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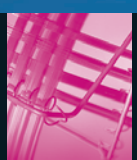

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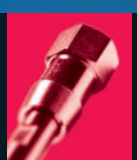

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Determination of Acrylamide in Water by Liquid Chromatography Coupled to Tandem Mass Spectrometry

Feng Jiali, Chen Dongyang, Wang Yihong, Li Bangrui, Zeng Dong, Zhang Hao, and Ding Li, Hunan Provincial Center for Disease Control and Prevention, Changsha, 410005, Hunan, China.

An analytical method based on liquid chromatography coupled to tandem mass spectrometry (LC–MS–MS) has been developed for the determination of acrylamide in water. To obtain clean extracts and low detection limits, an activated carbon cartridge was investigated for use in solid-phase extraction (SPE), and extraction conditions such as desorption solvent and elution volume were optimized by a series of experiments. High recoveries (99.1–99.8%) were obtained using the activated carbon solid-phase extraction cartridges with methanol as the eluent. This method could be applied to the quantification of acrylamide in environmental water samples.

Acrylamide (1), a known neurotoxin and putative human carcinogen, has been included among the substances to be monitored in drinking water. This compound has been regulated by the European Council Directive 98/83/EC (2) with a minimum quality requirement of 0.1 µg/L in drinking water. The main source of acrylamide to drinking water is the release of residual monomer from polyacrylamide coagulants used as a clarifier in raw water treatment.

As a result of its high solubility in water (2155 g/L at 30 °C) and its low levels in water, acrylamide is not easy to detect. There are very few reports on the determination of acrylamide in potable water At present, a frequently used method for the analysis of acrylamide relies on analyte derivatization and gas chromatographic (GC) separation (3). However, derivatization is often considered as time consuming, laborious, and can lead to a potential loss of analyte because of unstable or incomplete derivatization. Several groups have described a range of methods to quantify acrylamide by direct injection and reversed-phase ultraviolet high performance liquid chromatography (HPLC–UV) with a limit of detection (LOD) of 5 µg/L (4) and by solid-phase extraction (SPE) and gas chromatography coupled to mass spectrometry (GC–MS) analysis (5) to give limits of detection here. However, these methods are not sensitive enough for the analysis of low levels of acrylamide in water. The development of sensitive and reliable analytical methods for the quantification of acrylamide in potable water was considered as essential. The current study found that MS coupled to LC (6), using labelled acrylamide as the internal standard (IS), was now the most appropriate technique for the determination of acrylamide in water.

The purpose of this study was to develop a method for the determination of acrylamide in water at low levels. A reversed-phase LC–MS method based on a stable isotope dilution assay was developed for acrylamide analysis. Effectiveness of the enrichment process using the activated carbon cartridge for SPE was evaluated, and parameters such as desorption solvent and elution volume were optimized.

Experimental

Materials and Chemicals: Acrylamide (>99%) was provided by Dr. Ehrenstorfer GmbH, $^{13}C_3$-acrylamide was purchased from Cambridge Isotope Laboratories. All organic solvents were of HPLC-grade quality. Stock solutions of acrylamide and $^{13}C_3$-acrylamide were prepared by dissolving the compounds in methanol. These solutions were then appropriately diluted with water to prepare working standards.

The carbon SPE cartridges were from Varian (500 mg, 6 mL); Oasis MCX (150 mg, 6 mL) were from Waters; Supelclean ENVI-18 (500 mg, 6 mL) and Carb/NH_2 (500 mg, 6 mL) were from Supelco. Activated carbon was from Sinopharm Chemical Reagent Co.,Ltd.

Nitrocellulose syringe filters of 0.45 µm were purchased from Xiboshi. The water used was purified (18 MΩ quality) by a Milli-Q system (Merck Millipore).

KEY POINTS

- A new method has been developed for the determination of acrylamide in water at low levels.
- The limit of detection was 0.005 µg/L, and is lower than what has previously been reported.
- The method is sensitive, accurate, and reliable, and performs well at determining acrylamide in water.

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Figure 1: Chromatograms of (a) acrylamide and (b) $^{13}C_3$-acrylamide with a concentration of 5.0 µg/L.

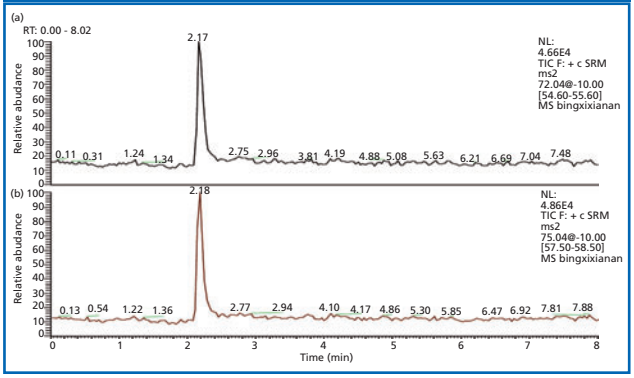
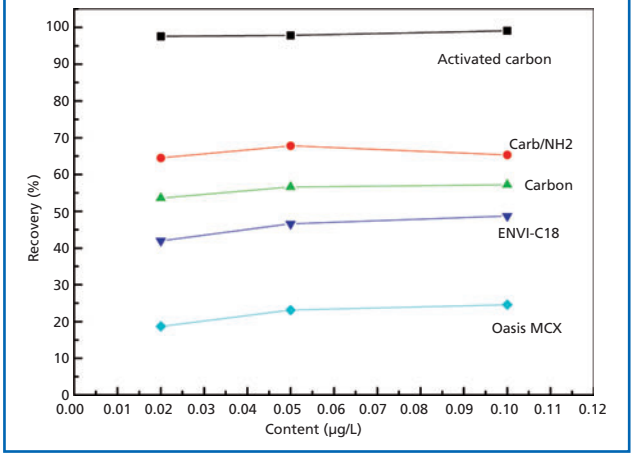


Figure 2: The recoveries of acrylamide with different cartridges.



River water was collected from Xiangjiang River.

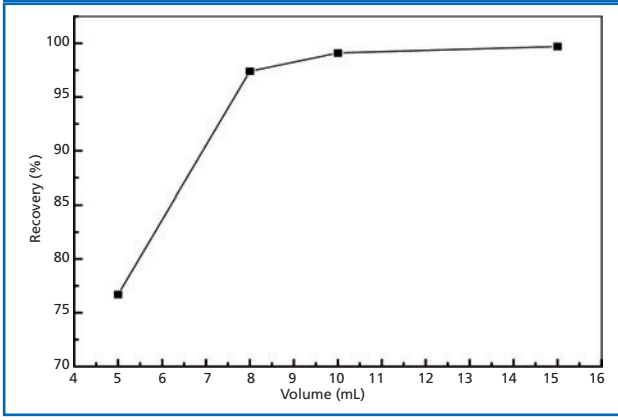
Chromatographic Conditions: The chromatographic separation of acrylamide was performed by reverse-phase LC using a 2.1 mm × 150 mm, 3.5-µm XBridge column (Waters). Optimum separation was achieved with an isocratic elution using methanol:water at a ratio of 10:90 as mobile phase at a flow rate of 0.2 mL/min. The sample volume injected was 10 µL.

MS Detection: MS–MS detection was performed on a TSQ Quantum Ultra triple quadrupole instrument (Thermo Fisher Scientific). Electrospray ionization (EI) in the positive ionization (PI) mode was used. The optimal source working parameters for monitoring positive ions were: Spray voltage: 4000 V; capillary temperature: 350 °C; Collision energy: 10 eV; sheath gas: 35 a.u; auxiliary gas: 10 a.u.

The MS detector operated in selected reaction monitoring (SRM) mode at *m/z* 72 and 75 for acrylamide and labelled $^{13}C_3$-acrylamide, and fragment ion at *m/z* 55 and *m/z* 58 were used for peak identity confirmation.

Sample Preparation: Prior to the extraction stages, samples were filtered through 0.45-µm nitrocellulose syringe filters. The cartridges were conditioned with 5 mL methanol followed by 5 mL of water. Each cartridge was loaded with 100 mL of filtered water, and then dried by nitrogen for 5 min. The target compounds collected on the cartridges were eluted with 10 mL of methanol. The extract was evaporated under a stream of nitrogen. Finally, acrylamide

Figure 3: The effect of elution volume on the recovery of acrylamide.



was dissolved in 1 mL of water and filtered as before and transferred into amber glass vials for LC–MS–MS analysis.

Quantification: Acrylamide was quantified using a linear calibration function that was established with standard solutions of acrylamide at concentration levels 1.00 µg/L, 5.00 µg/L, 10.0 µg/L, 50.0 µg/L, and 100 µg/L. In all instances, 5.0 µg/L of $^{13}C_3$-acrylamide internal standard was used for isotopic dilution quantification. The addition of a known level of internal standard to the samples at the beginning of the extraction process allowed the quantification of the analyte. This method gave more precise results because a correction of both extraction efficiency and changes in instrument performance were achieved.

Results and Discussion

Mass Spectrum of Acrylamide: An HPLC–MS–MS analytical method has been developed for the determination of acrylamide in water. The system was operated in positive electrospray and selected reaction monitoring mode. The detected ions of analyte and internal standard were $[C_3H_5NO]^+ = 72$ and $[^{13}C_3H_5NO]^+ = 75$, respectively. The daughter ions at 55 *m/z* and 58 *m/z* were acquired by the loss of a protonated amine in acrylamide and $^{13}C_3$-acrylamide, respectively. The dwell time was 1 s. An increase in dwell time led to a loss in linearity without a significant increase in sensitivity (7). The chromatograms of acrylamide and $^{13}C_3$-acrylamide are shown in Figure 1.

Optimization of SPE Procedure:

The Option of the Cartridge: A recovery assay was performed by adding 0.02 µg/L, 0.05 µg/L, and 0.10 µg/L of acrylamide to assess the extraction value. Five different SPE-cartridges with 10 mL methanol elution solvents in series were studied.

The results are shown in Figure 2. Activated carbon had the highest recovery for this application. The activated carbon has been frequently used in SPE of the polar micromolecules because of its wide specific surface area as well as reliable collection efficiency. Kawata et al. (8) reported the use of a granular activated carbon fibre as a device for SPE of eight hydrophilic organic compounds in environmental water. From these results, the activated carbon cartridge was selected as the extraction cartridge.

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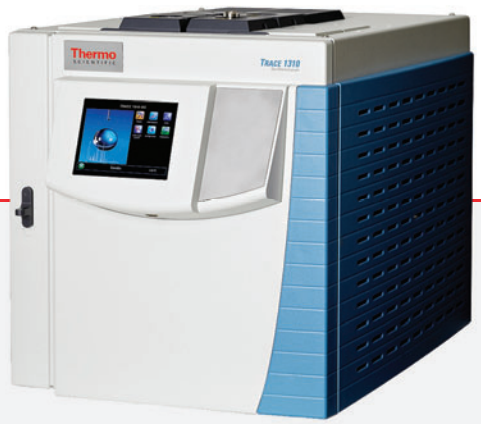

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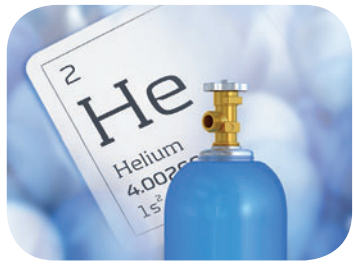

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Table 1: A comparison of different solvents on acrylamide recovery in water.

Solvent	Methanol	95% Methanol	90% Methanol	Acetonitrile	Chloroform
Recovery (%)	99.5	99.7	98.7	57.8	49.8

Table 2: The linear regression equations, correlation coefficients, and detection limits for acrylamide.

Analyte	Regression equation	Correlation coefficient	Limit of detection (µg/L)	RSD(%)
Acrylamide	Y = 0.0332X+ 0.011	0.9993	0.005	3.4

Table 3: The recovery yields of acrylamide (n = 6).

Sample	Spiked amount (µg/L)	After spiked (µg/L)	Recovery yield (%)
1	0.02	0.01982	99.1
2	0.5	0.4964	99.3
3	1.0	0.9980	99.8

Desorption Solvent: Desorption efficiencies for the target compound were determined by passing 100 mL of purified water spiked with 0.005 µg of the compound and $^{13}C_3$-acrylamide through the activated carbon cartridges. After the cartridges were dried, the compounds were eluted by using methanol, 95% methanol, 90% methanol, acetonitrile, and chloroform. The results are presented in Table 1.

The recoveries of 95% elution solvent reached 99.7% and were the highest. The performance of chloroform was the worst; it had only 49.8% recovery. The results matched the polar order of the solvents well. Because of the high polarity of acrylamide, water is a good choice as an elution solvent. However, as shown in Table 1, higher amounts of water did not significantly improve the recoveries, and instead prevented fast evaporation in the enrichment step. Therefore, methanol was recommended for the desorption solvent of the compounds from the extraction cartridge. Moreover, in SPE the elution volume is an important feature to take into account. The effects of different volumes of methanol on the recovery were studied. The elution curve (recovery [%] versus volume of elution) was established (Figure 3). As can be seen from the curve, the recoveries of acrylamide improved very little when the elution volume was more than 10 mL. For this reason, the optimum volume needed to remove the retained acrylamide quantitatively was set at 10 mL.

Method Performance: To check the performance of the method quality, parameters such as LOD, repeatability, and linearity range were established. The results are shown in Table.2.

LOD was determined as the amount of analyte that produced a signal-to-noise ratio (*S/N*) of 3:1, and was calculated using a blank sample (100 mL) spiked with very low amounts of acrylamide at the beginning of the extraction. The LOD value was 0.005 µg/L for a 100 mL sample. Repeatability was given as the relative standard deviation (RSD). Excellent repeatability of less than 3.4% was obtained for acrylamide.

The recovery yields of acrylamide in water were investigated with 0.02 µg/L, 0.5 µg/L, and 1.0 µg/L of standards compound in the water (blank). The results are shown in Table 3.

Sample Analysis: This method was applied for the determination of acrylamide in waters from Xiangjiang River. The results showed that acrylamide content of the water was lower than the LOD. Therefore, the concentrations of acrylamide in Xiangjiang River were estimated to be at an extremely low risk.

Conclusions

A method for the analysis of acrylamide has been developed. The method performs well at determining acrylamide in water where isotopically labelled internal standards are used. An improved purification and enrichment procedure was applied for the determination of acrylamide in water; recovery reached 99.8% and RSD was lower than 3.4%. This method could be applicable to the determination of acrylamide in water. Activated carbon is a very useful material and is widely used in environmental purification, such as air purification, sewage treatment, and solvent recovery,

References

(1) International Agency of Research on Cancer Monographs (IARC), vol.60, 1994, 389.
(2) European Council Directive 98/83/EC of 3 November 1998 on the Quality of Water Intended for Human Consumption, European Union, Brussels, Off. J. Eur. Commun. L330 (1998) 32.
(3) E. Tareke, P. Rydberg, P. Karlsson, et al., *J.Agric.Food Chem.* **50**(17), 4998–5006 (2002).
(4) M. Weideborg, T. Kallqvist, K.E. Odegrad, L.E. Sverdrup, and E.A. Vik, *Norway. Water Res* **35**(11), 2645–2653 (2001).
(5) A.F. Lagalante and M.A. Felter, *J.Agric.Food Chem.* **52**(12), 3744–3748 (2004).
(6) E. Bermudo, E. Moyano, L. Puignou, and M.T. Galceran, *Talanta* **76**(2), 389–394 (2008).
(7) S. Cavalli, S. Polesello, and G.Saccani, *J.Chromatogr.A* **1039** (1–2), 155–159 (2004).
(8) K. Kawata, T. Ibaraki, A. Tanabe, et al., *J. Chromatogr. A* **911**(1), 75–83 (2001).
(9) E. Bermudo, E.Moyano, L.Puignou, et al., *Analytica Chimic Acta* **559**(2), 207–214(2006).

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Problems with Large-Molecule Separations

John W. Dolan, LC Resources, Walnut Creek, California, USA.

Some attributes of large molecules make them behave differently from small molecules in reversed-phase separations.

Several readers have sent emails to me lately with questions and problems related to the liquid chromatographic separation of proteins, peptides, and other large molecules, so I've combined their questions into a discussion of some of the problems related to such separations. I'll use proteins as the model compounds, although the general behaviour of large molecules is similar. Reversed-phase liquid chromatography (LC) is the most popular separation technique for these molecules, and will be the focus of this month's "LC Troubleshooting" instalment. Reversed-phase LC, of course, is a denaturing technique, so it is good for analysis, but not for purification or recovery of intact molecules. For example, if you want to separate an enzyme from other compounds and then collect it for other uses, you'll want to preserve the activity of the enzyme. This means that the mobile phase must be sufficiently gentle that it does not denature the protein. Some techniques to accomplish this include ion exchange, gel permeation chromatography (GPC), hydrophobic interaction chromatography (HIC), and affinity chromatography, each of which use a nondenaturing aqueous mobile phase. Reversed-phase mobile phases for protein separations usually include acetonitrile, which will irreversibly denature the proteins.

When protein separations by reversed-phase LC were first being explored in the 1980s, some workers thought that the separation mechanism was completely different than that for small-molecule separations. Today, we know that is not the case — the same rules apply. But there are some aspects of the separation that we have to be careful of or we will not get the results we expect. Even today these "surprises" can confound workers new to the field. Let's look at some of the aspects of large-molecule separations that we need to pay attention to with reversed-phase LC.

Column Selection

Before starting any kind of LC separation, we need to pick a column. There are literally hundreds of reversed-phase columns to choose from, but we still need to make a wise choice. With small molecules, typically we start with a silica-based column with a C18 or C8 bonded phase. With large molecules, a C4 phase is a much more common choice. For small-molecule separations (for example, <1000 Da), the sample molecules are small enough to get between the bonded phase chains on the packing surface, so different chain lengths will effectively result in a different amount of chemically active surface area. Thus, we typically observe that a C18 phase will have retention factors that are perhaps 70% larger than those for a C8 phase, or that it takes approximately 5% more organic solvent to get the same retention time with a C18 phase as with a C8 phase. On the other hand, large molecules (for example, >10 kDa) are too large to penetrate the densely bonded phase, so they only "see" the tips of the bonded phase chains. This means that a C8, C18, and even C4 chain length "look" about the same to a large molecule. Another way to think of this is to visualize the column packing as a toothbrush, where the handle of the brush is the silica support and the bristles are the stationary phase molecules, fastened at one end. Small molecules might be thought of as grains of sand, which can penetrate into the densely packed bristles, whereas a large molecule might be more like a marble that sits on top of the tips of the bristles. So there isn't much effect of bonded phase chain length on retention with large molecules. Early in the development of such separations, a C4 chain length was chosen and has become the defacto standard for protein separations.

A second aspect of the column that is important is the size of the pores in the packing particles. The silica support is not a solid bead with the bonded phase on the outside, like the fuzz on a tennis ball. A better description would be a porous sponge, or better yet, a popcorn ball, where the pores are the spaces between the kernels. As a result, nearly all the surface area is within the particle, not on the outside. For this reason, the pores have to be large enough that the sample molecules can easily penetrate the pores. As a rule of thumb, we want the pore diameter to be at least three times the hydrodynamic diameter of the sample molecule. Columns used for small-molecule separations typically have pore diameters that range (between products) of 6–15 nm (60–150 Å), which are ample for free movement of small molecules in and out of the pores. Such pores, however, are too small for proteins, where pore diameters of 30–40 nm (300–400 Å) are more common. Larger-pore columns are available for size exclusion separations, but surface area is roughly proportional to the pore diameter, and retention to surface area, so excessive pore diameters may translate to insufficient retention.

Mobile-Phase Selection

Acetonitrile is usually chosen as the organic solvent for reversed-phase separation of proteins. Methanol could be used, but acetonitrile has the added advantage of transparency at low wavelengths (<220 nm), which often are necessary for detection. Trifluoroacetic

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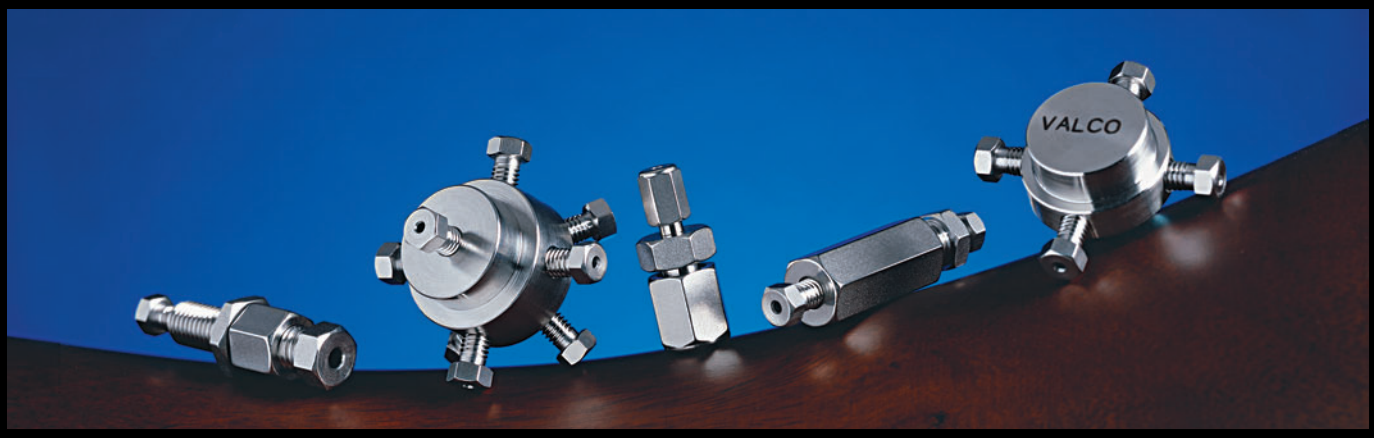

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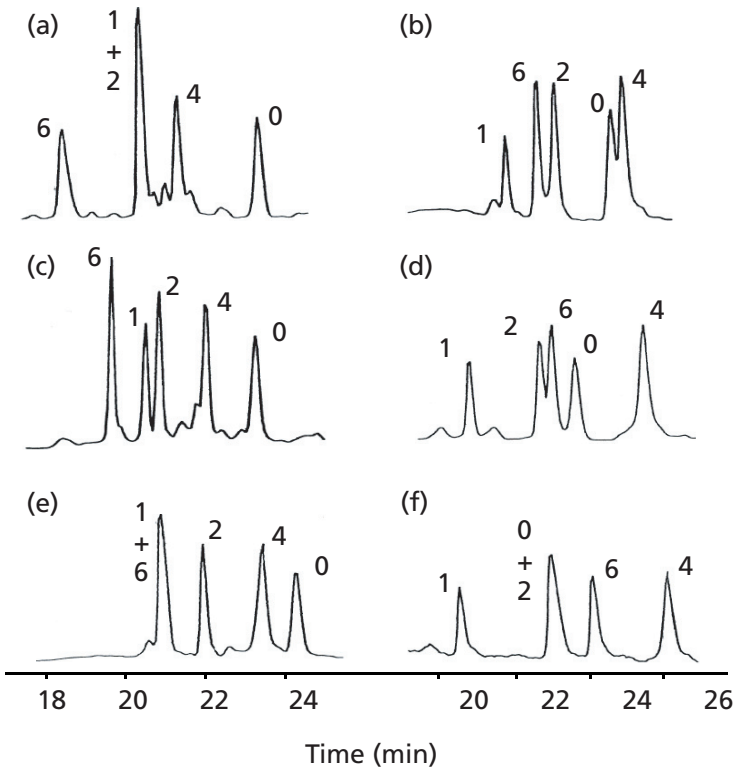
Figure 1: Effect of trifluoroacetic acid concentration on retention of basic peptides (the number of basic groups is indicated on each peak): (a) 0.02%, (b) 0.2%, (c) 0.05%, (d) 0.4%, (e) 0.1%, and (f) 0.8%. Adapted from reference 1.



acid is the preferred mobile-phase additive. At 0.1% trifluoroacetic acid in water for the A solvent and 0.1% trifluoroacetic acid in acetonitrile as the B solvent, a low pH (~2) and ion-pairing conditions are achieved. Fine-tuning of the concentration of trifluoroacetic acid may improve the separation, as is illustrated in Figure 1, where dramatic changes in retention order of a peptide sample are observed when the concentration of trifluoroacetic acid is changed over a range of 0.02% to 0.8%. However, most workers use 0.1% trifluoroacetic acid and don't vary it. Although trifluoroacetic acid–acetonitrile mobile phases are good for ultraviolet detection down to 200 nm, these mobile phases can cause ion suppression with mass spectrometric detection (LC–MS) when an electrospray ionization (ESI) interface is used. If such problems are encountered, LC–MS with an atmospheric pressure chemical ionization (APCI) interface may be a better choice because ion suppression is much less of a problem with APCI than ESI.

Gradient Conditions

The selection of the gradient conditions is the place where it is most common to run into problems when setting up a large molecule separation. It is unlikely that a separation will be achieved under isocratic conditions for large molecules, so gradients are standard. The gradient retention factor, k^*, is defined as:

$$k^* = (t_G F)/(1.15\, V_m \Delta\Phi S) \quad [1]$$

where t_G is the gradient time (in minutes), F is the flow rate (millilitres per minute), V_m is the column volume (millilitres), $\Delta\Phi$ is the gradient range (for example, 5–80% = 0.75), and S is a constant that is dependent on the molecular weight of the sample.

S can be estimated as follows:

$$S \approx 0.25\ MW^{0.5} \quad [2]$$

where MW is the molecular weight (in daltons).

To obtain "good" chromatography, we like to have $2 < k^* < 10$. Let's see how typical gradient conditions for a 400 Da, small molecule sample fit into this target region for k^*. Typical conditions might be a 150 mm × 4.6 mm column operated at 2 mL/min with a 5–95% B gradient over 20 min. In this case, $S \approx 0.25 \times 400^{0.5} = 5$, and the column volume is ~1.5 mL. So $k^* = (20 \times 2)/(1.15 \times 1.5 \times 0.9 \times 5) \approx 5$, which is in the middle of the desired $2 < k^* < 10$ range.

Let's try the same gradient conditions for larger molecules. This was what workers tried when such separations were first attempted, and you'll quickly realize the problem. We'll use a peptide with a molecular weight of 4000 Da and a 40 kDa protein, which give S values of 16 and 50, respectively. Using equation 1 and the same conditions as above, these translate into $k^* \approx 1.6$ and 0.5, respectively. For the peptide, this might give a marginal separation, but for the protein, the sample is eluted much too early. This would be analogous to "blasting" a sample off a column under isocratic conditions with a very strong mobile phase, where the sample travels through the column so quickly that it doesn't have adequate time to interact well with the column.

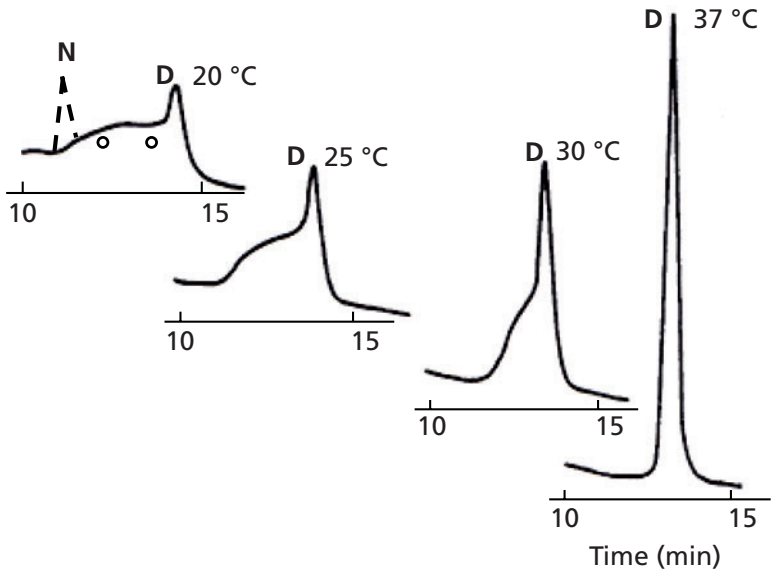
To compensate for the large S value, we need to adjust one or more of the other factors in equation 1 so that we can get a reasonable value of k^*. For example, with the protein, we could increase the gradient time to 60 min, which would increase k^* to ~1.5. Now you can see why large molecule separation methods tend to use long gradients.

Usually it is not necessary to use a full-range gradient with large molecules, so shortening the gradient range may be possible. For example, you might run a scouting gradient of 5–95% B in 60 min and observe the retention times of the components. From this, you can figure out approximately what %B is present when the first and last peaks are eluted, and then shorten the gradient range. Usually a range of 5–80% B is adequate, and a smaller range may be possible with some samples. For the present example, reducing the gradient range from 5–95% B to 5–80% B will increase k^* a bit more to ~1.9. Extending the gradient time to 70 min will bring k^* above 2.

Other Potential Problems

Sample diffusion in the mobile phase, within the stationary phase, and into and out of the packing pores plays a major role in peak broadening in LC. If the flow rate is too high, sample molecules can be swept through the column so fast that

Figure 2: Effect of temperature on peak shape for reversed-phase separation of ribonuclease. Dashed peak added to show approximate position of native (nondenatured) ribonuclease. See text for details. Adapted from reference 2.



slow diffusion causes broader peaks, lower column plate numbers, and, thus, lower resolution. Under ideal conditions that highlight diffusion effects, significant degradation of the chromatogram can be observed for increases in flow rate with small molecules and packing particle diameters (d_p) of ≥5 µm. However, with real samples under typical operating conditions we don't see any practical degradation of the chromatogram with a twofold flow rate change with particles with diameters ≤ 5 µm, and certainly not for diameters ≤ 3 µm. On the other hand, large molecules diffuse much more slowly, and therefore are also more sensitive to flow-rate changes. With small molecules, one way to speed up the separation while maintaining k^* constant is to both reduce the gradient time and increase the flow rate to keep the numerator of equation 1 constant. For example, a 20-min gradient at 1 mL/min will give the same k^* as a 10 min gradient at 2 mL/min, all other factors constant. For small molecules, this approach is usually successful. But for large molecules, care needs to be taken to be sure that the flow-rate change doesn't cause an increase in peak broadening that more than cancels the benefit of a shorter run. If you decide to use increased flow rate with large molecules, as might be done under ultrahigh-pressure liquid chromatography (UHPLC) conditions, be sure to look for this potential problem.

A final caution for the separation of proteins by reversed-phase LC has to do with molecular conformation. The native structure of proteins is complex and is important for biological activity. As was mentioned earlier, acetonitrile-based mobile phases denature proteins. A further complication is that different molecular conformations often have different chromatographic properties, which means that the same protein may have different retention times depending on its conformation. A difference in retention time for the same compound from run-to-run certainly is not a desirable property of a reliable method! An example of this is shown in Figure 2 for a sample of ribonuclease under reversed-phase conditions at different temperatures. The peak at ~13 min is the denatured form (D) of ribonuclease. The native form (N) does not make it through the column, but I've sketched it in with a dashed line in the 20 °C run. At 20 °C, the sample enters the column in the native conformation, but as it travels through the column, it becomes denatured. Molecules that are denatured at the column inlet travel through as a single denatured compound and show up as the 13-min peak. Other molecules travel different distances before they are denatured, and show up as the rounded baseline between the two peaks. By increasing the temperature of the column, the denaturing process takes place earlier, so that at 37 °C, all molecules are denatured at the inlet and travel through as a single denatured peak. The staff in our laboratory referred to chromatograms like the one at 20 °C as "bat-o-grams," because they looked a bit like Batman (eyes added for emphasis). Bat-o-grams are seen occasionally with proteins if they are not fully denatured before injection. It is best to make sure that the sample has been subjected to denaturing conditions before injection if you want to avoid the bat-o-gram problem.

Summary

We have seen that large molecules, such as proteins, obey the same rules that small molecules do in reversed-phase separations, but there are aspects of the separation that we need to pay careful attention to. Of particular importance is the requirement that the column packing pore diameter is sufficiently large that the sample molecules have free access to the pores and that the gradient conditions are adjusted so that k^* is not too small. Large molecules diffuse slowly, so it is wise to keep the flow rate low enough that they have time to diffuse in and out of the pores. Because reversed-phase mobile phases are denaturing, it is best to ensure that the sample is fully denatured before injection or protein denaturing on the column may cause peak shape and retention problems. For more information about the separation of peptides and proteins, references 3 and 4 are a good place to start.

References

(1) D. Guo, C.T. Mant, and R.S. Hodges, *J. Chromatogr.* **386**, 57 (2005).
(2) S. Cohen, K. Benedek, Y. Tapuhi, J.C. Ford, and B.L. Karger, *Anal. Biochem.* **144**, 275 (1985).
(3) L.R. Snyder, J.J. Kirkland, and J.W. Dolan, *Introduction to Modern Liquid Chromatography, 3rd ed.* (Wiley, Hoboken, New Jersey, USA, 2010), Chapter 13.
(4) R.L. Cunico, K.M. Gooding, and T. Wehr, *Basic HPLC and CE of Biomolecules* (Bay Bioanalytical Laboratory, Richmond, California, USA, 1998).

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New Gas Chromatography Products 2014

John V. Hinshaw, Serveron Corporation, Beaverton, Oregon, USA.

In this "GC Connections" instalment, I review gas chromatography (GC) instruments and accessories that were newly on display at the Pittsburgh Conference in March 2014, or were introduced to the marketplace in the preceding year.

The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) returned to Chicago's McCormick Place for its 65th annual meeting on 2–6 March 2014. This year, more than 16,200 conferees and exhibitor personnel attended Pittcon and the co-hosted Food Labs Conference. More than 36% were listed as first-time attendees, but the published statistics did not differentiate between Pittcon and Food Labs registrants. The exposition floor sited 935 exhibitors in a total of 1763 booths; 32 countries were represented. As was the case in 2013 in Philadelphia, the exhibition floor proved easier to navigate than in earlier conferences. This did not seem to be related to the size of the conference, which has not varied by more than about 10% in recent years, but rather the layout was well organized with many booths positioned in logical groups.

The technical programme was of high quality with 73 symposia, 10 awards, 84 oral sessions, plus posters and workshops making up a total of 2000 technical sessions. Emphasis was clearly on life science topics, which constituted 40% of the presentations. The short course program continued its high activity with 116 offerings.

Pittcon will journey to New Orleans, Louisiana, USA, in 2015 — a welcome respite from two consecutive years in colder northern locations. Heading to Atlanta, Georgia, USA, in 2016, conferees will enjoy a couple years of Southern hospitality until the conference swings back to Chicago, USA, in 2017.

This annual "GC Connections" instalment reviews gas chromatography (GC) instrumentation and accessories shown at this year's Pittcon or introduced during the previous year. For a review of new GC and liquid chromatography (LC) columns and related accessories, please see "Column Watch" in the April and May 2014 (1,2) issues of *LCGC Asia Pacific*, which are also available on-line at www.chromatographyonline.com/ColumnWatch.

The information presented here is based on manufacturers' replies to questionnaires, as well as on additional information from manufacturers' press releases, websites, and product literature on the past year's products. It is not based on actual use or experience of the author. During Pittcon, I took time to stroll around the convention aisles and see some of the new products first-hand as well as to discover a number of items that weren't covered by the questionnaires. Every effort has been made to collect accurate information, but because of the preliminary nature of some of the material *LCGC* cannot be responsible for errors or omissions. This column instalment cannot be considered a complete record of all new GC products introduced this year at Pittcon or elsewhere because not all manufacturers chose to respond to the questionnaire or attend the conference, nor is all of the submitted information necessarily included here because of the limited available space and the editors' judgment as to its suitability.

Gas Chromatography at Pittcon 2014

GC again displayed renewed vigour at this year's Pittcon technical sessions. As in previous years, work in comprehensive GC×GC continues to yield significant advances, while the older technique of moving thermal-gradient GC (TGGC) is finding a new niche. Of particular interest were two contributions in separate sessions by members of Milton Lee's group from Brigham Young University (Utah, USA) on the theory and practical applications of TGGC for microfabricated GC and for GC×GC (3,4) Originally investigated decades ago, TGGC helps focus and control the passage of analytes through a column to produce improved temperature-programmed separations. While the technique has not been adopted for commercialization in recent years, perhaps a new TGGC instrument will appear sometime in the future.

Table 1: Companies introducing new GC products.

Company Name
Agilent Technologies
Baseline Mocon
Bruker
CDS Analytical
EST Analytical
Gerstel
Hamilton
Markes International
OI Analytical/Xylem Analytics
Thermo Fisher Scientific
VUV Analytics

Table 2: New GC instruments.

Product	Company	Description
7000C triple-quadrupole GC–MS–MS system	Agilent Technologies	This system replaces the company's 7000B GC–MS–MS system and provides new capabilities. The 7000C system integrates new ion-source technology that was introduced earlier this year on the company's 5977 Series GC–MSD system. The new system has an instrument detection limit of 4 fg octafluoronapththalene. New integrated features shared by the 7890B GC and 7000C systems, plus new tools for Agilent's MassHunter software, support rapid method development, complete method optimization, and reduced energy consumption. In addition, the company offers an extensive multiple reaction monitoring (MRM) database of pesticides and environmental pollutants for the system.
9100 Series gas chromatograph	Baseline Mocon	Baseline introduced the 9100 Series gas chromatograph, an on-line fully integrated instrument that achieves laboratory quality analysis, according to the manufacturer. The GC system supports multiple detector options including a photoionization detector and a high sensitivity photoionization detector, plus a flame ionization detector and thermal conductivity detector to achieve low-level measurements into the sub-parts-per-billion range as well as high-level applications with percent concentrations. Supported applications include indoor and outdoor air quality monitoring, toxic gases in the workplace, environmental sources, fence lines, ambient air networks, trace impurities in specialty and industrial gases, and fast C_1–C_5 analyses. Key features include integrated GC data processing and a touch screen that eliminates the need for an external PC. The system incorporates multiple USB ports, remote access via network (LAN) connection, automatic calibration and diagnostics that ensure accuracy, internal data storage for current and historical data, and a compact footprint. The system supports automatic or manual calibration; packed, micropacked, or capillary columns as determined by application; a bench or rack mount configuration; *x*-purged or *z*-purged enclosures; five programmable relays; oven temperatures up to 120 °C; and four- to eight-point sampling options. It has a nominal weight of less than 30 lb (14 kg) and physical dimensions of 16.61 in. (42.2 cm) w × 5.74 in. (14.58 cm) h × 14.53 in. (40 cm) d. The unit can function at 0–40 °C with input voltages of 100–240 VAC. Other options and accessories include a digital input board with six contact-closure inputs; analog, voltage, or relay outputs; and built-in or external pump samplers.
Model 5500 fixed gas analyzer	CDS Analytical	The Model 5500 fixed gas analyzer is an add-on device for the company's trapping pyrolyzer systems that provides analysis of gases such as CO, CO_2, water, methane, and ethylene, among others, produced during pyrolysis of synthetic or natural polymeric materials. These fixed gases may not be fully resolved by a capillary GC, but they are useful for calculating British thermal unit (Btu) values and mass balance. The system adds a small chromatographic column and thermal conductivity detector to the outlet of the analytical trap, which are capable of analyzing fixed gases that are normally purged from the system before analysis. The prepackaged analyzer can be added to both new and existing systems. The system contains a Carboxen 1000 column, reference column, and thermal conductivity detector. A software package for the chromatography and A/D conversion is included. The software is preprogrammed to analyze for fixed gases, but users can change programming for other applications. The accessory uses a two-cell hot-wire TCD with switchable polarity with a programmable column heating rate up to 200 °C/min, a 1/8-in. (2-mm) i.d. × 9-ft. (3-m) long 60/80 mesh Carboxen 1000 column. The valve oven and TCD can be heated to 300 °C, and the flow rate can be set from 0 to 100 mL/min. The device incorporates a backlit light-emitting diode (LED) touchscreen user interface. It includes the gas injection port, A/D converter, and chromatography software.

Two other GC topics stood out: Alternative carrier gases and ionic liquid phases for GC. With the continuing uncertainty that surrounds helium supplies, the use of hydrogen or nitrogen as alternatives still garners much attention. Interest in hydrogen was evident throughout the exhibition as well as in a number of papers and posters. Recent developments seem to indicate that new supplies of helium from areas such as Qatar will ensure availability for the foreseeable future, but prices may remain high. Ionic liquid GC phases provide some unique selectivities and interest remains strong while applications of these materials expand to liquid–liquid and solid-phase micoextraction. See the related article on chromatography columns at Pittcon 2014 (2) for more details.

In the product realm, there were a few GC instruments introduced since Pittcon 2013. From Agilent Technologies, introduced at ASMS in 2013, the model 7000C triple-quadrupole GC–MS–MS system represents the high end of GC–MS with its advanced spectrometric capabilities and high sensitivity. During the past year, Agilent also introduced a hydrogen sensor and a large heated valve oven for its model 7890B GC system.

The 9100 Series gas chromatograph from Baseline Mocon is an on-line instrument that is reported to achieve laboratory-quality analysis for gas analysis across a wide range of applications. The model 5500 Fixed Gas Analyzer from CDS Analytical is an adjunct to the company's trapping pyrolysis systems for the analysis of low-molecular-weight gaseous by-products.

Table 3: New autosamplers and accessories.

Product	Company	Description
4100 Water/Soil sample processor	OI Analytical/ Xylem Analytics	OI Analytical's 4100 Water/Soil sample processor automates the handling and processing of samples in 40-mL volatile organic analysis (VOA) vials for purge-and-trap analysis of volatile organic compounds (VOC) in accordance with US EPA methods. The system efficiently processes up to 100 drinking water, wastewater, or soil samples. The system operates with single or dual OI Analytical Eclipse 4660 purge-and-trap instruments. The sample processor is equipped with a pneumatically-actuated cylindrical vial gripper that lifts and transports 40-mL VOA vials to and from water and soil sampling stations. The company's VOA Constrictor gripper mechanism surrounds and conforms to each vial ensuring reliable vial handling. The design of the VOA Constrictor gripper handles vials with all types of labels and eliminates dropped and broken vials. A built-in sensor enables the gripper to detect and confirm the presence of a vial or signal if a vial is missing. A new type of module for addition of internal standards, surrogate, and matrix spike standards is integrated into the 4100. This module uses electronically controlled high-speed injection valves to minimize usage of standard mixtures and decrease laboratory operating costs for expensive standards. The high-speed injection valves inject programmed volumes of standard without excess overfill volume or waste. Programming and operation of the 4100 is controlled by VOA View software. VOA View is a Windows-based graphical user interface (GUI) that enables users to program methods, sequences, and internal standard addition. VOA View visually depicts water and soil sample, the status of vials that have been processed, and details of the method being run. VOA View software enables a 4100 sample processor to communicate and operate concurrently with two purge-and-trap instruments for higher sample throughput and laboratory productivity.
Flex autosampler	EST Analytical	EST Analytical introduced the Flex autosampler series, which offers a variety of sampling modes for GC and GC–MS systems. According to the manufacturer, the base autosampler is compatible with any GC platform and can perform a wide variety of liquid injections as well as some basic sample preparation on-board. The system can be upgraded to perform headspace sampling with the addition of a six-position incubation station and heated syringe assembly. The sampling system is programmed through a drag-and-drop graphical interface that supports the development of complex sampling and sample preparation routines. Solid-phase microextraction (SPME) can be performed with the addition of an SPME conditioning station and syringe fibre holder. The Flex series is well suited for liquid injection, derivatization, standard addition, headspace, SPME, and sample preparation.
LV-20 standards addition module	OI Analytical/ Xylem Analytics	The OI Analytical LV-20 standards addition module is an option for the company's 4551A water autosampler. The LV-20 adds internal standards, surrogate, or matrix spike standards for GC and GC–MS analyses. The LV-20 is equipped with high-speed injection valves that minimize standard usage and decrease laboratory operating costs for expensive standards. The high-speed injection valves inject user-programmed volumes of standard without excess overfill volume and waste. Amber glass reservoirs protect standards from UV light and a dry-erase panel allows users to note what standards are in use and the date reservoirs were filled.
Model 7500 thermal desorption autosampler	CDS Analytical	The CDS 7500 thermal desorption autosampler is an extension of the company's 7400 purge-and-trap system that offers the ability for the user to change between thermal desorption (TD) tubes for air and VOA vials for soil or water analysis. The system features a pick-and-place robotic arm, three removable trays for a total of 72 samples, automated internal standard addition, the ability to desorb other manufacturers tubes, a sample-saver mode, and leak checking. The CDS 7500 autosampler is compatible with the company's Dynatherm 9300 Series and model 7000 E purge-and-trap system.

Bruker showed the GC-APCI II GC–MS interface, which can be combined with any Bruker ESI mass spectrometer, including the impact HD LC-QTOF system introduced at the 2013 ASMS conference. The interface makes the impact HD's unique LC–QTOF–MS capabilities — such as accurate mass screening or determination of elemental compositions — accessible for GC–QTOF–MS.

Markes International's Select-eV variable-energy ion-source technology for GC–MS allows the energy used for electron ionization to be changed on a sliding scale without loss of sensitivity. This allows the production of both classical mass spectra and repeatable soft-ionization spectra with reduced fragmentation and an enhanced molecular ion.

VUV Analytics displayed a new type of GC detector that collects vacuum-ultraviolet spectra of peaks as they are eluted for peak identification, deconvolution, and selectivity. The detector operates from ~120 to 240 nm, down to shorter wavelengths than the ~190-nm capability of routine diode-array LC detectors.

A number of autosamplers and related devices debuted this year. The model 4100 Water/Soil sample processor from OI Analytical automates the handling and processing of samples in up to 100 40-mL volatile organic analysis (VOA) vials for purge-and-trap analysis in conjunction with one or two of the company's Eclipse purge-and-trap

Table 4: New GC instrument accessories.

Product	Company	Description
GC-APCI II GC–MS interface	Bruker	The GC–APCI II system is designed to provide high GC–MS performance together with improved usability. According to Bruker, it allows better use of lab space and can save time during operation. Unidentified GC peaks can be routinely analyzed in ultrahigh-resolution (UHR) MS systems. The interface can be combined with any Bruker ESI mass spectrometer, including the impact HD LC-QTOF system introduced at the 2013 ASMS conference. The GC-APCI II makes the impact HD's unique LC–QTOF–MS capabilities — such as accurate mass screening or determination of elemental compositions — accessible for GC–QTOF–MS. The calibrant reservoir of the GC-APCI II interface enables software-controlled calibrant delivery. The calibrant can be added for data-file recalibration in each GC run. GC-APCI II system is an excellent solution for identification of unknowns in metabolomics and small molecule research. The redesigned ionization chamber provides very high sensitivity and low background in combination with well-resolved GC peaks, to deliver improved dynamic range and lower limits of quantitation (LLOQ) with up to 10-fold gains in sensitivity. The transfer line is compatible with the company's 436-GC and 456-GC systems.
Hot Injection and Trapping (HIT)	Gerstel	Gerstel's Hot Injection and Trapping (HIT) technique combines analytes from several headspace injections for each GC–MS run. HIT is performed using the company's MultiPurpose Sampler (MPS) with Thermal Desorption Unit (TDU) and Cooled Injection System (CIS), programmed-temperature vaporizer (PTV) type inlet. The system is easily switched between standard and HIT operation. The number of injections is specified in Gerstel's MAESTRO software under integrated control with the GC–MS system or under standalone control. Application examples include volatile organic compounds (VOC) such as flavour and off-flavour compounds in water and beverages to produce improved recovery and very low limits of detection (LOD).
Hydrogen sensor for the 7890B GC system	Agilent Technologies	This hydrogen sensor for the company's 7890B GC system provides the additional level of security many organizations require to use hydrogen in place of noncombustible carrier gases such as helium or nitrogen. The self-calibrating sensor will automatically execute a safe sequence for the shutdown of the GC system if even a small hydrogen leak is detected, which helps ameliorate such potentially hazardous situations in the laboratory. Along with the company's Programmable Helium Conservation Module, which implements "sleep-wake" modes for the Agilent 7890B GC system, the hydrogen sensor helps GC users deal with helium supply shortages and rising helium costs.
Large Valve oven for Series 7890B GC system	Agilent Technologies	The company's high-capacity external oven can be configured to support complex, multivalve ASTM International and European Standard (EN) GC applications. Engineered for thermal isolation from the GC oven, the oven provides a homogeneous isothermal environment for up to six positions for columns and valves. Its vertical design orientation facilitates maintenance. The Large Valve oven supports Agilent's standard multivalve analyzers such as refinery gas analyzers and natural gas analyzers with factory-tested methods and guaranteed chromatographic performance. The flexibility and configurability of the new external valve oven enable multiple ASTM and EN methods to be implemented on a single GC system, which provides a smaller laboratory footprint overall and more information from a single system.
S-Line Syringe	Hamilton	Hamilton's new S-line syringe for CTC PAL autosampler applications complements the company's existing C-Line and X-Type CTC PAL autosampler syringes. S-Line syringes are built for economic everyday use for GC and HPLC injection applications. The S-Line syringe is offered in both Gastight and Microliter versions. CTC PAL autosamplers are manufactured, marketed, and sold under different model names, and the Hamilton S-Line syringes can be used on all of them. According to the company, the fast injection speeds needed in GC analyses put considerable stress on syringe plungers, leading to broken syringes and unnecessary downtime. The Microliter version of the S-Line syringe overcomes stress problems in high-speed GC injections by incorporating a hand-fitted stainless steel plunger with a finely bored syringe barrel. The syringe has minimal drag force and optimal wear characteristics that maximize autosampler up-time. Gastight S-Line syringes incorporate a precision-machined PTFE plunger tip that creates a leak-free seal. The tip wipes the interior of the syringe barrel free of sample and minimizes sample carryover. This feature is particularly useful with heterogeneous samples because it reduces the chance that a deposit will occur and cause the plunger to freeze.
Select-eV technology for GC–MS	Markes International	Markes International's Select-eV variable-energy ion-source technology for GC–MS allows the energy used for electron ionization to be changed on a sliding scale without loss of sensitivity. This engenders the production of both classical mass spectra and repeatable soft-ionization spectra with reduced fragmentation and an enhanced molecular ion. The company explained that Select-eV allows any ionization energy from 70 to 10 eV to be selected as part of the operating method. At lower energies the molecular ion is enhanced, but structurally-significant fragments are also retained. This provides further confirmation of compound identity and distinguishes between compounds with spectra that are very similar at 70 eV. The full-spectral sensitivity of the company's BenchTOF time-of-flight mass spectrometers for GC is maintained or enhanced by the addition of Select-eV. The technology does not require reagent gases or source exchanges.

Table 4: New GC instrument accessories (*continued*).

Product	Company	Description
VGA-100 vacuum-UV detector	VUV Analytics	VUV Analytics displayed a new type of GC detector that collects vacuum-ultraviolet (VUV) spectra of peaks as they are eluted. The detector operates from ~120 to 240 nm, shorter wavelengths than the limits of routine LC detectors. It acquires spectra at rates up to 100 Hz, which in combination with its 80-µL flow-cell volume and operating temperature of up to 300 °C makes it compatible with faster gas chromatography. The system interfaces to many models of GC via existing mass spectrometer ports. The detector makes use of the spectral uniqueness of eluted compounds and chemical classes for selectivity, deconvolution, and goodness-of-fit metrics. It can use helium, nitrogen, argon, or hydrogen carrier gas. The deuterium lamp UV source has a stated lifetime of ~2000 h, while the need for a vacuum pump is eliminated by an internal purge-gas flow in the optical path. The company presented applications ranging from polyaromatic hydrocarbon (PAH) and xylene isomer identification to specialty gas contaminants and phosphine/H2S discrimination. Unique spectra were shown for pesticides, sulphur gases, and other compounds such as nicotine and acetonitrile.
Virtuoso vial labelling system	Thermo Fisher Scientific	Thermo Fisher Scientific's Virtuoso system prints indelible, detailed information directly on sample vials. The system's software provides automated download of sample lists, on-demand system diagnostics, and emergency sample labelling. Vial identification data can be input using the unit's touchscreen or directly from the lab network. Vial labelling takes approximately 5 s. The unit's sleeve holds up to 10 vials for autoloading. The company's testing has demonstrated an eightfold increase in productivity compared to conventional manual labelling techniques. Templates include standard required information, and users can customize them to suit their specific needs, including text, graphics, barcodes, and logos. The images resist conditions found in most labs, including solvents and handling. The vial identification system uses Thermo Scientific Virtuoso Vials for optimal performance, available in a variety of dimensions, glass colours, and closures.

instruments. The sample processor is equipped with a unique pneumatically actuated cylindrical vial gripper. Also from OI Analytical, the model LV-20 standards addition module is an option for the company's model 4551A water autosampler. The standards addition module adds internal standards, surrogate, or matrix spike standards for GC and GC–MS analyses.

The Flex autosampler from EST Analytical can perform a wide variety of liquid injections as well as some basic sample preparation on-board. This system can be upgraded to perform headspace sampling as well. And from CDS Analytical, the model 7500 thermal desorption autosampler is an extension of the company's model 7400 purge-and-trap system that offers the ability for the user to change between air-sample thermal desorption (TD) tubes and VOA vials for soil or water analysis.

Thermo Scientific's programmable Virtuoso vial printing system prints indelible, detailed information directly on sample vials in three text fields with a mini quick response (QR) barcode. Vial labelling takes approximately 5 s and the identification data can be input using the unit's touchscreen or directly from the lab network.

Gerstel's Hot Injection and Trapping (HIT) technique combines analytes from several headspace injections in a trapping or desorption procedure for single GC–MS runs. The technique integrates the capabilities of the company's MultiPurpose Sampler (MPS), Thermal Desorption Unit (TDU), and Cooled Injection System (CIS) inlet.

From Hamilton, the new S-line syringe for CTC PAL Autosampler applications complements the company's existing C-Line and X-Type CTC PAL autosampler syringes. S-Line syringes are built for economic everyday use for GC and high performance liquid chromatography (HPLC) injection applications.

Acknowledgements

I would like to thank the manufacturers and distributors that kindly furnished the requested information, which allowed a timely report on new product introductions over the past year. For those manufacturers who did not receive a preconference questionnaire this year and would like to receive one and be considered for early inclusion into the 2014–2015 new GC and related product introductions review, please send the name of the primary company contact, the mailing address, fax number, and e-mail address to Laura Bush, Editorial Director, *LCGC* and *Spectroscopy*, lbush@advanstar.com, Attn: 2015 New GC Products.

References

(1) R. Majors, *LCGC Europe* **27**(4), 196–207 (2014).
(2) R. Majors, *LCGC Europe* **27**(5), 255–265 (2014).
(3) H.D. Tolley, S.E. Tolley, A. Wang, M.C. Asplund, and M.L. Lee, "Properties of Thermal Gradient GC Separations", presented at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon 2014), Chicago, Illinois, USA, 2014.
(4) A. Wang, A.R. Hawkins, H.D. Tolley, and M.L. Lee, "Enhancing Separation Performance of Microfabricated Gas Chromatography Using Temperature Gradients", presented at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon 2014), Chicago, Illinois, USA, 2014.

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New HPLC Systems and Related Products Introduced at Pittcon 2014: A Brief Review

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Noteworthy high performance liquid chromatography (HPLC) and related products introduced at Pittcon 2014 in Chicago, Illinois, USA, and in the year prior are highlighted here. This instalment summarizes the technical aspects of new HPLC systems, modules, and chromatographic software as well as other product extensions. Also included is a section on several new mass spectrometers, which are viewed as HPLC detectors. The focus of this review is on innovative products and new features that have potential impacts on HPLC practitioners.

The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) is the world's largest conference and exposition on laboratory science. This year Pittcon was held at McCormick Place in Chicago, Illinois, USA, from 2–6 March 2014 (prior years held in Chicago were 2009 and 2007). Hosting Pittcon in Chicago makes plenty of sense because it is the third largest city in the United States and a major industrial centre in the Midwest. It has many renowned restaurants and excellent deep-dish pizza. However, holding a conference in the "Windy City" in early March is not without risks. I recall that on my way to Pittcon 2007, O'Hare International Airport was closed minutes after my plane landed because of a small snow storm. Moreover, the flights of two of my invited speakers were turned back that same day.

Things were pretty tough this year with the coldest weather on record in decades. Temperatures hovered around single digits with many snow showers, sleet, or freezing rain during the conference week. Somehow, folks from all over the world still managed to make it there. Pittcon 2014 attracted more than 17,000 attendees from over 90 countries. There were more than 2000 technical papers, including those from plenary lectures, invited or contributed symposia, workshops, posters, networking sessions, 114 short courses, and an enormous exposition with more than 900 vendors from over 30 countries displaying at ~1900 booths. Note that more than 20% of the attendees and exhibitors are non-US-based, making Pittcon a truly international conference.

Recent Trends in HPLC Equipment

Pittcon 2014 happened to be another lean year for new high performance liquid chromatography (HPLC) system introductions because all major manufacturers already had ultrahigh-pressure liquid chromatography (UHPLC) offerings in prior years (1,2). The global HPLC equipment market continues to be dominated by several major manufacturers, leaving scant pickings for any new entrants. The high cost of sales and marketing as well as the difficulties in setting up global service support further discourage any newcomers to the competitive and mature market. Nevertheless, the HPLC market remained quite active this past year with 27 "new" items reported in this review.

Table 1 lists new HPLC product introductions (arranged alphabetically by vendor names) at Pittcon 2014 or during the prior year, followed by descriptions and commentaries on each product, categorized as systems, modules (including several new mass spectrometer offerings), data systems, software, and related products (capillary electrophoresis [CE] and supercritical fluid chromatography [SFC]).

HPLC and UHPLC Systems and Line Extensions

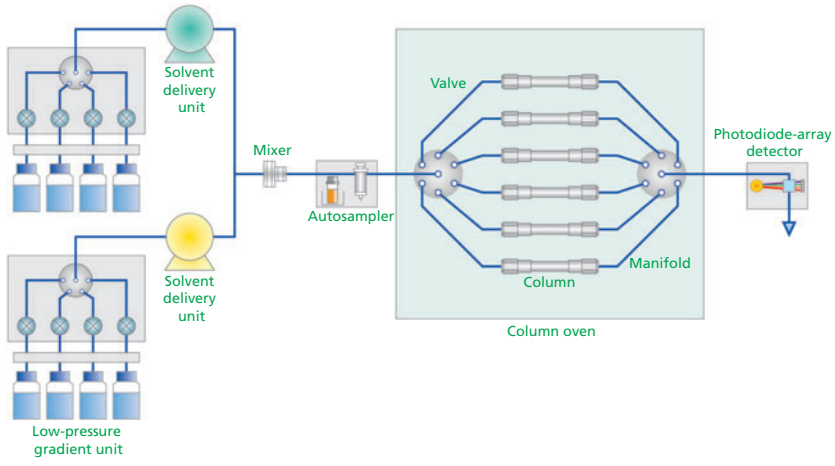
Several product extensions or specialized applications systems were offered at Pittcon 2014 or in the prior year. These are explained in more detail below.

Agilent 1260 Infinity LC and LC–MS Purification systems — Synthetic chemists often use a rapid generic HPLC–mass spectrometry (MS) or UHPLC–MS method to confirm the successful synthesis of a target compound. Once confirmed, a family of newly configured analytical-to-prep purification systems from Agilent can allow the chemists to start the purification of the synthesized compounds (from milligram to gram quantities) immediately without any method optimization or scale-up calculations, which is handled completely by Agilent's new Automated Purification software. Each of Agilent's 1260 Infinity LC-based purification systems is equipped with an analytical- or preparative-scale module — a pump (flow rate of 0.001–100 mL/min), an autosampler (injection volumes up to 10 mL), and

Table 1: Summary of new HPLC product introductions at Pittcon 2014 or in the year prior.

Vendor	New HPLC and Related Product	Description and Comments
AB Sciex (Danaher)	CESI 8000 system	Combines capillary electrophoresis (CE) with electrospray ionization (ESI) for MS for biologics characterization.
Advion	Expression compact mass spectrometer (Model S and L)	Lower-cost and highly portable single-quadrupole mass spectrometer designed for bench chemists for reaction monitoring and compound purification.
Agilent	Agilent 1260 Infinity LC and LC–MS Purification systems	A family of Agilent 1260 based analytical-to-prep systems designed for purification of synthetic compounds from milligram to gram quantity.
Agilent	1260 Bio-inert Multi-Detector SEC system	For advanced analysis of protein-based biopharmaceuticals using SEC with absolute molecular weight determinations with static and dynamic light scattering detection.
Agilent	Agilent Maintenance Wizard (MWiz)	Add-on software to Agilent's Lab Advisor that facilitates instrument diagnosis and maintenance by users.
Agilent	Intelligent System Emulation Technology (ISET) 3rd release	Facilitates method transfer across UHPLC and HPLC system platforms now supporting 1290 quaternary pump and allowing emulation of Waters and Shimadzu HPLC systems.
ACD	ACD/AutoChrom v. 2012	An automate method development software for Waters and Agilent HPLC and UHPLCs with improved user interface and UV–MS peak tracking.
Eksigent (AB Sciex/Danaher)	Ekspert microLC 200 system for LC–MS analysis	A specialized HPLC system for micro LC–MS and micro LC–MS–MS analysis.
EXCELLIMS	IA3100 HPLC-HPIMS	An ion mobility spectrometry (IMS) system for connection to any HPLC system to provide 2D orthogonal separations of complex samples and isomers.
JASCO	PR-2088 Prep SFC	A preparative supercritical fluid chromatography (SFC) system for use with 10–30 mm i.d. columns for sample purification.
Molnár-Institute	DryLab 2010 v 4.2	Now includes robustness module, knowledge management protocols, and Windows XP to Windows 8 support. It includes features such as peak match, 3D cube data display, and simultaneous optimization of three critical parameters.
Sedere	Sedex 90LT low-temperature evaporative light-scattering detector	Low-temperature nebulizer technology with new optical design that allows higher sensitivity (very low nanogram) and linearity performance. Compatible with UHPLC and SFC.
S-Matrix	Fusion AE LC method development QbD software v.9.7.0	For automated method development using design of experiment (DoE) software that supports Agilent and Waters systems. Fusion LC method validation (FMV) now supports experimental designs for both small and large molecules.
Shimadzu Scientific Instruments	Nexera X2 HPLC/UHPLC method scouting system	Easily configurable column and mobile-phase screening system for automated UHPLC method scouting using up to 96 unique separation conditions with enhanced software for system control.
Shimadzu Scientific Instruments	Crude2Pure (C2P) automated purification–powderization system	An automated compound isolation system based on an innovative "solid-phase trapping (polystyrene resin)" and evaporation process to allow multisample recovery of purified solids.
Shimadzu Scientific Instruments	UF-Amino Station: LC–MS amino acid analysis system	An automated amino acid analysis system using precolumn derivatization and LC–MS analysis that can quantitate up to 38 amino acids in food products with an analysis time of 9 min.
Shimadzu Scientific Instruments	Nexera SQUARE system	Nexera-based LC×LC and LC×LC–MS systems for separations and characterization of very complex samples.
Thermo Fisher Scientific	Corona Veo detector	Improves sensitivity of corona aerosol detection (CAD) by up to fivefold with new nebulizer and evaporation tube.
Thermo Fisher Scientific	Orbitrap Fusion Tribrid mass spectrometer	Tribrid MS system combines a quadrupole, a linear ion trap, and an orbital trap with a resolving power of up to 450,000 full width at half maximum (FWHM).
Thermo Fisher Scientific	Chromeleon chromatography data system 7.2 SR1	New release provides support for MS on Enterprise Environments, fraction collection control, and more "right-first time" analysis.
Tosoh Bioscience	EcoSEC high temperature GPC system	A third-generation GPC system for polymer characterization at temperatures up to 220 °C.
Waters Corporation	Acquity UPLC M-Class system	A UPLC system designed for nano- to microscale separations using UV and MS detection for samples of limited availability.
Waters Corporation	ionKey/MS System	The ionKey is an add-on microfluidic device for coupling a UPLC M-Class system to a Waters Xevo TQ-S MS system. It contains a 150-µm i.d. channel (column), an ESI interface, a heater, and all connections.
Waters Corporation	Acquity QDa detector	A compact and highly portable single-quadrupole MS system that can operate without a roughing pump.
Waters Corporation	Patrol UPLC Pilot Process Analysis system	A third-generation Waters process analyzer that automates sample extraction from a reactor, sample dilution, and analysis by UHPLC.
Waters Corporation	EmPower 3 chromatography data system (CDS) Feature Release 2 (FR2)	The leading CDS now supports 2D-LC, SFC, QDa, SQD, and TQD with enhanced mass spectral data processing and display in addition to compatibility with Linux Red Hat operating system. Ability to monitor status or user and system usage, and review and sign off on reports using wireless access from a tablet or smart phone.
Wyatt Technology	µDawn multiangle light-scattering (MALS) detector	A UHPLC detector for absolute molecular weight determination of polymers, biopolymers, proteins, and nanoparticles.

Figure 1: A schematic diagram of a Shimadzu Method Scouting System is shown here with details on specific modules and valving configuration. The system is designed for rapid column and mobile phase scouting by the selection of four A mobile phases, four B mobile phases, and six columns with a total of up to 96 combinations.



a fraction collector as well as various detector options such as Agilent's UV detector, evaporative light scattering detector, or 6100 MS system.

Agilent 1260 Infinity Bio-inert Multi-Detector size-exclusion chromatography system — This advanced size-exclusion chromatography (SEC) system is designed for the characterization and quality control of protein-based biopharmaceuticals such as monoclonal antibodies (mAbs). It features a titanium-based 1260 Infinity Bio-Inert Quaternary LC system with nonmetallic sample flow paths (ceramic or PEEK) with a static or dynamic light scattering detector for on-line determination of absolute molecular weights. This system is ideally suited for the quantitation and characterization of intact proteins, fragments, monomers, dimers, aggregates, and potential degradants.

Eksigent microLC200 for LC–MS — Eksigent (now part of AB Sciex under Danaher Corporation) introduces the Ekspert microLC 200 system for LC–MS and LC–MS–MS analysis. It consists of a pneumatic pressure-controlled micropump with a flow-rate range of 5–200 µL/min (without flow splitting) and a dwell volume of 3 µL.

Shimadzu Nexera X2 UHPLC Method Scouting system — This is a rapid column and mobile-phase

Figure 2: Overlaid chromatograms of injections of a dilute caffeine solution (10 µL of 8 ppm) using the Sedex S90 (S/N = 143, LOD = 1.7 ng) and Sedex S85 evaporative light scattering detectors. Column: 150 mm × 4.6 mm, 5-µm d_p Zorbax Eclipse XDB-C18; mobile phase: 80:20 water–acetonitrile; flow rate: 1.0 mL/min; column temperature: 20 °C; detector temperature: 40 °C.

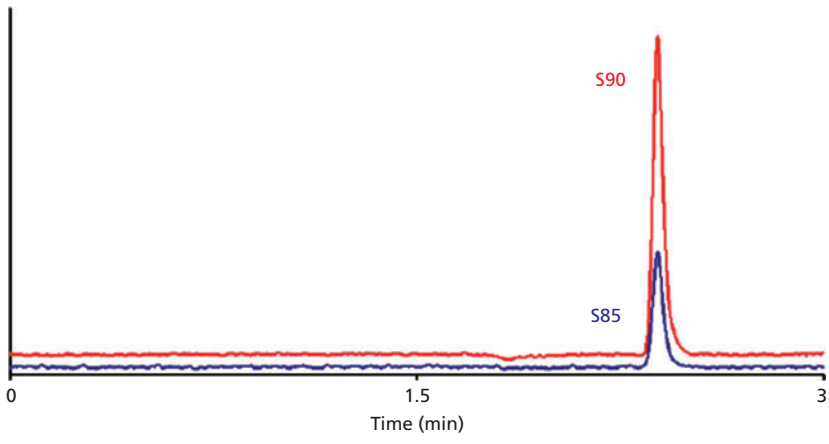
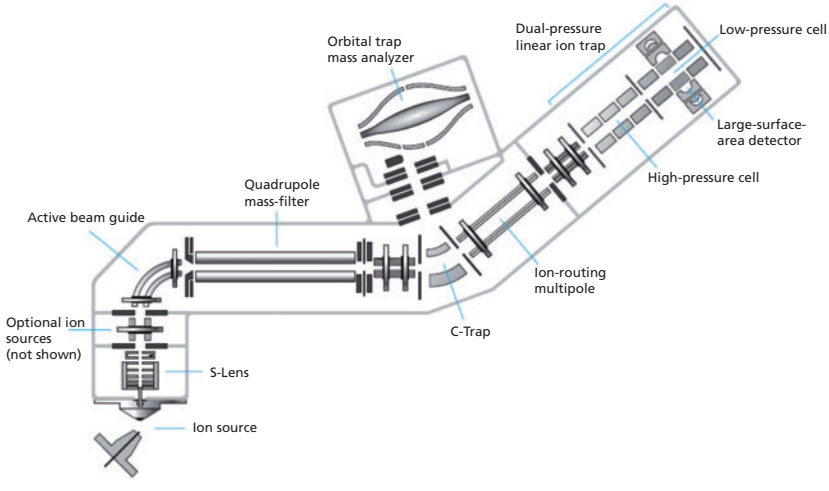


Figure 3: A schematic diagram of the ion path of the new Thermo Scientific Orbitrap Fusion mass spectrometer — a tribrid consisting of a quadrupole, an orbital trap mass analyzer, and a linear ion trap.



screening system designed for easy method scouting (development) by running up to 96 unique separation conditions (a combination of four A mobile phases (aqueous), four B mobile phases (organic solvents), and six different "orthogonal" columns. The system has an enhanced software package for system and work flow control. Figure 1 shows a schematic diagram of the Shimadzu Method Scouting System with the various HPLC modules and valving configurations for solvent and column selections. The system is ideal for automated column and mobile-phase screening and method optimization in laboratories specializing in HPLC method development.

Shimadzu Crude2Pure (C2P) Automated Purification/Powderization system — An add-on accessory to a Shimadzu preparative LC system that automates compound isolation based on an innovative "solid phase trapping (with polystyrene resin)" and evaporation process to allow for multisample recovery of purified solids (or highly concentrated solutions). The system includes enhanced software to visualize workflow and for system control.

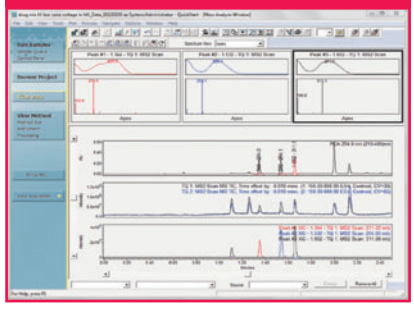
Shimadzu UF-Amino Station to support automated amino acid analysis system by LC–MS — The UF-Amino Station automates precolumn derivatization using phenylthiocarbamyl chemistry (PTC) followed by LC–MS analysis of 38 amino acids found in food products with an analysis time of only 9 min. Quantitation of 25 amino acids in 15 min is possible using UV detection. This compares favourably with the traditional method using ion-exchange chromatography with ninhydrin post-column reaction, which requires an analysis time of over 120 min.

Shimadzu Nexera Square system — Nexera-based LC×LC and LC×LC–MS systems that provide higher peak capacities through two-dimensional (2D) "orthogonal" separations of complex samples using either heart-cut or comprehensive mode. Instructive software is available for system control and qualitative or quantitative analysis.

Tosoh Bioscience EcoSEC High Temperature GPC system — This third-generation Tosoh high-temperature gel-permeation chromatography (GPC) system is intended for polymer characterization and is capable of precise determination of molecular weight averages and distributions and polydispersity of polyolefin and other organic polymers at column temperatures up to 220 °C. It is equipped with a dual-flow-cell refractive index detector, a preparative sample module, and a new line of high-temperature GPC columns.

Waters Patrol UPLC Pilot Process Analysis system — This is a new addition to the Waters family of at-line and on-line UPLC process analysis systems. Intended for use by analytical scientists supporting process development; it consists of a process sample manager and an Acquity UPLC system that automates sample extraction from a reactor, sample dilution, analysis and reporting, and communication to a laboratory information management system (LIMS) or distributed control system (DCS). The system is useful for real-time quantitative in-process monitoring and facilitates control of

Figure 4: A screen shot of Waters EmPower 3 Feature Release 2 showing the simultaneous display of chromatographic and UV and MS spectral data.



chemical reactions, fermentation, or bioprocessing.

Waters Acquity UPLC M-Class System — A UHPLC system that is designed for nano- to microscale separations using UV and MS detection for samples of limited availability. The system uses columns with internal diameters ranging from 75 µm to 1.0 mm packed with sub-2-µm materials, and operates at flow rates of 200 nL/min to 100 µL/min, and pressures of up to 15,000 psi. The system is ideally suited for proteomics, biomarker analysis, metabolic profiling, 2D separations, metabolite identification, and pharmacokinetic studies. The system is compatible with the company's new ionKey, a microfluidic device for direct coupling to a Waters Xevo TQ-S MS (and eventually to other MS platforms). The ionKey device contains a 150-µm i.d. channel (column), an electrospray ionization (ESI) interface, a heater, and all built-in connections.

HPLC Modules

Several new modules introduced this year are stand-alone UHPLC-compatible detectors for the analysis of nonchromophoric compounds. These are explained in detail below.

Sedex 90LT low-temperature evaporative light-scattering detector — This detector provides sensitive detection (very low nanogram) and gives a near-universal response for low- or non-chromophoric, nonvolatile, or semivolatile compounds. This unit uses an improved low-temperature nebulizer technology coupled to a new optical design to provide higher sensitivity (fivefold improvement compared to prior models) and better linearity (four orders of magnitude). It is compatible with UHPLC and supercritical fluid chromatography (SFC). Figure 2 shows overlaid chromatograms of the new model S90 versus that of the existing S85 detector on a caffeine solution showing a threefold increase in sensitivity.

Wyatt µDAWN multiangle light scattering (MALS) detector — This is the world's first UHPLC-compatible multiangle light scatting detector that is designed for absolute molecular weight determinations of polymers, peptides, proteins, biotherapeutics, quantum dots, and gold sols. The detector removes the need for column calibration and reference standards and has a much smaller flow cell (<10 µL versus 63 µL from a previous model)

Figure 5: A screen shot of Agilent's Maintenance Wizard showing details of the various modules in the HPLC system and a link to a video illustrating a maintenance procedure to the user.

for better compatibility with smaller UHPLC columns. This detector is complementary to the Optilab UT-rEX refractive index (RI) detector that was introduced in 2013.

Thermo Scientific Corona Veo Detector — The new Corona aerosol detector improves detection sensitivity by fivefold with a newly designed nebulizer. Other enhancements include easier front access to fluidic connections, exchangeable FocusJet nebulizer, electronic gas flow control, and the elimination of pressurized waste bottle because of the use of a new active drain pump.

EXCELLIMS IA3100 HPLC-HPIMS — The IA3100 ion mobility spectrometer can be added on to any HPLC system to provide 2D orthogonal separations of complex samples and isomers. This ion mobility spectrometer contains a splitter to provide flow of 2–10 µL/min to an electrospray ionization source that ionizes analytes separated by the HPLC, a drift tube containing a collision gas that provides an orthogonal separation mode based on molecular size and shape, and a Faraday plate detector.

Mass Spectrometers

MS has made significant impacts to scientific discoveries. Three new products are highlighted in this section because a mass spectrometer is often viewed as a HPLC detector. There appears to be two significant recent trends in MS equipment. First is the development of low-cost, highly portable single-quadrupole MS system, which allows the equipment to be used by nonspecialists for LC–MS, supporting flash purification and on-line reaction monitoring (3). A compact mass spectrometer, the 4000 MiD from Microsaic Systems (a chip-based MS) was introduced at Pittcon 2013. Second is the trend towards hybrid or tribrid high-resolution accurate mass MS, which provides an ideal tool for solving the most complex problems.

Advion Expression Compact mass spectrometer (Model S and L) — Advion, cofounded by Professor Jack Henion of Cornell University, is a major contract research organization (CRO) in bioanalytical testing. Advion introduced the Expression Compact mass spectrometer in 2011, targeting at-line, in-process reaction monitoring or target compound purification in synthetic organic chemistry laboratories. Two new compact single-quadrupole MS models CMS-S and CMS-L now support higher flow rates (up to 2 mL/min), scan rates (10,000 amu/s), and mass range (10–2000 amu).

Waters Acquity QDa detector — The Acquity QDa detector can operate without a roughing pump, and can work reasonable well using an integrated dry diaphragm pump. It is very compact (the size of a UV detector), has a mass range of 30–1250 amu with a scan rate of 10,000 amu/s, and is UHPLC- and SFC-compatible. The electrospray ionization (ESI) sensitivity is similar to those of other single-quadrupole mass spectrometers when operated with a roughing vacuum pump and is about fivefold less sensitive when used with the integrated diaphragm pump alone. Control and data display is through Waters Empower 2 FR5 to 3 or MassLynx 4.1.

Thermo Scientific Orbitrap Fusion MS — The development of the Orbitrap analyzer has been a story of luck, perseverance, occasional deep insights, and ultimate success. Within just a few years, this technology has virtually displaced Fourier transform ion cyclotron resonance (FT-ICR) as the benchmark for high-resolution accurate-mass MS. The Orbitrap Fusion MS system combines the best of quadrupole, orbital trap, and linear ion trap mass analyzers in a tribrid architecture that delivers excellent depth of analysis. It is capable of multiple fragmentation techniques (collision induced dissociation [CID], high-energy C-trap dissociation [HCD], and energy transfer dissociation, [ETD]), and a mass accuracy of <1 ppm with an impressive resolving power of up to 450,000 full width at half maximum (FWHM). This enables researchers in life sciences to analyze the most challenging low-abundance, high-complexity samples by allowing for effective identification, quantitation, and structure elucidation. Figure 3 shows the schematic diagram of the system's ion path.

Chromatography Data System

A chromatography data system (CDS) can be a standalone computer or a client-server based network. CDS typically also functions as a single-point system control in addition to data handling (peak integration, report generation, and data archival).

Waters Empower 3, Feature Release 2 — Waters Empower 3, introduced in 2010, is a leading chromatography data system, with a dominant position in client–server based networks. The current release supports 2D LC, SFC, Acquity QDa detector, single-quadrupole MS, and triple-quadrupole MS systems. It has enhanced connectivity to other vendors' chromatographic systems (HPLC, UHPLC, gas chromatography, ion chromatography, and SFC). This version offers an improved user interface for mass spectral data processing and display. It provides better support for pharmaceutical testing by allowing automatic identification of peaks (as impurity, degradant excipient), flagging of "out-of-specification" results, and naming unknowns as relative retention time (RRT) tags — all without the use of custom fields. Additional capabilities, such as allowing lab managers or users to monitor status or user and system usage, perform routine system administration, and review and sign off on reports, are available through regular network or wireless access from a tablet or smart phone. It now supports operation systems such as Linux Red Hat, Oracle Solaris, and Windows for

network servers. Figure 4 is a screen capture of EmPower 3 (FR 2) showing the simultaneous display of chromatographic and UV and MS spectral data.

Thermo Scientific Chromeleon 7.2 SR1 — This new release provides enhanced support for MS on Enterprise Environments, TSQ Quantiva/Endura, Q Exactive and Exactive Series, and MSQ for routine quantitative applications. It closes gaps of fraction collection, autodilution, and MSQ support from Chromeleon 6.8 and provides more "right-first-time" analysis by combining Chromeleon eWorkflows and Intelligent Run Control for more automated analysis including running samples right away using "one-click work flows".

HPLC Method Development and Other Software

Automated HPLC method development software has seen significant improvements in the last decade facilitating tasks such as column and mobile-phase screening, method optimization and validation, and robustness assessment (4–7). Some newer releases of method development and other HPLC-related software are summarized here.

ACD AutoChrom Method Development software v. 2012 — This software provides a complete automated method development capability for Waters and Agilent HPLC and UHPLC systems. It uses both automated experimental designs and predictive simulation in addition to sophisticated peak tracking functions using UV or MS data and an improved user interface. This latest release also integrates with the ACD/Spectrus database platform (5).

Molnár Institute DryLab 2010 v. 4.2 — DryLab, a predictive and visual modelling software, pioneered a modern software-assisted approach to HPLC method development. It was first introduced in 1986 by Snyder, Dolan, and Molnár (4). DryLab v. 4.2 offers simultaneous optimization of three measured critical parameters, resulting in a useful three dimensional (3D) cube data display, a new peak tracking tool (Peak Match), as well as a robustness calculation module, and a knowledge management protocol documenting method development strategies (6). The software is compatible with the Windows operating system (from XP to Windows 8).

S-Matrix Fusion AE QbD LC Method Development Software v. 9.7.0 — Over the last 10 years S-Matrix Corporation, a company specializing in design of experiments (DoE) software, has adapted its DoE and advanced modelling software to LC method development based on the principles of quality by design (QbD) (7). The latest release offers a multiparameter design space and proven acceptable range (PAR) graphics (from the International Conference on Harmonization Guidance, *ICH Q8(R2)*) integrated with its Monte Carlo robustness simulation engines, and extends

its automated LC experimentation capabilities to include all Waters and Agilent UHPLC systems under Empower and all Agilent UHPLC systems under ChemStation/OpenLab. This release also adds a full suite of automated validation experiments for both small and large molecule samples to its companion LC Method Validation module.

Agilent Intelligent System Emulation Technology (ISET), 3rd release — ISET facilitates method transfer from many UHPLC and HPLC platforms to the 1290 Infinity LC system to achieve the same chromatographic results without any change to the original method. This third release now also supports the 1290 Infinity Quaternary pump and allows emulation of additional Waters and Shimadzu UHPLC and HPLC systems.

Agilent Maintenance Wizard — The Agilent Maintenance Wizard is an add-on software module to Agilent's Lab Advisor, which enables "do it yourself" customers and self-maintainers to effectively perform maintenance on Agilent UHPLC and HPLC systems. The software guides users through the complete process of instrument maintenance, including assembly and disassembly, cleaning, parts exchange, and testing by an intuitive, structured, and easy to use wizard-like walk through. Descriptive and visualized instructions (such as animated PDFs and videos) help users every step of the way and provide accurate documentation of performed maintenance actions. Figure 5 shows a screen capture of the maintenance wizard with details of system configuration and a link to a maintenance video.

Other New Separation Systems

HPLC-related products discussed here include new production introductions of CE and SFC.

AB Sciex's CESI 8000 system for biologics characterization — This system is the first CE-related product launch from AB Sciex (AB Sciex separations business unit now combines Beckman Coulter's CE products and Eksigent's nano LC and micro LC systems). The system combines CE with electrospray ionization (ESI) for MS through a dynamic process in a single device (a porous tip design that allows ESI current to pull the analytes into the MS at very low flow, ~20 nL/min). The system is useful for the detailed CE–MS characterization of biologics and their digests (for analysis of identity, purity, heterogeneity, and stability).

JASCO PR-2088 Prep SFC — This SFC system is designed for use with 10-, 21-, or 30-mm i.d. columns for semipreparative to large-scale sample preparation and purification (hundreds of milligrams to tens of grams scale). It allows carbon dioxide delivery of up to 120 mL/min and supports interchangeable modifier pumps and various detection options (UV–vis, photodiode array, and circular dichroism). The company's SF-Nav software provides complete integrated control for flow rate, sample injection, and fractionation.

Personal Concluding Remarks

Many analytical chemists, including myself, come to Pittcon for professional and personal reasons. My schedule this year (my 15th consecutive year) included giving a two-day HPLC and UHPLC short course, attending the plenary lecture by Dr. Steven Carr on proteomics and Professor Mary Wirth's Dal Nogare Award address (on the separation of monoclonal antibodies using 0.5 µm colloidal particles), and chairing an invited symposium on quality control of biopharmaceuticals. My networking events included *LCGC*'s advisory board lunch (with the *LCGC* staff and more than 15 other board members), the Chinese American Chromatography Association (CACA) dinner event with 140 attendees (featuring a speech on portable HPLC by Professor Milton Lee of Brigham Young University), and the annual Pittcon presidential reception. I spent two days on the exposition floor, gathering confirmatory information for this article and witnessing first-hand, the newest trends in analytical instrumentation through the myriad innovative products on display. Every year at Pittcon, I personally witness the development of emergent analytical sciences and trends in instrumentation, as well as reacquaint myself with old colleagues and meet new friends. Somehow, Pittcon never fails to inspire us with new ideas as we return home with tired feet but renewed vigour to continue on with our respective journeys in analytical chemistry. We all look forward to warmer weather and a festive "post-Mardi Gras" Pittcon 2015 in New Orleans.

Summary

This review summarizes new HPLC product introductions (systems, modules, CDS, software, and related products) at Pittcon 2014 and in the prior year. It highlights innovative features of these new products from a user's perspective. Readers should refer directly to the manufacturers' sources for technical details and product specifications. My goal is to provide a summary and update of innovative HPLC products relevant to our readership, particularly to those who missed Pittcon this year. Note that new HPLC columns introduced at Pittcon are covered by Ron Majors' annual review (8,9). This year's coverage will appear in this issue and the May edition.

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References

(1) D. Guillarme and M.W. Dong, *Amer. Pharm. Rev.* **16**(4), 36–43 (2013).
(2) M.W. Dong, *LCGC Europe* **26**(4), 216–221 (2013).
(3) X. Bu, J. Yang, X. Gong, and C.J. Welch, *J. Pharm. Biomed. Anal.* **94**, 139–144 (2014).
(4) L.R. Snyder, *LCGC North Am.* **30**(4), 328–341 (2012).
(5) J.D. Stafford, T.D. Maloney, D.P. Myers, J.M. Cintron, B.C. Castle, *J. Pharm. Biomed. Anal.* **56**, 280–292 (2011).
(6) A.H. Schmidt and I. Molnar, *J. Pharm. Biomed. Anal.* **78–79**, 65–74 (2013).
(7) B. Debrus, D. Guillarme, and S. Rudaz, *J. Pharm. Biomed. Anal.* **84**, 215–223 (2013).
(8) R.E. Majors, *LCGC Europe* **26**(5), 277–291 (2013).
(9) R.E. Majors, *LCGC North Am.* **31**(5), 364–381 (2013).

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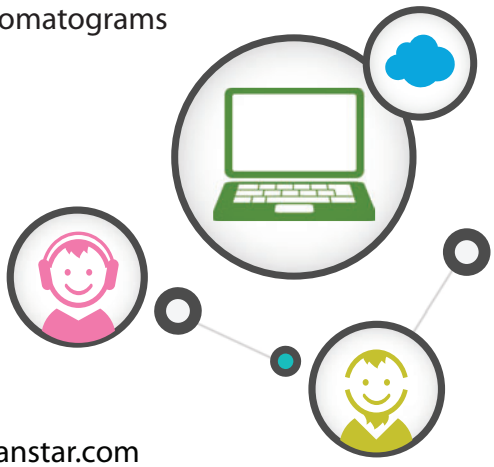

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UHPLC valves

Agilent 1200 Infinity Series Quick-Change Valves reportedly allow the automation of a wide variety of applications such as column or solvent selection, column regeneration, or sample cleanup and enrichment. The valves' design with separate valve heads and drives gives the flexibility to choose combinations that match a laboratory's individual application requirements.
www.agilent.com
Agilent Technologies, California, USA.

HPLC columns

Waters Corporation has introduced a line of 2.7-micron silica-based, solid-core particle columns for high performance liquid chromatography (HPLC). The company report that the columns run at lower pressures with high efficiency, giving flexibility to use longer column lengths to improve resolution or higher flow rates to improve instrument analysis time and increase throughput. The columns are available in C18+, C18, and HILIC chemistries.
www.waters.com
Waters Corporation, Massachusetts, USA.

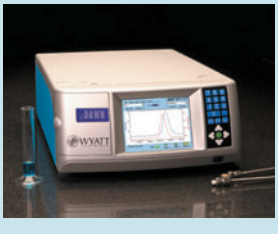
SPE micro-elution plates

Thermo Fisher Scientific has introduced SOLAμ solid-phase extraction (SPE) micro elution plates. The company report that the plates are designed to handle elution volumes as low as 25 µL, removing the blow-down stage of the SPE process. The macro-porous structure is designed for consistent sample and solvent flow through the SPE stationary phase, avoiding blockages caused by viscous biological samples.
www.thermoscientific.com/sola-spe
Thermo Fisher Scientific, California, USA.

Gas generators

F-DGSi Modular Alliance gas generators are available to supply hydrogen, nitrogen, and zero air. The generators are modular in design and can reportedly offer a solution specific to a laboratory; different combinations are available for single and multiple GC applications. The generators have a touchscreen display showing the status of the system with auto diagnostics.
www.f-dgs.com
F-DGSi, Evry, France.

UHPLC SEC–MALS detector



Wyatt has launched µDAWN, a multi-angle light scattering (MALS) detector that can reportedly be coupled to any UHPLC system to determine absolute molecular weights and sizes of polymers, peptides, proteins, or other biopolymers directly. To accommodate narrow peaks in UHPLC, the light scattering flow cell volume has been reduced from 63 µL to 10 µL. To minimize interdetector mixing, band broadening is under 7 µL.
www.wyatt.com
Wyatt Technology, California, USA.

Solid-core LC columns

ACE UltraCore is a new range of ultra-inert solid-core (superficially porous) particle UHPLC/HPLC columns from Advanced Chromatography Technologies. The company report that the range combines high efficiency and performance with low column back pressure. Available with SuperC18 and SuperPhenylHexyl bonding, these phases feature proprietary Encapsulated Bonding Technology (EBT), which results in excellent peak shape and superb phase stability across an extended pH range (1.5 to 11.0).
www.ace-hplc.com
Advanced Chromatography Technologies Limited, Aberdeen, Scotland.

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I ◯ Low-Pressure Chromatography
J ◯ Chiral Chromatography
K ◯ Affinity Chromatography
L ◯ Capillary Electrophoresis
M ◯ Solid Phase Extraction
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Sophisticated Antibody Analysis by GPC/SEC with RALS

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

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

Experimental

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

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

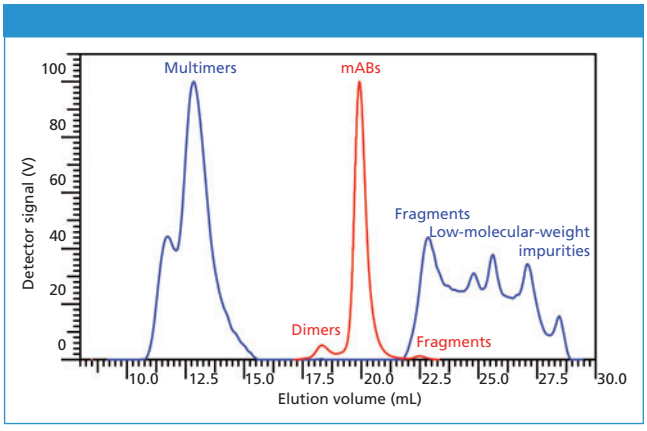

Figure 1: Separation range of the column combination. The red curve shows the UV signal of a full length antibody and its dimers plotted against the elution volume. The blue curve is the elugram of antibody fragments and their high level aggregates.

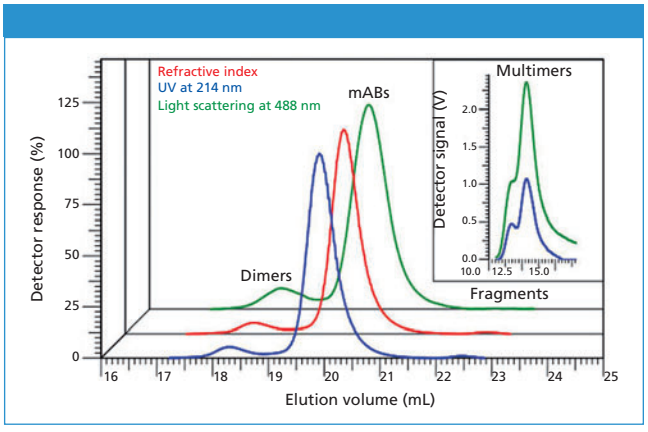

Figure 2: Sensitive analysis of antibody aggregates. The light scattering signal for the dimer is relatively high compared to that of the mABs because of molar mass dependency and provides improved sensitivity for the detection of high aggregates (inset).

Results

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

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

Conclusion

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

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In der Dalheimer Wiese 5, D-55120 Mainz, Germany
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Synthetic Rubbers: Polybutadiene

Wyatt Technology Corporation

Synthetic elastomers have replaced natural rubber to an astonishing degree, and account for more than 70% of the rubber used today. In the United States alone, 5 million tons of synthetic rubber are produced annually. The principal synthetic rubber elastomer is a copolymer of butadiene and styrene. The latex form of rubber and synthetic elastomers has applications in carpet and gloves, and coagulated latex is used for the production of tyres and mechanical goods. It is of critical importance to know the absolute molar mass and its distribution, as well as to gain insight into the conformation of synthetic rubber — which are indicative of the product's end-use performance.

Typically, polystyrene standards are used to estimate the molar masses of these polymers in size-exclusion chromatography (SEC) experiments, but by using a DAWN or miniDAWN multi-angle light scattering (MALS) detector, standards and column calibration are no longer needed. Here, the absolute molar mass and polydispersity, as well as the *rms* radius of two synthetic rubber samples, were measured directly using SEC combined with a DAWN.

The synthetic rubber samples were analyzed in toluene, and Wyatt Technology's Optilab was used as the refractive index detector for the SEC line. The refractive index increment dn/dc of polybutadiene in toluene is relatively low, and the dn/dc value of the butadiene/ styrene copolymer in toluene increases with the ratio of styrene present. In Figure 1, the molar mass and its distribution are determined absolutely — without using any standards or calibration routines.

In addition to the weight-average molar mass and the polydispersity, the DAWN can also determine the shape of the polymer by measuring the *rms* radius directly at each elution volume. It is well-known that butadiene forms highly branched polymers and that styrene forms linear polymers — and this is revealed by the DAWN.

Figure 2 shows a logarithmic plot of the molar mass versus the radius of the two synthetic rubber samples. The slope of such a plot is indicative of the shape of a polymer. A slope between 0.5 and 0.6 is usually found for linear polymers with a random coil conformation, while spheres have a slope of approximately 0.3. The values obtained for polybutadiene (0.25) and for the butadiene/styrene copolymer (0.38) indicate that this polybutadiene is more branched than the styrene/butadiene copolymer.

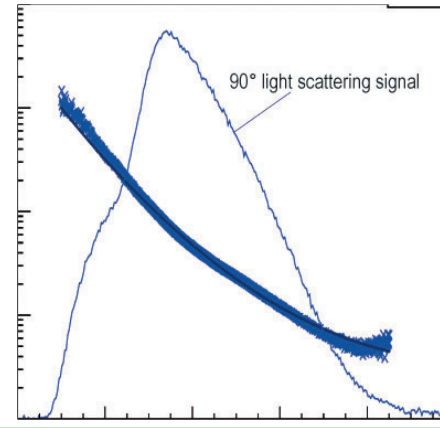

Figure 1: Molar mass versus elution time for the synthetic rubber sample superimposed upon the signal from one of the light scattering detector channels.

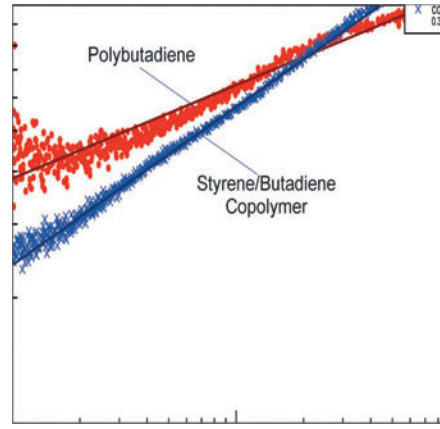

Figure 2: RMS radius versus molar mass ("conformation plot") for the two samples. The slope is indicative of the conformation (rod, coil, or sphere) of the molecule.

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