8 March 2023 Volume 19 Issue 3

The Column

the interactive e-publication for the global separation science industry

## Cover Story



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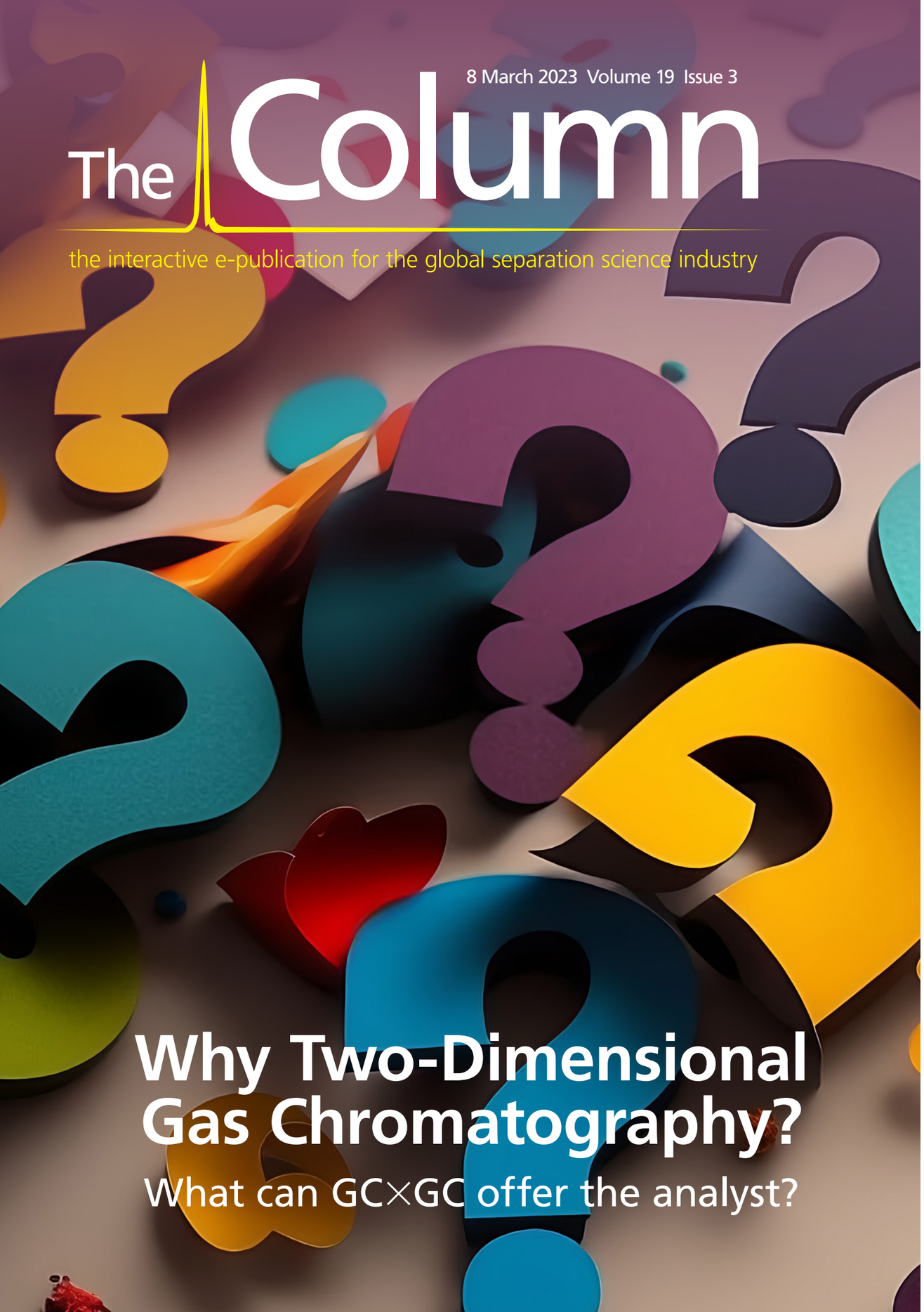

## Why Two-Dimensional Gas Chromatography?

### What can GC×GC offer the analyst?

## Cover Story



## Features



## Regulars

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# The *LCGC* Blog: GC×GC… Why Bother?

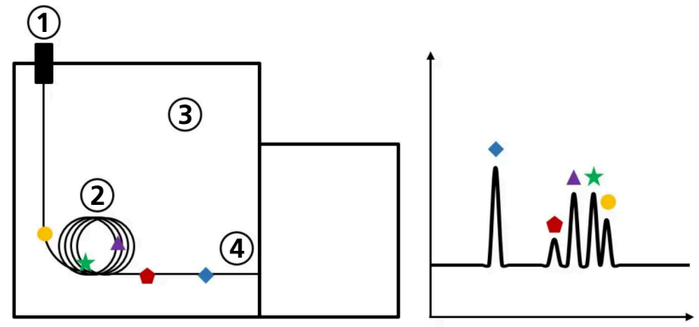
**Katelynn A. Perrault,** Chaminade University of Honolulu, Honolulu, Hawaii

**Comprehensive two-dimensional gas chromatography (GC×GC) is becoming increasingly popular but is still not used as commonly as it could be. That likely means that the technique is still not widely understood. This article is intended to begin demystifying GC×GC by presenting a simple explanation of how it works and its major benefits.**

I recently mentioned to a colleague that I have been doing research using comprehensive two-dimensional gas chromatography (GC×GC) since 2012. As 2023 arrives, this makes my love affair with the technique go back more than 10 years, which feels somehow significant. Time flies when you're having fun, right? I was actually captivated by the technique earlier than that, starting in 2011 when I attended the Multidimensional Chromatography Workshop in Toronto, Canada. In my early days using the technique, I truly thought to myself "Wow, this is going to be something that changes the game!" Honestly, I'm not sure what game I meant at the time, but I suppose it was the recognition that measurement science needed a really good nontargeted tool to screen complex samples.

A lot has changed with respect to GC×GC since I began using it. A large number of commercial options now exist, both in terms of instrument manufacturers and the hardware choices each manufacturer provides. Software for handling GC×GC data is improving significantly every year. The technique is emerging in many new research applications. As a result, awareness has also grown. If you are not using GC×GC, you may have heard about this technique before—maybe in a conference talk, in a magazine article, or in a journal publication. Undergraduate students across the United States and Canada are even starting to be introduced to it in upper-level chemistry curricula, from what I have gathered from some informal surveys and conference discussions.

**Figure 1:** Schematic of a one-dimensional gas chromatography instrument (left) with corresponding chromatogram (right).



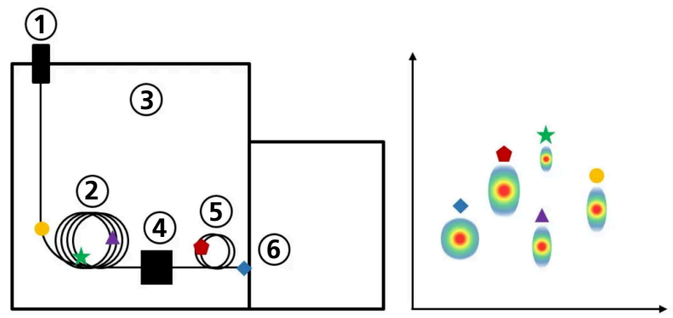

Despite the increase in GC×GC in the separation science and education communities, there are still many applications for which I wonder why GC×GC has not yet become common. Lately, my gut is telling me that we need to find more ways to "demystify" the technique. We need to be better communicators. We have to find a way to demonstrate when exactly GC×GC is going to be powerful vs. when not to use it. Often when I am teaching analytical chemistry, I refer to this as "knowing what the right tool is in your toolbox". In this blog entry, I hope to begin demystifying GC×GC by presenting my own simple explanation of how it works and its major benefits.

## How It Works

To understand GC×GC, one has to have a solid foundation in one-dimensional GC (1D-GC). In short, the main principles of 1D-GC are described below; the steps correspond to the numbers in Figure 1.

1. Sample is introduced at the inlet, which is held at a high temperature. Carrier gas (usually helium, hydrogen, or nitrogen) is used to sweep the injected vaporized sample onto the column.
2. The analytes from the sample travel through the column, which is coated with a stationary phase. Depending on the analyte's affinity for the stationary phase (related to its chemical structure and how it likes to interact with the stationary phase), the analytes will move through the column at different rates and be separated from one another.
3. The column is housed in an oven, which is often operated with a temperature ramp, meaning that the oven temperature increases over the course of the GC run.
4. Analytes ideally arrive at the detector one by one, after they are separated by

**Figure 2:** Schematic of a comprehensive two-dimensional gas chromatography instrument (left) with corresponding contour plot (right).



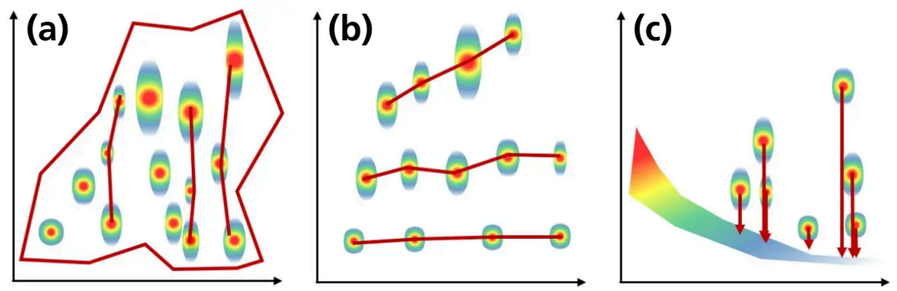

the column. The detector is able to read these eluted compounds and generate a chromatogram in which retention time is represented on the *x*-axis and detector response is represented on the *y*-axis. Peaks represent individual analytes from the sample.

However, the chromatogram shown in Figure 1 is often unrealistic for complex samples. Typically with complex mixtures of hundreds or thousands of compounds, analytes do not actually arrive at the detector one by one. Coelutions can occur, making it challenging for the detector to recognize which signal is related to which analyte. In the case of mass spectrometry detection, sometimes deconvolution can be used to resolve simple coelutions of two or three compounds; however, if you have five, six, seven compounds (or more) that are coeluted, deconvolution tools tend to fall short of resolving analyte peaks. If you are using a single channel detector such as a flame ionization detector, you don't even have access to deconvolution tools. This is where the value of GC×GC comes in. In the simplest possible terms, GC×GC allows more space to separate peaks from one another for samples that are much more complex and challenging to separate.

In GC×GC, most of the system components are the same as in 1D-GC, with the exception of two additional components—a modulator and secondary column. The process occurs as follows (Figure 2):

1. Sample is introduced at the inlet, which is held at a high temperature. Carrier gas sweeps the injected vaporized sample onto the column.
2. The analytes from the sample travel through the primary column, which is coated with a stationary phase. Depending on the analyte's affinity for the stationary phase, the analytes will move through the column at different rates and start to separate from one another.

**Figure 3:** Visual representation of the main benefits of GC×GC including (a) peak capacity and use of chromatographic space, (b) structured chromatograms, and (c) separation of signal from noise.




3. The column is housed in an oven, which is often operated with a temperature ramp, meaning that the oven temperature increases over the course of the GC run.
4. The modulator collects a small portion of effluent from the primary column (such as for 3 s) using a trapping mechanism based on thermal or flow regulation. This happens repeatedly during the entire run, sending a short plug of effluent onto the secondary column over and over.
5. Each small plug of effluent is separated in a rapid separation on the secondary column before the next plug is injected by the modulator. The secondary column has a different stationary phase and thus analytes will partition based on a retention mechanism that is different from that of the primary column. Sometimes, this secondary column is housed in a secondary oven with independent control to induce a temperature offset to enable this separation to occur more efficiently.
6. Analytes arrive at the detector at the end of the secondary column and are plotted in a contour plot, with the first-dimension retention time on the *x*-axis, the second dimension retention time on the *y*-axis, and detector response represented with colour on the *z*-axis. Each "blob" or "spot" on the contour plot represents an analyte.

The contour plots generated using GC×GC provide much more space for analytes to be separated because they can be separated on two dimensions on the plot. Although the general elution order in Figure 1 is similar to that in Figure 2, the actual space between each compound is increased across the plot. Although these figures represent a theoretical separation and are not based on real data, they demonstrate how some compounds (such as the green star and purple diamond) may be much easier to quantify if they are physically resolved and baseline separation is achieved between signals. This is a simple example with only five compounds; however, you can imagine a separation involving hundreds of compounds would have much more exaggerated improvements in separation when comparing 1D-GC and GC×GC results.



## The Benefits

Some of the "brag-worthy" benefits of GC×GC include the increase in peak capacity achieved (typically about 10× higher magnitude) and increased sensitivity. Increased sensitivity can come from focusing effects from thermal modulators or the separation of signal from background noise of the system, or both. In addition, due to the fact that two independent separation mechanisms are used, analytes are separated in two-dimensional space based on structural similarity. This effect has been dubbed with a few different names, including "structured chromatograms", "ordered chromatograms", or even the "roof tile effect". Essentially, compounds with similar functional groups will be eluted in a similar region of the plot and visual organization of them can be noticed based on their molecular weight or number of carbons. This can be beneficial especially when using a detector other than a mass spectrometer, such that structural information

can be inferred from the plot even without highly specific detectors.

In Figure 3, these phenomena are observed on theoretical plots.

1. Figure 3(a) demonstrates the use of the chromatographic space with an irregular polygon. The more space used, the more orthogonal the separation is considered to be. Increasing the orthogonality of the separation may be desirable, or it may not be necessary, depending on the analysis goal. Compounds that are eluted in the same vertical plane would typically have been coeluted in 1D-GC.
2. Figure 3(b) demonstrates the concept of a structured chromatogram. Using a nonpolar × polar column configuration, the row along the bottom would usually represent an *n*-alkane series in increasing order of carbon number from left to right.
3. Figure 3(c) demonstrates how chemical signal can be chromatographically separated from column bleed regions, increasing the overall detectability as a result of improved signal-to-noise ratio.

## Why Bother?

It goes without saying that I love GC×GC. This technique is now well-established, and we are starting to see it make waves in industry settings. Given that it is becoming more popular in industry, students should be aware of it, so I hope that we will see GC×GC emerge into more classroom teaching within higher education. I also hope that by communicating information about the utility of GC×GC, users will think critically about whether 1D- or 2D-GC is the right tool in their toolbox for any given application. When it comes to the real utility of GC×GC, in my opinion its power is best wielded when we want to:

- comprehensively discover all analytes in a complex sample;
- separate target analytes from a complex background;
- separate mixtures of compounds in a wide range of chemical classes;
- target compounds in complex mixtures that have a high dynamic range.

Being involved in the promotion of multidimensional chromatography as a valuable tool within our society has been a highlight of my career thus far, and something I don't plan to stop being involved in any time soon. I encourage you to contact me if you want to discuss whether GC×GC is right for your application!

## Interested in Learning More?

The Multidimensional Chromatography Workshop is held annually and helps new users of multidimensional separations get familiar with techniques and current research. This year's conference was held in Liege, Belgium, 30 January–1 February 2023. If you want to view highlights from the meeting, please visit www.multidimensionalchromatography.com for more details.



**Katelynn A. Perrault is an associate professor of forensic sciences and chemistry at Chaminade University of Honolulu, Hawaii. She specializes in the application of comprehensive two-dimensional gas chromatography for odour analysis applications, and mentors numerous undergraduate researchers as part of her integrated teaching and research programme. Her current interests include odour production from post-mortem microbes, development of GC×GC data processing workflows for dual-channel detection, promoting the adoption of GC×GC in the forensic sciences, and establishing a GC×GC curriculum to be taught in undergraduate chemistry classes.**

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# From the CEO

Welcome to the March issue of *The Column*! Our cover story this month comes from Katelynn Perrault from the Chaminade University of Honolulu in Hawaii, and she shares her love for two-dimensional gas chromatography (GC×GC). Despite its increased use in the separation science and education communities, there are still many applications where GC×GC has not yet become common. Katelynn wants to change that and presents a simple explanation of how it works and its major benefits to the analyst.

This month we spoke to "rising star" Anele Mpupa from the University of Johannesburg in South Africa, about his work in sample preparation using liquid chromatography (LC) with mass spectrometry (MS), and his focus on water quality monitoring of PFAS.

High-purity nucleotide triphosphates are essential to ensure efficient DNA and RNA synthesis. Researchers rely on purified nucleotide triphosphates as the building blocks for synthetic DNA and mRNA technology. However, commercially available sources of nucleotide triphosphates can be contaminated with mono-, di-, or even tetra-phosphates. The team from DuPont discuss how anion exchange resins can purify nucleotide triphosphates from crude nucleotide mixtures to achieve very high purity, thus ensuring efficient DNA or RNA synthesis.

With a focus on food, we take a look at the analysis of polar pesticides from fruit and vegetables using a high performance liquid chromatography (HPLC) column selectivity. The developed method proved to be precise, robust, and accurate for polar pesticides.

After a break of four years, the ANAKON conference is back! The event will take place in Vienna, Austria, from 11–14 April and will showcase new developments in instrumentation and analytical methodology, as well as important novel applications.

Happy reading!

**Mike Hennessy Jr.,**
President and CEO, MJH Life Sciences

# Knauer Announce Gender Pay Gap Surprise

Knauer (Berlin, Germany) has announced its gender pay gap across the company, and caused a small commotion. According to the latest figures from the company, the wage gap has not only disappeared but the women in the company now earn on average 1.36% more than their male counterparts.

Managing director Alexandra Knauer and equal opportunities officer Katharina Pohl

Managing director Alexandra Knauer said, "This is the smallest unadjusted gender pay gap we have ever had in the company. When we calculated it last year, our women still earned 2.2% less than the men. It is clear that this figure will always fluctuate, as it also depends on the gender distribution among skilled workers and management positions. Our guideline is the zero mark, and we are closer to it than ever."

Equal opportunity officer at Knauer, Katharina Pohl, added: "We are proud that we have one of the smallest gender pay gaps in Germany. At the same time, we know that there is always room for improvement. We will continue to work to ensure fair pay and equal opportunities for women and men and also people of different backgrounds."

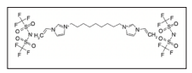
This is in stark reflection to women in Germany, who still earn an average of 18 percent less than men for the same work. Equal Pay Day is a symbolic day to raise awareness of the difference in earnings between women and men. If the current 18 percent is converted into days, women work for 66 days from the beginning of the year—that is, until 7 March 2023—unpaid.

For more information, please visit: **www.knauer.net**

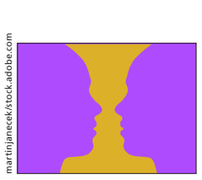
Photo Credit: Image courtesy of Knauer

# Peaks of the Month


- **The *LCGC* Blog: Allowable Changes to Chromatography Methods for HPLC – More Freedom for Chromatographers Granted by the Pharmacopeias?**—The new General Chapter does reflect the great work of the pharmacopoeias in helping chromatographers to use more modern technology for their regulated methods, speeding up analysis, and reducing waste. The latest revision allows us to adjust column dimensions for gradient separations, which is a significant advance. Although these freedoms are welcomed, one must be very mindful of the requirements or limits of the allowable changes and the verification work that underpins our demonstration of equivalent performance of the adjusted method. **Read Here>>**



- **Does High Polarity Mean High Retention on Stationary Phases in Gas Chromatography?**—This instalment of "GC Connections" examines the idea of stationary phase polarity in detail. The assumptions inherent in the most popular stationary phase polarity-evaluating systems—McReynolds constants and the polarity scale—are discussed. **Read Here>>**




- **Playing with Selectivity for Optimal Chiral Separation**—Chiral separation is challenging and requires thorough method optimization. Selectivity plays a critical role in chiral separation. In this study, various ways of achieving resolution by playing with selectivity are presented. **Read Here>>**


- **Analytically Speaking Podcast: Automating Advanced Chemometric Methods for Data Processing**—In this episode, we spoke to Professor Rasmus Bro, who is a full professor at the University of Copenhagen, about the world of data analysis used for spectroscopy and other analytical methods. Over the years he has worked on many aspects of chemometrics, developing numerous algorithms and methods such as fuzzy logic, deep learning, analysis of variance, and tensor modelling. He has received multiple awards in chemometrics and in the analytical sciences, and is the second most-cited scientist within the field of chemometrics with nearly 37,000 citations and an h-index of 78 (Google Scholar). Most of the algorithms and data sets he has worked on have been made publicly available on the internet. **Listen Here>>**



# News In Brief

## Agilent Technologies and Plasmion Collaborate

Agilent Technologies (Santa Clara, California, USA) and Plasmion (Augsburg, Germany) have established a marketing collaboration for the joint marketing of the Agilent Ultivo triple-quadrupole liquid chromatography–mass spectrometry (LC–MS) and LC/MSD iQ single-quadrupole LC–MS instruments and the Sicrit ion source made by Plasmion.

Plasmion has developed an ionization technology, called *Sicrit*, which can interface with these Agilent instruments using a standard mounting bracket.

"I am glad to see that our technology and products are recognized by such a strong partner like Agilent. The combination of the Sicrit ionization technology with Agilent mass spectrometers enables customers from lab and research to boost their efficiency, flexibility, and overall performance," said Dr. Thomas Wolf, CEO of Plasmion.

Through the agreement, the companies will market the combination of the instruments and ion source for nontraditional applications, such as the detection of volatile analytes without chromatography, in a direct mass spectrometry (MS) approach.

For more information, please visit:
**www.plasmion.de**

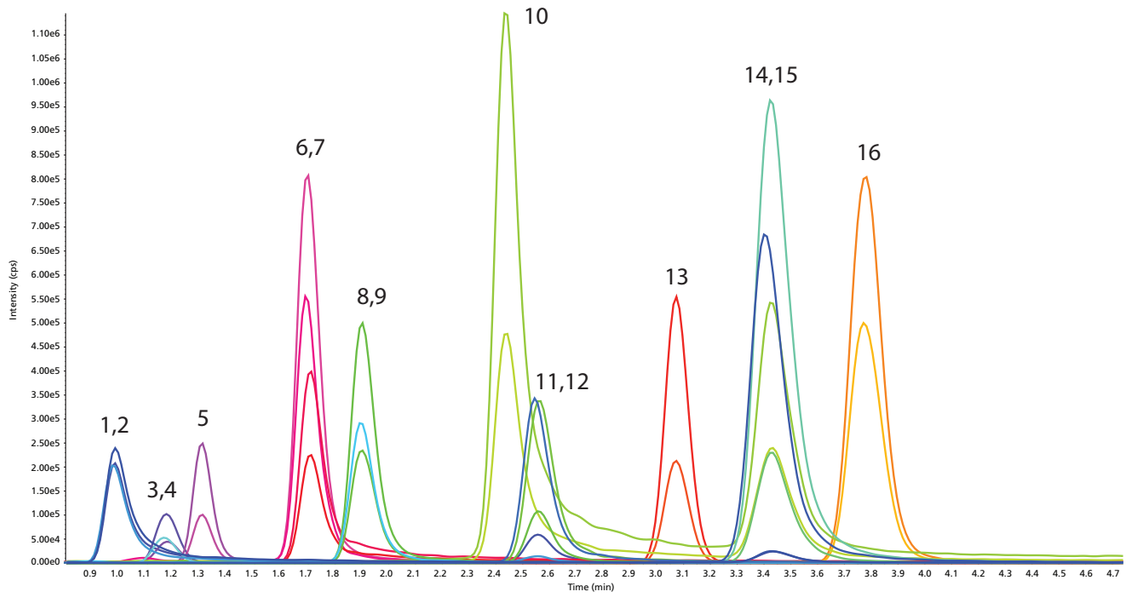
# Optimal Separation of Polar Anionic Pesticides from Fruits and Vegetables with HPLC Column Selectivity

**Richard F. Jack[1], Ramkumar Dhandapani[1], Luigi Margarucci[1], and Samuele Scurati[2],**
[1]Phenomenex, Torrance, USA, [2]DaSP, Italy

**The presence of ionic pesticides found in foods has increased in recent years. Because of their chemical properties their identification and quantification present unique analytical challenges compared to hydrophobic pesticides. Several approaches including ion-exchange chromatography (IEC), hydrophilic interaction liquid chromatography (HILIC), and ion chromatography (IC) have been developed to facilitate chromatographic resolution and to approach different matrices. This article presents a new column technology that uses reversed phase separation and offers excellent peak shape, resolution, and robustness for a wide variety of food matrices.**

The analysis of polar pesticides presents multiple challenges, including adequate retention, separation of critical pairs, and reproducibility, to name just a few. In addition, food matrices can add additional challenges due to the presence of complex matrix components including pigments, fats, and sugars that can interfere with the analyte of interest. Often, polar anionic analytes such as glyphosate will utilize QuEChERS (quick, easy, cheap, effective, rugged, and safe) or QuPPE

**Figure 1:** Separation of pesticides on the Luna mixed mode column.

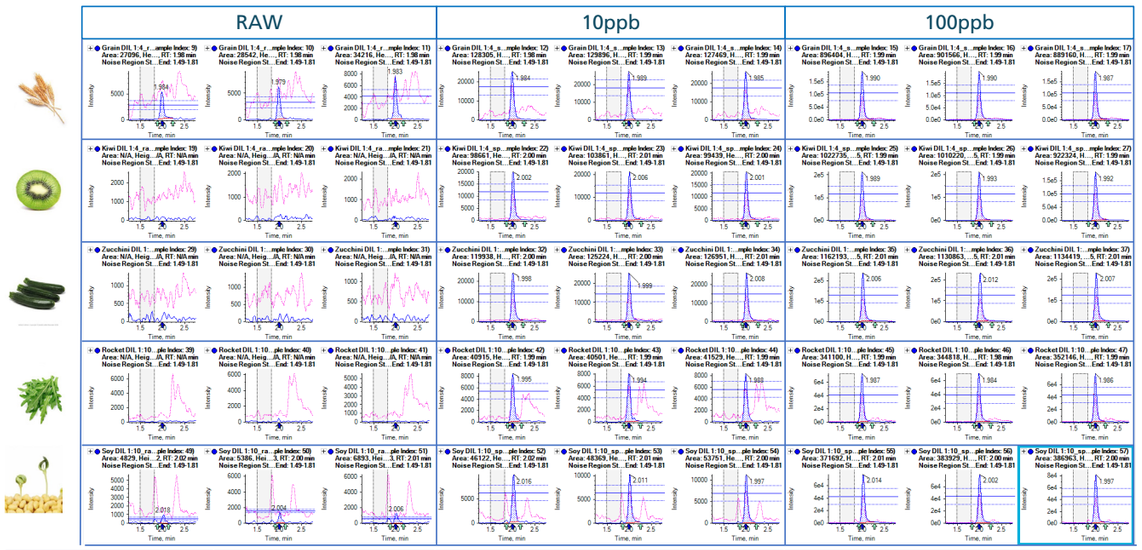



| Peak # | Analyte Name |
|---|---|
| 1 | AMPA |
| 2 | AMPA-C13N15 |
| 3 | MH-D2 |
| 4 | MH |
| 5 | Glifosinate |
| 6 | MPPA |
| 7 | Glyphoste |
| 8 | N-Acetyl-Glufoinate |
| 9 | N-Acetyl-Glufoinate-D3 |
| 10 | Phosphonic Acid |
| 11 | Ethephon-D4 |
| 12 | Ethephon |
| 13 | Chlorate |
| 14 | Fosetyl Al-D15 |
| 15 | Fosetyl Al |
| 16 | Perchlorate |

(quick polar pesticides) sample preparation techniques, followed by hydrophilic interaction liquid chromatography tandem mass spectrometry (HILIC–MS/MS) methods for chromatographic retention and separation. Historically, these methods are not user-friendly, and lack the reproducibility necessary for a commercial application. Glyphosate (N-[phosphonomethyl]glycine) is a widely used broad-spectrum systemic herbicide and crop desiccant. Previously, glyphosate was considered as safe and not toxic to humans (1–2). However, since the International Agency for Research on Cancer (IARC), a branch of the World Health Organization, classified glyphosate as a probable human carcinogen (3) it is now of concern. In this study, a unique selectivity that provides optimal separation of various anionic polar pesticide classes including glyphosate, chlorate, perchlorate, ethephon, phosphoric acid-based pesticides, and N-Ac-Glu pesticides will be presented. Phosphonic acid is a degradation product of the fungicide fosetyl aluminum (fosetyl-Al). This study will demonstrate robust polar pesticide analysis from several food matrices.

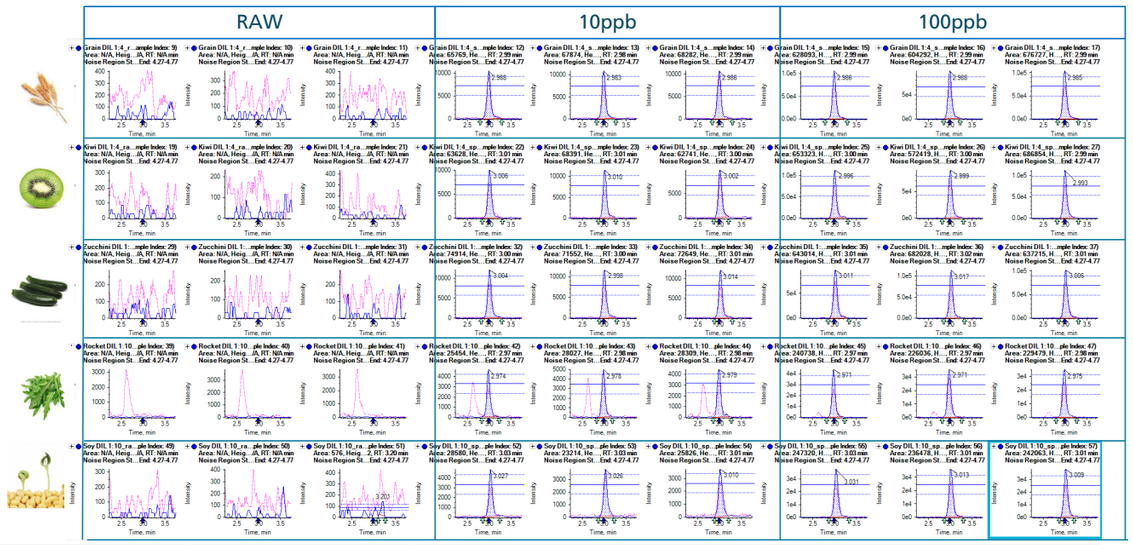
A mixed mode column with characteristics that make it amenable to both HILIC and reversed-phase chromatography has been introduced. These properties allow strong retention of polar compounds in HILIC and reverse-phase separation modes. This column is complementary for both reversed phase and normal mode, and is compatible in both 100% aqueous and 100% organic phases. The column allows the analyst to use a single column for both HILIC and reversed-phase modalities by reversing the gradient without changing the mobile phase.

**Figure 2:** Glyphosate.




| Matrix | RAW Mean | RAW CV | 10ppb Mean | 10ppb CV | 10ppb Accuracy | 100ppb Mean | 100ppb CV | 100ppb Accuracy |
|---|---|---|---|---|---|---|---|---|
| Grain DIL 1:4 | 3.27 | 14.7 | 14.46 | 2.32 | 111.9 | 96.67 | 2.56 | 93.4 |
| Kiwi DIL 1:4 | 0 | 0 | 10.07 | 3.95 | 100.7 | 106.57 | 10.85 | 106.57 |
| Zucchini DIL 1:4 | 0 | 0 | 10.75 | 3.99 | 107.5 | 99.02 | 1.86 | 99.02 |
| Rocket DIL 1:10 | 0 | 0 | 10.17 | 4.17 | 101.7 | 86.41 | 4.66 | 86.41 |
| Soy DIL 1:10 | 1.22 | 12.12 | 10.51 | 11.6 | 92.9 | 81.62 | 4.67 | 80.4 |

**Figure 3:** Ethefon.




| Matrix | RAW Mean | RAW CV | 10ppb Mean | 10ppb CV | 10ppb Accuracy | 100ppb Mean | 100ppb CV | 100ppb Accuracy |
|---|---|---|---|---|---|---|---|---|
| Grain DIL 1:4 | 0 | 0 | 9.43 | 2 | 94.3 | 89.12 | 5.8 | 89.12 |
| Kiwi DIL 1:4 | 0 | 0 | 9.09 | 4.68 | 90.9 | 89.28 | 9.23 | 89.28 |
| Zucchini DIL 1:4 | 0 | 0 | 10.23 | 2.35 | 102.3 | 91.6 | 3.73 | 91.6 |
| Rocket DIL 1:10 | 0 | 0 | 9.55 | 5.77 | 95.5 | 81.25 | 3.31 | 81.25 |
| Soy DIL 1:10 | 0.2 | 0 | 9.06 | 10.37 | 88.6 | 84.71 | 2.24 | 84.51 |

## Method

Normally, HILIC conditions are used for the analysis of extreme polar pesticides. The chromatogram in Figure 1 shows the separation of common pesticides including chlorate and perchlorate in less than 5 min using reversed-phase mode. Excellent peak shape and resolution was achieved for all compounds.

**Experimental Conditions:** particle size: 3 µm; dimension: 100 × 2.1 mm (Phenomenex); guard column SecurityGuard Ultra Cartridge (Phenomenex); mobile phase: A: 0.3% formic acid in water, B: 0.3% formic acid in acetonitrile; gradient: 0 min 2%B, 0.5 min 2%B, 6.0 min 20%B, 7.0 min 90%B, 9.0 min 90%B, 9.1 min 2%B, 12 min 2%B; flow rate: 0.3 mL/min; injection volume: 1 µL; temperature: 40 °C; MS/MS detector: Sciex 7500.

## Analysis of Pesticides from Food Matrices

In order to assess the effect of food matrices on column performance, sample extracts of spiked food extracts were tested. The sample preparation steps followed the "Quick Method for the Analysis of Numerous Highly Polar Pesticides in Foods of Plant Origin" (QuPPe-PO) (4). The food matrices tested were grain, kiwi, zucchini, arugula, and soy. The chromatograms (Figures 2 and 3) show the peaks for raw, 10, and 100 ppb spikes for each of the analytes from the matrices above after QuPPe-PO extraction. The peak shapes for both glyphosate and ethefon are both very sharp and well above baseline for accurate quantification. Figure 2 shows glyphosate recovery from raw matrix with an accuracy between 70–120%, even in a matrix (grain) that contains glyphosate at 3.27 ppb. A similar behaviour was observed for ethephon (Figure 3). The accuracy range is in agreement with that recommended by SANTE 11312/2021 (5). The same results were seen for all the pesticides tested in Figure 1 (data not shown).

## Results and Discussion

Column selectivity plays an important role

in providing enhanced chromatographic resolution for critical pairs. In addition, retention of extremely polar analytes is very challenging. This study has presented the optimal separation of polar pesticides using a mixed mode column. Polar interactions in the reverse-phase mode were used to obtain enhanced retention of polar pesticides. Baseline separation between phosphoric and phosphonic acid was achieved, avoiding false positive results in the determination of phosphonic acid. The chromatogram of standards shows excellent retention and selectivity for polar pesticides (Figure 1). Traditional reversed-phase columns do not retain analytes such as glyphosates, which can fall in the ion suppression zone in real samples and hence show as false positives or negatives. Enhanced polar selectivity from the un-endcapped silica base and the amide ligand provided excellent retention, which is evident from retention of the polar pesticides that range from 0.7 to 6.6 min. Sample extracts from grains, kiwi, zucchini, arugula, and soy were analyzed with this method, followed by spiking them with known concentrations of polar pesticides. The method proved to be precise, robust, and accurate for polar pesticides. Unlike traditional HILIC methods that suffer in terms of re-equilibration time and retention time shifting, this method provided more stable retention times and faster re-equilibration because the retention of very polar compounds was performed in reverse phase by utilizing polar interactions from the stationary phase (6). Thus, the developed method could be used in laboratories running routine polar pesticide analysis.

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including IC and high performance liquid chromatography (HPLC) systems, pumps, autosamplers, and a variety of detectors. Richard received his Ph.D. in biochemistry and anaerobic microbiology from Virginia Tech University in Blacksburg, Virginia, USA, and his master's in ecology from the University of Tennessee in Knoxville, Tennessee, USA.

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**Luigi Margarucci** began his career at Phenomenex in 2015 as a technical sales consultant. In 2017 he assumed the role of MS specialist and in 2020 he became head of training in the field of chromatography. Since 2021 he has held the role of technical manager for Italy. He was a research fellow at the University of Salerno, Italy, and in 2009 he moved to the University of Utrecht, Netherlands. To date, he is the author of 27 scientific publications, and has written and presented a large number of seminars related to analytical techniques such as HPLC, gas chromatography (GC), and solid-phase extraction (SPE).

**Samuele Scurati** received a degree in medicinal chemistry and a PhD in biochemistry in 2008. He then started his career as an application field marketing specialist for clinical and toxicology, developing new applications of LC–MS in the clinical market. In 2011 he became one of the founders of DaSP project, working as an application scientist to develop innovative LC–MS methods. He has published several scientific articles and participated in many national and international conferences.

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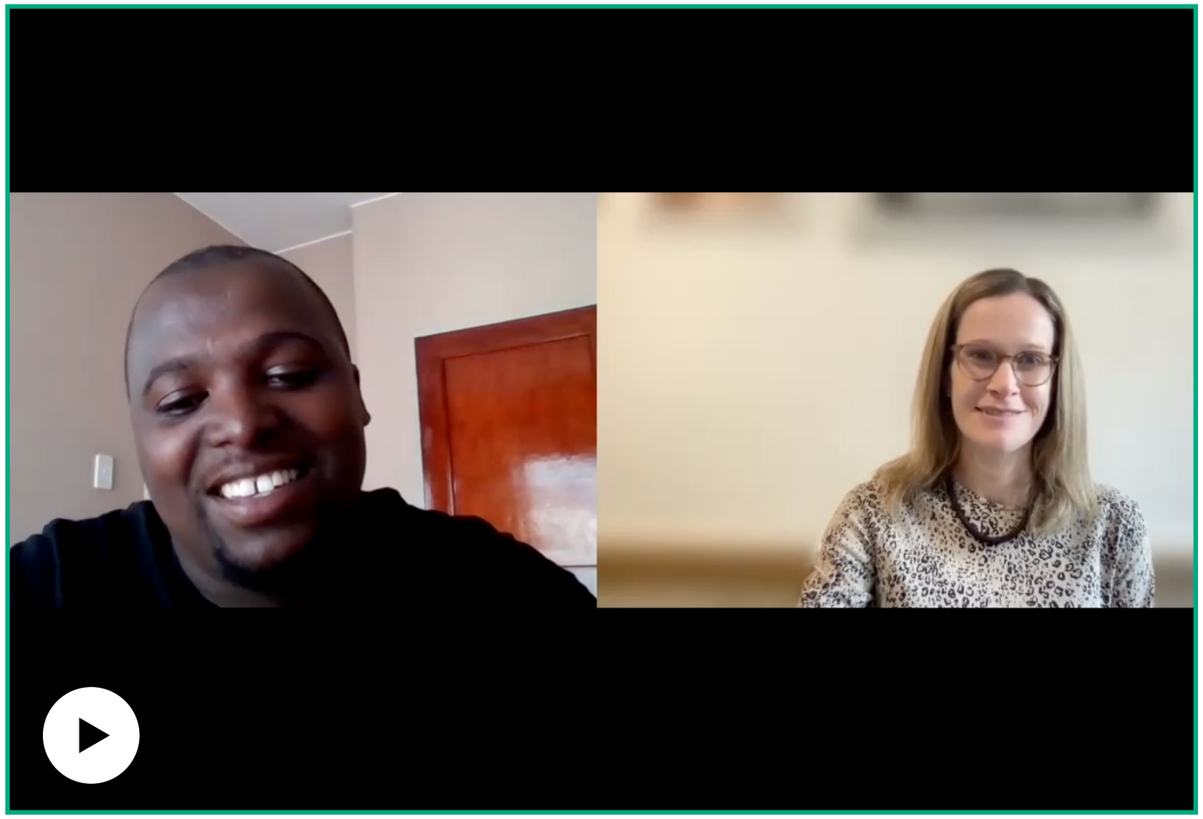
# Rising Stars of Separation Science: Anele Mpupa

**This month we interview Anele Mpupa from the University of Johannesburg in South Africa, about his work in sample preparation using liquid chromatography (LC) with mass spectrometry (MS), and his particular focus on water quality monitoring.**

*—Interview by Kate Jones*



To view our interview with our March Rising Star, Anele Mpupa, please **CLICK HERE**.

**Anele Mpupa** obtained his bachelor of science (chemistry and microbiology) and B.Sc. (Hons) in chemistry from Rhodes University, South Africa. Later, he completed his M.Sc. and Ph.D. in chemistry from the University of Johannesburg, South Africa. He received funding from the National Research Foundation (NRF) from 2017–2020 for his M.Sc. and Ph.D., respectively; he was also a recipient of the highly competitive Deutscher Akademischer Austauschdienst (DAAD) German academic exchange service for a four-month short stay research grant in Ulm University. He is currently a postdoctoral research fellow at the University of Johannesburg.

Dr Mpupa's research focuses primarily on water quality monitoring, the analysis and removal of emerging pollutants in water matrices using nanomaterials. Emerging pollutants are chemical substances that fall out of standard monitoring and regulatory programmes. These chemicals often include new generations of pharmaceutically active compounds, pesticides, surfactants, and personal care products among others. These chemicals occur at low concentration levels, making their monitoring a challenge. Therefore, his research focuses on the development of adsorbent materials for the determination of emerging pollutants in water matrices. Furthermore, some of the developed materials are tested for their potential for the mitigation of the pollutants in water, with special attention being paid to efforts using recycled feedstocks.

## Papers Mentioned in Interview


1. Mashile, G. P.; Mpupa, A.; Nomngongo, P. N. Magnetic Mesoporous Carbon/β-Cyclodextrin–Chitosan Nanocomposite for Extraction and Preconcentration of Multi-Class Emerging Contaminant Residues in Environmental Samples. *Nanomaterials* **20201**, *11*, 540. DOI: 10.3390/nano11020540
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3. Mpupa, A.; Nqombolo, A.; Mizaikoff, B.; Nomngongo, P. N. Beta-Cyclodextrin-Decorated Magnetic Activated Carbon as a Sorbent for Extraction and Enrichment of Steroid Hormones (Estrone, β-Estradiol, Hydrocortisone and Progesterone) for Liquid Chromatographic Analysis. *Molecules* **2022**, *27*, 248. DOI: 10.3390/molecules27010248


## RISING STARS OF SEPARATION SCIENCE

*LCGC*'s sister digital publication, *The Column*, will be running a series of interviews in 2023, featuring the next generation of separation scientists. Information for nominating a "rising star" can be found here: **bit.ly/3UFaS61**. Any questions about the submission process should be directed to Kate Jones, managing editor of *The Column*, at **kjones@mjhlifesciences.com**

Courtesy of TU Wien

# ANAKON 2023
# Event Preview

**ANAKON 2023 will be held in Vienna, Austria, from 11–14 April 2023. Here's a glimpse of what attendees can look forwards to.**

After a hiatus of four years due to the COVID-19 pandemic, the ANAKON conference series is back in 2023! The ANAKON conferences are the flagship events of the German, Swiss, and Austrian analytical chemistry communities and are jointly organized every other year by the Fachgruppe Analytische Chemie of the German Chemical Society (GDCh), the Division Analytical Sciences of the Swiss Chemical Society, and the Austrian Society of Analytical Chemistry (ASAC) within the Austrian Chemical Society (GÖCh). ANAKON 2023 will be organized in Vienna, Austria, from 11–14 April 2023 by the Conference Chairs Martina Marchetti-Deschmann, Erwin Rosenberg, and Victor U. Weiss at TU Wien. The conference venue is located right in the centre of Vienna, next to many of the city's landmark historical sights. Spacious and well-equipped lecture theatres and ample space for a company exhibition and poster displays guarantee vivid interaction of the participants during breaks and social events.

ANAKON 2023 is a conference open to all fields of analytical chemistry. It is intended to showcase new developments in instrumentation and analytical methodology, as well as important novel applications. A great variety of topics will be addressed at this conference, including—but not limited to—separation sciences, mass spectrometry (including ion mobility separation), miniaturization, microfluidics, imaging and lab-on-a-chip technologies, sample preparation, sensors, surface analysis, spectroscopy from the macro- to the nanoscale, and hyphenated techniques for more comprehensive analysis. The

application of the above-mentioned methods to the fields of bio- and clinical analysis, environmental analysis, and food analysis, and the application of analytical techniques in the determination of micro- and nanoplastics or in the field of cultural heritage science will be highlighted. In addition, ANAKON 2023 will also host the Austrian MassSpec Forum. Besides fundamental studies, many presentations at this embedded event will cover the omics field, showing the importance of combining separation techniques with mass spectrometry to dig deep into very complex samples.

The scientific programme will boast plenary as well as keynote lectures in which the state-of-the-art and innovative applications of analytical techniques will be presented by prominent speakers. Each keynote lecture opens a session in which contributed lectures will present further facets of the topic addressed by the headline speaker. The scientific programme of the conference will be entirely in English.

A particular highlight of the conference programme will be the Pregl-Award Session, during which the Fritz-Pregl Medal will be awarded; this is one of the most prestigious awards presented by the Austrian Society of Analytical Chemistry. This Pregl Session will also be organized to honour the 80th birthday of Wolfgang Lindner (University of Vienna)—an earlier recipient of the Fritz Pregl Medal. In addition to the designated Pregl Medal awardee of 2023, several other earlier recipients of the Pregl Medal will give keynote presentations at this session.

Selected presenters of poster contributions will have the chance to participate in the "science slam" in which they can present their poster in the form of a flash presentation. The best presentations will be awarded with poster prizes.

The scientific programme will be complemented by a delightful social programme throughout the conference and by a company exhibition that is located within the same space as the poster sessions and the coffee breaks.

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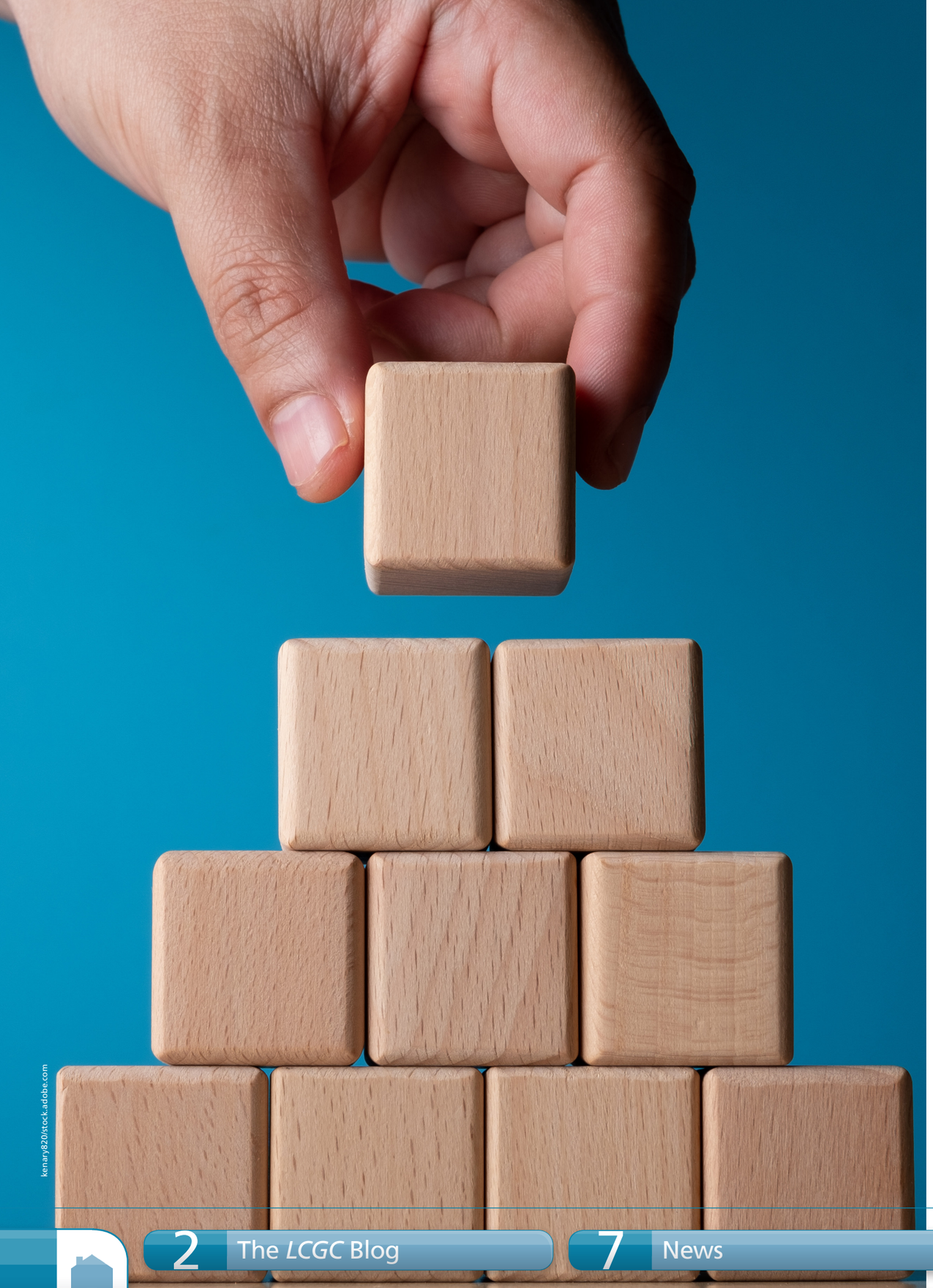

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# Purification of Nucleotide Triphosphates: Critical Building Blocks for mRNA Therapies

**Martin Deetz, Dorie Yontz, and Michael Ostrander,** DuPont, Wilmington, Delaware, USA

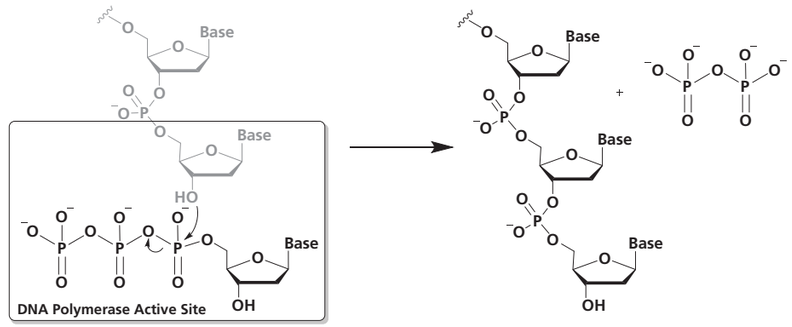
**High-purity nucleotide triphosphates are essential to ensure efficient deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis. Researchers rely on purified nucleotide triphosphates as the building blocks of messenger RNA (mRNA) technology, which is fundamental to applications such as COVID-19 vaccines. However, commercially available sources of nucleotide triphosphates can be contaminated with mono-, di-, or even tetra-phosphates. This article discusses the importance of high-purity nucleotide triphosphates and how fine mesh anion-exchange resins can be used to purify them.**

Nucleotides are the basic building blocks of life, consisting of a purine or pyrimidine base linked to a phosphorylated sugar. Nucleic acids are biopolymers formed from chains of nucleoside monomers that have been linked together with a polymerase enzyme. The polymerase enzyme then copies a nucleic acid template and assembles the corresponding base-paired nucleotide into the complementary nucleic acid chain.

This nucleic acid synthesis process involving nucleotide triphosphates and polymerase enzymes are used for both deoxyribonucleic acid (DNA) synthesis (such as biosynthesis or polymerase chain reaction [PCR] amplification) and ribonucleic acid (RNA) synthesis (such as *in vitro* transcription for messenger RNA [mRNA]).

The polymerase enzyme uses a nucleotide triphosphate to add to the 3′-end of the

**Figure 1:** DNA polymerase catalyzing the addition of deoxynucleoside triphosphate to the DNA chain.




growing nucleic acid chain, releasing pyrophosphate in the process (Figure 1). The polymerase enzymes utilize only the triphosphate form of the nucleotide as the substrate for nucleic acid synthesis. This is due to the lower relative transphosphorylation energy barrier for the triphosphate compared to the mono- or di-phosphate nucleotide (1). During *in vitro* nucleic acid synthesis, the purity of the triphosphate form of the nucleotide is directly correlated with the yield, as mono- and di-phosphate nucleotides are not functional substrates for the polymerase enzyme.

## Nucleotide Triphosphates in DNA Synthesis

A common method of DNA synthesis is PCR amplification (2). With PCR, a target sequence of DNA is amplified via the use of a thermally stable DNA polymerase enzyme, two oligonucleotide primers, and nucleotide triphosphates. With this technique, a thermal cycle dissociates the double stranded DNA, and the primers then attach to the target locations on the DNA. The DNA polymerase utilizes nucleotide triphosphates to copy the target region of each DNA strand. As described above, the substrate for the DNA polymerase enzyme is the deoxyribose nucleotide triphosphate monomers, as nucleotide mono- and di-phosphates will not be effectively incorporated by the enzyme.

## Nucleotide Triphosphates in mRNA Synthesis

mRNA therapeutics became part of the world's lexicon due to the mRNA vaccines developed to defend against SARS-CoV-2 during the COVID-19 pandemic. These vaccines appeared on the market quickly, yet the use of mRNA as a therapeutic route has been in development for decades (3). The underlying idea is to utilize *in vitro* transcribed mRNA and the body's own protein synthesis mechanism to produce beneficial proteins. In the case of mRNA vaccines, mRNA sends instructions for the cells to produce proteins that will trigger an immune response. For example, with SARS-CoV-2, the mRNA instructed ribosomes to produce the virus' spike protein (4).

mRNA vaccines are made through a process called *in vitro transcription* (IVT). During IVT, a DNA template—a linearized DNA plasmid with the instructions for the mRNA sequence—is combined with an RNA polymerase enzyme and nucleotide triphosphates and the mRNA molecule is assembled. In a second part of the synthesis, a capping group is added to the mRNA, which helps stabilize the molecule and initiate protein translation. Similar to above, the substrate for the RNA polymerase enzyme is the ribose nucleotide triphosphate monomers, as nucleotide mono- and di-phosphate will not be effectively incorporated by the enzyme.

## Importance of High Purity Nucleotide Triphosphates

As the polymerase enzyme relies on nucleotide triphosphates to create nucleic acids, the purity of the nucleotide triphosphate form is critical to performance. Using low-purity nucleotide triphosphate raw materials can result in low yield and low quality of the resulting nucleic acid.

Nucleotide triphosphates are commercially available from fine chemical suppliers, with the high-purity grades commanding a cost premium. However, even fine-chemical grade nucleotides can contain impurities, such as mono- and di-phosphate analogues, which can form during synthesis, shipment, and storage.

## Ion-Exchange Chromatography of Nucleotide Triphosphates

Ion-exchange chromatography (IEC) is designed to separate compounds based on differences in ionic charge. The technique relies on a polymeric resin that carries an ionic charge. Charged molecules and ions of the opposite charge will be attracted to the resin, while species with the same charge will be repelled. Species with a higher charge density will experience stronger attraction compared to those with a lower charge density. With the introduction of competing ions in the mobile phase, the weakly held ions can be selectively eluted from the resin. Anion-exchange chromatography (AEC) is very effective for the purification of nucleotides, as the triphosphates have a higher charge density than the mono- and di-phosphates.

Fine mesh ion-exchange resins are

**Figure 2:** Preparative separations of three different triphosphate nucleotide samples. (a) adenosine triphosphate, (b) cytidine triphosphate, and (c) uridine triphosphate.

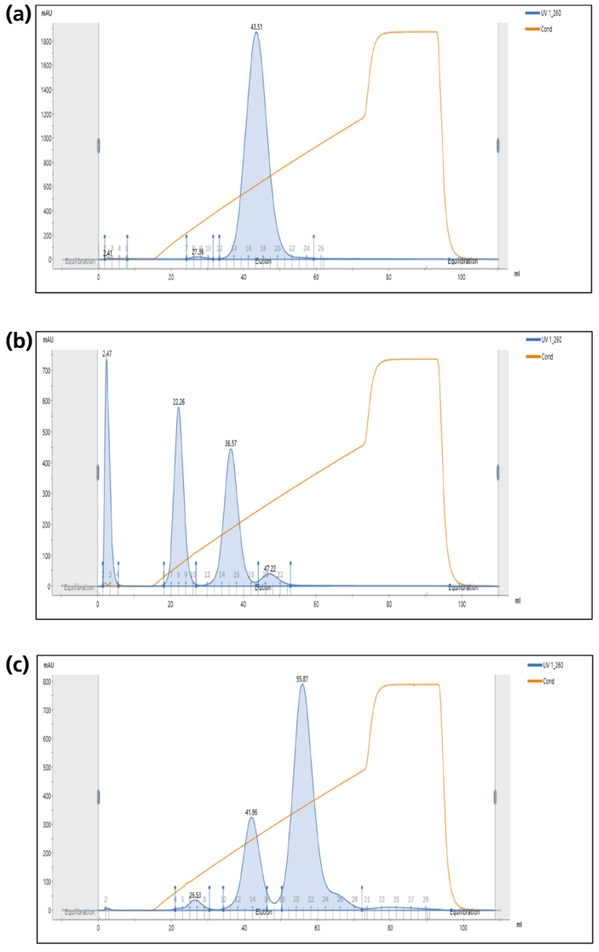
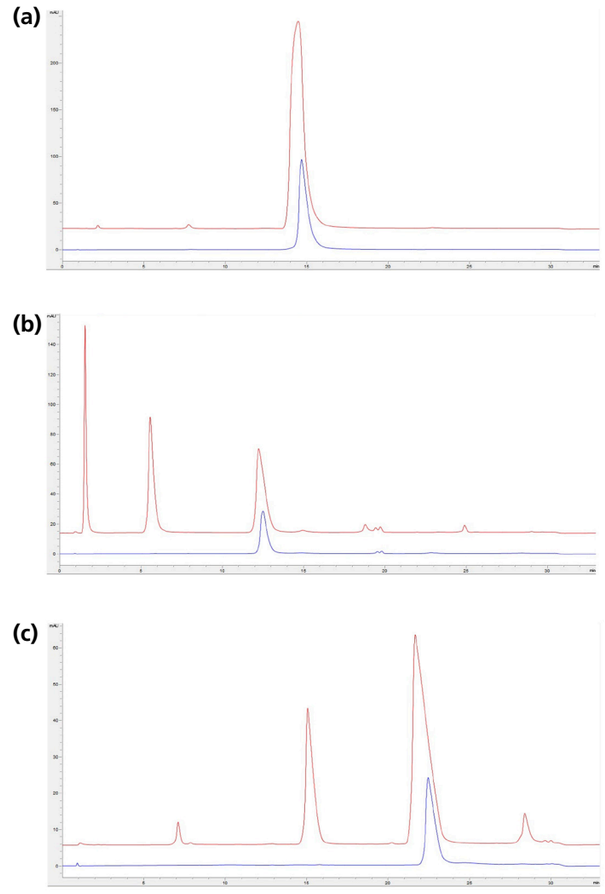



**Figure 3:** Analytical high performance liquid chromatography demonstrating the use of fine mesh ion-exchange resins to cleanly separate various phosphate forms. (a) adenosine triphosphate, (b) cytidine triphosphate, and (c) uridine triphosphate. Red traces are the crude material, blue traces are purity of the collected fraction after purification.




polymeric resins designed specifically for separating small, charged molecules, such as nucleotides. The anion-exchange resins used contain positively charged quaternary amine ligands that are strongly attracted to nucleotides. By introducing a mobile phase containing a competing salt, the nucleotides can be sequentially eluted from the weaker binding (monophosphate) to the stronger binding (triphosphate) nucleotide, resulting in high-purity nucleotide triphosphate monomers.

## Materials and Methods

Nucleotide triphosphates (adenosine triphosphate, cytidine triphosphate, and uridine triphosphate) were purchased from Thermo Fisher Scientific and used as received.

### Experimental Conditions:

*Preparative IEC:*

Resin: 2 mL DuPont AmberChrom 1 × 4 200–400 Cl fine mesh ion-exchange resin; buffer A: 0.02 M HCl, buffer B: 0.02 M HCl + 1 M NaCl; flow rate: 1 mL/min or 0.5 BV/

**Table 1:** Nucleotide purification, as received versus after ion-exchange chromatography

| Nucleotide | Triphosphate Purity (As Received) | Triphosphate Purity (After Ion-Exchange Chromatography) |
|---|---|---|
| Adenosine triphosphate (ATP) | 99.2% | 99.9% |
| Cytidine triphosphate (CTP) | 38.8% | 94.4% |
| Uridine triphosphate (UTP) | 69.9% | 99.6% |

min or 76.4 cm/h; injection: 0.2 mL of 10 mg nucleotide/mL solution; gradient %B: 0% for 5 CV, 0–60% over 30 CV, 100% for 10 CV

*Analytical IEC–HPLC:*

Column: 4 × 250 mm DNAPac PA200 (Thermo Fisher Scientific); buffers: A: 0.1 M NaOH, B: 0.25 M NaCl gradient, C: deionized water; flow rate: 1 mL/min, temperature = 23 °C; injection: "as received" samples: 1 µL of 10 mg nucleotide/mL solution. Fraction analysis: 10 µL injection; step 1: equilibrate the column with isocratic 10% A - 12% B + 78% C. Then inject 10 µL sample; step 2: isocratic 10% A - gradient 12% B to 60% B over 12.9 CV; step 3: isocratic 10% A - 12% B - 78% C for 1.3 CV

*Dynamic Loading Capacity Test*:

Column: DuPont AmberChrom 1 × 4 200–400 Cl fine mesh ion-exchange resin, 2 mL (10 × 26 OmniFit); loading buffer: 0.02 M HCl with 17.8 mg/mL crude ATP; flow rate: 75 cm/h, temperature = 25 °C, UV trace at 280 nm.

## Results

Preparative separations of three different triphosphate nucleotide samples are depicted in Figures 2(a–c). In Figures 2(b) and (c), the presence of the mono-, di-, and even tetra-phosphates make up a considerable amount of the mixture. By collecting only the target nucleotide peak, the high purity triphosphate can be separated from the mixture.

The analytical high performance liquid chromatography traces from the crude (red traces) and target peaks (blue traces) are shown in Figure 3(a–c) and demonstrate that fine mesh ion-exchange resins can cleanly separate the various phosphate forms of adenosine, cytidine, and uridine nucleotides, thereby providing high purity nucleotide triphosphates. As shown in Table 1, there was a significant increase in purity of the cytidine triphosphate from ~39% to ~94% in a single chromatography run. This technique was also able to polish the adenosine triphosphate from an initial purity of 99.2% to 99.9%. Thus, ion-exchange resins are demonstrably effective at improving purity across a range of nucleotide triphosphate molecules and purities.

## Conclusion

High-purity nucleotide triphosphates are essential to ensure efficient DNA and RNA synthesis. Researchers rely on purified nucleotide triphosphates as the building blocks for synthetic DNA and mRNA technology. However, commercially available sources of nucleotide triphosphates can be contaminated with mono-, di-, or even tetra-phosphates. Anion-exchange resins can purify nucleotide triphosphates from crude nucleotide mixtures to achieve very high purity, thus ensuring efficient DNA or RNA synthesis.

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Pennsylvania Convention Center, Philadelphia, Pennsylvania, USA

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**Website:** https://pittcon.org

## 11–14 April 2023

**ANAKON 2023**

Vienna, Austria

**Email:** office@anakon2023.at

**Website:** www.anakon2023.at

## 21–24 May 2023

**8th International Conference on Polyolefin Characterization (ICPC)**

Valencia, Spain

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**Website:** www.icpc-conference.org

## 18–22 June 2023

**HPLC 2023**

Düsseldorf, Germany

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The Column (ISSN 2050-280X) is the analytical chemist's companion within the dynamic world of chromatography. Interactive and accessible, it provides a broad understanding of technical applications and products while engaging, stimulating, and challenging the global community with thought-provoking commentary that connects its members to each other and the industries they serve.
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