INVESTIGATING DECOMPOSITION ODOR IN A TROPICAL CLIMATE WITH GC×GC–MS

APPLIED SCIENCES

New LC Columns and Accessories for 2020–2021

APPLICATIONS

Understanding and Achieving Low Limits of Detection in GC

OUR 2020–2021 REVIEW OF NEW SAMPLE PREPARATION PRODUCTS

NEW OPPORTUNITY FOR INDUSTRIAL–ACADEMIC PARTNERSHIPS IN SEPARATION SCIENCE

THE IMPACT OF SAMPLE INJECTION ON LC SYSTEM PERFORMANCE
The coagulation benefits of vitamin K1 (phyloquinone) are well-known, however, other congeners of the vitamin series are not as common (K2, K3, and K4), but equally contribute to a healthy disposition. K1 is abundantly found in leafy greens due to its role in photosynthesis. The absorption of K1 can be enhanced if a fat source like oil or butter is consumed with the greens, owing to the vitamin’s inherent lipophilicity. Vitamin K2, or menaquinone’s (MKs), are identified by their various isoprenoid sidechain lengths (4–13). The MK4 subtype, that is 4 isoprenoid repeating units, can be synthesized through normal gut bacteria, but the other subtypes are primarily produced through non-human gut bacterial modalities. Recently MK4 and MK7 have gained interest due to reports indicating an increase in bone health, dental health, and arterial plasticity as well as a decrease in cardiovascular disease and have been linked to an increase in adipose metabolism. Reports have indicated that an increase in MK7 can lead to better glucose homeostasis and offers a protection from the detrimental turnover of lipid and protein structures of the body. Unlike K1 and K2, which are naturally abundant, K3 and K4 are synthetically produced and have been successfully utilized in the inhibition of tumor growth while increasing the occurrence of apoptosis of such cells in multiple types of cancers including: leukemia, hepatocellular carcinoma, lung, breast, oral, bladder, bone, and prostate cancers. The addition of vitamin C to K3 (menadione) was shown to increase the efficacy of the anti-tumor properties through an increase in oxidative stress in tumor cells leading to cell apoptosis and an arrest of S phase cell division. K4 was similarly shown to have an analogous apoptotic pathway as K3 in its anti-cancer activity in prostate cancer cells. In addition to its use in cancer treatments, K4 has been used in the treatment of vitamin K deficiency bleeding (VKDB) in newborns.

With the growing utility of the K vitamin series of molecules, a need appears relevant for both food labeling of constituents and for research based separation of the natural and synthetic compounds of vitamin K. The fast simple method developed by Hamilton Company using the PRP-C18 5 µm 150 x 4.6 mm column allows for simple efficient identification of the whole vitamin series. The use of the ion-pairing agent, hexylamine acetate (HAA) provided the best separation with the most efficient peak height. HAA offers a good interaction between both the analytes of interest and the lipophilic PRP-C18 resin. To elute the analytes, the stronger chromatographic reversed-phase eluent, tetrahydrofuran (THF) was used. The devised method, though developed with UV detection, is compatible with the standard post column conditions that are typically used with the detection of vitamin K via fluorescence detection.


**Column Information**

<table>
<thead>
<tr>
<th>Packing Material</th>
<th>P/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP-C18 (5 µm)</td>
<td>79676</td>
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**Chromatographic Conditions**

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<tr>
<th>Gradient</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
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<tr>
<td>Injection Volume</td>
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<tr>
<td>Detection</td>
<td>UV at 254 nm</td>
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<tr>
<td>Dimensions</td>
<td>150 x 4.6 mm</td>
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<td>Eluent A</td>
<td>25 mM HAA pH 9.2</td>
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<tr>
<td>Eluent B</td>
<td>THF</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1.0 mL/min</td>
</tr>
</tbody>
</table>

**Absorbance (mV)**

![Graph](image-url)

**Compounds of Interest:**
1: Menadiol Diacetate
2: Menadione
3: Menaquinone-4 (MK4)
4: Phyloquinone
5: Menaquinone-7 (MK7)

**Author:** Adam L. Moore, PhD, Hamilton Company

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- Added utility to study ADCC activity of specific mAb glycoforms or modalities related to its Fc domain
- Analysis of fractions of the TSKgel FcR-IIIa-5PW column correlate with glycosylation and ADCC activity observed by the TSKgel FcR-IIIa-NPR column

Elution profile of Herceptin® biosimilar (left panel) on TSKgel FcR-IIIa-5PW and relative intensities from HILIC-MS analysis of released glycans from FcR fractions (right panel)

Download the application note: Monoclonal Antibody Characterization by Semi-Preparative FcR-Based Affinity Chromatography and HILIC-MS

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236 Investigating Decomposition Odor in a Tropical Climate by Comprehensive Two-Dimensional Gas Chromatography (GC×GC) Coupled with Mass Spectrometry (MS) and Flame Ionization Detection (FID)
Lena M. Dubois, David O. Carter, Julianne M. Byrne, Carlos Gutierrez, Jean-François Focant, and Katelynn A. Perrault
Decomposing animal tissue releases volatile organic compounds (VOCs), of interest in forensic science. We describe the use of GC×GC–qMS/FID retrofitted with a reverse fill/flush (RFF) flow modulator for analyzing these VOCs in a tropical climate.
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Where Has My Efficiency Gone?
Impacts of Extracolumn Peak Broadening on Performance, Part II: Sample Injection

Dispersion (broadening, or spreading) of analyte zones (peaks) outside of chromatography columns can seriously erode the resolution provided by good columns. In this installment, we focus on the contribution of the sample injection step to the total level of extracolumn dispersion in a liquid chromatography (LC) system. This contribution has both a physical component (that is impacted by injector design and configuration and method variables) and a chemical component (that depends on the difference between the mobile phase and the sample solvent compositions).

Dwight R. Stoll and Ken Broeckhoven

In last month’s installment of “LC Troubleshooting,” (1) we reviewed the basic concepts of extracolumn dispersion (ECD), and how the level of ECD associated with a particular instrument can impact the apparent quality of a separation (for example, as measured by resolution). We emphasized the point that smaller (that is, shorter and narrower) columns packed with smaller particles are more sensitive to the effects of ECD than larger columns packed with larger particles. We discussed ECD in an aggregate sense, without getting into details about the level of ECD that different liquid chromatography (LC) system components contribute to the total peak variance observed at the detector ($\sigma_{\text{observed}}^2$). After all, it is the total observed variance that directly affects the resolution of adjacent peaks in a chromatogram.

In this month’s installment, we begin to discuss in detail the contributions to the total observed variance made by specific system components. Figure 1 illustrates the contributions of different system components to the total observed variance, starting with the injection step and ending with the detection step. In principle, the total observed peak variance is simply the sum of the variances contributed by each of the system components. This is only rigorously correct if the dispersion in each element of the system is independent of the others (2), but, under most conditions, equation 1, shown in Figure 1, is accurate enough to guide method development and system optimization (3).

In this installment of “LC Troubleshooting,” we start by focusing on the variance associated with the injection of the sample into the mobile phase stream, and eventually the column. Different Approaches to Injection in Modern LC

A detailed overview of the operational principles of different types of high-performance liquid chromatography (HPLC) autosamplers was given recently by Steiner and associates in this magazine (4). In this article, we are mainly focusing on the extracolumn contributions to total peak variance associated with different injection approaches. Drawings of the two injector designs most commonly used in modern LC instruments are shown in Figure 2.

In the so-called fixed-loop approach (Panels a and b), sample is drawn from a vial by an automated syringe and subsequently transferred to a sample loop, in either a pushed loop mode, as illustrated in Figures 2a and 2b, or pulled through the needle directly into the sample loop (pulled loop design) (4). During the sample loading (“load” position), the desired sample is first drawn into the syringe. The loop—drawn in yellow in Figure 2—is at this point also not in the mobile phase flow path from the pump to the column. After fluidically connecting the syringe to the loop, the desired sample volume is pushed out of the syringe into the loop. Then, the valve is switched to the “inject” position; at this point, the sample that had been loaded into the loop is displaced from the loop into the column where sample components can be separated.

In the so-called flow-through approach shown in Figures 2c and 2d, also known as split-loop design (4), the mobile-phase flow path bypasses the injector needle in the “load” position, allowing the sample to be drawn from a vial into the needle using an automated syringe. Once the desired volume of sample has been drawn into the needle, the valve is switched to the “inject” position. This connects the needle to the mobile-phase flow path, pushing the sample through the needle seat capillary and into the column.
Each of these approaches has advantages and disadvantages compared to the other. One of the primary advantages of the fixed-loop approach is that there is the potential to decrease the variance associated with the injection step ($\sigma_{\text{injection}}^2$) if a small loop is used. A significant downside to this approach is that the injection volume is fixed, as the name suggests. Significantly changing the injection volume requires a physical change of the capillary used as the loop. It is possible to use partial filling of the loop, which does help to extend the practically useful range of injection volumes available with a fixed-loop. Conversely, one of the primary advantages of the flow through approach is that the injection volume can be changed over a wide range of volumes under software control, without changing any instrument components. However, this comes at the cost of increased ECD, since the flow path between the sample and the column includes a needle seat capillary that simply is not required in the fixed-loop approach. Some instrument vendors provide “low-dispersion” kits where this needle seat capillary has a lower inner diameter to reduce ECD, or have changed their instrument design such that the needle is integrated directly onto the injection valve, elimination this capillary entirely.

We note here that in two-dimensional liquid chromatography (2D-LC) separations, the interface that delivers fractions of first dimension (1D) effluent to the second dimension (2D) column for further separation is most similar to the fixed-loop injector shown in Figure 2. In this case, fixed-loops are used, and typically the interface valve has two positions that enable injection of sample from one loop into the 2D column while a second loop is filled with 1D effluent in parallel. The major difference with 2D-LC compared to the diagram in Figure 2 is that the sample loop is filled naturally as mobile phase flows out of the 1D column, rather than filling the loop with a syringe. Typically, the loops used in 2D-LC are much larger (for example, 40 to 80 µL) than those used in fixed-loop injectors for 1D-LC (typically less than 10 µL in UHPLC systems).

### Contributions of Injection Parameters to Peak Dispersion

In the simplest case of a fixed-loop injector with: 1) no seat capillary; and 2) an overfilled loop such that the distribution of sample inside the loop is homogeneous, the introduction of the sample into the mobile phase stream is conceptually close to introduction of a rectangular “sample plug”. For a truly rectangular plug, the contribution of the injection step to the observed peak variance (in volume units) would be given by equation 2:

$$\sigma_{\text{injection}}^2 = \frac{1}{12} \cdot V_{\text{injection}}^2 \quad [2]$$

where $V_{\text{injection}}$ is the volume of the sample loop itself (5). In practice, however, we observe variances that are usually much larger than those predicted by equation 2. A more general form of this expression replaces the factor 1/12 with $1/\theta_{\text{injection}}$ and forms equation 3

$$\sigma_{\text{injection}}^2 = \frac{1}{\theta_{\text{injection}}} \cdot V_{\text{injection}}^2 \quad [3]$$

For fixed-loop injectors operated in the full-loop mode, values of $\theta_{\text{injection}} = 4–8$ are observed in practice. The situation with flow-through injectors is a bit more complicated, due to the addition of the needle seat capillary in the flow path between the sample to be injected and the column. Recent work has shown that two distinct factors contribute to the total variance contributed by the injection process for flow-through injectors—a factor related to the physical volume of the injected sample, as in equation 3, and a factor related to hydrodynamic dispersion of the sample due to parabolic flow in the needle seat capillary (6). These contributions to the total injection variance can be expressed using equation 4:

$$\sigma_{\text{injection}}^2 = \frac{1}{\theta_{\text{injection}}} \cdot V_{\text{injection}}^2 + \sigma_{\text{hydrodynamic}}^2 \quad [4]$$

---

**FIGURE 1:** Equation 1 and illustration of the contributions of several LC system components to the total variance of peaks observed at the detector, $\sigma_{\text{observed}}^2$.

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Comparison of chromatograms obtained from injection of samples in (a) 30:70 acetonitrile:water, or (b) 70:30 acetonitrile:water. Conditions: column, 30 mm x 2.1 mm i.d. C18; injection volume, 40 µL; gradient elution from 50 to 90% acetonitrile from 0–15 s, with water as the aqueous phase; flow rate, 2.5 mL/min. Analytes are alkylphenols from acetophenone to hexanophenone. ACN = acetonitrile. For both samples loading (a and c) or injection (b and d) of samples into a column. Figures 2a and 2b show a fixed-loop injector; Figures 2c and 2d show a flow-through needle injector.

FIGURE 2: Drawings showing the flow paths through different injection systems during loading (a and c) or injection (b and d) of samples into a column. Figures 2a and 2b show a fixed-loop injector; Figures 2c and 2d show a flow-through needle injector.

FIGURE 3: Comparison of chromatograms obtained from injection of samples in (a) 30:70 acetonitrile:water, or (b) 70:30 acetonitrile:water. Conditions: column, 30 mm x 2.1 mm i.d. C18; injection volume, 40 µL; gradient elution from 50 to 90% acetonitrile from 0–15 s, with water as the aqueous phase; flow rate, 2.5 mL/min. Analytes are alkylphenols from acetophenone to hexanophenone. ACN = acetonitrile. For both subfigures, x-axis is Time (min), and y-axis is Absorbance (mAU at 254 nm).

where the $\sigma_{\text{Hydrodynamic}}^2$ term accounts for hydrodynamic dispersion in the needle seat capillary.

Careful measurements of $\sigma_{\text{Hydrodynamic}}^2$ made using a flow-through injector over a range of conditions show that for injection volumes less than about 2 µL, $\theta_{\text{injection}}$ is about 1 (6). In the range 2 < $V_{\text{injection}}$ < 10 µL, $\theta_{\text{injection}}$ increases roughly linearly with increasing $V_{\text{injection}}$, and then appears to plateau at a value of $\theta_{\text{injection}}$ ~ 8 for $V_{\text{injection}}$ > 10 µL.

The hydrodynamic component of the variance is related to the dimensions of the seat capillary, as well as the properties of the mobile phase and the molecular weight analyte through their effects on the diffusion coefficient of the analyte, as shown by equation 5:

$$\sigma_{\text{Hydrodynamic}}^2 \propto \frac{F^4L_{\text{Seat}}^4d_{\text{Seat}}^4}{D_{\alpha}}$$

where $L_{\text{Seat}}$ and $d_{\text{Seat}}$ are the length and diameter of the seat capillary, $F$ is the flow rate, and $D_{\alpha}$ is the diffusion coefficient of the analyte in the sample solvent. This equation shows why it is beneficial to reduce the length and diameter of the needle seat capillary in cases where reducing ECD is desired to avoid robbing a very good column of its inherent efficiency.

Readers interested in learning about these relationships in more detail are referred to recent literature on the topic (2,6).

The relationships in the preceding section provide relatively straightforward means to estimate the contribution of the injection step to the total, observed peak variance. However, up to this point in our discussion, we’ve made two important assumptions that are required for these expressions to accurately reflect what happens in a real system: 1) the separation is isocratic; and 2) the sample solvent is nominally identical to the mobile phase into which it is injected. When gradient elution is used, the situation changes substantially; this is one of the topics we will address in next month’s installment of “LC Troubleshooting.”

Of course, in real applications it is frequently unrealistic, or highly undesirable, to go to the trouble of matching the sample solvent to the mobile-phase composition, so it is important to acknowledge that the contribution of the injection step to the overall ECD of a system may be larger or smaller than that estimated, using equations 3 and 4 above in cases where the sample solvent is very different from the mobile-phase composition. In previous installments of “LC Troubleshooting,” we have addressed the sample solvent issues in some detail, but there has also been quite a bit of research published on this topic over the last few years.

Figure 3 shows an example of how simply inverting the ratio of organic solvent to water in the sample solvent can have a dramatic effect on a separation, making the difference between a chromatogram that looks quite nice and useful, and a chromatogram that is effectively useless (7). This example shows the effect on a reversed-phase separation; however similar effects can occur for most modes of LC separation, including hydrophilic-interaction chromatography (HLIC) (8).

In recent years, several groups, including our own, have worked to develop simulations that can predict both the negative
Solutions for Vaccine Characterization and Virus Research

Analyze key attributes with light scattering

Multi-angle and dynamic light scattering combine with size-exclusion chromatography or field-flow fractionation to provide essential tools for biophysical characterization. The recent focus on COVID-19 highlights the importance of SEC-MALS-DLS and FFF-MALS-DLS for analyzing viruses, virus-like particles, nanoparticle vectors for DNA or mRNA, glycoconjugates and protein antigens.

SEC/FFF-MALS-DLS couple size-based separation with independent determination of molar mass and size by a DAWN® light scattering instrument. Together with UV absorbance, the system quantifies additional key vaccine attributes: aggregation, oligomeric state of glycoproteins, degree of glycosylation, relative capsid content and virus physical titer.

To learn more about Wyatt’s unique solutions for vaccine characterization and development visit wyatt.com/vaccines
and positive effects of a mismatch between the sample solvent and the mobile phase. The published approaches for these simulations range from very detailed models that yield considerable insight into the origins of poor peak shapes like those observed in Figure 3b, to simpler approaches that are not quite as accurate, but are computationally much less intensive and can be calculated using a spreadsheet (9–12). The most recent of these articles by Pepermans and associates provided a very useful rule of thumb to help users anticipate whether or not they will encounter an effect like that shown in Figure 3; this guideline is expressed as equation 6:

\[ V_{\text{injection}} > V_m \ast k \]  

where \( V_m \) is the column dead time, and \( k \) is the retention factor of the analyte in the sample solvent (12). Specifically, equation 6 predicts that when this inequality is true, that distortion of the analyte peak will be so severe that part of the analyte will migrate all of the way through the column in the sample solvent, and breakthrough as a peak detected at the column dead time; most of the rest of the analyte will elute at the expected time based on its retention factor in the mobile phase. For example, for a column with a dead volume of 100 µL, if a sample solvent is used such that the analyte would have a retention factor of 0.5 if the sample solvent were used as the mobile phase, then a breakthrough peak will be observed at the column dead time for injection volumes larger than 50 µL. While equation 6 is very useful as a guide to avoiding problems of this kind, its use does require knowledge of the dependence of analyte retention on solvent composition; this is also true of the more detailed simulations described to date.

If the sample is dissolved in a mobile phase which has a higher elution strength than the initial mobile phase, the effect of this solvent mismatch can be mitigated by drawing mobile phase plugs with lower elution strength before or after the sample plug into the needle. Mixing of these weak solvent plugs with the sample solvent in the precolumn tubing and the column inlet causes the analyte bands to be compressed at the head of the column. This method is called “Performance Optimizing Injection Sequence” (13) and is based on the sandwich injection approach that was introduced to prevent sample precipitation in connecting tubing in polymer analysis (14). This technique was reported to be most useful for compounds in the range of 0.4 < k < 8, with the most benefit realized in the range 0.4 < k < 3 (13). Even if the sample solvent matches the initial mobile phase, this technique can significantly reduce contributions of \( \sigma_{\text{injection}}^2 \) and \( \sigma_{\text{solvent, pre-col}}^2 \) contributions to ECD (3). This technique can readily be applied with modern flow-through injectors using a injection program and an additional sample vial containing the weak solvent (3,13).

Summary
In this installment of “LC Troubleshooting,” we have discussed in some detail the impacts of injector design and configuration, as well as sample composition, on the contribution of the sample-injection step to the overall extracolumn dispersion (ECD) observed in a LC system. Understanding and estimating these effects can help us avoid situations where ECD seriously erodes the inherently high separation efficiency of a good column. In next month’s installment, we will focus on the contributions to ECD from other system components, including connecting tubing and detectors, and discuss differences in the impact of ECD on resolution when using either isocratic or gradient elution.

References

ABOUT THE COLUMN EDITOR
Dwight R. Stoll is the editor of “LC Troubleshooting.” Stoll is a professor and the co-chair of chemistry at Gustavus Adolphus College in St. Peter, Minnesota. His primary research focus is on the development of 2D-LC for both targeted and untargeted analyses. He has authored or coauthored more than 75 peer-reviewed publications and four book chapters in separation science and more than 100 conference presentations. He is also a member of LCGC’s editorial advisory board. Direct correspondence to: LCGCed@mmhgroup.com

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- Instagram: @lcgcmagazine
As in previous years, LCGC sent out a survey in late 2020 and early 2021 asking vendors to supply information on liquid chromatography (LC) columns and accessories launched after Pittcon 2020. Information for this article was obtained over the course of several months, and thus, it is possible that some information has been missed or misinterpreted. The reader is encouraged to check with specific vendor sites for additional products, as well as more detailed information on product usage and attributes.

The vendors that responded to the survey and their new LC products are listed in Table I. The products vary in targeted analyte type, as well as in their mode of chromatographic operation. The columns can be initially categorized as addressing small molecule or large molecule challenges. Within these categories, the products can be further separated based on specific modes of separation employed, including reversed-phase LC, hydrophilic-interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), size-exclusion chromatography (SEC), and ion-exchange chromatography (IEX). Chiral columns are treated as a separate category. In addition to new chromatographic columns, accessories related to LC are also addressed. Trends noted throughout the article are based on comparisons to yearly reports since 2016 (1–5).

In the 2020 LC product review, the number of vendors reporting new products as well as the overall number of products showed a sharp downturn over previous years. Table II provides a breakdown of products introduced in various categories over the previous five years plus this most recent year. The data suggests that the low numbers observed in the 2020 review was an anomaly, and not the beginning of a downward trend. The number of reporting vendors in 2021 is still low relative to some recent years; however, the number of unique column chemistries released is back on par with the previous five years of data. Please note that many products can be categorized in multiple areas, depending on usage. This is meant to be a basic guide that highlights trends only.

Small Molecule
Reversed-Phase/HILIC

The product offerings assigned to the small molecule category intended for reversed-phase and HILIC are listed in Table III. A total of 45 new column chemistries are shown with 43 reversed-phase columns, representing the largest single category in this review. It is clear from the list that superficially porous particles (SPPs) remain a substrate of choice to build phases upon while additional surface chemistries continue to be developed on fully porous particles (FPPs) as well. Continuing trends of recent years, “extended” C18 phases highlight the list where the C18 attributes are enhanced through additional interactions via “polar endcapping,” charged-surface modifications or through co-bonding with other functionalities. Columns specific to HILIC continue to show less activity in comparison to reversed-phase developments.

Advanced Materials Technologies (AMT) released two phases targeting specific classes of compounds. Halo PAH (polycyclic aromatic hydrocarbons), built on SPP, is described as a tri-functional C18 suggested for use in methods such as EPA 610, EPA 8310+2, and EU 15+1. AMT also launched Halo PFAS (perfluoroalkyl substances), a column suggested for EPA 533, EPA 537.1, and EPA 8327. This latter column is an endcapped octadecylsilane (ODS, C18) also bonded to an SPP support. AMT notes that both columns are “application-assured” through method qualified lot analysis. To complement the PFAS column, AMT has also developed the Halo PFAS Delay Column, which is described as a highly retentive alkyl phase. The delay column, placed...
upstream of the sample injector, acts to prevent background PFAS contamination from interfering with the PFAS analytes of interest.

Agilent introduced the AdvanceBio Peptide Plus column. Suggested for the detection of oxidized and deamidated peptides, the column is described as an endcapped “charged” C18 constructed on a SPP surface. The column is noted as being optimized for the separation of target peptides, impurities, and post translational modifications. The AdvanceBio EC-C18 column, another phase build on SPP supports, features polyether ether ketone (PEEK)-lined stainless steel hardware. The company promotes the column for the analysis of post-translational modifications (PTMs) of peptides, without the need for passivation for phosphorylated or methionine-containing peptides. The InfinityLab Poroshell 120 CS-C18, also released by Agilent, represents another charged-surface C18 phase built on SPPs. Described as a “hybrid endcapped C18,” the phase is purported to provide a highly pH-stable column (pH range 2–11). According to the company, the SPP particle modified to have a charged surface, provides better peak shape for basic compounds, formic acid compatibility for MS, reduced operating pressures, and increased speed of analysis compared to fully porous particle (FPP) options.

ChromaNik added new stationary phase chemistries to their SunShell (SPP-based) and their Sunniest (FPP-based) series of columns. SunShell Biphenyl is suggested for polar compounds, isomers, and pesticides. The SunShell C18 is now available in a 3.5 μm particles format, and is noted as a general purpose column. Sunniest Biphenyl, suggested for the same analytes as its SPP partner, provides a FPP-based option.

Fortis Technologies introduced a number of new phases based on SPP supports as well. SpeedCore Aqua is described as a

<table>
<thead>
<tr>
<th>Company</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced Materials Technology</td>
<td>Halo PFAS</td>
</tr>
<tr>
<td></td>
<td>Halo PFAS delay column</td>
</tr>
<tr>
<td></td>
<td>Halo PAH</td>
</tr>
<tr>
<td>Agilent</td>
<td>PEEK Lined PLRP-S column</td>
</tr>
<tr>
<td></td>
<td>AdvanceBio EC-C18 column</td>
</tr>
<tr>
<td></td>
<td>AdvanceBio SEC 1.9 μm column</td>
</tr>
<tr>
<td></td>
<td>AdvanceBio HIC column</td>
</tr>
<tr>
<td></td>
<td>AdvanceBio Peptide Plus column</td>
</tr>
<tr>
<td></td>
<td>InfinityLab Poroshell 120 CS-C18 (charged surface – C18)</td>
</tr>
<tr>
<td></td>
<td>Cannabis and hemp potency kits</td>
</tr>
<tr>
<td>ChromaNik Technologies Inc.</td>
<td>SunShell Biphenyl</td>
</tr>
<tr>
<td></td>
<td>SunShell C18, 3.5 μm</td>
</tr>
<tr>
<td></td>
<td>Sunniest Biphenyl</td>
</tr>
<tr>
<td>ColumnTek</td>
<td>Enantiocel AA (D)</td>
</tr>
<tr>
<td>Fortis Technologies</td>
<td>SpeedCore Aqua</td>
</tr>
<tr>
<td></td>
<td>SpeedCore C8</td>
</tr>
<tr>
<td></td>
<td>Bio Peptide</td>
</tr>
<tr>
<td>Horizon Chromatography Ltd.</td>
<td>Aurashell Amide 18</td>
</tr>
<tr>
<td></td>
<td>Aurashell C18 Ultra</td>
</tr>
<tr>
<td></td>
<td>Aurashell C18/AR</td>
</tr>
<tr>
<td></td>
<td>Horizon Amide 18</td>
</tr>
<tr>
<td>PharmaFluidics</td>
<td>μPAC CapLC</td>
</tr>
<tr>
<td>PSS GmbH</td>
<td>PSS MAB SEC column</td>
</tr>
<tr>
<td>Restek</td>
<td>Raptor Polar X</td>
</tr>
<tr>
<td>Thermo Fisher</td>
<td>Thermo Scientific MAbPac Capillary Reversed-Phase HPLC column</td>
</tr>
<tr>
<td>Tosoh</td>
<td>TSKgel UP-SW Aggregate</td>
</tr>
<tr>
<td>Waters</td>
<td>Acquity Premier columns</td>
</tr>
<tr>
<td></td>
<td>BioResolve SEC mAb guards and columns</td>
</tr>
<tr>
<td>Welch</td>
<td>Boltmate Core-Shell series</td>
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<tr>
<td></td>
<td>Ultisil Series HPLC columns</td>
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<tr>
<td></td>
<td>Xtimate Series HPLC columns</td>
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<tr>
<td>YMC</td>
<td>YMC BioPro HIC HT</td>
</tr>
<tr>
<td></td>
<td>YMC BioPro IEX MiniChrom columns</td>
</tr>
<tr>
<td></td>
<td>YMC Chiral ART Cellulose-SZ</td>
</tr>
<tr>
<td></td>
<td>YMC-Triart Prep Bio200 C8</td>
</tr>
</tbody>
</table>

TABLE I: 2020 LCGC new product survey responding vendors
polar-endcapped stationary phase, and is suggested for the extra retention of polar analytes. To provide additional options for retention of neutral, acidic and basic analytes, the company also introduced SpeedCore C8. SpeedCore BIO Peptide columns, based on a 160 Å SPP, were also developed. C18, C8, and C4 surface chemistries are available for peptide and small molecules.

### Table II: Trending data

<table>
<thead>
<tr>
<th>Year</th>
<th>Companies (Count)</th>
<th>Small Molecule (Count)</th>
<th>Large Molecule (Count)</th>
<th>Total (Count)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reversed-Phase</td>
<td>HILIC</td>
<td>Chiral</td>
<td>Ion-Exchange</td>
</tr>
<tr>
<td>2021</td>
<td>14</td>
<td>43</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2020</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2019</td>
<td>17</td>
<td>25</td>
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<td>2018</td>
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<tr>
<td>2017</td>
<td>27</td>
<td>17</td>
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<td>8</td>
</tr>
<tr>
<td>2016</td>
<td>18</td>
<td>25</td>
<td>18</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table III: Small molecule reversed-phase, ion-exchange (IEX) and hydrophilic interaction liquid chromatography (HILIC) columns

<table>
<thead>
<tr>
<th>Company</th>
<th>Product Name</th>
<th>Stationary Phase(s)</th>
<th>Chromatographic Mode</th>
<th>Particle Size(s) (µm)</th>
<th>Particle Type*</th>
<th>Dimensions (mm)</th>
<th>Comments#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced Materials Technology</td>
<td>Halo PFAS</td>
<td>C18</td>
<td>Reversed-phase</td>
<td>2.7</td>
<td>SPP</td>
<td>50 x 2.1 to 250 x 3.0</td>
<td>Recommended for EPA 533, EPA 537.1, EPA 8327 and emerging PFAS</td>
</tr>
<tr>
<td></td>
<td>Halo PFAS Delay Column</td>
<td>Highly-retentive Alkyl</td>
<td>Reversed-phase</td>
<td>2.7</td>
<td>SPP</td>
<td>50 x 3.0, 50 x 4.6</td>
<td>The delay column, placed upstream of the sample injector, is used to prevent background PFAS contamination</td>
</tr>
<tr>
<td></td>
<td>Halo PAH</td>
<td>Tri-functional C18</td>
<td>Reversed-phase</td>
<td>2.7</td>
<td>SPP</td>
<td>50 x 2.1 to 150 x 4.6</td>
<td>Recommended for EPA 610, EPA 8310, EU 15+1 and emerging PAHs</td>
</tr>
<tr>
<td>Agilent</td>
<td>Advance-Bio Peptide Plus</td>
<td>&quot;Charged&quot; C18</td>
<td>Reversed-phase</td>
<td>2.7</td>
<td>SPP</td>
<td>50 x 2.1 to 4.6 x 150</td>
<td>Optimized for the separation of target peptides, impurities and post translational modifications (PTMs)</td>
</tr>
<tr>
<td></td>
<td>AdvanceBio EC-C18</td>
<td>C18</td>
<td>Reversed-phase</td>
<td>2.7</td>
<td>SPP</td>
<td>50 x 2.1 and 100 x 2.1</td>
<td>PEEK-lined reversed-phase columns for analysis of PTMs</td>
</tr>
<tr>
<td>InfinityLab PoroShell120 CS-C18</td>
<td>&quot;Charged&quot; C18</td>
<td>Reversed-phase</td>
<td>2.7</td>
<td>SPP</td>
<td>50 x 2.1 to 150 x 4.6</td>
<td>Recommended for basic compounds using low ionic strength mobile phases</td>
<td></td>
</tr>
<tr>
<td>ChromaNik Technologies</td>
<td>SunShell Biphenyl</td>
<td>Biphenyl</td>
<td>Reversed-phase</td>
<td>2.6</td>
<td>SPP</td>
<td>30 x 2.1 to 150 x 4.6</td>
<td>Recommended for polar compounds, isomers and pesticides</td>
</tr>
<tr>
<td></td>
<td>Sunniest Biphenyl</td>
<td>Biphenyl</td>
<td>Reversed-phase</td>
<td>5</td>
<td>FPP</td>
<td>50 x 2.0 to 250 x 20</td>
<td>Recommended for polar compounds, isomers and pesticides</td>
</tr>
<tr>
<td></td>
<td>SunShell HILIC-5</td>
<td>Bare silica</td>
<td>HILIC</td>
<td>2.6</td>
<td>SPP</td>
<td>50 x 2.1 to 150 x 2.1</td>
<td>Suggested for polar compounds. Suitable for LC–MS,</td>
</tr>
<tr>
<td></td>
<td>SunShell C18</td>
<td>C18</td>
<td>Reversed-phase</td>
<td>3.5</td>
<td>SPP</td>
<td>50 x 2.1 to 250 x 4.6</td>
<td>General purpose column featuring low backpressure and high theoretical plates</td>
</tr>
</tbody>
</table>

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

Continued on Page 220
protein separations. The company notes that the pore structure has been optimized for peptides and proteins.

Horizon launched a number of new phases in their Aurashell (SPP) and Horizon (FPP) lines. Aurashell Amide 18 is described as an embedded amide group C18 suggested for basic and polar compounds. The company notes improved basic compound peak shapes and enhanced acidic compound retention with this phase. Aurashell C18 Ultra is touted as a “high pH C18.” The company claims improved column durability at elevated pH values. An additional phase in the Aurashell line, Aurashell C18/AR, was also released. This column features a unique C18/Phenyl chemistry that is said to enhance selectivity over conventional C18 phases for compounds containing aromatic functionalities. Horizon Amide 18 represents an embedded amide group C18 built on a FPP support. Similar to its SPP counterpart, this column is noted as improving basic compound peak shapes and enhancing acidic compound retention. The C18/Phenyl chemistry is now also available on a FPP surface with the launch of the Horizon C18/AR phase.

Waters launched a series of columns with the brand name Acquity Premier. Available in C18, phenyl-hexyl and C18-carbamate, the columns promise to provide maximum separation performance for metal-sensitive analytes. These columns utilize what the company refers to as “MaxPeak High Performance Surface” technology, which they claim significantly reduces unwanted interactions between analytes and metal surfaces. Available in 1.7 and 1.8 µm particle sizes with pore sizes ranging from 100 Å to 300 Å, the columns are suggested for oligonucleotides, acidic peptides, TCA cycle metabolites, phosphorylated compounds, and other metal-sensitive compounds.

Welch Materials launched several new series of columns over the past year. The Boltimate series is constructed on 2.7 µm, 90 Å SPP silica and is available with C18, phenyl-hexyl, pentafluorophenyl (PFP), and HILIC surface chemistry modifications. The company claims enhanced speed and resolution from this series of columns. The Ultisil series is based on FPP particles ranging in size from 1.8 µm to 10 µm and a pore size of 120 Å. With a number of different stationary phase options available (C18, C8, Phenyl, C4, C3, C1, cyano [CN], PFP, F-C8, and C30), the columns are suggested for small molecule reversed-phase separations and are noted as durable, workhorse columns. Lastly, the Xtrimte series containing C18, C8, C4, phenyl-hexyl, CN, and Polar-RP surface chemistries, is built on hybrid particles. According to the company website, “hybrid” refers to a unique 5 nm organic/inorganic polymer layer coated on the silica surface. Built on particle sizes ranging from 1.8 µm to 10 µm and with a pore size of 120 Å, the columns are suggested for small molecule reversed-phase separations and for use at extended pH range.

Hydrophilic-Interaction Chromatography (HILIC)
The introduction of HILIC phases over the past several years has waned. Aside
Pharmaceutical Roots: The Origins of Opioids

Part 2 of the Pharmaceutical Roots content series from LGC Mikromol, investigates the natural origins of opioids, and offers a deeper dive into their uses, risks, and mechanisms of action.

Named after Morpheus, the Greek god of dreams, morphine is a type of opiate pain medication. It has played a vital role in the history of chemical neuroscience since its isolation in the 19th century. Morphine belongs to a class of chemical compounds called alkaloids, which are produced by many organisms. What makes morphine particularly interesting is that it is only produced in significant amounts by one plant—*Papaver somniferum*, or the Opium Poppy.

THE HISTORY OF OPIUM

The opium poppy is a member of *Papaveraceae*, a large family comprising over 700 species of flowering plants. The plants vary wildly from flower to flower, but all have in common the production of a milky substance designed to protect the plant from herbivores known as latex. The latex of *Papaveraceae* have a rich phytochemistry and, when dried, the milky-white latex produced by *P. somniferum* turns into a sticky, brown resin known as opium.

Opium can be smoked, eaten, or drunk, and evidence of humans using opium dates back over 5000 years. Despite this long history, it was not until the 16th century that opium was used medicinally in the Western world. Paracelsus, a Swiss-German alchemist, promoted the use of laudanum (from laudere, “to praise”), an extract of opium mixed with alcohol, for many ailments. Many variations of laudanum followed, and the concoctions were available without prescription until the early 20th century.

ISOLATION OF MORPHINE AND OTHER ALKALOIDS

In the early 1800s, the German pharmacist Friedrich Sertürner began experimenting with opium, succeeding in isolating morphine. Morphine accounts for around 10% of the total alkaloid content of opium and was the first alkaloid ever extracted from both opium and plants. Other alkaloids later isolated from opium include papaverine, noscapine, codeine and thebaine. Papaverine was discovered in 1848 by Georg Merck and is used to treat erectile dysfunction. Noscapine was first isolated in 1817 and sometimes used as a cough suppressant. Papaverine and noscapine have very different structures from morphine, codeine, and thebaine, and are neither psychoactive nor addictive.
The French chemist Pierre Jean Robquet discovered codeine in 1832—he was also the isolator of noscapine. Codeine is less addictive and psychoactive than morphine or thebaine and is often used as a cough suppressant, or in conjunction with paracetamol as a painkiller. P. orientalis and P. bracteatum also produce thebaine, an important raw material for many opioids.

**FROM OPIATES TO OPIOIDS**

Opiates are the natural alkaloids obtained from the opium poppy. Unfortunately, many of these are highly addictive. Extensive research into semi-synthetic or synthetic opioids has been carried out, with hopes of finding a less addictive compound. As such, the opium family is a large one with semisynthetic opiate derivatives including oxycodone (from thebaine), hydrocodone (from codeine), and hydromorphone (from morphine) alongside opioid antagonists such as naloxone and naltrexone (both thebaine derivatives). There also exists a large number of synthetic opioids (almost 150) such as pethidine, fentanyl and methadone. Many of these compounds are less addictive but also less effective as analgesics, such as the cough suppressant pholcodine. Others, such as etorphine, are so potent they can be used to tranquilize elephants and walruses.

Perhaps the most infamous opioid is diacetylmorphine. In 1874, while on the hunt for a non-addictive morphine, English chemist Charles Wright boiled morphine and acetic anhydride, yielding diacetylmorphine. Wright carried out no further research on this compound and it was over 20 years later, in 1897, that German researcher Felix Hoffman re-discovered the acetylated substance. Bayer began marketing this new drug, calling it “Heroin” (based on the German “heroisch”, meaning heroic), as a non-addictive morphine-alternative cough suppressant. Of course, it was soon realized that heroin was twice as potent as morphine with extremely high addiction rates, and its sale and production was banned.

**LOOKING FORWARD**

Although total synthesis of morphine has been realised, methods are not efficient enough to meet global demand. Opium poppies are still cultivated around the world, but there is ongoing research into new, potentially more efficient methods of obtaining opiates, by way of biosynthesis.

For over ten years, researchers in Montreal, Canada, have been attempting the genetic modification of yeast to synthesise bioactive molecules. By reconstructing plant pathways mediating synthesis of morphine precursor (S)-reticuline, they have been able to increase the amount of precursor produced and provide a platform for morphine synthesis. In other studies in Japan, researchers have manipulated strains of *Escherichia coli* to synthesise thebaine from increased production of (R)-reticuline. Although they have a long way to go, both groups have the aim of scaling the these platforms up to industrial level. Until then, we will have to rely on opium poppies.
from one HILIC phase released in a series by Welch Materials, the only column launched specifically for HILIC over the past year was Restek’s Raptor Polar X. The proprietary stationary phase, described as a multimodal HILIC/ion-exchange SPP-based column, is suggested for a wide range of polar compounds, including polar pesticides (glyphosate), underivatized amino acids, and ultra-short chain PFAS analysis.

Preparative

Included with the small molecule listing is a preparative offering from YMC Co. Inc., the YMC-Triart Prep Bio200 C8. The column is suggested for the preparative purification of insulin and other peptides. The company notes that the C8 phase was designed for maximized loadability, recovery, and resolution of peptides. The company also claims that the wide usable pH range allows for

<table>
<thead>
<tr>
<th>Company</th>
<th>Product Name</th>
<th>Stationary Phase(s)</th>
<th>Chromatographic Mode</th>
<th>Particle Size(s) (µm)</th>
<th>Particle Type*</th>
<th>Dimensions (mm)</th>
<th>Comments#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fortis</td>
<td>Speed-Core Aqua</td>
<td><em>Polar Endcapped</em></td>
<td>Reversed-phase</td>
<td>2.6</td>
<td>SPP</td>
<td>Not disclosed</td>
<td>Suggested for polar compounds. Suitable for LC–MS.</td>
</tr>
<tr>
<td></td>
<td>Speed-Core Bio Peptide</td>
<td>C18, C8 and C4</td>
<td>Reversed-phase</td>
<td>2.6</td>
<td>SPP</td>
<td>Not disclosed</td>
<td>160 Å pore size suitable for high speed and high sensitivity preparation of larger peptides and proteins.</td>
</tr>
<tr>
<td></td>
<td>Speed-Core C8</td>
<td>C8</td>
<td>Reversed-phase</td>
<td>2.6</td>
<td>SPP</td>
<td>Not disclosed</td>
<td>Retention of neutral, acidic and basic analytes for LC and LC–MS</td>
</tr>
<tr>
<td>Horizon</td>
<td>Aurashell Amide18</td>
<td>Embedded Amide Group C18</td>
<td>Reversed-phase</td>
<td>2.7 and 5</td>
<td>SPP</td>
<td>Multiple lengths in 2.1, 3 and 4.6 mm diameters</td>
<td>Improved basic compounds peak shape and enhanced acidic compound retention</td>
</tr>
<tr>
<td></td>
<td>Aurashell C18 Ultra</td>
<td>C18</td>
<td>Reversed-phase</td>
<td>2.7 and 5</td>
<td>SPP</td>
<td>Multiple lengths in 2.1, 3 and 4.6 mm diameters</td>
<td>Enhanced surface protection improves column durability at elevated pH.</td>
</tr>
<tr>
<td></td>
<td>Aurashell C18/AR</td>
<td>C18 and Phenyl</td>
<td>Reversed-phase</td>
<td>2.7 and 5</td>
<td>SPP</td>
<td>Multiple lengths in 2.1, 3 and 4.6 mm diameters</td>
<td>Enhanced selectivity over C18 phases for compounds containing aromatic functionality</td>
</tr>
<tr>
<td></td>
<td>Horizon Amide 18</td>
<td>Embedded Amide Group C18</td>
<td>Reversed-phase</td>
<td>1.6, 3, 5 and 10</td>
<td>FPP</td>
<td>Multiple lengths in 2.1, 3, 4.6 and 10 mm diameters</td>
<td>Improved basic compound peak shape and enhanced acidic compound retention</td>
</tr>
<tr>
<td></td>
<td>Horizon C18/AR</td>
<td>C18 and Phenyl</td>
<td>Reversed-phase</td>
<td>1.6, 3, 5 and 10</td>
<td>FPP</td>
<td>Multiple lengths in 2.1, 3, 4.6 and 10 mm diameters</td>
<td>Enhanced selectivity over C18 phases for compounds containing aromatic functionality</td>
</tr>
<tr>
<td>Restek</td>
<td>Raptor Polar X</td>
<td>Proprietary</td>
<td>HILIC/ion-exchange</td>
<td>2.7</td>
<td>SPP</td>
<td>30 to 100 x 2.1, 50 x 3.0, 5 x 2.1 guard</td>
<td>Specifically designed for the analysis of a broad range of polar compounds</td>
</tr>
<tr>
<td>Waters</td>
<td>Acquity Premier C18</td>
<td>C18</td>
<td>Reversed-phase</td>
<td>1.7 or 1.8</td>
<td>Hybrid</td>
<td>50 to 150 x 2.1</td>
<td>Suggested for oligonucleotides, acidic peptides, TCA cycle metabolites and other metal sensitive compounds</td>
</tr>
<tr>
<td></td>
<td>Acquity Premier Phenyl-Hexyl</td>
<td>Phenyl-Hexyl</td>
<td>Reversed-phase</td>
<td>1.7 or 1.8</td>
<td>Hybrid</td>
<td>50 to 150 x 2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acquity Premier C18-Carbamate</td>
<td>C18 Carbamate</td>
<td>Reversed-phase</td>
<td>1.7 or 1.8</td>
<td>Hybrid</td>
<td>50 to 150 x 2.1</td>
<td></td>
</tr>
</tbody>
</table>

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

Continued on Page 221
**TABLE III (CONTINUED):** Small molecule reversed-phase, ion-exchange (IEX) and hydrophilic interaction liquid chromatography (HILIC) columns

<table>
<thead>
<tr>
<th>Company</th>
<th>Product Name</th>
<th>Stationary Phase(s)</th>
<th>Chromatographic Mode</th>
<th>Particle Size(s) (µm)</th>
<th>Particle Type*</th>
<th>Dimensions (mm)</th>
<th>Comments#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welch</td>
<td>Boltimate Core-Shell Series</td>
<td>C18, Phenyl-Hexyl, Pentafluorophenyl (PFP) and HILIC</td>
<td>Reversed-phase, HILIC</td>
<td>2.7</td>
<td>SPP</td>
<td>up to 150 in 2.1, 3 and 4.6 ID</td>
<td>Enhanced speed and resolution for small molecule analysis</td>
</tr>
<tr>
<td></td>
<td>Ultisil Series</td>
<td>C18, C8, Phenyl, C4, C3, C1, Cyano (CN), PFP, C30 and F-C8</td>
<td>Reversed-phase</td>
<td>1.8, 3, 5 and 10</td>
<td>FPP</td>
<td>Analytical to Preparative</td>
<td>Durable workhorse columns for small molecule, reversed-phase separations</td>
</tr>
<tr>
<td></td>
<td>Xtrimate Hybrid Series</td>
<td>C18, C8, C4, Phenyl-Hexyl, CN, Polar-RP</td>
<td>Reversed-phase</td>
<td>1.8, 3, 5 and 10</td>
<td>Hybrid</td>
<td>Analytical to Preparative</td>
<td>Durability with various selectivity options for small molecule, reversed-phase separations</td>
</tr>
<tr>
<td>YMC Co., Ltd.</td>
<td>YMC-Triart Prep Bio200 C8</td>
<td>C8</td>
<td>Reversed-phase</td>
<td>10</td>
<td>Hybrid</td>
<td>250 x 4.6</td>
<td>Designed for maximized loadability, recovery and resolution of peptides with a wide usable pH range</td>
</tr>
</tbody>
</table>

* FPP = fully porous (totally porous); SPP = superficially porous
# Comments supplied by vendors
alkaline cleaning-in-place (up to pH 12) to remove adsorbed proteins.

**Chiral Chromatography**

Table IV provides information on the two columns introduced this year intended for chiral separations. Interest in chiral stationary phases, as indicated by the number of new columns launched, has trended downward since a spike in activity in 2018. See Table II. ColumnTek LLC. introduced a new chiral stationary phase (CSP) Enantiocel AA (D) for the enantiomeric resolution of amino acids, hydroxy acids and amino alcohols. The company claims the column is an excellent chiral stationary phase for the direct resolution of α-hydroxy acids, such as DL-lactic acid. It also shows high utility for the chiral separation of free, underivatized amino acids with excellent peak shape. A new D-penicillamine chiral selector that is tightly bound to the packing utilizes ligand-exchange interaction for chiral recognition.

YMC Co. Inc. introduced the YMC Chiral ART Cellulose-SZ. The immobilized cellulose tris(3-chloro-4-methylphenylcarbamate) surface provides separations of chiral compounds, cis-trans isomers, and geometric isomers using a wide range of solvents offering greater flexibility for method development.

**Large Molecule**

New columns introduced since Pittcon 2020 intended for large molecule separations are provided in Table V. As stated earlier, large molecule analysis often requires multiple modes of separation for full characterization. As opposed to large molecule columns reported in 2020 that were all RP-based, this year large molecule columns utilizing RP, SEC, IEX and HIC were released.

**Reversed-Phase**

Two reversed-phase columns targeting large molecules were launched over the past year. Interestingly, both of these columns are based on polystyrene-divinylbenzene (PSDVB) particles. Agilent developed a PEEK-Lined PLRP-S column. The phase is described as being an “inherently hydrophobic based bead free of silanols and heavy metal ions.” High temperature and pH compatible, the column is suggested for oligonucleotide analysis and other large biomolecules. The company notes that the introduction of PLRP-S in PEEK-lined stainless hardware provides a column for large biological molecules free of non-selective biologic and metal interactions.

Thermo Fisher Scientific introduced the Thermo Scientific MAbPac Capillary Reversed-Phase HPLC column. Also based on PSDVB, the company claims the columns yield high sensitivity and high performance characterization of intact proteins for top-down proteomics applications. Additional applications are noted for monoclonal antibodies (mAbs) fragments, antibody drug conjugates (ADCs) and polyethylene glycol-added (PEGylated) proteins. The capillary format of the column is claimed to improve sensitivity, and achieve outstanding data results even with smaller sample volumes.

**Hydrophobic Interaction Chromatography (HIC)**

Hydrophobic interaction chromatography (HIC) is a valuable tool used to separate polar variants of proteins and study drug to antibody ratios (DAR) of ADCs. Two new columns in this category were released in 2020.

Agilent launched the AdvanceBio HIC column and suggests it for the analysis of mAbs, ADCs, and other recombinant proteins. The company notes that the hydrophobic coating provides new levels of hydrophobicity and selectivity to address these particularly challenging molecules.

YMC Co. Inc. introduced the YMC Bio-Pro HIC HT column. The product was designed specifically for DAR analysis of antibody drug conjugates, but also excels at HIC separations of proteins and mAbs. The company claims that the optimized butyl surface chemistry exhibits virtually no carryover while the 2.3 µm base particle offers high mechanical stability, enabling analysis at high flow rates, improving productivity by 2-3 times, while maintaining excellent resolution.

**Size-Exclusion Chromatography (SEC)**

Size-exclusion chromatography (SEC) resolves analytes based on molecular size and is often used as a complementary technique to reversed-phase analyses of proteins. Columns utilized in SEC are often characterized by strict control of pore size and by inert surface chemistry. Control of the pore size dictates how molecules of various sizes can diffuse into the pores while the inertness of the surface limits secondary interactions that may interfere with molecular size separation and determination. Four new SEC columns were introduced over the past year.

Agilent released the AdvanceBio SEC 1.9 µm column intended for pro-
The company notes that hydrophilic bonding prevents second-
ary interactions and protein adsorption. Columns are available in PEEK-lined
SS hardware.

**TABLE V: Liquid chromatography columns for large molecule separations**

<table>
<thead>
<tr>
<th>Company</th>
<th>Product Name</th>
<th>Stationary Phase(s)</th>
<th>Chromatography Mode</th>
<th>Particle Size(s) (µm)</th>
<th>Particle Type*</th>
<th>Dimensions (mm)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent</td>
<td>Advance-Bio HIC</td>
<td>&quot;Hydrophobic coating&quot;</td>
<td>HIC</td>
<td>3.5</td>
<td>FPP</td>
<td>30 x 4.6 and 100 x 4.6</td>
<td>Provides new levels of hydrophobicity and selectivity to address particularly challenging molecules such as monoclonal antibodies (mAbs), antibody drug conjugates (ADCs) and other recombinant proteins</td>
</tr>
<tr>
<td></td>
<td>Advance-Bio SEC 1.9 µm</td>
<td>Not disclosed</td>
<td>SEC</td>
<td>1.9</td>
<td>FPP</td>
<td>30, 150, and 300 x 4.6 in stainless steel hardware, 50 x 2.1 and 150 x 2.1 in PEEK hardware</td>
<td>High resolution and faster separation of aggregates and fragments</td>
</tr>
<tr>
<td>PSS GmbH</td>
<td>PSS MAB SEC</td>
<td>Polystyrene divinylbenzene</td>
<td>Reversed-phase</td>
<td>5</td>
<td>Polymeric</td>
<td>50 and 100 x 2.1</td>
<td>Suggested for large biologic molecules susceptible to non-selective biologic/metal interactions</td>
</tr>
<tr>
<td>Pharma-Fluidics</td>
<td>µPAC CapLC</td>
<td>C18</td>
<td>Reversed-phase</td>
<td>Micropillar Array</td>
<td>SPP</td>
<td>n/a</td>
<td>Suggested for high throughput omics analysis, host cell protein (HCP) monitoring and high sensitivity biopharma assays</td>
</tr>
<tr>
<td>PSS GmbH</td>
<td>PSS MAB SEC</td>
<td>Polystyrene divinylbenzene</td>
<td>Reversed-phase</td>
<td>4</td>
<td>Polymeric</td>
<td>150 x 1.5</td>
<td>Suggested for the characterization of intact proteins in top-down proteomics applications</td>
</tr>
<tr>
<td>Thermo Fisher Scientific</td>
<td>MAbPac Capillary Reversed Phase HPLC Column</td>
<td>Polystyrene divinylbenzene</td>
<td>Reversed-phase</td>
<td>3</td>
<td>FPP</td>
<td>150 x 4.6 and 300 x 4.6</td>
<td>Suggested for the quantitative determination of mAb aggregates and high molecular weight proteins and nucleic acids</td>
</tr>
<tr>
<td>Tosoh Bioscience</td>
<td>TSKgel UP-SW Aggregate</td>
<td>Diol</td>
<td>SEC</td>
<td>3</td>
<td>FPP</td>
<td>30, 150 and 300 x 4.6</td>
<td>Suggested for fast, high-throughput and high resolution separations of proteins and biopharmaceutical drugs such as mAbs and drug-to-antibody (DAR) analysis of ADCs</td>
</tr>
<tr>
<td>Waters</td>
<td>BioResolve SEC mAb</td>
<td>Diol</td>
<td>SEC</td>
<td>2.5</td>
<td>Hybrid</td>
<td>30, 150 and 300 x 4.6, 150 and 300 x 7.8</td>
<td>Suggested for mAb aggregate, monomer and fragment component resolution</td>
</tr>
<tr>
<td>YMC Co., Ltd.</td>
<td>YMC Bio-Pro HIC HT</td>
<td>Butyl</td>
<td>HIC</td>
<td>2.3</td>
<td>Polymeric</td>
<td>100 x 4.6</td>
<td>Suggested for fast, high-throughput and high resolution separations of proteins and biopharmaceutical drugs such as mAbs and drug-to-antibody (DAR) analysis of ADCs</td>
</tr>
<tr>
<td></td>
<td>YMC BioPro IEX MiniChrom</td>
<td>Sulfopropyl (cation-exchange) and quaternary amine (anion-exchange)</td>
<td>IEX</td>
<td>20, 30 and 75</td>
<td>Polymeric</td>
<td>20 and 100 x 8.0 and 100 x 11.3</td>
<td>Screening IEX resins for preparative scale-up applicable to proteins, mAbs, peptides and oligonucleotides purification/polishing</td>
</tr>
</tbody>
</table>

* FPP = fully porous (totally porous); SPP = superficially porous
# Comments supplied by vendors
The company claims application to a wide molar mass separation range and notes the columns are pre-equilibrated for direct use with light-scattering detection. These columns are also available in an optional bio-inert column hardware.

Tosoh introduced the TSKgel UP-SW Aggregate column for quantitative determination of monoclonal antibody aggregates, high molecular weight (MW) proteins and nucleic acids. The company claims the columns are ideally suited for applications such as (clinical) proteomics, metabolomics and biopharmaceutical analyses.

**Ion-Exchange Chromatography (IEX)**

IEX is another important tool for large molecule analysis. YMC Co. Inc. introduced the YMC BioPro IEX MiniChrom column set. The columns contain screening IEX resins for protein, mAb, peptide, and oligonucleotide purification and polishing. The columns, based on a polymethacrylate surface, are available with sulfopropyl (cation exchange) and quaternary amine (anion exchange) functionalization. The company notes high dynamic binding capacity, together with low non-specific adsorption and excellent recovery.

**Microfabricated Separation Devices**

Microfabricated columns are included with the large molecule column listing. Several years ago PharmaFluidics launched their first microchip chromatography device containing what the company terms as “perfectly ordered pillar arrays” that are manufactured using lithographic techniques. Since that introduction, PharmaFluidics has released different effective length nanoflow columns and a trapping column to enable fast sample clean up and enrichment of samples prior to injection on to their analytical columns. This year the company released the µPAC CapLC column that expands the use of ordered pillar array technology into the capillary flow regime. The new product offers flow rate versatility between 1 and 15 µL/min at moderate pressures which enables short gradient separations. The company claims the columns are ideally suited for applications such as (clinical) proteomics, metabolomics and biopharmaceutical analyses.

**Accessories**

Accessories are important products that facilitate and often enable liquid separations. The lone products introduced in the past year that fit this category are Agilent’s Cannabis and Hemp Potency Kits. The kits contain consumables required for potency (cannabinoid) analysis in cannabis flower and hemp. The company notes added convenience from a single part number kit that includes sample preparation products, sample containment, and HPLC columns and supplies for analysis.

**Conclusions**

Following the dramatic drop off of products released relating to liquid chromatography last year, it was refreshing and reinvigorating to report an increase of the numbers to near-normal levels in 2021. The most striking observation from this year is the high activity in small molecule, reversed-phase column development. Similar to past years, the introduction of alkyl phases with extended or alternative selectivity has highlighted new surface chemistry in this category. C18 phases with the incorporation of polar interactions through endcapping alternatives, the addition of co-functionalities such as phenyl and the use of “charged” surfaces have all been noted in this review. Phases continue to be constructed on both fully porous and superficially porous supports. The use of “hybrid” particles, either surface modified via polymer deposition or by incorporation of organic moieties into the silica framework, continues its upward trend. It was also refreshing to report completely new lines of columns from several lesser-known vendors. For large molecules, a return to products covering a number of different modes of chromatography, each providing important information toward the full characterization of these complex molecules is noted. Columns for SEC actually outnumbered those developed for reversed-phase modes in this category. Here again, modern particle technologies, such as SPP, hybrid and polymeric supports, continue to enable new features and increased capabilities for chromatographers to exploit.

**References**


**ABOUT THE AUTHOR**

David S. Bell is a director of Research and Development at Restek. He also serves on the Editorial Advisory Board for LCGC and is the Editor for “Column Watch.” Over the past 20 years, he has worked directly in the chromatography industry, focusing his efforts on the design, development, and application of chromatographic stationary phases to advance gas chromatography, liquid chromatography, and related hyphenated techniques. His main objectives have been to create and promote novel separation technologies and to conduct research on molecular interactions that contribute to retention and selectivity in an array of chromatographic processes. His research results have been presented in symposia worldwide, and have resulted in numerous peer-reviewed journal and trade magazine articles. Direct correspondence to: LCGCedit@mmhgroup.com
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- Intact and subunits
- Intact using HIC
- Aggregates and fragments
- Charge variants
- Peptide mapping
- Glycans
- Protein titer
Since 1870, Sartorius has been on the cutting edge of weighing technology and innovation. In 1970, the company created the world’s first electronic balance, and in 1994 the first monolithic weight cell in 1994. Fast forward to 2009 and the Cubis® II, the first fully configurable balance series, was introduced. And within a few years, a high-capacity micro balance was added with 60 million divisions, which was the highest resolution laboratory balance on the market at the time. Today, Sartorius’ Cubis II platform offers a completely configurable, high-performance portfolio of both laboratory-weighing hardware and software for scientists in R&D and analytical laboratories.

Ensuring Data Integrity and Compliance
The pharmaceutical industry is faced with many challenges, and one of the biggest challenges is data integrity. In fact, 65% of all warning letters issued by the FDA in 2017 were related to data integrity issues—from not documenting batch-related activities and backdating of data to transferring data incorrectly or an insufficient audit trail (1).

To address these issues, Attributable, Legible, Contemporaneous, Original, and Accurate (ALCOA) principles are incorporated into the Cubis II balance, offering a full form of compliance to adhere to all necessary data integrity regulations. With a pharmaceutical package, the Cubis II offers users all of the technical controls necessary to support compliance with common regulations such as FDA’s (21 CFR Part 11) and EU’s (Annex 11). These controls include audit trail, safe data transfer, backups, time synchronization, e-signatures, access control and user management, and alibi memory.

The audit trail is essentially a tamper-protected timestamp electronic log file that allows reconstruction of events related to the creation, modification, or deletion of records. Data can easily be displayed, filtered, recorded, and exported in various ways. Additionally, the system can automatically execute time-controlled actions for backups. Users are able to upload data onto a file share or export the data to other systems. Archiving is also easily done within full compliance.

The system enables the protection of electronic records from any sort of manipulation by saving all files together with a calculated MD5 checksum, which is stored in the audit trail files or in a separate MD5 file. An accurate timestamp is essential for trusting records. The Cubis II balance supports automatic time synchronization via a network time protocol (NTP).

Electronic signatures can be used to sign the final report via the electronic signature feature for all weighing processes. The combination of both the username and a password is fully compliant with (21 CFR Part 11).

Additionally, the Cubis II balance provides two options for complete user management with access control: A local user management that can be configured in accordance with your password policy, or the password rules for (21 CFR Part 11) compliance can easily be implemented by integrating the system into your company’s own domain to allow for a single sign-on. And in this case, the company-defined password rules are implemented automatically.

From Hardware to Software
Beyond compliance, the Cubis II platform has fully customizable hardware, software, and connectivity solutions.

From the weighing module standpoint, there are up to 46 different weighing modules that can best fit user parameters and preferences. Additionally, there are seven different models of draft shields to choose from. Depending on balance type and needs, there are unique features to support a range of balance performance and designs. Users can choose between an advanced and an essential user interface display based on what is being weighed as well as the applications and requirements.

Software benefits include flexibility and modularity, cost effectiveness, and a future-proof nature. There are more than 60 optional software applications that are clustered into four different QApp packages organized according to the applications and functions necessary in the laboratory. Users only need to select the QApps software necessary for their workflows and can pick and choose between different packages to fulfill specific demands. Packages come preinstalled on the balance with the option to add more later, and once the software is purchased and installed, free updates or upgrades are included with every balance in the Cubis II portfolio for the lifetime of the product.

Intelligence in the Laboratory
To put the advantages of the Cubis II balance platform into perspective, here are a few practical examples using the automatic motorized leveling function, gesture control, climate monitoring, status center notification, and stabilization time
to aid the performance of measuring samples and weighing them in a pharmaceutical or medical-device environment.

The automatic motorized leveling function is a convenient feature that avoids the burdensome process of repeatedly having to prepare the balance. This is especially beneficial for applications in which hazardous samples might be employed in a fume hood. The user does not have to worry about leveling the balance because it is done automatically.

Gesture control enables users to automatically have the balance recognize up to four different gestures that can be used and positioned near the balance directly in the flow of motion at their workspace via the gesture sensor. This feature allows users to open and close the automatic draft shield or start the ionizer and other functions, enabling users to work conveniently and efficiently within their workspace. This is especially helpful when working in a hands-free environment where samples are simultaneously manipulated and weighed.

The built-in climate sensor monitors all influencing variables, including temperature, pressure, and humidity. This is critically important when trying to achieve highly accurate results in a pharmaceutical or medical-device setting.

Status center notification allows users to see all balance information and environmental conditions at a glance. Functions such as calibration, leveling, temperature and humidity settings, air pressure, and service functions are all centralized into one dashboard. In the event of a warning alert, a detailed help message is sent, and support is available immediately so that the problem can be identified and corrected.

When a major manufacturer in the stent industry compared results from the Cubis II balance against results from another manufacturer, they noticed they were getting 20% faster performance with the Cubis II balance system because of faster stabilization times. The Cubis II helped improve this medical-device manufacturer’s laboratory testing efficiency, especially when they worked with larger quantities of solutions, standards, and materials.

Conclusion
The Sartorius Cubis II balance platform combines customization and compliance to support pharmaceutical laboratory-weighing applications, allowing users to align with their unique demands—from compliance requirements to maximizing operational efficiencies and experimental outcomes.

Reference

Wallace Harvey
Regional Business Manager
Laboratory Weighing Division
Sartorius

Industry Insights, a paid program
New Sample Prep Products and Accessories

This yearly report on new products introduced in the preceding year, since March 2020, covers sample preparation instrumentation, supplies, and accessories.

Douglas E. Raynie

I recall last year’s PittCon in Chicago. There was talk going around about a novel virus beginning its spread across the United States and the world. I don’t recall anyone wearing masks, yet. Handshakes were prevalent, though often reluctantly, and some offered fist bumps instead. Hand sanitizer was everywhere.

In the next week or two, our world changed.

Presumably due to the pandemic, the number of new sample preparation technologies introduced in the past year appeared to be down compared with previous years. Our annual review of sample preparation products covers the previous year. In late 2020, the LCGC staff submitted a survey to vendors of sample preparation products. Responses to this survey are compiled in this review and were fewer than in the past, as are new product introductions during the past twelve months noted via direct mailings, e-mail, and other marketing means. Additionally, a keyword search (using the terms “sample preparation equip,” “extraction equipment,” “blend/grind/mix/shake/stir,” “evaporators/evaporation,” “filtration and purification,” and “pipets/pipetters”) of the online PittCon 2021 vendor list was run, but attending a virtual conference is not the same as physical attendance.

Most product introductions were aimed at improved performance of existing technologies or aimed at specific applications. Sorbents and accessories for solid-phase extraction led the way, as in previous years.

This review is presented in three sections. First, solid-phase extraction (SPE) sorbents and products are discussed. Next, instrument-based sample preparation technologies are presented. Finally, attention is turned to other sample preparation accessories and supporting technologies. To assist the reader with some of the details behind these new products, each section presents a tabular summary of the associated products. In all cases, the new products we uncovered are presented in the annotated table, while the text highlights particularly worthwhile products.

Solid-Phase Extraction

Agilent Technologies focused on specialized sorbents which they used in a variety of application areas. Their Cannabis and Hemp Potency Kits performs the stated application using a 4-mm, 0.45-μm pore regenerated cellulose syringe filter; sample preparation consumables, and liquid chromatography column for potency analysis in cannabis flowers and hemp products. The Agilent BondElut Lipid uses a size exclusion and hydrophobic interaction sorbent in 1-mL cartridges or 96-well plates for lipid analysis, lipid profiling, or lipidomics. The lipid analysis products are based on their existing BondElut EMR-Lipid media.

Continuing the theme of novel configurations of SPE, Biotage brought their Mikro Solid Phase Extraction Microelution Plates, featuring hydrophobic and mixed-mode sorbents to market. These 96-well microelution plates feature five different media: wettable hydrophobic; wettable hydrophobic plus strong cation exchange; wettable hydrophobic plus strong anion exchange; wettable hydrophobic plus weak cation exchange; and wettable hydrophobic plus weak anion exchange. The combination of the high capacity of the microelution plates and the low elution volume required may lead to an increased concentration of analyte in the elution solvent.

CDS Analytical continued development of their acquisition of the Empore product line. They also feature a 96-well plate with combined reversed-phase and cation exchange modes (Empore 6041 SDB-RPS Standard 96-well plate). The same styrene divinylbenzene–sulfonate sorbents are also found in 6-mL two-layer cartridge format. A final new product is the EZ-Trace, a manual, vacuum-controlled extraction workstation. The independent channel design allows precise flow control to prevent cross-contamination, and the workstation is applicable with a number of U.S. Environmental Protection Agency (EPA) methods.

For several years, DPX Technologies has been at the forefront of the pipette tip approach to SPE. This year, they advanced the field even further with the introduction of Tip-on-Tip (ToT) technology. ToT utilizes a top conductive tip with a bottom filtration tip, and can be employed in filtration, cleanup, and SPE modes. In the filtration mode, called INTip filtration, the sample is aspirated in the upper tip, which is then fitted to the filtration tip, and the sample is dispensed. This mode allows for high-throughput protein precipitation, particulate filtration, or β-glucuronidase removal. Figure 1a demonstrates an example of the protein precipitation mode using acetonitrile to crash blood proteins. The cleanup mode is compatible with a number
of different sorbent chemistries, including a newly released size-exclusion chromatography product. As with the blood protein precipitation example, one strength of the ToT is its advantages with viscous systems. With applications requiring specific sorbent-sample interactions, the SPE filtration tip is used. One advantage claimed for this approach is cost effectiveness relative to alternative methods using magnetic beads. A summary of the SPE mode is shown in Figure 1b.

Two instruments for SPE workflows were announced this year. Thermo Fisher Scientific launched the fully automated AutoTrace 280 PFAS system for the determination of per- and polyfluoroalkyl substances (PFAS) in drinking water by EPA method 537.1. The simultaneous processing of up to six samples takes 2-3 h, but requires only 15 min of operator intervention. The Waters Otto SPEcialist Positive Pressure Manifold is a programmable, semi-automated system for SPE using 96-well plates or larger (1, 3, and 6 mL) cartridges. The pressure profile of each channel is tracked to ensure workflow consistency.

Table I provides a summary of each of these SPE products.

### Automated Sample Preparation Instrumentation

Volatiles analysis, whether headspace sampling or following thermal desorption, was a feature of the sample preparation instruments introduced this year. Agilent Technologies introduced the Agilent 8697 Headspace Sampler, which is claimed to be the first headspace sampler with integrated gas chromatography (GC) communication. Features include a microchannel-based electronic pneumatic control (EPC) module with automatic pressure compensation and valve-based sampling. Sample racks can be exchanged during sample processing. Alternate carrier gases are allowed via an isolated flow path.

The CDS Analytical 7550S system is a stand-alone 72-position thermal desorption autosampler. Sample split, thermal slicing, and programmable tube conditioning are new features of the system.
### TABLE I: Solid-phase extraction products

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Product Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent Technologies</td>
<td>Cannabis and Hemp Potency Kit</td>
<td>Regenerated cellulose syringe filter and various consumables for cannabinoid analysis using 4-mm diameter, 0.45-micron pores. Single product number for entire kit.</td>
</tr>
<tr>
<td>CDS Analytical</td>
<td>BondElut Lipid Extraction</td>
<td>Size exclusion and hydrophobic interaction sorbent available in 40 mg, 1 mL cartridges or 60 mg, 2 mL 96-well plates. Highly selective and efficient extraction of lipids.</td>
</tr>
<tr>
<td>Biotage</td>
<td>Empore EZ-Trace SPE Workstation</td>
<td>Manual, vacuum-controlled SPE workstation with independent channel design for precise control and no cross-contamination. Flow switching allows for separate collection of aqueous and organic wastes.</td>
</tr>
<tr>
<td></td>
<td>Empore 4341HD2 SDB-RPS 10 mm/6 mL 2-layer SPE Cartridge</td>
<td>Mixed-mode styrene divinylbenzene and sulfonate cation exchange cartridge disks. A prefilter prevents particulates and macromolecules from interrupting solvent flow. Disk SPE cartridge format for high flow and high capacity with lower elution volumes.</td>
</tr>
<tr>
<td></td>
<td>Empore 6041 SDB-RPS Standard 96-well Plate, 1.2 mL</td>
<td>Mixed-mode styrene divinylbenzene and sulfonate cation exchange 96-well plates. A prefilter prevents particulates and macromolecules from interrupting solvent flow. A patented collar design prevents cross-contamination. High flows, high capacity, and reduced elution volumes.</td>
</tr>
<tr>
<td>D PX Technologies</td>
<td>INTip Size Exclusion Chromatography</td>
<td>Dry resin gel filtration media within a pipette tip with 5000 Da and 1500 Da molecular mass cutoffs available. Automates group separations without the need for centrifugation or positive pressure manifolds.</td>
</tr>
<tr>
<td></td>
<td>INTip Filtration</td>
<td>Tip-on-Tip technologies combines a lower filtration pipette tip and an upper wide bore conductive tip which aspirates sample solutions, is placed onto the filtration tip, and dispenses the sample in a clean, particulate-free manner. A variety of porosities are available with the filtration tip.</td>
</tr>
<tr>
<td>Thermo Fisher Scientific</td>
<td>AutoTrace 280</td>
<td>Single instrument automates all steps of EPA method 537.1 for determination of per- and polyfluoroalkyl substances in drinking water. Can be configured to accommodate cartridges with 1-, 3-, or 6-mL plungers or 47 mm disks.</td>
</tr>
<tr>
<td>Waters Corporation</td>
<td>Otto SPEcialist Positive Pressure Manifold</td>
<td>Facilitates processing of 96-well plate or cartridge SPE. Use of positive pressure improves robustness, reliability, and reproducibility. Software tracks and documents pressure profiles for consistency between users.</td>
</tr>
</tbody>
</table>

### TABLE II: Automated sample preparation products

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Product Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent Technologies</td>
<td>8697 Headspace Sampler</td>
<td>Headspace autosampler with integrated GC communication. A microchannel-based EPC module with automated pressure compensation and valve-based sampling allows for improved precision. An isolated carrier flow path accommodates alternate carrier gas use and safely vents vials.</td>
</tr>
<tr>
<td>CDS Analytical</td>
<td>75505 Thermal Desorption Autosampler</td>
<td>A stand-alone 72-position thermal desorption autosampler with sample split, thermal slicing, and programmable tube conditioning.</td>
</tr>
<tr>
<td>Fritsch Milling &amp; Sizing</td>
<td>Pulverisette 11 Knife Mill</td>
<td>Homogenization of a variety of sample types with cryogenic option for difficult samples. Sample volumes from 40 to 1400 mL. Dishwasher and autoclave compatible.</td>
</tr>
<tr>
<td></td>
<td>Analysette 22 Next</td>
<td>Green laser light (532 nm) extending measurement range from 0.01 to 3800 μm with wide light scattering angles. Wet dispersion with quick rinse cycles and suspension stability via temperature and pH adjustment.</td>
</tr>
<tr>
<td>Gerstel</td>
<td>Thermal Extraction and Desorption GC–MS</td>
<td>TGA and thermal desorption processes are decoupled from each other. Uses sample sizes up to 100 mg. An IS standard for microplastics is under development.</td>
</tr>
<tr>
<td>Schivo Medical</td>
<td>Nano Litre Dispense Module</td>
<td>Contactless dispensing from single nanoliter up to full syringe volumes in a single dispensing action. Can dispense live cells and a variety of viscosities for MS introduction. Droplets can be dispensed at rates up to 20 Hz.</td>
</tr>
</tbody>
</table>
The Gerstel TED-GC/MS system combines thermal desorption and sample pyrolysis with GC-mass spectrometry (MS). Large sample capacity renders the instrument amenable for the characterization of microplastics by separating thermogravimetric analysis and solid-phase concentration steps. The system was developed in partnership with the Bundesanstalt für Materialforschung und-prüfung (Federal Institute for Materials Research and Testing) in Berlin, Germany.

Fritsch Milling and Sizing continued its development of particle milling and sizing equipment. The Pulverisette 11 is a knife mill for the particle size reduction and homogenization of moist, oily, fatty, dry, soft, brittle, ductile, and fibrous samples from a variety of application areas. Capacity for cryogenic grinding and sample volumes from less than 40 mL to 1400 mL are features of the equipment. Dishwasher- and autoclave-safe sample compartments are other primary benefits of the knife mill. Fritsch also launched the Analysette 22 Next for particle sizing from 0.01 to 3800 μm. A static green laser is a feature of the optical bench and the instrument has minimal moving parts. Measurements can be made in less than 10 s.

The nano Litre Dispense Module (nLDM) is a sample introduction and microarray preparation systems from Schivo Medical. It allows noncontact dispensing from single nanoliter volumes up to full syringe capacities at rates up to 20 Hz. Software control allows precise control of individual droplet sizes. Live cells and solutions of a range of viscosities may be dispensed.

Table II provides a summary of these automated sample preparation instruments.

### TABLE III: Sample preparation accessories and related products

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Product Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC Direct</td>
<td>Syringe Filters</td>
<td>4-, 13-, and 25-mm diameter syringe filters for chromatography.</td>
</tr>
<tr>
<td>Microsolv Technology</td>
<td>Autosampler Vials</td>
<td>Polypropylene vials have no ionic leachables, so they are compatible with ion chromatography and capillary electrophoresis. Reduced adsorption of polar compounds.</td>
</tr>
<tr>
<td>Pall</td>
<td>Syringe Filters</td>
<td>Water-wettable PTFE syringe filters, centrifugation devices, well plates, and membrane disks. The hydrophilic and inert media offer minimal protein binding and low levels of UV-absorbing contaminants.</td>
</tr>
<tr>
<td>AcroPrep 24-well Filter Plates</td>
<td>24-well, 7 mL filter plates with a variety of membrane options designed for protein purification and 0.2 μm sterile filtration.</td>
<td></td>
</tr>
<tr>
<td>Supelco</td>
<td>Smart SPME</td>
<td>Smart chip-based SPME fibers monitors fiber stroke count, usage dates, temperature exposure, phase chemistry, and lot number with robust configuration compatibility with PAL II autosampler.</td>
</tr>
</tbody>
</table>

**Sample Preparation Accessories and Related Products**

The field of sample preparation is so broad, and there are any number of established and emerging technologies identified as sample preparation, that commercial developments and new product introductions are often in seemingly scattered areas. An attempt is made here to unify these varied product offerings.

**Filtration**

HPLC Direct launched a line of syringe filters with 4, 13, and 25 mm diameters.

A syringe filter featuring water-wettable PTFE was developed by Pall. This hydrophilic, chemically inert membrane accommodates aqueous, acidic, basic, and organic solutions for minimal protein binding and low levels of UV-absorbing contaminants. In addition to syringe filters, the material is also used in centrifugal devices, well plates, and membrane devices. Pall also offers Acro-Prep 24-well filter plates. The seven mL filter plates offer a variety of membrane options, including 0.2-μm sterile filtration media.

**Glassware**

Microsolv Technology introduced polypropylene autosampler vials for chromatography. The two mL vials have no ion extractables to allow compatibility with ion chromatography and capillary electrophoresis.

**Solid-Phase Microextraction**

Smart technology has become pervasive throughout modern devices and is now entering the realm of solid-phase microextraction (SPME). Supelco has developed a new smart chip which monitors fiber stroke count, usage dates, temperature exposure, phase chemistry, and lot number for traceability and increased productivity. The Smart SPME format is compatible with the PAL II GC autosampler.

Table III provides a summary of these sample preparation accessories and related products.

**Conclusions and Future Directions**

As the pandemic slowly winds down and the business community opens back up, it will follow that extraction research will continue and commercial development and marketing will follow. A return to in-person conferences and visits with sales representatives will help drive awareness. The specific technologies, however, will be a “wait and see,” depending on the technologies and the ability of vendors to quickly commence development activities.

**ABOUT THE COLUMN EDITOR**

Douglas E. Raynie

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Going Low: Understanding Limit of Detection in Gas Chromatography (GC)

Limit of detection (LOD) is among the most important and misunderstood analytical variables of merit for both instruments and analytical methods. In this installment, we review the history and fundamentals for determining and reporting LOD for analytical instruments and methods. We also discuss the International Union of Pure and Applied Chemistry (IUPAC) and propagation of errors methods used for calculating LOD and explain the limitations of the IUPAC method in modern chromatography. We used simulated data to discuss the implications of the calculation method, and talk about the reported LOD values and our understanding of the sources of experimental uncertainty and variability in LOD determinations.

Nicholas H. Snow

Limit of detection (LOD) is among the most calculated and most misunderstood analytical figures of merit for instruments and methods. We are first exposed to LOD in undergraduate analytical chemistry textbooks. LOD is among the most reported figures of merit in validated methods, literature articles, and advertising literature. The International Union of Pure and Applied Chemistry (IUPAC) defines LOD as “the smallest concentration or absolute amount of analyte that has a signal significantly larger than the signal from a suitable blank (1).” As a result, this statement informs us that LOD can be expressed in concentration or mass units and that the analyte must produce a signal that can be deemed greater than a blank by a statistical test. In practice, a “signal significantly larger” means that the analyte signal should have a value two or three times the blank.

The best way to determine LOD is to measure it directly by preparing standards at appropriately low concentrations and analyzing them on the instrument or running them through the method procedure. Analysts should know that experimental errors involved in the standard preparation and dilution procedure as well as the inherent 33–50% relative variance that is inherent in a measurement where the signal is only two or three times the instrumental noise in the blank will factor into the LOD measurement. This means that LOD values should be reported to one significant digit only. Reporting LOD to more than one significant digit is one of the most common mistakes seen throughout the literature.

LOD is usually calculated rather than measured directly. The classical methods for calculating LOD were developed using spectroscopic and electrochemical instruments in the 1960s and 1970s (2–4). Chromatographers have generally adapted these methods to gas chromatography (GC) and high performance liquid chromatography (HPLC), although there are fundamental differences between spectroscopic and chromatographic instruments.

In 1983, Long and Winefordner provided a review and critical discussion of LOD calculation methods that still provides useful insights nearly 40 years later (5). Their article is still cited as additional reading material for the discussions of LOD found in the instrumental analysis textbooks of today (6,7). More recently, in 2015, Krupcik and others provided a detailed statistical analysis of LOD and limit of quantification (LOQ) calculations in gas chromatography–flame ionization detection (GC-FID) and comprehensive two-dimensional gas chromatography–flame ionization detection (GC×GC-FID) analyses (8). They evaluated methods based on the classical IUPAC method and using signal-to-noise (S/N) ratios and found significant differences between LOD and LOQ calculated by each method in both GC and GC×GC. The results of LOD determinations are highly dependent on both the calculation method used and on how any standards used for the analysis are prepared. At a minimum, for LOD to be comparable between methods and instruments and repeatable between experiments, the calculation and the standard preparation should be reported in much more detail than is typical in the chromatographic literature.
In the remainder of this article, we focus on a critical evaluation of the classical IUPAC definition for LOD, applied to chromatography, because this is the most widely practiced method. In determining LOD, most authors cite the classical IUPAC calculation equation, shown in equation 1, which states

\[ C_L = \frac{ks_B}{m} \]  

where \( C_L \) is the limit of detection, usually expressed in concentration units, \( s_B \) is the standard deviation of the signal generated by multiple blank measurements, or in the case of a chromatographic analysis, the signal for multiple data points recorded along the baseline, \( m \) is the slope of the calibration curve, and \( k \) is usually two or three, depending on the specific LOD definition chosen. This has been a matter of debate over the years. Long and Winefordner recommend \( k = 3 \) as the lowest value that provides a statistically significant difference between the signal and the noise. I agree with this as LOD should be reported conservatively, as we will see below, and \( k = 3 \) provides over 90% confidence that the signal is not noise. If performing LOD determinations for method validation in a regulated environment, the definition recommended by the regulating authority should be used.

Before going into more details of LOD calculations, it is educational to look at data showing very low signals and instrumental noise. Figure 1 shows four plots, each with very low signal. Figure 1a shows 100 data points of simulated random instrumental noise, generated by the random number generator function in Microsoft Excel. This could be interpreted as 5 s of the noise generated by a flame ionization detector, with no analyte passing through the detector, operating at a sampling rate of 20 Hz. Every 20 values on the x-axis represents 1 s of data collection. The y-axis data are random numbers between 0–10.

![Simulated chromatograms showing noisy baselines](image)

**FIGURE 1:** Simulated chromatograms showing noisy baselines: (a) baseline only, (b) baseline with a peak representing \( k = 2 \), (c) baseline with a peak representing \( k = 3 \), and (d) baseline with a peak representing \( k = 10 \).

**TABLE I:** X- and Y-data and statistical calculations for a calibration curve as shown in Figure 2, based on the simulated noise data shown in Figure 1.

<table>
<thead>
<tr>
<th>X-data</th>
<th>Y-data</th>
<th>Y-data with noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>55</td>
<td>56.65</td>
</tr>
<tr>
<td>10</td>
<td>105</td>
<td>105</td>
</tr>
<tr>
<td>20</td>
<td>205</td>
<td>202.95</td>
</tr>
<tr>
<td>40</td>
<td>405</td>
<td>388.8</td>
</tr>
<tr>
<td>80</td>
<td>805</td>
<td>788.9</td>
</tr>
<tr>
<td>120</td>
<td>1205</td>
<td>1192.95</td>
</tr>
<tr>
<td>160</td>
<td>1605</td>
<td>1637.1</td>
</tr>
</tbody>
</table>

Slope (\( m \)) 10.12

\( S_m \) 0.114

Intercept (\( i \)) -4.06

\( S_i \) 9.47

\( R^2 \) 0.999
As seen in Figure 1a, the noise is random. The baseline would be expressed as a signal of 5, the midpoint of the noise, which ranges from 0 to 10. Figure 1b shows a small peak that is 1 s (20 data points) wide with the peak height representing $k = 2$, a common usage in LOD calculations. Note that the peak does not appear symmetrical, as the random noise still comprises much of the signal. This is typical of actual experimental data for analyses performed on samples at or near the LOD. Both peak area and peak height determinations for this peak would have large amounts of experimental uncertainty. This leads to the important point made earlier that LOD should never be reported to more than one significant digit. Any additional claimed precision in an LOD determination is meaningless.

Figure 1c shows a 1 s wide peak representing $k = 3$ in equation 1, the recommended value. The peak, while still noisy, looks more like a traditional chromatographic peak. The peak height and peak area are still subject to experimental uncertainty approaching (1/3) or 33%, still making this a one significant digit estimate. One result of this is that the peak height or peak area for small signals, in the same order of magnitude of the noise, should be reported with one or two significant digits, no matter how many digits the data system provides in the peak table. Furthermore, the retention time, reported as the peak maximum, may also be more variable than implied by the number of digits provided by the data system.

Figure 1d shows a 1 s wide peak representing $k = 10$ in equation 1, a typical value for LOQ that is often determined when validating a method or when determining analytical figures of merit. This looks more like a useful chromatographic peak and the peak height and area are now subject to experimental uncertainties around 10%, making this a two-significant digit determination in many cases. However, the peak is still subject to significant impact from the random instrumental noise. LOQ is typically stated as the lowest concentration or mass for which quantitation would be effective and $k = 10$ is often used with equation 1 to determine this value.

Figure 2 is a calibration plot showing sample data, typical of detection limit calculations, and Table I shows the x- and y-data and the statistics for the regression line. The calculations were performed using Microsoft Excel and the statistical calculations were performed using the LINEST function. The y-intercept of five for the line was chosen from the average baseline values shown in Figure 1 and is illustrative of an instrument with a non-zero baseline. The slope of 10 was chosen as an illustration and provides data over a range of approximately 50–2000 of our arbitrary units, or about 1.5 orders of magnitude, typical of many calibration curves, and with the lowest point near the LOD. The random number generator function in Microsoft Excel was used to superimpose noise of +/-5% on each of the y-data points. The blue dotted line represents the original noise-free y-values, and the gray dots represent the same values with the random noise added. Note that they do not perfectly overlap. We all know that on a calibration curve, the best fit line and the data points do not always match up, yet by using Equation 1 to calculate LOD, we assume that they do.

In Table I, the x-data points are shown, along with the y-data without noise and the y-data with noise. Below these are the statistical results for the slope and intercept, their standard errors and the $R^2$ value, based on the noisy data.

As seen in equation 1, the IUPAC equation is dependent on the amplitude of the baseline noise, which can be measured directly from the baseline of the chromatogram, preferably in a region near the peak of interest and the slope of the calibration curve. For this example, Figure 1a was used as the baseline noise. Note that equa-

**TABLE II**: Results of five propagation of errors method determinations of LOD using equation 2 with randomized (±5%) noise on the calibration curve.

<table>
<thead>
<tr>
<th>Determination</th>
<th>$S_b$</th>
<th>$S_m$</th>
<th>$i$</th>
<th>$m$</th>
<th>$S_m$</th>
<th>$C_i$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>5</td>
<td>-10</td>
<td>10.4</td>
<td>0.079</td>
<td>3</td>
<td>0.999</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>5</td>
<td>6.6</td>
<td>10.2</td>
<td>0.080</td>
<td>3</td>
<td>0.999</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>5</td>
<td>3.9</td>
<td>9.80</td>
<td>0.047</td>
<td>2</td>
<td>0.999</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>5</td>
<td>11.2</td>
<td>-0.54</td>
<td>0.135</td>
<td>4</td>
<td>0.999</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4.9</td>
<td>-1.5</td>
<td>10.3</td>
<td>0.058</td>
<td>2</td>
</tr>
</tbody>
</table>

**FIGURE 2**: Calibration plot showing (blue dots) ideal data, and (gray dots) data with ±5% noise superimposed on the Y-values.

**Calibration Curves**

**TABLE II**: Results of five propagation of errors method determinations of LOD using equation 2 with randomized (±5%) noise on the calibration curve.
tion 1 does not provide any specification on the region of the calibration curve that is used or its relationship to the ultimately determined LOD. More importantly, there is no accounting for experimental uncertainty in the slope of the calibration curve. The LOD determination is only as good as the instrument and the standards used to measure it.

An alternate approach, discussed by Long and Winefordner and based on similar principles to the IUPAC equation method that employs propagation of errors accounting for experimental uncertainty in the calibration curve or standard preparation, is described by equation 2:

$$C_L = \frac{1}{k} \left( s_y^2 + s_x^2 + (\bar{x})^2 s_y^2 \right)$$

where the propagation of errors method includes terms for experimental uncertainty in both the slope ($s_m$) and y-intercept ($s$) of the calibration curve. The need to include slope uncertainty is clear; this cannot be assumed to be zero. Intercept uncertainty accounts for a non-zero baseline, as does the presence of a term for the amplitude of the baseline itself ($s_B$). In short, all experimental values that impact LOD determination are included. Equation 2 reduces to the IUPAC method (equation 1) if the data are background corrected, providing a baseline of zero and the uncertainties on the slope and intercept values determined by linear regression. Those calculations are difficult, time-consuming, and prone to mistakes, if performed by hand. Further, analog instrumentation was often much noisier than today’s digital systems, making instrumental noise more relevant than today’s digital systems, making instrumental noise sources more common than the experimental uncertainty in standard preparation using volumetric glassware.

Thinking back to the context in which the classical IUPAC equation was developed, it is important to consider that hand calculators did not exist and calculators or software that would easily perform any kind of linear regression would not calculate uncertainties on the slope and intercept values determined by linear regression. Those calculations are difficult, time-consuming, and prone to mistakes, if performed by hand. Further, analog instrumentation was often much noisier than today’s digital systems, making instrumental noise sources more common than the experimental uncertainty in standard preparation using volumetric glassware. Today, the reverse situation is much more likely. Instrumental noise is much lower, especially when multidimensional detectors such as tandem mass spectrometry (MS/MS) are used, making uncertainty in the standard preparation much more important in the LOD calculation.

There is much more to determining a LOD than simply looking up the IUPAC equation or other formula required by a regulatory body, whether that is examining the noise on a chromatogram, making a calibration curve, determining the slope, or calculating a value. We see that the LOD is dependent on several variables, including the instrumental noise and sensitivity, the uncertainty in the sensitivity and the calculated y-intercept of the calibration curve. At the very least, for LOD values to be comparable between instruments and methods, the calculation method should be clearly stated and sample calculations, including the uncertainties in the blank and calibration curve, should be provided in method reports or publications.

**References**


Investigating Decomposition Odor in a Tropical Climate by Comprehensive Two-Dimensional Gas Chromatography (GC×GC) Coupled with Mass Spectrometry (MS) and Flame Ionization Detection (FID)

During decomposition of mammalian remains, volatile organic compounds (VOCs) are released into the environment. Comprehensive two-dimensional gas chromatography (GC×GC) assists in analyzing these VOCs with its increased separation power and sensitivity. This study analyzes the temporal changes in decomposition VOCs from human analogs in a tropical climate. For this specific application, the use of a gas chromatograph coupled with a quadrupole mass spectrometer (qMS) and simultaneous flame ionization detector (FID) retrofitted with a reverse fill/flush (RFF) flow modulator is reported for the first time. The method was used to examine postmortem processes to monitor chemical changes from pig carcasses. Approximately 30 compounds were tentatively identified from the decomposing remains, representing consistent trends in the decomposition VOC profile at a new location. As interest increases worldwide in collecting VOC samples as evidence in forensic casework, this type of instrumentation could become a valuable tool in crime laboratories.

Lena M. Dubois, David O. Carter, Julianne M. Byrne, Carlos Gutierrez, Jean-François Focant, and Katelynn A. Perrault

The decomposition process of human and animal remains involves major changes and initiates the production of many by-products. Some of these by-products are organic in nature and originate from biochemical reactions that break down macromolecules within biological tissue. Because of enzymatic reactions and microbial processes, the molecules gradually break down, resulting in a large number of volatile organic compounds (VOCs). In forensic science, this collection of VOCs is referred to as decomposition odor, and can be used in forensic tracing for various purposes. For instance, decomposition odors can be detected to support the presence of a body at a particular location (1). It has been hypothesized that their presence can also be correlated to the length of time since death when estimating the postmortem interval (PMI) (2). This type of information is important in establishing a series of events in the circumstances surrounding a death of a human or animal, which can be critical information in a forensic investigation. Furthermore, the improved understanding of the formation and evolution of decomposition odor is of high interest to handlers of cadaver-detection dogs to improve training practices in search and rescue operations (3).

From a chemical point of view, decomposition odor represents a complex mixture of gaseous molecules and its complete characterization requires a powerful analytical technique. In the early stages of decomposition odor research, one-dimensional gas chromatography coupled to mass spectrometry (1D GC–MS) was most often used (4,5). However, the limited separation power was challenged by the complexity of these samples and complete resolution of the compounds was often not achieved, which can lead to significant challenges in understanding the compositions of decomposition odor, the trends in compound abundance over the duration of the decomposition process, and implications of decomposition odor production given different external variables. Ultimately, using 1D GC–MS is problematic because it hinders the ability of this work to reach critical areas of need that rely on understanding decomposition odor, such as helping to locate missing persons or homicide victims.

In recent years, a general shift from 1D-GC to comprehensive two-dimensional gas chromatography (GC×GC) has been observed in this field (6–8). The general shift to comprehensive two-dimensional gas chromatography (GC×GC) happened because of the superior peak capacity, sensitivity, and selectivity of GC×GC (9). In addition, GC×GC instrumentation allows more effective management of the large dynamic range encountered in decomposition VOC studies (9). This development has been followed with more accurate concentration measurements and higher confidence in decomposition VOC identification. Currently, decomposition odor is largely studied using GC×GC in research laboratories, which may be partly because of the lengthy and challenging process of adapting a novel analytical technique to the needs of a modern forensic science laboratory. In addition, cost and time investment for method validation through laboratory accreditation, as well as acquisition and maintenance costs, might impede its implementation. However, recent technical advances led to commercial development of new GC×GC equipment configurations with some of them having the potential to be a tool in forensic laboratories (8). In this study, a GC×GC instrument with a quadrupole mass spec-
trometer (qMS) and simultaneous flame ionization detector (FID) with a reverse fill/flush (RFF) flow modulator was employed to analyze VOCs emitted by decomposing human analogs.

In the past, the decomposition VOC profile has been studied on different continents, including locations in Europe, North America, and Australia. However, most studies are performed in humid subtropical or humid continental climates. Table I lists location and climate zones according to the Köppen-Geiger classification (10) for all longitudinal decomposition studies, with more than one replicate investigating the VOC profile from human or animal remains. In spite of some local climatic differences, some general trends could be established between these studies (11,12). However, the local environment heavily impacts decomposition outcomes; therefore, it is important to understand decomposition odor evolution in a variety of climate zones. The purpose of this study was to perform the first decomposition odor study in a tropical region using comprehensive two-dimensional gas chromatography-single quadrupole mass spectrometry and a flame ionization detector (GC×GC–qMS/FID).

Specifically, the tropical savanna climate experienced in Honolulu, Hawaii, was chosen. Honolulu is a contrasting location to areas that have been previously studied because of its year-round, consistently warm temperatures and its minimal temperature difference throughout the day. The tropical savanna is the second-most common climate type in the world (following hot desert), and these regions experience many climate events, such as tropical storms, and these types of climate events contribute to a need for forensic search and recovery. Therefore, it is very important to improve our understanding of decomposition odor in these regions. This study reports on a GC×GC–qMS/FID-based method that was applied to examine the postmortem decomposition process to monitor chemical changes of surface deposited pig carcasses on a decomposition facility in a tropical climate.

Materials and Methods

Decomposition Trial

This research was conducted in April 2019. The trial was performed using three swine (Sus scrofa domesticus) carcasses, each weighing approximately 30 kg. The pigs were part of the same breeding program; therefore, they were sufficiently similar in terms of body composition to represent biological replicates. The carcasses were purchased from a licensed abattoir postmortem. Therefore, an animal ethics approval was not required because the experimental subjects were not killed specifically for the purposes of research. The carcasses were transported to the field site within an hour of death. They were placed in an outdoor decomposition site located in Palolo Valley, Honolulu, Oahu, Hawaii, previously described by Chun and others (28). This particular site constantly experiences a tropical savanna climate (10). The vegetation at the moderately steep site is representative of a tropical savanna ecosystem on Oahu; it is rocky and dominated by guinea grass (Megathyrsus maximus) with night blooming cereus (Hylocereus undatus), aloe (Aloe spp.), and carrion plants (Stapelia gigantea). Figure 1 shows an aerial view of the outdoor decomposition site. Three control sites were designated, containing the same soil and vegetation but no decomposing remains.

<table>
<thead>
<tr>
<th>Köppen-Geiger Class</th>
<th>Description</th>
<th>Locations Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Tropical rainforest</td>
<td>Honolulu, HI, USA (Aw) (Current Study)</td>
</tr>
<tr>
<td>B</td>
<td>Desert</td>
<td>San Marcos, TX, USA (Cwa) (13,14)</td>
</tr>
<tr>
<td>C</td>
<td>Humid subtropical</td>
<td>Knoxville, TN, USA (Cwa) (15,16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sydney, AUS (Cfb) (9,17–20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Belgium (Cfb) (21–23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Athens, GR (Csa) (24,25)</td>
</tr>
<tr>
<td>D</td>
<td>Humid continental</td>
<td>Klein Schneen, DE (Dfb) (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oshawa, ON, CA (Dfb) (26,27)</td>
</tr>
<tr>
<td>E</td>
<td>Tundra</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Aw: Tropical wet and dry climate; Cwa: Monsoon-influenced humid subtropical climate; Cfb: Temperate oceanic climate; Csa: Hot-summer Mediterranean climate; and Dfb: Warm-summer humid continental climate

VOC Sampling of Pig Carcasses

A stainless steel hood (dimensions, 122 x 91 x 56 cm; volume, 622 L) was placed over the pig carcasses. After an equilibration time of 15 min, VOCs in the air above each of the three pig carcasses were col-

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**TABLE I:** Köppen-Geiger class, description of the climate zone, and location of longitudinal decomposition odor studies performed on human or animal remains with more than one replicate.
lected by active sampling. For this purpose, a constant flow air pump was attached to the sampling port of the hood. VOCs were collected onto sorbent tubes containing Tenax TA and Carbograph 5TD. A flow rate of 100 mL/min was used for 10 min, pumping a total volume of 1 L of headspace gases through the sorbent tubes. Starting from the day of the deposition (Day 0, notated as D0), samples were taken every second day for two weeks (D0, D2, D4, D6, D8, D10, D12, and D14).

The same experimental design was used to collect samples from the headspace above three control sites on each sampling day. Control sites were located in a similar region to the experimental samples, with representative soil and vegetation, but did not contain decomposing remains. Control sites were located upslope of the carcass samples to prevent any contamination from groundwater, and were also located away from areas where carrion plants occasionally flowered. Carrion plants did not flower during the course of this experiment, but sample collection occurred in an isolated manner with the stainless steel hood to prevent this source of potential impact. All sample collection tubes were capped with long-term storage caps and stored in an air-tight, sealed glass jar for transport to and from the field site. Field blank samples were collected on each sampling day by opening a tube at the site for 10 s and recapping. The field blank was stored alongside the samples and represented any compounds that would be present from the sampling protocol that are not attributed to the site itself. Further details of this sampling procedure and the necessity for implementing these quality assurance measures are detailed in the literature (19).

All sample tubes were injected with a deuterated internal standard (1 µL of 100 ng/µL chlorobenzene-d₅, Sigma Aldrich) in high performance liquid chromatography (HPLC)-grade methanol (J.T. Baker). A C₇-C₃₀ saturated alkane standard (Sigma Aldrich) was injected using 1 µL of 100 ng/µL of hexane (Sigma-Aldrich) prior to the first sample injection and immediately following the last sample injection to enable the calculation of linear retention indices.

**GC×GC–qMS/FID Analysis**

Sample analysis was carried out using a Unity-xr thermal desorber (Markes International Ltd) and a Thermo Scientific Trace 1300 gas chromatograph and flame ionization detector with an ISQ 7000 single quadrupole with an insight flow modulator (SepSep Analytical Ltd). The qMS data were used to tentatively identify compounds comparing the mass spectra to the National Institute of Standards and Technology (NIST) library and the FID data for quantification (8,29).

Each sample underwent a two-step desorption: Primary desorption of the sample took place with a trap flow of 50 mL/min was used for 10 min, pumping a total volume of 1 L of headspace gases through the sorbent tubes. Starting from the day of the deposition (Day 0, notated as D0), samples were taken every second day for two weeks (D0, D2, D4, D6, D8, D10, D12, and D14).

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Each sample underwent a two-step desorption: Primary desorption of the sample took place with a trap flow of 50 mL/min and split flow of 20 mL/min at 300 °C for 5 min following a 1-min nitrogen dry purge. The sample was re-condensed at −10 °C on a general purpose carbon cold trap (Markes International Ltd). The cold trap was then rapidly heated for secondary desorption at 320 °C for 3 min following another 1-min nitrogen dry purge.

An Rxi-624Sil MS column (30 m × 0.25 mm i.d. × 1.4 µm film thickness) and a Stabilwax (5 m × 0.25 mm i.d. × 0.25 µm film thickness) were used in the first and second dimension, respectively (Restek Corporation). The flow rate in the first dimension column was 1 mL/min, the auxiliary gas flow was 20.14 mL/min, and the flow rate in the bleed line (5 m × 0.1 mm i.d.) was 1.03 mL/min. The loop dimensions were 0.53 mm i.d. × 1133 mm, resulting in a loop volume of 25 µL. The modulation period was 2.5 s and the flush...
time was 100 ms. The flow rate in the second dimension column was 17.9 mL/min. The flow was split with a ratio of 4.5:1 between the FID and mass spectrometer.

The GC oven started at an initial temperature of 65 °C and was increased to a final temperature of 250 °C at a rate of 3 °C/min and held for 5 min. The transfer line and the ion source temperature were held at 280 °C. The qMS was operated in full electron ionization (EI) scan mode with a mass range of 40–300 m/z operating with a rate of ~41.5 scan/s. The FID was operated with 350 mL/min ultra-zero grade air (Airgas), 4 mL/min ultra high purity nitrogen as makeup gas (Airgas), and 35 mL/min ultra high purity hydrogen (Airgas). The temperature of the FID was set at 250 °C. The acquisition rate of the FID was 120 Hz. Instrument control was performed using Chromleon 7 version 7.2.9 (Thermo Scientific).

**Data Processing**

Data acquisition was performed for both data sets using the Thermo Scientific Chromeleon CDS software. GC–qMS data were processed with the same software. GC×GC–qMS .raw files were exported, converted to the .cdf format, and imported into the ChromSpace software (SepSolve Analytical Ltd) for processing. GC×GC-FID files were exported as .cdf files and imported into the ChromSpace software (SepSolve Analytical Ltd) for processing.

**GC×GC–qMS**

Dynamic baseline correction was performed on imported .cdf files with a peak width of 0.4 s. Stencils for the peaks of interest were obtained by applying the curve-fitting algorithm for peak integration with a 3-point Gaussian smoothing function. Stencils are defined regions on the contour plot where peaks are searched for, similar to retention windows in 1D-GC data. The minimum peak area was 5000, the minimum peak height was 50000, and the minimum peak width was 0.001. Parameters for peak merging included a tolerance of 2%, an overlap of 2%, an intensity of 2%, and a correlation of 0.5. Subpeak apex windows for fronting and tailing were set to 2% for both low and high peak maxima. Peak response for qMS data is recorded in counts.

**GC×GC–FID**

Top-hat baseline correction was used on imported .cdf files using a peak width of 0.4 s. Stencils obtained from GC×GC–qMS data processing method were transferred to FID files and the stencil was transformed manually to align over FID peaks. Peak detection was performed using the local regions of interest produced by these stencils with a minimum peak area of 1, a minimum peak height of 0, and a minimum peak width of 0. FID peak areas, normalized using the area of the internal standard, were used to examine temporal trends between samples. Peak response for FID data is recorded in pA.

**Results and Discussion**

**Chemical Profile**

Figure 2 displays two chromatograms as examples of a typical VOC profile of the fresh stage (A) and active decay stage (B) of decomposition. For each sampling day, the VOC profile was characterized and compounds were assigned to one of the major chemical classes (acids, sulfur-containing compounds, ester, nitrogen-containing compounds, heterocyclic, alcohol, ketone, aldehyde, and other). Figure 2 demonstrates the complexity of the initial fresh profile, which largely contributes to the environmental organic matter in the soil and vegetation surrounding the remains, with some contributions made by the skin microbiota. Figure 3 displays the temporal changes between day 0 and day 14 of the trial. Figure 4 provides a more detailed view of acids, and of sulfur- and nitrogen-containing compounds. The chemical classes reported in this study have been previously reported as part of the decomposition profile (30). However, organic matter is present in all soil and therefore various compound classes show elevated levels also at control sites. Because of the fact that alkanes and aromatics were also present at high levels in the control samples and followed a similar trend, they were not included in these figures. Temporal trends appeared to exist for acids, sulfur-containing compounds, ester, and nitrogen-containing compounds. In Canada, Stadler and others analyzed VOCs from surface-deposited pig carcasses and found an increase of sulfur-containing compounds during the early stage of decomposition (26,27). In Greece, Agapiou and others reported a peak of sulfur-containing compounds on the first day of their trial with buried pig carcasses (24). Forbes and Perrault in Australia also monitored a higher presence of various sulfur-compounds; however, no values for their relative abundance are provided (17). The increase of acids in this study around day 6–8 (see Figure 4) is in accordance with the findings of Stadler and others (26). How-
Temporal change of (a) acids, (b) sulfur-, and (c) nitrogen-containing compounds in samples and controls during the period of trial.

These observations are in agreement with what DeKeirsschieter and others reported, which was that a similar increase of VOCs was observable during the active decay stage and the advanced decay stage when investigating decomposition VOCs from pig remains in Belgium (21). These findings indicate that some trends can be associated with the decomposition stage, independent from the geographical location. Again, a detailed comparison is difficult because of differences in experimental parameters and data format reported in publications. Thus, the observation of comparable trends demonstrates the portion of the decomposition odor profile that transcends these differences in analytical parameters.

It is important to note that decomposition VOCs have been proposed as PMI estimation tools in the past. However, the majority of studies that comment on temporal trends for PMI estimation are primarily focused on using VOCs as a tool for differentiating between stages of decomposition that can be visually observed. To use specific decomposition VOCs as biochemical markers for postmortem interval, a much deeper understanding of differences in odor production under varied circumstances is required. As far as decomposition odor, the possibility of using decomposition VOCs as biochemical markers becomes a stronger possibility, and could provide a valuable tool to operationalize odor analysis for death investigation purposes.

**Field Design**

Besides some major differences between humans and pigs, in the field of decomposition odor many research groups have used pig carcasses because they are considered to be adequate analogs (31) and because of the similarities between pigs and humans regarding the body composition, the gut flora, and the lack of heavy fur (32). It was only recently that the first full longitudinal field trial was performed comparing the odor from humans and pig carcasses placed in the same field environment at the same time (33). The use of human analogs mainly occurs because of two reasons: First, the use of pig carcasses reduces legal, ethical, logistical, and economical obstacles; second, and more importantly for this study, it allows for very similar carcass replicates, and it controls confounding factors that are especially important in the phase of method development and comparison. This study used pig carcasses to demonstrate the comparability of the instrumentation with previous studies. In the experimental design, an attempt was made to control for as many internal or external variables to ensure adequate biological replicates. However, validation studies with human remains are still desirable to support the hypothesis that the results for pig and human remains are comparable.

**Instrument Configuration**

A variety of different instruments and experimental settings have been reported for the analysis of decomposition VOCs. The most popular configuration for GC×GC analysis uses a thermal modulator and a time-of-flight (TOF) mass spectrometer for the detection and identification of analytes.
retrofitted onto a pre-existing 1D-GC instrument and equipped with dual channel detection using a qMS instrument and a FID. This is a cost-effective alternative with the realistic potential for the adoption of GC×GC in routine forensic analysis, because laboratories are being provided with the potential to convert their pre-existing instruments into GC×GC instruments. However, there are currently no validated methods for the analysis of decomposition VOCs. Importantly, no studies have been performed in different laboratories to demonstrate inter-laboratory repeatability. Additionally, there are no reference standards for decomposition odor for researchers to use in comparing results between laboratories. Therefore, a direct comparison of this new configuration remains challenging. In addition, differences between the results presented herein and those from other studies in literature may not be based solely on the physical aspect of the instrumentation being used, but also on the way the produced data were processed. For advancement in our knowledge of decomposition odor, it would be valuable to take these points under consideration for future work. The lack of standardization across the field can be a hindrance to using decomposition odor as a valuable forensic tool.

**Conclusion**

In this study, the decomposition VOC profile on Oahu, Hawaii, using a retrofitted GC instrument with a RFF flow modulator and equipped with dual-channel detection using a qMS instrument and a FID, was established while taking into account environmental variables. Temporal trends were observed for acids, sulfur-containing compounds, esters, and nitrogen-containing compounds. Acids increased in the beginning of the trial, reaching their highest abundance around day 6–8 and representing a significant part of the advanced decay VOC profile. Sulfur-containing compounds increased even more rapidly in the beginning ([day 2]; combined with esters, they greatly contributed to the active decay VOC profile. Nitrogen-containing compounds rose to a maximum abundance around day 8 and showed an increased presence in the active and the advanced decay VOC profile.

Even though it is difficult to compare the results among different studies because of the use of different methods and instrumentation, trends in the evolution of some major chemical classes were consistent with results from studies conducted in other climate zones. Overall, this study demonstrates the robustness of the technique and, in addition, it indicates that identifying the major VOCs consistently produced by decomposition is possible in various geographical locations and forensic scenarios. However, to provide more comparable data, some of the variables between studies need to be standardized; eventually, this would allow inter-laboratory comparison studies to be performed.

**References**


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An Opportunity for Industrial–Academic Partnerships

Kevin A. Schug

In April 2020, on the heels of the pandemic shutdown, the price of crude oil fell to a negative value for the first time ever, sending ripples through an oil and gas industry that had more ebbed than waned in the United States over the past 10 to 15 years. Going forward, there is a generally positive sentiment about the importance of oil and gas extraction to our collective energy economy, but the downturn has created significant introspection across the industry.

While companies lick their wounds and decide their next moves, an important concept called environmental and social corporate governance (ESG) has come greater into focus. The hydrocarbon extraction and processing industry as a whole has certainly come under increasing scrutiny for its potential environmental implications (especially, greenhouse gas emissions) in the past several years. The truth of the matter is that some companies are more progressive and responsible with their actions towards documenting, limiting, and mitigating deleterious environmental and social impacts than others.

Even if the fine details about how it is regulated have not been fully formulated, ESG promises to involve some overall metric to rank the environmental and social responsibility efforts and actions of companies. Favorable metrics will be necessary to attract investment dollars in the industry going forward.

It is worth mentioning that, while ESG is discussed very prominently in the hydrocarbon processing industry (HPI), it is a concept that is permeating corporate America, as a whole. A closer look would likely find that virtually all major corporations now have some executive sustainability officer who is focused on reducing waste and improving environmental and social sustainability. While our research has primarily been involved with assessing the potential environmental impacts of upstream oil and gas production from shale, inevitably we also became involved in the midstream logistics of handling the product, and more importantly the wastewater byproducts. In most shale plays, the volume of water byproduct (so-called “produced water”) far exceeds the volume of hydrocarbons extracted from an oil and gas well. We have worked with small and large companies to assess the effectiveness of different produced-water treatment technologies—the goal being to recycle or reuse the treated water, rather than to simply dispose of it underground, where it has been shown to cause unwanted seismic activity.

The success of these technologies will ultimately be driven by their cost, throughput, performance, and robustness.

That said, going forward, downstream HPI activity is likely a bigger opportunity for industrial–academic partnerships. Downstream HPI activity deals with turning raw products into those that can be used by the consumer, including fuels, plastics, and other feedstocks. A prominent way a company can improve its ESG is to reduce reliance on virgin feedstocks and instead, to recycle used products. For example, an abundance of used plastic products can be broken back down into their constituent monomers and used anew.

This concept screams for more development in chemical measurements and analysis. As researchers develop new catalysts, which can be used to selectively decompose plastics, those catalysts need to be characterized (for example, pore structure, porosity, chemical make-up). The next level of complexity comes from the wide range of different plastics desired to be processed. Plastics are essentially reduced into a range of pyrolysis oils. Imagine the varying heteroatom content present both in the plastic feedstock and the finished pyrolysis oil. Different heteroatoms can have particularly deleterious effects on catalyst performance. Depending on the desired use of the recycled feedstock, not only the heteroatom content, but also the olefin and aromatic content of the product oils are desired to be determined.

The downstream HPI market, especially that associated with plastics recycling and driven heavily by a desire for improved ESG, is expected to double in the next 30 years. This is a major opportunity for academia to re-engage in the HPI, through the development of novel hardware and software solutions. Gas chromatography literally got its start in HPI. Instead, now think multi-dimensional separation and detection systems implemented for routine process monitoring. It is no small challenge, but it is one modern analytical chemists are ready to address. On the flip side, HPI companies are looking for an edge and will certainly have significant budgets associated with establishing themselves as a responsible leader in the industry through ESG.

The time should be right for the developing of more industry–academic partnerships in HPI, and the driving force is to achieve overall greater sustainability. This feels like it could be a “win” all around for everyone and the environment.
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