis, gel permeation chromatography (GPC), and amino acid analysis. Agilent's 1260 Infinity II Bio-Inert system is a titanium-based system suited for biomolecule analysis. The 1260 Infinity II Multi Detector GPC system is offered with an optional viscometer, refractive index, or light scattering detectors for organic polymers. Agilent's Amino Acid Analysis (AAA) system uses automated precolumn derivatization with ortho-phthaldialdehyde (OPA) and fluorenylmethyloxycarbonyl chloride (FMOC) reagents and UV or fluorescence detection. An example chromatogram for the analysis of both primary and secondary amino acids in a cell culture media is shown in Figure 1 (8).

Buchi Labortechnik AG has introduced the Pure C-850 FlashPrep System, an all-in-one, dual-use flash or prep HPLC system for purification of organic compounds. This system includes both hardware and dedicated software for purification projects up to 100 mL/min and 4300 psi or 300 bar with UV or evaporative light-scattering (ELSD) detection.

Shimadzu Scientific Instruments made a significant impact at Pittcon 2019 with the introduction of a new compact Nexera Series UHPLC system with higher productivity and performance as well as automation features such as auto startup and shutdown, auto diagnostics and recovery, and mobile phase monitoring. The system is capable of injecting a sample every 7 s and can accommodate ~17,000 samples with its new plate changer. Key components of the Nexera UHPLC series include the mobile phase monitor mentioned above, the SPD-40, SPD-40V, or SPD-M40 absorbance detector, the LC-40 series solvent delivery unit, the SIL-40 series autosampler, and a new slim-line column oven.

Shimadzu also introduced the Nexera Bio UHPLC (9000 psi or 600 bar). This system features inert materials resistant to high-salt mobile phases, such as a carbon-coated pump head, gold-plated ferrules, stainless steel-clad PEEK tubing, and a ceramic injection needle.

Shimadzu also introduced the Hemp Analyzer, a dedicated HPLC platform dedicated for quantitative analysis of cannabinoid content in hemp. This system includes hardware, software, consumables, and application notes featuring three proven methods dedicated to cannabinoid analysis in hemp.

Waters updated its quaternary and binary Acquity UPLC systems (I-Class Plus, H-Class Plus, and H-Class Plus Bio) with a reduced system dispersion of 7 to 12 µL and a diminished dwell volume of 75 to 400 µL. Furthermore, improvements to the system in the solvent degasser, in sample heating and cooling, as well as in novel sampling needle surface treatments, have greatly improved the analytical performance of these systems.

Waters also introduced the BioAccord System, an integrated LC–MS system for biopharmaceutical analysis based on the Acquity I-Class Plus and the new Acquity RDa TOF-MS system (7000 amu and mass resolution of 10,000). The BioAccord is capable of automated workflows for intact mass and subunit analysis, peptide mapping, and released glycan assays. The system provides a new level of user experience featuring a one-button start-up for power on, pump down, and to initial system setup for any trained chromatographer to generate accurate mass spectrometry data (9). This system is designed to use mass spectrometry data and informatics (Waters Unify Scientific Information System) to simplify the characterization of complex biopharmaceuticals for development and quality control laboratories.

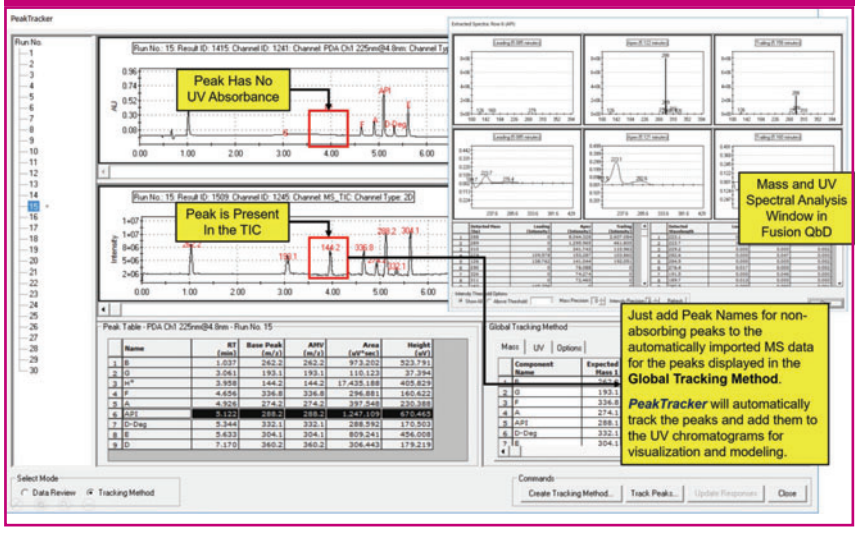
New HPLC Modules

Applied Separations' Zephyr high-pressure pump is a unique preparative pump module that supports mass flow control capable of 330 mL/min flow at a pressure of up to 900 bar for isocratic or multistep gradient operation.

Tosoh Bioscience introduced the $Lens_3$ MALS detector, a new multiangle laser light scattering detector compatible with HPLC and UHPLC for absolute measurements of molecular weights of polymers based on the radii of gyrations of particles in the range of 2 to 50 nm. This detector integrates the best of both MALS and low-angle light scattering (LALS).

Wyatt Technology introduced DAWN, a new multi-angle light scattering instrument with optional embedded dynamic light scattering detector for determination of absolute molar mass, size, conformation, and conjugation of macromolecules (proteins and polymers) and nanoparticles. DAWN is configured for HPLC whereas microDAWN is the version used for UHPLC. MiniDAWN is used for characterization of macromolecules and nanoparticles up to 50 nm in radius. Wyatt also introduced Optilab and ViscoStar, a differential refractive index detector and a differential viscometer, respectively, for HPLC.

Figure 4: A screenshot of the PeakTracker user interface, illustrating some of the new functionalities showing UV and MS data within S-Matrix's Fusion Quality by Design (QbD) software for HPLC method development.



Both detectors are available as micro versions for UHPLC.

New Mass Spectrometers (MS)

Bruker introduced a high-resolution trapped ion mobility MS instrument for analysis of isomeric compounds with a mass resolution of ~200. Ion mobility MS is particularly powerful when used in conjunction with a high-accuracy MS system for characterization of complex samples containing isomeric sugars and lipids.

The PerkinElmer QSight 400 series is a high-sensitivity triple-quadrupole LC–MS system with StayClean and dual source (electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) technology for robust high-throughput analysis. It is offered with PerkinElmer's QSight LX-50 UHPLC instrument with a binary pump (18,000 psi or 1250 bar), a dual-needle autosampler, and column oven.

Shimadzu Scientific Instruments is aggressively increasing its MS product portfolio and now offers a new LC–MS 9030 QTOF instrument that uses patented technologies to deliver both high resolution and accurate mass. Innovations include high-efficiency ion guides, proprietary UFgrating, iRefTOF, and UF-FlightTube technologies. Mass ranges are 10–2000 amu for the quadrupole and 10–40,000 amu for TOF. Mass resolutions are 0.8 u and 30,000 full width at half maximum (FWHM), with a mass accuracy of <1 ppm.

Shimadzu is also stepping up its game in the Clinical Laboratory Automation Module by offering the CLAM-2030 for LC–MS. The CLAM-2030 is a fully automated sample preparation module for Shimadzu's Nexera X2 UHPLC instruments and family of triple-quadrupole MS instruments (the 8060, 8050, 8045, and 8040) for blood, urine, serum, and plasma samples. Supported functions include dispensing of samples and reagent, derivatization, stirring, filtering, heating, and sample transfer to autosamplers. An optional module configuration is available for automated toxicological screening that includes supported protocols for a 161-analytes panel. Figure 2 shows an automated workflow schematics for the CLAM-2030 in the analysis of serums with supported functionalities against that of a traditional manual workflow (10).

Thermo Scientific introduced the Orbitrap ID-X Tribrid MS consisting of a quadrupole (50–2000 amu), an orbital ion trap (up to 500,000 mass resolution and a scan rate of 30 Hz), and a dual-cell linear ion trap designed for small-molecule identification and structure elucidation.

Thermo Scientific also introduced the Q-Exactive UHMR, an orbital ion trap hybrid system with a mass range of 350–80,000 *m/z*, a mass resolution to 200,000, a scan rate of 12 Hz, and a mass accuracy of 3 ppm.

In addition, Thermo Scientific introduced the ISQ EM single-quadrupole mass spectrometer with a heated ESI, APCI, or a dual source. It has an extended mass range of 10–2000 amu, a scan rate of 20,000 Da/s, and mass accuracy of <0.1 Da. Control and data handling are by the Chromeleon 7.2 CDS.

Waters introduced the Renata DX Screening System, an in-vitro diagnostic device designed for dried blood analysis based on flow-injection MS/MS. The Renata DX incorporates the XevoTQD IVD MS, the Acquity UPLC I-Class IVD Binary Solvent Manager, and the 3777C IVD Sample Manager with appropriate MS data and application software. The RenataDX Screening System is manufactured as a U.S. FDA Class I medical device.

The Waters Dart QDa System is an automated sampling system for the Waters compact QDa single-quadrupole mass spectrometer using a direct analysis in real time (DART) ion source for direct introduction of solid samples. It can perform rapid fingerprinting of foods and food ingredients and verify sample authenticity and adulteration.

New Chromatography Data Systems (CDS)

Agilent launched the new OpenLAB CDS in 2015 with an improved user interface, data handling, and regulatory compliance features required in pharmaceutical, food, and environmental laboratories. The current version of OpenLAB 2.3 supports additional LC and LC–MS functionalities (MS peak purity and diode array data tools), advanced reporting, e-signature capabilities, and direct connections to enterprise content management (OpenLAB 3 ECM for multivendor connectivity), laboratory information management systems (LIMS), using a sample scheduler, and electronic laboratory notebooks (ELN). The latest ChemStation Edition provides

specific instrument control of Agilent's other instruments such as GC, capillary electrophoresis (CE), and 2D-LC systems.

Thermo Scientific's Chromeleon 7.2 CDS now supports instrument control and data processing for Thermo Scientific's LC–MS and GC–MS systems for single-quadruple, triple-quadrupole, and Exactive Series Orbitrap systems. It supports infrastructure as a service (IaaS) cloud deployment, reducing the resources needed for training and laboratory operation. This CDS offers an extensive toolset for enhanced regulatory compliance, and automated workflow solutions support in a global network. Figure 3 shows a screenshot of the Chromeleon 7.2 displaying total ion chromatograms (TIC) and mass spectral plots. Chromeleon XPS Open Access is a simplified user interface for walk-up and multiuser access by non-chromatographers. The Waters Empower 3 CDS has been updated with enhanced regulatory compliance features, an easier peak integration algorithm, and new configuration tools for system administrators.

HPLC Method Development Software

ACD/Labs introduced the ACD/Method Selection Suite, which streamlines HPLC and UHPLC method development by combining physicochemical property predictions with method optimization tools to define better starting conditions, refine key separation parameters, and estimate retention times.

S-Matrix has introduced two new capabilities for its Fusion QbD HPLC method development software: PeakTracker and Rs–Map Response. PeakTracker automates, optimizes, and simplifies the use of photodiode array UV and MS data for LC and LC–MS method development. The "Rs–Map Response" feature uses the retention time and peak shape parameter modelling technologies to predict *United States Pharmacopeia* (*USP*) resolution from retention and peak shape data. Figure 4 shows a screenshot illustrating some of the new functionalities of PeakTracker within the Fusion QbD software.

Solvents and Gases for HPLC and MS

Leman Instruments introduced gas generators to provide high-purity nitrogen and hydrogen (99.999%) at a flow rate up of 250 or 500 mL/min for GC and MS instruments.

Merck KGaA (MilliporeSigma) introduced a new line of ultrapure LC–MS-grade solvents (LiChrosolv Brand, acetonitrile, methanol, and water) that have lower levels of particulate and chemical contaminants, including lower levels of polyethylene glycol (PEG).

Other Separation Systems

Postnova introduced the EAF2000 Electrical Flow FFF series, a separation system based on the principle of electrical and asymmetrical field-flow fractionation (FFF) for the separation of particles, polymers, and proteins. Separation by particle size and particle change based on electrophoretic mobility can be achieved. Shimadzu Scientific Instruments introduced a new Prep SFC system in collaboration with the Emerging Technologies Consortium to support purification in drug discovery and other industries.

Summary

This instalment summarizes new HPLC and MS products introduced at Pittcon 2019 and in the prior year and describes modern trends of these products and the market for them.

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A Q&A

Next-Gen Multi-Angle Light Scattering

Daniel Some, PhD
Principal Scientist
Wyatt Technology

New HPLC/UHPLC product line offers more robust measurements and increased uptime.

In March 2019, Wyatt Technology Corporation launched its next-generation of online multi-angle light scattering (MALS), refractive index, and differential viscometry detectors for high performance liquid chromatography (HPLC) and ultrahigh-pressure liquid chromatography (UHPLC) systems. LCGC recently asked Dan Some, PhD, Principal Scientist at Wyatt Technology, about the advancements made in Wyatt's product line for absolute macromolecular characterization.

LCGC: Can you explain what is size-exclusion chromatography (SEC)-MALS and why it is of interest to protein and polymer scientists?

Some: SEC-MALS couples online multi-angle light scattering detection and other online detectors (such as refractive index and differential viscometry) to size-exclusion chromatography. With this technique, the only purpose of the SEC column is to separate the different molecules from each other. The actual characterization of the molecules takes place solely within the detectors, which allows absolute characterization to be performed. This method does not depend on the retention time within the column, the conformation of the molecule, or a molecule's interactions with the column. Thus, in SEC-MALS we do not encounter the errors of typical analytical SEC where reference molecules are run even though they might (and often do) behave differently on the column than your molecules.

This technique allows us to analyze monodispersed molecules, such as proteins, or polydispersed macromolecules, such as heterogeneous polymers, to determine their molecular weight, size, conformation, and branching ratio. The oligomeric state of proteins in native solution can be determined, resulting in a much better understanding of the essential biophysical properties of the macromolecules than can be obtained from analytical SEC.

LCGC: What would you say is new and improved in Wyatt's DAWN, Optilab, and ViscoStar products launched in March 2019?

Some: In March, we launched a re-envisioned product line of the DAWN, Optilab, and ViscoStar online detectors for SEC with multi-angle light scattering. While these detectors offer the same industry-leading sensitivity, range of measurements, and other features

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"Across all these products—DAWN, Optilab, and ViscoStar—the key added value is enhanced productivity arising from the new Smart Services Platform. The platform includes the System Ready Monitor and System Health Indicators, ensuring users do not waste runs due to sub-optimal chromatography conditions."

that our customers are used to for maximum characterization of their macromolecules, the new products have a sexy, new modern look and feel. For example, there is a large capacitive touchscreen that allows users to interact more intuitively with the instrument and access the information that they need from the front panel. The instruments also have improvements in serviceability and maintainability, achieved by making them more modular. In fact, individual modules can be swapped out on-site. In addition, CheckPlus software performs a full diagnosis and sends those diagnostics to an engineer at Wyatt for a more in-depth look. Depending on what the engineer decides, a technician can come on-site and swap out the modules with very little downtime.

LCGC: What are some of the newest innovations in the DAWN line, which has been Wyatt's flagship product for 37 years?

Some: In previous generations, we worked on improving the technical specifications, getting higher sensitivity, expanding the range of measurements, and adding user interface improvements. Key in the new generation of DAWN detectors is the built-in intelligence that assists users in knowing when their SEC-MALS system is ready for optimal measurements, when the noise level is low enough, and when the system is fully equilibrated. In addition, swappable flow cells allow for a new flow cell to be swapped in without the need for laser alignment. Opto-mechanics are more robust, and modifications to the optical design further reduce stray light. Dedicated slots for the WyattQELS dynamic light scattering module have been added so that, rather than sacrificing one of the MALS angles as with the previous models, WyattQELS gets its own slot, and the software automatically identifies into which angle the user has placed the WyattQELS optical fiber.

LCGC: What do you see as the main value to customers in the updated product line?

Some: Across all these products—DAWN, Optilab, and ViscoStar—the key added value is enhanced productivity arising from the new Smart Services Platform. The platform includes the System Ready Monitor and System Health Indicators, ensuring users do not waste runs due to sub-optimal chromatography conditions. The platform's self-diagnostics and CheckPlus instrument log application permit remote evaluation by our service team, and full on-site repair service.

LCGC: What can Wyatt offer to those who use UHPLC?

Some: microDAWN is the multi-angle light scattering online product for use with UHPLC. microOptilab is the refractive index detector UHPLC, and microViscoStar is the differential viscometer for UHPLC. Users can get the complete range of characterization of molecular weight, size, and conformation, with all the benefits of UHPLC, which means faster runs, lower sample consumption, lower mobile phase consumption, and enhanced productivity.

LCGC: Where can readers go to learn more about SEC-MALS technology and applications?

Some: The best place to start is our website, which is www.wyatt.com, and there we have information about the theory of SEC-MALS, light scattering, and other technologies. Folks can learn about the various solutions that the instruments offer, the applications they provide, the different types of analytes that can be analyzed, the industries served, and the products' features and benefits. There is also an extensive library of webinars that can be viewed to learn more.

With a long history in scientific instrumentation, Wyatt Technology is a leader in light scattering instruments, accessories, software and services for determining the properties of macromolecules and nanoparticles in solution.

New Liquid Chromatography Columns and Accessories for 2019

David S. Bell, Column Watch Editor

Our annual review of new liquid chromatography (LC) columns and accessories, introduced at Pittcon and other events.

This article covers liquid chromatography (LC) columns and accessories commercially released after Pittcon 2018 through this year's conference held in Philadelphia, Pennsylvania, USA. *LCGC* once again sent out a survey in early 2019, asking vendors to supply information about products launched after Pittcon 2018. Other areas of interest, such as gas chromatography (GC), chromatographic instrumentation, and sample preparation, will be covered elsewhere. Given that information for this article is obtained over the course of many months, it is very possible that some information has been missed. The reader is encouraged to check with specific vendor sites for additional products, as well as more detailed information regarding what is presented in this article.

The vendors that responded to the survey with high performance liquid chromatography (HPLC) and ultrahigh-pressure liquid chromatography (UHPLC) columns are listed in Table 1. This year, as in past, the product range is highly varied. The entries can be initially categorized as targeting small molecules or large molecules. Within these categories, the products can be further separated based on the type of particle to which the phases are bonded. Broadly speaking, products from this year were based on superficially porous particles (SPP) or fully porous particles (FPP). Lastly, the products are identified by the specific modes of chromatography (such as reversed-phase or size-exclusion) they employ. Trends noted throughout the article are based on comparisons to previous yearly reports (1–3).

Trends noted throughout the article are based on comparisons to previous yearly reports.

Columns for Small-Molecule Separations

Reversed-Phase and HILIC: The product offerings assigned to the small-molecule category, intended for reversed-phase and hydrophilic interaction liquid chromatography (HILIC), are listed in Table 2. A total of 17 new entries, including multiple column chemistries within a new product line, are shown. The new columns are split nearly evenly between SPP and FPP architectures.

Advanced Materials Technology (AMT) extended its portfolio of SPP phase offerings by launching a C30 chemistry on a 160 Å pore size particle and a biphenyl phase on its 90 Å support. C30 chemistries provide shape selectivity that often results in improved separation of long-chain, structurally related isomers. The company notes that the phase is designed to deliver fast separations, and is 100% aqueous compatible. Biphenyl phases are noted for providing pi-pi interactions, as well as shape selectivity, and often find application in the analysis of pain management drugs, cannabinoids, pesticides, and steroid analyses. AMT claims that the biphenyl phase is also 100% aqueous compatible.

Fortis Technologies and MilliporeSigma also launched new reversed-phase products based on SPP supports. Fortis introduced an RP18-Amide and a C18-PFP, while MilliporeSigma produced a C30 and a polar-modified C18, or AQ-C18, phase. Amide phases often show improved peak shape for basic analytes, and provide alternate selectivity through hydrogen bond interactions (4). Mixed C18 and pentafluorophenyl (PFP) stationary phases provide alternative selectivity to the standard alkyl phase alone. As previously mentioned, C30 columns offer more shape selectivity relative to C8 and C18 phases, and are typically touted as 100% aqueous compatible. MilliporeSigma notes that the C30 phase is suitable for the separation of hydrophobic, structurally related compounds, such as lipids and carotenoids, among others. Polar modification of an alkyl phase can be used to eliminate so-called "dewetting" of the media under highly aqueous

Table 1: Companies that responded to the 2019 *LCGC* LC column survey

Company	Product
Advanced Materials Technology, Inc.	Halo 90 Å Biphenyl
	Halo 160 Å C30
	Halo 1000 Å Diphenyl
Agilent Technologies	AdvanceBio HIC
	Agilent 1260 Infinity II Bio-inert LC equipped with the 1260 Infinity II DAD with Bio-inert flow cell
Daicel Corporation	Daicel DCpak PBT
	Daicel DCpak P4VP
	ChiralPak IH
Develosil	Develosil UHPLC Column 1.6 µm
Fortis Technologies Ltd	SpeedCore RP18-Amide
	SpeedCore C18-PFP
Imtakt USA	Cadenza CX-C18
	Dacapo DX-C18
	Metal-Free
	Intrada SEC
MilliporeSigma	BioShell IgG 1000 Å Diphenyl
	BioShell IgG 1000 Å C18
	BioShell A160 Peptide Phenyl-Hexyl
	Ascentis Express 160 Å C30
	Ascentis Express 90 Å AQ-C18
Phenomenex	Lux i-Amylose-3
	Luna Omega SUGAR
	SecurityLINK UHPLC Fingertight Fittings
	bioZen WCX
Regis Technologies	Reflect Polysaccharide Chiral Columns
Shimadzu Scientific Instruments	Shim-pack Velox LC Columns
	Shim-pack Arata
	Shim-pack Bio Diol and IEX
SiliCycle	SiliaChrom Plus HPLC columns
	Chrom Palladium Scavenger guard column
Thermo Fisher Scientific	Thermo Scientific ProPac Elite WCX LC column
	Thermo Scientific Dionex CarboPac PA200 anion-exchange column
	Thermo Scientific Dionex IonPac AS31 anion-exchange column
	Thermo Scientific Dionex IonPac AS32-Fast-4µm anion-exchange column
Tosoh Bioscience	TSKgel FcR-IIIA-NPR
Waters Corporation	BioResolve RP mAb Polyphenyl Column
	BioResolve SCX mAb Column

conditions. The modification may also provide alternative selectivity when compared to the standard alkyl phase, typically in the form of added retention for polar analytes.

Shimadzu Scientific Instruments introduced a new line of columns based on SPP design. The Shim-pack Velox columns surface chemistries include a couple of C18s, a biphenyl, a pentafluorophenylpropyl (PFPP), and a HILIC phase. The line also consists of 1.8-, 2.7-, and 5-µm particle sizes. According to the company, the series of columns combines highly efficient core–shell particle technology with a wide range of surface chemistries, providing the best opportunity for optimal resolution in a wide variety of applications and challenging separations.

New stationary phases and full product lines continue to be built on FPP supports as well. Daicel Corporation has introduced two interesting polymerically coated silica columns during the past year. The Daicel Dcpak PBT is a polybutyleneterephthalate surface that is noted by the company as generating novel selectivity. The other phase is a poly(4-vinylpyridine) phase that is said to possess robust performance. Daicel informed that these phases can be used in LC and in supercritical fluid chromatography (SFC).

Imtakt USA continued to build its FPP-based product line with the introduction of a polar-endcapped C18, noting that the phase has general purpose, can be run at high pH, and provides alternative selectivity to standard C18 phases. Imtakt also introduced a polyether ether ketone (PEEK)-lined stainless steel hardware line of columns named Metal-Free. The company noted that 17 stationary phases, in multiple dimensions and particle sizes, are now available in this hardware. PEEK-lined stainless steel hardware is commonly used to reduce or eliminate undesired interactions of analytes, such as chelating compounds, with metal surfaces.

Develosil released a new UHPLC column line based on fully porous, 1.6-µm particles. The line contains many of the standard surface chemistries, such as C18,

Table 2: Reversed-phase and hydrophilic Interaction liquid chromatography (HILIC) columns

Company	Product Name	Stationary Phases	Chromatographic Mode	Particle Size(s) (µm)	
Advanced Materials Technology, Inc.	Halo 160 Å C30	C30	Reversed-phase	2.7	
	Halo 90 Å Biphenyl	Biphenyl (dimethylbiphenylsilane)	Reversed-phase	2.7	
Agilent Technologies	AdvanceBio MS Spent Media	Proprietary zwitterionic	HILIC	2.7	
Fortis Technologies	SpeedCore RP18-Amide	RP18-amide	Reversed-phase	2.6 and 5	
	SpeedCore C18-PFP	C18-PFP	Reversed-phase	2.6 and 5	
MilliporeSigma	Ascentis Express 90 Å AQ-C18	Polar modified C18	Reversed-phase	2.0, 2.7, and 5	
	Ascentis Express 160 Å C30	C30	Reversed-phase	2.7	
Shimadzu Scientific Instruments	Shim-pack Velox columns	SP-C18, C18, biphenyl, PFPP, HILIC	Reversed-phase and HILIC	1.8, 2.7, and 5	
Daicel Corporation	Daicel Dcpak PBT	Polybutylene terephthalate	Reversed-phase	3 and 5	
	Daicel Dcpak P4VP	Poly(4-vinylpyridine)	Reversed-phase, Normal phase, SFC	3 and 5	
Develosil	Develosil UHPLC columns	C30, C18, C8, C1, and HILIC	Reversed-phase and HILIC	1.6	
Imtakt USA	Cadenza CX-C18	Polar endcapped C18	Reversed-phase	3 and 5	
	Metal-free	17 stationary phases	not defined	2.5, 3.0, and 5.0	
Phenomenex	Luna Omega SUGAR	Amide polyol/amine	HILIC	5	
Regis Technologies	Evoke C18 columns	C18	Reversed-phase	1.8, 3, 5, and 10	
Shimadzu Scientific Instruments	Shim-pack Arata columns	C18	Reversed-phase	2.2	
SiliCycle Inc.	SiliaChrom plus preparative	C18, C8, C4, phenyl, PFP, bare silica, cyano, diol, amide, SAX, and SCX	Reversed-phase, normal-phase, ion-exchange	5 and 10	

* FPP = fully porous (totally porous) particles; SPP = superficially porous particles;
† Comments supplied by vendors

C8, C1, C30, and a HILIC phase. Regis Technologies introduced Evoke C18 columns to the market. According to the company, Evoke C18 columns are packed with high purity silica, and endcapped using a unique gas-phase technology. The process purportedly leads to improved surface coverage and shielding of acidic silanol groups for a highly inert, hydrophobic surface. The company reports excellent separation kinetics and unique selectivity, in comparison to other C18 phases.

	Particle Type*	Dimensions (mm)	Comments†
	SPP	Capillary to analytical	Highly reproducible bonded phase coverage, resulting in fast, highly efficient, rugged separations with high shape selectivity
	SPP	Capillary to analytical	The Halo biphenyl offers a combination of hydrophobic, aromatic, and polar selectivity, for applications in pain management drugs, cannabinoids, pesticides, clinical, steroids, and electron-poor analytes
	SPP	50 × 2.1 to 150 × 2.1	A novel zwitterionic phase retains highly charged compounds, with excellent peak shape, even in high pH or high temperature conditions
	SPP	Capillary to analytical	Sharp peak shapes, very high efficiencies >200 k, use on normal 400-bar LC systems. Orthogonal selectivity.
	SPP	Capillary to analytical	Sharp peak shapes, very high efficiencies >200 k, use on normal 400-bar LC systems. Orthogonal selectivity to standard L1 columns.
	SPP	20 × 2.1 to 250 × 4.6	C18 (polar modified octadecyl) suitable for the separation of polar compounds under reversed-phase conditions
	SPP	20 × 2.1 to 250 × 4.6	Suitable for the separation of very hydrophobic, long chain, and structurally related compounds, like fat-soluble vitamins, lipids, and carotenoids
	SPP	30 × 2.1 to 250 × 4.6	The series of columns combines highly efficient core–shell particle technology with a wide range of surface chemistries, to provide the best opportunity for optimal resolution
	FPP	50 × 2.1 to preparative	Robust polymeric phase with novel selectivity
	FPP	50 × 2.1 to preparative	Polymeric phase with robust performance compared to monomeric vinyl pyridine phases
	FPP	35 × 2 to 150 × 2	UHPLC column line with conventional phase chemistries
	FPP	Capillary to preparative	General all-purpose C18, high pH, isomers, alternative selectivity
	FPP	10 × 2.0 to 250 × 2.0	Peek-lined stainless steel hardware
	FPP	50 × 2.1 to 250 × 4.6	Unique selectivity designed for HILIC mode application and separations
	FPP	Analytical to preparative	Evoke C18 Columns are optimized for high efficiency separations and excellent column-to-column reproducibility
	FPP	50 × 2.0 to 150 × 3.0	Columns for basic drug analysis
	FPP	50 × 50 to 250 × 100	Exact same phases as SiliaChrom Plus analytical columns for smooth and surprise-free scale-up from analytical to preparative format

Shimadzu Scientific Instruments released an additional product based on FPP supports. The Shim-pack Arata is described as a C18 built on a 2.2-µm particle. Though details are limited, the company claims the phase provides excellent peak shape for basic compounds, while maintaining good peak shape for acids.

Also included in this list is a line of preparative columns from SiliCycle. The SiliaChrom Plus preparative line includes various common stationary phases covering reversed-phase,

Table 3: Ion-exchange chromatography columns for small-molecule separations

Company	Product Name	Stationary Phases	Particle Sizes (µm)	Particle Type*	
Thermo Fisher Scientific	IonPac AS32-Fast-4 µm anion-exchange column	Alkanol quaternary ammonium ion	4	FPP (polymeric)	
	IonPac AS31 anion-exchange column	Alkanol quaternary ammonium ion	6	FPP (polymeric)	
	CarboPac PA200 anion-exchange column	Quaternary ammonium ion	5.5	FPP (polymeric)	

*FPP = fully porous (totally porous) particles; SPP = superficially porous particles
† Comments supplied by vendors

Table 4: Chiral stationary phases

Company	Product Name	Stationary Phases	Particle Sizes (µm)	Particle Type*	
Daicel Corporation	ChiralPak IH	Immobilized amylose tris (S)-α-methylbenzylcarbamate	2, 3, 5, and 20	FPP	
Phenomenex	Lux i-Amylose-3	Amylose tris(3-chloro-5-methylphenylcarbamate)	2.7	FPP	
Regis Technologies	Reflect polysaccharide chiral columns	Amylose tris(3,5-dimethylphenylcarbamate), cellulose tris(3,5-dimethylphenylcarbamate), and cellulose tris(3,5-dichlorophenylcarbamate)	3, 5, 10, and 20	FPP	

*FPP = fully porous (totally porous) particles; SPP = superficially porous particles; † Comments supplied by vendors

normal-phase, and ion-exchange, on both 5- and 10-µm particles. The company claims smooth scale-up from their SilicaChrom analytical columns.

HILIC, which is often used for the retention and separation of polar compounds, has shown steady growth in terms of columns released over the past several years. Only two new HILIC surface chemistries were reported this year, however. Phenomenex introduced the Luna Omega Sugar column, which is described as an amide polyol–amine HILIC phase. The company notes that the novel nitrogen-containing stationary phase greatly increases retention and selectivity for sugars and sugar alcohols under HILIC conditions.

Agilent Technologies also introduced AdvanceBio MS Spent Media columns. The product is a proprietary zwitterionic HILIC phase that retains highly charged compounds with excellent peak shape. The company also notes amino acid separations using this column, without the need for derivatization. There were a few other HILIC phases mentioned within new column lines introduced this year, but it is apparent that development of new HILIC stationary phases has recently slowed down.

Ion-Exchange Chromatography: Products released this year for small molecule ion-exchange chromatography are presented in Table 3. Ion-exchange chromatography exploits strong interactions between opposite charges of a surface and an analyte. For the analysis of small ionic compounds, polymeric supports are often modified to carry permanent (strong cation and anion-exchange) or variable (weak cation and anion-exchange) charge, that can be used to interact with and separate analytes with the opposite charge.

The products in this category were all developed by Thermo Fisher Scientific. The Dionex IonPac AS32-Fast-4µm anion-exchange column is reported to be useful for the separation of low polarity anions, polysulfonated aromatics, aromatic dyes, pigments, polythionates, persulfate, and perchlorate. The phase is built on a polymeric ethylvinylbenzene, crosslinked with 55% divinylbenzene with an alkanol quaternary ammonium ion modification. The second column is Dionex CarboPac PA200 anion-exchange column. The company notes that the column provides predictable and high-resolution gradient separation of complex carbohydrates, such as oligosaccharides. This phase is also built on a ethylvinylbenzene, crosslinked with 55% divinylbenzene polymer; however, the surface is modified with a quaternary ammonium ion. Lastly, the company introduced the Dionex IonPac AS31 anion-exchange column. This column shares the same polymeric support with the first two with an alkanol quaternary ammonium ion modification. The company reports

	Dimensions (mm)	Comments†
	2 × 150 and 4 × 150, plus guards	Separation of low polarity anions, polysulfonated aromatics, aromatic dyes, pigments, polythionates, persulfate, and perchlorate
	250 × 2, plus guard	Unique column selectivity allows faster analysis time, while still separating all 9 haloacetic acids from matrix ions
	250 × 0.4 and 250 × 1, plus guards	Column provides predictable and high-resolution gradient separation of complex carbohydrates, such as oligosaccharides

	Dimensions (mm)	Comments†
	Analytical and preparative	Robust immobilized phase with extended application domain
	Analytical and preparative	Chiral stationary phase with unique enantioselectivity for a wide range of chiral molecules
	Analytical and preparative	Unique, proprietary phase coverage claimed to provides excellent peak shape and improved resolution versus leading chiral phases

unique column selectivity that allows faster analysis time while still separating all nine haloacetic acids from matrix ions.

Chiral Chromatography: Table 4 provides information on columns introduced this year intended for chiral separations. There are only three entries noted this year, which is far fewer than what was reported in 2018. It is also interesting to note that all of the chiral phases released since Pittcon 2018 were developed on FPPs, whereas the previous year products were largely focused on adopting SPP technology.

Daicel Corporation introduced ChiralPak IH, which is an immobilized tris(S)-a-methylbenzylcarbamate chiral stationary phase (CSP). The company claims that the immobilized phase is robust, and exhibits an extended application domain over coated phases. Phenomenex launched the Lux i-Amylose-3 CSP. The column is based on amylose tris(3-chloro-5-methylphenylcarbamate) modification, and is noted as having unique enantioselectivity for a wide range of chiral molecules.

Regis Technologies released a new line of CSPs called Reflect polysaccharide chiral columns. The columns are available in analytical and preparative dimensions and particle sizes, the latter ranging from 3 to 20 µm. With three surface chemistries, Amylose tris(3,5-dimethylphenylcarbamate), cellulose tris(3,5-dimethylphenylcarbamate), and cellulose tris(3,5-dichlorophenylcarbamate), the line is suitable for a wide range of chiral compounds. The company claims that a unique, proprietary phase coverage provides excellent peak shape and improved resolution versus leading chiral phases.

Columns for Large-Molecule Separations

Reversed-Phase: The complexity of large molecules necessitates the use of multiple modes of separation to fully characterize them. New columns introduced since Pittcon 2018 intended for the separation of large molecules (herein loosely defined as peptides and larger) are provided in Table 5. As noted from this list, there have been developments in reversed-phase, size-exclusion chromatography (SEC), ion-exchange, hydrophobic interaction chromatography (HIC), and affinity chromatography over the course of the year. It is interesting to note that all of the columns intended for large molecule, reversed-phase separations have been constructed using SPP technology.

Advanced Materials Technology, MilliporeSigma, and Waters Corporation all introduced columns intended for reversed-phase separation of large molecules based on SPP technology. Of the five different phases introduced, four contain aromatic modifications that provide alternative selectivity to alkyl-based phases. The trend of developing alternative selectivity for large molecules is similar to the

Table 5: Columns for separating peptides, proteins, and amino acids

Company	Product Name	Stationary Phase(s)	Chromatographic Mode	Particle Size(s) (µm)	Particle Type*	
Advanced Materials Technology	Halo 1000 Å Diphenyl	Diphenyl	Reversed-phase	2.7	SPP	
MilliporeSigma	BioShell A160 Peptide Phenyl-Hexyl	Phenyl-hexyl	Reversed-phase	2.7	SPP	
	BioShell IgG 1000 Å C18	C18	Reversed-phase	2.7	SPP	
	BioShell IgG 1000 Å Diphenyl	Diphenyl	Reversed-phase	2.7	SPP	
Waters Corporation	BioResolve RP mAb Polyphenyl	Polyphenyl	Reversed-phase	2.7	SPP	
Imtakt USA	Intrada SEC	Substituted diol	SEC	3	FPP (hybrid)	
Shimadzu Scientific Instruments	Shim-pack Bio Diol	Diol	SEC	2, 3, and 5	FPP	
Phenomenex	bioZen WCX	Linear carboxylate polymer	Ion-exchange	6	Nonporous PS-DVB polymer	
Waters Corporation	BioResolve SCX mAb	Strong cation exchanger	Ion-exchange	3	FPP (polymeric)	
Agilent Technologies	AdvanceBio HIC	Proprietary	HIC	3.5	FPP	
Tosoh Bioscience LLC	TSKgel FcR-IIIA-NPR	Modified recombinant Fcg receptor IIIa protein	Affinity	5	FPP (polymeric)	

* FPP = fully porous (totally porous); SPP = superficially porous
† Comments supplied by vendors

ongoing trend for reversed-phase small molecule separation tools. AMT and MilliporeSigma launched 1000 Å diphenyl phases. The large pore size is purported to improve peak shapes, especially for very large proteins, and the diphenyl phase is intended to provide alternative selectivity.

MilliporeSigma also released a 1000 Å C18 column for the separation of monoclonal antibodies (mAbs), antibody–drug conjugates (ADCs), antibody fragments, and other large proteins. The company also released an addition to their BioShell A160 Peptide line of columns with a phenyl-hexyl modification. The company notes that the phase provides alternative selectivity for peptide mapping as compared to C18 and cyano stationary phases.

Waters Corporation launched a polymeric version of an aromatic phase, called BioResolve RP mAb Polyphenyl, also targeting mAbs and ADCs. The company reports that the phase provides improved chromatography performance, beneficial selectivity, reduced carryover, and friendlier conditions for interfacing with mass spectrometry (MS).

Size-Exclusion Chromatography: Size-exclusion chromatography (SEC) is a technique that aims to resolve analytes based on molecular size, and is often used as a complementary technique to other modes of separation for the characterization of large molecules. Columns utilized in SEC are often characterized by strict control of pore size and by inert surface chemistry to minimize analyte–surface interactions.

Matching the number of offerings reported in 2018, two new SEC phases were introduced during the past year. Imtakt USA introduced a 3-µm substituted diol modified silica column, intended for the separation of large biologicals or synthetic polymers. The company notes that the phase is designed to work with MS-friendly eluents.

Shimadzu Scientific Instruments released Shim-pack Bio Diol in 2-, 3- and 5-µm particle sizes. The company offers multiple pore sizes as well, and claims the columns are effective for the analysis of

	Dimensions (mm)	Comments†
	Capillary to analytical	Alternate selectivity for large biomolecules
	20 × 2.1 to 250 × 4.6	BioShell A160 Peptide Phenyl-Hexyl offers an alternative selectivity for peptide mapping and proteomic applications to the C18 and CN phase chemistries
	20 × 2.1 to 250 × 4.6	Separation of monoclonal antibodies, antibody–drug conjugates, antibody fragments, and large proteins with molecular weights ≤500 kDa
	20 × 2.1 to 250 × 4.6	Separation of monoclonal antibodies, antibody–drug conjugates, antibody fragments, and large proteins with molecular weights ≤500 kDa with additional selectivity
	50 × 2.1 to 150 × 4.6	For reversed-phase separations of intact or sub units of monoclonal antibodies (mAbs) and antibody–drug conjugates (ADCs)
	250 × 1.0 to 250 × 20	Designed to work with MS-friendly eluents
	300 × 4.6 and 300 × 8	Effective for analysis of aggregates and fragments of biopharmaceuticals
	50 × 4.6 to 250 × 4.6	Separation of acidic and basic variants for proteins
	50 × 2.1 to 100 × 4.6	For scientists who need to characterize, monitor, and QC charge variants of mAb-based therapeutics during discovery, development, and manufacturing
	30 × 4.6 and 100 × 4.6	Optimized hydrophobicity for performing HIC separations with lower salt
	75 × 4.6	First FcRγ affinity chromatography analysis column for antibody drugs

protein aggregates and fragments of biopharmaceuticals.

Ion-Exchange Chromatography: In large-molecule characterization, ion-exchange chromatography is an essential tool to analyze for charged variants, and complements information gained from reversed-phase and SEC analyses. Since Pittcon 2018, two new ion-exchange chromatography columns intended for large-molecule analysis have been launched.

Phenomenex released bioZen WCX, which is described as a linear carboxylate polymer grafted onto a nonporous polystyrene-divinylbenzene (PS-DVB) polymeric bead, that effectively separates acidic and basic protein variants.

Waters Corporation introduced the BioResolve SCX mAb column for the analysis of charge variants of mAb-based therapeutics. This column, also based on a nonporous polymeric support, utilizes a strong cation exchange moiety to enable charge separations.

Hydrophobic Interaction Chromatography: Hydrophobic interaction chromatography (HIC) is another powerful and complementary tool for the characterization of large molecules. HIC makes use of the interactions between hydrophobic regions of the protein, with weakly hydrophobic ligands attached to the stationary phase. Retention is governed by the concentration of salt in the mobile phase. HIC traditionally utilizes high concentrations of nonvolatile buffers, rendering it incompatible with mass spectrometric detection. Significant effort has been made over the past few years to render HIC more MS-compatible (5).

Agilent Technologies launched AdvanceBio HIC, which is a proprietary stationary phase built on a 3.5-µm FPP support. The company claims that the phase was designed to exhibit optimized hydrophobicity for performing HIC separations with lower salt concentrations.

Affinity Chromatography: Affinity chromatography is a separation technique based on highly specific interactions between two partners. Through the immobilization of one partner to a solid surface, the other partner can be effectively

"fished" out of a complex mixture with high specificity (1). Affinity chromatography is yet another complementary tool employed to fully characterize large molecules.

Although affinity columns were absent from the 2018 report, affinity has resurfaced in 2019 with one entry from Tosoh Bioscience. Tosoh introduced the TSKgel FcR-IIIA-NPR column, a polymeric-modified recombinant Fcγ receptor IIIa protein, 5-µm, nonporous, affinity-based phase selective for N-glycosylated immunoglobulins. The column is intended for the fast evaluation of antibody-dependent cellular cytotoxicity (ADCC) activity of monoclonal antibodies, cell line screening in early R&D, biosimilar–originator comparison, upstream development–optimization, and monitoring of glycoengineering.

Large-molecule separations continue to be an area of interest. The need for multiple chromatographic techniques for characterization of these complex systems provides a wide landscape for product invention and introduction.

Accessories

Accessories are important products that enable and often facilitate liquid separations. Since Pittcon 2018, two LC accessories have been launched.

Phenomenex recently released the SecurityLink UHPLC finger-tight fitting system. The system is reported by the company to simplify system and column connections, while providing consistent performance through Torque Limiting Technology, which prevents column damage from overtightening. Once the perfect connection has been made through finger tightening, the SecurityLink fitting offers a haptic "click" to confirm that optimum torque has been reached. This ensures a consistent connection each and every time, and prevents over- or under-tightening that may cause column or performance issues.

SiliCycle introduced the SiliaChrom Palladium Scavenger guard column to protect HPLC columns from residual palladium often found in synthesis samples. Palladium scavengers are grafted on silica gel, and packed into guard cartridges to effectively reduce palladium concentrations to single-digit ppm levels, protecting the valuable analytical column.

Conclusions

Columns intended for both small-molecule and large-molecule separations continue to be developed. For small molecules there was an even split of phases built on FPP and SPP technologies. Efforts using SPP designs seemed to be focused on alternative reversed-phase selectivity offerings. Companies are, however, continuing to fill out and build new product lines on FPP architecture. There were few new HILIC phases introduced this year as compared to years past, which may indicate a saturation of the market.

Large-molecule separations continue to be an area of interest. The need for multiple chromatographic techniques for characterization of these complex systems provides a wide landscape for product invention and introduction. Reversed-phase columns based on SPP technology dominate the offerings. A trend of offering alternative selectivity for large-molecule separation using aromatic stationary phases is noted. This is a similar trend to small-molecule reversed-phase analyses. Products designed for SEC, ion-exchange chromatography, HIC, and affinity were also introduced.

Notably absent from the list of products introduced this year is in the area of micro- and nanoscale separation devices. These devices were prevalent in the 2018 report, and noted as a potential trend for the future. It will be interesting to watch for a revival in years to come.

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Although *LCGC* has made every attempt to include every submission in the series of review articles, it is possible that some have been missed. If there have been omissions or if you want to be sure to be included in the 2020 review series, please contact Laura Bush, the Editorial Director of *LCGC Europe*, at lbush@mmhgroup.com.

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Looking into **Lipids**

Lipidomics is one of the youngest branches of "omics" research. Maria Fedorova from Leipzig University, in Leipzig, Germany, discusses the latest trends and challenges in lipidomics research and highlights how innovative bioinformatics solutions are addressing data handling issues in this evolving field.

Interview by Alasdair Matheson, Editor-in-Chief, *LCGC Europe*

Q. What is the definition of lipidomics?

A: Lipidomics is the large-scale study of diversified molecular species of lipids, with the aim of addressing the identification and cellular and tissue distribution of lipids as well as their related signalling and metabolic pathways in a variety of organisms.

As with any "omics" study, lipidomics aims to describe the whole variety of lipid species and to provide knowledge on their diversity, distribution, and concentration, which can then be used for further systems biology and systems medicine data integration.

Lipidomics is probably the youngest addition to the family of classical "omics" studies, which includes genomics, proteomics, transcriptomics, and metabolomics. A lot of effort is currently directed at providing the inventory of natural lipidomes. It might sound surprising, but only a limited number of organisms or tissue-specific lipidomes have been characterized so far. In fact, for human tissues, the blood lipidome is probably the only in-depth characterization that has been verified by different laboratories.

Another active area in lipidomics research is the identification of lipid markers that can be associated with human health and pathologies. The dynamic nature of lipids and their deep involvement in a variety of functional activities makes them very attractive biomolecules for diagnostic, prognostic, and therapeutic applications.

Q. What are the main aims of your research group?

A: Our group focuses on the development and optimization of analytical and bioinformatics solutions for high-throughput lipidomics with the aim of studying human metabolic disorders. We would like to have the tools to perform a deep lipidomics profiling of human tissues to create reference lipidomes and integrate the data on existing lipid species in genome-scale metabolic models to describe the whole set of biochemical reactions driven by corresponding enzymes (genes).

This type of deep lipidomics profiling requires a combination of several analytical and computation strategies to ensure the high quality of the data and cannot be called *high-throughput*. However, having the whole set of lipid species in the tissue (or at least the majority of it) integrated using systems biology and systems medicine tools would allow us to design high-throughput and robust analytical solutions suitable for translation to the clinic.

Another focus of our group is the characterization of oxidized lipids derived using reactions catalyzed by dedicated enzymes, for example, cyclooxygenase and lipoxygenase, or by free-radical-driven oxidation in conditions generally classified as oxidative stress or redox imbalance (1–3).

These modified lipids represent the fraction of what we call *epilipidomes*, a subset of lipidomes derived by modifying the native lipids. Indeed, similar to epigenomes and proteoforms, which have been shown to play significant regulatory roles on other "omics" levels, modified lipids perform fine-tuning of metabolic and signalling functions. Relatively well studied at the level of free fatty acids (eicosanoids and prostaglandins), oxidative modifications of phospholipids and triglycerides (TGs) are not currently well understood.

The oxidation of fatty acyl chains changes the physicochemical properties of lipids, which causes them to function differently. However, from an analytical perspective, this also requires the optimization of new separation methods and mass spectrometry (MS)-based protocols. We are working on methods that combine liquid chromatography (LC) (mostly reversed phase) and tandem mass spectrometry (MS/MS) to specifically detect, identify, and quantify oxidized lipids in human lipidomes connected to pathologies associated with chronic inflammation where redox dysregulation plays a significant role (4–8).

Q. What are the main challenges facing separation scientists involved in lipidomics and how are they being overcome? Are there any recent developments in lipidomics from an analytical perspective that you think are particularly innovative?

A: Lipids are difficult to analyze because (i) they have very different physicochemical properties and require different extraction and separation methods), (ii) they are usually present at very different concentrations in biological samples so require methods with a wide dynamic range, and (iii) we actually still do not know the whole variety of lipid species and thus their physicochemical properties in natural lipidomes. This makes it difficult—if not impossible—to have an "all-in-one" analytical solution capable of detecting and identifying all the different lipid species in a given biological system.

The combination of separation techniques with modern MS instruments capable of high-resolution, mass accuracy, sensitivity, and speed has

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significantly improved the dynamic range in lipidomics analysis. This offers us the possibility of identifying hundreds of lipid species from natural lipidomes on the fatty acyl level in one LC–MS/MS analysis. However, the development of analytical tools to define fatty acyl chain positions, for example, *sn*-1 versus *sn*-2 in phospholipids, remains challenging for high-throughput applications.

A lot of progress was recently achieved for methods to define double bond positions in esterified fatty acyl chains. Several methods based on different gas-phase fragmentation mechanisms, such as ozone-induced dissociation (OzID) and ultraviolet photodissociation (UVPD), as well as chemical derivatization strategies, such as the Paterno-Büchi reaction or epoxidation, offered the possibility to identify isomeric lipid species (9–14).

The separation of some structural isomeric lipids, such as bis(monoacylglycero)phosphates (BMP) and phosphatidylglycerols (PG), remains challenging as well. Methods based on chemical derivatization (methylation), ion mobility spectrometry (IMS), and separation using zwitterionic hydrophilic interaction liquid chromatography (HILIC) stationary phases using optimized concentrations of ammonium acetate were recently demonstrated (15–17).

Q. There are three major chromatography techniques used in lipidomics: normal phase, reversed phase, and HILIC. Are there distinct application areas where these individual categories of chromatography are being used in lipidomics?

A: As the majority of the lipidomics studies rely on the on-line coupling of LC to electrospray ionization (ESI)-MS, normal-phase chromatography is a less popular method because of the low compatibility of the mobile phase components with ESI (18–20).

Both reversed phase and HILIC are used very widely in lipidomics. Reversed phase remains the most popular choice for lipidomic profiling because of the ability to separate multiple lipid species within the same lipid class based on the length and number of double bonds in fatty acyl chains (21,22). HILIC provides lipid class-based separation and can be the optimal choice for lipid quantification using class-specific internal standards that would closely coelute with multiple lipid species within corresponding lipid classes (21). Moreover, the complementary nature of reversed phase and HILIC separation mechanisms makes the combination of both techniques a very attractive choice for deep lipidomics profiling, especially when dealing with the lipidomes characterized by a wide range of polarities and lipid concentrations.

Q. Can you comment on method selection and practical considerations for the choice of stationary phases and mobile phases for normal phase, reversed phase, and HILIC in lipidomics?

A: In my opinion, the choice of the stationary phase chemistry should be defined by the lipidome that needs to be analyzed. As a result of the differences in the polarities, as well as the range of concentrations for lipids from different classes, there would be no universal choice for LC stationary phases.

Recently we performed a comparison of five reversed-phase columns with different stationary phase surface chemistry (C18 versus C30), types of stationary phase particles (fully porous particles [FPP] versus. solid-core particles [SCP]), and particle size (1.9 µm versus. 2.6 µm versus. 3.0 µm) using the same mobile phases and tandem mass spectrometry method to resolve the human blood plasma lipidome (23). We demonstrated that not all C18 columns are efficient for lipid chromatography and selection should not be based entirely on particle size. Thus, pore size, as well as surface area, can play a significant role for stationary phases with the same surface chemistry. Columns with fully porous sub-2-µm particles and solid-core 2.6-µm particles usually perform well.

The choice of the surface chemistry depends on the polarity of the studied lipidome. Thus, a C18 stationary phase is recommended for the analysis of lipidomes of intermediate polarity, for example, a plasma lipidome with a relative high content of both phospholipids and triacylglycerol and cholesteryl esters, while C30 columns would be more suitable for samples with a high content of long chain hydrophobic lipids, for example, adipose tissue.

Gradient elution using water–acetonitrile–isopropanol is probably the most popular mobile phase used for reversed-phase chromatography in lipidomics. Methanol can also be included into the eluent system. In HILIC, unbound Si-based columns are the most popular for lipid separation. Among polar-bonded phases are polyvinylalcohol- and dihydroxypropyl-modified silica stationary phases. Recently, interesting applications of zwitterionic HILIC columns have been demonstrated (16). The majority of mobile phases consist of acetonitrile and aqueous buffers (ammonium formate and acetate), supplemented with minor amounts of isopropyl alcohol (IPA), methyl tert-butyl ether (MTBE), methanol, or other polar, water-miscible solvents.

In general, the selection of mobile phase—including suitable additives and their concentrations—is crucial in lipidomics. For example, terminal phosphate groups in some lipid classes interact with stainless steel material in the flow path of the high performance liquid chromatography (HPLC) systems leading to peak tailing. This effect can be eliminated by adding phosphoric acid in the samples or by substituting all HPLC tubing to PEEK material. Another significant challenge in optimizing the mobile phase for optimal separation of complex lipidomes is the different dissociation states of phospholipids at different pHs. The coexistence of a single lipid in a charged, ionized state together with its neutral form would result in peak broadening and tailing. Thus, one should tune the pH of mobile phase to ensure uniform distribution of dissociation state for different lipid classes.

Q. Supercritical fluid chromatography (SFC) is also used to a lesser extent. When is SFC useful?

A: The application of SFC for lipidomics analysis has shown a high potential over the last decade. SFC combines the advantages of both gas chromatography (GC) and HPLC (low back pressure, solubility of analytes, and good kinetic performance) resulting in high efficiency and short separation times. Ultrahigh-performance SFC separation using sub-2-µm unmodified and functionalized silica stationary phases coupled on-line to ESI-MS was used for the analysis of complex lipidomes as well as the separation of lipids within different lipid classes (24,25). For example, the separation of 30 lipid classes within 6 min was recently demonstrated by the group of Michal Holčapek (26).

Q. Are comprehensive chromatography techniques commonly used in lipidomics?
A: Several very interesting examples of comprehensive chromatography for lipidomics studies have been published. Off-line and even on-line coupling of two orthogonal separation techniques illustrated deep lipidomics coverage (27–32). However, routine application of comprehensive chromatography techniques is not common. Off-line combinations of two chromatographic techniques, such as HILIC and reversed phase (which is the most popular orthogonal system), are relatively easy to perform, but do not provide high-throughput. Automated on-line coupling would provide the most robust solution. However, instrumentation, such as an ultrahigh-pressure liquid chromatography (UHPLC) system with two sets of pumps, would require additional investment, and the analytical workflow would still need to be optimized to ensure full capacity for both separation modes. Instead deep lipidomics profiling usually relies on the combination of different lipid extraction methods, as well as fractionation using solid-phase extraction (SPE) and liquid–liquid extraction (LLE) protocols.

Q. Big data is always a concern in any "omics" field. You recently published two papers related to data handling using open source software: LipidHunter and LPPtiger. What solutions do these offer separation scientists?
A: As I mentioned previously, lipidomics is the youngest addition to the classical "omics" family and computational solutions to support high-throughput analytical workflows are much less developed compared to transcriptomics, proteomics, and metabolomics. One of the main bottlenecks remains reliable high-throughput identification of lipids from LC–MS/MS datasets. With this in mind, we developed LipidHunter as an open source software to identify lipids from LC–MS/MS datasets obtained using data-dependent acquisition (33). When we started in lipidomics, we went through hundreds of tandem mass spectra manually to learn how we can confidently identify lipids. When we got tired of doing this manually, we created this software to do it for us. This software repeats all the steps of lipid identification one would do during manual identification, but much faster and keeps the whole identification process very transparent and traceable. The first version of this software only dealt with phospholipid identifications, but when we started to work with adipose tissue lipidome we extended it to glycerolipids and the second version is now freely available (https://github.com/SysMedOs/lipidhunter).

LPPtiger (LPP stands for lipid peroxidation products) is a software tool for the analysis of oxidized phospholipids (https://bitbucket.org/SysMedOs/lpptiger). Oxidized phospholipids represent a very interesting fraction of epilipidome. However, the identification from LC–MS/MS cannot be directly translated from native phospholipids. With this software, we implemented several new algorithms including *in silico* prediction of oxidized epilipidome from native lipidome provided to the software. To perform

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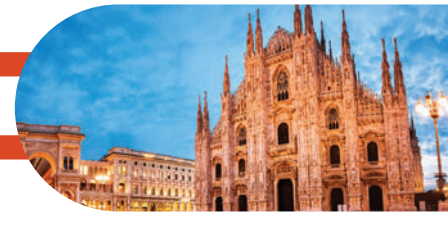

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data-driven prediction of oxidized lipids rather than simple enumeration of oxygen atoms to unsaturated fatty acyl chains, we conducted the meta-study on the available literature describing mechanisms of oxidation for polyunsaturated fatty acids and used the information from more than 170 publications to integrate these data in metabolic networks representing oxidation pathways for ten polyunsaturated fatty acids (PUFAs). LPPtiger relies on these metabolic networks to perform data-driven *in silico* oxidation (34).

Q. Are the any other "big data" solutions being adopted in lipidomics?

A: There are several other very good open source computational solutions supporting lipid identification from LC–MS/MS datasets including LipidBlast, MSDIAL, and Lipostar just to name a few (35–37).

Further directions supporting big data integration in lipidomics should provide tools for pathway mapping and network integration of lipidomics data. The LipidMaps consortium brings different aspects of lipidomics studies together and provides an integrative platform for lipid analysis (38).

Q. Are there any developments in sample preparation in lipidomics that are worth commenting on?

A: Once again, the choice of extraction method depends on the biological matrix and lipid species variety and concentrations present in the sample. Folch-, Bligh and Dyer-, and MTBE-based methods are very popular, as well as the butanol–methanol extraction (BUME protocol) (39).

Q. Adipose tissue lipidomes are currently a main focus of research. Why are these molecules important and what analytical strategies are used to analyze these analytes?

A: The role of adipose tissue in human physiology was reconsidered after the discovery of adipokines and their role in the regulation of human metabolism and immune responses (40–42). Adipose tissue metabolism was correlated with insulin sensitivity status as well as chronic inflammation accompanying numerous human pathologies, changing our view on the role of adipose as an inactive lipid storage organ to the active regulator of whole-body metabolism. Together with liver and all lipoproteins in blood, adipose tissue is among the most crucial organs in lipid trafficking, distribution, regulation, and metabolism.

However, it still remains largely unknown which exact lipid species are present in adipose tissue. We know it contains massive amounts of triglycerides, but are they all the same? Which fatty acyl chains are esterified and de-esterified? What is the dynamic of this process and how is it regulated?

Currently we have identified over 1000 individual TG lipids in human white adipose tissue (data unpublished). Why do we have such a large variety of individual species of TGs? What is the difference in lipidomes of white adipose tissue from different depots, for example visceral versus subcutaneous, and different insulin sensitivity states? We try to answer these questions by combining dedicated analytical workflows with systems biology tools to provide an integrative fatty acid-centric view on adipose tissue metabolism.

For example, to uncover the diversity of adipose tissue lipidome, we combined several extraction and fractionation (SPE and LLE) methods followed by nuclear magnetic resonance (NMR), thin-layer chromatography (TLC), HILIC–MS/MS, and reversed-phase LC–MS/MS on C18 and C30 columns (data unpublished). Furthermore, to ensure high-quality, reliable identification of lipids from multiple measurement platforms, we used a combination of three different lipid identification software tools, which allowed us to compose the reference lipidome of human white adipose tissue, including over 1600 lipid molecular species. All these data are currently used for integration into genome-scale metabolic models specific for adipose tissue. The availability of a high-quality in-depth characterized lipidome described by means of a genome-scale metabolic (GEM) model will provide us with the possibility to understand disease-associated metabolic changes in lipidomes profiled using more targeted techniques applied for a large number of human adipose tissue samples.

Q. What other projects are you working on at the moment?

A: We are also looking into the lipotoxicity effects connected with ectopic lipid accumulation in cardiac cells. Using a cell culture model of mild nitroxidative stress, we demonstrated the formation of lipid droplets in cardiac cells accompanied by the accumulation of oxidized lipids (43). In collaboration with the group of Dolores Perez-Sala in CSIC Madrid (Spain) and the group of Professor Spengler at the University of Giessen (Germany), we combined confocal fluorescent microscopy, LC–MS/MS-based lipidomics, and single-cell matrix-assisted laser desorption–ionization (MALDI) imaging to understand the distribution of lipids and their oxidized forms upon lipid droplets formation and associated the dynamic of these droplets with autophagy-lysosomal degradation pathway.

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Maria Fedorova studied biochemistry at Saint Petersburg State University, in Saint Petersburg, Russia, and obtained her Ph.D. at the Faculty of Chemistry and Mineralogy, at Leipzig University, in Leipzig, Germany. She is a group leader at the Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, at the University of Leipzig, Germany. Her research is focused on the development and optimization of chromatography and mass spectrometry methods for the analysis of lipids and their modified forms. Her group works on implementation of high-throughput LC–MS methods in discovery lipidomics targeting in-depth identification and quantification of human lipidome in a variety of tissues. By combining lipidomics data with investigating related proteins and protein post-translational modifications via a systems medicine approach, she aims for a deeper understanding of pathophysiology of obesity, insulin resistance, type II diabetes, and cardiovascular disorders.

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www.chromatographyonline.com/LCGCSocialMedia

Liquid chromatography

Shimadzu's new Nexera series LC-40 offers groundbreaking technology in terms of intelligence, efficiency, and design, according to the company. The Nexera ultrahigh-performance liquid chromatograph series incorporates artificial intelligence as analytical intelligence, allowing systems to detect and resolve issues automatically. The series reportedly makes laboratory management simple by integrating IoT and device networking, enabling users to easily review instrument status, optimize resource allocation, and achieve higher throughput.
www.shimadzu.eu
Shimadzu Europa GmbH, Duisburg, Germany.

HILIC columns

Hilicon offers a broad range of hydrophilic interaction liquid chromatography (HILIC) products to separate polar compounds. Three column chemistries in UHPLC and HPLC, iHILIC-Fusion, iHILIC-Fusion(+), and iHILIC-Fusion(P), provide customized and complementary selectivity, excellent durability, and ultralow column bleeding, according to the company. The columns are suitable for the LC–MS analysis of polar compounds in "omics" research, food and beverage analysis, pharmaceutical discovery, and clinical diagnostics.
www.hilicon.com
Hilicon AB, Umeå, Sweden.

LC columns

Based on a sulfonated, cross-linked styrene-divinylbenzene copolymer, Eurokat columns are available in several ionic forms. One advantage of this particular cation exchanger is the application of organic solvent-free methods. According to the company, the columns are also eco-friendly, as well as cost-efficient during application and very long-lasting. They are reportedly the ideal choice for the analysis of sugars, organic acids, and alcohols.
www.knauer.net/columns
Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany.

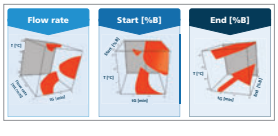
SFC columns

Highly robust YMC-Triart columns are a great choice for SFC, according to the company. Available with different selectivities (diol, PFP, C18, hybrid-silica), particle sizes (1.9-, 3-, 5-µm), and dimensions, the columns cover the full range of applications from analytical to (semi)preparative scale. Full SFC compatibility has been officially certified by an independent institute.
https://ymc.de/sfc-columns.html
YMC Europe GmbH, Dinslaken, Germany.

Volumetric dispenser

A one volumetric precision dispenser for aggressive liquids and solvents, the dispenser has a programmable volume from 10 mL to 1100 mL and can be repeated to pump several litres in portions or repeatedly fill vessels. The process is operated using a touch panel. No computer is required, the user can type in the desired volume and it will withdraw the volume.
www.biotechfluidics.com/products/pumps/volumetric-precision-dispenser/
Biotech AB, Onsala, Sweden.

Method modelling software



Molnár-Institute's DryLab software has a 35-year history in scientific method modelling. Using a DoE of 12 input runs, the software integrates the theory of solvophobic interactions and linear solvent strength (LSS) to predict the movements of peaks, selectivity changes, and retention times of any multidimensional design space. The software's automation module creates method sets in the most economic and ecologic order, executes runs, and acquires results from the CDS. Mass and other integrated data are retrieved and ambiguity in peak tracking is reduced to a minimum.
www.molnar-institute.com
Molnár-Institute, Berlin, Germany.

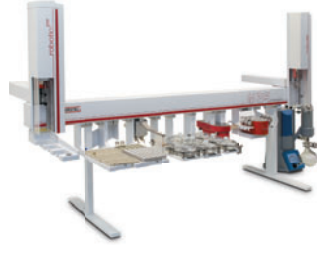
FID gas station

The VICI FID gas station combines the reliability of the VICI DBS hydrogen and zero-air generators into one compact and convenient package.
Available in high and ultrahigh purity for all GC detector and carrier gas applications. The generator is available in two styles: flat for placement under a GC, or the Tower. Available in H_2 flow ranges up to 1 L/min and 10.5 bar.
www.vicidbs.com
VICI AG International, Schenkon, Switzerland.

Multi-angle static light scattering

Introducing the next generation DAWN multi-angle static light scattering (MALS) detector for absolute characterization of the molar mass and size of macromolecules and nanoparticles in solution. DAWN offers high sensitivity, a wide range of molecular weight, size, and concentration, and a large selection of configurations and optional modules for enhanced capabilities, according to the company.
https://www.wyatt.com/dawn
Wyatt Technology, Santa Barbara, California, USA.

GC–MS



An automated GC–MS-based system determines 3-MCPD, 2-MCPD, and glycidyl fatty acid esters in edible oil meeting the requirements of standard ISO, AOCS, and DGF methods. Samples are automatically prepared and analyzed, including analyte derivatization and evaporation of excess reagent and solvent for best limits of determination and system stability.
www.gerstel.com
Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany.

LC accessories

Restek has expanded the company's line of liquid chromatography accessories for chromatographers. High-quality couplers, fittings, unions, tees, and crosses; PEEK and stainless steel tubing; mobile phase maintenance and safety products, including bottle tops, valves, filters, and spargers are now available.
www.restek.com/LCacc
Restek Corporation, Bellefonte, Pennsylvania, USA.

Crimping and decapping

The CR-1000 is a crimping and decapping machine for all types and sizes of vials and caps. The force is adjustable according to the type of vials, caps, and septum. According to the company, the machine is simple to use, offers reproducible crimping, and is suitable in any type of room and for any power supply socket.
The CR-1000 reportedly provides an average rate of 400 vials/h. The company are looking for partnership in Europe so don't hesitate to visit their website.
www.sertir.fr
Action Europe, Sausheim, France.

Electrochemical detector

The Decade Elite from Antec Scientific is designed as an easy-to-use electrochemical detector that can integrate with any LC system on the market, according to the company. The system can reportedly handle fast eluting peaks in (U)HPLC and deliver fast stabilization from dedicated flow cells. When used with the SenCell, the system is a highly sensitive electrochemical detector.
www.AntecScientific.com
Antec Scientific, Zoeterwoude, Netherlands.

Sample automation

Markes' new Centri multitechnique platform is an advance in sample automation and concentration for GC–MS, according to the company, and offers four sampling modes: HiSorb high-capacity sorptive extraction, headspace, SPME, and thermal desorption. The company reports analyte focusing allows increased sensitivity in all modes, state-of the-art robotics increase sample throughput, and sample re-collection allows repeat analysis without having to repeat lengthy sample extraction procedures.

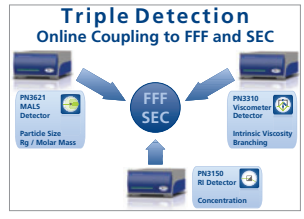
http://chem.markes.com/Centri
Markes International Ltd., Llantrisant, UK.

Sample prep

LCTech has introduced an automated system designed to clean up samples that need to remain melted in PCB and dioxin analysis. Three specifically designed heating zones keep the sample liquid from sample vial to the first column. The DEXTech Heat, which is based on the established DEXTech Pure system, processes difficult samples, such as stearin or PFADs. Excellent automated, reliable results, without clogging, are produced, according to the company.

www.LCTech.de
LCTech GmbH, Obertaufkirchen, Germany.

Triple detection



Postnova has introduced the Triple Detection for thermal field-flow fractionation (FFF) and GPC/SEC. Triple Detection is the combination of multi-angle light scattering (MALS), viscosity detection, refractive index detection, and UV detection. In a single separation experiment, Triple Detection provides molar mass distribution, molecular size distribution, and molecular structure (branching, composition) of polymers, biopolymers, polysaccharides, proteins, and antibodies.

www.postnova.com
Postnova Analytics GmbH, Landberg, Germany.

Lab equipment

Trackman Connected is a tablet with accessories and applications that make pipetting on 96- and 384-well plates faster and more reliable, reportedly improving efficiency at the bench by tracking pipetting tasks. Designed to communicate with Pipetman M Connected via Bluetooth, the tablet interacts in real-time with the pipette and guides users through their protocol with PipettePilot.

www.gilson.com
Gilson, Middleton, Wisconsin, USA.

GC

GL Sciences's CryoFocus-4 is a GC cryogenic trap, used to refocus analytes on the column. The technology was developed using knowledge learned with Optic-4, a multi-mode inlet for gas chromatography. Cooling is done using either CO_2 (-50 °C) or LN_2 (-150 °C). Low temperature trapping is combined with a fast heating rate (60 °C/s). The result, according to the company, is very sharp peaks, and improved separation of volatile and semivolatile compounds.

www.glsciences.eu
GL Sciences B.V., Eindhoven, Netherlands.

Chromatography software

DataApex has launched a new version of Clarity Chromatography software. Clarity version 8 comes with a graphically enhanced user interface, improvements in MS and GLP options, and new control modules. According to the company, Clarity brings easy operation, user support, and optional extensions for various applications, such as PDA, MS, GPC, NGA, and many more. A free demo is available from DataApex's website.

www.dataapex.com
DataApex, Prague, Czech Republic.

5th International Workshop on Electrochemistry–Mass Spectrometry (ELCHEMS^5 2019)

The **5th International Workshop on Electrochemistry–Mass Spectrometry (ElCheMS^5)** will be held **11–12 June 2019** at the **University of Münster, Germany**, and is hosted by the group of Uwe Karst and Martin Vogel. The topics covered span all instrumental aspects of electrochemistry–mass spectrometry (EC–MS), including fundamentals, novel hardware and software, integration of analytical separation techniques, and miniaturized and on-chip approaches for EC–MS. Current applications of EC–MS will form the second major aspect of the meeting, including the simulation of metabolic and advanced oxidation processes, preparative scale EC–MS, the generation of reactive metabolites and their reaction products, and EC–MS in protein and peptide chemistry. The organizers are expecting to see a continuing trend towards the (semi)-preparative use of EC–MS for the generation of milligram-scale amounts of substances to allow further characterization by nuclear magnetic resonance (NMR) and other spectroscopic techniques. Additionally, the trend towards automated approaches will address the challenge to investigate larger numbers of samples within a reasonable time scale. ElCheMS^5 will feature tutorial-style lectures by leading experts, ensuring it is an ideal event for novices in the field. Contributed oral and poster presentations by the attendees form the backbone of the workshop, and there is ample time to discuss all presentations. Coffee breaks and a barbecue on the evening of 11 June 2019 with beer, steaks, and bratwurst provide further opportunities to meet the experts in person and to share and acquire knowledge. The intense exchange between academia, instrument manufacturers, and chemical and pharmaceutical industries is another established feature of the meeting, which will be continued. Submissions for oral presentations should be submitted by **20 May 2019**, and poster submissions by **1 June 2019** to the following address: ElCheMS2019@wwu.de **Website:** www.uni-muenster.de/Chemie.ac/en/karst/workshops/elchems.html

"Separation Science: Driving Pharmaceutical Development and Manufacture" The Chromatographic Society Annual Spring Symposium

The Chromatography Society's (ChromSoc) annual Spring Symposium **"Separation Science: Driving Pharmaceutical Development and Manufacture"** will take place on **9 May 2019** in **Sunderland, UK**. The programme will involve presentations from academics and pharmaceutical industry analysts on the UK development and manufacturing context, the scope of applications, the role of pharmacopoeia, the effective use of liquid chromatography (LC) in quality control, bioanalysis, and biopharmaceuticals, and examples of the use of modern LC technology in development and manufacturing. Before the meeting, analytical scientists supporting pharmaceutical development and manufacturing were invited to comment on the most important unmet analytical needs they are facing. Arising from this, for the main part of the afternoon session, speakers, and in particular vendors, will be set the task of demonstrating how the following identified challenges might be met:

- The sheer number and variety of molecular entities coming through development and manufacture;
- The range and variety of formulations now being encountered;
- The need for automation in sample preparation for development and manufacture;
- Taking greater utilization of preparative LC in pharmaceutical development and manufacture;
- Training.

These themes will take up a large part of the afternoon session and at the close of the meeting a panel discussion will be held to consider whether these challenges are being satisfactorily addressed. **Registation:** https://goo.gl/meK4UT

6–7 May 2019
Method Development for the Separation of Therapeutic Proteins (Biopolymers)
Molnár-Institute, Berlin, Germany
E-mail: trainings@molnar-institute.com
Website: http://molnar-institute.com/fileadmin/user_upload/Training/SeminarRegistrationForm.pdf

12–17 May 2019
International Symposium on Capillary Chromatography (ISCC) and the GC×GC Symposium
Fort Worth, Texas, USA
E-mail: info@isccgcxgc.com
Website: www.isccgcxgc.com

13–17 May 2019
5th Workshop on Analytical Metabolomics
Aristotle University, Thessaloniki, Greece
E-mail: info.metabolomics@gmail.com
Website: http://biomic.web.auth.gr/workshop2019

22–23 May 2019
3rd International Conference and Exhibition on Petroleum, Refining, and Environmental Technologies (PEFTEC 2019)
Rotterdam, Netherlands
E-mail: info@ilmexhibitions.com
Website: www.ilmexhibitions.com/peftec/

17–18 June 2019
Analytical Quality by Design
Naarden-Bussum, Netherlands
E-mail: info@kantisto.nl
Website: www.kantisto.nl/index.php/agenda/30-agenda-items/38-analytical-quality-by-design

18–20 June 2019
LABWorld China 2019
Shanghai New International Exhibition Center (SNIEC), Shanghai, China
E-mail: salesoperations@ubm.com
Website: www.pmecchina.com/labworld/en

16–20 June 2019
48th International Symposium of High Performance Liquid Phase Separations and Related Techniques (HPLC 2019)
Milano-Bicocca University, Milan, Italy
E-mail: hplc2019@effetti.it
Website: www.hplc2019-milan.org

Please send any upcoming event information to Lewis Botcherby at lbotcherby@mmhgroup.com

The Essential Guide to Electron Ionization in GC–MS

The "must know" details of which all electron ionization (EI) gas chromatography–mass spectrometry (GC–MS) users should be aware.

In an electron ionization (EI) source, analyte ions in the gas phase encounter a stream of thermionic electrons with 70 electron volts (eV) of energy, emitted from the surface of a heated metal filament. The energy from these electrons is transferred, in part, to an analyte (no collisions are involved!), which causes the ejection of an electron from, and atom within, the analyte molecule, forming a radical (odd electron) cationic species:

$$M + e^- \xrightarrow[(70\ eV)]{} M^{+\cdot} + e^- + e^- \quad [1]$$

(?eV) (thermal, low energy, from analyte)

The amount of energy required to remove an electron from smaller organic molecules typically ranges from 8 to 12 eV, and any excess energy imparted by the ionizing electron may cause bond breakage and the formation of fragments (note that not all of the remaining electron energy is necessarily transferred to the analyte molecule).

The site of ionization is perhaps the first consideration when attempting to better understand the ionization process, and, in general, the following series indicates the energy required (and therefore favourability) of ionization site:

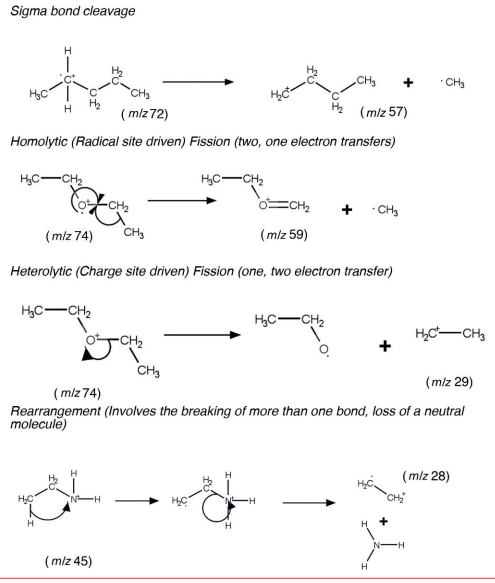
$$\sigma \text{ nonbonding} < p < \sigma \text{ (bonding)} \quad [2]$$

So, if an analyte were to contain a heteroatom, for example, containing nonbonding electrons, one might first begin to elucidate the spectrum obtained by assuming that the charge is cited on the heteroatom, and so on.

In electron ionization mass spectrometry, the intensity of the spectral signal for a molecular ion or fragment is dependant upon the energetic favourability of that species being formed, which is usually closely linked to the ability of the resulting ion to stabilize the charge which it carries, as well as the stability of the radical or molecular product (that is, the stability of all products must be considered). For this reason, highly unsaturated or aromatic species, which are able to stabilize charge, tend to have their most intense fragments at the higher molecular weight end of the spectrum (the right-hand end), and alkane species, which are less able to stabilize the charge on the molecular ion, tend to fragment more readily, and the most intense fragments will lie to the lower molecular weight end (the left-hand end of the spectrum).

Identifying the molecular ion within the spectrum is important, as it provides the molecular weight of the analyte, which is obviously very helpful for analyte identification. If a very weak molecular ion is suspected, one may reduce the energy of the ionizing electrons, from 70 eV down to around 25 eV, before signal intensity becomes too weak to distinguish from noise. This tends to promote the intensity of the molecular ion, and helps us confirm the suspected molecular ion.

Figure 1: "Typical" mechanisms and pathways with which fragments are formed.



When considering the nature of the fragments formed, and thus the chemical nature of the analyte, there are some "typical" mechanisms and pathways with which fragments are formed, typically to stabilize the charge on the analyte molecule, see Figure 1.

Remember that only the charge products are seen within the mass spectrometer, as we cannot guide the neutral species through the mass spectrometer towards the detector.

There are many more "tools" that can be used in spectral interpretation and those described above represent just some the fundamental considerations. For further information see: https://www.chromacademy.com/mass-spec-training.html

More Online:
Get the full tutorial at
www.CHROMacademy.com/Essentials
(free until 20 May).

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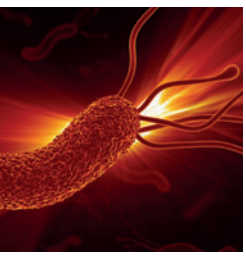
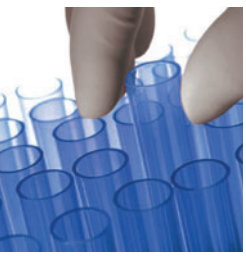
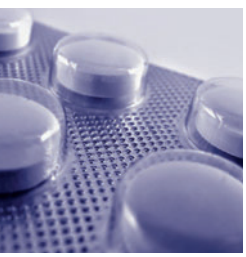
Decade Elite

Electrochemical detector for any (U)HPLC system







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