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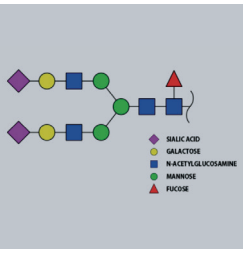

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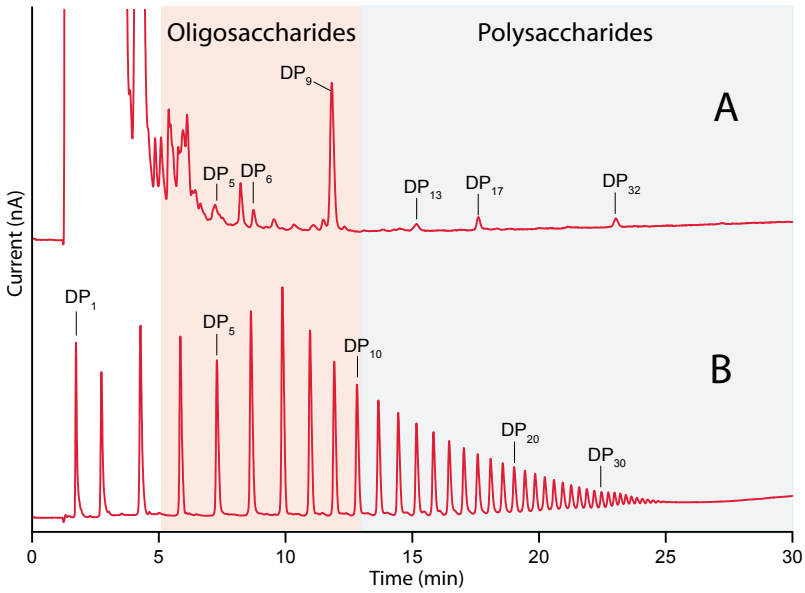

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Grinias speaks about his background, teaching philosophy, students, and lab equipment.

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Around the World with The Multidimensional Chromatography Workshop

Dwight Stoll talks with Katelynn Perrault Uptmor, Pierre-Hugues Stefanuto, and Petr Vozka about the multidimensional chromatography workshop, better known as the MDCW for short.

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NOTE FROM THE CEO

DEAR SCIENCE ENTHUSIASTS,

Greetings from the team at *LCGC International*!

It's with great pleasure that we present to you our April 2024 issue, packed with insightful content and cutting-edge research from the world of chromatography.

Without further ado, let's dive into the highlights of this month's edition.

In our first installment of the column "LC Troubleshooting," penned by Dwight Stoll and James Grinias, we explore the fundamental similarities between electronic circuits and flow in LC systems. Aptly titled "Treat It Like a Circuit, Part I: Essentials of the Similarities Between Electronic Circuits and Flow in LC Systems," this featured cover piece promises to be an enlightening read, offering new insights into understanding flow phenomena in chromatography.

Next up, in "GC Connections," Nicholas Snow presents an annual review of the latest developments in gas chromatography products introduced between 2023 and 2024. Stay updated with the newest advancements in the field!

Moving on to "Perspectives in Modern HPLC," Michael W. Dong takes us through a brief review of new HPLC systems and related products introduced in the past year. From high-performance liquid chromatography to mass spectrometry, this article offers a comprehensive overview of the latest innovations.

Our application article delves into environmental analysis, focusing on the small-molecule composition of Voacanga africana seeds using LADI-MS. Will Wetzel interviews Rabi Musah, revealing her fascinating research on this topic.

In a peer-reviewed article titled "Validation of HPLC-DAD Based Stability Indicating Assay for Ornidazole in Periodontal Polymeric Hydrogel," authors Gopal Prasad Agrawal, Mohammed Ageeli Hakami, and colleagues present a meticulously designed and validated HPLC method for quantifying ornidazole in oral gel formulations, offering valuable information for quality control laboratories.

Last but not least, our feature article by Bob Pirok, "Resolving Separation Issues with Computational Methods," kicks off a series focusing on data analysis in chromatography. Dive into the intricacies of retention time and learn how to extract valuable information from chromatograms using these computational techniques.

Additionally, we're excited to introduce two comprehensive guides for our readers: the *Biopharmaceutical and Gene Therapy Terminology Guide*, and the *Chromatography Terminology Guide.* These resources, accessible on the LCGC website (see the QR codes to the left), aim to enhance your understanding of key concepts in these scientific fields.

We hope you find this issue as engaging and informative as we do.

Happy reading! ■

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

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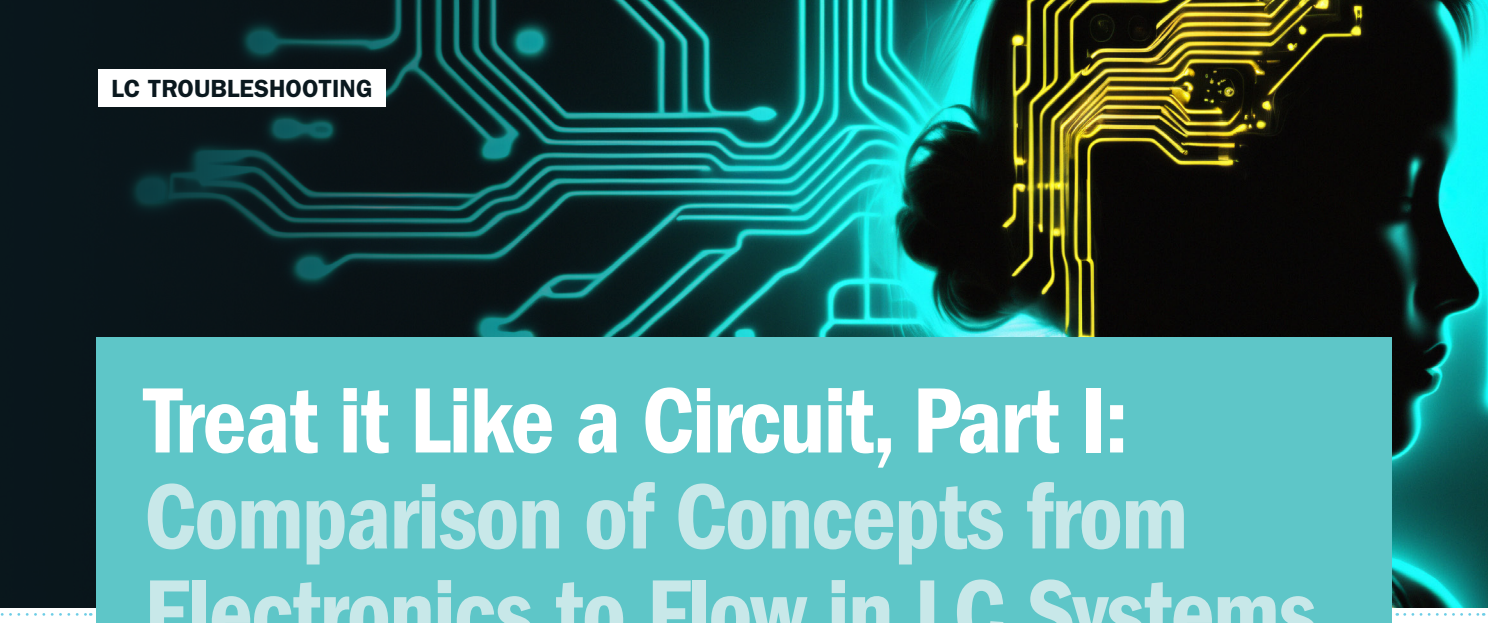

Treat it Like a Circuit, Part I: Comparison of Concepts from Electronics to Flow in LC Systems

Dwight R. Stoll and James P. Grinias

The analogy that electrons flowing in wires is like water flowing through a tube can be remarkably effective for teaching and learning about fluid flow in liquid chromatography (LC) systems. In this installment, we will go over the basics of this analogy, as well as explicitly discuss the phenomena observed in the two cases and the physical laws that we can use to rationalize and predict the behavior that we see. In the second installment in this series, we will then use this knowledge to demonstrate how these ideas can be used to troubleshoot problems when they arise in LC systems.

IN MY INTERACTIONS *with liquid chromatography (LC) users from various backgrounds and levels of training and experience, I observe that perceptions about how flow and pressure "work" in LC systems vary dramatically from one user to the next. In some cases, I see that the fluidics of the LC system are treated as a black box, without any concrete sense for what the driving forces are, which variables are dependent on others, and in what ways. In other cases, I hear people talk about the behavior of the fluidics in ways that are simply incorrect. Neither of these situations is helpful when it comes to troubleshooting a system that has some flow or pressure related problem. In discussing these observations with Jim Grinias, professor of Chemistry at Rowan University, I found that we agree on two points: 1) many LC users would be better troubleshooters if they had a better understanding of how the fluidics of LC systems "work"; and 2) that there is great value in borrowing concepts from electronics that describe the flow of electrical current in circuits to help us understand and predict the flow of liquids in LC systems. Thus, I've asked Jim to join me in writing a two-part series focused first on fundamental concepts in Part I, and then the application of those concepts to real problems in LC systems in Part II.*

~ Dwight Stoll

Opening Thoughts

The technical backgrounds of LC users are remarkably diverse. We think this is actually a testament to the incredible flexibility and utility of LC as an analytical technique. Of course, there are LC users who have taken multiple courses in analytical chemistry, and maybe even have an advanced degree in analytical chemistry or chemical engineering. But, there are probably just as many (maybe even more) LC users with no formal training in separation science in their background. For example, most biology degree programs don't require a course in analytical chemistry, and yet there are many biologists using LC every day, simply because LC is a very powerful tool for answering a variety of biologically-oriented questions—for example, how do the metabolites in my cell line change in response to a chemical stimulus? In our discussions in preparation for this installment, we realized that the average LC user has probably had more exposure to concepts in physics, including electricity and magnetism, than concepts in hydraulics; therein lies at least some of the value in drawing comparisons between the flow of charge in electrical systems, and the flow of fluids in LC systems. If we can leverage knowledge and concepts from prior experiences with electricity and apply them to understand how LC systems work, we will be better prepared to diagnose and resolve problems encountered with LC systems.

Please note that this discussion is framed using terms in a way that is meaningful for practicing chromatographers, and is not intended to be an in-depth treatment found in an electronics textbook or physics lecture. As a result, some field-specific misnomers may occur, but these do not affect the utility of the central idea of treating electronic and fluidic circuits similarly in the context of LC troubleshooting. Readers interested in diving into these topics in more detail are referred to respected texts in the field (1,2).

Definitions, Relationships, and Concepts

In both electrical and fluidic systems, there has to be a driving force that results in the movement of matter from one place to another. In electrical systems, we speak of

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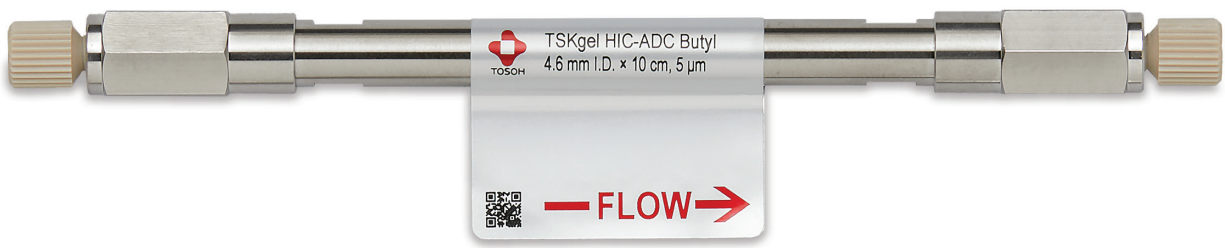

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TABLE I: Comparison of concepts in electrical and fluidic systems

Electrical System	Fluidic System
$\Delta V = I \cdot R$ [1] known as "Ohm's Law" where: ΔV ="voltage drop" I = electrical current R = electrical resistance and: $R = \frac{\rho \cdot L}{A}$ [2] where: ρ = resistivity of the material L = of the resistor A = cross-sectional area of the resistor	$\Delta P = \frac{8 \cdot \eta \cdot L \cdot F}{\pi \cdot r^4} = \frac{8 \cdot \pi \cdot \eta \cdot L \cdot F}{A^2}$ [3] known as "Poiseuille's Law" where: ΔP ="pressure drop" F = flow rate $\frac{8 \cdot \eta \cdot L}{\pi \cdot r^4} = \frac{8 \cdot \pi \cdot \eta \cdot L}{A^2}$ = "flow resistance" [4] and: η = fluid viscosity L = tube length r = tube radius

voltage differences between two points, or *voltage drops*, that result in the movement of electrical charge (that is, *current*) between the two points. Similarly, in fluidic systems, we speak of *pressure drops* that result in the movement of fluid (that is, the molecules that make up the fluid; we simply refer to this as the *flow*). In both cases, it is instructive to think of matter (electrons or molecules) moving from an area of high potential energy to an area of lower potential energy, and the voltage or pressure drop are ways of quantifying the energy gradients. Once we accept the idea that these drops (voltage or pressure) result in the movement of matter (electrons or molecules), then a number of other useful comparisons can be made between the two systems.

Comments on These Relationships and Their Implications

How to Think About the Unifying Relationship: Proportional vs. Inversely Proportional

Simply writing down the expressions in Table I is one thing. More important is how we think about them and apply them in practice when working with real systems. The magnitude of the flow, whether the flow involves movement of electrons or fluids, is directly proportional to the energy difference. Anything we do to increase the energy difference will immediately result in an increase in the flow (if we don't change anything else). This relationship is most familiar when thinking about electrical systems where it is usually the voltage drop that is nominally fixed (as in the case of a battery). Here, when we connect a battery to a circuit having a certain resistance, current will flow in the circuit in response to the voltage drop. Since the voltage of the battery is fixed, anything we do to increase or decrease the resistance in the circuit (for example, changing the length of the resistor) will immediately result in a decrease or increase in the current in the circuit.

Another way of thinking about this formulation is that an energy difference will result from a certain flow dictated by the operator of the system. Moreover, for a given flow rate, the energy difference that results is dependent on the resistance in the circuit. Anything we do to decrease the resistance in the circuit will immediately result in a decrease in the energy difference if all other variables are held constant in addition to the flow. This relationship is most familiar when thinking about fluidic systems where it is usually the flow that is nominally fixed, as in the case of an LC pump with a user-defined flow rate. Here, when we turn on the pump connected to tubing and other components with a certain flow resistance, we will see the energy difference develop. We refer to this as the pressure drop, which is typically measured at the pump, but this is really a measure of the pressure difference between a measurement point in the pump and atmospheric pressure (typically taken as zero for practical purposes). In this case, since the flow is fixed (as a setting in the pump), anything we do to increase or decrease the resistance in the flow path (for example, changing the length of connecting tubing) will immediately result in a decrease or increase in the measured pressure drop.

Details of (Flow) Resistance

Equations 2 and 4 in Table I provide a more detailed perspective on the factors that dictate resistance to flow in electrical and fluidic systems. Here it is convenient to consider the simplest cases in each system. In the electrical case, we can consider a wire connecting a voltage source to ground, which has a certain length, cross-sectional area, and resistivity (that is, a property of the material the wire is made of). In the fluidic case, we can consider a piece of tubing connecting the outlet of a fluid pump to a waste container open to the atmosphere, where the tubing has a certain length and cross-sectional area associated with the inside, open part of the tube. In the fluidic case, the important material property is the viscosity (think of this as how "thick" the fluid is; peanut butter is very viscous, whereas hexane is not) of the fluid flowing through the tubing. We see that in both cases the resistance is directly proportional to the first power of the length, and the first power of the material property. Making the wire or tube longer or shorter will increase or decrease the resistance. Similarly, changing the material properties such that the resistivity or viscosity increases or decreases will increase or decrease the resistance.

Careful readers will notice that there is an important difference between the resistances in the electrical case (the aforementioned equation 2) and the fluidic case (equation 4, also in Table I), namely that in the former case the resistance is inversely proportional to the first power of the cross-sectional area of the wire, and in the latter case the resistance is inversely proportional to the square of the cross-sectional area of the tube. This difference results from the no-slip boundary condition (that is, the velocity of the fluid is zero at the wall of a tube) that is required in fluidic circuits (3). But, in both cases, increasing or decreasing the diameter of the wire or tube will decrease or increase the resistance.

Behavior of Systems Having Multiple Elements

Analysis of Voltage Drops and Pressure Drops

In real electrical and fluidic systems, there are usually multiple elements (for example, multiple segments of tubing joined together, usually of different lengths and diameters),

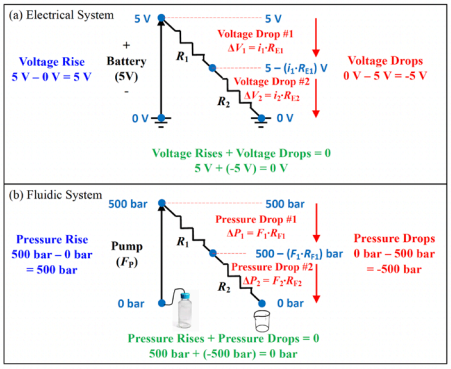

FIGURE 1: Illustration of the application of Kirchhoff's Voltage Law to (a) a simple electrical system, and (b) by analogy to the pressure rises and drops encountered in an LC (fluidic) system.

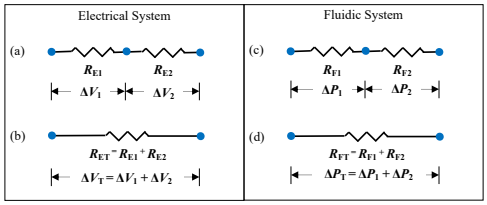

FIGURE 2: Illustration of the relationships between resistances of connected elements in (a,b) electrical systems and (c,d) fluidic systems, as well as the voltage or pressure drops across the elements.

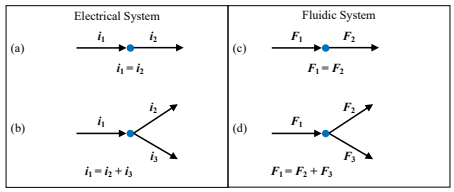

FIGURE 3: Illustration of the application of Kirchhoff's Current Law to a simple electrical system involving (a,b) components connected at junctions, and (c,d) comparable illustrations of junctions in a fluidic system.

and we need a way to analyze these systems so that we know what to expect from them, which in turn gives us the ability to recognize when something is wrong. Two invaluable tools used in analysis of electrical circuits are Kirchhoff's Laws, one for voltage, and one for current. Kirchhoff's Voltage Law asserts that the sum of the "voltage rises" and "voltage drops" around a loop must be zero. Here, by "loop," we are referring to a closed electrical circuit. In Figure 1a, we see that both ends of the simple circuit are connected to ground, so this is a closed circuit. Starting from the ground point on the left, we encounter a voltage rise at the battery. Then, when current flows through the electrical resistor R_{E1}, there is a voltage drop from 5 V by the amount $i_1 \cdot R_{E1}$, where i_1 is the amount of current. This same current (that is, $i_1 = i_2$; see below for more discussion on this point) then flows through the second electrical resistor R_{E2} to ground, and the voltage drops further by the amount $i_2 \cdot R_{E2}$ to 0 V at ground.

Figure 1b shows the analogous concepts applied to the fluidic system we encounter in an LC instrument. We replace the battery with a pump that produces a user-defined flow rate F_P. As explained above, this flow will result in a pressure measured at the pump. As the mobile phase flows through downstream elements with fluidic resistance the pressure drops first by the amount $F_1 \cdot R_{F1}$, and further by the amount $F_2 \cdot R_{F2}$ as the mobile phase proceeds toward the

waste container that is typically at atmospheric pressure (for our purposes here, this is "fluidic ground"). As in Figure 1a, notice here that $F_1 = F_2$ (unless there is a leak!). Also note that we have used the shorthand R_F here to indicate fluidic resistance, but this is really $\frac{8 \cdot \pi \cdot \eta \cdot L}{A^2}$, as in equation 4.

What Happens When Elements are Connected?

We only concern ourselves here with one situation involving connected components, illustrated in Figure 2, which we refer to as elements connected in series. Elements connected in parallel are also very important in some specific situations in LC, and these will be discussed in next month's installment. As with the analysis of voltage and pressure drops discussed above, if we understand how systems behave when multiple elements are connected in different ways, we can make predictions about how we expect the systems to behave and observe when things don't look right. First, Figure 2a shows two electrical resistors connected in series. If we imagine what would be the resistance of a single element needed to replace the two connected resistors (see Figure 2b; in electronics, this is called a *Thévenin equivalent*), the key idea is that the resistances of two resistors connected in series simply add. Similarly, the voltage drops across each resistor also add such that the voltage drop across the single resistive element (that is, R_{ET} in Figure 2b) is the sum of the two individual voltage drops ΔV_1 and ΔV_2. Figures 2c and 2d show the comparable diagrams in the fluidic case. We see that the fluidic resistances connected in series add such that the resistance of the single equivalent element (R_{FT}) is the sum of the two individual resistances R_{F1} and R_{F2}. Also, the pressure drops across the two elements add such that the pressure drop across the single resistive element is the sum of the two individual pressure drops. We will see in next month's installment that this fact is very useful when troubleshooting pressure problems.

Kirchhoff's Current Law

Kirchhoff's Current Law, also known as the *junction rule*, describes the behavior currents in electrical systems. It is useful to think of it as an expression of the concept of *conservation of charge*. Specifically, the rule asserts that the sum of the currents into any junction (the blue dots in Figure 3) must be exactly equal to the sum of all currents exiting the junction. That is, all the charge that flows into the junction must flow out of the junction. In Figure 3a, where there is only one current in and one current out, $i_1 = i_2$. If we add a second conductor connected to the junction on the "out" side carrying a current i_3, then the rule tells us that $i_1 = i_2 + i_3$. More interesting to chromatographers is the idea that this same kind of rule applies to flows entering and exiting a junction in an LC system. In this case, the fundamental concept in play is the idea of *conservation of mass*. In Figure 3c, all the molecules of fluid entering the junction must also exit the junction, so that mass is conserved, and $F_1 = F_2$. This is the most common situation in LC systems. However, if we add a third tube carrying fluid at the flow rate F_3, connected to the outlet side of the junction, then we see that $F_1 = F_2 + F_3$. We will see that this relationship is particularly useful when making predictions about how a passive flow splitter works, as will be discussed in next month's installment.

Looking Ahead

Having discussed the essential relationships between flow and pressure in fluidic systems, we are now well positioned to apply these principles to important practical situations encountered in LC systems. In next month's installment of "LC Troubleshooting," we will demonstrate how these ideas can be used to systematically troubleshoot common problems related to flow and pressure, and introduce freely available calculation tools that can be used to make predictions about flows and pressures based on inputs of other practical variables such as tubing length and diameter, solvent composition, and temperature. We will also use the ideas discussed here to understand how a passive flow splitter works, and make predictions about split ratio for a given configuration of tubing lengths and diameters used in the splitter. Finally, we will discuss the pressure drop across an LC column containing particles. In this case, equation 3 does not apply, and we need to make some adjustments to the flow-pressure relationship to accurately predict these pressure drops.

Summary

In this installment, we have discussed the idea that concepts from electronics can be used to help understand the relationship between flow and pressure in LC systems. When analyzing electrical systems, Kirchhoff's Laws concerning voltages and currents can be used to rationalize the behavior of these systems and make predictions about how things are expected to change when a component of the system is adjusted. Analogous relationships can be used when analyzing fluidic systems, which enable us to make predictions about how pressure and flow will change when we make an adjustment to an element of the system, such as changing the diameter of a piece of tubing. In next month's installment, we will put these relationships and knowledge to work, demonstrating how it all can be used to troubleshoot common problems related to pressure and flow that arise when using LC systems. ■

This article has additional supplemental information only available online.
Scan code for link.

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From Pittcon 2024 and Beyond: New Gas Chromatography Products to Watch This Year

Nicholas H. Snow

I am pleased to present our annual review of new products in gas chromatography, introduced between spring 2023 and spring 2024. Pittcon convened in sunny San Diego, California, from February 24–28, 2024, and continued the trend of trade shows becoming smaller over the past several years. The intimacy of the show allowed me to easily converse with many colleagues and vendors who I see in-person once a year. The personal interactions, collaboration, and new ideas were more than worth the cost and effort of travel. Many of the major vendors were not present; I hope that they will reconsider attending and marketing at Pittcon and other conferences both as a means for "waving the flag" and for personal interaction and collaboration with colleagues and decision makers. The smaller conference opens up new venues and opportunities, with future meetings in Boston, Massachusetts, San Antonio, Texas, and back in San Diego.

PITTCON 2025 will be held from March 1–5 in Boston, Massachusetts. Although travel funding and opportunities are tight, I challenge everyone to start or resume attending professional conferences this year. Also, consider participating in a local or regional chromatography discussion group or local section of the American Chemical Society (ACS) meeting. In the remainder of 2024 and early 2025, I plan to attend at least four meetings, including the American Chemical Society in Denver, Colorado in August 2024, the Eastern Analytical Symposium in Princeton, New Jersey in November, 2024 and Pittcon in Boston in March, 2025. I have used the rise of the virtual conferences to attend additional meetings that I would not have attended otherwise due to the travel costs. Coming out of the pandemic, current global economic conditions were extremely difficult for conference organizers and for the many vendors, contractors, and economies that conferences support. They still need your help, even more than last year. Conference and meeting attendance is still the best way to improve skills, learn about new techniques, train, and educate yourself.

For a no-cost virtual experience, look out for the 2024 ChromTalks, in June, sponsored by *LCGC International* and CHROMacademy, its online training platform.

Gas chromatography (GC) continues to have a strong presence on the conference circuit and in the literature. Topics such as cannabis analysis, biofuels, food and beverages, forensics, pharmaceuticals, and general applications of both GC and two-dimensional gas chromatography (GC×GC) are seen in a robust publication space. GC itself is trending toward being less the driver of research than its application to scientific problems. The most effective online search for GC today probably begins with phrases such as "GC of _____" with your analyte or sample of interest filling in the blank.

TABLE I: Companies mentioned in this review

Buck Scientific
GL Sciences
Gow-Mac
Hyperchrom SA
Jeol
LECO
Lucidity
PAC
Restek
Shimadzu
Yoke Instruments

The information presented in this article is based on vendors' responses to questionnaires and additional information from press releases, websites, and product literature, not on actual

TABLE II: New Instruments and Systems in 2023–2024

Company	Product Name	Description
HyperChrom	Hyper-Fast Flow-Field Thermal Gradient GC	• Developed by Peter Boeker at Bonn University • Based on a thermal gradient principle, a standard, 4-m capillary column is sheathed in a metal sleeve • The temperature differential along the column between the injector and detector vastly increases the number of theoretical plates experienced by the sample components • Runtimes are reduced by a factor of 10–20 times • Most applications on HyperChrom involve runtimes and cycle times of between 1–2 min. • Throughput is thus increased by greater than an order of magnitude
Shimadzu	Brevis GC-2050	• Ultra-compact design, 35% reduction in footprint • Can be configured with a variety of detectors and sample introduction techniques, including dual-line configurations • Uses industry-standard columns and consumables • Smaller oven to reduce thermal mass and heat loss, reducing power consumption by 30% • 350-mm system width • Supports up to two injection ports and three detectors • Compact GC supports simultaneous operation of two analytical lines • Reduced helium usage via carrier gas saver mode • Remote display monitoring and control • Simplified maintenance and tool-less column installation • Quick, efficient automatic conditioning • Allows use of hydrogen as the carrier gas
Shimadzu	GCMS-QP2050	• Advanced automated technology in a compact footprint • Easy-to-use system • Can be partnered with Shimadzu's high-end Nexis GC-2030 or compact Brevis GC-2050 to meet customers' performance and space requirements • New DuraEase ion source reduces downtime by enabling maintenance in only 1 min., but also maintains the way for new stability and sensitivity throughout its lifetime • Easy sTop and Vacuum control from the front GC panel makes maintenance software free • Long life filament, lasts about five times longer than standard filaments • High-speed scanning (30,000 AMU/s) • Interface was redesigned for optimized temperature distribution and to suppress or remove the adsorption of high boiling compounds or analytes, leading to more repeatable results • New detector with the latest noise suppression technology and high amplification performance • Alternative carrier gas ready out-of-the-box—all stainless-steel internal plumbing allows for immediate hydrogen connection
Yoke Instruments	GC-7860 Series	• 8-inch touch screen • Manual and full EPC control for nitrogen, air, helium, hydrogen, argon • Heating rate up to 80 °C/min. • 450 °C maximum temperature • Supports double oven and double rear opening modes • 6-way external event control • Up to three detectors: TCD, HTCD, FID, FPD, ECD, NPD, ZD, PDHD, PID, AID • Inlets include split/splitless, packed, valve, autosampler, headspace, thermal desorption, purge and trap • Multi-dimensional chromatography available

use or experience of the author. Every effort has been made to collect accurate information, but because of the preliminary nature of some of the material, *LCGC International* cannot be responsible for errors or omissions. This column cannot be considered a complete record of all new GC

TABLE III: New Stationary Phases Introduced in 2023-2024

Company	Product	Description
GL Sciences	InertCap 1 and 5 HT	• InertCap 1HT is a non-polar high-temperature column bonded 100% dimethlypolysiloxane • Compounds are eluted in order of increasing boiling point • InertCap 1HT is specially processed for operation up to 400 °C with a high-temperature polyimide coated fused silica tubing • InertCap 5HT is a low-polarity, high-temperature column bonded with 5% Diphenyl (equiv.) – Dimethylpolysilphenylene siloxane • It is specially treated for operation up to 400 °C using high-temperature polyimide-coated fused silica tubing, allowing for the elution of high-boiling-point compounds and achieving excellent peaks

TABLE IV: New Sampling Devices and Detectors for 2023–2024

Company	Product	Description
PAC	Sulfur or Nitrogen Chemiluminescence Detector (SCD/NCD)	• On-board gas filtration system to deliver high gas purity and save bench space by removing the need to install a separate unit for cleaning incoming gas • On-board validation using the new G-Cal permeation device for detector sensitivity validation and detector troubleshooting (especially allowing users to separate out the detector from the complex GC application) • Separation of the furnace units from the control box allows for users to pick from sulfur only, nitrogen only, or a combination unit meaning each system takes up the same amount of bench space whether needing one element or both • Updated ceramic pyrotubes with new dimensions that help optimize the vacuum, give lower detection limits, and lengthen the time between maintenance of the detector • New GC column flow variation compensation in the form of a furnace base gas control to help remove the variability of incoming flow to the base of the detector that happens with normal oven ramping • Suggested Applications ASTM D5623 (trace sulfur components in light petroleum liquids), ASTM 5504 (sulfurs in natural gas and gaseous fuel), ASTM D7011 (thiophene in benzene), ASTM D7807 (boiling range of hydrocarbons and sulfur components), ASTM D5303 (trace carbonyl sulfide in propylene gas)

products introduced in the past year because not all vendors responded to the questionnaire, nor is all the submitted information necessarily included here, because of the limited available space and the editors' judgment as to its suitability. Table I provides a listing of the vendors highlighted in this review, some of whom are lesser known in the areas they are listed, showcased new products this year, or both. For a more complete picture of the GC space today, I encourage you to also review the three previous years' reviews (1–3).

In new instruments, trends seen over the past few years toward smaller, more automated systems, systems that are more specialized, and spectrometric detectors continue this year. New smaller-footprint products are seen from **HyperChrom**, **Shimadzu,** and a newcomer from China, **Yoke Instruments**. **Lucidity** has increased its product line in support of its mini-GC product, and small footprint GCs are still available from reliable standbys **Gow-Mac** and **Buck Scientific**. Table II shows a list of new, complete instruments introduced over the past year. All these systems are designed with optimizing and simplifying workflows, operation, and maintenance. One concern is that systems are becoming so automated and convenient to use that training and education may be viewed as less necessary, a seemingly reasonable view until something goes wrong. They represent a range from a portable instrument about the size of a small briefcase, to a bench-top instrument to a range of options for gas chromatography–mass spectrometry (GC–MS) and gas chromatography–tandem mass spectrometry (GC–MS/MS).

Stationary phases continue to be updated by most of the vendors, with nearly all making incremental improvements or showing new applications in their online application note libraries. New stationary phases, from **GL Sciences**, highlighted in Table III, illustrate this incremental trend. Using classical polydimethysiloxane stationary phases and new polyimide coatings they extend the range of fused silica columns to 400 °C. Users are encouraged to contact and keep up with your column vendor; new phases and improvements are happening continuously.

In 2023–2024, after a strong several years, there were relatively few new developments in developments in new accessories, sampling devices, and detectors. These continue to trend toward simplifying analysis and making chromatographers' work easier. A new sulfur and nitrogen chemiluminescence detector was offered by **PAC**, summarized in Table IV. **VUV Analytics** continues to improve its small-footprint LUMA vacuum ultraviolet detector, making it now compatible with most major vendors' data systems. **LECO** continues to develop its Precision Flow Modulator, making GC×GC more accessible and simpler to use. For more information on sample preparation, see the specific sample preparation article by Doug Raynie in *LCGC International* next month. As with the GC reviews, it is worthwhile to review the past two years as well for a complete picture of sample preparation products (4,5). Table IV provides a listing of products released in the past year that illustrate this trend. Many of the accessories are instrumental improvements in detectors and sampling devices.

Software and online resources continue to evolve along with the

new technology and upgrades to existing platforms. **Shimadzu** and **Jeol** highlight the addition of artificial intelligence (AI) to assist in method development and spectral interpretation in their GC–MS and GC–MS/MS systems. With the emergence of AI throughout society and science, it is worthwhile to inquire of any software and instrument control vendor, where they stand with implementing AI capabilities. **Restek** continues to develop and improve its online GC and LC method development tools, Pro-RZGC and Pro-EZLC. These are both simple to use and worth a look, even if you just like to play around with seeing the effects of changes in conditions on separations.

Our emergence from the pandemic provides more opportunities to continue education and training. Besides numerous short courses associated with Pittcon and other major conferences, many additional training and educational resources are available for 2024. The pandemic has resulted in much growth in online training and seminar offerings by vendors and training organizations, so check with your vendor. Opportunities for in-person short courses, seminars and training are moving again and are offered by most major instrument vendors and through many conferences and organizations, including the American Chemical Society, Eastern Analytical Symposium (Princeton, New Jersey, November 2024), local and regional chromatography discussion groups and, of course, Pittcon. *LCGC International*'s online training system, CHROMacademy, is continuously adding content and is again hosting ChromTalks, a virtual symposium in June 2024.

As GC moves through 2024 and beyond, development and innovation are ongoing and include all areas: sampling and supplies, new instruments, columns, detectors, data systems, and educational opportunities. I look forward to more innovation and advancements in the coming years and I hope to see many of you at EAS 2024 in my home state of New Jersey, or at Pittcon 2025 in Boston. ■

This article has additional supplemental information only available online.
Scan code for link.

ABOUT THE AUTHOR

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is the Founding Endowed Professor in the Department of Chemistry and Biochemistry at Seton Hall University, and an Adjunct Professor of Medical Science. During his 30 years as a chromatographer, he has published more than 70 refereed articles and book chapters and has given more than 200 presentations and short courses. He is interested in the fundamentals and applications of separation science, especially gas chromatography, sampling, and sample preparation for chemical analysis. His research group is very active, with ongoing projects using GC, GC–MS, two-dimensional GC, and extraction methods including headspace, liquid–liquid extraction, and solid-phase microextraction.

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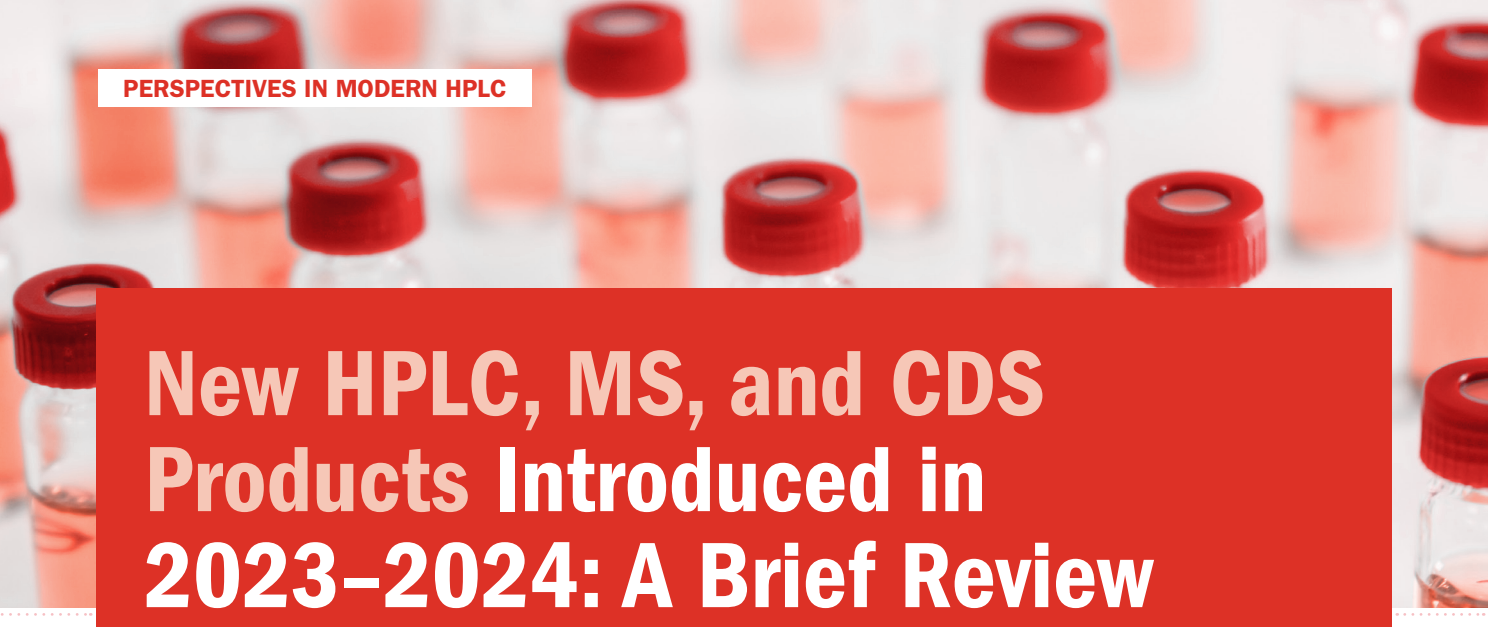

New HPLC, MS, and CDS Products Introduced in 2023–2024: A Brief Review

Michael W. Dong

This article describes newly introduced high-performance liquid chromatography (HPLC), mass spectrometry (MS), chromatography data systems (CDS), and related products in 2023–2024. It summarizes their technical specifications and significant user benefits.

THE 75TH PITTCON was held at the San Diego Convention Center, California, on February 24–28, 2024, for the first time on the West Coast. San Diego, a city of 1.3 million, is renowned for its moderate year-round temperatures and outstanding tourist attractions (including Gaslamp Quarter, Balboa Park, the San Diego Zoo, Sea World, La Jolla, Coronado, and Legoland), and academic and industry centers (UC San Diego, Scripps Institute, Salk Institute, Qualcomm, and Illumina). Its proximity to Los Angeles and the availability of many moderately priced hotels make it an ideal conference venue. The Pittcon organizer recently changed the meeting locations from a four-city rotation of Philadelphia, Chicago, Atlanta, and New Orleans to a new city list of San Diego, Boston, and San Antonio. We hope exhibitors and attendees will embrace these exciting locations.

HPLC and MS New Product Introduction 2023–2024

Table I lists new product introductions ordered alphabetically by the vendor's name, categorized as high-performance liquid chromatography (HPLC) systems, modules, mass spectrometry (MS), chromatography data systems (CDS), and other software. Each product's innovative features and benefits are briefly described, including a summary of pertinent specifications. The footnotes of Table I contain the definitions of common acronyms used in the table and text.

HPLC and UHPLC Systems (Including IC, CE, and Preparative LC)

This section describes new HPLC, ultra-high-pressure liquid chromatography (UHPLC), ion chromatography (IC), capillary electrophoresis (CE), and supercritical fluid chromatography (SFC) systems and their salient features, benefits, and technical specifications.

Agilent

1290 Infinity II Bio Online LC System

The 1290 Infinity II Bio Online LC System blends high-performance and biocompatible features for comprehensive analysis and real-time monitoring in pharmaceutical bioprocessing. It offers advanced control over process analytical technology (PAT) needs. The system facilitates precise biomolecule separations and analyses alongside real-time process monitoring, optimizing processes quickly to save time and resources. Agilent's online monitoring software allows remote monitoring and control of chromatography systems, providing alerts and data analysis with minimal effort.

InfinityLab GPC/SEC Solutions

The GPC/SEC system offers a comprehensive characterization of macromolecules using specialized instruments, columns, standards, and software, including advanced multidetectors such as multi-angle light scattering, micro refractive index detection, and improved GPC software (WinGPC) on the OpenLab CDS. The system can handle high-temperature GPC (up to 220 °C).

ProteoAnalyzer System

This parallel CE analysis system separates 10–240 kDa proteins with automated CE-SDS (12 capillaries). A streamlined workflow labels proteins with fluorescent tags detected with a charged-coupled device (CCD) camera for quantitation. It provides an all-in-one analysis with powerful software (ProSize data analysis).

Axcend

Reaction Monitoring Solution

Axcend's compact Focus LC for process analytical technology (PAT) can be placed adjacent to a reaction vessel, enabling scientists to monitor chemical reactions under near-real-time conditions. The miniaturized LC is compatible with the Agilent OpenLab Chemstation edition and the DataApex Clarity CDS.

Knauer

Azura UHPLC 1240 Bar System

The new 1240-bar UHPLC system has a flow range of 0.1–5.0 mL/min and targets high-throughput UHPLC and MS/MS applications.

The 1st Annual Rising Stars of Separation Science Awards celebrates the achievements of early-career separation scientists who are propelling the field field forward.

The 2024 awardees will be honoured at the **ISC conference Liverpool, United Kingdom on Monday 7 October 2024** followed by an evening celebration at the **Cavern Club**.

Rising Stars of SEPARATION SCIENCE
LCGC INTERNATIONAL™

2024 Categories

- Liquid Chromatography
- Gas Chromatography
- Life Science (Pharmaceutical)
- Life Science (Biopharmaceutical)

Submission deadline: 1 June 2024

Submit Your Nomination

ISC2024
LIVERPOOL, 6-10 OCTOBER 2024

TABLE I: Summary of new HPLC, MS, and CDS products in 2023-2024

Manufacturer	HPLC, MS, and CDS	Description
Agilent	Agilent 1290 Infinity II Bio Online LC System	The 1290 Infinity II Bio Online LC System blends high-performance and biocompatible features for comprehensive analysis and real-time monitoring in pharmaceutical bioprocessing.
	InfinityLab GPC/SEC Solutions	The GPC-SEC system offers a comprehensive characterization of macromolecules using specialized instruments, columns, standards, and software.
	ProteoAnalyzer system	This parallel CE analysis system separates 10-240 kDa proteins with automated CE-SDS (12 capillaries).
	Revident LC/Q-TOF	The Revident LC–QTOF revolutionizes the identification process with automated reinjection and sophisticated diagnostics for MS/MS applications.
	OpenLab 2.7	The OpenLab CDS version 2.7 offers innovations that drive higher productivity in the chemical, energy, and pharmaceutical markets.
Axcend	Reaction Monitoring Solution	Axcend's compact Focus LC enables scientists to monitor chemical reactions under near-real-time conditions.
Elsci	Elsci Peaksel	Peaksel is an HPLC processing software for batch processing and high-throughput experiments.
Knauer	Azura UHPLC 1240 bar system	The new 1240-bar UHPLC system has a flow range of 0.1–5.0 mL/min and targets high-throughput UHPLC and MS/MS applications.
	Dosing pump 4.2S compact pump	The Azura P 4.2S compact pump has a pressure rating of 400 bars and supports flow rates of up to 50 mL/min.
	Azura FC 6.1 Fraction collector	The FC 6.1 is a small, versatile fraction collector for FPLC and HPLC applications.
Shimadzu	Nexera Prep	The Nexera Prep is a scalable modular automated preparative LC system designed for flexible purification workflows from analytical to semi-prep scale.
	Nexera Analytical and Prep SFC	The Nexera analytical and preparative SFC supports fully automated online sample preparation, analysis, and fractionation.
	CLAM-2040	The Shimadzu CLAM-2040 is a fully automated sample preparation module for LC–MS with built-in quality control functionalities and specification alerts.
	OAD TOF system	The QTOF LC–MS utilizes oxygen attachment dissociation (OAD) and allows the positional analysis of carbon-carbon double bonds in lipids.
	LabSolutions MD	A software solution for HPLC or UHPLC method development that uses QbD principles.
	LabSolutions Insight Biologics	LabSolutions Insight Biologics is a software for oligonucleotide characterization offering a complete workflow from data acquisition to analysis and reporting.
	LabSolutions Sync	LabSolutions Sync is an external control software synchronizing third-party software for sample pretreatment units with Shimadzu LC and LC–MS.
Shine Qingdao	CIC-300+ Dual-channel IC	The CIC-300+ Dual-channel IC allows simultaneous cation and anion analysis.
	CIC-D120+ IC	The CIC-D120+ IC is a reagent-free instrument with intelligent operation and real-time monitoring functions.

Shine Qingdao Shenghan Chromatograph Technology

CIC-300+ Dual-channel IC

The CIC-300+ Dual-channel IC allows simultaneous cation and anion analysis. It has a built-in eluent generator, vacuum degassing, consumables monitoring, and real-time monitoring functions with auto-ranging conductivity detection.

CIC-D120+ IC

The CIC-D120+ IC is a reagent-free instrument with intelligent operation and real-time monitoring functions, including leak detection, automated startup and shutdown, and auto-ranging conductivity detection.

Shimadzu

Nexera Prep

The Nexera Prep is a scalable modular automated preparative LC system designed for flexible purification workflows from the analytical to the semi-prep scale (1 mg to 2 g, with flow rates from 0.1 to 150 mL/min). The system is equipped with multiple detector options.

Nexera Analytical and Prep SFC

The Nexera analytical and preparative SFC and supercritical fluid extraction system supports fully automated online sample preparation, analysis, and fractionation for high recovery preparative purification.

Thermo Scientific

Inuvion Ion Chromatograph

The new Thermo Scientific Dionex Inuvion IC makes ion analysis simpler and intuitive for routine analysis with an intelligent design, automated startup and shutdown routines, built-in diagnostics, and reagent-free operations. It is a compact IC on a flexible and upgradable platform.

Waters

Alliance iS HPLC System

The Alliance iS HPLC System (intuitive simplicity) is a next-generation HPLC system with a quaternary pump and a UV absorbance detector that reduces common laboratory errors, operator training requirements,

and method transfer time. The Alliance iS HPLC System improves pharmaceutical quality control laboratories' productivity and data quality through its intelligent design, intuitive touchscreen operation, built-in error-reducing features, and intelligent method translation apps. The system was introduced in March 2023. Updated specifications include a gradient delay volume <1000 mL, a flow range of 0.001 to 10 mL/min, maximum pressure of 10,000 psi, a pH range 1 to 13, a flow accuracy of ±1%, compositional accuracy of ±0.5%, an injection precision of <1.0% to <0.25% depending on injection volumes using flow-through needle design, and sample carryover of ≤0.002%.

HPLC Module (Including Sample Preparation Module)

Knauer

Dosing Pump 4.2S Compact Pump

The Azura P 4.2S compact pump is the successor to the P 2.1 and P 4.1 pump family. The pressure rating is 400 bars and supports flow rates up to 50 mL/min.

Azura

FC 6.1 Fraction Collector

The FC 6.1 is a small, versatile fraction collector for fast protein liquid chromatography (FPLC) and HPLC applications. It can be used for flow rates from 0.1 to 250 mL/min and offers space for a main rack and a small side rack. The main rack can hold 3 x 96 well plates, 165 x 1.5/2 mL tubes, 99 x 15 mL tubes, or 30 x 50 mL tubes. The side rack offers space for 3 x 250 mL bottles.

Shimadzu

CLAM-2040

The Shimadzu CLAM-2040 is a fully automated sample preparation module for LC–MS with built-in quality control functionalities and specification alerts. The 2040 module automatically performs all processes necessary for analyzing biological samples, including sample and reagent pipetting, mixing, heating, vacuum filtration, and transferring the extracted sample to the LC–MS system autosampler. It can prepare up to three samples simultaneously and deliver a result every 2.6 min under optimized conditions.

Thermo Scientific

Vanquish Refractive Index Detector

The Vanquish refractive index detector (RID) extends detection to non-chromophoric compounds such as carbohydrates, lipids, or polymers. The new detector saves bench space and offers leak detection and safe handling.

Waters

DynaPro ZetaStar

The ZetaStar DLS (dynamic light scattering) instrument measures dynamic, static, and electrophoretic light scattering. It provides data on size and size distribution, zeta potentials (charge), particle concentrations, turbidity, and stability indi-

TABLE I (CONTINUED): Summary of new HPLC, MS, and CDS products in 2023–2024

Manufacturer	HPLC, MS, and CDS	Description
Syft Technologies	Syft Tracer	Syft Tracer is a direct injection selected ion flow tube MS (SIFT-MS) for high-throughput volatile organic compounds or inorganics analysis.
	Syft Tracer Pharm11	Syft Tracer Pharm11 is a complete solution for high-throughput automated workflows in pharmaceutical applications.
Thermo Fisher Scientific	Inuvion ion chromatograph	The Inuvion IC makes ion analysis simpler and more intuitive for routine analysis.
	Vanquish refractive index detector (RID)	The Vanquish RID extends detection to non-chromophoric compounds such as carbohydrates, lipids, or polymers.
	Orbitrap Astral MS	The Orbitrap Astral is a quadrupole-orbitrap-Astral MS that combines faster throughput, deeper coverage, and higher sensitivity with accurate and precise quantitation.
	Neoma MS/MS MC ICP-MS	Neoma MS/MS MC-ICP-MS is a high-resolution double-focusing multi-collector ICP-MS that offers better sensitivity and precision for isotope analysis by wet or dry plasma.
	Chromeleon 7.3.2	The Chromeleon 7.3.2 CDS delivers new features and enhancements for higher efficiency and stability, particularly regarding MS data processing.
Waters	Alliance iS HPLC System	The Alliance iS HPLC System is a next-generation HPLC designed for routine QC labs, combining intuitive operation with improved system robustness, capable of reducing human errors by up to 40%.
	DynaPro ZetaStar	The ZetaStar instrument measures dynamic, static, and electrophoretic light scattering.
	Xevo TQ Absolute IVD MS	The Xevo TQ Absolute IVD MS is a compact TQ MS that offers improved sensitivity, system uptime, reduced running costs, and lower LOQs with six orders of dynamic range.
	Targeted MS Imaging Solution	Combines DESI XS with Xevo TQ Absolute for a first-to-market MS Imaging solution, enabling highly sensitive compound visualization at a faster speed.
	Clinical Diagnostics Solution – MassTRAK Solutions	MassTRAK IVD Systems allow instrument customization to provide accurate results while maintaining regulatory compliance in clinical analysis using LC–MS/MS.
	MRT Resolution Enhancement Mode (REM)	An extension of the core capability of the MRT (Multi Reflecting TOF) analyzer technology with a REM provides a 50% increase in mass resolution.
	Empower 3.8.0 & 3.8.1	Waters Empower 3.8.1 introduces enhanced technical controls to reduce human bias during result generation. Additionally, it reinforces data integrity, enhances security, and adds over 20 enhancements that further reduce risks.
	Bioprocess Walk-Up Solutions	The Bioprocess Walk-Up Solutions is a streamlined interface and dedicated workflows with automated sample preparation for critical attribute measurement during biologics process development.

Definition of acronyms: SQ (single quadrupole), TQ (triple quadrupole), TOF (Time of Flight), Q-TOF (quadrupole-Time of Flight), FWHM (Full width at half maximum), DDA (data-dependent acquisition), DESI (Desorption Electrospray Ionization, CE (capillary electrophoresis), IC (ion chromatography), GPC/SEC (gel permeation chromatography and size exclusion chromatography), UPLC (ultra-performance liquid chromatography), SFC (supercritical fluid chromatography), FPLC (Fast protein liquid chromatography), ICP (Inductively Coupled Plasma), RID (refractive index detector), UV (ultraviolet), PDA (photodiode array), ELSD (evaporative light scattering detector), AI (artificial intelligence) IVD (Invitro diagnostics), DLS (dynamic light scattering), QbD (Quality by Design), CDMO (Contract Development Manufacturing Organization), GMP (Good Manufacturing Practice), 21 CFR Part 11 (21 Code of Federal Regulations Part 11), USP (United States Pharmacopeia), pptv (part per trillion by volume), LOQ (limit of quantitation), kDa (kilo Dalton), and fg (femtogram).

cators of nanoparticles and biological drug products. It can be paired with the ARC HPLC autosampler to support the expanding needs of biopharma formulation and development laboratories. The ZetaStar instrument helps scientists in gene therapy, vaccines, mRNA, and protein analysis in developing more stable formulations.

Mass Spectrometers (MS)

Agilent

Revident LC/Q-TOF

The Revident LC/Q-TOF revolutionizes the identification process with smart automated reinjection and sophisticated diagnostics for MS/MS applications with multiple workflow capabilities, combining excellent isotope fidelity, mass accuracy, and dynamic range. The three common workflows include Intelligent Reflex, targeted MS/MS for suspect confirmation, and iterative MS/MS.

Shimadzu

OAD TOF System

The quadrupole time-of-flight (Q-TOF) LC–MS utilizes oxygen attachment dissociation (OAD). This proprietary Shimadzu fragmentation technology allows the positional analysis of carbon–carbon double bonds in lipids and other organic compounds. Collision-induced dissociation (CID) is also possible with this system, and users can seamlessly switch between OAD and CID for analysis.

Syft Technologies

Syft Tracer

Syft Tracer is the next generation of real-time, direct injection selected ion flow tube MS (SIFT-MS) optimized for high-throughput volatile organic compounds or inorganics analysis. The system uses microwave discharge for ion generation and MS/MS detection and quantitation. The mass range is 10–400 amu, with low sensitivity.

Syft Tracer Pharm11

Syft Tracer Pharm11 is a complete solution for high-throughput, *21 CFR Part 11* compliant, automated workflows in pharmaceutical contract development and manufacturing organization (CDMO) applications.

Critical quality tests for drug impurities like nitrosamines, ethylene oxide, residual solvents, formaldehyde, and others can be performed in real time. The system can perform headspace extraction, dissolution in aqueous solvents, or non-polar alternatives, and direct sampling from powder or solid headspace.

Thermo Scientific

Orbitrap Astral MS

The Thermo Scientific Orbitrap Astral is a quadrupole-orbitrap-Astral MS that combines faster throughput, deeper coverage (for proteomics sequencing), and higher sensitivity while delivering accurate and precise quantitation. It has a mass range of 40–6000 *m/z* (or up to 8000 with the biopharma option), a dynamic range of >5000 in a single microscan, a mass resolution of 480,000 (fwhm) in the Orbitrap analyzer, and 80,000 in the Astral analyzer, an MS/MS mass accuracy of <3 ppm in the Orbitrap analyzer and a <5 ppm in the Astral analyzer, a scan rate of 200 Hz in the Astral analyzer, and single-ion sensitivity of 50 fg of reserpine with the Astral analyzer.

Neoma MS/MS MC ICP-MS

Neoma MS/MS MC-ICP-MS is a high-resolution double-focusing multi-collector ICP-MS that filters out the noise for better sensitivity and precision for isotope analysis by wet or dry plasma.

Waters

Xevo TQ Absolute IVD MS

The Xevo TQ Absolute IVD MS is a compact triple quadrupole (TQ)-MS that offers improved sensitivity, system uptime, reduced running costs, and lower limits of quantitation (LOQs) with six orders of dynamic range.

Targeted MS Imaging Solution with DESI XS and Xevo TQ Absolute

The DESI XS MSI is typically applied for mapping small molecules directly from tissue sections and, combined with TOF-MS, is ideally suited for untargeted discovery analysis. On the other hand, TQ-MS systems are renowned for their sensitivity, speed, and quantitative robustness for targeted applications using multiple reaction monitoring (MRM) modes of acquisition, and they are widely adopted for drug quantitation. Combining the two technologies, a remarkable and unique technique emerges to perform sensitive, fast, and quantitative MSI directly on the surface.

Clinical Diagnostics Solution – MassTRAK Solutions

MassTRAK IVD systems allow instrument customization to provide accurate results while maintaining regulatory compliance in clinical analysis using LC–MS/MS with an I-Class Acquity UPLC coupled to Xevo and TQ-S micro or TQ Absolute IVD systems. They support columns, reagents, sample handling, automation, and informatics solutions.

MRT Resolution Enhancement Mode (REM)

Recent updates for the Select Series MRT were introduced to improve specificity and utility for UPLC–MS/MS metabolomics, drug discovery applications, and mass spectrometry imaging experiments. The MRT System now offers a new resolution enhancement mode (REM) that provides 50% higher resolution, making it capable of 300,000 fwhm resolution and a 3X faster scan rate,

while maintaining its parts-per-billion (ppb) mass accuracy.

Chromatography Data System (CDS) and Other Software

Agilent

OpenLab 2.7

The latest release of OpenLab CDS version 2.7 offers innovations that drive productivity to a higher level in the chemical, energy, and pharmaceutical markets, including calibration curve improvements, integration optimizer enhancements, centralized unattended printing, and cloud-based help and learning.

Elsci

Peaksel

Peaksel is an HPLC processing software for high-throughput experiments. It has a browser-based interface to facilitate collaboration and data sharing with Spotfire-like visualizations to eliminate the need for Microsoft Excel. It has templates to promote standardization, an application programmatic interface to enable automation, and batch-processing capabilities for high-throughput workflows.

Shimadzu

LabSolutions MD

LabSolutions MD is a software solution for HPLC or UHPLC method development using analytical quality by design (AQbD) principles. It improves the efficiency of analytical method development by screening for optimum parameters, such as columns and mobile phases, using design of experiments (DoE). An AI-driven automated gradient optimization function also tests and predicts the best gradients to achieve the user's set parameters, such as resolution and tailing factor. LabSolutions MD works with LC, SFC, and most detectors, including UV, photodiode array (PDA), evaporative light scattering detector (ELSD), and single quadrupole MS (SQMS).

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Thermo Scientific

Chromeleon 7.3.2

The Chromeleon 7.3.2 CDS delivers new features and enhancements for higher efficiency and stability, particularly regarding MS data processing. It guides operators with a customizable user interface and reporting engine and streamlines the handling of custom variables. It simplifies the support of good manufacturing practices (GMP) guidelines to ensure data integrity and traceability.

Waters

Empower 3.8.0

The new features of Waters Empower 3.8.0 include views of audit trails and user types, calculations based on USP <621> and EP 11, assignable privileges, Empower method validation signature/workflow optimization, intensity label levels for the spectrum index plot and the mass analysis plot, and Alliance iS HPLC system functionality suite with eConnect column technology.

Empower 3.8.1

Waters Empower 3.8.1 includes a suite of enhancements that highlight compliance, data quality, and security. The new features include technical controls for data processing, minimizing human bias during result generation. Audit trail entries have been fine-tuned to ensure data integrity by locking channels manually versus with a signature.

Bioprocess Walk-Up Solutions

The Bioprocess Walk-Up Solutions is a streamlined interface for dedicated workflows with automated sample preparation and LC–MS analytics. The interface allows a combination of the Andrew+ Pipetting Robot and the BioAccord LC–MS System to provide rapid sample processing from bioreactor systems for high-quality results with minimal user interaction.

Summary and Commentaries

This installment provides an overview of new separation science products introduced in 2023–2024. It summarizes innovative features, key benefits, and pertinent specifications of these new HPLC, MS, CDS, and other software products.

I have attended Pittcons for four decades, and Pittcon 2024 remained a rewarding and inspiring experience. Unfortunately, the number of exhibitors was significantly reduced post-pandemic, and several major manufacturers (Agilent, Thermo Fisher Scientific, Sciex, and Waters) were absent from the exhibition hall this year. I want to appeal to all instrument manufacturers to reconsider returning to the most prominent American laboratory conference to benefit laboratory scientists and consumers seeking to purchase new equipment, software, and consumables. I hope the Pittcon organizing committee will continue to deliver a conference of increasing relevance to the analytical science community and instrument manufacturers. I fervently hope to see more manufacturers at the exhibition halls of Pittcon 2025 in the city of Boston, the new pharmaceutical hub in America. ■

Acknowledgments

The author thanks the marketing staff of all manufacturers who provided timely responses to the *LCGC* questionnaires. A special thanks to my reviewers for providing timely technical and editorial inputs to this article: Alice Krumenaker of Hovione, Mengling Wong and Bifan Chen of Genentech, Alec Valenta of Thermo Fisher Scientific, Janice Foley of Waters, He Meng of Sanofi, Leon Doneski of Arcutis Biotherapeutics, Giorgia Greco of KNAUER, Emanuela Gionfriddo of U. Buffalo, and Stanislav Bashkyrtsev of Elsci. Their input is invaluable for the accuracy and clarity of this article.

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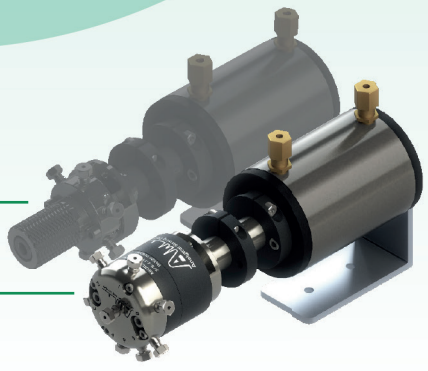

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Resolving Separation Issues with Computational Methods: What Is the Retention Time, Exactly?

Bob W.J. Pirok

OUR ATTENTION is typically focused on chromatography in the laboratory, yet ultimately, a liquid chromatograph (LC) or gas chromatograph (GC) only produces a signal—a chromatogram—from which information still must be distilled. Fortunately, software tools can generally help us quite well with our contemporary data sets. However, as separation technology improves, both the challenges and the need for methods to obtain information from the data increase. In this series of articles, we will discuss different aspects of data analysis and learn how to extract different pieces of information from our chromatograms, while also understanding the assumptions and risks used to help us interpret the value of the information. We start with simple, yet important, information bits, which we will tie together in future articles. In this way, we can continue solving separation problems beyond the limits of our chromatographs. Each article contains a reference to a web tutorial where the gained knowledge and skills can be practiced and applied to a real signal (1).

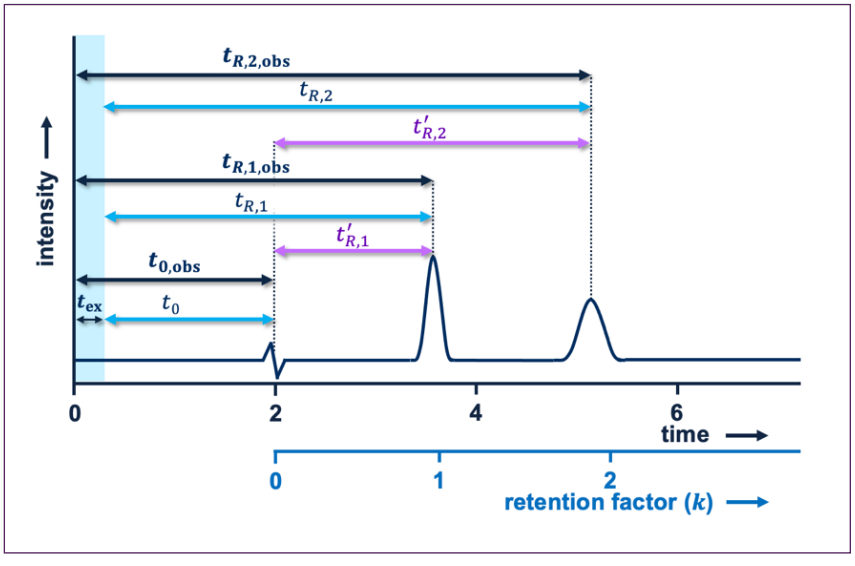

FIGURE 1: Schematic example of a chromatogram that presents different presentations of the retention time.

Relevance of the Retention Time

In this first article, we inspect the determination of the retention time, which is—next to the peak area—arguably the most basic information of interest from a peak. Its most elementary application is the qualitative identification of a known compound by means of reference information. For example, if our analyte of interest is usually eluted at 3.7 min for a given method, then we tend to identify it in a new measurement by looking for a peak at that specific time point.

The retention time is also of importance for fundamental research, including (i) the prediction of retention times in method development; (ii) retention modeling to characterize selectivity; and (iii) assessing the performance of chromatographic systems. In future arti-

cles, we learn about how data processing affects the retention time and subsequent properties of our signal (see Figure 1).

How do we determine the retention time? To understand our computational task, it is first relevant to refresh our perspective on what the retention time actually represents for our situation. The *retention time* is the time it takes for the analytes to be transported through a chromatographic column.

At this point, it is important to realize that the recorded chromatogram reflects the time at which the analytes are observed at the detector ($t_{R,\mathrm{obs}}$), not at the exit of the column. For fundamental studies of retention, it is thus important to subtract extra-column time (t_{ex}) from the observed retention time. The t_{ex} may be easily obtained by simply removing the column and measuring the elution time of an analyte. However, for most practical applications, its value is so small that it may reasonably be ignored. In some cases, the net retention time (t'_{R}) is used where the dead time (t_0 or t_{m}) is subtracted. Other cases use the relative retention time, where the retention time of one compound is compared to another.

From the moment that we inject our analytes, various band broadening processes occur as the analytes migrate through the chromatographic system. This results in our chromatographic peak, which never represents a singular discrete retention time, but rather a distribution of retention times. This is depicted in the chromatogram shown in Figure 2b, which highlights one of the peaks.

The most convenient method to determine the retention time is by simply regarding the apex, or maximum, of the peak (Figure 2b, Point I). This is especially correct when the peak is symmetrical, as is the case with the well-known Gaussian distribution (also known as *normal distribution*).

Intuitively, the peak apex may be found by finding the maximum value of a peak. This can be literally the time associated with the point of the peak with the highest *y*-value and is typically identified by functions that seek data points that are larger than (multiples of) its neighbors. Alternative methods include taking the derivative and identifying when it crosses zero, finding the minimum of the second derivative, and fitting a bell curve to the signal and interpolating its maximum. The strategy highly hinges on the characteristics of the chromatogram and the degree of robustness required.

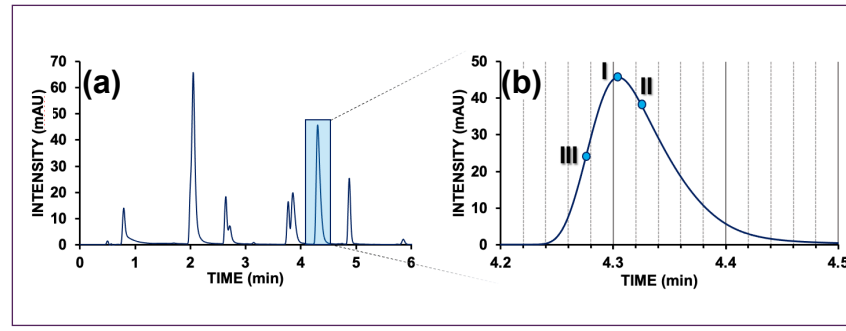

FIGURE 2: (a) Example of a small molecule separation by reversed-phase. (b) Highlighted peak with different retention time definition points references on the peak. See text for explanation. AU = arbitrary units.

However, peaks in chromatography are—especially in the case of LC—rarely symmetrical. An arguably more appropriate method to determine the retention time is by computing the mean of the chromatographic distribution (Figure 2b, Point II), which represents its center of gravity. The most accurate way to obtain this value is through the use of the first statistical moment (equation 1).

The first normalized statistical moment (m_1) is equal to the integral of the product of the peak signal and time (the gross first moment, M_1), divided by the integral of the peak (that is, its area, M_0).

$$m_1 = \frac{M_1}{M_0} = \frac{\int_{-\infty}^{+\infty} t\, h(t)\, dt}{\int_{-\infty}^{+\infty} h(t)\, dt} \qquad [1]$$

The advantage of the first-moment method is that it accounts for any peak asymmetry, but the challenge is that it is not easily and robustly obtained. We will see in a future installment that the statistical moments rely heavily on a correct definition of the start and end of the peak, which is especially tricky when peaks are partially co-eluted. This explains why it is more robust and, thus, more common to use the apex of the peak.

Finally, a rare determination of the retention time considers the start or frontal slope of the peak (Figure 2b, Point III). Although this is never useful for calculating the retention factor, it can sometimes be useful in cases of preparative separations, when the column is intentionally overloaded. Here, the interest is to identify when the peak is starting to elute so that it may be fractionated.

Having determined the retention time correctly, you are now able to compare the value between chromatograms or against reference standards. You may also consider tables or databases of retention values. However, in that case, it is essential to transform the retention time to dimensionless parameters that are much less dependent on experimental conditions, such as column dimensions and flow rates. ■

Reference

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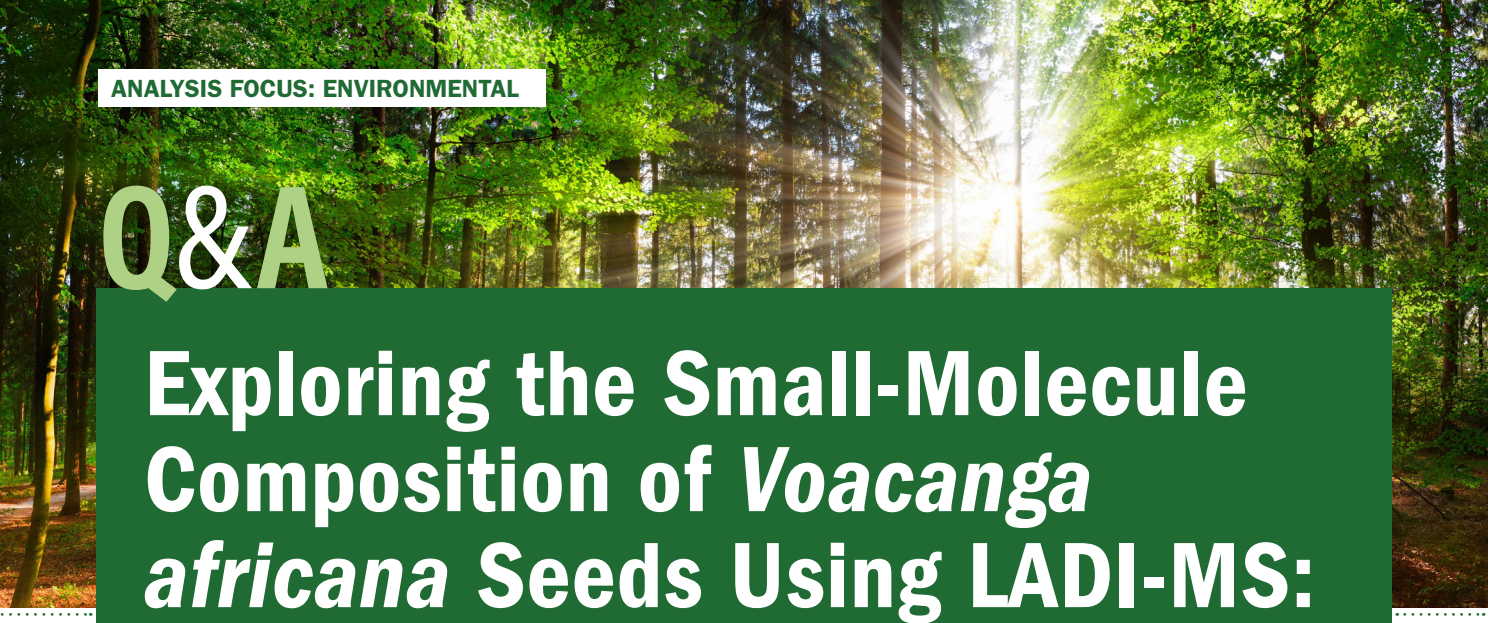


Q&A

Exploring the Small-Molecule Composition of *Voacanga africana* Seeds Using LADI-MS: An Interview with Rabi Musah

Will Wetzel

Plant seeds are a source of medicinal components used in human therapeutics. As a result, it is important that extraction methods are developed that can remove the bioactive compounds used for these therapeutics without damaging the plant tissue. In this "Focus on Environmental Analysis" column, *LCGC* sat down with Dr. Rabi Ann Musah from the University of Albany, who is currently researching methods to protect seed viability while isolating specific compounds. Musah's team used a technique called laser ablation direct analysis in real-time imaging–mass spectrometry (LADI–MS) to analyze the distribution of molecules, such as alkaloids, in *Voacanga africana* seeds.

PLANTS PLAY AN essential role on Earth. They are critical cogs in several ecosystems that help moderate the planet's climate. Plants have a unique versatility about them that allow them to be valuable in various functions. Apart from regulating ecosystems, plants help produce oxygen (which is necessary for many living organisms to live), serve as a primary source of food for most living organisms and as key ingredients for the development of essential pharmaceutical drugs, help prevent soil erosion, and provide some living organisms with a habitat. It is essential, therefore, that plants thrive on earth, and that requires ensuring that their seeds remain viable for growth and development.

However, what makes this challenging is the presence of bioactive compounds in plants, which could damage plant tissue if certain plant parts are removed. Plant seeds, because they often contain the medicinal compounds plants have, offer an alternative way to extract the compounds used in human therapeutics without sacrificing the integrity of the plant. Rabi Ann Musah, PhD, a professor of chemistry at the University of Albany, State University of New York, is exploring ways to help protect seed viability through optimizing the isolation of specific compounds (1,2). However, optimal accomplishment of this task requires knowing the spatial distribution of the molecules within the plant tissue (1). Musah and her team recently used an ambient mass spectral imaging technique called laser ablation direct analysis in real-time imaging–mass spectrometry (LADI–MS) to determine the distributions of molecules, including alkaloids, in *Voacanga africana* seeds (1).

Musah sat down with *LCGC* to discuss her findings and the importance that her research has on environmental sustainability and human development.

Q Could you explain the significance of utilizing plant seeds as a renewable resource for medicinal natural products in comparison to other plant parts? How does this approach contribute to sustainability?

Over the millennia, humans in all parts of the world have utilized plants to treat disease. The therapeutic benefit of plants is a consequence of the presence of bioactive compounds. Plant parts often used in traditional medicine for this purpose include leaves, bark, and roots. However, a disadvantage of harvesting these plant parts is that it can severely damage or completely destroy the plant. For example, removing bark exposes vulnerable plant tissue to disease-causing microbes, and the harvesting of roots can result in the sacrifice of an entire plant. It is also often the case that the presence of medicinal compounds occurs only after the plant has reached sexual maturity, which, for trees, can take several decades to achieve. Therefore, reliance on the aerial parts or the roots of trees for the acquisition of medicines may be unsustainable because the loss of the plants outpaces the ability to replenish them in a timely manner. In contrast, most plants produce a high volume of seeds at regular intervals, making the seeds a renewable resource.

It is not unusual for the seeds to contain compounds identical to or structurally related to medicinal compounds found in other plant parts. To the extent that they do, seeds can serve as a renewable reservoir of medicinal natural products, the utilization of which contributes to sustainability by eliminating the need to sacrifice whole plants to isolate useful molecules. Furthermore, their harvest is non-destructive to the plant.

Q The study mentions the use of laser ablation direct analysis in real-time imaging–mass spectrometry (LADI–MS) (2,3) to reveal spatial distributions of molecules within *V. africana* seeds (1). What advantages does this technique offer in understanding the distribution of compounds compared to conventional methods?

Conventional imaging mass spectrometry (MS) methods include matrix-assisted laser desorption/ionization–mass spectrometry imaging (MALDI–MSI) and desorption electrospray ionization–mass spectrometry imaging (DESI–MSI), among others. Although these approaches are immensely powerful, their application for the analysis of hard or porous plant tissues in particular is extremely challenging. For some methods, a high vacuum requirement results in the loss of small molecules, including the secondary metabolites that serve as medicinal compounds. This means that during the analysis, the presence of such molecules in the sample would be entirely missed. When the material being analyzed is porous, it can be difficult to achieve the reduced pressures required to perform the analysis. Sample preparation requirements can also be very challenging because the brittleness of the samples makes it difficult, if not impossible, to generate suitably thin slices without the samples fragmenting in the process. If the application of a matrix is a requirement of the technique, the method development, including matrix optimization, presents significantly time-consuming bottlenecks. For approaches that require solvent, sample wetting can distort the spatial distributions of detected compounds, which can lead to an erroneous interpretation of the results.

Laser ablation direct analysis in real-time imaging–mass spectrometry (LADI–MS) is a technique recently pioneered in my research group to circumvent some of the aforementioned challenges and facilitate interrogation of the spatial distributions of plant secondary metabolites contained within brittle, porous or hard plant tissues (3). LADI–MS is performed under ambient conditions without the need for any vacuum. This enables low mass range molecules that would otherwise evaporate under high-vacuum conditions to be readily observed. It accommodates samples with uneven surfaces that can be of up to 10 cm^2 in area with depths of up to 2 cm. No solvent is required, and thus, the small-molecule spatial distributions observed are not distorted by the presence of solvent.

Q What special sampling or analysis issues did you encounter in this research?

An issue that we encountered was the lack of availability of authentic chemical standards against which the chromatographic and mass spectral characteristics of detected tentatively identified alkaloids could be compared for the purpose of establishing structural confirmations. Because of their structural complexity, the generation of authentic standards through organic synthesis was not possible, as this would have entailed the launching of several multi-step synthesis projects that would likely span several years. For this reason, several of our identifications were based on tentative matching of EI fragmentation patterns for compounds contained within the NIST database. We would have liked to also analyze standards by gas chromatography–MS (GC–MS) to validate their retention times, as well as perform multistage mass spectrometry (MSn) analysis to confirm structure identifications.

Q Did you apply any specialized chemometrics or software for imaging or data analysis?

For our analyses, we used the imaging software Iolite (Elemental Scientific Inc., Omaha, NE) to couple the mass spectral data files to the file output of the laser system to generate the ion images.

Q What potential impact could the discovery of 31 new compounds in *V. africana* seeds have on medicinal research or drug development, particularly concerning opioid use disorder or other health conditions?

This work identified 31 compounds in *V. africana* seeds that included carbazole, sesquiterpenoid, carboline, monoterpene lactone, aromatic alcohol, and indole alkaloid compound classes. For any of these that are not commercially available and are of interest for medicinal natural product drug development or other purposes, *V. africana* seeds could be utilized as a source of these compounds. This could be especially beneficial for acquiring biologically active indole alkaloids. Studies have revealed that the indole alkaloid ibogaine, which has been isolated from genus *Voacanga* plants, may hold promise for the treatment of opioid use disorder (OUD) and other chemical addictions, and there is also interest in determining the potential of other structurally related compounds present in the plant as either treatments for OUD or precursors for the synthesis of ibogaine. Having knowledge of not only which indole alkaloids are present in the seeds, but also where they are localized, enables the efficient development of optimized extraction protocols for the isolation and purification of these compounds. Having greater access to these compounds could greatly facilitate progress on research and development of OUD treatments.

Q The study highlights the localization of fatty acids in the embryo and alkaloids in the seed endosperm (1). How might this information change or optimize the process of isolating specific compounds from these seeds?

The location information of compounds of interest could greatly streamline and enhance the efficiency of the process of isolating them. For example, most of the compounds of interest were found in either the embryo or endosperm of the seed. Therefore, a tumbling process could first be used to remove the outer seed coat. This would serve as an easy "pre-concentration" step that would also reduce the amount of solvent needed to isolate the compounds.

Q How can leveraging the spatial distribution of compounds within plant tissues, as demonstrated in this study, contribute to environmentally sustainable practices in the extraction of medicinal compounds (1)?

By first removing the outer seed coat using large, mechanical tumblers, the chemically rich endosperm and embryo can be exposed. This avoids processing plant tissues that do not contain the material of interest, which in turn reduces time and cost. It also minimizes the waste and overuse of solvent.

Q Considering the findings of this study, what strategies or methods could be employed to enhance the efficiency of secondary metabolite isolation from *V. africana* seeds while minimizing the need for harvesting other plant parts?

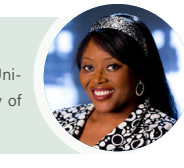
Whenever compounds of interest can be found within seeds, what we essentially have is a living manufacturing process by which molecules can be made available. This circumvents the need to isolate compounds from other plant parts, which is advantageous because isolating molecules from the roots, stem, leaves and bark not only has the potential to destroy the plant itself, but could also result in exposure of the plant tissue to pathogens that may themselves destroy the plant.

Q In the context of pharmaceutical or medicinal applications, how might the localization of specific compounds within distinct parts of the seed impact the formulation or development of drugs derived from these natural products?

The localization of compounds in distinct seed parts confers several advantages for their isolation and purification, providing that the spatial distribution of compounds is known. One of the challenges associated with the isolation of medicinal compounds or the semi-synthesis of medicinal compounds from plant-derived precursors is that a lot of waste can be generated when entire plant organs are needlessly processed, rather than only processing those segments where the compounds of interest are localized and concentrated. This can result in the generation of large amounts of biohazardous plant-derived and solvent waste. When the locales of target molecules in the plant parts are known, cost-effective and environmentally benign preprocessing steps, such as the mechanical removal of the seed coat and other tissue layers, can reduce the mass of material that would then need to be processed by solvent. This streamlines the isolation process while also saving time and yielding materials that, by mass, contain a higher relative concentration of the compounds of interest. ■

This article has additional supplemental information only available online. **Scan code for link.**

ABOUT THE INTERVIEWEE



Rabi Ann Musah, PhD
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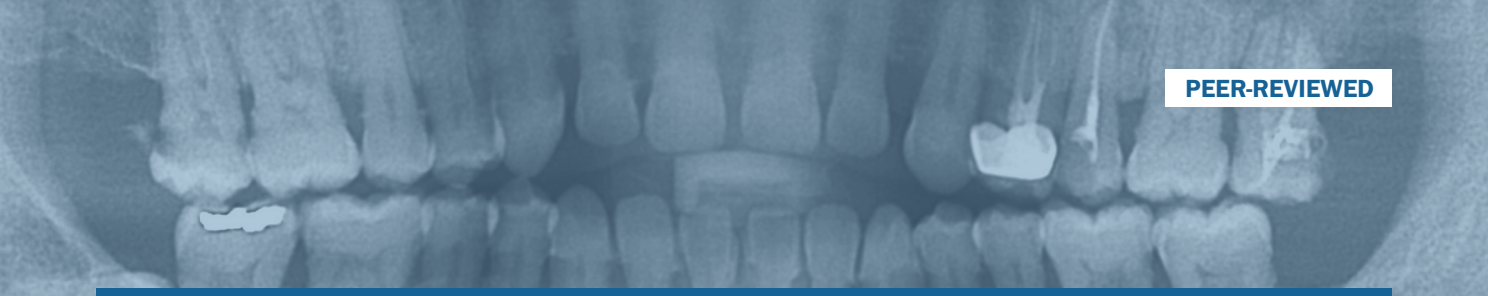

Validation of HPLC-DAD Based Stability Indicating Assay Method for Ornidazole in Periodontal Polymeric Hydrogel: Robustness Study using Quality by Design (QbD) Approach

Gopal Prasad Agrawal, Mohammed Ageeli Hakami, Farhan R. Khan, Prabhat Kumar Upadhyaya, Anuj Garg, and Meenakshi Bajpai

In the present study, a gradient reversed-phase high-performance liquid chromatography (RP-HPLC) method has been designed and validated to quantify Ornidazole (OZ) in the marketed formulation (oral gel) with the application of QbD. The proposed HPLC method utilizes an RP C18 column (4.6 x 250 mm, 5 µm). The mobile phase contained HPLC grade Solvent A (water) and Solvent B (acetonitrile) and ran at a flow rate of 1 mL/min in a gradient mode. The linearity was plotted in an OZ range of 1–12 µg/mL and the correlation coefficient was found to be 0.9998. The limit of quantification, as well as the limit of detection, has been recorded as 0.70 µg/mL and 0.23 µg/mL. Inter- and intra-day precision (RSD) percentages were reported respectively within a range of 0.262–0.589 and 0.179–0.879. At 80, 100, and 120 percent different OZ levels, the average accuracy was 99.55, 99.58, and 99.92, respectively. The result of the study indicates that the OZ gel estimate HPLC method has good sensitivity, reproducibility, accuracy, and precision. This method may be used for the estimation of OZ in a gel formulation in quality control laboratories.

ORNIDAZOLE (OZ) IS A 5-nitroimidazole derivative and is used as an antimicrobial drug. OZ is used to treat stomach, urinary tract, intestine, and genital infections because of anaerobic and protozoa bacteria (1,2). Ornidazole is also widely used to treat periodontitis (3–5). Nitro gr. (OZ) interacts with microbial DNA and inhibits nucleic acid synthesis (2). The microorganisms adhere to the tooth's surfaces and affect the supporting tissues of the teeth (3–5). Thereby, treatment of periodontitis mainly focuses on the use of antimicrobials and the reduction of bacterial growth. The molecular formula of OZ is $C_7H_{10}ClN_3O_3$ (MW is 219.62 g/mol), and its molecular structure is depicted in Figure 1. It is a crystalline white to pale yellow powder. The OZ melting point is 88–91 °C and very slightly soluble in ether, practically insoluble in water, and freely soluble in methanol (6).

Forced degradation studies are a scientific necessity and also a regulatory requirement in various countries during the drug development process. Here, OZ was exposed to various stress study conditions according to ICH guidelines and USP for determining the inherent stability of the drug (7,8). The reverse-phase high performance

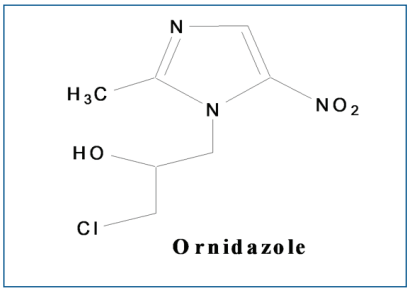

FIGURE 1: Structural formula of OZ.

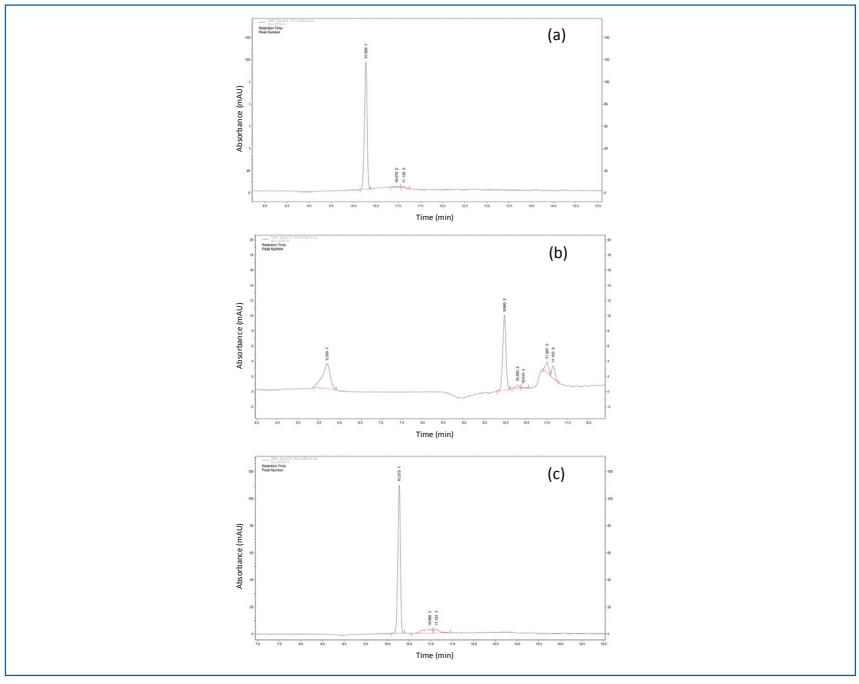

FIGURE 2: (a) Chromatogram of OZ after degradation by HCl, and (b) OZ after degradation by NaOH, and (c) OZ after degradation by H_2O_2.

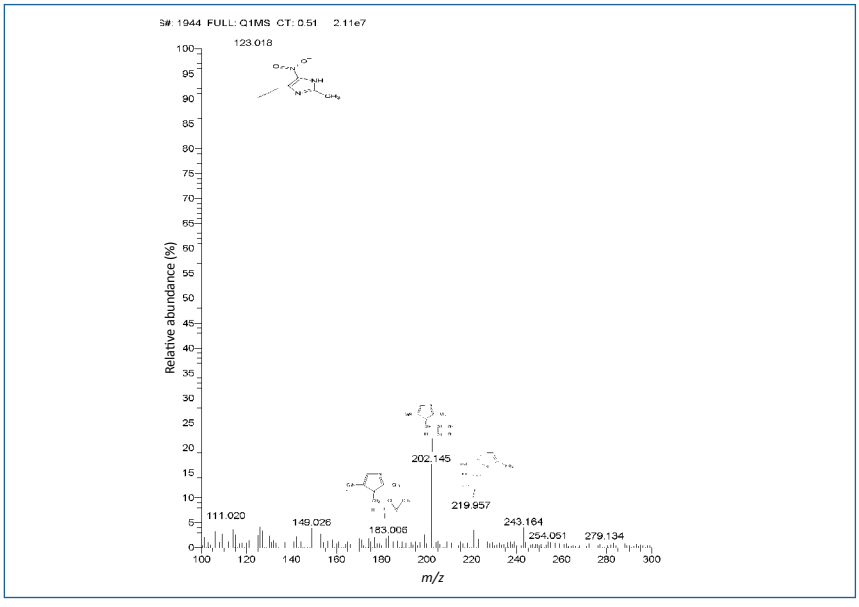

FIGURE 3: MS spectra of the degraded sample of OZ.

liquid chromatography (HPLC) technique for checking its suitability has been designed and verified during stress studies to generate degradation products and process-related impurities of OZ.

Many techniques for estimating OZ are described in the literature, such as high-performance thin layer chromatography (HPTLC) (9), ultraviolet (UV) and HPLC (10), isocratic HPLC (11–24), gradient HPLC (25–27), ultrafast LC (UFLC) (28), and bioanalytical (29) methods. In the present study, a reversed-phase liquid chromatography (RPLC or RP-HPLC) technique has been designed and proven to quantify Ornidazole in the marketed formulation (oral gel). This is the first time report that an HPLC technique has been utilized to estimate Ornidazole in the formulation of oral gel, as per ICH Q2 guidelines with the application of quality by design (QbD).

A systematic approach was applied under QbD (30) like design dpace to optimize critical quality attributes and critical method attributes. A QbD concept is important to make a visual "design space" in which the method is robust. The design space is the multidimensional combination where interaction of input variables is established to give assurance of quality (31–34).

Experimental

Chemicals and Reagents

OZ (percentage purity 99.8% w/w) was purchased from a supplier (Yarrow Chem, Mumbai). Methanol was purchased by Merck Life Science Pvt. Ltd., Mumbai (Batch no. SI5SF65571). HPLC water (Batch no. 220517) and acetonitrile (Batch no. 260617) was purchased by CDH, New Delhi. A gel of OZ was purchased from the market name Ornigreat Gel 10 gm (Mankind Pharma Ltd).

Instrument

HPLC

The Agilent Technologies 1260 Infinity II HPLC system (Agilent Technologies) with quaternary pump (G7111A 1260 Quat Pump VL) coupled with photodiode array detector (PDAG7115A) has been employed in the analysis. Data processing was performed on the software package (Agilent OpenLAB version A.02.02). Different analytical columns were used, like Agilent Eclipse plus C18 (Lot No. B16178) and Shiseido Capcell Pak C18MG-II S-5 (Lot No. BSII33) dimension (4.6 × 150 mm with size particle of 5 µm). The column oven (G7116A 1260MCT) and Rheodyne manual injector (G1328C 1260 Man. Inj.) with a 20 µL loop were used in the HPLC system.

LC–MS/MS

Liquid chromatography was achieved with a Shimadzu scientific instrument (UPLC, LC-30AD, Shimadzu Corporation) with a column, ACE C18 (100 mm x 4.6 mm i.d., 5 µm particle size) maintained at 35 °C, using solvent A, comprising water (90%)

and solvent B acetonitrile (10%) with 0.1% v/v formic acid in gradient mode. The flow rate was set at 0.45 mL/min. The total analysis time was 5.50 min. The ionization and detection were carried out on a triple quadrupole mass spectrometer, Shimadzu LCMS-8050 (Shimadzu Corporation), equipped with electrospray ionization operated in positive polarity using multiple reaction monitoring (MRM).

Chromatographic Conditions

The chromatographic separation has been done on Agilent Technologies' 1260 Infinity II Quaternary HPLC system using an Agilent Eclipse Plus C18 column (4.6 mm × 250 mm, 5 µm), and the temperature was maintained at 27 °C in a thermostat. To develop a linear, precise, sensitive, and suitable HPLC method for the estimation of OZ in gel concentration form many chromatographic conditions were applied. In the isocratic mode, peak shape was not symmetrical and theoretical plates were very low. Finally, mobile phase composition in gradient mode with water (solvent A) as well as acetonitrile (solvent B) in different ratios as per the time program was applied. A flow rate of 1 mL/min was adjusted. The mobile phase has been measured at 0.0 min–10% B, 10 min–90% B, and at 20 min–10% B, and reserved up to 5 min for column equilibrium. The column elution was monitored at 319 nm in wavelength variable detector (PDA). A PTFE pore-size filter 0.22 µm (Millipore, USA) was employed for filtration and sonic the mobile phase before use. The temperature of the column has been kept at 27 °C.

Standard Solution

To create the standard solution, 10 mg of OZ standard was weighed accurately. The weighed quantity of OZ standard was transferred to a clean 10 mL volumetric flask to dissolve the drug in HPLC-grade methanol. The solution was diluted to get a 1 mg/mL concentration. Using a micropipette, the solution was diluted to get a standard 5 µg/mL solution concentration.

Assay of the Marketed Product (Ornigreat Gel 1% w/w)

A portion of the gel (10 mg equivalent to OZ) was properly weighed and further transferred to a flask; the drug was extracted with methanol, then sonication was done for 10 min. The extract was filtered through a membrane filter (0.22 µm) and the filtrate was put into a beaker of 100 mL further volume was added up to the level. The methanolic extract of the dosage form was diluted to get final concentrations (5 µg/mL) and then treated as described previously. The recovery of OZ in dosage form was calculated from the corresponding calibration graph.

Stability-Indicating Study (35)

Forced degradation studies under different conditions were carried out on OZ gel according to the following conditions:

OZ gel equivalent to 10 mg of the drug was weighed properly and then put into a volumetric flask of 100 mL. Further OZ gel

TABLE I: Results of precision of OZ using the proposed HPLC method.

	Inter-day Precision			Intra-day Precision		
	Concentration (µg/mL)	Mean ± SD (*n* =3)	RSD (%)	Concentration (µg/mL)	Mean ± SD (*n* =3)	RSD (%)
Assay-1	4	3.980 ± 0.035	0.879	4	3.990 ± 0.013	0.331
	5	5.001 ± 0.009	0.179	5	5.003 ± 0.009	0.467
	6	5.982 ± 0.034	0.572	6	6.006 ± 0.016	0.262
Assay-2	4	3.991 ± 0.029	0.721	4	4.003 ± 0.024	0.589
	5	5.015 ± 0.023	0.464	5	5.010 ± 0.028	0.565
	6	5.988 ± 0.035	0.582	6	6.005 ± 0.022	0.361

TABLE II: Results of robustness test for OZ.

Std	Run	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3	Response 4
		A: Flow Rate (mL/min)	B: Column Temp. (°C)	C: % of ACN in Mobile Phase	D: Wavelength (nm)	Tailing Asymmetry	Retention Time (Min)	Peak Area	Theoretical Plate
8	1	1.1	29	92	321	1.17	10.21	426326.7	143505.26
2	2	1.1	25	88	321	1.04	10.35	425535.7	143548.67
1	3	0.9	25	88	317	1.24	10.38	425415.7	143579.02
7	4	0.9	29	92	317	1.18	10.19	426414.8	143356.06
3	5	0.9	29	88	321	1.27	10.36	425210.9	143453.33
5	6	0.9	25	92	321	1.34	10.16	425448.7	143394.66
4	7	1.1	29	88	317	1.15	10.22	425960.8	144069.33
6	8	1.1	25	92	317	1.21	10.26	425519.3	143400.66

was exposed to two different strengths of HCl (0.1 N and 1.0 N), kept aside for 12 h in dark at room temperature, then neutralized with NaOH, which had the same normality as HCl. A pH meter is used before analysis to verify that pH has 7.0 as a value. The same concentration of the drug in the gel was exposed to 1 N HCl and heated at 70 °C for 6 h. The stress study in alkaline conditions was also performed using different strengths of NaOH solutions (0.1 N and 0.5 N) for 6 h in dark at room temperature then neutralized which had the same normality as HCl, and a pH meter is used before analysis to verify that pH has 7.0 as a value. The oxidative degradation studies were performed using hydrogen peroxide (3% v/v) to expose the OZ gel (equivalent to 10 mg of OZ) further. The strength of H_2O_2 was increased up to 30% v/v to the same strength of the OZ gel at room temperature for 8 h in dark. To end the reaction, the distilled water of 5 mL was mixed in. 20 µL of the degraded solutions were injected in triplicates, and the chromatograms were performed under chromatographical conditions, as stated.

OZ gel (equivalent to 1 mg/mL) was treated for thermal degradation at 60 °C for 48 h. Photolytic degradation was also performed to expose the OZ gel to white fluorescent light (1.2 million lux) near UV fluorescent light (200 w/m^2) for 10 days. After the withdrawal of each sample from the different studies, they were subjected to a filter through a syringe filter (0.22 µm) and diluted to HPLC grade methanol to achieve a 5 µg/mL concentration of the drug.

Validation Method

The HPLC method was validated for the estimation of OZ as per ICH Q2 guidelines (ICH Q2, 2005).

Specificity and Selectivity

The specificity was defined by the ICH Q2 guideline as a knack to identify the analyte in the presence of their impurities. For selectivity and specificity of the method, the standard solution and sample solutions were examined to verify the interference and separate degrading impurities in the OZ peak region.

Linearity

The standard OZ solution at diverse levels (1–12 µg/mL) was determined by injecting solutions in triplicate. Peak area versus respective concentrations, a graph was plotted, and the equation and correlation coefficient were obtained using linear regression analysis.

Precision

A concentration (5 µg/mL) of the standard solution was injected in six replicates for determining system and method precision.

The OZ was prepared in three different levels (80, 100, and 120%) in triplicate, and OZ solutions were estimated within a day and three days later to get the inter-day and intra-day precision data.

Accuracy as Recovery

As per the guidelines ICH (8) and USP (7), accuracy was determined by the addition of pre-analyzed samples of OZ with a standard drug at diverse concentrations (80, 100, and 120%), and these solutions were made in triplicate further samples were suitably diluted and analyzed.

LOD (Limit of Detection) and LOQ (Limit of Quantification)

According to ICH guidelines, the following equations: LOQ = 10σ/S; LOD = 3.3σ/S were used to calculate LOD and LOQ for a developed method of OZ where the standard deviation is denoted by σ as well as calibration curve slope by S. The LOQ was analyzed at lower concentration of OZ with precision and accuracy.

Stability in Analytical Solution

In two different conditions, the stability studies of standard and sample solutions were investigated at 4 h of time intervals till 48 h. The standard and sample solutions of OZ were kept without light protection at room temperature and refrigerator (2–8 °C). The OZ concentration was determined in standard and sample solutions and % RSD was calculated.

Robustness

The organic content of the mobile phase was changed by ±2% v/v. Some are deliberated changes in the method were applied and discussed below. A QbD approach was applied for optimizing chromatographic conditions. For the DoE studies, four variables were selected:

1. Organic % in mobile phase (88–92);
2. Flow rate (0.9–1.1 mL/min);
3. Wavelength (317–321 nm);
4. Column temperature: 25–29 °C

and four responses were observed:

1. Tailing asymmetry;
2. RT;
3. Peak area; and
4. Theoretical plate.

Results

Chromatographic Conditions

The mobile phase was selected water (Solvent A): acetonitrile (Solvent B) ratio 90:10 in gradient mode with a 1 mL/min flow rate. At 0–9 min, the percentage of Solvent B was set to 10% by volume and at 10–19 min it increased to 90% by volume, thereafter, reserved for the initial condition up to 25 min. The retention time for OZ was 10.29 min and a run time of a total of around 25 min.

Stressed Studies

In acidic conditions (0.5 N HCl), the sample was withdrawn after 1 h and analyzed. The sample was not degraded and no impurities were observed in the chromatogram. But in the case of 1 N HCl after 4 h, there were two new peaks (Figure 2a) observed at retention time at 10.97 min and 11.13 min, with RRT 1.07 and 1.10, respectively.

The alkaline condition (0.5 N NaOH) of the drug was initially degraded within 15 min along with two peaks (IV-1.07 and V-1.08) were observed. Then, further drug completed degraded with three new peaks (RRT I-0.55, II-0.97 and III-1.01). The peak purity of all PDA samples showed that the primary peak of ornidazole was not co-eluting peaks (Figure 2b), and thus, the stability-indicating nature of the technique was established, (which is obtained in acidic conditions) and a new highest peak was observed at 9.98 min as a major degraded product. In 1 N NaOH, the peaks were observed to be the same, but the drug was completely degraded. The further depredated sample was analysed by LC–MS/MS. Many degradation products were found in mass spectra (Figure 3). The *m/z* values of major degradants are 123.018, 183.006, and 202.145, the last two were reported (Bakshi et al., 2001) as epoxide and diol forms of OZ. In the chromatogram (Figure 2b), these two degradants were observed at RT 5.70 and 9.98 min. The base peak was observed at *m/z* value 123.018 in the MS spectra after cleavage of the side chain in OZ.

In oxidative conditions (1% H_2O_2), the drug was degraded in a very low amount during 6h. Further, the drug was determined in 10% H_2O_2 in the same time there was drug degraded up to 20.4% w/w along with two peaks observed (RRT 1.07 and 1.08) in the chromatogram (Figure 2c).

The photolytic degradation of the drug was found after 2 d at about 6% w/w. After 5 d, samples were analyzed, and the drug was found to be 95% w/w. Further, the sample was analyzed for 10 days. Then, the drug remained at almost 93.1% w/w, and no other peak of degradation products (except OZ) was observed.

After thermal degradation, the drug was found at 24 h about 91.6% w/w. After 48 h, samples were analyzed again, and the drug was found to be 88.5% w/w with a single peak of OZ at 10.29 min.

Selectivity and Specificity

A blank (solvent), OZ standard and its formulations (gel), chromatograms showed a single peak at a retention time of 10.29 min, which revealed that in the OZ peak region, no other peak was seen. The peak was discovered in OZ gel to be pure and can be identified unequivocally as shown in Figure 4 (3D view of OZ Peak; this and all subsequent figures are accessible through the QR code at the end of the article). The chromatogram depicts that the gradient method was highly specific for separating the drug from degradation impurities I, II, III, IV, and V with the RT of 5.70, 9.98, 10.43, 11.00, and 11.15 min in Figure 2b.

Linearity

Excellent linearity revealed a calibration curve range of 1–12 µg/mL and a correlation coefficient of 0.9998 with a linear regression line equation of $y = 91349x - 24980$ was identified.

Precision

Precision is the variance of findings in repeated analyses under similar experimental conditions of the homogenous sample. The technique has been verified by performing method, system, inter-day, as well as intraday precision. The findings of method and system precision were respectively 0.478 and 0.325 percent RSD. The results are given in Table I.

Accuracy, as Recovery

The recovery data showed that at three levels <2% was (RSD percent). The average method accuracy at 3 distinct ranges of 80, 100, and 120 percent was 99.55, 99.58 and 99.92. The values of accuracy were found within a range.

LOQ and LOD

The S/N ratio (signal-to-noise) of LOD and LOQ was determined to be 0.23 µg/mL and 0.7 µg/mL with high precision as well as accuracy. The relative standard deviation in percent was less than 2%.

Stability in Analytical Solution

The result of the room temperature stability of standard OZ and sample solutions was constant. The cumulative percentage RSD of 0.46 was 0.89 at refrigeration condition (2–8 °C). Both solutions of OZ were stable up to 48 h at 25 °C.

Robustness

The robustness was established by deliberate diverse chromatographic conditions by changing the column temperature, flow rate, detection wavelength, column make, and composition of organic solvent within given ranges from actual chromatographic settings.

DoE Evaluation and Design Space

Factorial DoE has been performed with study type 2 levels, level 2 factorial, and four factorial equations mode.

The equations are given below:

Tailing Asymmetry: $-1.15A - 0.51B - 0.40C + 0.86D + 0.5\,AB + 0.45AC - 0.85AD$

Retention Time: $-0.125A + 0.43B - 0.84C - 0.84C - 0.32D - 0.475AB + 0.725AC + 0.325AD$

Peak Area: $2131A - 676.17B + 2602.12AC - 4059.9D + 1178AB - 2218.5AC + 3883AD$

Theoretical Plate: $1852.13A - 1849.41B + 823.59C + 693.18 + 1973.87AB - 1076.13AC - 822.42AD$

Where: *A*: Flow rate (mL/min); *B*: Column Temperature (°C); *C*: Percentage of Organic solvent (%); *D*: Wavelength (nm)

The factorial DoE results are summarized in Table II and QbD plots were applied for evaluation. Figure 5 illustrate the DoE contour plots.

In the design space based on the DoE set of experiments, the water (88–90%) in solvent A facilitated the separation of peaks. A column temperature of 25–27 °C is suggested for the separation of peaks. Considering the peak shape of all of the impurities and OZ, a flow rate of 0.9–1.0 mL/min and a column temperature of 27 °C were selected and have a positive impact on theoretical plates. The flow rate and mobile phase ratio Solvent A 90% and Solvent B 10% had resistance to change in peak area as well as the symmetry of the peaks. The design space results

were uploaded in design expert software and the plot was drawn and depicted in Figure 5.

Discussion

The researchers (36) found that the drug remained unstable in every situation like acidic conditions, alkaline media, acidic medium, as well as oxidative stress in presence of light. OZ (I) decomposes to an epoxide (II) and is transformed into diols in alkaline conditions (III). In the presence of a base, the intramolecular SN2 reaction causes ring closure. The mechanism of degradation of OZ depicts in Figure 6.

Conclusion

For the analysis of OZ in a gel formulation, a selective and gradient HPLC technique has been validated with the application of QbD. The chromatographic conditions were optimised with the application of QbD, and the method was developed to be reliable, rapid, and reproducible. The findings of peak purity revealed that the OZ peak is pure and that no peak merges during retention. Particularly, the findings showed that the technique used to estimate OZ in the gel dosage form. The suggested HPLC technique is very precise, sensitive, as well as reproducible. Finally, it could be used for the routine analysis of quality control laboratories. ■

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Conflict of Interest

The authors have no conflict of interest.

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HPAEC-PAD Analysis of Oligo– and Polysaccharides as Fraud Marker in Honey

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Nearly half of the honey imported into Europe (46%) and a significant portion in America (70%) are suspected to be adulterated. This application note describes the use of High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) as a sensitive analytical tool for the detection of honey fraud.

2013 EU REPORT concerning food safety listed honey as one of the top 10 food products at risk of food fraud (1). Honey is particularly susceptible to fraud due to its substantial price difference with alternative sweeteners, such as sugar syrups. Regulatory frameworks, for example the EU Honey Directive 2001/110/EC (2) or the USDA CID A-A-20380 (3), specify compositional parameters of authentic honey. These parameters include the level of mono– and disaccharides in the honey, along with other parameters such as moisture content, diastase activity, and amino acid content. Despite these strict regulations, instances of fraudulent honey remain undetected and therefore require additional authenticity testing parameters. A recent report highlights the significance of oligo– and polysaccharides as additional parameters for determining honey authenticity (4).

This application note describes a method for the profiling of oligo– and polysaccharides in honey samples using HPAEC-PAD. The method is based on gradient separation using the new SweetSep™ AEX200 high-resolution anion-exchange column. The AEX200 stationary phase consists of highly monodisperse 5 µm ethylvinylbenzene-divinyl-benzene copolymer particles coated with quaternary amine functionalized nanoparticles (5). As a proof of principle, an authentic honey and intentionally adulterated honey sample were analyzed and the obtained carbohydrate profiles were compared.

Experimental Conditions

HPAEC-PAD analysis of oligo– and polysaccharides in honey was performed according to the conditions and gradient program described in Tables I and II, respectively. A 50 g/L stock standard of maltodextrin (Dextrose equivalent 4—7) was prepared in 95/5 (v/v%) water/acetonitrile. The working standard of maltodextrin was prepared by serial dilution of the stock standard with DI water, to a final concentration of 0.5 g/L. The working standard solution was filtered over a 0.22 µm PES (Polyethersulfone) syringe filter prior to analysis.

An authentic avocado honey (geographical origin: Mexico) and rice syrup samples were received from a third party. The samples were made by dissolving 1.25 g samples in 50 mL DI water (25 g/L). Subsequently, the honey sample was filtered over a 0.22 µm PES syringe filter into the vials for injection. The rice syrup sample is further diluted 10× with DI water to a final concentration of 2.5 g/L before injection. An adulterated honey sample was prepared by mixing the solution of the original

TABLE I: HPAEC-PAD Conditions

System	ALEXYS™ Carbohydrate Analyzer – quaternary low pressure gradient (Antec Scientific)
Detector	DECADE™ Elite electrochemical detector (Antec Scientific)
Columns	SweetSep™ AEX200, 4×200 mm column, 5 µm (Antec Scientific)
Mobile phase	A: 100 mM NaOH B: 100 mM NaOH + 500 mM NaOAc Eluents prepared & blanketed with Nitrogen 5.0
Flow rate	0.7 mL/min
Pressure	about 180—200 bar
Injection	10 µL
Temperature	30 °C for separation, 35 °C for detection
Flow cell	SenCell with Au WE, stainless steel AE and HyREF (Pd-hydrogen) RE, AST 2
Potential waveform	E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s
I-cell	about 0.3 µA
ADF	0.5 Hz
Range	5 µA/V

TABLE II: Gradient program

Time (min)	%A	%B	Description
0	92	8	Gradient elution and detection
30	10	90	
30 - 35	10	90	Column clean-up and regeneration
35 - 50	92	8	Equilibration to starting conditions

honey sample (25 g/L) with the solution of rice syrup (2.5 g/L) in a 9:1 ratio. The adulterated honey sample consisted of a final concentration of 22.5 g/L of honey and 0.25 g/L of rice syrup, corresponding to an adulteration level of approximately 1.1%.

Results

A chromatogram of a 10 µL injection of the 0.5 g/L maltodextrin standard is presented in Figure 1. Peak assignments were established based on the injection of glucose (DP_1), maltose (DP_2), and maltotriose (DP_3) standards (data not shown here). A high-resolution separation of each degree of polymerization (DP) up to DP_{40} was achieved within 25 minutes.

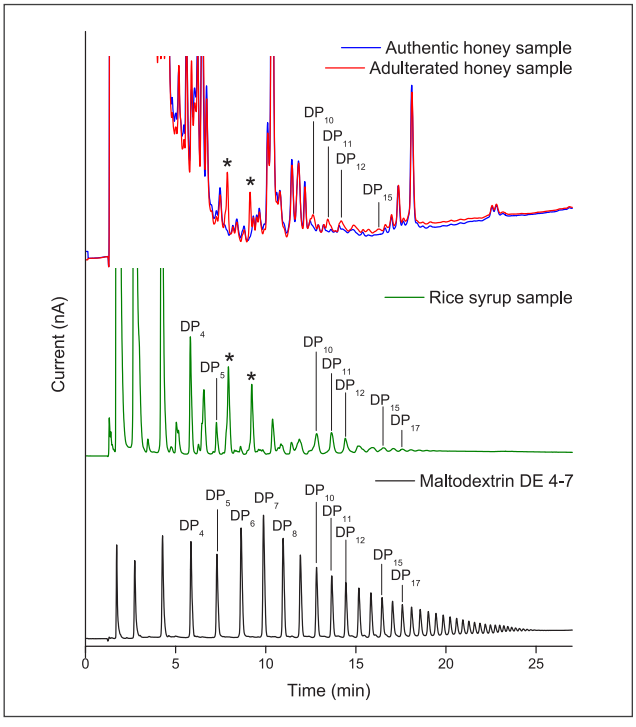

FIGURE 1: Overlay of chromatograms of a 10 µL injection of the 0.5 g/L maltodextrin standard (black trace), 2.5 g/L rice syrup sample (green trace), authentic avocado honey sample (blue trace), and avocado honey sample adulterated with 1.1% rice syrup (red trace). Peaks marked with (*) are from the rice syrup.

The authentic honey sample consists of various oligo- and polysaccharides, ranging from DP_5 up to about DP_{17}. Notably, the chromatogram of the honey sample exhibits multiple peaks between t = 5 minutes to t = 10 minutes which do not correspond to any of the maltodextrin standard peaks. These unidentified peaks may represent different types of oligosaccharides.

Rice syrup is a popular adulterant originated from C3 plants (plants with Calvin photosynthesis cycle) and shares a similar carbon isotope profile with sugars in natural honey. Consequently, honey adulteration using rice syrup is more difficult to detect by another analytical method, such as IRMS (Isotope Ratio Mass Spectroscopy) or NMR (Nuclear Magnetic Resonance Spectroscopy) (6). The chromatogram of the rice syrup sample (Figure 1) shows the presence of oligo– and polysaccharides, some of which match the oligo– and polysaccharides in the maltodextrin standard, specifically DP_4, DP_5, and DP_{10}—DP_{17}. The oligosaccharide peaks marked with asterisks in the rice syrup chromatogram do not match the DP_6 - DP_8 peaks found in maltodextrin.

The chromatogram of the adulterated honey sample, overlaid in Figure 1, demonstrates the challenges associated with detecting fraudulent honey. Based on the chromatogram in Figure 1, the oligo– and polysaccharide profiles of the adulterated honey sample closely resemble those of the original honey sample. However, a comparison between the original and the adulterated sample shows several signs of adulteration by the addition of rice syrup. Two additional peaks, marked with asterisks, appeared only in the adulterated sample. In addition, several other peaks corresponding to the DP_{10}–DP_{12} malto-oligosaccharides of the rice syrup are more prominent in the adulterated sample. These results highlight the potential of HPAEC-PAD to detect honey adulteration by syrup addition at levels of as low as 1%, based on the oligo- and polysaccharide contents.

Conclusions

The new SweetSep™ AEX200 anion-exchange column offers high-resolution separation of oligo– and polysaccharides up to DP_{40} within 25 minutes, enabling fast profiling of these larger carbohydrates in honey samples using HPAEC-PAD. The analysis of an authentic and adulterated avocado honey sample demonstrates the method's ability to detect low-level adulterations of honey by addition of starch-based syrups (rice, wheat, etc.). Therefore, making HPAEC-PAD a potentially valuable tool to detect fraudulent honey products based on the oligo– and polysaccharide contents.

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Two–Step CBG Purification: Combined Matrix Removal and Peak Recycling

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Introduction

Due to the progressive decriminalization of cannabis for recreational and medicinal use, the properties of this plant have received increasing attention. Its two most well-known cannabinoids are THC (Δ^9-trans-tetrahydrocannabinol) and CBD (cannabidiol). CBG (canabigerol) is less popular than CBD, but it is becoming increasingly important. Like CBD, CBG offers a variety of potential health benefits, such as antibacterial, antimicrobial, and antidepressant effects (1,2).

The purification of CBG and other cannabinoids from cannabis extracts is a challenging task because of the complexity of the matrix. Additionally, the chemical structure and physical properties are often similar, making it difficult to separate them (Fig. 1) (1,2).

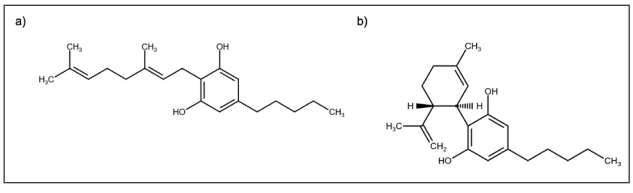

FIGURE 1: Structural formula of a) CBG and b) CBD.

Therefore, specific methods and techniques are required to ensure accurate separation. Recycling chromatography is an ideal method for effectively separating two closely eluting peaks, such as CBG and CBD. It increases the length of the column bed by redirecting the peaks of interest through the column multiple times. A peak recycling system was upgraded to remove impurities from the sample by parking the target peaks and reinjecting the peaks back into the system for peak recycling over the two columns.

Sample Preparation

An ethanolic extract of CBG rich cannabis sample was prepared. After decarboxylation and winterization, ethanol was removed, and the raisin was stored at 4°C. The CBG-rich cannabis extract was diluted in 100% ethanol to the indicated concentration

Results

A peak recycling system was upgraded with two additional valves to enable a peak parking step for matrix removal (step 1) before the peak recycling step (step 2) (Fig. 2).

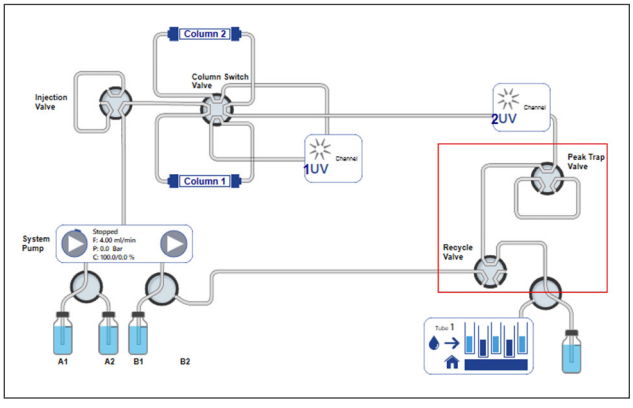

FIGURE 2: PurityChrom6 visualization of peak recycling system. Red rectangle: system upgrade with two additional valves.

The peak trap valve can be switched between two positions, allowing the flow to bypass the trap or pass the peak into the trap, where the peak is parked in a loop or column (Fig. 3).

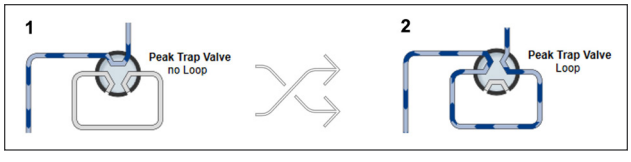

FIGURE 3: Peak trap valve. 1: Flow bypasses the peak trap. 2: Flow passes the peak trap.

The recycle valve can be switched between two positions to change the system from normal (position 1) to recycle mode (position 2) (Fig. 4).

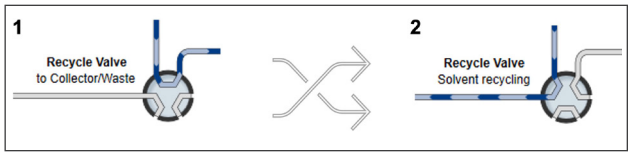

FIGURE 4: Recycle valve. 1: Flow goes to the collector/waste. 2: Flow is being recycled.

Step 1: Matrix Removal by Peak Parking

The sample is injected, passing both columns and UV1 before passing UV2 (Fig. 2). Here, the start of the major peak is

detected, the peak trap valve switches to position 2, and the flow passes through the peak trap where the peak is captured (Fig. 3). When the peak end is detected, the peak trap valve is switched back to position 1, bypassing the peak trap (Fig. 3).

In Fig. 5, the chromatogram of the cannabis sample passing UV1 and UV2 and the trapped area by the peak trap valve is shown.

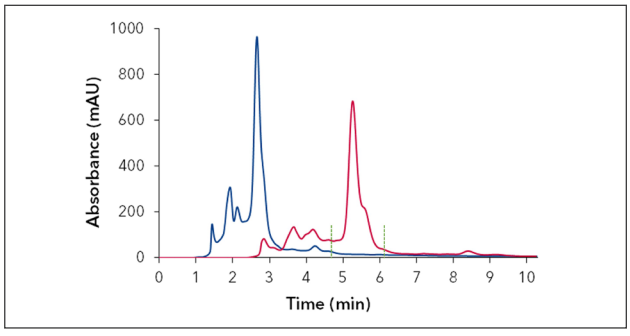

FIGURE 5: Chromatogram from recycling system; green-dashed line: manual switching peak trap valve; blue: absorbance UV1; red: absorbance UV2; 4 ml/min; 228 nm; 100 µl cannabis extract 10 mg/ml.

Step 2: Peak Recycling

After the matrix is removed, the parked peak is reinjected into the system and recirculated with the flow. As the absorbance of the peak in the UV1 decreases, the position of the column switch valve is changed, taking the delay volume between the detector and the valve into account. The switching cycle is repeated until the target resolution is reached, or the peak broadening exceeds one column volume. Then, an automated fractionation was carried out by using a threshold set for the UV2 (Fig.6).

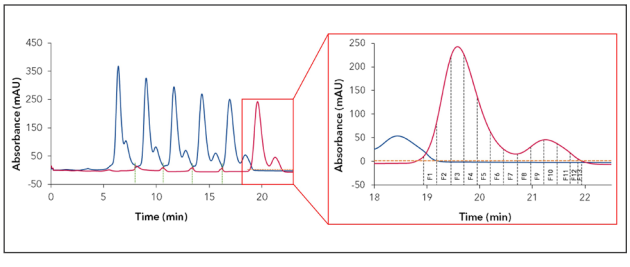

FIGURE 6: Chromatogram from recycling system; green-dashed line: manual switching peak trap valve; blue: absorbance UV1; red: absorbance UV2; orange dash line: threshold UV2

Fractions 1 to 13 were analyzed by the analytical method to calculate the amount and purity of CBG and CBD (Tab. 1).

The analysis showed that fractions 1 to 7 contain CBG with a purity of 100%. After fraction 7, the CBG purity decreases due to the presence of CBD. In contrast, the purity of CBD increases. Based on the amount and purity of CBG and CBD in each collected fraction, the selection of fractionation windows in which CBG and CBD can be fractionated with the highest recovery or purity is possible.

TABLE I: Analysis of fractions (100 µl injection with 10 mg/ml cannabis extract).

	CBG		CBD		Others	
	Amount [µg]	Purity [%]	Amount [µg]	Purity [%]	Amount [µg]	Purity [%]
Sample	63.20	80.78	12.48	15.95	2.56	3.27
F1	1.63	100.00	-	-	-	-
F2	9.39	100.00	-	-	-	--
F3	19.41	100.00	-	-	-	-
F4	18.10	100.00	-	-	-	-
F5	10.43	100.00	-	-	-	-
F6	4.77	100.00	-	-	-	-
F7	1.93	100.00	-	-	-	-
F8	0.89	65.33	0.47	34.67	-	-
F9	0.37	13.39	2.41	86.61	-	-
F10	0.15	4.08	3.58	95.92	-	-
F11	-	-	2.82	100.00	-	-
F12	-	-	0.85	100.00	-	-
F13	-	-	0.20	100.00	-	-

Conclusion

An optimized recycling system was used to remove matrix components from CBG-rich cannabis extracts, which enabled the separation of CBG and CBD. The peak trap valve was used to remove the early and late eluting matrix components. The recycle valve was used for peak recycling. From a 100 µl sample injection (10 mg/ml cannabis extract), approximately 67 µg CBG was purified to 100% purity.

The results demonstrate the simplicity and benefits of the optimized recycling system. Depending on the target, the method can be adapted to other target compounds from different matrices.

References

(1) De Luca, C.; et al. Green Cannabigerol Purification Through Simulated Moving Bed Chromatography. *Green Anal. Chem.* **2023,** 100066.

(2) Nachnani, R.; et al. The Pharmacological Case for Cannabigerol. *J. Pharmacol. Exp. Ther.* **2020,** *376* (2), 204–212.

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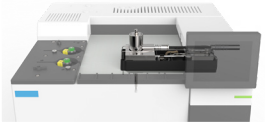

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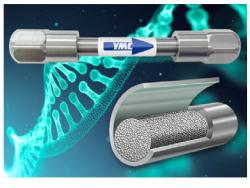

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