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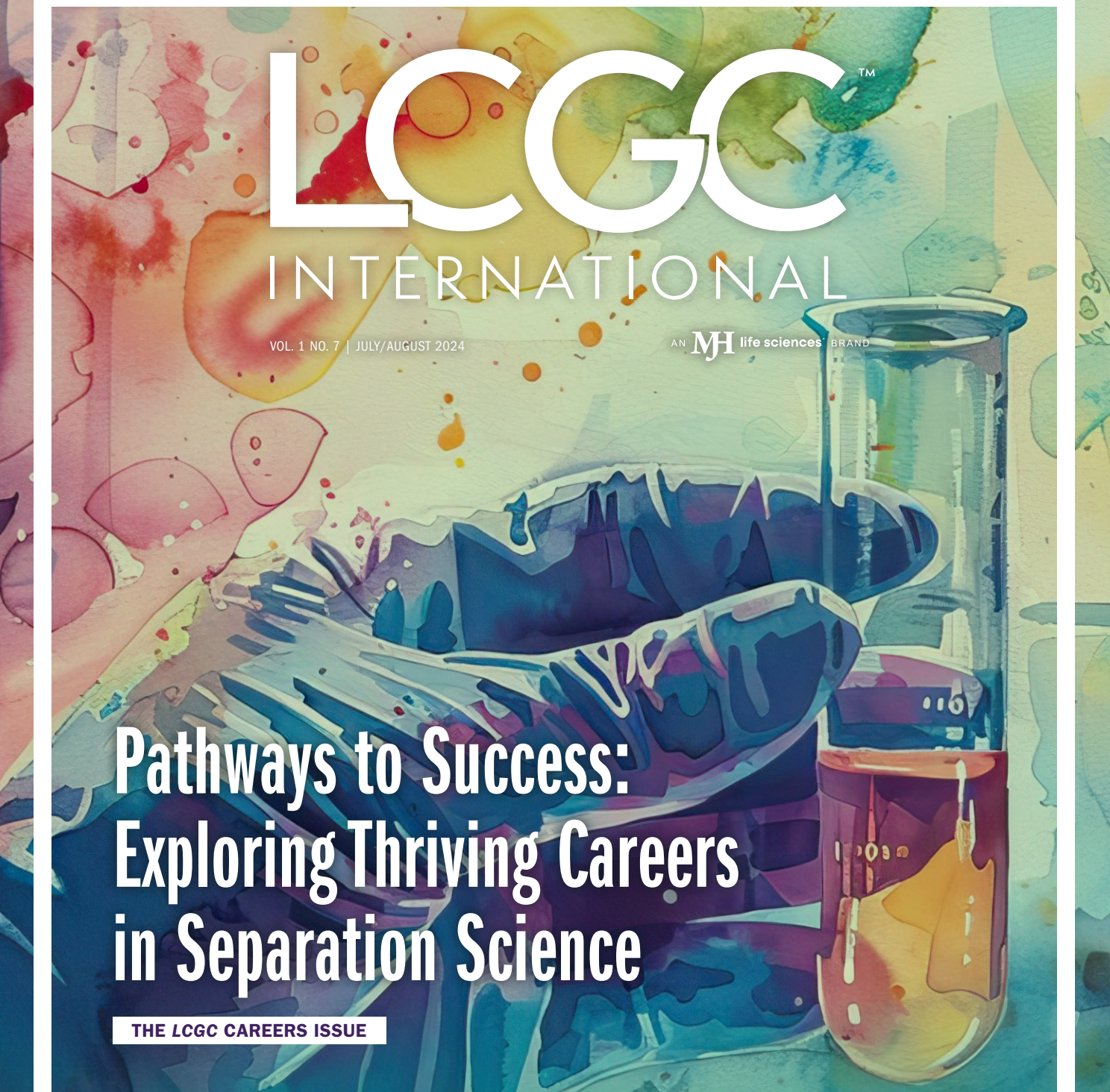

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CONTENTS

JULY/AUGUST 2024 | VOLUME 1 | NO. 7

COVER STORY

COLUMNS

SPECIAL SECTION

ON THE COVER

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Executive Editor of *LCGC International*, Alasdair Matheson, spoke to Jef Focant from the University of Lieges regarding his HTC-18 presentation.

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The Biggest Analytical Challenges in PFAS Testing

Tarun Anumol, global environment market manager for Agilent Technologies, spoke with *LCGC International* about how the company is addressing PFAS analysis challenges with its clients.

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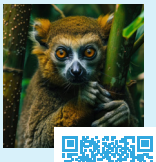

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Enhancing Lemur Breeding in Captivity with GC-MS

Researchers at the University of Wolverhampton have utilized gas chromatography-mass spectrometry (GC-MS) in their efforts to create new scent enrichments in their efforts to trigger mating behaviors of zoo-housed lemur species.

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For the Love of Separation Science

Podcast co-hosts Dr. Dwight Stoll and Dr. James Grinias talk with Dr. Andre de Villiers, Professor of Chemistry at Stellenbosch University in South Africa.

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NOTE FROM THE CEO

WELCOME TO THE July-August 2024 issue of *LCGC International!*

We're thrilled to bring you our annual "Salary Survey and Career Issue," packed with insights and knowledge to enhance your professional journey in separation science, as well as a mix of compelling columns and in-depth feature articles that we hope will both inform and inspire you.

Our "LC Troubleshooting" column, "Chromatographic Isolation of Sensitive Compounds: Challenges and Solutions," explores the intricacies of liquid chromatography (LC), and offers practical strategies to minimize compound degradation during the isolation process. This is essential reading for anyone looking to refine their technique and ensure the integrity of their samples during analysis.

Next, in the "Column Watch" section, we present "Development of a Standardized Protocol for the Classification of Column Sets in Comprehensive Two-Dimensional Gas Chromatography." This article explores the classification of GC×GC column sets based on polarity differences, known as orthogonality. The authors introduce the Century Mix, a standard reference mixture, to aid in the characterization of these column sets, providing a valuable resource for those working with GC×GC systems.

Our "GC Connections" column, "From Gas to Gas: Fundamentals of Static Headspace Extraction-Gas Chromatography," covers the fundamentals of headspace extraction, emphasizing the importance of chemical equilibria in achieving accurate and reliable results. These insights make this a must-read for those utilizing gas chromatography in their analyses.

Turning to our feature articles, we delve into the world of careers in analytical chemistry and chromatography with "The State of Jobs in Analytical Chemistry: A Deep Dive into the Latest Trends in Salaries, Jobs, and Career Growth in Chromatography." This informative article examines current salary trends, popular job roles, and strategies for standing out in a competitive job market. Insights from experts at the American Chemical Society and other industry groups add depth to this essential career guide.

Finally, we explore the importance of mentoring in "Unlocking Potential: The Crucial Role of Mentoring in Advancing Analytical Science." This article features reflections from key opinion leaders and the *LCGC International* Editorial Board members on the mentors who have shaped their careers. Their stories and advice provide a roadmap for finding and fostering impactful mentor–mentee relationships.

We hope you find this issue both enlightening and practical as you navigate your career in analytical chemistry. As always, we value your feedback and look forward to your thoughts on this edition.

Warm regards and happy reading! ■

Mike Hennessy, Jr.
President & CEO, MJH Life Sciences®

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Chromatographic Isolation of Sensitive Compounds: Challenges and Solutions

Fuh-Rong Tsay, Derek Henderson, Fatima Naser Aldine, Dwight Stoll, and Imad A. Haidar Ahmad

Liquid chromatography (LC) can be used to isolate compounds of interest from mixtures for further analysis, using techniques such as mass spectrometry and nuclear magnetic resonance (NMR). The conditions of such isolation methods can lead to degradation of the isolated compounds of interest, which is obviously a major problem if a goal of the isolation is to identify the compound of interest. In this installment of "LC Troubleshooting," we discuss strategies that can be used to minimize the likelihood of compound degradation in the isolation process.

PREPARATIVE CHROMATOGRAPHY is often essential in industrial settings to isolate highly pure target compounds from complex samples for purposes including structure identification, activity testing, quantitative analysis, mass spectrometric (MS) analysis, reaction monitoring, and developing analytical standards. Depending on the concentration of the target compound in the sample and the amount of purified material needed, the isolation can be carried out at the analytical scale (µg to mg), while preparative scale purification is required for larger quantities (> gram). Several challenges are frequently encountered during this type of isolation, including sample complexity, poor peak shapes, scalability, productivity, solvent and buffer selection, isolating purity and recovery, and barriers to automation. In this article, we discuss purification of a target impurity performed at low temperature to promote the stability of the target compounds. Compared to separations carried out at higher temperatures, the chromatographic profile observed under low temperature conditions shows excessive peak broadening. However, chromatographic performance is sacrificed in the interest of reducing degradation of the target compound. In this particular case, the temperatures of the autosampler, column compartment, and the fraction collector were all set to 5 °C. The highly aqueous fractions were dried using a rotatory evaporator operated at low temperature and pressure, while an azeotrope was added to facilitate water removal.

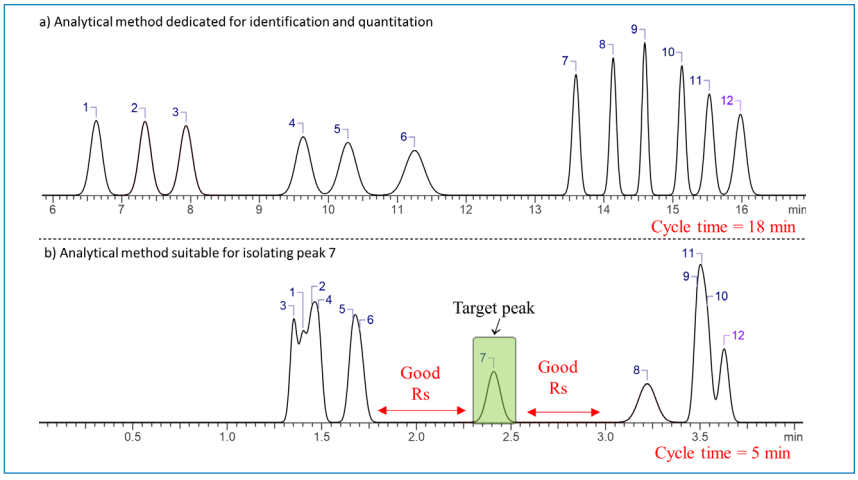

FIGURE 1: Simulated chromatogram for the separation of a warfarin mixture. (a) Analytical method suitable for quantitating and identifying all the individual peaks in the sample; (b) Method suitable for isolating peak 7.

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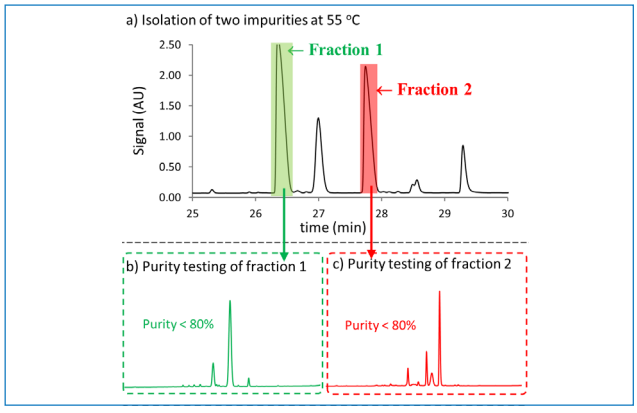

FIGURE 2: Isolation of two peaks of interest at 55 °C. (a) Separation of crude sample with two fractions collected as indicated by the shaded regions. Chromatographic conditions: Sample–66.7 mg/mL in 17% water (H_2O)/83% acetonitrile (ACN); Column–Ascentis Express C18; Detection–UV absorbance at 210 nm; Flow rate–0.6 mL/min; Mobile phase–0.05 % trifluoroacetic acid in H_2O (A) and 0.05 % trifluoroacetic acid in ACN; (b) Gradient conditions–5 %B (2.4 min), 5–65 %B (36.6 min), 65–95%B (0.1min), 95 %B (5min), 95%–5 %B (0.1 min), 5 %B (6 min); (b) Purity of fraction 1; (c) Purity of fraction 2. Chromatographic conditions: Column–Waters Acquity UPLC BEH C18, 150 mm x 2.1 mm i.d., 1.7 µm; Detection–UV 210 nm; Flow rate–0.5 mL/min.; Mobile phase–0.05% methanesulfonic acid in 98:2 (v/v) H_2O:IPA (A) and 98:2 (v/v) ACN:IPA (B). Gradient conditions–5 %B (0.5 min), 5–65 %B (7.5min), 65–95%B (0.1 min), 95 %B (1 min), 95–5%B (0.01 min), 5 %B (1 min).

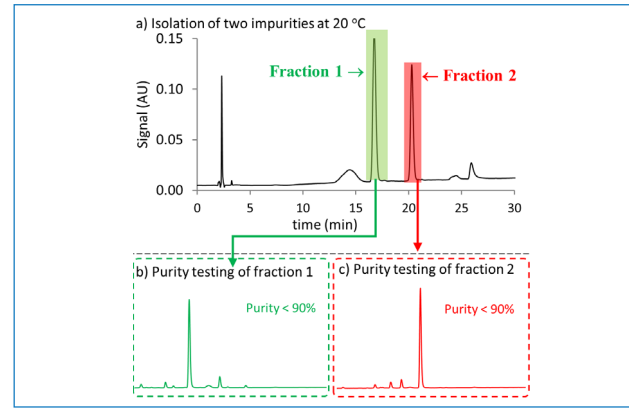

FIGURE 3: Isolation of two peaks of interest at 20 °C. (a) Separation of crude sample with two fractions collected as indicated by the shaded regions. Chromatographic conditions: Sample–66.7 mg/mL in 17% water/83% ACN; Column–Ascentis Express C18; Detection–UV absorbance at 210 nm; Flow rate–0.6 mL/min.; Mobile phase–0.05 % trifluoroacetic acid in H_2O (A) and 0.05 % trifluoroacetic acid in ACN (B); Gradient conditions–29 %B (3 min), 29-38.5 %B (18 min), 38.5-95%B (0.01min), 95 %B (6min), 95%-29 %B (0.01 min), 29 %B (6 min); (b) Purity of fraction 1; (c) purity of fraction 2. For chromatographic conditions of b and c, please see to Figure 2.

separation performance, as measured by metrics such as resolution or analysis time. There are several variables we can consider adjusting, including selection of stationary phase, selection of mobile phase solvents, buffer types and pH, column temperature, column dimensions, particle size, and gradient elution parameters (for example, gradient slope). Contemporary workflows very often involve the use of software designed for the purpose of systematically adjusting one or more of these variables as a means of guiding the method development process. We often prioritize conditions that enable detection of the largest number of analytes in a mixture with good efficiency and adequate resolution between individual components. An example of this is shown in Figure 1a, where there is adequate resolution between all the components of the mixture in a reasonable analysis time (1). The ultimate goal of a method like this is to separate the components of a mixture for identification and quantification of individual components, which can be implemented in applications such as quality control, forensic analysis, pharmaceutical analysis, research and development, and process optimization. An analytical method could be further validated and used for decades, especially in the pharmaceutical industry, for purity analysis, content uniformity, and impurity monitoring.

Chromatographic methods can also be developed for isolating target components from a complex mixture at the analytical scale (that is, µg–mg amounts). During method optimization in this case, the emphasis is on maximizing the resolution in the vicinity of the peak of interest while also reducing the method cycle time to maximize productivity. An example of this is shown in Figure 1b for the same sample as in Figure 1a. Here, resolution of compounds 1–6 and 9–12 is sacrificed in

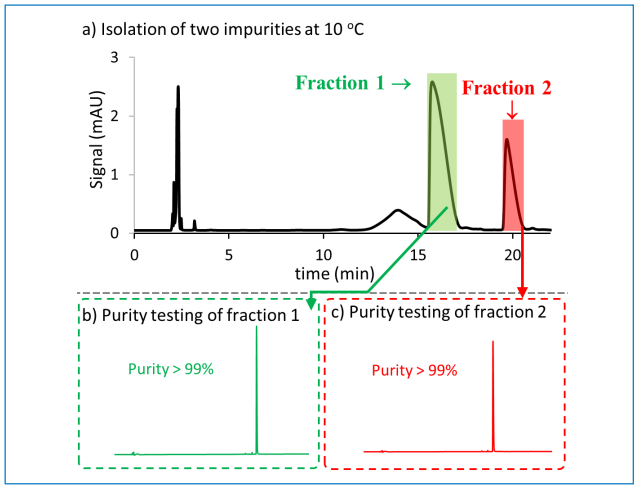

FIGURE 4: Isolation of two peaks of interest at 10 °C. (a) Separation of crude sample with two fractions collected as indicated by the shaded regions. Chromatographic conditions: Sample–66.7 mg/mL in 17% water/ACN; Column–Ascentis Express C18; Detection – UV absorbance at 210 nm; Flow rate–0.6 mL/min.; Mobile phase–0.05 % trifluoroacetic acid in H_2O (A) and 0.05 % trifluoroacetic acid in ACN (B); Gradient conditions–29 %B (3 min), 29–38.5 %B (18 min), 38.5-95%B (0.01min), 95 %B (3min), 95%–29 %B (0.01 min), 29 %B (4 min); (b) Purity of fraction 1; (c) Purity of fraction 2. For chromatographic conditions of b and c, see Figure 2.

the interest of very cleanly isolating compound 7. Maximizing resolution in the vicinity of the target compound increases the loading capacity for crude sample material, and maximizes purity and recovery. When developing an isolation method, our choices of stationary and mobile phases' chemistries are also more limited, because we need to anticipate the possibility of scaling the method up (for example, larger diameter columns and higher flow rates) and the need to remove large volumes of solvent from the collected isolate fraction(s). The use of volatile buffers and stationary phases that are commercially available in large diameter formats facilitates scaling up the method while generating highly pure isolates.

Sample Stability During Isolation

Drying isolates collected following preparative separations can pose challenges because of several factors including instability of isolated compounds, large volumes of collected materials, the presence of salts or nonvolatile buffering agents, and compound reactivity. Some situations involve more than one of these factors, severely complicating the drying process and the isolation of pure target materials (2–4). In the work discussed here, the isolation of two compounds at the milligram scale was required to establish their structures. Given the high concentrations of both compounds in the crude sample and the low amounts needed for further characterization work, the isolation was performed at the analytical scale. An analytical method was developed using Ascentis Express C18 using 0.05% trif-

luoracetic acid in water and acetonitrile at 55 °C, as shown in Figure 2a, with the peaks of interest shaded in green and red. The isolated fractions were assessed for purity before drying as shown in Figures 2b and 2c, and the purity was clearly less than 98% (a desired purity level at this stage). Several peaks were observed in analyses of the isolated fractions indicating significant degradation of the target impurities during the collection step.

To assess the possibility that degradation of the target impurities was promoted by the 55 °C temperature, the temperatures of the autosampler, column compartment, and fraction collector were dropped from 55 °C to 20 °C. The chromatogram obtained following these changes is shown in Figure 3a. It is clear that fewer peaks are observed, and in lower abundance, in Figure 3a compared to Figure 2a, which suggests that degradation of the target impurity was occurring during the chromatographic separation itself. Figures 3b and 3c show chromatograms for the fractions collected from the separation at 20 °C, where it is evident that the purity is significantly higher (> 90%) than for the fractions collected from the separation at 55 °C. However, the obtained purity is still less than the target level of 98%, which is required for establishing molecular structure and further characterization using other techniques. To further troubleshoot this issue, the temperature was further dropped to 10 °C, and the obtained chromatogram is shown in Figure 4a. The peak broadening observed here is due to operating the column at low temperature (5); however, the purity of the isolated peaks is higher than 99% (Figures 4b and 4c), confirming again that the degradation of the target impurities observed in the previous isolations at 55 and 20 °C occurs primarily during the fraction collection process itself.

The materials isolated at 10 °C meet the target purity level before drying, as shown in Figures 4b and 4c. It is critical to keep the samples at a temperature as close as possible to freezing to minimize degradation. Given that the collected fractions contain a lot of water, this poses a challenge of finding a suitable procedure for drying. Lyophilization was used in a first attempt to dry the material; however, this was not successful. Next, a rotary evaporator was used at low pressure (5 mbar), while maintaining the temperature of the water bath at 6 °C after using dry ice to cool the bath initially. To facilitate the removal of the last amounts of water from the material, small amounts of acetonitrile were added to establish an azeotrope. The final dried material produced using these conditions met the purity requirement and was successfully used for further characterization of the impurity compounds.

Summary

In this installment of "LC Troubleshooting," we have discussed work aimed at analytical scale milligram-level isolation of two impurities present in a crude reaction mixture. The chromatographic method chosen for isolation at low temperature (10 °C) shows inferior chromatographic performance compared to the original method developed at 55 °C. However, sacrificing performance as measured by traditional chromatographic metrics was critical to avoid degradation of the target compounds during the isolation process. In the final step, the collected fractions were dried by rotary evaporator at low temperature and pressure, with the addition of acetonitrile to the highly aqueous fractions, thereby establishing an azeotrope to facilitate faster drying.

This work is also a helpful reminder that we must always consider the possibility of material degradation during the analysis when developing analytical methods. We usually look for conditions (for example, mobile phase, column, and temperature) that provide the best selectivity and maximum number of observed peaks. However, it is important to keep in mind that some of the observed peaks could be generated in situ because of the influence of buffer or temperature on compound instability. ■

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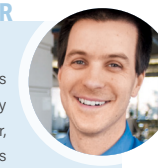

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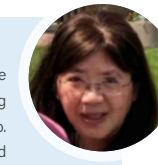

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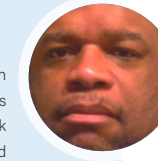

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is a Research Director within the Discovery Chemistry and Externalization department at Amgen, leading the Synthetics Separations team. His expertise is focused on method development, in-silico modeling, advanced analytical techniques, and purification.

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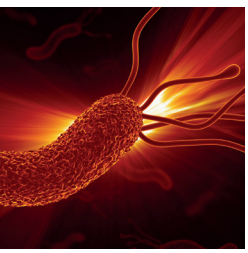

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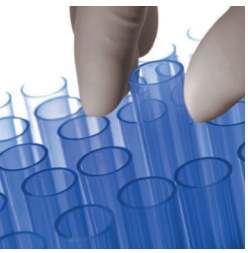

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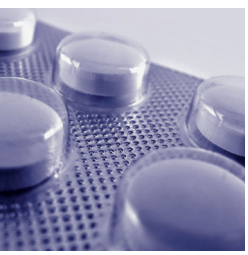

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Development of a Standardized Protocol for the Classification of Column Sets in Comprehensive Two-Dimensional Gas Chromatography

J.-M. D. Dimandja, J. Archer, J. Casanova, E. Posenecker, P.-H. Stefanuto, J.-F. Focant, D. Struk, P. Hesketh, C. Heist, and K. Rimmer

One-dimensional gas chromatography (1D-GC) stationary phases are generally classified according to their relative polarity into non-polar, semi-polar, and polar columns. In comprehensive two-dimensional gas chromatography (GC×GC), it is the polarity difference between the two tandem-assembled stationary phases that determines the selectivity of the column ensemble. This polarity difference is called *orthogonality*, and GC×GC column sets can be broadly categorized into four groups based on the direction of the serial coupling between the primary and secondary columns. A significant portion of the GC×GC column sets in use today are operated in *forward-orthogonality* mode, which means that the secondary column is more polar than the primary column. A growing number of reported GC×GC applications operate in *reversed-orthogonality* configurations, where the secondary column is less polar than the primary column. Very few examples exist of *non-orthogonal* column sets because of the fact that there is limited additional selectivity that the secondary column can offer over the separation that has already been achieved in the primary column when the two phases are either identical or close in polarity. The fourth group of GC×GC column sets, called *hybrid orthogonality*, involves the coupling of stationary phases with peculiar selectivity differences that manifest themselves in the bi-dimensional separation plots. In this work, we are presenting a normalized approach to GC×GC column set characterization that is based on the use of a reference mixture of standards called *the Century Mix*. The Century Mix contains 100 chemical probes of different functionalities that span a reasonable range of volatilities and polarities to capture the selectivity profile of any GC×GC column set. The Century Mix also contains important chemical probes that are used for 1D-GC column characterization (such as the Grob mix and the Rohrschneider/McReynolds compounds) to make some connections between the 1D-GC building block columns and the GC×GC column sets. We finally also outline some other important metrics of comparison that should be taken into consideration to assess the overall performance of a GC×GC system.

THE GOAL OF ANY chromatographic method is the full separation of all analytes of interest in the shortest period of time possible, with good peak shape attributes. Therefore, column selection is a very important decision in the method development workflow, and the classification of stationary phases is helpful in this process. In one-dimensional gas chromatography (1D-GC), the selectivity and performance characteristics of a stationary phase are provided through commonly used mixtures, such as the Rohrschneider/McReynolds compounds or the Grob test compounds (1,2). These mixtures contain chemical probes that provide information on the relative polarity of a stationary phase, where *polarity* is defined as the combined effect of a variety of specific interactions (electrostatic dipole, hydrogen bonding, electron donor-acceptor, and ionic or covalent bond formation) that are taking place between the solute and the stationary phase.

Selectivity in comprehensive two-dimensional gas chromatography (GC×GC) offers the advantage of increased separation power through the combined association of the properties of two coupled stationary phases. In addition, GC×GC separations are structured, and the two-dimensional (2D) contour plot chromatograms can be thought of as maps in which two principal gradients are displayed—the primary dimension is the *volatility gradient axis*, and the secondary dimension is the *polarity gradient axis*. This is why GC×GC chromatograms often feature discernible patterns of compounds with similar functional groups, which can be very useful in non-targeted analysis for the putative identification of unknown compounds before they can be confirmed with an analytical standard (3).

The exhaustive classification of all possible GC×GC column sets is not a practical undertaking because of the sheer number of possible combinations that exist. However,

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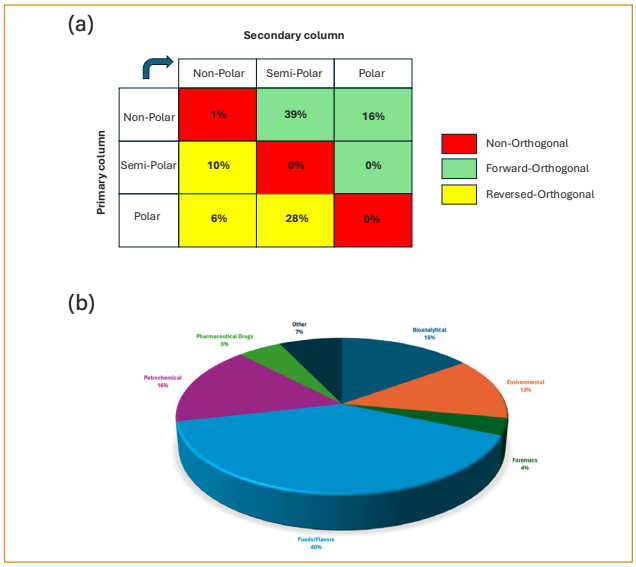

FIGURE 1: 2023 GC×GC peer-reviewed publication survey results: (a) Column-set classification; (b) Application area classification.

GC×GC column sets can be categorized into groups according to the relative polarities of the stationary phases that are being coupled, as shown in Figure 1a. A survey of 140 peer-reviewed publications from 2023 (using the keywords "comprehensive two dimensional gas chromatography" in SciFinder Scholar) shows that a significant portion of GC×GC (55%) projects are operated in "forward" (or "normal") orthogonality mode, where the primary column is less polar than the secondary column. These column combinations include the non-polar/semi-polar (39%), non-polar/polar (16%), and the semi-polar/polar (0%) sections in Figure 1a. However, a significant increase in the number of "reversed" orthogonality configurations (semi-polar/non-polar, polar/non-polar, and polar/semi-polar) is noted (44%), which is more than triple the output from the last review by Focant and Stefanuto where this type of survey had been reported (4). This is a positive trend to observe because it means that GC×GC method developers are starting to use more of the possible selectivity that is available. Figure 1b shows a pie chart of application areas from the same survey data. Food and flavor methods seem to be on the rise (40% of the reported work), with a healthy balance between petrochemical, environmental, and bioanalytical applications. It should also be noted that forward and reversed orthogonality column sets are used in all these application areas, and the choice of the column set in GC×GC is more dependent on the nature and the diversity (in terms of functional groups) of the analytes to be separated than on the application area.

It has been generally accepted that the quality of a separation in GC×GC is dependent on the polarity difference (termed *orthogonality*) between the primary and secondary columns, and it is not surprising that the column sets that are classified as non-orthogonal

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TABLE I: Overview of the Century mix compound composition.

Compounds	Function
Alkanes	Mark the direction of the primary column separation gradient. Hexane(C_6) to Eicosane (C_{20}) range.
Aromatic hydrocarbons	Mark the direction of the secondary column separation gradient. Benzene (1-ring) to Phenanthrene (3-ring) range.
Homologous compounds	Markers of the elution order in the secondary column separation gradient. Ten series of homologous compounds include ketones (C_6 to C_{12}), primary alcohols (C_5 to C_{12}), secondary alcohols (C_5 to C_{10}), FAMES (C_5 to C_{13}), alkyl benzenes (C_7 to C_{15}), alkenes (C_8 to C_{11}), alkyl acetates (C_5 to C_8), carboxylic acids (C_4 to C_8), Lactones (C_6 to C_9), and phthalates (C_{10},C_{12}, and C_{14}).
Grob mix and McReynolds compounds	Markers of selectivity metrics connected to 1D GC.
Miscellaneous compounds	Selected marker compounds connected to a variety of application areas (bioanalytical, environmental, foods and flavors)

TABLE II: Retention index information of the generation of a predictive GC×GC plot. See text for more details.

Compound Name	Homologous Series Class	Retention Index Information		ΔI
		DB-17	VF-200	
Phenanthrene	Aromatic Hydrocarbon	2151	2054	-97
Naphthalene	Aromatic Hydrocarbon	1397	1335	-61
Benzene	Aromatic Hydrocarbon	764	726	-38
Nonylbenzene	Alkylbenzene	1692	1651	-42
Toluene	Alkylbenzene	871	838	-34
I-Decene	Alkene	1012	1006	-6
I-Octene	Alkene	809	804	-5
Hexane	Alkane	600	600	0
Eicosane	Alkane	2000	2000	0
I-Pentanol	Primary Alcohol	862	860	-1
I-Decanol	Primary Alcohol	1380	1387	7
Hexanoic acid	Carboxylic Acid	1137	1141	4
Octanoic acid	Carboxylic Acid	1325	1341	16
2-Pentanol	Secondary Alcohol	782	794	12
2-Decanol	Secondary Alcohol	1294	1313	20
Butanoic acid, methyl ester	FAME	823	852	30
Dodecanoic acid, methyl ester	FAME	1636	1680	45
Dimethyl phthalate	Phthalate	1771	1808	37
Dipropyl phthalate	Phthalate	2084	2136	51
2-Hexanone	Ketone	907	983	76
2-Dodecanone	Ketone	1515	1622	107
Y-Hexalactone	Lactone	1308	1449	141
y-Nonalactone	Lactone	1619	1801	182

(non-polar/non-polar, semi-polar/semi-polar, and polar/polar in Figure 1a) are not as highly used as the other combinations. However, demonstrations of non-orthogonal GC×GC systems do exist, and seem warranted when an on-column conversion of target analytes (because of a thermal or chemical process) into a related species is part of the analytical strategy for the method. Marriott and associates have recently reported the use of an Rxi-1 MS/DB-5 column combination for the monitoring of pyrethrin isomers in thermal isomerization reactions (5). At the other end of the polarity scale, Delmonte and associates demonstrated in 2013 the use of an SLB-111/SLB-111 column set for the separation of fatty acid methyl esters through the use of on-column hydrogenation in the modulator (6). It should also be noted that when columns with very different polarities are connected, the peak shapes of the analytes on one stationary phase can be affected on the other stationary phase, thus reducing the effective separation as well as the detectability limits. For these reasons, it is important to recontextualize our understanding of the orthogonality and selectivity concepts in GC×GC. It is our firm belief that there are more relevant column combinations to explore, and the goal of this work is the development of a standard mixture of compounds to help better categorize the grid of possible GC×GC column combinations.

The Century Mix

Even though petroleum-based samples (such as gasoline, diesel, or jet fuel) have been used for years to characterize GC×GC systems, it would be much more desirable to have a more standardized mixture for more effective comparisons. In 2003, an 80-compound mixture was developed for this purpose that was an extension of the Grob mix to better cover the 2D chemical space (7). This mixture, which was called the *Phillips mix* in honor of the late Prof. John B. Phillips (the inventor of GC×GC), has been progressively amended over the years for a variety of reasons, such as the removal of compounds that may reduce the stability of the overall mixture or the addition of compounds to capture a more reasonable portion of the 2D chemical space. The currently proposed mixture contains 100 compounds, and it is

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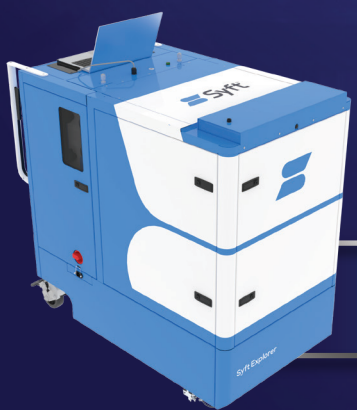

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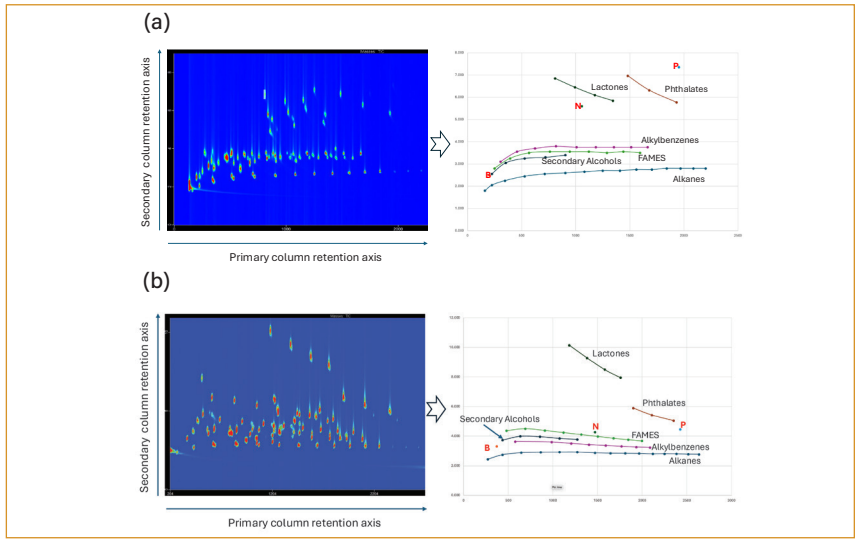

FIGURE 2: GC×GC contour plot chromatograms of two forward-orthogonality column sets, with accompanying peak-apex plots in which some of the homologous series are identified (for the aromatic hydrocarbon compounds, B is benzene, N is naphthalene, and P is phenanthrene). Retention times for the primary and secondary axes are in seconds. (a) Rxi-5Sil MS/Rxi-17Sil MS column combination; (b) ZB-5 MS/VF-200 column combination.

called the *Century mix* (see Table I for an outline of the compounds in the mixture in terms of their functions). Even though this mixture is not currently commercially available, an interagency collaboration effort supported by the U.S. Food and Drug Administration (FDA) and the National Institute of Standards and Technology (NIST) is underway for the development of this mixture to standardize instrument performance metrics in light of the many different variations of GC×GC instruments that now exist on the market today.

An important component of the use of this standard mixture is the normalization of operating conditions. At the current stage in the development, thermally modulated instruments are being used, and the parameters reported here are for these systems. The mixture is intended for use on all GC×GC systems, and an interlaboratory study is being planned for this evaluation. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Injections were performed on a split/splitless system (using a 100:1 split), and the injection volume was 1 µL. The primary column dimensions were 30 m in length, with a 250 µm i.d. and a 0.25 µm film thickness. The secondary column dimensions were two meters in length, with a 250 µm i.d. and a 0.25 µm film thickness. The primary column temperature program started at an initial temperature of 40 °C for 2 min, then ramped up at a rate of 5 °C/min to 230 °C where it was held for 10 min. The analysis run time was 50 min, and the secondary column oven was programmed at a rate 5 °C higher than the primary column oven. The modulator temperature offset was 15 °C higher than the primary oven, with a hot pulse time of 0.6 s. The modulation period was adjusted based on the elution time of the most retained analytes to avoid any wraparound peaks. The transfer line temperature into the time-of-flight mass spectrometer (TOF-MS) was 280 °C, and spectra were collected at a rate of 100 spectra/sec over a range between 35 and 500 amu. The ion source temperature was 225 °C, and the electron ionization energy was 70 eV.

Figure 2a shows a contour plot chromatogram of the Century mix on a forward orthogonality column set in which the primary column is an Rxi-5Sil MS primary column (30 m, 250 µm i.d., 0.25 µm film) connected to an Rxi-17Sil MS (2m. 250 µm i.d., 0.25 µm film) secondary column. The primary column separation range is marked by the alkanes (eluting at the bottom of the chromatogram) between C_6 (hexane) and C_{20} (eicosane). The direction of the secondary column gradient is marked by the aromatic hydrocarbons (benzene, naphthalene, and phenanthrene), which are eluted in increasing second dimension time, which indicate that secondary column polarity is higher than the first dimension polarity. The selectivity of the column set can be described by the elution order of the homologous series of compounds. In this case, the elution order goes as follows: alkanes < alkenes < secondary alcohols < primary alcohols < FAMES < carboxylic acids < ketones < alkylbenzenes < lactones < phthalates. The peak shapes of all the compounds in the mixture seem to be reasonably symmetrical, which indicates an acceptable separation. In addition to these basic comparison parameters, the other probes in the Century mix are used to establish connections to the 1D-GC metrics as well as other performance metrics (such as peak shape and efficient use of the two-dimensional chemical space), but these will not be discussed in detail in this paper.

Figure 2b shows the contour plot chromatogram of the Century mix on a different forward orthogonality column set than the one shown in Figure 2a. The primary column in this case is a ZB-5MS column (30 m, 250 µm i.d., 0.25 µm film) that is connected to a VF-200 (2 m, 250 µm i.d., 0.25 µm film) secondary column. Even though both columns belong to the "non-polar/semi-polar" category, the 2D separation gradient that is produced in Figure 2b is not identical to the one in Figure 2a, meaning that the selectivity of the second column set is slightly different than the first column set. One noticeable indicator of the selectivity difference is the elution order of the homologous series, which is as follows: alkanes < alkenes < alkylbenzenes < primary alcohols < secondary alcohols < carboxylic acids < FAMES < ketones < phthalates < lactones. It is interesting to note this elution order change for columns in the same classification category because it indicates that selectivity in GC×GC is not locked into a given general class of stationary phases, and that these gradients should be investigated in a little more detail. It will also be interesting to investigate the additional changes to the selectivity that can be produced with different operating parameters now that we can monitor

the differences between chromatograms of all target analytes in the Century mix.

Predictive GC×GC Chromatograms

Several groups have worked on predictive modeling in GC×GC over the past few years (8–12). In this paper, we would like to describe a very simple method to generate predictive GC×GC plots that might be helpful in looking at combinations of columns that may not have been attempted yet, but that may present some interesting selectivity options. The only requirement for this method is the generation of two chromatograms on the stationary phases of interest at the operating conditions described in Section 2 of this paper. These chromatograms will be obtained for a given set of target analytes of interest, and a set of alkanes to calculate retention index values for each compound, as shown in Table II. The predictive chromatogram is a representation of the temperature-programmed retention index of the first column and the specific index (ΔI) in the second dimension, which is obtained by subtracting the retention index of the first column from the retention index of the second column. The predictive GC×GC plots are representations of relative positions in the 2D plane (not exact retention times), and they can be helpful in determining which column combinations may offer some interesting selectivities. These plots can also provide a valuable visual estimation of the occupation of the chemical space for a set of target analytes when comparing different column sets. The value of these predictive plots is significant for individuals evaluating the potential utility of GC×GC in laboratories that do not have access to an instrument.

Figure 3a shows a predictive plot for a "hybrid" orthogonality column set, which was obtained with an Rtx-17 first dimension column and a VF-200 second dimension column (this plot uses the data that was generated in Table II for the full set of Century Mix compounds). Both of these columns are considered semi-polar, and traditionally would not be thought of as "orthogonal" enough to be useful in GC×GC. From the ΔI data in Table II, it is noteworthy that there are values above and below the alkanes, which is not commonly observed in GC×GC chromatograms. Forward orthogonality systems typically have the alkanes at the bottom of the chromatogram (least retained), whereas reversed orthogonality systems have the alkanes at the top end of the chromatogram (most retained). However, in 2012, Seeley and associates demonstrated an example of a separation using two semi-polar stationary phases (DB-210/HP-50 combination) that was able to separate fatty acid methyl ester (FAME) impurities from petroleum samples (13). The FAME compounds were selectively distanced from the rest of the petroleum sample at the bottom of the chromatogram, which was a very unusual result. Using the Century mix compounds, we observe a similar result, except in this case it is the homologous series of alkylbenzenes and alkenes that elute below the alkanes. The FAME compounds actually elute above the alkanes because of the fact that the trifluoropropyl stationary

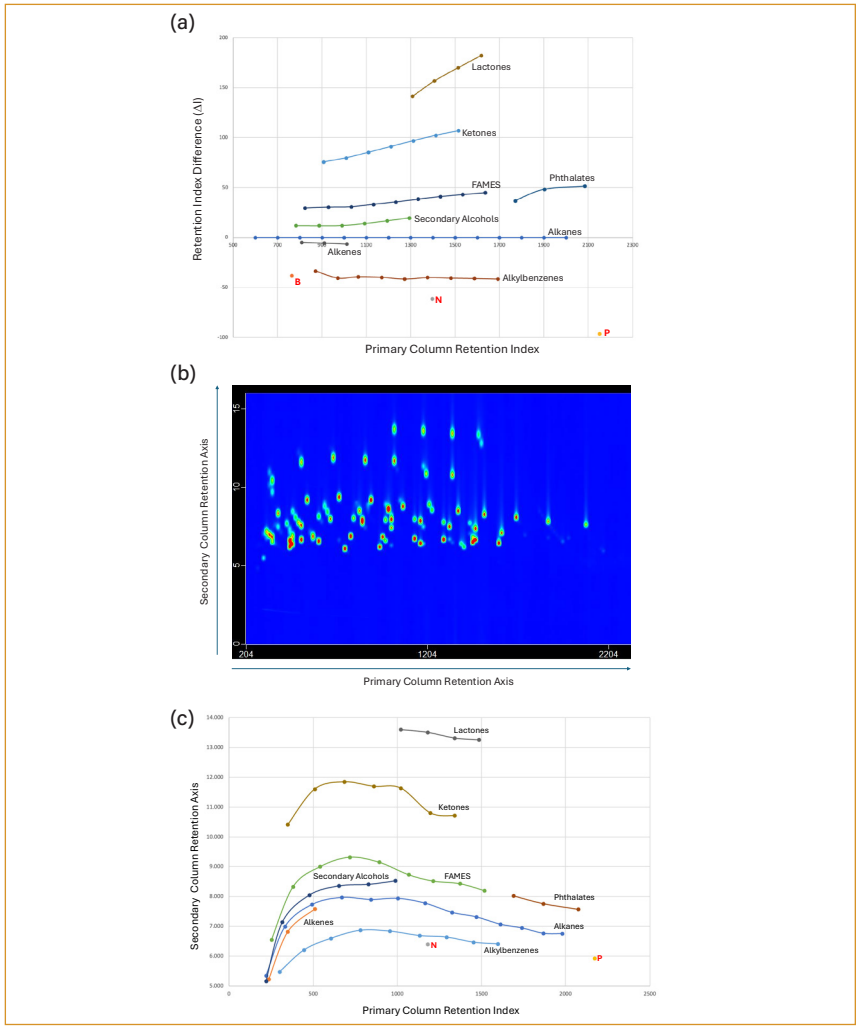

FIGURE 3: GC×GC Century mix analysis on a reversed-hybrid orthogonality column set. (a) Predictive plot in which some of the homologous series are identified (for aromatic hydrocarbon compounds, B is benzene, N is naphthalene, and P is phenanthrene); (b) Peak-apex plot in which some of the homologous series are identified. Retention times for the primary and secondary axes are in seconds; (c) Contour plot chromatogram. Retention times for the primary and secondary axes are in seconds.

phase is the secondary column and gives reversed selectivity to the Seeley column set, in which the trifluoropropyl stationary phase was used as the primary column.

The elution order of the rest of the homologous compound series is also correctly predicted in the plot (shown in Figure 3b). An actual contour plot of the GC×GC chromatogram is shown in Figure 3c, although the identity of the target analytes is harder to discern, because of the presence of many of the other compounds besides the homologous series of compounds. In anticipation of the unusual retention patterns that are to be expected in previously untried column combinations, we have prepared a set of confirmation mixtures that can be run separately in order to isolate specific functionalities when necessary. These subsets of the full mixture will also be important when using single channel detectors (such as flame ionization detectors [FIDs]) that do not provide the additional information on peak identity that a mass spectrometer (MS) is capable of generating. An internal standard (Quinoline) is also an available compound that can be added to the Century mix to generate signal ratio information that can be helpful when comparing the signal intensities from different detectors. This type of information is valuable when evaluating the potential of newly synthesized stationary phases, such as the polymeric ionic liquid phases recently reported by Ryoo, Anderson, and associates (14).

Conclusion

A better understanding of the separation gradients at play in the second dimension of the GC×GC chemical space is necessary to improve our analytical strategies for method development. We hope that the development of this standardized mixture will be helpful in advancing our knowledge of column selectivity, and we plan to investigate more column sets to gain further insight into the relationship between orthogonality and separation efficiency in GC×GC. We also firmly believe that a database of retention index information obtained at standard operating conditions needs to be developed on a variety of stationary phases in order to help with the task of column selection. Information on metrics such as peak asymmetry and peak width at a nominal concentration should also be available in this database to help with the development of the next generation of predictive modeling for GC×GC. ■

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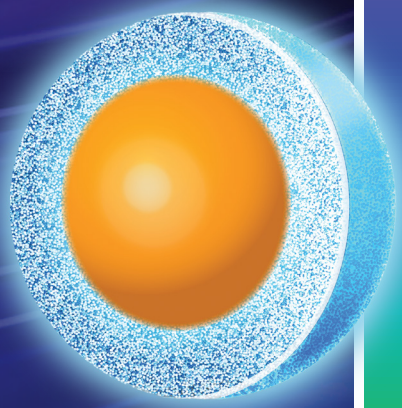

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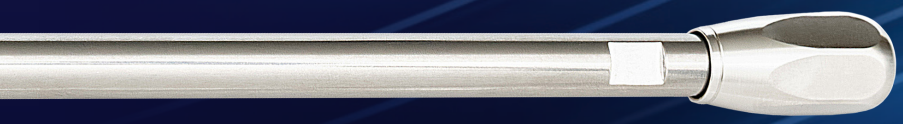

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From Gas to Gas: Fundamentals of Static Headspace Extraction-Gas Chromatography

Nicholas H. Snow

Headspace sampling is one of the staple sample introduction techniques for gas chromatography (GC) because it involves the injection of samples already in the vapor phase into the instrument. In this installment, we discuss the fundamentals of headspace extraction, including static versus dynamic extraction, establishing equilibrium in the vial, consequences of the partition coefficient, temperature, pressure, and transfer to the gas chromatograph. We see how, with consideration of the fundamental chemical equilibria involved, headspace extraction with GC can be one of the simplest yet most powerful and versatile chromatographic techniques.

HEADSPACE SAMPLING or extraction involves the collection of an aliquot of the vapor present, usually in a closed vial, above a liquid or solid sample placed within the vial. Not surprisingly, headspace sampling is among the most popular sampling or sample preparation methods for gas chromatography (GC), since, following the vaporization of sample components in the vial, the vapor is then injected or transferred to the gas chromatograph as vapor, eliminating the need for a liquid-based injection system (1,2). Most modern headspace sampling instruments still use the classical split or splitless inlet to complete sample transfer to the column.

Headspace extraction is usually performed in one of two ways. In *static headspace extraction* (SHE), a sample is placed in a vial, the vial is sealed (and possibly pressurized), and the sample-vial system is brought to equilibrium at a chosen temperature. Once equilibrium is established, an aliquot of the vapor within the vial is collected, either using a gas tight syringe or an automated set of valves and transferred to the gas chromatograph for analysis. SHE is most often used for routine applications where analyte concentrations in a typical liquid matrix are in the high part-per-billion (ppb) range or higher.

For lower concentration samples, *dynamic headspace extraction* (DHE), commonly called purge and trap, is often used. In this case, gas, usually helium or nitrogen, is continuously passed through the sample and over a sorbent trap, where vaporized analytes are sorbed and collected. Analytes in the tap are then desorbed into the gas chromatograph by flowing carrier gas through the trap and heating it. While SHE is an equilibrium technique, DHE uses the drive to equilibrium, as described in LeChatelier's Principle, and can qualitatively remove all the analyte from a sample. This allows determination of much lower analyte concentrations. Purge and trap is often used in ultratrace analysis of contaminants in drinking water and similar applications. Going forward, this discussion will focus on SHE.

The fundamentals of SHE are discussed in detail in the classic work by Ettre and Kolb, which, while written nearly 20 years ago, is still timely (3). They provide details about the theory and fundamentals of SHE, along with instrumental details and practical hints regarding vials, septa, and operational techniques.

Figure 1 shows a simple diagram of a setup for SHE-based analysis. In a typical instrumental setup, once a sample is loaded into a vial, the vial is then temperature- and pressure-equilibrated using heating and carrier gas to mildly elevated temperature and pressure. Care should be taken to ensure that the temperature is high enough to facilitate vaporization of analytes, but not too much that there is either vaporization of the solvent or liquid matrix or degradation of the analytes.

New users can get started with SHE very easily. All that is needed for a simple experiment with limited temperature control are a vial and a gas tight syringe. Simply place the sample in the vial and use the gas tight syringe to collect an aliquot of the vapor in the vial headspace, then inject the collected vapor into the gas chromatograph. This method is implemented in an

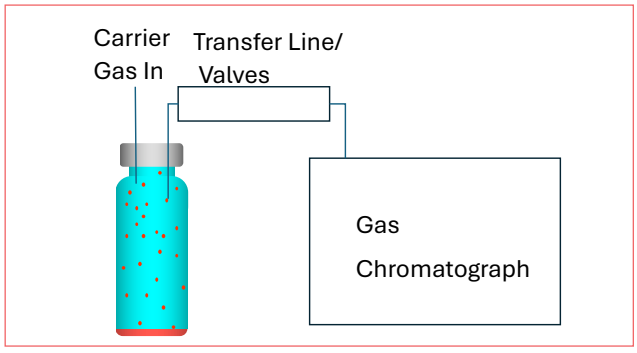

FIGURE 1: Simplified diagram of an instrument for SHE. The heated and pressurized vial is directly connected to the gas chromatograph using a transfer line and a series of valves to control the injected sample volume.

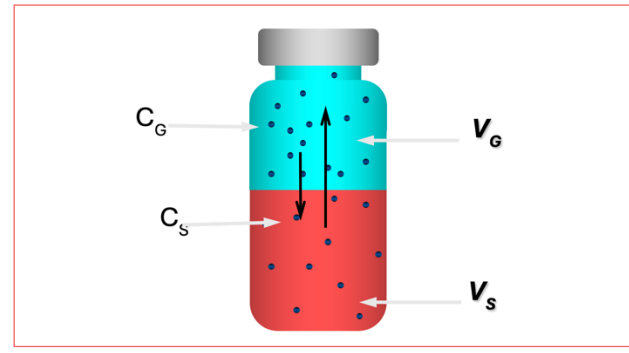

FIGURE 2: Diagram of a sealed vial for SHE. Red represents a liquid sample; blue represents the headspace; dots represent analyte. In this vial, there are 14 dots in the headspace and 10 dots in the sample, representing a partition coefficient (*K*) of 10/14 or 0.7.

automated version with temperature control of both the vial and syringe on today's rail-based autoinjectors.

Instrumental SHE devices are available from several vendors, and they generally operate on similar principles. The vial is heated and pressurized with carrier in a small oven with precise controls on the temperature and pressure. Through a series of timed valves and a transfer line, the vial is connected to a classical split or splitless inlet. The pressurized vial is opened to the inlet through the transfer line for a precisely controlled time to transfer an aliquot of the headspace to the gas chromatograph.

Figure 2 shows a diagram of a vial, prepared for SHE. The vial contains an aliquot of sample solution and is sealed at the top, usually using a septum and crimp or screw top. When allowed to stand long enough, the interior of the vial will come to an equilibrium condition, with masses of solvent and analyte vapor in the headspace above the solution determined by the partition coefficient of the equilibrium process.

Equation 1 shows the simple chemical equation and equilibrium constant expression that determines the concentration of analyte in the headspace above a sample solution. Note that this is very similar to the mobile phase–stationary phase equilibrium expression seen in gas chromatography and in other phase transfer equilibria such as liquid-liquid extraction. In this equilibrium constant expression, the partition coefficient is inverted when compared to gas chromatography. In gas chromatography, the solution phase is in the numerator (product) and the vapor phase is the denominator (reactant); in this equation, the vapor phase is in the numerator and the solution phase is in the denominator.

$$A\,(sol'n) \Leftrightarrow A\,(g) \qquad K = \frac{[A(g)]}{[A(sol'n)]} \qquad [1]$$

However, for method development in SHE, Kolb and Ettre derived an expression, seen in equation 2, that includes the partition coefficient, but it is expressed in a consistent manner with GC, with the solution phase in the numerator and the vapor phase in the denominator.

Equation 2 relates the conditions within a vial for SHE and the peak area obtained at the end of the analysis. The final peak area is proportional to the initial concentration of the analyte in the sample phase divided by the sum of the partition coefficient and the phase ratio. In SHE, the phase ratio is the same as for GC, the ratio of the vapor phase to the solution phase.

$$A \,\alpha\, \frac{C}{K+\beta} \qquad [2]$$

We can use Equation 2 and the fundamentals of solution-vapor equilibrium to examine several method development-related situations in SHE, including the impact of partition coefficient, phase ratio (sample size), temperature, and pressure. In equation 2, a proportionality symbol is used to account for variations due to conditions in the gas chromatograph that may influence the peak area, such as inlet conditions, detector choice, and response factor.

Temperature

As we learned in introductory chemistry courses and in the fundamentals of gas chromatography, increased vial temperature moves solution-vapor equilibria, as shown in equation 1, to the vapor phase. For the partition coefficient seen in equation 2, this would make the denominator smaller, decreasing the partition coefficient (making the denominator in the fraction smaller), increasing the value of the fraction, and therefore increasing the resulting peak area. This makes intuitive sense; higher vial temperature results in more analyte in the vapor, resulting in a higher peak area and all other variables being equal.

A second consideration with temperature is solute-solvent or matrix intermolecular interactions, commonly called *matrix effects.* If these effects are strong, temperature may have a lower-than-expected impact on vaporization and peak area. Essentially, strong interactions between the analyte and the matrix reduce the impact of temperature on the partition coefficient, reducing the impact on the final peak area. An interesting consequence of matrix effects can occur when non-polar solutes are dissolved in polar solvents at low concentrations. In this case, matrix effects can enhance vaporization as the non-polar solute is repelled by the polar solvent.

Phase Ratio and Partition Coefficient

This leads to a discussion of the relationship of the partition coefficient and the phase ratio, seen as a sum in the denominator of equation 2. In most SHE methods, the phase ratio, volume of the vapor divided by volume of the liquid, varies between about 1–20. Consideration of the phase ratio together with the partition coefficient can be very helpful in method development. If the partition coefficient has a similar order of magnitude to the phase ratio, often seen in the situations of weak interactions or matrix effects between solute and matrix, or with volatile analytes, then the phase ratio will impact the peak area, with a larger phase ratio increasing the denominator and reducing the eventual peak area. In this situation, the phase ratio should be minimized, but this will require larger sample volume.

If the partition coefficient is much larger than the phase ratio, the case with low volatility analytes or strong matrix effects, then the phase ratio has little effect on the final peak area. Beware that a very small sample volume with a volatile solvent can result in much of the solvent evaporating if the vial is heated, affecting reproducibility. If the partition coefficient is very low, much lower than the phase ratio, the situation with highly volatile analytes, then the phase ratio has a large impact on the peak area. In this case, sample volume should be very carefully controlled, as small variations in the volume will lead to variation in the phase ratio, leading to variation in the final peak area.

Vial Pressure

Pressure in the vial has a minor influence on the partition coefficient and no influence on the phase ratio. As we learned in introductory chemistry, adding an inert gas, such as the

carrier gases nitrogen or helium, does not change the equilibrium position of the reaction at constant volume. However, higher pressure in the vial may cause more of the vapor to be sampled into the transfer line in a given sampling time.

In SHE, the vial temperature, pressure, phase ratio, concentration of the analyte solution, and matrix effects all combine to determine the concentration of analyte in the vapor phase within the vial, the headspace. The analyte vapor concentration in the headspace then determines the mass of analyte, and the peak area is eventually determined in the gas chromatographic analysis. For reproducible quantitative analysis in SHE, like other extraction techniques, care should be taken to ensure that the two-phase system in the vial has reached dynamic equilibrium between the solution and vapor prior to sampling the vapor phase and injecting into the gas chromatograph. Failing to achieve equilibrium in an extraction system is the leading cause of reproducibility problems with analytical methods involving extraction, including SHE.

Conclusions

Static headspace extraction (SHE) is among the most common sampling and sample preparation techniques used in combination with gas chromatography. Conceptually, SHE is very simple: inject a sample of the vapor above a liquid or solid in a sealed vial. In practice, there are several method development considerations, based on solution or sample-vapor equilibrium, temperature, pressure and volume of the sample and vapor phases in the vial. Users can start with SHE by using a simple vial and gas tight syringe and can progress to fully automated instrumental configurations. Many autoinjectors include SHE capabilities as an option. ■

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The State of Jobs in Analytical Chemistry: A Deep-Dive into the Latest Trends in Salaries, Jobs, and Career Growth in Chromatography

Will Wetzel

The editors of *LCGC International* explore how chemists can set themselves apart in an ever-competitive job market, highlighting the latest data in salary and employment statistics.

THE MARKET FOR ANALYTICAL chemists remains strong despite the current ongoing challenges in the broader labor market. Jobs in chemistry and material scientists are projected to see 6% growth through 2032, higher than average, according to the Bureau of Labor Statistics (BLS) (1). There will be approximately 7200 job openings for chemists and materials scientists per year during this period of time, according to BLS (1). As older workers look toward retirement, younger workers will have new opportunities as they enter the workforce (1).

However, despite all these positive trends, analytical chemists, and workers in general, are dealing with a unique set of challenges in the current marketplace, and it is influencing their salaries, opportunities for advancement, and overall job satisfaction. Anecdotal evidence suggests that some analytical laboratories are still struggling to retain workers. Fewer technicians are specializing in separation science and tools are becoming more automated, meaning technicians are often less specialized.

This turnover could also be related to outside causes, such as inflation. To keep up with the rising cost of living, many workers are seeking to grow their income by finding another job. Nearly half (45%) of workers planning to switch jobs cited needing a higher income to make ends meet (2).

In this article, the editors of *LCGC International* dive deep into the latest employment trends and salary data for analytical chemists, providing tips on how employees can find new roles and opportunities at any stage in their career.

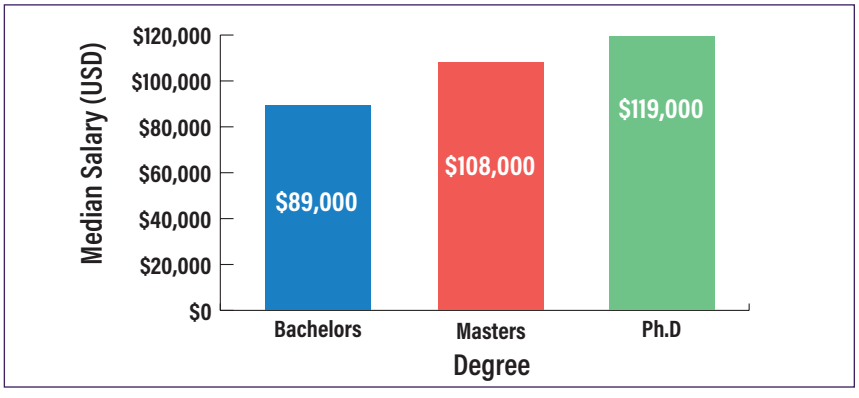

FIGURE 1: The American Chemical Society (ACS) 2023 data for analytical chemists based on degree. Data is presented in United States dollars (USD).

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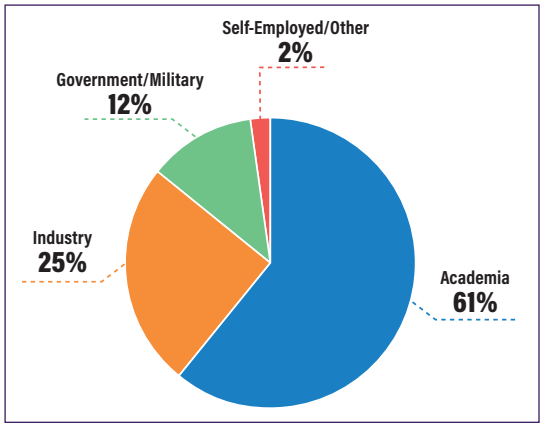

FIGURE 2: The American Chemical Society (ACS) 2023 data showing what fields analytical chemists are working in.

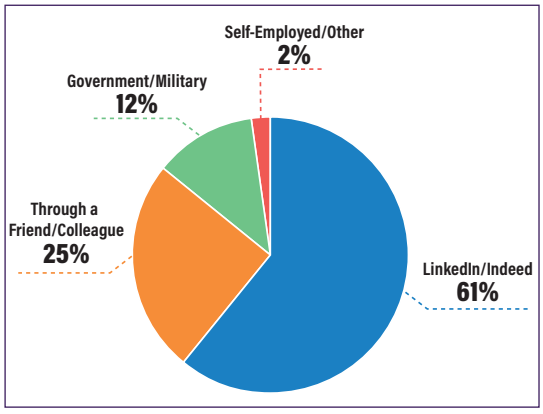

FIGURE 3: The American Chemical Society (ACS) 2023 data showing the most popular job search methods for analytical chemists.

Salary Trends and Data

Salaries for analytical chemists fluctuate based upon where the job is located, but across all employment sectors, pay for analytical chemists are faring well. In 2022, the American Chemical Society (ACS) surveyed 125,048 scientists affiliated with ACS to better understand pay in the industry (3). The data revealed that the median salary back in 2021 was, on average, $105,000, representing an approximately 7% increase from 2021 (3).

However, although salaries have risen, pay increases have not kept up with the rate of inflation. The ACS 2022 survey showed that salaries dipped in relation to the rate of inflation (3). The Royal Society of Chemistry (RSC) also reported a similar trend in the United Kingdom. Just 18% of their survey respondents said employers offered them a salary increase that matched or surpassed the rate of inflation, whereas 62% of respondents reported that they received a below-inflationary pay increase (4).

Currently, one of the big trends that analytical chemists are observing is how salaries compare between workers who received degrees in analytical chemistry and those who simply work in the analytical chemistry field. The median salary for those working in analytical chemistry is $89,000 with a bachelor's degree, $120,000 with a master's degree, and $131,000 with a PhD. However, the median salary for those receiving degrees in analytical chemistry is $92,000 with a bachelor's degree, $108,000 with a master's degree, and $119,000 with a PhD (Figure 1). This data suggests that a worker specializing in analytical chemistry in school does not necessarily come out ahead of the worker who landed in the analytical chemistry field without the specialization. The ACS survey also showed that across the board, workers with bachelor's degrees, master's degrees, and doctorates saw similar gains in overall salary (3).

A gender pay gap still exists in analytical chemistry. Men continue to make more than women on average, said LaTrease Garrison, chief operating officer (COO) of the ACS. However, according to Garrison, analytical chemist salaries for men and women are moving closer to equilibrium.

"Those that identify as male continue to make more than others, but the gap is decreasing," Garrison said. Data collected by Zippia also enforces this point; examining data from 2022, it is estimated that women make 98% of what men make in analytical chemistry, which shows that compared to other industries, analytical chemistry may be closer to pay equity (4).

Another factor that was examined in the survey is worker's sense of well-being and whether there was a strong correlation between workers feeling good about their well-being compared to their income. The RSC survey examined whether respondents were happy with their day-to-day life. About half of respondents (49%) said they were tired, while 33% felt stressed; meanwhile, 41% said they feel positive, and 30% were reported as feeling happy (5).

Zoe Ayers, president of the Royal Society of Chemists (RSC) analytical science community council, explained that the data showed that worker happiness is correlated to their financial situation.

"Data from the RSC's Pay and Reward 2023 report makes it clear that there is an inherent link between well-being and peoples' financial situation," Ayres told *Chemistry World* (5). "We also know that when scientists find themselves in a supportive environment this enables better science. With the ongoing cost-of-living crisis in the United Kingdom, ensuring scientists are being paid a fair, living wage can really help alleviate stress and anxiety over money worries, and improve well-being."

Job Market Trends

Analytical chemists often seek out roles in academia, industry, and government. In 2023, among the respondents to the ACS survey who selected analytical chemistry as their line of work, the majority (61%) were in academia. A further 25% were in industry, 12% were in government or military organizations, and 2% were either self-employed or in another field (Figure 2).

Of all ACS survey respondents in 2023, 10% cited obtaining a degree in analytical chemistry, and 53% of those people describe their current job as relating to analytical chemistry. For those in analytical chemistry industry careers, 37% described their job function as analytical services, and 25% described their job function as R&D applied research.

Although analytical chemists work across the country, several states, especially those with a greater population, stand out regarding having the most analytical chemists live and work in their state. Compiling data from 2021 to 2023, the ACS survey data showed

that California, Massachusetts, Ohio, Pennsylvania, and Texas had the greatest number of individuals with degrees in analytical chemistry. The states with the greatest number of respondents whose current job was described as analytical chemistry from 2021–2023 are California, Pennsylvania, Massachusetts, and Ohio.

Finding Jobs and Demonstrating Key Skills

Despite the current economic challenges, the job market for analytical chemists remains strong. However, there are numerous challenges that analytical chemists must navigate.

One is automation and its impact on jobs. Because of automation, it is becoming harder for workers to show their competency in conducting routine analysis (5). Instead, the ability to troubleshoot and having the background knowledge in using sophisticated instrumentation has gained a greater importance (6).

Because instruments are increasingly becoming more sophisticated as the technology develops, this trend is opening new opportunities for analytical chemists. For example, because of regulatory requirements that laboratories must comply with, quality assurance specialists are in high demand, along with quality control experts (6).

"For the past three years, there has been a slight increase in the number of individuals whose work specialty is analytical chemistry and people in the workforce with a bachelor's degree is growing," Garrison said.

The data from the ACS survey shows that 73% of those working in analytical chemistry had a PhD, compared to 64% of overall survey respondents.

To find a job, Garrison reported that individuals who obtained their degree in analytical chemistry and recently joined the workforce ($n = 42$) stated the most effective job search methods are websites, such as LinkedIn and Indeed (43%) and informal channels through knowing a colleague or friend (17%). For individuals whose work specialty is analytical chemistry ($n = 61$), they reported similar responses, with 56% selecting websites, 21% informal channels, and 7% faculty advisors (Figure 3).

For 2023 graduates who work in analytical chemistry, they indicated that participating in academic research (29%), summer undergrad research (21%), ACS student affiliates (12%), and internship/co-op experience (11%) during their academic tenure helped them join the workforce working in their field. ■

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Unlocking Potential: The Crucial Role of Mentoring in Advancing Analytical Science

Aaron Acevedo

LCGC International recently asked analytical scientists in industry and academia to share how mentorship has impacted their careers.

MANY SUCCESSFUL PEOPLE seek out the help of different mentors throughout their careers. The vast majority (92%) of Fortune 500 companies have mentorship programs, according to a 2023 report from MentorcliQ (1), which also noted that companies with mentoring programs increased their headcounts by approximately 4%, compared to an average headcount decrease of 33% for companies without these programs.

There's a reason why so many employers offer mentorship opportunities—data shows its key to individual success. Approximately 75% of executives credit their success to the help of a mentor, and 90% of employees with a mentor report being happy at work, according to an article in *Harvard Business Review (2)*. In analytical science specifically, mentorship is even more important, as early career scientists work to navigate a competitive job market.

Recently, the editors of *LCGC International* asked separation scientists at a variety of different points throughout their careers to share their experience with mentorship. In this article, you'll find advice from Elizabeth Neumann of the University of California, Davis; Emanuela Gionfriddo of the University at Buffalo; Bob Pirok of the University of Amsterdam; Ronald E. Majors, a consultant and formerly of Agilent Technologies; Michael D. McGinley of California State University, Long Beach; and Jim Grinias of Rowan University, on how to be an effective mentor and where to find a mentor at any stage in your career.

Who is a mentor that has impacted your career? How did you meet them, and what made them a great mentor?

NEUMANN: I'm very blessed in my life to have multiple people I call mentors. All of them have had such huge impacts in my life. The most prominent one is probably my undergrad professor, Touradj Solouki, Professor of Chemistry at Baylor University. He took the time to explain to me how graduate school worked, and he encouraged me to apply. He told me, based on my CV and my resume, what places I should be going to, and he believed in me, even when I didn't necessarily believe in myself. I think how I met him was that I just happened to be in his class; we connected in personality and interests, and he saw potential in me that I didn't even see in myself.

Another important mentor I've had is my graduate advisor, Jonathan Sweedler. He taught me how to be an analytical chemist in a rigorous way. How to pride myself on good research, to think creatively, to not be afraid to go after research avenues that other people think are too difficult to achieve. He has always valued things like mental health and well-being in a person. When I met him, I really liked him, and I really liked his story. He's a trained nuclear magnetic resonance (NMR) spectroscopist, but he switched to mass spectrometry (MS) in the middle of his assistant professorship. That was something I really admired in him—his ability to take chances and to be fearless in the pursuit of scientific integrity and achievement.

GIONFRIDDO: I had several mentors in my career, each with a unique contribution to my personal and professional development. A common characteristic among my mentors is that they never sugarcoated reality for me. They made me aware of the challenges that a career in academia poses and supported my efforts in achieving my goals, all while offering precious advice on how to build resilience, grit, overcome challenges, and how to sit back and enjoy the little victories along the way.

PIROK: I would say there can be no single mentor. I think you learn a lot of different lessons from different people throughout life that all can have a significant impact on your career. Mind you, they do not all have to be from your work environment. It can be from your partner or friends, who tell you to mind your energy, which, at crucial moments, allows you to perhaps take a step back and prevent burnout. It can also be a person who continuously support you, or even a reviewer that burns down your work and challenges you to do better. I guess logical people to mention are my promoters, Peter Schoenmakers and Ron

Peters, who taught me the basics of science and chromatography, or Arian van Asten, who never leaves an opportunity to burn my idea down, while always having my back (yes, you can do both simultaneously).

However, the person I most identify as a mentor is Dwight Stoll. I met him for the first time at—I think—the HPLC 2017 meeting in Prague, Czech Republic. I was a very enthusiastic PhD student who was presenting his work on a two-dimensional–liquid chromatography (2D-LC) separation system for the analysis of nanoparticles. Long story short, Stoll complimented me, but also quickly showed skepticism towards certain aspects. I will never forget it, because usually—out of politeness—people are very indirect and positive about the work. Since then, he has given me input on some of the crucial steps in my career.

MAJORS: In my career, there were four key people who, at different stages of my development, influenced my decision to become a chemist, specifically an analytical chemist. First was Mrs. Rogers, my junior year high school chemistry teacher at Roosevelt High in Fresno, California. She recognized my keen interest in chemistry and encouraged me to go to the next level. She made chemistry sound exciting in both the lecture room and in the laboratory. She kept me as a laboratory tech in my senior year where I helped prepare solutions and other chemicals for the chem laboratory and encouraged me to help (or at least try) to instill a love of chemistry to the junior students.

Second was George Kaufmann, an inorganic chemistry professor at my undergrad school of Fresno State in California, who encouraged me to do research in my freshman year in his laboratory and urged me to give talks at regional American Chemical Society (ACS) student affiliate meetings. Those talks gave me the confidence to stand up in front of a group to deliver a scientific lecture. Third was Ray Bremner, also of Fresno State. He was my quant professor and saw my interest in analytical chemistry, so he encouraged me to do research in this area and instilled in me the importance of sampling and sample preparation. Finally, there was L.B. "Buck" Rogers, my Purdue University graduate supervisor, who taught me how to think outside the box and how to do the critical experiments necessary to prove one's hypothesis. He also instilled in me the importance of writing up my results, telling me that, if you can't put in writing what you have accomplished, then nobody will ever know what you have done and contributed to science. All four of these teachers influenced my decision to become an analytical chemist.

MCGINLEY: Mark Zukowski. Mark ran the inflammatory disease program at Amgen in the late 1980s through the mid-1990s. Mark was not my supervisor, but was senior director and a key opinion leader in Amgen's early days. What made him a great mentor was, beyond just his scientific expertise, which was considerable. Zukowski knew how to motivate and

teach people. He built a team of excellent scientists and leaders who worked collaboratively to achieve great results together. Many of us who he mentored moved on to major leadership roles throughout the biopharmaceutical industry.

GRINIAS: One of the primary mentors that had a major impact on my career is Jim Jorgenson, who was my doctoral advisor at the University of North Carolina at Chapel Hill. We met during my graduate student recruitment weekend in Chapel Hill, and had a great conversation about the opportunities that might exist pursuing research in his group. He provided me with several of the foundational capillary ultra-high performance liquid chromatography (UHPLC) research papers that were published in the late 1990s and early 2000s. After reading them, I knew that I wanted to pursue a research career that involved capillary-scale LC separations. We worked together for five years, and I think the thing that I appreciated the most about him as a mentor was that he provided the right balance of guidance and freedom to explore the field of chemical separations without getting too lost. He let my colleagues and I pursue our individual interests, and was always there to provide help when needed.

What are pieces of career advice that your mentors gave you that helped grow your career?

PIROK: The most important one is to carefully consider what questions your data does answer and which they don't Furthermore, be wary of having your desire for a successful story, paper, or method cloud the actual message that the data demonstrates. A nice example is a retention modelling study we did together in 2020 (3). Initially, we based our conclusions on our own data set. Stoll told me to stop for a second and take a step back. He challenged us to consider measuring new data with a higher precision. Indeed, it turned out that some of the conclusions were affected, and it really improved the paper.

GRINIAS: The best piece of career advice that Jorgenson gave me related to the future of our field: there are lots of reasons that we need to separate chemical components within complex mixtures, from miniaturized targeted analyses up to large industrial scales. We may not know the exact application or specific mechanisms that will be utilized, but there will always be a need for separation science. I have passed on this same information to my students when they inquire about the importance of chromatography research experience in their careers.

How do you find a good mentor? Why is it important to have one?

NEUMANN: One step I usually suggest is that you should be available to find a mentor and to be mentored. That means you're willing to take advice, you're willing to self-reflect, and you're willing to see areas where you can improve. You'd be surprised how many people aren't ready to hear how to improve. Not like horrible criticism, but rather, how do you improve as an individual? You must be open to that kind of feedback. Further, I think being available for networking is big so you can find out what you want from a mentor before you even approach any potential mentors.

Once you have an idea of what mentoring you need, it becomes much easier to target people. Part of being in a mentor–mentee relationship is that it is a two-way street. You must give about as much as you're receiving, and I think young people underestimate how much they can give to mentors. I think one should consider, are you ready to be mentored? And if you are, what kind of mentoring do you need? Then, look for a society that provides either a list of people who are ready to mentor, and try to network at conferences, in your department, or at departmental events.

GIONFRIDDO: That's a difficult answer, since I believe my mentors found me. In general, I believe the basis for a good mentor-mentee relationship is sharing the same values and work ethic and being open to listening to each other. It is also important for a mentee to learn how to take tough advice and for mentors to try to build on the strengths of the mentee while working on addressing their shortcomings. A good mentor is someone who can inspire you to dream more, learn more, do more, and become more, especially when you don't know yet what your full potential can be.

MCGINLEY: I would suggest looking for the experts, ones that others look upon for advice. This person must also have integrity and compassion for others; what good is advice and guidance from someone who is morally bankrupt? The key is to ask for help from experts, which may require some humility. Based on their response and interest in your success, that will determine if you want them to be your mentor.

PIROK: Contact the person—even if you don't know the person well—who has given you useful (critical) feedback, or who thinks very different than you do. This does require you to be open to criticism. I feel we far too often are too sensitive to criticism because we perceive it negatively. Of course, it is not always that great to hear something negative about your work, especially if they are right, but it is precisely this reason why you should have someone like that near you. To attract this, you need to radiate that you are open to it. Remember that people usually only do this if they feel you are worth it. It is a compliment to you.

MAJORS: Try to find someone who has had experience in life that you want to pursue. For example, an academic who has never worked in industry or consulted for industry may not be able to transfer that knowledge that you need to be successful at the entry level, since they have never experienced a real work environment. If you are already in an industrial position, try to align yourself with a people person who truly wants you to be successful as a potential lifelong employee.

GRINIAS: The key thing to look for is someone who has a position that you may be aspiring to, or perhaps more importantly, has mentored other people into positions like one that you may be pursuing. In addition to that, it is generally a good idea to find people with which you have a good rapport and are accessible when you may be seeking advice.

Where can someone who is new to the industry look to find a mentor?

NEUMANN: If you identify as a woman, there is Females in Mass Spectrometry (FeMS). There's also the American Chemical Society (ACS), which has a lot of mentoring initiatives for young women. They focus on women in chemistry. There's also Annual Biomedical Research Conference for Minoritized Scientists (ABRCMS), which is for minority scientists. If you identify with one of those minority populations, a lot of those larger chemistry and analytical chemist societies will have mentoring programs to help these individuals persist in science. So that's more of a formalized way to look for mentoring. The Society for Mass Spectrometry has some as well. Pittcon has a couple resources as well.

The easiest place to look is just locally in your department; lots of faculty members like mentoring, and I have lots of friends and interested industry people that also really appreciate the opportunity to talk to others. I encourage young students especially to think about what jobs they want and to do informational interviews, where you email someone with no other goal than to learn about who they are, how they got where they are, and what their job is. You'll find a lot of mentoring opportunities through these informational interviews.

You can usually start this process by cold emailing. Some people won't respond, but some will, and through this, you'll learn. What jobs do you like? What aspects of these jobs are true? Are there things you're learning about jobs that you never knew? As you meet people, you'll find people you click with, and then those are the people that make fantastic mentors. Those organic relationships get fostered through these small interactions that build up over time. ■

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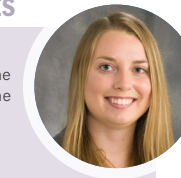
ABOUT THE INTERVIEWEES



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Analysis of Xylazine and Adulterants in Biological Samples

Emily Eng, Forensic Technical Specialist at UCT

This application note details a robust and effective method for the simultaneous analysis of fentanyl, fentanyl analogs, xylazine, and other common adulterants by SPE and LC-MS/MS.

Introduction

Xylazine is a veterinary sedative that has been emerging as a popular adulterant. It is most commonly seen with powders and tablets containing fentanyl.[1,2] Drug powders or tablets can be "cut" with other substances, some of which have their own pharmacological effects, known as adulterants. It is becoming increasingly popular for fentanyl samples to be adulterated with xylazine. This drug combination is commonly called "Tranq".[1] Although xylazine is not an opioid, its use with fentanyl is having a significant impact on the opioid epidemic.

UCT Part Numbers:

CSDAU206: Clean Screen® DAU 200 mg, 6 mL
SCS27-DA1021: SelectraCore® DA Column 100 x 2.1 mm, 2.7 µm
SCS27-DAGDC21: SelectraCore® DA Guard Column 5 x 2.1 mm, 2.7 µm
SLGRDHLDR-HPOPT: Selectra® Direct Connect Guard Holder

Instrument Method

LC-MS/MS System	Shimadzu Nexera LC-30AD with MS-8050
Column Temperature	40°C
Flow Rate	0.4 mL/min
Injection Volume	5 µL
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	Methanol
Gradient Program	Conc. B 5% (0 min) - 45% (3-7 min) - 100% (8-9 min) - 5% (9-12 min)

SPE Procedure

Sample Prep: Urine: Add 1 mL urine sample + 500 µL MeOH (optional) + 2.5 mL of 100 mM phosphate buffer pH 6.0 + ISTDs
Note: Include a hydrolysis procedure to recover conjugated analytes
Blood: Add 0.5 mL whole blood sample + 3 mL of 100 mM phosphate buffer pH 6.0 + ISTDs
Condition: (a) 1 x 3 mL MeOH (b) 1 x 3 mL DI H20
(c) 1 x 3 mL 100mM phosphate buffer pH 6.0
Load: Load sample at 1-2 min/mL
Wash: (a) 1 x 3 mL 100 mM HCl in DI H2O (b) 1 x 3 mL MeOH

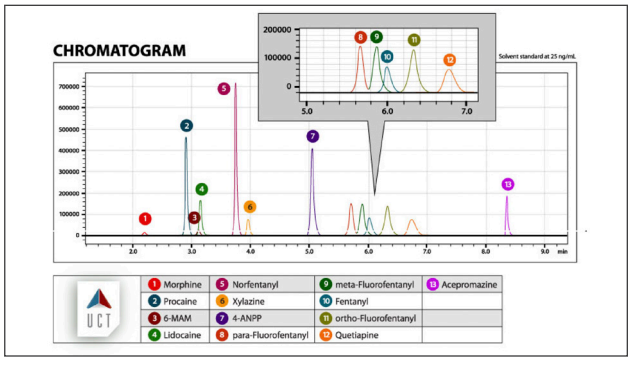

FIGURE 1: Chromatogram of a solvent standard mix at 25 ng/mL.

Dry: Dry for 5-10 minutes at full vacuum or pressure
Elute: 1 x 3 mL DCM:IPA:NH_4OH (78:20:2) or MeOH:NH_4OH (98:2)
Evaporate: (a) Evaporate eluate at 40°C, starting at 5 psi and increasing the pressure slowly over 30 minutes
Reconstitute: (a) Reconstitute samples in 1 mL of 95:5 H_2O:MeOH or other appropriate solvent and volume

Results

TABLE I: Analysis of QC samples prepared at 5 ng/mL, 25 ng/mL and 80 ng/mL.

n=3	Blood	Urine
Recoveries	83% - 113%	88% - 119%
Matrix Effects	(-25%) - 14%	(-25%) - 17%
RSD	1% - 20%	2% - 20%

*Recoveries were calculated using a pre- and post-spike sample technique. Matrix effects were calculated by comparing post-spike samples and solvent standards. Acepromazine not included.

References

(1) Congressional Research Service (2023, February 2) Xylazine: Considerations for Federal Control. CRS Insight. https://www.everycrsreport.com/
(2) Holt, Andrew C, et. al. (2023, January 6) Widespread Distribution of Xylazine Detected Throughout the United States in Healthcare Patient Samples. Journal of Addiction Medicine. https://journals.lww.com/journaladdictionmedicine.

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The supremacy of bioinert coated hardware in oligonucleotide analysis using YMC Accura BioPro IEX QF columns

Kirstin Arend & Ann Marie Rojahn, *YMC Europe GmbH*

This application note shows the analysis of a short RNA oligonucleotide using a bioinert coated YMC Accura BioPro IEX QF column and the corresponding conventional PEEK column. Significant improvements in peak shape and recovery can be observed with the YMC Accura BioPro IEX QF column.

OLIGONUCLEOTIDES CAN BE USED to modulate gene expression, which provides a huge potential in treatment of a wide range of diseases. The field of therapeutic oligonucleotides is rapidly growing, demanding robust and sensitive analytical methods to ensure product quality and safety.

Anion exchange chromatography (AEX) is a suitable tool for oligonucleotide analysis. However, oligonucleotides tend to exhibit poor peak shape, leading to low recovery. One major reason is their adsorption to the column hardware. Tedious passivation with precious sample provides only insufficient improvement. Reproducible analyses can be achieved by using bioinert coated hardware, as provided by YMC Accura BioPro IEX QF columns.

Experimental Conditions

For the analysis of a 21mer RNA sample a YMC Accura BioPro IEX QF column as well as the corresponding column with PEEK hardware were chosen. The stainless-steel column body and its frits of YMC Accura hardware are equipped with a bioinert coating, which tends to be less hydrophobic than PEEK which is commonly used for bio applications.

Additionally, the robust bioinert coating used on YMC Accura hardware is 130 to 320-fold thicker compared to similar hardware concepts making it more durable. A long-term inertness against sensitive substances is ensured. Figure 1 shows a schematic cross-section of a YMC Accura BioPro IEX QF column.

Chromatographic conditions can be found in Table 1.

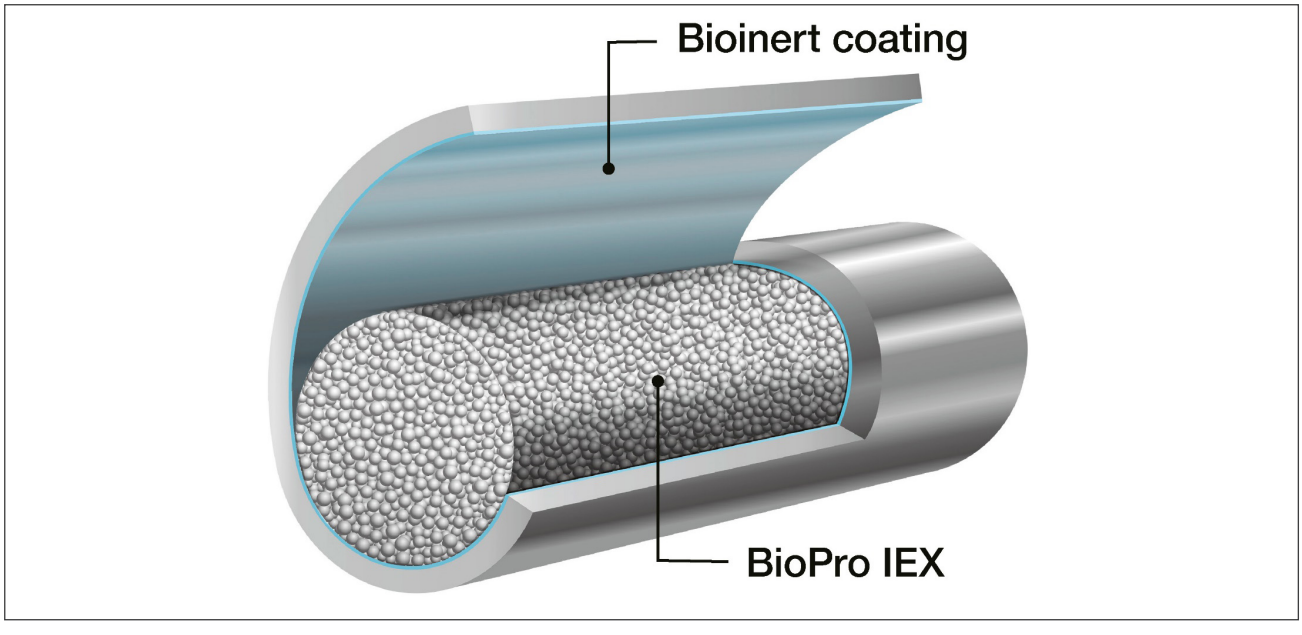

FIGURE 1: Schematic cross-section of a YMC Accura BioPro IEX QF column.

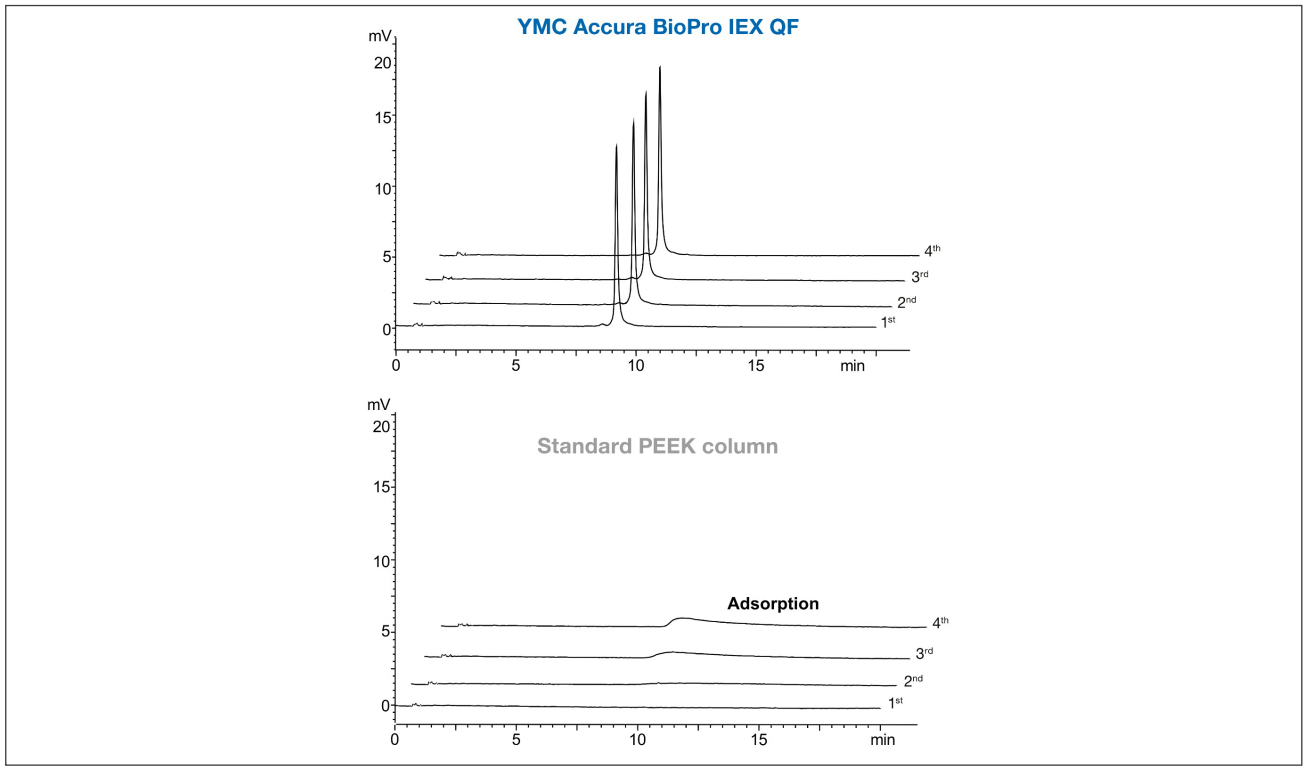

FIGURE 2: Successful analysis of a 21mer RNA using the YMC Accura BioPro IEX QF (top) in comparison to a standard PEEK column (bottom).

Results

The analysis of the 21mer RNA showed strong adsorption when using a standard PEEK column (see Figure 1). Even consecutive injections did not significantly improve the analysis. After four injections only a small broad peak is obtained. In contrast, with a bioinert coated YMC Accura BioPro IEX QF column, superior peak shape and excellent recovery were achieved from the first injection. Consecutive injections show that peak shape and recovery remain stable.

TABLE I: Chromatographic conditions.

Columns:	YMC Accura BioPro IEX QF (5 µm) 100 x 4.6 mm ID (bioinert coated hardware) BioPro IEX QF (5 µm) 100 x 4.6 mm ID (standard hardware)
Part Nos.:	QF00S05-1046PTC QF00S05-1046WP
Eluent:	A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M $NaClO_4$
Gradient:	25–40%B (0–15 min), 40%B (15–20 min)
Flow rate:	1.0 mL/min
Temperature:	60°C
Detection:	UV at 260 nm
Injection:	4 µL (5 nmol/mL)
Sample:	21mer RNA
System:	bioinert HPLC

Conclusions

The combination of the rigid stationary phase BioPro IEX QF with the bioinert coated YMC Accura hardware enables reliable results from the first analysis. Since the bioinert coating is less hydrophobic than commonly used PEEK, no adsorption of the oligonucleotide occurs, so that no preconditioning is necessary to achieve excellent recoveries.

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High-Throughput and Sensitive HPLC Analysis of Lipids Used in LNP Formulations with Evaporative Light Scattering Detection

Mareike Prüfer, Giorgia Greco, *KNAUER Wissenschaftliche Geräte GmbH*

LIPID NANOPARTICLES (LNPS) are used to administer mRNA vaccines against Covid-19 and are considered as a crucial factor in ending the pandemic. Moreover, these nanoparticles are being researched as a potential delivery system for active pharmaceutical ingredients that can combat infectious diseases, cancer, and genetic disorders. KNAUER is the global leader in the production of impingement jet mixing systems for the formulation of LNPs and provides HPLC systems which can be used for the analysis and quality control of LNP formulations. Here, we show the development of analytical methods for high-throughput analysis of the lipid composition of LNPs. The lipids can be analyzed with a fully porous or a core-shell phase in gradient or isocratic mode. The separation was speed up to a 2 min method. KNAUER AZURA® UHPLC system coupled to evaporative light scattering detection provided high sensitivity, with LODs in the range of 0.8–3 µg/ml (4–15 ng) and LOQs of 0.9–4 µg/ml (5–32 ng).

Sample Preparation

The lipids used in two LNP formulations for mRNA vaccines were provided as lipid nanoparticle exploration kits LNP-0315 and LNP-102 by Cayman Chemical. All analytes were dissolved in gradient grade ethanol or provided as a solution. Mixtures and dilutions were prepared in ethanol:water 90:10 v/v.

Results

Method development was started with the core-shell phenyl-hexyl column and overview gradients containing 10 mM ammonium acetate as a modifier. At first, the lipids used for the covid-19 vaccine from Biontech-Pfizer were separated: DSPC, Cholesterol (Chol), ALC-0315 and ALC-0159 (LNP-0315 mix). With a methanol-water gradient, the lipids did not elute completely (Fig. 1). With a water-acetonitrile gradient, complete elution and separation of all four lipids was reached, but the peak shape was not satisfactory (Fig. 2).

When methanol was replaced by acetonitrile in a gradient, the peak shape was much improved (Fig. 3). The organic solvents apparently have to be combined to achieve optimal separation. An even better peak shape is achieved when using mixtures of acetonitrile and methanol as an eluent. Because most of the lipids elute during the isocratic hold, it was possible to optimize an isocratic method for this separation as well (Fig. 4). After optimization of the ELSD evaporation temperature (ET), the isocratic method offers good separation as well as high sensitivity for all lipids (Fig. 4).

All methods are also suitable for the separation of the lipids of the Moderna Covid-19 vaccine (DSPC, Chol, DMG-PEG(2000), SM-102). With slight alterations, separation was also successful using the fully porous column. The fastest separation and highest sensitivity were reached for the isocratic method used with the fully porous column.

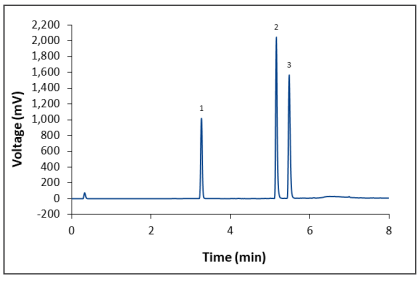

FIGURE 1: Separation of lipids using core-shell phenyl-hexyl column and water-methanol overview gradient (80 % → 95 % methanol in 4 min). Column temperature (CT) = 50°C, evaporation temperature (ET)= 45°C. 1: Chol; 2: DSPC; 3: ALC-0315.

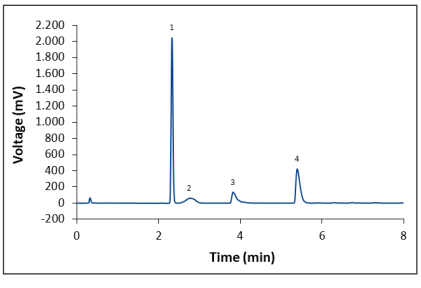

FIGURE 2: Separation of LNP-0315 mix using core-shell phenyl-hexyl column and a water-acetonitrile overview gradient (70 % → 90 % in 4 min). CT = 50°C; ET= 45°C. **1**: Chol; **2**: ALC- 0159; **3**: DSPC; **4**: ALC-0315.

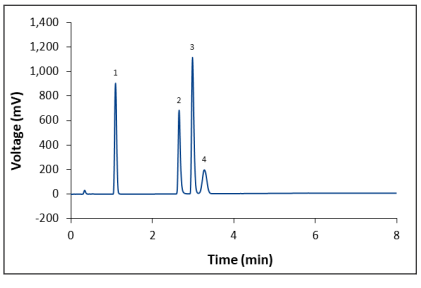

FIGURE 3: Separation of LNP-0315 mix using core-shell phenyl-hexyl column and gradient M2, CT = 50°C; ET = 45°C. **1**: Chol; **2**: DSPC; **3**: ALC-0315; **4**: ALC-0159.

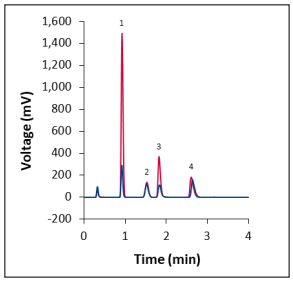

FIGURE 4: Optimization of ET with method M1 for the core-shell column. Blue: ET = 45°C; Red: ET = 35°C. Sample: LNP-0315 mix (dilution 1:2). **1**: Chol; **2**: ALC0159; **3**: DSPC; **4**: ALC-0315.

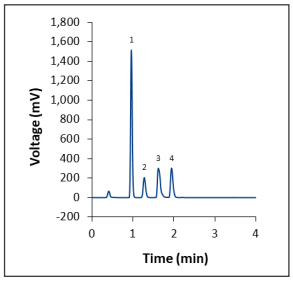

FIGURE 5: Separation of LNP-0315 mix with fully porous phenyl-hexyl column and isocratic method M3. **1**: Chol; **2**: ALC-0159; **3**: DSPC; **4**: ALC-0315.

Quadratic calibration of all final methods resulted in good fits with $R_2 \geq 0.999$ for all analytes. The limit of detection (LOD) and limit of quantification (LOQ) were approximated linearly from the peak heights at the three lowest detected levels and the ASTM noise measured in blanks. A signal-to-noise ratio (S/N) of S/N = 3 for LOD and S/N = 10 for LOQ were assumed (Tab. 1). LOQs were in the range of 0.9–3.5 µg/ml. The comparison with results from literature shows that the reached LODs and LOQs are sufficiently low. In a study on lipid stability in LNPs reported by Merck, Cholesterol and DSPC were analyzed using HPLC separation and a charged aerosol detector. With the same injection volume (5 µl), the LOQs were 36 µg/ml and 25 µg/ml, respectively, and therefore one order of magnitude higher than shown here [1].

TABLE I: Limit of detection (LOD, S/N = 3) and limit of quantification (LOQ, S/N = 10) for lipid detection with method M3.

	LOD (µg/ml)	LOQ (µg/ml)
Cholesterol	0.8	0.9
ALC-0159	2.2	3.5
DSPC	2.3	2.9
ALC-0315	2.5	3.7
SM-102	2.5	3.3
DM-PEG 2000	2.1	3.5

Conclusion

Two phenyl-hexyl columns were successfully used for the separation of lipid mixtures for LNP formulations. Acetonitrile and methanol are both needed as eluents for good peak shape and complete elution. The lipids can be separated with gradient methods which replace methanol by acetonitrile during the gradient or in a faster, isocratic method which is equally suitable for analysis of LNP lipid content. For each analyte, LOD and LOQ were similar for all methods. The ELSD provides high sensitivity for all tested lipids.

Materials and Methods

TABLE II: Chromatographic parameters of final methods.

general	flow rate:	0.4ml/min			
		eluent A: deionized water	eluent B: acetonitrile	eluent C: 200 mM ammonium acetate	eluent D: methanol
M1 (isocratic)	column:	core-shell	column temp.:	55 °C	
	time program	eluent A:	eluent B:	eluent C:	eluent D:
	0-4 min	10 %	55 %	5 %	30 %
M2 (gradient)	column:	core-shell	column temp.:	55 °C	
	time program	eluent A:	eluent B:	eluent C:	eluent D:
	0 min	5 %	0 %	5 %	90 %
	4 min	5 %	90 %	5 %	0 %
	8 min	5 %	90 %	5 %	0 %
	13 min	5 %	0 %	5 %	90 %
M3 (isocratic)	column:	fully porous	column temp.:	50 °C	
	time program	eluent A:	eluent B:	eluent C:	eluent D:
	0-4 min	5 %	72 %	5 %	18 %

TABLE III: Instruments.

Instrument	Description	Article No.
pump	AZURA P 6.1L, LPG	APH39EA
autosampler	AZURA AS 6.1L	AAA50AA
detector	SEDEX 100LT	A0754-6
thermostat	AZURA CT 2.1	ATC00
core-shell column	Kinetex® 1.7 µm phenyl-hexyl 100 Å; 2.1x50 mm (Phenomenex Part No 00B-4500-AN)	-
fully porous column	ChromCore 1.8 µm phenyl-hexyl 120 Å; 2.1x50 mm (Nanochrom Part No A311-018012-02105S)	-
software	ClarityChrom 9.0.0	A1670

Reference

(1) Kinsey, C., Lu, T., Deiss, A., Vuolo, K., Klein, L., Rustandi, R. R., Loughney, J. W. Electrophoresis 2022, 43, 1091–1100.

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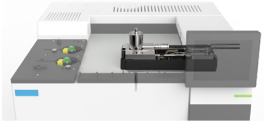

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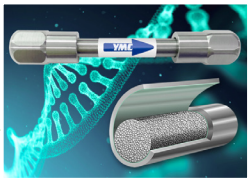

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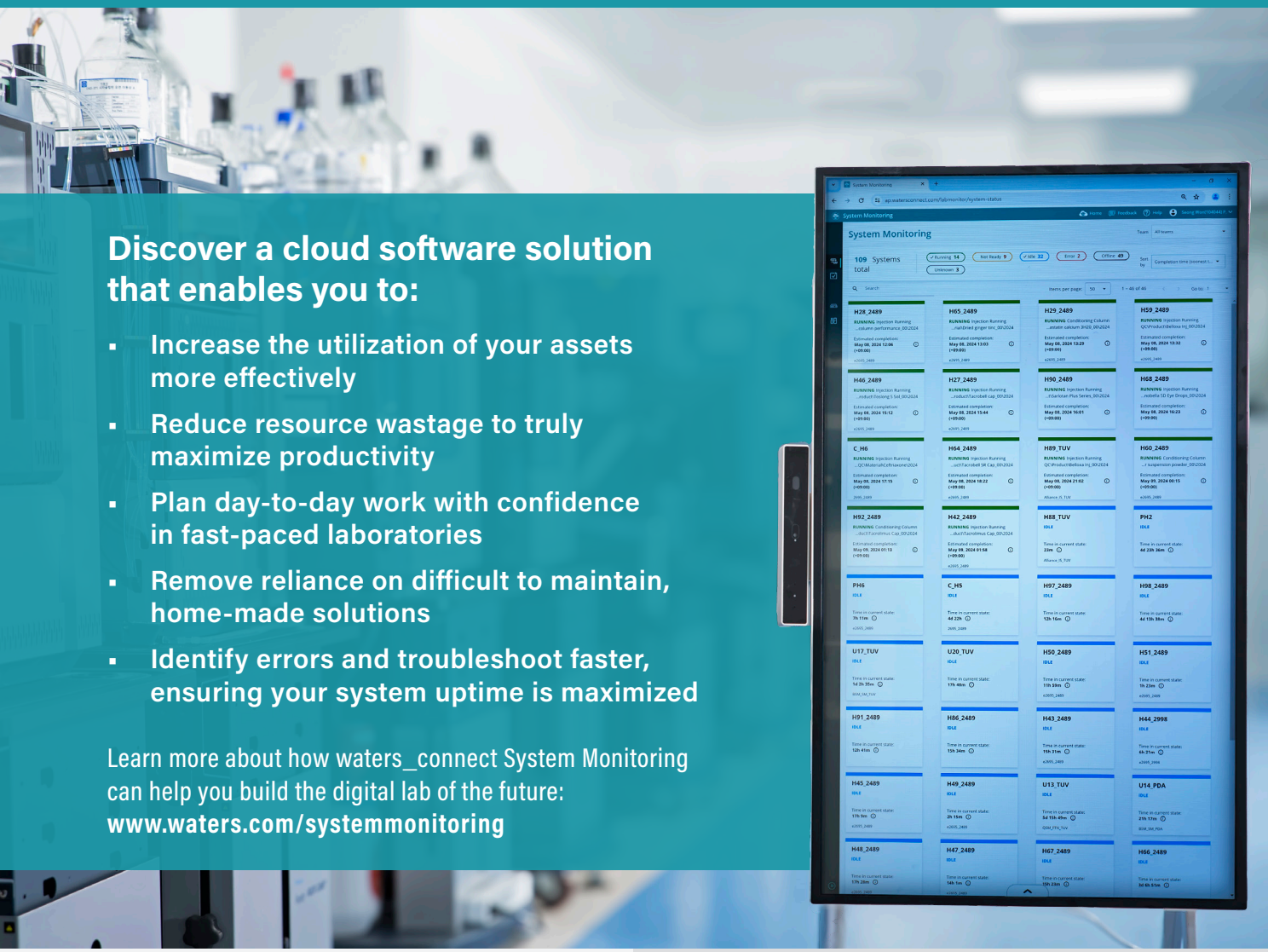

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