On Target for Non-Targeted Analysis

Advances in LC–HRMS and data processing tools
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In this instalment of “Column Watch”, a simple method for profiling free naphthenic acids in produced water by GC–MS is proposed.

“Practical HPLC Simulator”: A Useful Freeware for Learning HPLC

Michael W. Dong

The “practical HPLC simulator” is a freeware program that uses actual separation data to demonstrate the chromatographic principles under isocratic or gradient conditions and the impacts of instrumental, column, and mobile phase parameters on method performance.

Determination of Organophosphate Esters in Water Samples Using Gas Chromatography–Mass Spectrometry and Magnetic Solid-Phase Extraction Based on Multi-Walled Carbon Nanotubes

Xiyue Wang, Yuanyuan Tian, Lili Lian, Hao Zhang, Bo Zhu, Wenxiu Gao, and Dawei Lou

A method based on GC–MS coupled with magnetic SPE with multi-walled carbon-nanotube-coated Fe₃O₄ as adsorbent was developed for the analysis of four organophosphate esters (OPEs) in ambient water samples. The method was validated for its comparable sensitivity and potential application prospect.

Retention Factor is Independent of Pressure in LC, Right?

Trevor Kempen and Dwight R. Stoll

Understanding when the effect is likely to be large enough to affect resolution is valuable for troubleshooting unexpected results that arise during both method development and the execution of established methods.

Non-Targeted Food Analysis: How HRMS and Advanced Data Processing Tools Address the Current Challenges

LCGC Europe spoke to Christine Fisher about the challenges and solutions associated with developing non-targeted food analysis methods, why data quality is so important, and how data processing software and algorithms are helping to tackle the current challenges in food analysis.

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PEER REVIEW

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This article looks at the benefits of combining dynamic headspace sampling (DHS) with capillary GC–TOF-MS as a tool for untargeted analysis of aroma compounds in food and beverages.
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October Update

In this issue of LCGC Europe, we spoke to Christine Fisher from the US FDA about the challenges and solutions associated with developing non-targeted food analysis methods, why data quality is so important, and how data processing software and algorithms are helping to tackle the current challenges in food analysis.

This month’s peer-review article presents a method for the determination of organophosphate esters (OPEs) in water samples using gas chromatography–mass spectrometry (GC–MS) and magnetic solid-phase extraction (MSPE). Known as common flame retardants and plasticizers, some OPEs have been found to have carcinogenic properties, and so their continued monitoring is of the utmost importance.

LC Troubleshooting discusses the effect of pressure on retention in LC. As pressures above 400 bar continue to be routine, it is important to understand what the effect on resolution is likely to be to aid in troubleshooting any unexpected results.

Naphthenic acids (NAs) are naturally occurring organic acids derived from crude oil. Recent research has shown that there is an increasing demand for methods to monitor the occurrence of NAs in complex mixtures, such as wastewater, to address the environmental issues that currently surround the petroleum industry. In response to this, this month’s Column Watch presents the development of a successful method for qualitative analysis of free NAs.

Perspectives in Modern HPLC discusses the use of a practical LC simulator as a learning and teaching tool. Teachers and students alike can perform virtual HPLC experiments under isocratic or gradient conditions applicable to real instrumentation and method development.

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Mike Hennessy Senior,
Chairman and Founder, MJH Life Sciences™
Organophosphate esters (OPEs) are common flame retardants and plasticizers and are used in the production of plastic foam, electronics, furniture, textiles, and construction materials (1,2). It has been reported that the widespread application of OPEs has led to their becoming an emerging pollutant. Most OPEs are primarily combined with the product through physical action. Therefore, they are easily released into the surrounding environmental media using volatilization, abrasion, and even dissolution. They have been detected in surface water (3,4), groundwater (5), wastewater (6,7), atmospheric particulate matter (8, 9), and dust (10). The previous studies found that some commonly used OPEs have carcinogenicity, neurotoxicity, and hepatoxicity, which could threaten human health and ecosystems (11,12). Exposure to triphenyl phosphate (TPhP) disturbed hormone levels and sperm quality in fish (13). Tributyl phosphate (TnBP) and Tris (2-ethylhexyl) phosphate (TEHP) showed strong irritation to the skin (1).

In recent years, the United States, the European Union, and other countries around the world have set up relevant laws and regulations to limit the use of OPEs in domestic

A method based on gas chromatography–mass spectrometry (GC–MS) coupled with magnetic solid-phase extraction (SPE) with multi-walled carbon-nanotube-coated Fe$_3$O$_4$ as adsorbent was developed for the analysis of four organophosphate esters (OPEs) in ambient water samples. The magnetic multi-walled carbon nanotube composites were prepared by hydrothermal synthesis and characterized with scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FT-IR), and superconducting quantum interference device (SQUID) magnetometry. The extraction and desorption conditions, such as adsorbent dosage, adsorption time, eluent type, and eluent volume, were studied. The adsorbent was used to extract analytes within 50 min. The limit of detection was in the range of 0.038–1 μg/L and the limit of quantitation was between 0.10 and 3.59 μg/L. The method was applied to analyze organophosphate esters in environmental water samples. A 72.5–89.1% recovery was obtained by analyzing spiked samples with low-, medium-, and high-concentration analytes. The relative standard deviations were less than 10%. The method has comparable sensitivity and accuracy and can be successfully used to detect organophosphate esters in environmental water samples.

**KEY POINTS**

- The MSPE–GC–MS method based on Fe$_3$O$_4$ @SiO$_2$ MWCNTs was developed for the analysis of OPEs in water samples.
- The presented method had the advantage of preferable recoveries for both hydrophilic and hydrophobic OPEs, and low consumption of organic solvent.
- The method validation proved its comparable sensitivity and potential application prospect.

Organophosphate esters (OPEs) are common flame retardants and plasticizers and are used in the production of plastic foam, electronics, furniture, textiles, and construction materials (1,2). It has been reported that the widespread application of OPEs has led to their becoming an emerging pollutant. Most OPEs are primarily combined with the product through physical action. Therefore, they are easily released into the surrounding environmental media using volatilization, abrasion, and even dissolution. They have been detected in surface water (3,4), groundwater (5), wastewater (6,7), atmospheric particulate matter (8, 9), and dust (10). The previous studies found that some commonly used OPEs have carcinogenicity, neurotoxicity, and hepatotoxicity, which could threaten human health and ecosystems (11,12). Exposure to triphenyl phosphate (TPhP) disturbed hormone levels and sperm quality in fish (13). Tributyl phosphate (TnBP) and Tris (2-ethylhexyl) phosphate (TEHP) showed strong irritation to the skin (1). In recent years, the United States, the European Union, and other countries around the world have set up relevant laws and regulations to limit the use of OPEs in domestic
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products. Organizations and institutions worldwide have also begun to pay close attention to the issue of environmental pollution from OPEs, bringing about much research on the subject.

There are many kinds of pretreatment methods for OPE extraction in water samples, such as liquid–liquid extraction (LLE) (14), membrane-assisted solvent extraction (MASE) (6), solid-phase extraction (SPE) (3,15), and solid-phase microextraction (SPME) (16,17). LLE and SPE are the most commonly used pretreatment methods. However, these methods are cumbersome and require a large amount of organic solvent for pretreatment of the extraction column. Moreover, LLE and SPE methods have poor recoveries for polar and volatile (for example, trimethyl phosphate [TMP], Tri(2-chloroethyl) phosphate [TCEP]) or hydrophobic (for example, TEHP) compounds (15,18,19). Magnetic solid-phase extraction (MSPE) has been developed as a new type of sample preparation technique and has received considerable attention. MSPE uses magnetic or magnetizable material as the sorbent for the extraction or preconcentration of the target analytes (20). The method also overcomes the column plugging problem that often occurs in SPE, greatly simplifying the SPE process and enhancing the extraction efficiency. Currently, MSPE is widely used for qualitative and quantitative analysis of various organic pollutants and inorganic metal ion contaminants in an environmental sample when combined with high performance liquid chromatography (HPLC) or gas chromatography (GC) (21–23). This makes the method ideal for application in the field of biological separation and environmental analysis. However, MSPE is currently rarely used for OPEs analysis.

Instrumental analysis of OPEs is mainly conducted using the GC and nitrogen--
phosphorous detector method (GC–NPD) (4), GC–mass spectrometry (GC–MS) (17,23), and liquid chromatography–tandem mass spectrometry (LC–MS/MS) (14,24). Among them, GC–NPD offers poor stability, requiring regular replacement parts, while the sample matrix can affect the electrospray ionization source of LC–MS/MS. In this article, a method based on MSPE–GC–MS for the analysis of OPEs in river water samples was developed. The MSPE and GC–MS detection conditions were investigated. The method offers a wide linear range and good sensitivity and recovery for both polar and nonpolar OPEs. It can be successfully used to detect OPEs in environmental water samples.

**Materials and Method**

**Materials:** Organophosphate esters (TnBP, TCEP, TPhP, and TEHP) were purchased from AccuStandard (AccuStandard Inc.). Their full names, abbreviations, chemical structures, and octanol-water partition coefficient ($K_{ow}$) are shown in Table 1. Ethyl acetate and multi-walled carbon nanotubes were obtained from the Aladdin Reagent Company. Tetraethyl orthosilicate (TEOS), polyethylene glycol, $\text{FeCl}_3\cdot6\text{H}_2\text{O}$, aqueous ammonia (25 wt% aqueous $\text{NH}_3$), and ethylene glycol were the products of the Tianjin Damao Chemical Reagent Factory. The OPEs stock solution with a concentration of 10 mg/mL was prepared with ethyl acetate and was stored in a refrigerator at 4 °C. According to experimental requirements, other solutions were diluted by using the stock solution.
solution. Unless specifically mentioned, ultrapure water was used in all experiments.

**Instruments:** The analysis was performed using an Agilent 7890 gas chromatograph coupled to an M7-300EI single quadrupole mass spectrometer (GC–MS) system. The morphology of the prepared material was observed by using a Tecnai-G20 transmission electron microscope (FEI) and a JSM-7500F scanning electron microscope (Japan Electronics). Magnetic measurements were performed by using a superconducting quantum interference device (SQUID) and a magnetometer (VSM, Quantum Design). The functional groups of the synthesized adsorbent were measured using a Fourier-transform infrared spectroscopy (FT-IR) spectrometer (920, Tuopu). Water was purified through a Milli-Q system (Millipore).

**Preparation of MSPE Sorbent:**

**Synthesis of Fe$_3$O$_4$:** Magnetic nanoparticles were synthesized by using a solvothermal method (25). First, 1.35 g of FeCl$_3$•6H$_2$O was added to a beaker containing 40 mL of ethylene glycol. The solution was then stirred for 15 min. Second, 3.6 g of anhydrous sodium acetate and 1.0 g of polyethylene glycol were added to the mixed solution. Stirring was continued even after the formation of a uniform yellow thick liquid. The sample was then transferred to a 50-mL polytetrafluoroethylene autoclave, which was sealed and placed in an oven at 190 °C for 8 h. The new-solid sample was then cooled to room temperature and the Fe$_3$O$_4$ nanoparticles were separated with the magnet. After washing three times with ultrapure water and anhydrous ethanol, the sample was vacuum dried at 60 °C for 2 h. The Fe$_3$O$_4$ black powder was obtained.

**Synthesis of Fe$_3$O$_4$@SiO$_2$ Microspheres:** SiO$_2$-coated Fe$_3$O$_4$ microspheres were prepared via a modified Stöber synthesis (26). First, 0.1 g Fe$_3$O$_4$ nanoparticles were added to 50 mL HCl (0.1 mol/L) and the solution underwent ultrasound treatment for 10 min. After washing with distilled water, the Fe$_3$O$_4$ was added to a three-necked round bottom flask containing 80 mL of absolute ethanol, 20 mL of water, and 1 mL of aqueous ammonia. The solution was mixed well through mechanical agitation. A 0.03 g measure of tetraethyl orthosilicate (TEOS) was added dropwise and stirred at room temperature for 6 h. Finally, the product was separated with a magnet, washed three times with distilled water and absolute ethanol, respectively, and vacuum dried at 60 °C for 2 h. The Fe$_3$O$_4$@SiO$_2$ microspheres were obtained.

**Synthesis of Fe$_3$O$_4$@SiO$_2$ Multi-Walled Carbon Nanotubes:** Fe$_3$O$_4$@SiO$_2$ (0.004 g) and multi-walled carbon nanotubes (MWCNTs) (0.016 g) were

---

**TABLE 1: The full names, abbreviations, chemical structures, and $K_{ow}$ of four OPEs**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>Log $K_{ow}$</th>
<th>Substituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tri-n-butyl phosphate</td>
<td>TnBP</td>
<td>4.00</td>
<td>$R_1 = R_2 = R_3 =$</td>
</tr>
<tr>
<td>Tri(2-chloroethyl) phosphate</td>
<td>TCEP</td>
<td>1.44</td>
<td>$R_1 = R_2 = R_3 =$ Cl</td>
</tr>
<tr>
<td>Triphenyl phosphate</td>
<td>TPhP</td>
<td>4.70</td>
<td>$R_1 = R_2 = R_3 =$</td>
</tr>
<tr>
<td>Tri(2-ethylhexyl) phosphate</td>
<td>TEHP</td>
<td>9.49</td>
<td>$R_1 = R_2 = R_3 =$</td>
</tr>
</tbody>
</table>

---

**TABLE 2: Calibration results by MSPE**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>Linear Dynamic Range (μg/mL)</th>
<th>$R^2$</th>
<th>LOD$^a$ (μg/L)</th>
<th>LOQ$^b$ (μg/L)</th>
<th>RSD$^c$ (%)</th>
<th>n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>TnBP</td>
<td>8.45</td>
<td>0.1–10</td>
<td>0.9974</td>
<td>0.45</td>
<td>1.51</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>TCEP</td>
<td>9.34</td>
<td>0.1–10</td>
<td>0.9990</td>
<td>0.56</td>
<td>1.35</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>TPhP</td>
<td>14.28</td>
<td>0.1–10</td>
<td>0.9914</td>
<td>1.00</td>
<td>3.59</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>TEHP</td>
<td>14.73</td>
<td>0.1–10</td>
<td>0.9973</td>
<td>0.038</td>
<td>0.10</td>
<td>10.4</td>
<td></td>
</tr>
</tbody>
</table>

$^a$LOD: Detection limit (signal to noise = 3)

$^b$LOQ: Limit of quantitation (signal to noise = 10)

$^c$RSD: Relative standard deviation
added to deionized water. The mixture was sonicated for 15 min to ensure complete self-assembly of MWCNTs onto Fe₃O₄@SiO₂ nanoparticles. A black homogeneous liquid was formed.

**Sample Preparation by MSPE:** Several elements of extraction, such as adsorbent dosage, adsorption time, elution solvent, and elution time, were studied. During MSPE, 20 mg Fe₃O₄@SiO₂ MWCNTs were dispersed to 20-mL pure water solution spiked with four OPEs (10 μg/L), which was subjected to a constant-temperature 25 °C water bath with vibration mixing for 50 min. After the oscillation extraction, Fe₃O₄@SiO₂ MWCNTs that had absorbed four OPEs were separated by a magnet. The supernatant was discarded. A 1400 μL measure of the ethyl acetate was then used to desorb the OPEs by ultrasonic treatment for 20 min. The obtained elution solution containing OPEs was aspirated off with nitrogen. The residual sample was dissolved with 200 μL ethyl acetate and then analyzed by GC–MS.

**GC–MS Analysis:** The GC conditions were as follows. A 30 m × 0.32 mm, 0.25-µm HP-5 column (Agilent, J&W) was used for separation of the target analytes. The carrier gas was helium (purity ≥ 99.999%) and the flow rate was 1.2 mL/min. For the heating

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### TABLE 3: Recoveries and precision of OPEs after being spiked in actual water samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Added (μg/L)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
<th>n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>TnBP</td>
<td>2.5</td>
<td>79.1</td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>80.9</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>82.2</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>TCEP</td>
<td>2.5</td>
<td>85.3</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>87.6</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>89.1</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>TPnP</td>
<td>10</td>
<td>78.8</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>80.9</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>TEHP</td>
<td>2.5</td>
<td>72.5</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>75.1</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>77.3</td>
<td>5.9</td>
<td></td>
</tr>
</tbody>
</table>

- No quantified
program, the initial temperature was 70 °C, heating at 15 °C/min to 200 °C, then at 10 °C/min to 280 °C (where the temperature was held for 5 min). The inlet temperature was 280 °C and the interface temperature was 280 °C. The splitless mode was adopted and the injection volume was 1 μL.

The mass spectrometry conditions were as follows: EI ion source, energy = 70 eV, temperature = 230 °C, quadrupole temperature = 150 °C, and the data acquisition mode was a selected ion monitoring (SIM) scan. The SIM ions (m/z) of TnBP were 99, 155; TCEP were 63, 249; TPhP were 326, 770; TEHP were 99, 113.

Results and Discussion
Characterization of Fe₃O₄@SiO₂ MWCNTs: The preparation procedure of Fe₃O₄@SiO₂ MWCNTs is shown in Figure 1. The structure and composition were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and FT-IR. Figure 2(a) displays the SEM image of the pristine MWCNTs. It can be seen that the MWCNTs are long with a rather smooth surface and curly shape. With the help of references 27 and 28, and the SEM and TEM images of Fe₃O₄@SiO₂ MWCNT nanocomposites shown in Figure 2(b)–2(d), it was found that the material is nearly in a core–shell structure, with black solid spheres showing Fe₃O₄ and a surface coating of a thin layer of SiO₂ (Figure 2[c] and 2[d]). The SiO₂ shell modified by the Stöber method not only protected the Fe₃O₄ magnetic shell from O₂ oxidation in the air but also prevented Fe₃O₄ agglomeration. Almost all the Fe₃O₄@SiO₂ nanoparticles were preferentially adhered to the surfaces of MWCNTs, indicating that the MWCNTs had been successfully modified (Figure 2[b]).

The FT-IR spectra of the Fe₃O₄@SiO₂ MWCNT composite are shown in Figure 3(a). The characteristic absorption peak of Fe–O in Fe₃O₄ is near 631 cm⁻¹ (29). In the vicinity of 1045 cm⁻¹, the stretching vibration characteristic absorption peak of Si–O–Si can be observed, indicating that SiO₂ was successfully modified on the surface of Fe₃O₄ (30). The bands around 1630 cm⁻¹ can be assigned to the stretching vibration peak of C = C in MWCNTs (31). These results confirmed that the Fe₃O₄@SiO₂ MWCNT magnetic composites were successfully prepared.

A magnetometer was used to study the magnetic properties of the adsorbent. Figure 3(b) shows the magnetization curve (hysteresis loop) of Fe₃O₄@SiO₂ MWCNT nanoparticles. The saturation magnetization was 7.68 emu/g. The curve is smooth and symmetrical at the origin, and no remanence (residual magnetism) was found, indicating superior magnetic character.

Effect of the Amount of Magnetic Adsorbent: A different mass of magnetic adsorbent (5–30 mg) was added to a 20 mL spiked solution to study OPE extraction efficiency. The results are shown in Figure 4(a). The peaks of OPEs significantly increased with the increase of adsorbent amount within the range of 5–20 mg. When the adsorbent added was greater than 20 mg, the OPE extraction efficiency decreased. It might be that excessive adsorbent enhanced the adsorption capacity between the adsorbent and the analytes and resulted in incomplete

# Table 4: Comparison of analytical methods for OPEs detection

<table>
<thead>
<tr>
<th>Extraction Technique</th>
<th>Matrix</th>
<th>Extraction Condition</th>
<th>Detection</th>
<th>LOD (ng/L) Injection Volume</th>
<th>Recovery (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSPE</td>
<td>River water</td>
<td>Sample volume: 0.02 L, Solvent: 1.4 mL</td>
<td>GC–MS</td>
<td>38–1000 (1 μL)</td>
<td>72.5–89.1</td>
<td>This study</td>
</tr>
<tr>
<td>LLE</td>
<td>Wastewater, Surface water</td>
<td>Sample volume: 1L, Solvent: 10 mL</td>
<td>PTV–GC–MS</td>
<td>0.1–3 (40 μL) (LOQ)</td>
<td>28–128 (TCEP28)</td>
<td>(36)</td>
</tr>
<tr>
<td>SPE</td>
<td>Drinking water</td>
<td>Sample volume: 1 L, Solvent: 10+12 mL</td>
<td>LC–MS/MS</td>
<td>0.5–1 (10 μL)</td>
<td>70–99 (TEHP: 38.4)</td>
<td>(18)</td>
</tr>
<tr>
<td>SPE</td>
<td>River water</td>
<td>Sample volume: 0.2 L, Solvent: 9+9 mL</td>
<td>LC–MS/MS</td>
<td>2–6 (10 μL)</td>
<td>70–110 (TEHP: 40–76)</td>
<td>(15)</td>
</tr>
<tr>
<td>SPME</td>
<td>River water, Tap water</td>
<td>Sample volume: 0.01 L</td>
<td>GC–NPD</td>
<td>1.4–135.6</td>
<td>76.4–112.4</td>
<td>(4)</td>
</tr>
<tr>
<td>MASE</td>
<td>Wastewater</td>
<td>Sample volume: 0.1 L Solvent:1 mL</td>
<td>LC–MS/MS</td>
<td>1–25 (LOQ)</td>
<td>63–98 (TCEP 5)</td>
<td>(6)</td>
</tr>
</tbody>
</table>
elution of the analytes. Therefore, the amount of magnetic adsorbent added was set at 20 mg.

**Effect of Extraction Time:** Extraction time is one of the most important parameters in MSPE. The effect of extraction time on the adsorption of OPEs was determined by testing extraction times of 20–80 min. The results are shown in Figure 4(b).

With the extension of time, the extraction efficiency gradually increased but then decreased after 50 min. It was speculated that volatilization of eluent caused sample loss and decreased the extraction efficiency. Based on the above experimental results, 50 min was chosen as the best extraction time.

**Effect of Eluent:** The eluent is also an important factor affecting extraction efficiency. The effects of methanol, acetonitrile, 1:1 methanol–acetonitrile (v/v), and ethyl acetate on the OPE extraction efficiency were investigated, respectively. The results are shown in Figure 5(a). The ethyl acetate solution was the most efficient eluent. This result is consistent with previous literature (32–34) and so the ethyl acetate was chosen as the eluent.

**Elution Volume and Elution Time:** The influence of the eluent volume on the extraction efficiency of OPEs was also investigated. An eluent volume of 1400 μL was sufficient to obtain better extraction efficiency. More elution had almost no effect on the extraction results but extended the concentration time. Therefore, a volume of 1400 μL ethyl acetate was used to elute the sample.

Additionally, the influence of the elution time on the extraction efficiency of OPEs was also investigated. With ultrasonic assistance, OPEs were eluted quickly and efficiently from the MSPE adsorbent surface. As is shown in Figure 5(b), when elution time was extended over 20 min, extraction efficiency decreased, so 20 min was chosen as the elution time.

**Method Evaluation:** Under these optimal conditions, the detection limit (LOD), the limit of quantitation (LOQ), recovery, and precision were used to evaluate the developed MSPE–GC–MS method. The instrumental linearity was performed by detecting OPE standard solutions with 0.1, 0.5, 1, 2, 5, and 10 μg/mL. Good linearity with coefficient $R^2 = 0.9914–0.9990$ was obtained in this range (Table 2). The LOD and LOQ—defined as three times and ten times signal-to-noise—was achieved by analyzing four spiked OPEs in a pure water sample. The LODs of TnBP, TCEP, TPhP, and TEHP were 0.45, 0.56, 1, and 0.038 μg/L, respectively. The LOQs of TnBP, TCEP, TPhP, and TEHP were 1.51, 1.35, 3.59, and 0.1 μg/L, respectively. In pure spiked water solutions, the average recoveries of TnBP, TCEP, TPhP, and TEHP were 84.3%, 90.7%, 83.8%, and 79.4%, respectively. According to previously reported results (35), the high extraction efficiency can be attributed to the fact that the OPEs can interact with the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ MWCNT adsorbents via a Brønsted acid-base interaction, van der Waals forces, and a $\pi-\pi$ electron donor-acceptor (EDA) interaction between the OPEs and the functionalized groups of the adsorbents.

**Actual Water Sample Detection:** To determine the applicability of this method, water from the Songhua River (Jilin Province, China) was selected for testing. First, water samples were centrifuged at 10,000 rpm for 10 min to remove sediment, and the supernatant was passed through a 0.45 μm filter. Then the water sample was processed as per section named “Sample Preparation by MSPE” and extracted OPEs were analyzed with GC–MS. No OPEs were detected. OPEs were then added to Songhua River water samples to make the concentrations of 2.5, 10, and 25 μg/L. The recoveries of OPEs spiked at the low, middle, and high concentrations were 72.5–89.1%, with mean relative standard deviations (RSDs) ranging from 3.4% to 9.7% (Table 3). There were inorganic salts, organic matter, microorganisms, and some impurities found in the Songhua River water. The extraction efficiency of the adsorbent therefore had a certain impact. However, recoveries of four OPEs in environmental water were acceptable.

**Compared With Other Methods:** The MSPE–GC–MS method established in this paper was compared to other methods.
It was shown that MSPE–GC–MS had preferable recoveries for both polar and hydrophobic OPEs (TCEP and TEHP), and required less organic solvent compared to LLE and SPE methods (18, 36). However, the LOD and LOQ of this method were higher than previous studies. This could possibly be attributed to the smaller sample volume used in this study. The SPME method was a solvent-free sample preparation method, which also had a lower LOD (4). The recovery of polar TCEPs in MASE was only 5%. Moreover, the MAE method was time-consuming, and the cost of membrane is higher (6).

Conclusions
In this study, Fe3O4@SiO2 MWCNT magnetic composite was prepared and used to analyze organophosphate esters in water samples in combination with GC–MS for the first time. This method requires only 20 mg of magnetic adsorbent, isothermally oscillated for 50 min, 1400 μL of eluting solvent, and 20 min of elution time. The method has the advantages of good recoveries, a wide linear range, low consumption of organic solvents, and low preparation cost of magnetic composite materials. This method could be successfully used to extract and analyze organophosphates (TnBP, TCEP, TPhP, and TEHP) in environmental water samples.

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This work was supported by the Project of Science and Technology Development of Jilin Province (No. 20190303116SF and 202002008JC), the Research and Development Project for Industrial Technology of Jilin Province (No. 2020C028-1), the Talents Project for Innovation and Entrepreneurship of Jilin province (No. 2020030), and the Project of Science and Technology of the Education Department of Jilin Province (No. JJKH20210242KJ).

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Conflict of Interest
There are no conflicts of interest to declare.

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Retention Factor is Independent of Pressure in LC, Right?

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The retention factor (k) is a valuable measure of retention in chromatography because it is independent of several method variables, including flow rate and column length. In reversed-phase liquid chromatography (LC), the retention factor is also nominally independent of operating pressure for small nonpolar molecules at low pressure. However, as the field moves in the direction of routine use of pressures well above 400 bar, and biomolecule separations become more prevalent, the effect of pressure on retention should not be overlooked. Understanding when the effect is likely to be large enough to affect resolution is valuable for troubleshooting unexpected results that arise during both method development and the execution of established methods.

In the first few decades of the development of high performance liquid chromatography (HPLC), there was quite a lot of theoretical consideration given to the relationship between pressure and analysis time in LC. Several researchers, including Knox (1), Poppe (2), and Guiochon (3), provided us with ways to think about optimizing the speed of LC separations—for a given maximum pressure that can be generated by a LC pump, what are the best choices for parameters such as particle size, column length, and flow rate? During this same time period, however, there was very little discussion of the effect of operating pressure on retention in LC. It wasn’t until the 1990s that researchers started looking at this relationship closely, but the introduction of commercial LC instrumentation that could reliably operate well above 400 bar in the early 2000s led to many published studies in this area over the past 15 years or so.

In my own laboratory, we are increasingly encountering situations where the effect of pressure on retention cannot be ignored, in part because we are doing more work these days with large biomolecules where such effects are more obvious. For this installment of “LC Troubleshooting”, I’ve asked my student Trevor Kempen to join me to provide some background on this topic, and then use experimental data from reversed-phase separations of small molecules to illustrate when the effect of pressure on retention could complicate method development, or even produce changes in selectivity during routine operation of an established method.

\[
k = \frac{n_{A,sp}}{n_{A,mp}} \quad [1]
\]

A little algebra yields the more commonly used relationship between k, retention time (t_r), and the column dead time (t_m):

\[
k = \frac{t_r - t_m}{t_m} \quad [2]
\]

At this point, we can illustrate the interest in k. Suppose that at a flow rate of 1 mL/min we observe a peak A with a retention time of 4 min, and we know that the column dead time at this flow rate is 1 min. Using equation 2, we would calculate that \( k = 3 \). Now, if we double the flow rate, the retention time of peak A will decrease by a factor of two; it simply takes half the time for the analyte to move through the column if the mobile phase is moving twice as fast. The new retention time for peak A will be 2 min, and the new dead time will be 0.5 min. From the point of view of retention time, the retention of peak A has decreased greatly. However, putting these numbers into equation 2, we find that the retention...
factor is still 3.0; it has not changed, even though we doubled the flow rate. Similar arguments can be made to show that, in principle, variables including column length, column diameter, particle size, and flow rate have no direct effect on retention factor. This point is exceedingly valuable in a variety of situations in LC, not the least of which is troubleshooting problems or unexpected observations during method development. For example, the ideas above lead us to expect that when we move from a 50-mm long column to one that is 150-mm long, keeping all other variables constant, retention times of our analytes will increase by a factor of three, but the retention factor will remain unchanged. If this is not what we observe, then something is not right—perhaps the stationary phase in the two columns is different, or there is a leak in the system due to the higher pressure drop generated by the longer column. Again, the idea that the retention factor is independent of certain variables is central to many of the ways that we think about how LC separations work. Although not placed in the same category as other variables such as flow rate and column length, pressure is usually not considered in textbooks as having a major effect on retention, again probably because this was not a major focus of research prior to the early 2000s. However, research by several groups is yielding an increasingly quantitative picture of how and when pressure does, in fact, affect retention factor in significant ways. Given that these effects roughly increase in magnitude with increasing molecular size of the analyte, the effects can be dramatic (for example, retention changes greater than 100% when moving from 100 to 1000 bar), and very important for biomolecule and other macromolecule separations.
Brief Review of Recent Research in This Area

In an early study in this area in 1997, McGuffin and Chen (5) measured the dependence of the retention factors of a homologous series of fatty acids on pressure, and found that $k$ increased from 9.3 to 24.4% over the range of pressure from about 100 to 333 bar. In the publication describing these results, they proposed the following expression for the dependence of retention factor on pressure ($P$) in LC that has been adopted by most subsequent publications:

$$\ln\left(\frac{k}{k_{\text{ref}}/}\right) = -\frac{\Delta V}{R T} P + \ln\left(\frac{\beta}{\beta_{\text{ref}}/}\right)$$

where $R$ is the gas constant, $T$ is temperature, $\Delta V$ is the difference between the molar volume of the analyte in the stationary phase and mobile phase environments, and $\beta$ is the phase ratio. The subscript ref indicates a reference condition at some specific pressure. If equation 3 accurately describes the effect of $P$ on $k$, then a plot of $\ln(k)$ vs. $P$ should be linear, with a slope of $-\frac{\Delta V}{R T}$ and a $y$-intercept of $\ln\left(\frac{\beta}{\beta_{\text{ref}}/}\right)$. In most published results of this kind for reversed-phase LC separations, the slopes of such plots are positive, which means that the $\Delta V$ terms are negative. This is usually interpreted as reflecting a decrease in the solvation of the analyte as it moves from the mobile phase to the stationary phase (6).

In reversed-phase LC, the magnitude of the $\Delta V$ term has been shown to be condition-specific and dependent on a large number of variables, including:

- mobile-phase properties, such as organic solvent content and buffer pH (6)
- stationary-phase properties:
  - ligand chain length for bonded stationary phases (6–8)
  - ligand hydrophilicity (6,7,9)

---

**TABLE 1: Changes in retention factor in response to pressure for different probe compounds**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Percent Change in $k$ for $\Delta P = 500$ Bar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nortriptyline</td>
<td>30.9</td>
</tr>
<tr>
<td>N-butylaniline</td>
<td>-17.7</td>
</tr>
<tr>
<td>Propiophenone</td>
<td>5.3</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.2</td>
</tr>
<tr>
<td>4-n-butylbenzoic acid</td>
<td>19.1</td>
</tr>
</tbody>
</table>

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  • Ionization state (6,12,13)
  • Flexibility/rigidity (8,11)

Interestingly, the dependence of retention on pressure in hydrophilic interaction chromatography (HILIC) separations is usually the opposite of that observed in reversed-phase LC separations; that is, $\Delta V$ is usually positive (6,9).

**Experimental Data Illustrate Potential Consequences**

Different research groups have used various experimental approaches to study the effect of pressure on retention in LC. One approach is to vary the flow rate through the column, which will change the pressure that the column experiences during separations. The major upside of this approach is that it is experimentally convenient and easy to do. The major downside is that changing the flow rate will also change column temperature because of frictional heating, and this temperature change, in turn, will affect retention, making interpretation of observations more difficult. Åsberg, Fornstedt, and coworkers have demonstrated the use of column water jackets to minimize this complication (13), but this is not very convenient experimentally. The other commonly used approach is to add different lengths of restriction capillaries (for example, adding a 50 µm internal diameter (i.d.) capillary when operating at 1 mL/min will significantly raise the pressure the column experiences) between the column outlet and the detector. The major upside of this approach is that it does not require a flow rate change, and therefore the column temperature will not change significantly due to changes in frictional heating. The major downside of this approach is that it requires a physical change of the restriction capillary in the flow path between the column and detector to produce a change in the pressure, which is not very convenient and prone to irreproducibility.

For this type of study in our laboratory, we have primarily used the restriction capillary approach, but with one twist. We mounted several capillaries with different lengths or diameters on a valve normally used for column selection in a LC instrument. We can then modulate the flow rate by changing the position of the selection valve, in turn places a different restriction capillary in the flow path between the column outlet and detector. An illustration of this type of setup is shown in Figure 1.

The dependence of retention factor on pressure for some simple small molecules under reversed-phase separation conditions is shown in Figure 2. Among the five molecules represented are two neutrals (toluene and propiophenone), one weak acid (4-n-butylbenzoic acid), one relatively strong base (nortriptyline; $pK_a$ of protonated amine is about 10.5), and one relatively weak base (N-butylaniline; $pK_a$ of protonated amine is about 5.0). As expected from the data of other groups found in the literature, the strongest dependencies (that is, the steepest slopes) are observed for the more dipolar molecules (here, the acids and bases) (6,10). The dependence for the small, neutral toluene is measurable but very weak. The dependencies for the weak acid and strong base are both strong and positive. Interestingly, the dependence for the weak base (N-butylaniline) is also quite strong, but negative. This, too, is consistent with observations discussed in the literature, where other groups have suggested that this result can be explained by the differential shifts in the dissociation constants of the different acids and bases involved (here, ammonium and formate in the mobile phase, in addition to the analytes) in response to changes in pressure (6,14). These dependencies are quantified as percent change in retention factor per 500-bar increase in pressure in Table 1.

**What Does This All Mean for Practical Work?**

The chromatograms that yielded the retention factors plotted in Figure 2 are shown in Figure 3. Including uracil, there are six compounds in the test mixture. We see that at the lowest pressure of 53 bar, only five peaks are observed; in this instance, N-butylaniline and propiophenone have been coeluted. As the pressure is increased, the resolution of this pair of peaks improves to the point where they are baseline resolved at the highest pressure of 491 bar. This dramatic shift results from the fact that the dependencies of retention factor on pressure have opposite signs for these two compounds. On the other hand, the opposite is observed for nortriptyline and N-butylaniline. At low pressure, they are well resolved, but as pressure is increased, resolution is lost, and, at the highest pressure, the nortriptyline is barely observable as a shoulder on the N-butylaniline peak.

We could make a pretty long list of different scenarios where changes in retention factor with changes in pressure on the order of those shown here could significantly impact both method development and separation performance during routine execution of an established method. Just a couple of examples will suffice to make the point here. In the case of method development, it is common to do a selectivity screen using short columns with different stationary phases to find one or a few phases that look promising in terms of the
selectivity needed to solve a particular separation problem. In a next round of optimization, using a longer column close to the length that will likely be used in the final method will result in higher operating pressures, unless the flow rate is deliberately lowered when using the longer column so that the pressure does not change. In this scenario, if the retention of the compounds under study is sensitive to pressure, significantly different selectivity will be observed in the next round of optimization compared to what was observed during the initial stationary phase screen. This could be either a positive change or a negative one (that is, more or less resolution), but in either case it could be an unexpected change that may be difficult to interpret.

In the case of an established method, an example of a change in the instrument that could precipitate a pressure-induced change in method performance is a change in the connecting tubing between the outlet of the column and the detector. For example, unknowingly replacing a 120 μm i.d. capillary with a 75 μm i.d. capillary could easily lead to a change of 50 bar experienced by the column. For many methods involving separation of a few compounds and resolution to spare, such a change will hardly be noticeable. However, in methods with crowded chromatograms, several critical pairs, and highly pressure-sensitive compounds, such a change could quickly become a big problem. Of course, making a change in the diameter of connecting tubing such as in this example is typically not recommended, but mistakes like this do happen, and it is helpful to be aware of the consequences that could follow from such a change.

**Summary**

In this instalment of “LC Troubleshooting”, we have discussed the fact that, in reversed-phase LC, the operating pressure that the column experiences can significantly affect analyte retention factors. We briefly reviewed recent research in this area, which has shown that \( \ln(k) \) is linearly dependent on \( P \), with a slope that is related to the difference between the molar volumes (\( \Delta V \)) of the analyte in the mobile- and stationary-phase environments. Sometimes these effects are too small to be noticed in chromatograms obtained at different pressures, but, in other cases, relative shifts in retention can significantly increase or decrease resolution of neighbouring peaks. These effects increase in magnitude with increasing size and dipolarity of the analyte, and can be quite dramatic for large biomolecules and other macromolecules. Understanding when these effects can occur is an important asset in the “LC Troubleshooting” toolbox (15).

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**References**

Unlocking the Power of 3D Absorbance Data

Peak deconvolution with photodiode array (PDA) detectors using a unique software function can separate peaks that are not resolved on-column, yield better detection results, and minimize method development and analysis time.

**LCGC**: What’s the difference between 2D UV-vis data and 3D PDA data?

**DOMANSKI**: Two-dimensional UV-vis data describes the total absorbance of a sample versus time for a single chosen wavelength producing a chromatogram. This type of data only shows that a compound has some absorbance at the wavelength of observation but can’t provide much more information. There is no way to tell if we are observing the compound at a wavelength of high or low absorbance or to predict what the compound might be. When we collect 3D PDA data, for each moment in time during the experiment, we can observe and record intensity across a range of wavelengths. This provides useful spectrum information that can determine maxima that yield the best limits of detection for the compounds of an assay or be used for traditional PDA data analysis such as cosine-vector peak purity.

**LCGC**: Beyond these uses, how does peak deconvolution unlock the full potential of PDA data?

**DOMANSKI**: While traditional PDA analysis tools may use a portion of the spectrum information available, peak deconvolution uses all the data and applies accepted mathematical techniques to separate peaks that are not resolved on-column. This can allow for detection of coeluting impurities in a potency assay, indicate an unexpected coeluting reaction product, or allow for characterization of a hard-to-separate degradant.

**LCGC**: What is the key to peak deconvolution, and how did this function get developed?

**DOMANSKI**: At the heart of our PDA peak deconvolution function is a sophisticated peak-fitting and spectrum-fitting algorithm that uses the multivariate curve resolution – alternating least squares (MCR-ALS) approach to determining solution sets, which is often used in benchtop spectroscopy applications such as chemometrics and observation of reaction kinetics. Because the technique can work with complex multi-component systems, it is ideal for LC-PDA data, where a single experiment may contain many peaks, each with associated
spectral data. Making the leap from accepted technique to implementation in software was done in conjunction with Eisai Co. Ltd., a leading global pharmaceutical manufacturer. Their goal was to accelerate therapeutic development by investing in technologies that would reduce analysis time and increase awareness of coelution-related issues at every stage of the process.

**LCGC:** How does MCR-ALS deconvolution compare to other forms of peak purity assessment?

**DOMANSKI:** In contrast to traditional cosine-vector peak purity or purity-angle assessment, MCR-ALS deconvolution uses the entire data set from the defined time and wavelength domain to calculate peak shape and spectrum information for each identified chromatographic feature. If users have multiple impurities, up to five peaks can be identified within a single deconvolution segment, and up to 12 segments can be set in each chromatogram. So not only can coelution be spotted, but the potential impurities can be characterized with the same tools as any other peak, including the reconstructed spectrum.

**LCGC:** Can the deconvoluted peaks be integrated, and are reconstructed spectra accurate?

**DOMANSKI:** The short answers are yes, and yes. With regards to integration, we have studied a three-component system of common pharmaceutical drugs under ultra-high-performance liquid chromatography (UHPLC) conditions that result in partial separation and elution in less than 20 seconds. The resulting chromatogram produces peaks and valleys, but none of the peaks are baseline resolved. We then compared the results of PDA peak deconvolution with the traditional integration strategy of splitting the peaks with a vertical drop from the bottom of the valley to baseline and with separate injections of each standard.

Traditional valley-to-valley definition of the unresolved peaks led to as much as 13.1% deviation from the single standard injection, whereas using the deconvolution result, peak area was much more accurately assigned to the peaks, with the greatest error versus single standard injection being 5.6%. For the same three-component system, the reconstructed spectra were compared with the observed spectra from the single standard injections. For all the reconstructed spectra, the similarity was 0.9998 or greater, indicating a near perfect match to empirical data.

**LCGC:** With these capabilities, where do you see PDA peak deconvolution fitting into pharma and biopharma workflows?

**DOMANSKI:** With its ease of implementation, this technique can potentially replace complex multi-dimensional separation or time-consuming low-flow, high-capacity columns that cannot provide rapid results in a user-friendly way. As International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) and other regulatory bodies continue to tighten requirements for impurities detection, PDA peak deconvolution is an additional arrow in the quiver of analysis with its ability to detect low-level coeluting impurities. By identifying these species at the beginning of drug development, time can be saved throughout the downstream process. For early-stage high-throughput screening, PDA peak deconvolution could allow for faster injection cycles while maintaining the quality of data.

In a medicinal chemistry setting, deconvolution could be used in UHPLC reaction monitoring to look for hidden peaks or coeluting intermediates or be applied to re-injections of purification fractions as a secondary check of purity. As the drug-development cycle continues, any in-silico testing that uses absorbance can be augmented by using PDA peak deconvolution to provide information on coeluting peaks without the need to spend additional time on LC method development. And for groups that may have experience with PDA peak deconvolution, such as analytical method research and development, the ability to use this powerful technique integrated directly into the instrument software provides unprecedented access and ease.

**LCGC:** Is Shimadzu planning future development of this technique?

**DOMANSKI:** We are developing a new generation of the PDA peak deconvolution algorithm that will improve performance in terms of sensitivity and reliability for some of the most difficult use cases. Our international team is working in cooperation with pharma users to optimize the feature for lower detection limits, even when there is minimal on-column separation.

For more information, visit SolutionsForPharma.com.
Profiling Free Naphthenic Acids in Produced Water Using Gas Chromatography Coupled to High Resolution Accurate Mass Spectrometry

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Naphthenic acids (NAs) are naturally occurring organic acids found in crude oils. NAs are one of the main classes of acids found in produced water (PW). In the petroleum industry, the need to stabilize oil–water emulsions leads to a demand for reliable methods for compositional analysis of these emulsions. Gas chromatography coupled to mass spectrometry (GC–MS) is one of the most important techniques for NA profiling. Traditional methods employ a derivatization step to improve the volatility and detectability of NAs. In this work, we propose a simple method for profiling free NAs in PW by GC–MS. Eight analytical columns were carefully selected by evaluating metrics, such as normalized analyte response and select chromatographic parameters. The best-performing columns were used to analyze NAs extracted from a real-world PW sample. The analytes were detected by Fourier-transform orbital ion trap mass spectrometry using chemical ionization in the negative mode (NCI). The combination of the selected columns and the enhanced signal-to-noise ratio (S/N) attained by the GC-NCI-high resolution, accurate-mass mass spectrometry (HRAM–MS) method made it possible to detect and identify several homologous series of acidic oxygenated compounds ranging from aliphatic to aromatic analytes.

Produced water (PW) is the wastewater that comes out of the ground along with oil and gas during oil and gas extraction (1,2). PW is a complex mixture of organic and inorganic matter. Its exact composition depends on several factors, such as the location and age of the reservoir, its geological formation, the type of hydrocarbon produced, and the recovery methods employed (such as water injection) (1). The oil components in PW may be found in a dispersed or dissolved form (Figure 1) (3), and it has been reported that more than 1000 chemical components have been detected in PW samples. Examples of organic species include aromatic hydrocarbons such as benzene, toluene, ethylbenzene, xylene (BTEX), phenols, and naphthenic acids (NAs).

NAs are generally defined by the petroleum industry as naturally occurring organic acids derived from crude oil, comprising an acid-extractable fraction of petroleum including aliphatic, aromatic, and cyclic compounds, as well as organic components containing heteroatoms (oxygen, nitrogen, and potentially sulfur atoms) (4,5). However, the chemical definition restricts the molecular coverage of NAs to cyclic organic acids. NAs are described by the general molecular formula \( C_nH_{2n+2}O_Z \), where \( Z \) is a negative and even integer. The \( Z \)-value is proportional to the double-bond equivalent (DBE). For instance, linear and branched acids exhibit DBE values of 1 (\( Z = 0 \)), monocyclic acids show DBE values of 2 (\( Z = -2 \)), bicyclic acids exhibit DBE values of 3 (\( Z = -4 \)), tricyclic acids show DBE values of 4 (\( Z = -6 \)), and monoaromatic acids exhibit DBE values of 5 (\( Z = -8 \)).

NAs are surface-active compounds, participating in the formation and
stabilization of water and oil emulsions (4,6). It was reported that the structure of NAs plays an important role in regulating the interfacial tension of emulsions (7). In addition, NAs can significantly impact the environment because of their toxicity (4,5). Recent studies indicate that larger NA species exhibit higher toxicological potential than smaller ones (8,9). In this context, there is an increasing demand for methods to monitor the occurrence of NAs in complex mixtures, such as wastewater, to address operational problems and environmental issues that currently plague the petroleum industry.

A variety of analytical methods have been proposed for characterizing NAs. However, there is still much space to standardize and develop new methods to improve sensitivity, precision, and accuracy (10). Peak or signal overlap can occur in the instrumental measurements because of sample complexity, and because over 1500 species with a broad range of molar mass have been detected in a single NA mixture (11). Also, some species are detected at trace levels (12), making it necessary to include a preconcentration step prior to instrumental analysis. In this context, gas chromatography coupled to high resolution accurate-mass mass spectrometry (GC–HRAM–MS) is an interesting tool for analyzing NAs because of the improved (peak and mass) resolving power and sensitivity (13,14).

Derivatization reactions are common in GC–MS methods to profile NAs to increase their volatility (Henry...
TABLE 1: Columns phases and geometries evaluated for the analysis of free organic acids

<table>
<thead>
<tr>
<th>#</th>
<th>Column Name</th>
<th>MAOT (°C)</th>
<th>Stationary Phase</th>
<th>Geometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TR5</td>
<td>360/370</td>
<td>Poly(dimethylsiloxane) with 5% phenyl monomer incorporation</td>
<td>30 m × 0.25 mm i.d. (0.1 µm)</td>
</tr>
<tr>
<td>2</td>
<td>Supelcowax10</td>
<td>260/280</td>
<td>Polyethylene glycol</td>
<td>30 m × 0.25 mm i.d. (0.25 µm)</td>
</tr>
<tr>
<td>3</td>
<td>SLB IL60i</td>
<td>280</td>
<td>1,12-Ditripropylphosphoniumdodecane bis(trifluoromethanesulfonylimide)</td>
<td>30 m × 0.25 mm i.d. (0.2 µm)</td>
</tr>
<tr>
<td>4</td>
<td>SLB IL76i</td>
<td>270</td>
<td>Tri(tripropylphosphoniumhexanamido) triethylamine bis(trifluoromethanesulfonylimide)</td>
<td>30 m × 0.25 mm i.d. (0.2 µm)</td>
</tr>
<tr>
<td>5</td>
<td>Watercol 1460</td>
<td>260</td>
<td>Tri-(tripropylphosphoniumhexanamido)-triethylamine trifluoromethanesulfonate</td>
<td>30 m × 0.25 mm i.d. (0.2 µm)</td>
</tr>
<tr>
<td>6</td>
<td>RXI-17Sil MS</td>
<td>340/360</td>
<td>Poly(dimethylsiloxane) with 50% phenyl monomer incorporation</td>
<td>30 m × 0.25 mm i.d. (0.25 µm)</td>
</tr>
<tr>
<td>7</td>
<td>RTX-200MS</td>
<td>320/340</td>
<td>Poly(trifluoropropylsiloxane)</td>
<td>30 m × 0.25 mm i.d. (0.25 µm)</td>
</tr>
<tr>
<td>8</td>
<td>RXI-1301Sil MS</td>
<td>320</td>
<td>Poly(dimethylsiloxane) with 6% cyanopropylphenyl monomer incorporation</td>
<td>30 m × 0.25 mm i.d. (0.25 µm)</td>
</tr>
</tbody>
</table>

MAOT = maximum allowed operating temperature (in °C)

The careful combination of adequate column chemistry and the selectivity of negative chemical ionization (NCI) mode for compounds with labile hydrogens made it possible to detect more than 100 species of NAs in a real-world PW sample, proving to be a simpler and faster method compared to methods that use derivatization. Furthermore, this approach also reduced the generation of hazardous residues.

Material and Methods

Reagents and Solutions: The cyclopentanecarboxylic acid (CAS: 3400-45-1), cyclohexanecarboxylic acid (CAS: 98-89-5), benzoic acid (CAS: 65-85-0), cyclohexaneacetic acid (CAS: 5292-21-7), cyclohexanecarboxylic acid (CAS: 828-51-3), 1-naphthoic acid (CAS: 86-55-5), and myristic acid (CAS: 544-63-8) (Sigma-Aldrich) were used for column evaluation. A working solution containing the analytes at 0.5 mg/L was used to determine the chromatographic parameters. A 10 mg/L working solution of 1-decane (Sigma-Aldrich) was used to determine the normalized peak areas of the analytes. The technical mixture of NAs (Sigma-Aldrich; CAS: 1338-24-5; batch: BCBS3204V) was
used to prepare a simulated wastewater sample to evaluate the proposed method. All solutions were prepared using 2,2,4-trimethylpentane (isooctane) (Sigma-Aldrich). A real-world sample of PW was provided by the Leopoldo Américo Miguez de Mello Research and Development Center (Cenpes, Petrobras). Methylene chloride and sodium sulfate (Synth) were employed during sample preparation.

**Sample Preparation:** The simulated sample was prepared by spiking the aqueous matrix with the technical mixture of NAs at 0.5 µg/mL. The aqueous phase exhibited a sodium chloride concentration of 10% (m/v) to simulate the composition of a real PW sample. The aqueous phase was acidified to a pH value of 2 to ensure that all NAs were found in the neutral form for liquid–liquid extraction.

Liquid–liquid extraction (LLE) was performed with 5 mL of the PW sample and aliquots of 5 mL of methylene chloride. The extraction was repeated three consecutive times. The organic phases were pooled in a round-bottom flask with sodium sulfate. The volume of the extract was reduced to 1.5 mL of isooctane. This procedure was applied to both samples, simulated and real-world PW.

**Column Evaluation:** A total of eight columns (Restek Corporation, MilliporeSigma, Sigma-Aldrich, Supelco, and Thermo Fisher Scientific) were evaluated for analyzing free NAs, as shown in Table 1. All experiments employed the same GC method. The column selection was performed in a Trace 1310 gas chromatograph coupled to an ISQ transmission quadrupole mass spectrometer (Thermo Fisher Scientific). A Triplus RSH autosampler fitted with a 10 µL syringe was used to inject 1 µL of the sample. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The split–splitless (SSL) injector fitted a 4.0 mm i.d. precision liner (Restek Corporation) and was operated at 250 °C and a split ratio of 15:1. The GC oven was programmed as follows: isotherm at 100 °C for 5 min, followed by heating from 100 to the maximum allowed operating temperature (MAOT) at 6 °C/min—except for n-decane wherein the initial oven temperature was 50 °C. The MS transfer line and ion source temperature were 260 and 250 °C, respectively. Electron ionization (EI) was performed at 70 eV and 50 µA of emission current. The full MS spectrum was acquired with a mass range of 80 to 500 Da. A full width at half maximum (fwhm) resolving power of 3 × 10^6 and the ion injection time (IT) was set to automatic.

**Data Processing:** Xcalibur (Thermo Fisher Scientific) was used for instrument control and data collection. The normalized peak areas were determined by dividing the peak area of the analyte by the peak area of the n-decane.
For group-type analysis, the software Composer 2.0 (Sierra Analytics) was used. The parameters employed for elemental assignment were processing (without a signal-to-noise [S/N] filter); ion properties (allow adducts [H]); matching policy (5 ppm mass tolerance window, \( m/z \) range 80 to 500, and 0.01% of minimum abundance); heuristic rules mode (use hydrocarbon rules); and element range (Pass 1: maximum four oxygen; Pass 2: maximum four nitrogen; Pass 3: maximum four sulfur; and Pass 4: maximum three oxygen, three nitrogen, and three sulfur, in addition to the C and H ranges). Each assigned class was confirmed by matching of the fine isotopic pattern, peak shape restriction using the extracted ion chromatograms (EIC), and with the available analytical standards.

### Results and Discussion

**Column Evaluation:** Column selection is likely the most important step in method development. NAs are organic acids that exhibit Henry’s constant of \( \sim 8.56 \times 10^{-6} \) atm m\(^3\)/mol (4), which requires phases with adequate MAOT values to perform highly efficient separations. Also, the presence of the acidic moieties may likely influence peak symmetry, which warrants careful evaluation because little to no data are available for free NAs. In this context, it is important that column selection addresses unwanted analyte adsorption and peak shape. Unwanted adsorption can be estimated by comparing the normalized areas of the analytes, whereas the peak shape

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parameter</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclopentane carboxylic acid</td>
<td>Peak area</td>
<td>15.0</td>
<td>5.1</td>
<td>2.6</td>
<td>2.4</td>
<td>2.9</td>
<td>9.6</td>
<td>6.0</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>( T_e ) (°C)</td>
<td>109.1</td>
<td>205.5</td>
<td>152.9</td>
<td>180.2</td>
<td>204.8</td>
<td>113.7</td>
<td>105.5</td>
<td>121.9</td>
</tr>
<tr>
<td></td>
<td>( A_s )</td>
<td>0.5</td>
<td>1.8</td>
<td>2.3</td>
<td>1.8</td>
<td>1.4</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Cyclohexane carboxylic acid</td>
<td>Peak area</td>
<td>35.5</td>
<td>11.3</td>
<td>6.3</td>
<td>5.9</td>
<td>10.8</td>
<td>25.0</td>
<td>13.0</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>( T_e ) (°C)</td>
<td>124.2</td>
<td>218.7</td>
<td>166.4</td>
<td>189.3</td>
<td>214.1</td>
<td>130.6</td>
<td>119.1</td>
<td>138.1</td>
</tr>
<tr>
<td></td>
<td>( A_s )</td>
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<td>2.3</td>
<td>1.5</td>
<td>2.3</td>
<td>2.1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>Peak area</td>
<td>23.9</td>
<td>ND</td>
<td>6.5</td>
<td>ND</td>
<td>ND</td>
<td>53.6</td>
<td>12.2</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>( T_e ) (°C)</td>
<td>130.6</td>
<td>ND</td>
<td>196.3</td>
<td>ND</td>
<td>ND</td>
<td>144.9</td>
<td>126.2</td>
<td>148.2</td>
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<td></td>
<td>( A_s )</td>
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<td>ND</td>
<td>1.4</td>
<td>ND</td>
<td>ND</td>
<td>0.5</td>
<td>0.7</td>
<td>0.7</td>
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<tr>
<td>Cyclohexane acetic acid</td>
<td>Peak area</td>
<td>36.3</td>
<td>11.7</td>
<td>7.4</td>
<td>6.6</td>
<td>12.8</td>
<td>15.0</td>
<td>15.9</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>( T_e ) (°C)</td>
<td>137.3</td>
<td>229.3</td>
<td>178.0</td>
<td>197.5</td>
<td>223.0</td>
<td>152.7</td>
<td>131.3</td>
<td>151.4</td>
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<tr>
<td></td>
<td>( A_s )</td>
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<td>2.3</td>
<td>1.8</td>
<td>1.7</td>
<td>2.1</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Cyclohexane butyric acid</td>
<td>Peak area</td>
<td>41.7</td>
<td>14.7</td>
<td>1.7</td>
<td>9.6</td>
<td>16.8</td>
<td>45.3</td>
<td>18.6</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>( T_e ) (°C)</td>
<td>167.2</td>
<td>250.0</td>
<td>201.1</td>
<td>215.2</td>
<td>238.8</td>
<td>171.9</td>
<td>158.1</td>
<td>179.4</td>
</tr>
<tr>
<td></td>
<td>( A_s )</td>
<td>0.6</td>
<td>3.2</td>
<td>1.4</td>
<td>1.5</td>
<td>1.2</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Cyclohexane pentanoic acid</td>
<td>Peak area</td>
<td>29.4</td>
<td>23.4</td>
<td>13.7</td>
<td>11.6</td>
<td>68.4</td>
<td>27.0</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( T_e ) (°C)</td>
<td>180.7</td>
<td>250.0</td>
<td>209.1</td>
<td>222.1</td>
<td>ND</td>
<td>184.4</td>
<td>170.2</td>
<td>192.2</td>
</tr>
<tr>
<td></td>
<td>( A_s )</td>
<td>0.6</td>
<td>2.0</td>
<td>1.0</td>
<td>1.2</td>
<td>ND</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>1-Adamantane carboxylic acid</td>
<td>Peak area</td>
<td>29.2</td>
<td>10.6</td>
<td>16.5</td>
<td>11.7</td>
<td>26.2</td>
<td>54.8</td>
<td>20.8</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>( T_e ) (°C)</td>
<td>183.0</td>
<td>250.0</td>
<td>217.4</td>
<td>229.1</td>
<td>250.0</td>
<td>195.2</td>
<td>173.6</td>
<td>194.3</td>
</tr>
<tr>
<td></td>
<td>( A_s )</td>
<td>0.6</td>
<td>2.5</td>
<td>1.3</td>
<td>0.6</td>
<td>1.4</td>
<td>0.6</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>1-Naphthoic acid</td>
<td>Peak area</td>
<td>18.7</td>
<td>ND</td>
<td>16.4</td>
<td>5.0</td>
<td>ND</td>
<td>44.6</td>
<td>16.8</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>( T_e ) (°C)</td>
<td>183.0</td>
<td>ND</td>
<td>16.4</td>
<td>5.0</td>
<td>ND</td>
<td>44.6</td>
<td>16.8</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>( A_s )</td>
<td>0.7</td>
<td>ND</td>
<td>250.0</td>
<td>248.9</td>
<td>ND</td>
<td>221.7</td>
<td>195.6</td>
<td>ND</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>Peak area</td>
<td>13.3</td>
<td>10.6</td>
<td>10.8</td>
<td>4.0</td>
<td>34.2</td>
<td>34.2</td>
<td>11.4</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>( T_e ) (°C)</td>
<td>208.1</td>
<td>250.0</td>
<td>220.7</td>
<td>229.1</td>
<td>244.5</td>
<td>205.0</td>
<td>193.7</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>( A_s )</td>
<td>0.6</td>
<td>2.1</td>
<td>1.4</td>
<td>1.7</td>
<td>1.5</td>
<td>0.7</td>
<td>0.7</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = compound not detected. \( A_s \) = asymmetry factor (1.0 Gaussian shape, <1 net fronting, >1 net tailing). \( T_e \) = elution temperature. The peak area reported is the normalized value.
may influence the overall peak capacity of the separation (19). In this work, we evaluated the role of the column chemistry (Table 1) for analyzing the nine model analytes.

The first parameter evaluated in the method development was the MAOT, which dictated the highest temperature that may be employed during separation without deteriorating the stationary phase (chemical breakdown or phase pooling). As seen in Table 1, all polysiloxane-based phases exhibited superior values of MAOT compared to polyethylene glycol (PEG) and ionic liquid (IL)-based phases. Polysiloxane-based phases may be interesting choices for analyzing free NAs because they enable more efficient separations for less-volatile compounds. For instance, columns 2, 4, and 5 exhibited the highest retention of the late eluted compounds (Table 2). Conversely, the elution temperatures of the peaks were much lower than the MAOT of the column when using columns 1, 3, 6, 7, and 8. Consequently, this result narrowed the list of interesting columns for profiling free NAs.

Next, selectivity of the GC column was evaluated. Currently, there are two main approaches to describe the selectivity according to the polarity number or the solvation properties (20). The solvation parameter model (SPM) accounts for different interactions that may occur between the stationary phase and the analyte (solute). The model for retention of an analyte by gas and liquid chromatography is given by equation 1:

$$\log k = c + eE + sS + aA + bB + I$$  

[1]

where \(k\) is the retention factor for a specific compound; the uppercase letters are the solute descriptors; and the lowercase letters are the system constants. The system constants characterize the solvation properties of the phase and may be defined as the capability of establishing \(e\) (electron lone pair interactions); \(s\) (dipole-type interactions); \(a\) and \(b\) (hydrogen-bond basicity and acidity, respectively); and \(i\) (dispersive interactions and cavity formation). The \(c\) term is related to the phase ratio of the column (20). The solvation properties of the evaluated columns were estimated from previous reports (20–22) by considering phases with similar chemistries, as shown in Table 3. Although this approach might not accurately represent the actual solvation properties of the columns evaluated, it was acceptable to provide a solid discussion of the general trends observed in this report.

Columns 2 and 4 exhibited the largest values for the hydrogen-bond basicity, which is expected to generate the highest retention factors for NAs because of the presence of an acidic moiety in the analytes, as shown in Figure 2. In fact, the largest retentions (Table 2) were observed for columns 2, 4, and 5 whereas the retention factor of the analytes on column 5 were the highest values reported in this study. For instance, the use of the PEG-based phase (column 2) resulted in elution temperatures nearly 80 °C higher than that of polydimethylsiloxane-based phases (column 1). Although the system constants are not available for column 5, one can speculate that likely the \(a\)-system constant should be higher than that of columns 2 and 4 because of the presence of amide groups in the phosphonium-based IL, which is also supported by the consistently large values of retention factors.

Overlapped peaks like cyclohexanepentanoic acid/1-adamantanecarboxylic acid (columns 1 and 4) and 1-naphthoic acid/myristic acid (column 7) were observed. Four overlapped peaks were detected using column 8, namely cyclohexaneacetic acid/benzoic acid and cyclohexanepentanoic acid/1-adamantanecarboxylic acid. These results seem to indicate that columns with
higher $a$-values can generally provide baseline resolution of the NAs. However, careful evaluation of column 4 shows that other solvation properties may also play an important role ($e$ and $s$) in the separation of NAs. Furthermore, excessive large $a$-values shifted the elution temperature of the analytes towards the column’s MAOT, therefore narrowing the volatility range of the resulting method.

All columns exhibit asymmetric peaks. The largest asymmetry values were found for column 2 followed by columns 4 and 5. Column 3 exhibited values like polysiloxane-based phases, despite being an IL-derived phase. Most importantly, the largest normalized peak areas were found for columns 1, 3, 6, and 7. This result generally indicates reduced unwanted analyte adsorption during the separation (23,24). The selection of the best-performing columns (3, 6, and 7) was determined after careful consideration of the following criteria: column inertness, selectivity, and the MAOT of the column. Thus, the selected columns were used for profiling free NAs in PW samples.

### Produced Water Analysis:

The selected columns were used in analyzing two samples, a technical NA mixture and a real-world PW sample. Soft ionization was selected to assign the adduct ion, allowing for reliable attribution of the elemental composition of the corresponding peaks in the chromatogram. The analyte response was evaluated using both NCI and positive chemical ionization (PCI). The largest response factors were obtained in NCI. Also, the contribution of hydrocarbons (interfering compounds) was much lower in NCI compared to PCI. Hence, NCI was selected for the analysis of the free NAs.

The qualitative results showed that the total number of peaks detected using columns 6 and 7 was considerably larger than that of column 3 (Table 4). Similarly, the number of peaks with the general formula $C_{x}H_{y}O_{z}$ ($O_{2}$ class) was also consistently higher using columns 6 and 7 compared to column 3. The results obtained with columns 6 and 7 indicate the presence of NAs with carbon numbers ranging from $C_{z}$ to $C_{16}$, with DBE values from 1 ($Z = 0$) to 8 ($z = -14$). Representative compounds of monocyclic, adamantane, and aromatic acids are illustrated in Figure 3. These results showcased the importance of proper column selection for profiling free NAs in produced water. Although the current method was successfully applied to the detection of acids found in the range of low $\mu$g/mL, there are many additional sample preparation strategies under investigation to further improve the sensitivity of the method (25).

### Conclusions

In this work, a method for qualitative analysis of free NAs was successfully developed. Method development included careful column selection based on selectivity, chemical inertness, and chromatographic performance. The three best-performing columns (RXI-17Sil MS, RTX-200MS, and SLB-IL60i) allowed the profiling of NAs, but the most informative chromatograms were obtained with polysiloxane-based phases. Important classes of acids ($O_{2}$ class) were detected in the range of low $\mu$g/mL, including monocyclic acids, adamantane acids, and aromatic acids. The hope is that this proof-of-concept study illustrates the importance of column selection during GC method development.

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“Practical HPLC Simulator”: A Useful Freeware for Learning HPLC

Michael W. Dong, Perspectives in Modern HPLC Editor

The “practical HPLC simulator” is a freeware program that uses actual separation data to demonstrate the chromatographic principles under isocratic or gradient conditions and the impacts of instrumental, column, and mobile phase parameters on method performance.

In March 2020, the University of Geneva suspended all face-to-face courses and practical works during the pandemic. We began to teach through distance learning and developed an Excel spreadsheet to simulate the chromatographic analyses that our students in the laboratory performed. Previously, we acquired a database of ~4500 separation experiments (37 molecules, four columns, two organic modifiers, four mobile phase pH, two temperatures, and two gradient times) to allow the extrapolation of the chromatographic behaviours of the compounds. However, hundreds of hours of work were still required to integrate all the experimental data with the fundamental chromatographic equations to allow reasonable simulation.

We decided to offer this useful education tool to the scientific community as freeware from the School of Pharmaceutical Science’s website. The program has been downloaded 4000 times since its launch in November 2020. The program has received positive feedback from teachers and users, and we hope that this freeware will help the global practitioners to better understand the myriad intertwining factors in high performance liquid chromatography (HPLC).

Dr. Davy Guillaume
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About HPLC
High performance liquid chromatography (HPLC) is a widely practiced analytical technique used in research and product quality assessments (1,2). Although many analytical chemists learn HPLC in universities by taking separation science classes, most practitioners acquire working knowledge from their laboratory colleagues or by performing assays. Others learn from textbooks (1–5), short courses at national meetings (6), online content providers (7,8), and training sessions by equipment manufacturers or consumables suppliers. Nevertheless, most learning platforms are fee-based and have pros and cons that may not satisfy the needs of individual learners.

I recently came across an HPLC simulation program from the University of Geneva that offers a free learning platform for practicing scientists in augmenting their understanding of basic HPLC concepts. This article provides insights on how to get started and a case study on its use.

An Introduction and How to Download the Program
The Practical HPLC Simulator v.1.0 is a free excel spreadsheet with macros that provides simulated chromatograms from several separation data sets. Dr. Davy Guillaume developed the program at the University of Geneva’s School of Pharmaceutical Sciences with contributions from Balazs Bobaly and Jean-Luc Veuthey. This program can be downloaded from the following link: https://ispso.unige.ch/labs/fanal/practical_hplc_simulator:en

Figure 1 shows the main menu and the download links. The user should click on the blue button to download the spreadsheet with Macro and an accompanying document called the “Virtual HPLC work document” (brown button) that explains the terms and procedures.

A Demo Example
Once downloaded, the program allows the user to enter a data set on the column, the mobile phases, and the instrument settings and then observe the effects of these variables on the selected chromatographic examples. The user can “hover” the mouse pointed above the red triangle to view more information on the parameter and suggested values. The
user can explore freely and revert all entries to the original set by pressing the reset button on the top right corner of the main menu. Here is a step-by-step demonstration example that allows the user to get familiar with the simulation process:

1. Enter “UHPLC” system, mobile phases, column, and operating parameters (see Figure 2). The explanations and suggestions for each parameter are automatically given as .xls comments while entering the parameters.

2. Do simulations using data on separation option on mix #5 (salbutamol and impurities).

3. The resulting simulated chromatogram and method performance data are shown in Figure 3.

4. The user can continue to explore by altering parameters such as mobile phase A (MPA), mobile phase B (MPB), ultraviolet (UV) cell volume, monitoring wavelength, column parameters, such as length (L), internal diameter (i.d.), particle size (d_p), bonded phase chemistry, injection volume (V_inj), sample diluent, tubing dimensions, gradient or operating conditions, and dwell volume (V_d).

5. The resulting chromatogram and method performance data can be observed, such as retention time, resolution, peak width, asymmetry, peak height, peak capacity (P_c), and signal-to-noise ratio (S/N).

6. Significant contributions to the extracolumn volume are also calculated.

Rationales for the Initial Column Choice
Figure 4 shows the initial choice of column dimension (L and i.d.), d_p, and bonded phase. These parameters represent a rational starting point for initial method development used in a generic gradient method approach described elsewhere (9). The rationales for selection are as follows:

- C18 bonded phase is by far the most common stationary phase used in pharmaceutical analysis.
- The 50 mm x 3.0 mm column size represents an effective choice for initial method development, capable of fast separations with reasonable resolution.
- The sub-3-μm particle (2.7 μm) is a compromise choice for quality control applications, balancing efficiency performance and compatibility with HPLC and ultrahigh-performance liquid chromatography (UHPLC) equipment.

The “Standard Parameters” and the Resultant Chromatographic Performance
Figure 5 displays a screenshot of the standard parameters and the resulting chromatographic performance. These “standard parameters” constitute a convenient starting point of our simulation process.
The rationales for selecting these parameters are as follows:

- An acidic MPA is generally used to separate acids and bases with good peak shapes by controlling the ionization state of the analytes. A low pH of 2.7 using 0.1% formic acid is a common MPA in pharmaceutical analysis (10).

- A UV cell volume (1 µL) is needed for a short UHPLC column to minimize extracolumn band broadening (2).

- A time constant of 50 ms and a fast acquisition rate of 5 Hz are reasonable parameters for fast separations, affording enough data point collection without increasing too much the size of the instrumental files.

- The selection of column parameters, such as dimension (50 mm × 3.0 mm) and dp (2.7 μm), is based on the intended application described earlier. A column temperature of 35 °C is a “default” for column ovens without Peltier cooling.

- Sample mix #5 (salbutamol, a very common receptor active in bronchodilators against asthma and its four impurities) is selected as a relevant case study in pharmaceutical analysis.

- An injection volume of 2.0 µL at a sample concentration of 0.5 mg/mL is found to yield sufficient method sensitivity (limit of quantitation to 0.05%) for this pharmaceutical assay.

- Gradient parameters, such as the gradient range and the gradient time or the time for the gradient segment (t_G), are important considerations for separation resolution and P_c. Note that sample analysis time is dependent on t_G plus the column equilibration time. A broad gradient of 5–95% acetonitrile at a t_G of 2 min is a common parameter set used in high-throughput screening applications (2).

- Flow rate (F) is an important parameter to optimize analysis time and overall peak resolution. An optimum flow rate based on dp and column i.d. should be selected. A higher F is preferred in gradient separations for faster analysis time. The optimum F for the selected column is ~1 mL/min (2).

- A short length of small i.d. connecting tubing is preferred for UHPLC. A 50 mm, 100 μm i.d. connection tubing is optimum for UHPLC without excessive pressure drop.
Figure 5 also shows output data, including retention time, peak asymmetry, peak heights and peak area, and S/N ratio, as well as additional information on $R_c$ and the contribution to extracolumn volume.

The Simulation Sequence Used for Demonstrating the Effect of Operating Parameters on Method Performance

After establishing the “standard conditions” and examining the resulting chromatogram for the impurity analysis in mix #5, let’s examine a sequence of simulation results from changing vital parameters, such as $t_G$, gradient range, UV cell volume, outlet connection tubing i.d., detection time constant, and MPA pH. After each alteration, we can observe the method performance parameters, such as $R_s$, analysis time, $P_c$, and sensitivity, as shown in Figures 6–11.

Figure 6 shows the effect of extending $t_G$ from 2 min to 10 min to increase $P_c$ and $R_s$. $t_G$ controls the total analysis time, which includes any purging step and column equilibration time (2 min in the following discussion). As shown in the comparison below, the increase in $P_c$ and $R_s$ is dramatic but at the expense of a longer analysis time of 12 min (even though all peaks elute before 2 min). Note that the total time is the gradient time plus column equilibration time, which is about 2 min.

- Figure 5: $t_G = 2$ min, analysis time = 4 min, $P_c = 92$, $R_{1,2} = 1.43$
- Figure 6: $t_G = 10$ min, analysis time = 12 min, $P_c = 164$, $R_{1,2} = 3.33$

Figure 7 shows the effect of a narrower gradient range to increase $R_s$ without impacting analysis time. One way to increase $R_s$ without impacting analysis time is to choose a narrower gradient range of 5–30% B compared to the broad range of 5–95% B while maintaining a $t_G$ of 2 min. A narrower range would increase the $R_s$ of a specific range of solute hydrophobicity and is often used to increase resolution around the active pharmaceutical ingredient (API) peak in purity assays of pharmaceuticals (11).

- Figure 5: Range = 5–95% B, $t_G = 2$ min, analysis time = 4 min, $P_c = 92$, $R_{1,2} = 1.43$
- Figure 7: Range = 5–30% B, $t_G = 2$ min, analysis time = 4 min, $P_c = 63$, $R_{1,2} = 2.97$

Figure 8 shows the effect of replacing a small UV flow cell (1.0 μL) with a larger flow cell (10 μL). This simulation illustrates the effect of extracolumn broadening from using an HPLC-type UV flow cell with UHPLC-type columns. Note the substantial reduction of $P_c$ and the $R_s$ of the first two peaks.

- Figure 5: $t_G = 2$ min, analysis time = 4 min, $P_c = 92$, $R_{1,2} = 1.43$
- Figure 7: $t_G = 2$ min, analysis time = 4 min, $P_c = 63$, $R_{1,2} = 1.43$
- Figure 8: $t_G = 2$ min, analysis time = 4 min, $P_c = 69$, $R_{1,2} = 1.05$
Figure 7: The effect on a narrower gradient range of 5–30% B to increase \( R_s \) without impacting analysis time.

Figure 8: The effect of using a larger HPLC-type UV flow cell of 10 \( \mu \)L on \( P_c \) and \( R_s \).

Figure 9: The effect of using a larger i.d. outlet connection tubing (250 \( \mu \)m vs. 100 \( \mu \)m) on \( P_c \) and \( R_s \). Similar to the band broadening effect of using a bigger UV flow cell, a large i.d. outlet connection tubing can be deleterious to \( P_c \) and \( R_s \), as shown in Figure 9. A longer connection tubing can have a similar broadening effect. In contrast, larger inlet tubing is more tolerable in gradient analysis because of the “band focusing effects” in reversed-phase LC (2).

- Figure 5: \( t_0 = 2 \) min, analysis time \( = 4 \) min, \( P_c = 92, R_{1,2} = 1.43 \)
- Figure 6: \( t_0 = 2 \) min, analysis time \( = 4 \) min, \( P_c = 92, R_{1,2} = 1.43 \)
- Figure 7: \( t_0 = 4 \) min, analysis time \( = 2 \) min, \( P_c = 92, R_{1,2} = 0.94 \)
- Figure 8: \( t_0 = 1.44 \) min, analysis time \( = 4 \) min, \( P_c = 93, R_{1,2} = 2.28, S/N = 682 \)
- Figure 9: \( t_0 = 2 \) min, analysis time \( = 4 \) min, \( P_c = 62, R_{1,2} = 0.94 \)

Figure 10 shows the effect of a slower detector time constant (500 ms) to \( P_c \) and \( R_s \) for fast eluting peaks, which can be substantial.

- Figure 5: \( t_0 = 2 \) min, analysis time \( = 4 \) min, \( P_c = 92, R_{1,2} = 1.43 \)
- Figure 10: \( t_0 = 2 \) min, analysis time \( = 4 \) min, \( P_c = 46, R_{1,2} = 0.67 \)

Figure 11 shows the effect of MPA pH at 7.0 on retention time and sensitivity at 220 nm. Replacing MPA of 0.1% formic acid at pH 2.5 with phosphate buffer at pH 7.0 increases both retention time and \( R_s \) since the basic analytes are less ionized (therefore less polar and more hydrophobic). Another advantage is a sevenfold increase of sensitivity at 220 nm (S/N of 682 vs. 90) because of the higher UV transparency of the phosphate buffer (lower UV cut-off). However, there is a loss of MS compatibility when using phosphate buffer.

- Figure 5: analysis time \( = 4 \) min, \( t_0 = 0.9 \) min, \( P_c = 92, R_{1,2} = 1.43, S/N = 90 \)
- Figure 11: analysis time \( = 4 \) min, \( t_0 = 1.44 \) min, \( P_c = 93, R_{1,2} = 2.28, S/N = 682 \)

Summary and Conclusions

Table 1 summarizes the effects on method performance from the various parameter change.

In this article, we demonstrated the use of a practical LC Simulator as a learning and teaching tool. We provided the rationales for adopting this common UHPLC column, operating, and instrument parameters as the “standard” conditions. We demonstrated how these operating parameters may affect the separation in a case study on impurity testing by observing the resulting chromatograms and method performance.
performance. The simulation case study sequence examines the effect of $t_0$, narrow gradient range, detector cell volume, the diameter of outlet tubing, detector time constant, and mobile phase pH and transparency.

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The Special Requirements of Biomolecules in HPLC Analysis

Biomolecules such as proteins, antibodies, or viral vectors are becoming increasingly important therapeutics, as evidenced by clinical trials and ultimately drug approvals. But they are also significantly different from small molecules, which affects their high-performance liquid chromatography (HPLC) analysis.

LCGC EU: Why is there currently a focus on biomolecules in the pharmaceutical landscape?  
KRUMM: This is not a recent development. The first biotechnologically produced molecule was approved in 1982, recombinant insulin. Since then, we do have more molecules, different kinds of molecules. Beyond peptide hormones, such as insulin, there are recombinant enzymes, monoclonal antibodies (mAbs), and molecules derived from those. More recently, we have nucleic acid-based therapeutics.

Secondly, it got easier to fine-tune those molecules thanks to genetic engineering. We can either increase the efficacy or get rid of undesired characteristics and side effects, which are a few reasons why we are seeing an uptick in interest.

LCGC EU: Biomolecules and small molecules both employ HPLC for their analysis—is there a big difference?  
KRUMM: Yes, there is. The most obvious one is size. Small molecules are around 100 - 200 Daltons, whereas proteins are 100 - 200 kilodalton. A pore size of 6 nanometers might be sufficient for separating small molecules, whereas bigger molecules, such as mAbs, require bigger pores in order to enter them and interact with the stationary phase. For mAbs, this is 25 - 30 nanometers whereas gene-delivery particles need 100 nanometers or larger pores.

But it’s not only the size that is different: biomolecules are more complex molecules. For example, proteins are not just one string of amino acids that we analyze; there is a secondary structure, such as alpha helices and beta sheets, that fold together to a tertiary structure. This means we look at the surface of the molecule and analyze that. Apart from that, the complex structure of the molecule is very sensitive to the pH. For instance, an acidic condition tends to have more aggregates for protein samples. Also, the pressure from HPLC and especially ultra-high-performance liquid chromatography (UHPLC) analysis may harm the structure of a biomolecule. This is less of a problem for smaller molecules and smaller proteins or peptides, but the bigger the molecule, the more impact it has. mAbs, which are 150 kilodaltons, increase aggregation at pressures of 300 bar. Organic solvents also impact on the structure of the biomolecules. Proteins are denatured at too-high organic solvent concentrations. As such, we need to consider methods that work at aqueous conditions.

Other factors include heterogeneity within our samples due to post-translational modifications, for example. We also have a higher range of process-related impurities that come from the production process, meaning from the cells that produce the protein we want to analyze. All these things—the
post-translational modifications, the conformation, and the process-related impurities have an impact on the efficacy and/or the safety of our pharmaceutical product, which is why they need to be analyzed.

**LCGC EU:** Which separation modes meet these requirements for biomolecule characterization and impurity determination?

**KRUMM:** We need gentle and close-to-physiological conditions to analyze the protein as it is in our formulation. The best way to do this is with size-exclusion chromatography (SEC). It works with an isocratic elution at a modest pH and salt concentration around room temperature. It’s not only gentle with proteins, but it also allows us to analyze aggregates, which are immunogenic and need to be determined in the sample as well as fragmented protein.

Different sizes are not the only thing we are interested in. There are other types of chromatography required to analyze further characteristics and impurities. Ion-exchange chromatography (IEC) works with aqueous conditions. It starts with a no-salt concentration and elutes at high salt-concentrations, i.e., with an increasing salt gradient. IEC separates molecules as to the charge, which is important because biomolecules have different charge variants. This can be a result of post-translational modifications, oxidations, or reductions.

The third chromatographic mode that is typically used as an orthogonal method is hydrophobic interaction chromatography (HIC). It also works at aqueous conditions, starting with high-salt concentrations down to low-salt concentrations for elution. HIC, as the name says, separates molecules according to their hydrophobicity. In that regard, it’s similar to reverse-phase chromatography, though it does not require an organic solvent in the mobile phase. HIC is also able to detect aggregates, oxidations, and conformational changes, which alter the hydrophobic surface of molecules.

**LCGC EU:** Once separated, molecules need to be detected—are there also differences?

**KRUMM:** Yes and no. We are familiar with UV detection thanks to small molecules and biomolecules. But we are limited in the wavelengths used for proteins, 280 nanometers. This is where the aromatic side chains of proteins absorb. If this is not possible, proteins can be detected at 230 nanometers, where the peptide bond absorbs light; for nucleic acid-based drugs, it’s 260 nanometers, which is used for detection.

Small molecules also taught us about mass spectrometry (MS), which is used to analyze glycosylations and the precise mass of sample components, but we need to use volatile buffers. Methods such as hydrophilic-interaction chromatography (HILIC) or reversed-phase chromatography are directly compatible with MS but are less used for biomolecules with the exception of glycan analysis. For other biomolecules, we have the possibility to use SEC with volatile salts and buffers. Bigger molecules also means they are amenable to detection by multi-angle light scattering (MALS). It is very beneficial to detect aggregates as the technique is particularly sensitive for large molecules, making it possible to detect aggregates at very low concentrations.

**LCGC EU:** What does the future hold for biomolecule analysis?

**KRUMM:** The future will probably be designed by the technology itself, which is evolving. One of the latest methodologies is the attempt to mimic an interaction of biomolecules with receptors as they would take place in the body itself. One example is receptors binding the Fc parts of mAbs. These Fc receptors are placed onto an HPLC column, and antibodies bind or not, which allows us to analyze whether they would do so in the body. And therefore, we don’t only have to look at the structure and the physical characteristics of a biomolecule, but we have an indicator of the function and efficiency of the molecule directly from an HPLC analysis.

Another driver is the development of new biomolecules that need to be analyzed—look at gene delivery particles such as lipid nanoparticles, virus-like particles, or AAVs: they are much bigger than what we analyzed before. We already know that we can separate them using SEC, but they pose questions that we didn’t have to answer before: is there cargo in a gene delivery particle? We are trying to solve this using MALS, which gives the size of the molecule and its molecular weight. We will probably be surprised by what the future brings.

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Non-Targeted Food Analysis: How HRMS and Advanced Data Processing Tools Address the Current Challenges

Q. Why are non-targeted methods necessary for food analysis?
A: Liquid chromatography–high-resolution mass spectrometry (LC–HRMS) non-targeted methods can detect and identify new, unknown, and/or unexpected compounds and can be instrumental in pinpointing unsafe contaminants in food. They are often used in screening applications, such as olive oil authentication (1), or in response to adverse events, such as when milk and milk products were contaminated with melamine in China in 2008 (2), with Chinese authorities later reporting that approximately 53,000 infants suffered illnesses, with 13,000 hospitalizations and four deaths. They can also be used for retrospective analyses where previous data can be mined for newly discovered compounds of concern to determine if these chemicals have been detected previously, for example, retrospective analysis for transformation products of detected pesticides and veterinary drugs (3). Non-targeted methods are also useful in nutrient analyses and foodomics because they offer a more complete view of sample composition.

Q. What novel challenges have emerged that non-targeted methods are suited to solving?
A: The challenges we face today are not novel or different from the ones we faced a decade ago, but they have become more common, partly because of the globalization of the food supply which enables the year-round availability of a highly diverse array of foods regardless of the local climate/weather conditions. Currently, the FDA is using targeted methods for food sample analyses to verify that foods, domestic and imported, meet all applicable food safety standards. However, when targeted methods are not available, non-targeted analyses allow us to detect and identify new, unknown, and/or unexpected compounds.

Q. The techniques used in non-targeted analysis (NTA) generate a lot of data and this is especially true when analyzing a complex food matrix. What issues arise when this much data is generated?
A: Non-targeted datasets are extremely information-rich, which is incredibly powerful, but requires the use of software and processing tools to efficiently mine the data. Therefore, to ensure that the data and results generated are trustworthy, it is important to develop and implement quality control procedures that can be used to provide confidence in the sample preparation procedures, instrument operation, software, processing tools, and settings. In addition, NTA detects hundreds to thousands of compounds in a single food sample, most of which are inherent to the food, so we need tools and methods...
to quickly flag compounds of interest for further investigation or identification.

**Q.** Your recent paper focused on specific data processing tools that are currently available for NTA/suspect screening analysis (SSA) (1). What are some of the challenges that data processing tools can overcome?

**A:** Data processing tools are generally used to prioritize data and molecular features for further investigation, reduce false positives, and/or putatively identify detected compounds. Given that we typically detect thousands of molecular features in a single food sample, prioritizing features of interest helps us focus our identification efforts more efficiently. For example, for food safety applications we have used chemometric approaches, such as principal component analysis (PCA) or differential analysis, among many others, to highlight compounds and/or samples that need further scrutiny. These would be things such as compounds that are present in a suspect sample compared to a control sample.

Suspect screening analysis is one of the quickest ways to putatively identify sample components, where detected masses, molecular formula, and/or fragmentation spectra are screened against compound databases. But there can be tens to thousands of molecular structures with the same molecular formula, resulting in a high propensity for false positives. Retention time prediction, tandem mass spectrometry (MS/MS), and ion mobility spectrometry (IMS) can provide additional structural information to help eliminate some of these false positives and/or determine which functional groups are likely to be present in the molecular structure. In addition, suspect screening approaches are limited by incomplete databases. However, tandem mass spectrometry tools, such as in silico MS/MS, similarity searches, and molecular networking can be used to help bridge this gap.

**Q.** What is data quality and why is it so important?

**A:** Data quality refers to the accuracy, precision, reproducibility, and reliability of the collected data. In non-targeted analysis, data quality includes the observed mass accuracy and measured isotopic abundance of detected compounds, the chromatographic separation of compounds, for example, resolution and peak shape, and, of course, the number of mass spectra collected across chromatographic peaks, the reproducibility of detected compounds, retention times, and intensities across replicates, and so on. Like targeted methods, collecting quality data is necessary to obtain reliable and meaningful results. The amount of data generated and our reliance on processing software makes data quality particularly important for non-targeted analyses. Using pooled samples and/or large standard mixtures are two good examples of quality assurance/quality control (QA/QC) measures that can be employed to determine and demonstrate data quality (4,5). Ensuring good data quality is critical for attaining high-throughput analysis.

**Q.** What other measures would you recommend for obtaining high-quality data for non-targeted analysis?

**A:** Regardless of the methods used, it is important to implement quality control procedures to properly assess data quality. For example, we have developed a non-targeted standard quality control (NTS/QC) mixture containing 89 compounds covering a wide range of physicochemical properties, such as molecular weights 126–1100 Da, estimated log $K_{ow}$ range -8 to 8.5, amenable to electrospray ionization in positive and/or negative modes.
and diverse chemical classes (4). We successfully implemented the NTS/QC to measure critical data quality parameters, including observed mass accuracy within 3 ppm, isotopic ratio accuracy where most compounds matched with a score greater than 0.6 out of 1, and peak height reproducibility where greater than 94% of compounds had less than 20% relative standard deviation. This procedure also highlighted areas for improvement in our method. For example, we could improve the separation of early eluting polar analytes, and we found that background and/or matrix interferences were responsible for poor isotopic ratio matches for some compounds. It is also good practice to use a quality control mixture to monitor instrument performance over time to indicate if a new column is necessary or if the mass spectrometer requires cleaning. It is also important to randomize samples and to analyze blanks and pooled samples multiple times throughout the sample queue to check for carryover and drifts in instrument performance.

Q. There is a wide array of data processing software and algorithms available, the selection of which can impact on the results of a study. Are there any efforts being made to standardize these into a workflow for specific fields of study? And if so, what are the challenges towards achieving this?

A: There are a number of ongoing efforts to standardize and harmonize aspects of non-targeted analysis workflows, including those by several working groups such as the Metabolomics Quality Assurance and Quality Control Consortium (mQACC) (6), the Norman Network (7), and Benchmarking and Publications for Non-Targeted Analysis (BP4NTA) (8). One of the biggest challenges for standardization is broad applicability of non-targeted analysis even within a given field. For example, within food analysis, workflows used to investigate food safety, quality, and authenticity are often distinct from those used to investigate foodomics and nutrition. In addition, no single method is capable of detecting and identifying everything, as demonstrated by the EPA’s Non-Targeted Analysis Collaborative Trial (ENTACT) (9,10), so standardizing a single method will inherently exclude some of the chemical space that may be of interest. It is also important to consider that standardizing non-targeted workflows may result in stifled creativity and advancement of the field.

I think there are certainly applications and/or aspects of workflows that could benefit from standardization, such as thorough reporting of NTA methods and results. For example, using the NTA Study Reporting Tool (SRT) developed by BP4NTA (8). Other applications that could benefit from standardization would be QA/QC best practices, with efforts by mQACC (6) being notable, and quality control mixtures for performance assessment (4). However, standardization of all non-targeted workflows is likely not realistic and could be a detriment to the field.

Specifically considering processing workflows, a recent study found that there was only ~10% overlap of reported detected compounds using different software to process the same dataset (11). While this result is likely a combination of the different algorithms and chosen settings for each software, it is certainly striking and emphasizes the need for standardization efforts. Hindering these efforts, however, is software accessibility. Vendor-specific software is often very powerful and user-friendly, but can often only work with vendor-specific data and may be cost-prohibitive for some users. Open-source software gets around these drawbacks and can be more flexible but often requires more programming knowledge and/or linking of multiple algorithms to generate a full workflow. This increased flexibility makes the tools more powerful but also more difficult to standardize, although there have been efforts to do this in metabolomics (12).

Q. What exactly are molecular networks and what uses do they have?

A: Molecular networks rely on MS/MS data, where fragmentation spectra are generated by the dissociation of precursor ions in the mass spectrometer via collisions with gas molecules. These spectra are often searched against spectral libraries of known compounds to aid in compound annotation and identification. However, spectral libraries are not comprehensive. Molecular networks address this gap by grouping detected compounds into molecular families based on the degree of similarity between their fragmentation spectra. Often similar chemical structures generate similar fragment ions. This is useful for annotating and identifying unknowns and can also be used to highlight compounds of interest. For example, when investigating specific compound classes, molecular networks can help indicate which detected molecular features belong to that class. Similarly, in food safety applications, molecular networks could be used to indicate which compounds do not require further identification efforts because they are grouped with sugars or flavonoids and so forth. This means they are likely inherent to the food. Molecular networks can also help reduce false positive candidates for identification purposes.

Q. Machine learning approaches are increasingly common. What considerations must be taken before utilizing such tools? And what advice would you give to those who are looking to start using them?

A: A considerable risk of machine learning approaches is an over-interpretation of the
Machine learning models can also be sensitive to small changes in the data. While this is often a strength in medical imaging/diagnostic applications, this can potentially limit applications with mass spectrometry data. For example, background ion signals change with different mobile phases, columns, tubing, instrument setups, and so on. Here, it can be difficult to reliably apply the same machine learning model to classify samples over time and between laboratories because the model may differentiate samples based on changes in background ion signals as opposed to the chemical composition of the samples.

A good approach is to learn what types of applications machine learning works well for and why, and consider the limitations when deciding how best to implement it into desired workflows. Quality control procedures, such as known spiked compounds, should be used to test these models and ensure they are fit-for-purpose. Machine learning models are extremely powerful, and as more training data becomes available the performance and reliability should improve. I am excited to see how machine learning impacts non-targeted analysis in the future!

Q. What are you currently working on?
A: Most of my current projects aim to improve different aspects of non-targeted workflows. In one example, I’m developing a method for introducing calibrant ions into the source of our instrument during heated electrospray ionization (HESI) to enable automatic lock-mass calibrations to improve the measured mass accuracy of observed compounds, especially during queues lasting several days to weeks. This should also lead to better putative compound identifications. I am also working to further develop a standard mixture that can be used to evaluate non-targeted method performance, which we hope will be commercialized to increase accessibility for other non-targeted analysis researchers. I am investigating methods to improve identification of fluorine-containing compounds and to reduce false positive candidate matches using a combination of annotated data, such as retention time prediction, tandem mass spectrometry data, and so forth. Additionally, I am a member and co-chair for the BP4NTA working group, where I am collaborating with researchers in other fields to develop recommendations for the non-targeted community, including performance assessments of non-targeted data and results.

Q. Regarding the Benchmarking and Publications for Non-Targeted Analysis (BP4NTA) group, have you published any information so far and where can scientists go to find out more?
A: The BP4NTA group currently has two manuscripts in the review process. One of these serves as an introduction to the group and the types of challenges we are working on. The other introduces a tool called the “Study Reporting Tool” (SRT), which lists information that is useful to report in NTA studies so that these complicated methods can be fully understood and thus made reproducible. The SRT also includes a scoring system to help evaluate studies during manuscript and proposal review. I am also currently working with other BP4NTA members on a manuscript discussing performance assessments for non-targeted methods. If you would like more information or are interested in joining BP4NTA, please visit the website: https://nontargetedanalysis.org. Here, you can find a breadth of reference material regarding non-targeted analysis workflows, including defined terms, publications in the field, commonly used software tools, libraries/databases, and the SRT. We also include a list of sub-committees and their contact information in case you would like to learn more about our specific activities.

We have a blog where we post BP4NTA-relevant news and NTA job postings, and a “contact us” section where you can express interest and give feedback. We are a very welcoming group and love hearing new ideas on how we can develop tools, improve workflows, and tackle challenges that can help the broader NTA community.

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8) https://nontargetedanalysis.org/
Deep Well Plate
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www.microplates.com/draft/kingfisher-compatible-96-well-microplate/
Porvair Sciences Ltd, Wrexham, UK.

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www.shimadzu.eu
Shimadzu Europa GmbH, Duisburg, Germany.

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iSPE-HILIC cartridges and 96-well plates are designed for HILIC (hydrophilic interaction liquid chromatography) sample preparation in solid-phase extraction (SPE). They are particularly useful for the enrichment and purification of polar compounds, such as glycans, glycopeptides, and phospholipids, in proteomics, glycomics, and lipidomics.

www.hilicon.com
Hilicon AB, Umeå, Sweden.

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www.wyatt.com
Wyatt Technologies, Santa Barbara, California, USA.

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www.ionbench.com
ionBench, Sens, France.

High Performance Pulse Dampers
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https://www.knauer.net/
Knauer Wissenschaftliche Geräte, Berlin, Germany.
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