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Effects of Sample Diluents and Sample Matrices on Residual Solvents in Pharmaceuticals Analyzed with Static Headspace Gas Chromatography

Yu (Yon) Wang, Jianchen (Jessie) Wang, and Prasad Panzade

In static headspace gas chromatography (HS-GC) sample diluents and sample matrices can affect analytical method sensitivity, accuracy, and interferences. By applying the rules revealed by this study, these problems can be avoided.

Liquid Chromatography

Troubleshooting for Two-Dimensional Liquid Chromatography: Breakthrough in the Second Dimension

Dwight Stoll

A common problem encountered in the development of 2D-LC methods is that the first dimension mobile phase properties can negatively affect the quality of subsequent second dimension separations. This installment reviews the origin of this problem and discusses potential solutions.

Image Credit: tashas/stock.adobe.com
June Update

Welcome to the May/June issue of LCGC Asia Pacific. This month’s cover story covers an important environmental issue: the contamination of surface waters with per- and polyfluoroalkyl substances (PFAS). The analysis of PFAS is a constantly evolving field of research, and in this interview two leading researchers, Stefan Van Leeuwen and Bjorn Berendsen, discuss the benefits of using a novel hyphenated approach involving fragment ion flagging incorporated with liquid chromatography high-resolution mass spectrometry.

LC Troubleshooting describes practical steps that chromatographers using two-dimensional liquid chromatography can take to overcome problems associated with the composition of the mobile phase in the first dimension adversely affecting the quality of the separation in the second dimension.

GC Connections investigates the value of multidimensional mass spectrometry (MS/MS). The use of full-scan analysis in MS/MS makes the detector universal, whereas the use of selected ion monitoring and multiple reaction monitoring makes the detector selective and noise-free to allow quantitative analysis at the femtogram level.

The use of metal organic frameworks (MOFs) in sample preparation has increased dramatically recently. Sample Preparation Perspectives looks at current trends in the role of MOFs in sample preparation and the application areas they are being used in.

This month’s peer-review article examines the effects of common sample diluents and matrices of drug substances on residual analyte solvents using headspace gas chromatography. Applying the rules of these effects can improve method sensitivity and accuracy and also avoid interferences.

LCGC is a multimedia platform that helps chromatographers keep up to date with the latest trends and developments in separation science and supports them to perform more effectively in the workplace. Keep updated with our print and digital content by visiting the global website (www.chromatographyonline.com), subscribing to our newsletters, and attending our wide range of educational virtual symposiums.

Mike Hennessy Senior, Chairman
Static headspace gas chromatography (HS-GC) has been widely used for analysis of residual solvents in pharmaceuticals (1–3). In closed headspace vials, the sample mixture is composed of sample diluents (also called sample solvents), samples, and analyte solvents that are to be tested. The analyte solvents are dissolved in the sample mixtures (the liquid phase) before partitioning into the gas phase. The peak response of a solvent is directly proportional to its gas phase concentration, which is dependent on its initial concentration in the liquid phase, and eventually dependent on its concentration in the sample. Many factors can affect the partitioning processes of solvents (4,5). Adjusting diluents by adding water to organic solutions to increase peak responses for nonpolar solvents and by adding inorganic salts to aqueous solutions to increase peak responses for polar solvents have been reported (4–7). The effects of several diluents on peak responses of some residual solvents have also been reported (8).

Sample matrices also have impacts on solvent peak responses. The effects of sample matrices on some residual solvents have been studied (9). The counts of hydrogen (H) bond acceptors and donors in compounds have been used to explain the causes of matrix effects. However, the principles of diluent effects and matrix effects on residual solvents analyzed with HS-GC are still not clear.

Effects of Sample Diluents and Sample Matrices on Residual Solvents in Pharmaceuticals Analyzed with Static Headspace Gas Chromatography

Yu (Yon) Wang, Jianchen (Jessie) Wang, and Prasad Panzade, Apotex Inc, Toronto, Ontario, Canada

The effects of sample diluents, including Dimethyl sulfoxide (DMS), N,N-dimethylacetamide (DMA), and N,N-dimethylformamide (DMF), and the effects of sample matrices of a drug substance on 16 residual analyte solvents were studied using static headspace gas chromatography (HS-GC). The results showed that analyte solvents with polarities higher than those diluents had higher peak responses in DMA or DMF than they had in DMS, and vice versa. From DMS to DMA, diluent effects were approximately linearly proportional to the values of solvent polarity relative to DMS. The tendencies and magnitudes of diluent effects were mainly dependent on the polarities of analyte solvents and diluents. As sample amounts increased, analyte solvents exhibited various positive or negative matrix effects. The tendencies and magnitudes of matrix effects were mainly dependent on the polarities of analyte solvents, diluents, and samples and were further affected by sample solvation processes. Applying the rules of effects of sample diluents and sample matrices can improve method sensitivity and accuracy, and avoid interferences as well.

**KEY POINTS**

- The effects of sample diluents and sample matrices on residual solvents were analyzed using HS-GC.
- Analytical method sensitivity, accuracy, and interferences can be affected.
- This article details the rules to follow to avoid any issues.
In this study, effects caused by sample diluents and sample matrices were analyzed. From the perspective of polarities, interactions between diluents, analyte solvents, samples, and sample solvation processes were analyzed to reveal the rules governing diluent effects and matrix effects. The use of the acquired understanding of diluent effects is then illustrated in a troubleshooting example.

**Experimental**

**Materials and Methods:** All of the chemicals and reagents used were common inventories. A GC 7890

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**TABLE 1:** Peak areas of analyte solvents in different diluents and their diluent effects when dimethyl sulfoxide (DMS) was replaced by dimethylacetamide (DMA) or dimethylformamide (DMF)

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Polarity*</th>
<th>Polarity Relative to DMS</th>
<th>DMS Peak Area</th>
<th>DMA Peak Area</th>
<th>%Change (Diluent Effect)</th>
<th>DMA Peak Area</th>
<th>%Change (Diluent Effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.762</td>
<td>0.318</td>
<td>357</td>
<td>525</td>
<td>47.1</td>
<td>611</td>
<td>71.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.654</td>
<td>0.210</td>
<td>345</td>
<td>428</td>
<td>24.0</td>
<td>495</td>
<td>43.2</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>0.617</td>
<td>0.173</td>
<td>236</td>
<td>259</td>
<td>9.7</td>
<td>306</td>
<td>29.7</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>0.546</td>
<td>0.102</td>
<td>439</td>
<td>448</td>
<td>2.1</td>
<td>516</td>
<td>17.7</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.460</td>
<td>0.016</td>
<td>573</td>
<td>629</td>
<td>9.8</td>
<td>579</td>
<td>1.1</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>0.444</td>
<td>0</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>t-Butanol</td>
<td>0.389</td>
<td>-0.055</td>
<td>730</td>
<td>624</td>
<td>-14.5</td>
<td>713</td>
<td>-2.3</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.355</td>
<td>-0.089</td>
<td>1849</td>
<td>1657</td>
<td>-10.4</td>
<td>1508</td>
<td>-18.4</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>0.309</td>
<td>-0.135</td>
<td>361</td>
<td>302</td>
<td>-16.4</td>
<td>337</td>
<td>-6.6</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.228</td>
<td>-0.216</td>
<td>1317</td>
<td>982</td>
<td>-25.5</td>
<td>943</td>
<td>-28.4</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>0.207</td>
<td>-0.237</td>
<td>777</td>
<td>615</td>
<td>-20.9</td>
<td>606</td>
<td>-22.0</td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>0.117</td>
<td>-0.327</td>
<td>748</td>
<td>500</td>
<td>-33.1</td>
<td>509</td>
<td>-31.9</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.111</td>
<td>-0.333</td>
<td>3103</td>
<td>2043</td>
<td>-34.2</td>
<td>2124</td>
<td>-31.5</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.099</td>
<td>-0.345</td>
<td>572</td>
<td>335</td>
<td>-41.5</td>
<td>355</td>
<td>-38.1</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>0.009</td>
<td>-0.435</td>
<td>1806</td>
<td>919</td>
<td>-49.1</td>
<td>1046</td>
<td>-42.1</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>na</td>
<td>na</td>
<td>1154</td>
<td>591</td>
<td>-48.8</td>
<td>671</td>
<td>-41.9</td>
</tr>
<tr>
<td>Cyclohexene</td>
<td>na</td>
<td>na</td>
<td>2750</td>
<td>1526</td>
<td>-44.5</td>
<td>1685</td>
<td>-38.7</td>
</tr>
</tbody>
</table>

**Key:** na: not available; *: polarity values cited from reference 10; polarity relative to dimethyl sulfoxide was the value of the solvent polarity subtracted by the polarity of dimethyl sulfoxide; peak area was mean of peak areas from four standard injections and was used for %Change calculations.
A series instrument with a 7697A headspace autosampler and a flame ionization detector (FID) was used, along with a 75 m × 0.53 mm, 3.0-µm capillary column DB-624 (Agilent Technologies) for analysis. Data acquisition and analysis were performed by Empower software (Waters).

**Chromatographic Parameters:**
The parameters for the headspace autosampler were as follows: The temperature for the oven was kept at 80 °C, the loop at 170 °C, and the transfer line at 175 °C. The times for the GC cycle, vial equilibration, pressure equilibration, and injection were 45, 30, 0.10, and 0.5 min, respectively. The vial fill mode was “fill to pressure” with the fill pressure at 10 psi. The loop fill mode was “default”; shake level was at “7”.

The loop size was 1.0 mL, and a 20 mL headspace vial was used. For the GC parameters, the carrier gas was helium, and the constant flow mode had a flow rate of 5.0 mL/min. The inlet was a volatile interface inlet with a split ratio of two and the temperature was set at 180 °C. For the GC oven program, the initial temperature was set at 40 °C and held for 20 min before being increased to 140 °C at a rate of 10 °C/min, and held for 1 min. It was later further

---

**FIGURE 2:** Relationships of diluent effects to solvent polarity relative to dimethyl sulfoxide when the diluent was changed from dimethyl sulfoxide to dimethylacetamide.
increased at a rate of 30 °C/min to 230 °C and held for 6 min. The FID temperature was set at 250 °C, with the hydrogen flow at 40 mL/min, the air flow at 400 mL/min, and the make-up gas (helium) at 25 mL/min.

**Standard Solution Preparation:** Standard solutions containing 16 analyte solvents with concentrations of ~120 µg/mL each of methanol, ethanol, acetone, 1-propanol, isopropyl alcohol (IPA), t-butanol, acetonitrile, dichloromethane (DCM), ethyl acetate, and benzene, ~40 µg/mL each of tetrahydrofuran (THF), cyclohexene, and toluene, and ~10 µg/mL each of ethyl ether, cyclohexane, and n-hexane were prepared in dimethyl sulfoxide (DMS), N,N-dimethylacetamide (DMA), or N,N-dimethylformamide (DMF), separately.

**Headspace (HS) Vial Preparation:** Next, 2.0 mL of the diluent, standard solutions, or samples plus 2.0 mL of the diluent or spiking standard solutions were put into a 20-mL size HS vial and sealed for use. For the purpose of this experiment, one injection per vial was made.

**Results and Discussion**

**Effects of Different Sample Diluents on Common Residual Solvents:** Standard solutions prepared in DMS, DMA, or DMF were injected. Figure 1 illustrates the representative GC chromatogram of 16 analyte solvents in DMS. Figure 1 shows that the peaks of all analyte solvents were well resolved. There were no significant interferences for any solvent, including the diluents. The peak retention times of the same analyte solvent in different diluents of DMS, DMA, or DMF were technically identical.

Peak responses of analyte solvents in different diluents of DMS, DMA, or DMF were tabulated and appear in Table 1. Compared to peak responses of solvents in DMS, it was found that some solvents had higher peak responses and others had smaller peak responses in DMA or DMF. These up or down changes on peak responses of analyte solvents caused by different diluents were diluent effects.

There are two aspects of the characteristics of diluent effects: the direction (up or down) of the change and the magnitude of the change. The term, ±%Change, in peak response was introduced to describe these properties when the diluent was changed. It was calculated by the equation below:

\[
\text{% Change} = \frac{\text{Peak response in DMA or DMF} - \text{Peak response in DMS}}{\text{Peak Response in DMS}} \times 100
\]

The calculated diluent effects (±% change in peak responses) on all analyte solvents after DMS was replaced by DMA or DMF are listed in Table 1. It was shown that the direction of change (positive or negative) and the magnitude of diluent effects varied from solvent to solvent. For example, methanol saw a 47.1% increased peak area, and n-hexane obtained a 49.1% decreased peak area in DMA than in DMS. By relating diluent effects to polarities, it was found that after DMS was replaced by DMA, methanol, ethanol, 1-propanol, IPA, and acetonitrile had higher polarity values and had positive diluent effects. On the other hand, t-butanol, acetone, ethyl acetate, dichloromethane, ethyl ether, benzene, toluene, cyclohexane, cyclohexene, tetrahydrofuran, and n-hexane had smaller polarity values and had negative diluent effects. When DMS was replaced by DMF, the diluent effects were similar to those seen with DMA, for all analyte solvents.

In chemistry, it is common knowledge that polar attracts polar and nonpolar attracts nonpolar. When two solvents are mixed, they will attract each other more strongly when their polarities are closer. DMS, DMA, and DMF have intermediate polarity values, with DMS being greater than DMA and DMF (10). Applying this knowledge to static headspace analysis, it can be inferred that solvents with polarity values higher than...
that of DMS will be more strongly trapped in the liquid phase of DMS than that of DMA. Consequently, such solvents will produce a lower concentration in the gas phase and smaller peak responses in DMS than in DMA. In contrast, solvents with polarity values lower than that of DMS will be less strongly trapped in the liquid phase of DMS than that of DMA. Thus, such solvents will yield higher concentrations in the gas phase and larger peak responses in DMS than in DMA. The results shown in Table 1 match the above hypothesis correctly. Therefore, it was concluded that diluent effects were highly dependent on the polarities of analyte solvents and diluents.

To describe the relationships of polarities to diluent effects, the concept of solvent polarity relative to diluents was introduced. This value is the difference in polarity between a solvent and a diluent.

![FIGURE 4: (a) Linear regression results for linearity studies of benzene with significant interferences. (b) Linear regression results for linearity studies of benzene after eliminating self-contamination.](image)

### TABLE 2: Sample matrix effects on analyte solvents with different sample amounts

<table>
<thead>
<tr>
<th>Sample Weight</th>
<th>100 mg</th>
<th>200 mg</th>
<th>400 mg</th>
<th>600 mg</th>
<th>800 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solvents</strong></td>
<td>%Change (Matrix Effect)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>12.5</td>
<td>14.0</td>
<td>20.2</td>
<td>22.2</td>
<td>27.1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12.2</td>
<td>13.6</td>
<td>19.3</td>
<td>20.8</td>
<td>25.7</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>15.7</td>
<td>17.2</td>
<td>22.9</td>
<td>23.9</td>
<td>30.4</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>10.6</td>
<td>11.9</td>
<td>16.8</td>
<td>17.6</td>
<td>22.5</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>9.2</td>
<td>10.5</td>
<td>15.1</td>
<td>16.1</td>
<td>19.6</td>
</tr>
<tr>
<td>1-Butanol</td>
<td>8.9</td>
<td>10.1</td>
<td>14.3</td>
<td>15.5</td>
<td>19.2</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>5.1</td>
<td>6.3</td>
<td>6.3</td>
<td>11.2</td>
<td>13.7</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>2.3</td>
<td>2.6</td>
<td>3.8</td>
<td>3.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>2.7</td>
<td>2.8</td>
<td>3.6</td>
<td>2.9</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Group C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td>3.6</td>
<td>2.4</td>
<td>1.3</td>
<td>-1.7</td>
<td>-3.3</td>
</tr>
<tr>
<td>Benzene</td>
<td>2.1</td>
<td>1.3</td>
<td>0.6</td>
<td>-1.5</td>
<td>-3.0</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>0.2</td>
<td>-1.2</td>
<td>-3.3</td>
<td>-6.4</td>
<td>-9.3</td>
</tr>
<tr>
<td><strong>Group D</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclohexene</td>
<td>-1.5</td>
<td>-3.0</td>
<td>-5.8</td>
<td>-9.0</td>
<td>-12.3</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>-3.4</td>
<td>-4.3</td>
<td>-7.1</td>
<td>-10.1</td>
<td>-13.7</td>
</tr>
<tr>
<td><strong>Group E</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>-3.2</td>
<td>-2.5</td>
<td>-2.8</td>
<td>-3.8</td>
<td>-5.8</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>-6.8</td>
<td>-5.2</td>
<td>-5.6</td>
<td>-6.5</td>
<td>-8.9</td>
</tr>
</tbody>
</table>

**Key:** Five standard injections, three spiked sample preparations, one un-spiked sample preparation, the peak area of the spiked sample was corrected by un-spiked sample, use mean of peak areas for %Change calculations.
calculated by subtracting the solvent polarity by the diluent polarity. The values of solvent polarity relative to DMS for analyte solvents are listed in Table 1 as well. For the values of solvent polarity relative to DMS, solvents possessing positive numbers had positive diluent effects. Coincidently, solvents possessing negative numbers had negative diluent effects. Furthermore, solvents with larger absolute values of positive numbers, such as methanol, had larger magnitudes of positive diluent effects. Solvents with larger absolute values of negative numbers, such as n-hexane, had larger magnitudes of negative diluent effects when DMS was replaced by DMA, and vice versa.

Figure 2 shows the correlation between %change and the values of solvent polarity relative to the polarity of DMS for 14 analyte solvents. It was found that there was an approximately linear correlation between them. This relationship provides a simple approach for using the polarities of solvents and diluents to predict the approximate diluent effects when DMS is replaced by DMA.

Based on the above analysis, it can be inferred that adding less-polar additives (modifiers) to existing diluents, such as adding DMA to DMS, will reduce the overall polarity of diluents, and then will increase the volatility of polar solvents and decrease the volatility of nonpolar solvents. Conversely, adding more polar additives, such as adding water to DMS, will increase the overall polarity of diluents, and then will reduce the volatility of polar solvents and increase the volatility of nonpolar solvents. Correspondingly, based on the polarities of diluents and analyte solvents and using the rules of diluent effects, enhanced peak responses of residual solvents can be achieved by properly choosing or adjusting diluents to improve method performance, such as method sensitivity.

Matrix Effects on Residual Solvents: Sample matrix effects are defined as the change in peak responses of analyte solvents in given diluents before and after adding samples. A standard solution and samples spiked with the same standard solution are usually used to study matrix effects. A standard solution of 16 residual solvents prepared in DMS and an organic drug substance (with 11 H-bond acceptors and four donors) were used in this study. Triplicates of spiked samples with sample amounts of 100, 200, 400, 600, and 800 mg were prepared and consecutively injected.

Similar to diluent effects, the %change in peak response was used to indicate characteristic properties of matrix effects (the positive or negative change and the magnitude of change) on solvents. It was calculated by the equation below:

\[
\% \text{Change} = \frac{\text{Peak response of spiked sample} - \text{Peak response of spiking Std}}{\text{Peak response of spiking Std}} \times 100
\]

The matrix effects on all tested solvents with different sample amounts are summarized in Table 2. As with diluent effects, matrix effects also varied from solvent to solvent. For example, methanol yielded 12.5% and 27.1% matrix effects, and n-hexane yielded -6.8% and -8.9% matrix effects with sample amounts of 100 mg and 800 mg, respectively.

Based on the characteristics of matrix effects, five different types of matrix effects were classified, and all tested solvents were sorted into different groups. As sample amounts increased, group A solvents (methanol, ethanol, 1-propanol, IPA, acetonitrile, t-butanol, and dichloromethane) had significant positive effects with increasing trends, group B solvents (acetone and ethyl acetate) had slightly positive effects with no trends, group C solvents (toluene, benzene, and THF) had slightly positive effects at lower sample amounts and gradually turned to negative effects at higher sample amounts with decreasing trends, group D solvents (cyclohexene and cyclohexane) had negative effects with decreasing trends, and group E solvents (ethyl ether and n-hexane) had negative effects with no trends. These changing trends of matrix effects along with sample amounts were statistically confirmed by p-values with a threshold of 0.05. To clarify the trends and characteristics of different types of matrix effects, using one solvent from each group, %change versus sample concentrations is graphically illustrated in Figure 3.

Just as with diluent effects, the organic sample worked as the polarity-modifying additives; it modified the overall polarities of sample mixtures. After adding samples, group A and B solvents with polarity values from 0.228 to 0.762 gained positive matrix effects, indicating that their volatilities increased. Meanwhile, group D and E solvents, which were nonpolar or close to nonpolar, yielded negative matrix effects, indicating that their volatilities decreased. All these results confirmed that the overall polarity of sample mixtures was reduced by
samples. Thus, polarity continued to play an important role in molecular interactions between diluents, analyte solvents, and samples to produce matrix effects. Group C solvents were at the transitional points of matrix effects changing from positive (groups A and B) to negative (groups D and E). Group C solvents had decreasing trends and negative effects at high sample amounts; they were similar to group D and E solvents. Therefore, it can be inferred that for all levels of sample amounts, group C solvents should have the same negative direction of matrix effects caused by the reduced polarity of sample mixtures as group D and E solvents.

When solvents (like water and ethanol) mix or dissolve, the solvation process reduces their partial molar volumes (11). Similarly, as samples are dissolved in the diluent, the sample solvation process also reduces the actual diluent volume available to interact with analyte solvents, resulting in higher concentrations in the liquid phase, and subsequently higher peak responses and positive matrix effects. Therefore, the overall matrix effects are affected by both polarities and sample solvation processes.

As a conclusion, matrix effects, the results of molecular interactions between diluents, analyte solvents, and samples, are mainly dependent on their polarities and closely related to sample solvation processes. Given the complexity of molecular interactions in sample mixtures, further work will be needed to reveal the details of matrix effects.

Matrix effects will cause biases for method accuracy when using external standard calibration. The use of proper internal standards, correction factors, or standard addition approaches can compensate for these matrix effects. **Troubleshooting Example: Using Diluent Effects for Benzene Interference Investigations:** Benzene is classified as a toxic solvent and its allowed maximum residual amount in samples is 2 ppm (1–3). For headspace analysis, if using 100 mg of sample and 2.0 mL of the diluent, the benzene concentration of corresponding 100% specification level was only 0.1 µg/mL, which produces a very small peak. Benzene analysis is very sensitive to interferences. There were a few special cases
of benzene interference in our laboratories; their contamination sources had been undiscovered until they were linked to diluent effects. One example was a linearity study of benzene, ethanol, and toluene in a method validation. The correlation coefficients ($R^2$) for ethanol, toluene, and benzene were 1.00, 1.00, and 0.87, respectively. The acceptable criteria for linearity were $R^2 \geq 0.98$. Benzene linearity failed the acceptable criteria, as shown in Figure 4(a). It was shown that some solutions had unexpected higher peak responses, indicating that benzene contamination occurred.

Investigations ruled out many possible causes, such as contamination from glassware, dilution errors, or solvent coelution. The method used the mixture with DMS and water as diluents. DMS and water are polar, and benzene is nonpolar. As discussed above for diluent effects, benzene is likely to evaporate from solutions prepared with DMS and water. If this were the cause, evaporation losses of benzene would yield smaller peak responses rather than the higher ones observed in this study.

All solution preparations were performed in a fume hood for safety purposes. Checking the working surroundings, it was noticed that used pipettes were also stored inside the same fume hood. The benzene in the residual solutions contained in used pipettes could evaporate and potentially migrate into diluents. Subsequent solutions prepared with these diluents could yield higher peak responses than originally expected.

To verify this hypothesis, two sets of linearity solutions were prepared. The first set was prepared in the same manner as before and still produced failing results. The second set was prepared by removing all used pipettes out of the fume hood and produced much improved results, with $R^2 = 1.00$, as shown in Figure 4(b). Thus, the above hypothesis for benzene self-contamination was confirmed. In addition, the common practice in laboratories of using beakers to add diluents into flasks that already contained solutions of residual solvents could also cause a similar issue.

The issue that happened to benzene could possibly occur to ethanol and toluene as well. Notwithstanding, they did not produce failing results. That was because they either had closer polarities and better compatibility with the diluents and the issue did not occur, or they had higher concentrations, and the possible biases from this issue were not big enough to affect their results significantly.

Understanding and using the theory of diluent effects was the key for success in troubleshooting this problem. This study can provide an example for laboratory investigations for similar issues and a guide for good laboratory practices to avoid interferences, especially for benzene analysis.

**Conclusion**

In pharmaceutical analysis, when residual solvents are analyzed by static headspace gas chromatography, both sample diluents and sample matrices can produce significant impacts on peak responses of residual solvents. In our study, the tendencies and magnitudes of diluent effects were shown to be mainly dependent on the polarities of residual solvents and diluents. The tendencies and magnitudes of matrix effects were mainly dependent on the polarities of residual solvents, diluents, and samples and further on sample solvation processes. Applying the rules of diluent effects and matrix effects can improve method sensitivity and accuracy, and avoid interferences as well.

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Two-dimensional liquid chromatography (2D-LC) separations can pose troubleshooting challenges not normally encountered in conventional high performance liquid chromatography (HPLC) separations. A common problem encountered in the development of 2D-LC methods is that the first dimension mobile phase properties can negatively affect the quality of subsequent second dimension separations. In this instalment the origin of this problem and potential solutions are discussed.

For more than three decades, this “LC Troubleshooting” column has focused on solving problems encountered in liquid chromatography methods involving a single column. Since the late 1970s, researchers have been working to develop two-dimensional liquid chromatography (2D-LC), which typically involves two different columns (1). For decades, 2D-LC has been used extensively and successfully in several specific application areas, including proteomics and polymer separations.

Most recently, however, the number of commercially available options for off-the-shelf instrumentation for 2D-LC has increased. Because of this, users in a broad array of application areas have begun deploying 2D separations more widely (2). Given the steady increase of active users deploying 2D-LC, it is now time to dedicate some instalments of “LC Troubleshooting” to problems encountered in 2D-LC. Although it is true that many of the troubleshooting topics in the context of conventional HPLC separations also apply to 2D-LC (for example, best practices for mobile-phase preparation are nominally the same for one-dimensional (1D)- and 2D-LC), it is also true that there are challenges that are unique to 2D-LC and deserve focused attention. Readers interested in learning much more about state-of-the-art 2D-LC separations are referred to several recent review articles (1–3). In the following paragraphs, I provide a brief review of the concept of 2D separation, and then discuss one of the common problems encountered by users developing 2D-LC methods: breakthrough peaks in the second dimension.

Two-Dimensional Separations: The Basic Idea

People are interested in 2D-LC separations because the 2D format provides a chance to resolve analytes that otherwise would not be resolved by a single column in conventional 1D-LC. This can be useful for separating very complex mixtures, such as tryptic digests of proteins where the analyst tries to extract as much information from the sample as possible. However, 2D separations are also useful for samples that are not so inherently complex, but contain several analytes that are difficult to separate. For example, samples of several different enantiomer pairs may be difficult to separate using a single chiral column, but may be relatively easy to separate in a 2D format using a combination of achiral and chiral columns (4).

There are several different modes or ways of executing 2D-LC separations. Among these, the single heartcut mode—denoted LC-LC—is the simplest in terms of the instrumentation, methods, and data analysis involved. The goal of such a separation is illustrated in Figure 1. A first dimension...
D) separation results in three clusters of unresolved peaks. The first cluster contains a peak corresponding to the analyte of interest (blue peak). Current commercial instrumentation and software make it straightforward to target this peak and instruct the instrument to transfer a portion of effluent from the 1D column containing the peak (along with the overlapping pink and green peaks) to a second dimension (2D) column for further separation. Then, provided a 2D column and separation conditions are chosen that are complementary to the 1D conditions, the analyte of interest can be separated from the compounds it overlapped with in the 1D separation, enabling accurate quantitation and a qualitative analysis free from interferents.

There are a number of ways that instrumentation can be configured to support such a separation, but one example of the interface between the 1D and 2D separations is shown in Figure 2. The interface valve has two positions, and the valve is toggled to either have the 1D column effluent flow to waste in regions of the separation that don’t require additional 2D separation (position B), or to collect the 1D effluent in the sample loop (position A). At the end of this sampling period, the contents of the loop are effectively injected into the 2D column for further separation by switching the valve from position A to B.

Mobile-Phase Mismatch Can Lead to Breakthrough Peaks

As indicated in the preceding section, an important determinant of successful 2D-LC separations is the degree of complementarity of separation conditions (that is, the separation selectivities should be different) used in the first and second dimensions. Sometimes, this can be realized without the conditions used in the 1D separation negatively impacting the quality of the 2D separation as measured by chromatographic efficiency or detection sensitivity. For example, many applications of 2D-LC in the field of proteomics involve a 1D separation based on a cation-exchange mechanism and a 2D separation that relies on a reversed-phase mechanism. In the case of 2D-LC, peptides are eluted from the 1D column in effluent composed mainly of a buffered aqueous solution. This is highly favourable for the 2D separation in the sense that relatively large volumes of this effluent can be injected into the 2D column without negatively impacting the widths or shapes of 2D peaks. However, a favourable relationship between the operating conditions used in the two dimensions is not inevitable. In other cases, the properties of the effluent from the 1D column can have a dramatic and negative effect on the quality of the 2D separation. We refer to problems of this type as originating from mismatch between the mobile phases used in the two dimensions. An example of this is where we use a hydrophilic interaction liquid chromatography (HILIC) separation in the first dimension and a reversed-phase LC separation in the second dimension. In general, HILIC separations tend to involve mobile
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phases containing much more than 50% acetonitrile, whereas reversed-phase LC separations of compounds that can be reasonably retained under HILIC conditions tend to involve mobile phases containing much less than 50% acetonitrile. If HILIC separation is used in the first dimension and reversed-phase LC separation is used in the second dimension, this then sets up a situation where a large volume of 1D effluent containing analytes of interest and more than 50% acetonitrile is injected into the reversed-phase LC column running with a mobile phase containing less than 50% acetonitrile.

A Variety of Solutions to Help

While the mobile-phase mismatch problem described above is certainly not new to the 2D-LC community, its

Figure 3 shows an illustration of the local environment inside the 2D column during the injection of 1D effluent into the 2D column. In principle, this injection step is not different from what happens in conventional 1D-LC. However, problems can arise in 2D-LC when the volume of effluent from the 1D column is not very small (less than 1%) relative to the volume of the 2D column itself (which is common in 2D-LC). I like to emphasize the impact of this difference by saying that in 2D-LC the 1D column effluent becomes the 2D mobile phase during the injection step. If analytes are weakly retained by the 2D column in the 1D effluent, then it hardly matters what the analyte retention is in the 2D column in the 2D effluent, the mobile phase—retention during the injection step will be determined primarily by the properties of the 1D effluent. Therefore, such conditions can very easily lead to terrible chromatographic outcomes in the second dimension. Figure 4 shows the experimental results from recent work by my research group that was aimed at developing 2D-LC separations of monoclonal antibodies (mAbs) using HILIC and reversed-phase LC separations in the first and second dimensions, respectively (5). In this instance, the influence of the 1D effluent on the 2D separation is so severe that most of the analyte mass injected into the 2D column breaks through in the dead volume of the column, and only a small fraction of the mass is retained and eluted with normal looking peaks. This type of outcome is, of course, highly undesirable from the point of view of both quantitative and qualitative uses of 2D-LC.

A Variety of Solutions to Help

While the mobile-phase mismatch problem described above is certainly not new to the 2D-LC community, its
significance in practical 2D-LC method development and the importance of developing solutions for the problem to the continued growth of the technique have garnered a lot of attention in recent years. A broad perspective on the significance of the problem in different application areas and for possible combinations of different separations modes has been discussed in detail by Pirok and co-workers (6). The good news is that currently there are commercially available technologies that can be used to address the problem, and other potential solutions are being explored by various research groups across the globe (7). Currently, two of the most effective commercially available solutions rely on interventions that disrupt the situation illustrated in Figure 3 that can lead to poor outcomes like that shown in Figure 4(b), by adjusting the composition of the 1D effluent prior to injection of that material into the 2D column for further separation. In an approach known as **at-column dilution (ACD)**, a pump is used to add diluent to the 1D column effluent as it exits the column, thereby adjusting the properties of the effluent prior to loading into the 2D column (8). In a different approach known as **active solvent modulation (ASM)**, valve technology is used to enable temporary adjustment of the properties of the 1D effluent prior to injection into the 2D column (9). Figure 4 shows a representative result from our own work aimed at demonstrating the positive impact of an intervention like this on the quality of the 2D separation in a situation where the impact of the mobile-phase mismatch is severe if left unchecked. A 1D HILIC separation of the fragments of the monoclonal antibody atezolizumab after treatment with the IdeS enzyme and reduction with dithiothreitol is shown in panel (a). Adding a 2D reversed-phase LC separation either without or with ASM produces the 2D chromatograms shown in panels (b) and (c), respectively. Separating these protein fragments by HILIC requires a 1D mobile phase with about 70% acetonitrile, and a 2D mobile phase with about 30% acetonitrile for reversed-phase LC separation. When there is no mitigation of the effects of this mobile-phase mismatch, the 70% acetonitrile in the 1D effluent causes severe breakthrough of protein peaks in the dead volume of the 2D column due to very low reversed-phase LC retention in a high acetonitrile environment. These breakthrough peaks are highlighted by the red rectangle in Figure 4(b). On the other hand, ASM enables a reduction in the acetonitrile level to below 30% in the sample that is injected into the 2D column, thereby completely eliminating the breakthrough, as shown in Figure 4(c).

**Summary**

Currently, it is most important that 2D-LC users recognize that breakthrough in the second dimension of 2D-LC separations can be a severe problem, particularly in cases where the degree of mismatch between the mobile phases used in the two dimensions is large. Currently, ACD and ASM are two popular and commercially available solutions that address many, but not all, of these challenges. There are many other potential “homemade” solutions, and several research groups are exploring other potential solutions involving membrane and sorbent-based approaches, to name a few (7, 10). Given the importance of this problem it seems likely that we will see other commercial solutions emerge in the not-too-distant future.

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Flying High with Sensitivity and Selectivity: GC–MS to GC–MS/MS

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Mass spectrometry (MS), often termed mass selective detection, is the most powerful detector available for gas chromatography (GC). Multidimensional mass spectrometry (MS/MS) takes mass selective detection to another level on benchtop systems, offering both universal and selective detection along with low detection limits. In this instalment of “GC Connections”, we review the fundamentals of MS/MS and how they relate to MS as a detector for GC. We see how using full-scan analyses can make the detector universal and how by using selected ion monitoring and multiple reaction monitoring the detector can be so selective and noise-free that femtogram quantitative analysis is commonplace. We then examine some scenarios that should lead analysts to consider using GC–MS/MS to solve complex problems.

High sensitivity and selectivity are among the most important goals of any chromatographic method development or optimization process. Instruments, stationary phases, and detectors are usually chosen with one or both of these goals in mind. In gas chromatography, mass selective detectors (MSDs or mass spectrometers) have been used for decades to provide both high selectivity and high sensitivity. Capillary gas chromatography coupled to mass spectrometry (GC–MS) is a straightforward, yet powerful coupling of the selectivity of GC with the high sensitivity and option of universal or selective detection of MS. Traditional GC–MS provides multiple dimensions of separations and low detection limits in benchtop or smaller instruments.

Before flying into the details of MS/MS, we should briefly review some of the terminology specific to MS as a detector for GC. Mass selective detectors operate in two modes. The first mode is full-scan, in which spectra are continuously collected in quadrupole systems at rates usually up to 10–20 spectra per second depending on the mass range selected. The second mode is selected ion monitoring (SIM), in which one or more individual ions are monitored. The data can be obtained in three forms:

• A total ion chromatogram (TIC) is the sum of all signals that reach the detector and is a demonstration of nearly universal detection. The full mass spectrum can be obtained at any point on the chromatogram.
• An extracted ion chromatogram (EIC) is obtained from the TIC by choosing one or more individual masses and extracting these from the full data set. This allows both universal and selective detection in a single experiment, since the ion chosen for analysis can be characteristic of a single compound or compound class.
• Selected ion monitoring (SIM) is obtaining a TIC in which the detector is set to monitor only one or a few ions. If a spectrum is selected from the TIC, it will only show the few ions that were selected when the experiment was set up.

There are several common GC–MS and GC–MS/MS instruments. Single dimension, classical GC–MS is mainly performed using quadrupole mass analyzers. Ion trap, a derivative of quadrupole instruments, and time-of-flight (TOF) are also used for specific analyses. Quadrupole-based systems are simpler and less expensive; GC–TOF-based systems offer the highest sensitivity and much greater mass precision and accuracy.

GC–MS/MS can be achieved through several configurations, with a wide range of capability and complexity. The most common of these is GC–triple quadrupole-MS (GC–TQMS), while GC–ion trap-MS (GC–ITMS) and GC–quadrupole time-of-flight MS (GC–QTOF-MS) are also available. A brief discussion of the evolution of ion trap and triple quadrupole mass analyzers over the years is provided in the brochure by Huebschmann.
Professors Chris Enke and Rick Yost, inventors of the TQMS, have provided two excellent video interviews discussing the development of the technique in detail (2,3).

Figure 1 shows a block diagram of the detector on a GC–TQMS system compared to a traditional GC–MS system. Both are available in benchtop configurations. The main difference between the two systems is the presence of three quadrupole mass filters on the GC–TQMS system and one on the GC–MS system. Both systems use a transfer line with a capillary direct interface into the ion source and classical electron ionization ion source between the GC and the mass analyzer. Both operate with the ion source and mass analyzer at high vacuum and use a classical electron multiplier to detect ions that pass through the mass analyzer. As described in more detail below, the first quadrupole (Q1) performs in the same manner as the single quadrupole in traditional GC–MS, selecting the ions that are ultimately passed to the electron multiplier detector. It can operate in either full-scan or selected ion monitoring modes. The second quadrupole (Q2) is used as a medium for collision induced fragmentation of ions passed through the first quadrupole to produce new fragments, and the third quadrupole (Q3) is used to select and analyze these new fragments.

MS/MS is among the most flexible of all detectors, as it operates in several modes. In traditional GC–MS, full-scan MS provides a nearly universal detector; any analyte that can be ionized within the ion source can be detected. SIM-MS is a highly selective detector; the signals for the chosen ions are the only ones recorded. SIM is used for quantitation as the reduction in the signals being monitored versus full-scan also reduces the noise, increasing the signal-to-noise (S/N) ratio and therefore lowering the detection limit.

Figure 2 shows how the most common modes of quadrupole MS and MS/MS detection work. Full-scan and SIM single quadrupole detection are seen in the left side of the figure, in the ion source and Q1 images. The ion source generates ions including many masses; the quadrupole can either pass all of them (full-scan) or selected ions (SIM). Triple quadrupole MS offers even more flexibility, since two additional quadrupoles are employed, as seen in Figure 2. Note that in both GC–MS and GC–MS/MS, classical electron ionization (EI) is by far the most commonly used...
A GC–MS/MS system can be operated exactly as a single quadrupole system. The second and third quadrupoles can be set to pass ions through without any further separation or reaction. This is often the first step in developing a method or transferring one to GC–MS/MS as it provides a traditional total ion chromatogram and traditional mass spectra of the analytes as a starting point.

In a product ion scan, the first quadrupole (Q1) can be set for selected ion monitoring, as in traditional GC–MS. Collision-induced ionization then occurs in the second quadrupole (Q2) to generate further fragmentation of the chosen ion, providing additional fragmentation that is analyzed by scanning the third quadrupole (Q3). This is a powerful tool for qualitative analysis since larger fragments from a traditional single dimension mass spectrum can be further fragmented to aid in confirming the correct structure. A product scan is also used for choosing transitions in initial method development for multiple reaction monitoring.

In a precursor ion scan, Q1 can be operated full scan, which passes all of the fragments generated in the ion source; think of traditional MS without the detector, with all of the resulting fragments reionized in Q2 and a single fragment chosen for monitoring in Q3. This is very similar to SIM in traditional GC–MS and is useful for quick quantitative analysis method development.

In multiple reaction monitoring (MRM), the most sensitive mode for quantitative TQMS, both Q1 and Q3 are set for single ion analysis. Based on the full-scan mass spectrum or a product ion scan, a fragment from Q1 is chosen and then, based on the further fragmentation in Q2 as seen by scanning Q3, a single fragment from Q3 is chosen. This provides possibly the ultimate selectivity, as the possibility of two compounds, even closely related isomers, having the same transitions from precursor ion fragment to product ion fragment and the same (or close)
retention time in the column decreases greatly. MRM also provides very low detection limits by significantly reducing noise in both dimensions.

Figure 3 shows the well-known mass spectrum for caffeine, which provides an example for the utility and power of MRM. The mass transitions that generate the signals seen in the mass spectrum are also provided. In MRM, this full-scan spectrum is the starting point for method development, either determined experimentally or obtained from the literature. As this is an election ionization spectrum, it is good practice to interpret the spectrum, using traditional spectral interpretation rules at this point for a full understanding of the structural elements and decomposition reactions that generate each of the fragments. There are multiple excellent tutorial books relating to election ionization mass spectral interpretation available (4,5). Should there be additional spurious peaks, these can be identified at this point so they do not cause confusion later.

In traditional single dimension GC-MS, the full-mass spectrum is used to identify the analyte. Quantitation is performed using either extracted ion chromatograms obtained by selecting one or more individual masses from the total ion chromatogram or by selected ion monitoring. SIM offers lower detection limits by reducing noise as most of the mass signals seen in the full-scan spectrum and their accompanying noise are eliminated.

In MRM, further advantage is taken of this noise reduction. One or more peaks from this initial mass spectrum is then chosen for further fragmentation. Usually this is the largest (base) peak, or it can be another strong signal that may be more characteristic of that compound. With this mass chosen, a second experiment is performed with Q1 operating in SIM mode to pass the one ion, termed a Precursor ion, with that ion being reionized in Q2, with the resulting new fragments, termed product ions, passed to Q3 operating in scan mode as seen in the Figure 2 product scan, to generate a mass spectrum of the fragment. One or more ions from this product spectrum can then be chosen for the final quantitative method. Any of the transitions seen in Figure 3, such as those from mass 194 to mass 109, are termed MRM transitions and are often reported in the literature for completed methods. Any of the transitions can be used with one for quantitation and additional transitions for confirmation. Selectivity is generated...
because, instead of looking at individual masses which may not be unique to a compound, this is looking at transitions, which are very unique, especially when multiple transitions are used.

The ability to obtain spectra from precursor ions and to perform MRM leads to three situations in which GC–MS/MS is especially useful:

1. Targeted analysis of a few analytes, in which you know the identity of the analyte or analytes, for which extreme sensitivity or low detection limits are required and/or the sample matrix is highly complex.

2. Simultaneous targeted analysis of many analytes whose chromatographic peaks are not fully resolved.

3. Untargeted analysis in which the matrix is complex, analyte chromatographic peaks are overlapped, and the additional qualitative information about fragmentation is needed.

Analysis of extremely low levels of emerging contaminants in environmental water samples is an example of the first case. These appear at very low levels and are the result of human activity. One application, freely available online, shows analysis of several steroids in water at low parts per billion (ppb) and parts per trillion (ppt) levels using solid-phase microextraction (SPME) coupled to GC–MS/MS (6). In this work, the sample preparation and detection were optimized but the chromatography was not, so it also illustrates the second problem: the analyte peaks are not resolved by the chromatography but are easily resolved using their differing MRM transitions. In a more extreme example, over 300 pesticides were extracted from apples and determined simultaneously in a single run using GC–MS/MS (7). This work illustrates the second and third cases: there are many analytes, the analysis can be either targeted or untargeted, and the sample matrix is a complex food sample.

MS/MS provides the ultimate in detection for capillary gas chromatography. It can be both universal (full-scan) or selective (SIM or MRM) and it is highly sensitive with detection limits of femtograms readily available. MS/MS is especially suited to the most difficult separation and detection problems. The trade-off of this capability is capital and ongoing expense. MS/MS detectors are expensive, with fully loaded systems costing hundreds of thousands of U.S. dollars and have higher ongoing costs than traditional GC–MS systems, requiring special training to operate and needing additional maintenance compared to GC and GC–MS systems. The high sensitivity and low detection limit of MS/MS makes it especially sensitive to laboratory conditions such as clean carrier gases and careful sample preparation. Errors in sample preparation and contamination issues are often amplified when instrumental noise from the detector is lowered, so special care in sample preparation and laboratory management of GC–MS/MS is required. The unmatched combination of sensitivity with the ability to be both selective and universal makes MS/MS the most powerful detection tool available for capillary gas chromatography.

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Next Generation Sorbent-Based Extractions with Metal-Organic Frameworks

Douglas E. Raynie, Sample Preparation Perspectives Editor

Metal-organic frameworks (MOFs) are coordination networks consisting of a metal ion linked with organic ligands. The resulting three-dimensional structures create pores that can be exploited for a number of chemical processes, including analytical extractions. The resulting sorbent-based extraction systems have several advantages, notably selectivity. The use of MOF in extraction has exploded in the last two or three years. In this article, we take a look at the current state of the art regarding analytical extractions utilizing MOF, including a description of what MOF are, their preparation, principles of use, advantages, and application areas.

The field of sample preparation and analytical extractions faces a somewhat unique contradiction. On one hand, analysts are seeking high levels of selectivity during these preliminary stages. That is, there is a desire to isolate our analytes of interest to the exclusion of everything else prior to the actual analysis. On the other hand, advances in chromatography, mass spectrometry, and spectroscopy over the past couple of decades have allowed us to characterize samples of increasing impurity. Significant increases in gaining selectivity during the sample preparation steps of an analysis are gained with the use of selective adsorbents. These extractions include solid-phase extraction (SPE), solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE), dispersive extractions (including QuEChERS, matrix solid phase dispersion [MSPD], and dispersive SPE [dSPE]), and a host of similar techniques related to sorbent-based methods. Our last sample preparation trends survey, conducted five years ago (1), demonstrated steadily increasing use of these techniques. Historically, these sorbent-based extractions featured use of either chromatography stationary phases or general sorbents, like silica, carbon, or alumina. More recently, specialized sorbents are being used for sorbent-based extractions, such as molecularly imprinted polymers, restricted access media, crown ethers, and others.

Last year at this time (2), we reported on the major sample preparation advances in the previous year. One major advance, still in an emerging state, was the use of metal organic frameworks (MOFs) for extraction. This was highlighted by one of our thought leaders and featured prominently in an Analytical Chemistry fundamental review (3). During this past year, the use of MOFs in analytical extractions has exploded from the emerging state to the breakthrough phase. We can see in Figure 1 an almost exponential growth in the annual number of publications, as reported with the Scopus database using the search terms “metal organic frameworks” and “extraction”, over the past decade. In 2020, nearly 250 articles were reported, compared with just 8 in 2011! With this explosive growth, there must be some distinguishing features surrounding the use of metal organic frameworks (MOFs) for extraction. In this “Sample Preparation Perspectives” instalment, we explore this phenomenon with a focus on the past year or two.

Metal Organic Frameworks
MOFs are coordination polymers or highly ordered crystalline structures, typically two- or three-dimensional, composed of metal cations or clusters connected with...
coordinating organic ligands (4,5). They are mesoporous with pores in the 2–50 micrometer diameter range. MOFs can have a surface area in the thousands of m²/g with a high number of pores and functional groups. Self-assembly of the metallic moieties with multifunctional organic ligands containing nitrogen and/or oxygen comprise the MOF. The first permanently porous MOF were reported in 1995 (6). The coordination between the metallic component and the organic ligand is described by the hard/soft acid/base (HSAB) theory. Because of the chemical nature of the MOFs, intermolecular forces, including electrostatic interactions, Van der Waals forces, hydrophobic interactions, π–π interactions ion exchange, Lewis acid-base, chelation, hydrogen bonding, and coordination can adsorb analytes from various mixtures during an extractive procedure (7–9).

Synthesis of MOF can be by a variety of routes, including slow evaporation, covalent assembly, chemical co-precipitation hydrothermal, solvothermal, microwave-assisted method, mechanochemical, and electrochemical techniques (10,11). Because of these synthetic procedures, there is wide latitude in creating MOFs with crystalline properties, tunable porosity, and surface areas in the range of 2000–7000 m²/g. Additionally, variable pore volumes can be created, and uniform porous structures achieved, with high thermal and mechanical stability (8). MOFs functionalized with ionic liquids are even being produced (12). Given the number of available functional groups and metal ions or clusters available, it is conceivable that the potential number of available MOFs to be created is infinite.

**Extraction Modes**

Essentially all modes of sorbent-based extractions have been performed with MOFs, including the in-tube and in-syringe approaches to SPE. This is because of their key features. MOF sorbents have high tunable porosity and surface area, designable structures, internal functionalities, and outer surfaces available for molecular interactions. MOFs also have thermal and mechanical stability, structural cavities, and uniform active sites (13,14). The MOFs can be included as part of polymer matrices, or in the pores of organic monoliths. As a result of these properties, MOFs have been used in conventional and dispersive SPE, though compaction and flow irregularities with cartridge SPE seem to lead to the dispersive approach being favoured. With dispersive SPE, the MOFs are rather easily dispersed with the sample matrix and recovery of the MOFs via phase separation is often straightforward. SPME and SBSE are also popular approaches for using MOFs during analytical extractions. One unique opportunity of MOFs is via magnetization of the metal component. After mixing the MOFs, frequently as nanoparticles, with the sample, recovery can be quite simple. Analyte enhancement factors using MOFs is large, often in the thousands. Extraction efficiencies with MOFs are similar to other sorbents and the extraction configuration; that is, the ratio of MOF to sample amount and analyte concentration, sample volumes, surface area, identify and volume of sample and eluent solvents, flow rates, extraction times, ionic strength, and sample phase can all play important roles in extraction efficacy, as they would in conventional and dispersive SPE, SPME, or SBSE. Thus, the selectivity, solvent use, recovery, and other advantages of these techniques still hold.

**Applications**

Given all of the stated advantages of MOFs and their explosive growth in the literature, one can expect that application of MOFs in sorbent-based extractions are manifold. These applications are found with both liquid and
solid samples and, while not exclusive, are found primarily in the biological, environmental, and food areas. Table 1 summarizes applications found in recent reviews (5,8,10,11,13,15–17).

A few key observations are gleaned from this table. Conventional SPE, SPME, and SBSE techniques using MOFs are somewhat evenly distributed, yet are the more minor approaches to MOF extractions. It is the approaches that take special advantage of the unique properties of MOFs that make up the majority of the applications. These approaches are dSPE and use of magnetic MOF. In the biological field, drugs of interest included antibiotics, nonsteroidal anti-inflammatory drugs, penicillins, and methamphetamine in urine, milk, and tissues. Additional biomolecules isolated from these matrices include estrogens and hormones, carbohydrates, peptides, proteins, and aflatoxins. Each of the major environmental contaminants of recent interest are extracted with MOF, namely endocrine disruptors, most types of herbicides and pesticides, phthalate esters, parabens, polychlorinated biphenyls and polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, polyfluorinated compounds in environmental waters and wastewater, soils and sediments, body fluids, fruits and vegetables, and related sample types. Fruits, vegetables, beverages, edible oils, and meats were investigated for herbicides and pesticides, hormones, and aflatoxins.

### Conclusions and Future Prospects

An emerging type of sorbent material, MOFs, are presented for use in conventional and dispersive

<table>
<thead>
<tr>
<th>Mode</th>
<th>Analyte</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPME, SPE, dSPE</td>
<td>Antibiotics</td>
<td>Water, milk, soil, sediments</td>
</tr>
<tr>
<td>dSPE</td>
<td>Anti-inflammatory drugs</td>
<td>Water, urine</td>
</tr>
<tr>
<td>dSPE</td>
<td>Fungicides</td>
<td>Water, soil</td>
</tr>
<tr>
<td>In-tube SPE, magnetic, dSPE</td>
<td>Fluoroquinolones</td>
<td>Water, foods, tissue</td>
</tr>
<tr>
<td>SBSE</td>
<td>Benzylpenicillin</td>
<td>Biological samples, milk</td>
</tr>
<tr>
<td>SBSE</td>
<td>Phytohormones</td>
<td>Fruits</td>
</tr>
<tr>
<td>SBSE, magnetic, SPME, dSPE</td>
<td>Phthalate esters</td>
<td>Foods, oils, water, beverages</td>
</tr>
<tr>
<td>SBSE, magnetic</td>
<td>Organophosphorus pesticides</td>
<td>Water, fruits</td>
</tr>
<tr>
<td>SBSE, dSPE</td>
<td>Triazine herbicides</td>
<td>Water, fruits, vegetable oils, foods</td>
</tr>
<tr>
<td>SBSE, dSPE</td>
<td>Parabens</td>
<td>Cosmetics, plasma, milk, urine</td>
</tr>
<tr>
<td>SBSE</td>
<td>Sulfonylurea herbicides</td>
<td>Water</td>
</tr>
<tr>
<td>SBSE, SPME, magnetic</td>
<td>Polychlorinated biphenyls</td>
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</tr>
<tr>
<td>SBSE</td>
<td>Caffeine</td>
<td>Beverages, urine</td>
</tr>
<tr>
<td>SBSE</td>
<td>Azo dyes</td>
<td>Water</td>
</tr>
<tr>
<td>SBSE</td>
<td>Carvediol, haloperidol</td>
<td>Water, plasma</td>
</tr>
<tr>
<td>Magnetic, SPME, dSPE, SPE</td>
<td>Polycyclic aromatic hydrocarbons</td>
<td>Water, soil, plasma, urine, meats, smoke, beverages, blood</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Neonicotinoid insecticides</td>
<td>Water, melons, vegetables</td>
</tr>
<tr>
<td>Magnetic, dSPE</td>
<td>Pheny lure herbicides</td>
<td>Fruits, soil, water, beverages, vegetable oils</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Flunitrazepam</td>
<td>Beverages</td>
</tr>
<tr>
<td>Magnetic</td>
<td>N-linked glycan</td>
<td>Egg proteins, serum</td>
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<tr>
<td>Magnetic</td>
<td>Peptides</td>
<td>Serum</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Chlorophenols</td>
<td>Water, beverages, vegetables</td>
</tr>
<tr>
<td>Magnetic, SPME, dSPE</td>
<td>Organochlorine pesticides</td>
<td>Water, vegetables</td>
</tr>
<tr>
<td>Magnetic, SPE</td>
<td>Carbamates</td>
<td>Water, fruits, vegetables, beverages</td>
</tr>
<tr>
<td>Magnetic, SPE</td>
<td>Endocrine disrupting compounds</td>
<td>Water, juice, tissue</td>
</tr>
<tr>
<td>Magnetic, SPME</td>
<td>Pyrethroids</td>
<td>Vegetables, water</td>
</tr>
<tr>
<td>Magnetic, dSPE, SBSE</td>
<td>Hormones</td>
<td>Water, urine, cosmetics, vegetables, sewage</td>
</tr>
<tr>
<td>Magnetic, dSPE</td>
<td>Benzyolurea insecticides</td>
<td>Beverages, grains, water, honey, fruits</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Anionic surfactants</td>
<td>Water</td>
</tr>
<tr>
<td>Magnetic, dSPE</td>
<td>Estrogens</td>
<td>Water, urine, milk</td>
</tr>
<tr>
<td>SPME</td>
<td>BTEX</td>
<td>Water, urine, smoke</td>
</tr>
<tr>
<td>SPME, magnetic, dSPE, SPE</td>
<td>Phenols</td>
<td>Water, beverages, plants, honey</td>
</tr>
<tr>
<td>SPME</td>
<td>Odorants</td>
<td>Water</td>
</tr>
<tr>
<td>SPE</td>
<td>Nucleoside diols</td>
<td>Cells</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Bisphenols</td>
<td>Serum</td>
</tr>
<tr>
<td>Magnetic, dSPE</td>
<td>Triazole fungicides</td>
<td>Vegetables, plasma</td>
</tr>
</tbody>
</table>
TABLE 1 (CONTINUED): Representative application areas of extractions performed using MOFs

<table>
<thead>
<tr>
<th>Mode</th>
<th>Analyte</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic</td>
<td>Bactericides</td>
<td>Fruits, vegetables</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Pacitaxel</td>
<td>Plasma</td>
</tr>
<tr>
<td>Magnetic, dSPE, SPE</td>
<td>Sulfonamides</td>
<td>Meat, water, milk, honey, meats</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Tetracycline</td>
<td>Water</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Nitroaromatics</td>
<td>Water, smoke</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Haloacetic acids</td>
<td>Urine, meats</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Perfluoro compounds</td>
<td>Water, milk, fruits, vegetables</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Alkaloids</td>
<td>Urine, plants</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Anthraquinones</td>
<td>Beverages</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Glycoproteins</td>
<td>Serum</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Trypsins</td>
<td>Tissues</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Phosphopeptides</td>
<td>Milk, serum</td>
</tr>
<tr>
<td>dSPE</td>
<td>Neurotransmitters</td>
<td>Biologicals</td>
</tr>
<tr>
<td>dSPE</td>
<td>Gallic acid</td>
<td>Urine, plasma, water</td>
</tr>
<tr>
<td>dSPE</td>
<td>5-Nitroimidazoles</td>
<td>Water</td>
</tr>
<tr>
<td>dSPE</td>
<td>Amphenicols</td>
<td>Water</td>
</tr>
<tr>
<td>dSPE</td>
<td>Glucocorticoids</td>
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</tr>
<tr>
<td>SPE</td>
<td>Phenoxyacetic acid herbicides</td>
<td>Vegetables</td>
</tr>
<tr>
<td>SPE</td>
<td>Nitrobenzene</td>
<td>Water</td>
</tr>
<tr>
<td>dSPE</td>
<td>Sialic acids</td>
<td>Serum</td>
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<tr>
<td>dSPE</td>
<td>Domoic acid</td>
<td>Shellfish</td>
</tr>
<tr>
<td>dSPE</td>
<td>Aflatoxins</td>
<td>Food</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Penicillins</td>
<td>Milk</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Polybrominated diphenyl ethers</td>
<td>Milk, plasma, serum, water</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Polysaccharides</td>
<td>Algae</td>
</tr>
<tr>
<td>Magnetic</td>
<td>DNA</td>
<td>Blood, bacterial cells</td>
</tr>
<tr>
<td>SPE</td>
<td>Sulfamono-methoxine</td>
<td>Soil, sediment</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Aristolochic acid</td>
<td>Vegetation</td>
</tr>
<tr>
<td>dSPE</td>
<td>Methamphetamine</td>
<td>Urine</td>
</tr>
</tbody>
</table>

SPE, SPME, SBSE, and, especially, magnetic extractions. These MOFs are characterized by high surface area, controlled and tunable porosity, high stability, and significant functionalization. Applications to biological, environmental, and food samples abound. Analytical extraction with MOFs as a field is only about a decade old and growing rapidly. Consequently, continued use of MOFs for new application areas, including industry standard and regulatory methods, are the obvious growth area. Currently, there are no commercial MOF extraction materials and consumables available; such commercialization will help drive these additional applications.

References

ABOUT THE COLUMN EDITOR

Douglas E. Raynie is a Department Head and Associate Professor at South Dakota State University, USA. His research interests include green chemistry, alternative solvents, sample preparation, high-resolution chromatography, and bioprocessing in supercritical fluids. He earned his Ph.D. in 1990 at Brigham Young University, Utah, USA, under the direction of Milton L. Lee. Raynie is a member of LCGC’s editorial advisory board. Direct correspondence to: amatheson@mjhlifesciences.com
Q. Why are per- and polyfluoroalkyl substances (PFAS) gaining so much attention at the moment and why is the analysis of these analytes important?

Stefan Van Leeuwen: PFAS are a class of chemicals that are extremely stable and resistant to degradation. Their surfactant properties give these chemicals unique characteristics for application in a wide array of products and processes. PFAS are used as surfactants for water and dirt repellency of clothing and furniture, as surfactants in aqueous fire fighting foams, for grease- and water-proofing food contact materials, and in many other applications. Unfortunately, their persistency and toxicity has become a major concern for society. The European Food Safety Authority (EFSA) recently evaluated that some specific PFAS show adverse effects on the immune system at low levels (1).

BB: Several pollution incidents in the USA, Europe, and Asia involving PFAS manufacturing plants polluting river water and drinking water, among other incidents, has brought the PFAS problem to the surface. Policy makers are now putting regulatory measures in place to regulate PFAS in products, the environment, food, and drinking water, and to stimulate the search for alternative chemicals.

Q. What techniques have been used to analyze these compounds historically, and do they have any limitations?

BB: The attention for these compounds started off in the early 2000s, with the targeted analysis of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). Labs, including Wageningen Food Safety Research (WFSR), mainly used liquid chromatography (LC) with triple quadrupole (QqQ) mass spectrometry (MS) to achieve the desired selectivity and sensitivity (2,3). Gas chromatography tandem mass spectrometry (GC–MS/
We challenged ourselves by searching for PFAS in surface waters that were suspected to contain relatively low levels of PFAS.

MS) methods were used for the highly fluorinated volatile PFAS. Over the years other PFAS were added and today some 20–40 compounds are analyzed routinely.

SVL: In the meantime, it became clear that PFAS is a very diverse group of chemicals, covering very different chemical families (4). Today, more than 4700 PFAS have been registered in an Organisation for Economic Co-operation and Development (OECD) database (5), and several of these lead to environmental and human exposure through food and drinking water. Moreover, several PFAS can actually degrade abiotically and biotically through pathways, yielding unknown intermediates. With these developments, the need for identification of unknowns became more apparent.

Q. Your team recently developed a method for the nontargeted analysis of per- and polyfluoroalkyl substances in surface water using liquid chromatography high-resolution mass spectrometry (LC–HRMS) with fragment ion flagging (FIF)?

SVL: That’s right. Together with a student, Thijs Hensema, we have developed this approach, as we felt that current approaches for nontarget identification of unknown PFAS had some drawbacks that we wanted to get around. We called the approach fragment ion flagging (FIF) (6).

Q. What is fragment ion flagging and what benefits does it offer the analyst for this particular application?

BB: FIF is a method for the identification of unknown PFAS, by looking at the fragmentation patterns of these substances. Specific characteristic fragments of PFAS will then be used as a lead towards structural elucidation of the parent PFAS. We have developed and tested this FIF workflow, and the analyst can easily follow this stepwise procedure for identification of unknown PFAS. FIF can be used in any HRMS system (coupled to GC or LC) that allows the use of all ion fragmentation (AIF) and data independent acquisition (DIA).

Q. What sample preparation technique did you use?

SVL: Most studies so far were conducted on samples with relatively high levels of PFAS, for example, downstream from a manufacturing plant. We wanted to investigate the samples with background contamination that were likely to have lower contamination levels. To do so, we used solid-phase extraction (SPE)—in our case a weak anion exchange SPE column—to isolate and concentrate anionic PFAS. This provided us with a 1000-fold concentration factor. In the future, we will expand this further to other isolation modes, such as cationic and mixed-mode isolation.

Q. What is novel about your approach and what benefits does it offer the analyst for this application compared to other techniques?

SVL: The methods applied so far for identification of unknown PFAS were based on full-scan feature selection, such as homologue series detection, mass defect filtering, and intensity threshold filtering. Although these approaches have proven benefits, they come with some drawbacks. Homologue series detection only works in cases where the unknown PFAS are part of a homologue series. PFAS that do not fit in that definition may be missed out from the identification. Mass defect detection works well for highly fluorinated PFAS, but the mass defect will be counteracted if multiple hydrogens are present in a molecule. Threshold filtering is a way to reduce the number of signals; however, one may overlook small but relevant signals.

BB: After some brainstorming, we realized that we should make use of the typical fragments that originate from the ionization and fragmentation of several PFAS. This overcomes some of the drawbacks of the previously mentioned approaches. We have developed the FIF workflow and this includes five steps. First, we performed AIF and a full scan with the system we used solid-phase extraction (SPE)—in our case a weak anion exchange SPE column—to isolate and concentrate anionic PFAS. This provided us with a 1000-fold concentration factor. In the future, we will expand this further to other isolation modes, such as cationic and mixed-mode isolation.

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used. Second, we assessed the data screening for the typical fragments $C_n^1F_{2n+1}$, $C_n^2F_{2n+1}$, $C_n^3F_{2n+3}$, $C_n^4F_{2n+7}$, $C_n^5F_{2n+11}$ and $C_n^6F_{2n+15}$ O or neutral loss. The third step was to select the corresponding precursor ion in the full-scan data, followed by step four, recording of the product ion mass spectrum of the precursor ion, and finally, perform the structure elucidation.

**Q. What were the main obstacles you had to overcome and how did you overcome them?**

**BB:** We challenged ourselves by searching for PFAS in surface waters that were suspected to contain relatively low levels of PFAS. As a result, we observed many interfering background signals and selection of relevant signals for PFAS was hampered. By applying selective sample preparation to clean and concentrate the sample, we were able to increase the signal intensity. By comparing sample extracts with different dilution factors, we were able to eliminate background signals that originated from the instrument and solvents used. We must also acknowledge that FIF does not guarantee that all PFAS present in a sample will be detected, but we were able to detect and identify several PFAS in the tested samples, of which some have never been reported before.

**Q. What were your main findings?**

**SVL:** With the FIF methodology, we detected 40 structures in Dutch surface water, and we tentatively identified four new structures that had not been reported before. Next to that, we identified fragments of what we believe are the larger molecular analogues of GenX (hexafluoropropylene oxide-dimer acid [HFPO-DA]). These are heavier analogues (hexafluoropropylene oxide-timer acid [HFPO-TrA], hexafluoropropylene oxide-tetramer acid [HFPO-TeA], and so on) that we have not previously detected in the regular targeted analysis. To me, this shows the strength of the FIF identification approach.

**BB:** For some of the PFAS structures, the final step of unambiguous identification by comparing with a reference standard could not be achieved due to the lack of such a reference standard. This problem is faced more generally by nontarget identification methods for pollutants, metabolites, and so on. Ideally, this will be solved by the synthesis of many new reference standards, but that is a very resource-demanding task.

**Q. Are you planning to develop this research further?**

**SVL:** Yes, we want to develop this process further, as there are still some challenges ahead. The process of data handling is quite labour-intensive and could benefit from automation, which will stimulate a broader application. Furthermore, for many of the 4700 PFAS compounds, no reference standards are available, which hampers quantification. This is not a unique situation for PFAS, but broadly for nontarget analysis, and we will investigate how to perform approximate quantification of these compounds where we don't have access to reference standards.

**BB:** We are also aiming to apply FIF to food samples, which introduces an additional challenge as such samples are much more diverse and require more intensive sample cleanup.

**Q. Could FIF be useful for other environmental (or food) applications?**

**SVL:** We demonstrated FIF on surface water, and I'm confident that FIF will also work on other environmental matrices. Our next step is to apply it to foods, particularly those where foods were derived from animals exposed to PFAS, such as fish from Dutch rivers. Moreover, one can think of packaged foods where they are wrapped in food contact materials (FCMs) that have been treated with a wide range of PFAS in the past to make them water- and greaseproof.

**Q. Will FIF become an important technique in PFAS analysis?**

**BB:** PFAS contaminants will stay with us for a long time, and the need for identification tools will therefore remain. With FIF, a valuable identification approach is added to the nontarget identification toolbox, which will also be applicable to other food and environmental contaminant fields.

**References**

**Nitrogen Generator**
The VICI DBS HP Tower Nitrogen Generator produces 24/7 on-demand supply of high-purity nitrogen with flow rates from 500 to 4000 m/min, purity up to 99.999%, less than 0.1 ppm, and THC pressure up to 5 bar. The generator can be placed close to the instrument, which reportedly eliminates the need for long gas lines from external cylinder supplies.

[www.vici-dbs.com](http://www.vici-dbs.com)
VICI AG International, Schenkon, Switzerland.

**TOC Analyzer**
Shimadzu’s TOC-1000e is designed for pure water applications and reportedly has the world’s smallest and lightest cabinet. It provides high sensitivity detection, making it ideal for fields requiring high-purity water applications, such as precision manufacturing, pharmaceuticals, and semiconductors.

[www.shimadzu.eu](http://www.shimadzu.eu)
Shimadzu Europa GmbH, Duisburg, Germany.

**HPLC Columns**
YMC-SEC MAB columns are specially designed for the HPLC analysis of antibodies, their fragments, or aggregates by size-exclusion chromatography. According to the company, they are ideal for the high-resolution analysis of both the high- and low-molecular-weight species. The silica-based YMC-SEC MAB columns ensure excellent lot-to-lot reproducibility and full compatibility with light scattering detection due to their high inertness.

[www.ymc.co.jp](http://www.ymc.co.jp)
YMC Co., LTD., Kyoto, Japan.

**GC Generators**
The Precision Hydrogen Trace generators are designed for GC carrier gas use and detectors requiring hydrogen fuel gas, such as FID and FPD. With three flow rate models (250 cc/min, 500 cc/min, and 1200 cc/min), one generator is capable of supplying multiple GC instruments and can be stacked with nitrogen and zero-air Precision models for a “space-saving” GC gas solution with no need for cylinders.

[www.peakscientific.com](http://www.peakscientific.com)
Peak Scientific, Inchinnan, UK.

**MS Kit**
The Sciencix CTS-21568 PM Kit for Sciex mass spectrometer models 4500/5500/6500 helps maintain optimal performance and comes with a complete nebulizer rebuild kit (including capillary electrode, PEEK tubing, tensions spring, and fittings), heater gas and curtain gas O-rings, air filters, and lint-free cleaning swabs for source and quadrupole.

[www.sciencix.com](http://www.sciencix.com)
Sciencix, Inc., Burnsville, Minnesota, USA.

**Pharmaceutical Reference Standards**
Discover the LGC Mikromol range of more than 5000 API, impurity, and excipient reference standards, each accompanied by a comprehensive CoA detailing characterization, and with a growing portfolio accredited to ISO 17034, according to the company.

LGC, Middlesex, UK.
**Phospholipid Removal**  
The Microlute PLR from Porvair Sciences is a 96-well microplate that provides effective removal of phospholipids and proteins with high levels of reproducibility from plasma and serum samples while maintaining maximum recovery of target analytes. According to the company, Microlute PLR microplates enable you to increase the sensitivity and integrity of your UHPLC and HPLC methods.

[www.microplates.com/microlute-plr](http://www.microplates.com/microlute-plr)  
Porvair Sciences Ltd, Wrexham, UK.

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**Absolute Molar Mass and Size**  
The microDAWN can be coupled to any UHPLC-SEC system to determine absolute molecular weights and sizes of polymers, peptides, or proteins directly, without resorting to column calibration or reference standards. According to the company, the microDAWN produces minimal band broadening to maintain the narrow peaks typical of UHPLC aggregates.

[www.wyatt.com/microDAWN](http://www.wyatt.com/microDAWN)  
Wyatt Technologies, Santa Barbara, USA.

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**SEC Columns**  
PSS MAB, for size-exclusion chromatography (SEC) of monoclonal antibodies, is the latest addition to the PSS column family. Analytical and semi-micro columns covering a wide molar mass range and pre-equilibrated for light scattering detection are available. Bio-inert coated hardware is also optional for separations that are required to be metal-free.

[www.pss-polymer.com](http://www.pss-polymer.com)  
PSS GmbH, Mainz, Germany.

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**CPC System**  
The Verity CPC Process System performs silica-free chromatography for industrial purification. According to the company, this system is an advanced CPC solution for annually processing tons of cannabis samples with high returns for both mainstream and minor cannabinoids. The system is designed to comply with the highest regulatory and safety standards.

[www.gilson.com](http://www.gilson.com)  
Gilson, Wisconsin, USA.

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**GC Autosampler**  
The HT2800T is HTA’s multi-functional autosampler that combines the functions of an autosampler for liquid, static headspace, and SPME in a single unit. Compatible with GC and GC–MS systems of any brand or model. According to the company, HT2800T features cutting-edge technologies that allow usability optimization and excellent analytical performance.

[www.hta-it.com](http://www.hta-it.com)  
HTA s.r.l., Brescia, Italy.

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**Electrochemistry-MS**  
The Roxy Exceed is a new generation potentiostat dedicated to online coupling of electrochemistry with mass spectrometry (MS). The system supports DC, scan, and pulse mode and can be controlled from any LC–MS system. The instrument is suitable for predicting drug metabolism and for MS proteomics.

**Enzyme for High-Throughput Applications**

UCT’s highly concentrated Abalonase Ultra features enzymatic activity levels upwards of 150,000 units/mL. According to the company, this 3× concentrated β-glucuronidase can be used for real-time and room temperature hydrolysis for high-throughput drug-screening applications.


UCT Inc., Bristol, Pennsylvania, USA.

**UHPLC HILIC Columns**

iHILIC-Fusion and iHILIC-Fusion(+) have two lines of 1.8-µm UHPLC HILIC columns with different surface chemistries. According to the company, these columns provide customized and complementary selectivity, ultimate separation efficiency, and ultra-low column bleeding. The columns are particularly suitable for LC-MS-based applications for the analysis of polar compounds.

[www.hilicon.com](http://www.hilicon.com)

Hilicon AB, Tvistevägen, Umeå, Sweden.

**Method Translator**

Pro EZLC method translation software makes it possible to scale down an existing LC method to a smaller column format so that users can speed up run time, increase sample throughput, and reduce solvent use, according to the company. The user can input current column dimensions and method conditions, then specify the dimensions of the new column that they want to try.

[www.restek.com/Pages/Pro-EZLC-Method-Translator](http://www.restek.com/Pages/Pro-EZLC-Method-Translator)

Restek Corporation, Bellefonte, Pennsylvania, USA.

**EAF4 System**

Postnova’s simultaneous electrical and asymmetrical flow field-flow fractionation (EAF4) system is designed to enhance separation and characterization of biopharmaceutical, environmental, and nanomaterials. In an EAF2000 system, electrical and cross-flow fields are applied simultaneously, enabling separations by particle size and particle charge based on electrophoretic mobility to characterize complex proteins, antibodies, and viruses, as well as environmental and charged nanoparticles or polymers.

[www.postnova.com](http://www.postnova.com)

Postnova Analytics GmbH, Landberg, Germany.

**Dynamic Headspace System**

The Dynamic Headspace System (DHS 3.5) holds up to four times more sorbent, resulting in improved recovery, accuracy, and limits of quantitation, according to the company. Standard 3.5” tubes can be used for trapping. The DHS 3.5, Thermal Desorber TD 3.5+, and MultiPurpose Sampler MPS can process 120 samples in one run. The optional DHS large holds 250, 500, and 1000 mL containers.

[www.gerstel.com](http://www.gerstel.com)

Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany.

**Micro-Pillar Array Columns**

The 200 cm µPAC column is the best choice for comprehensive proteomics, while the 50 cm µPAC column is suitable to perform higher throughput analyses with shorter gradient times, according to the company. The µPAC Trapping columns were developed with the same morphology as the analytical columns to meet customer needs for peptide sample enrichment.

[www.pharmafluidics.com](http://www.pharmafluidics.com)

PharmaFluidics, Ghent, Belgium.
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