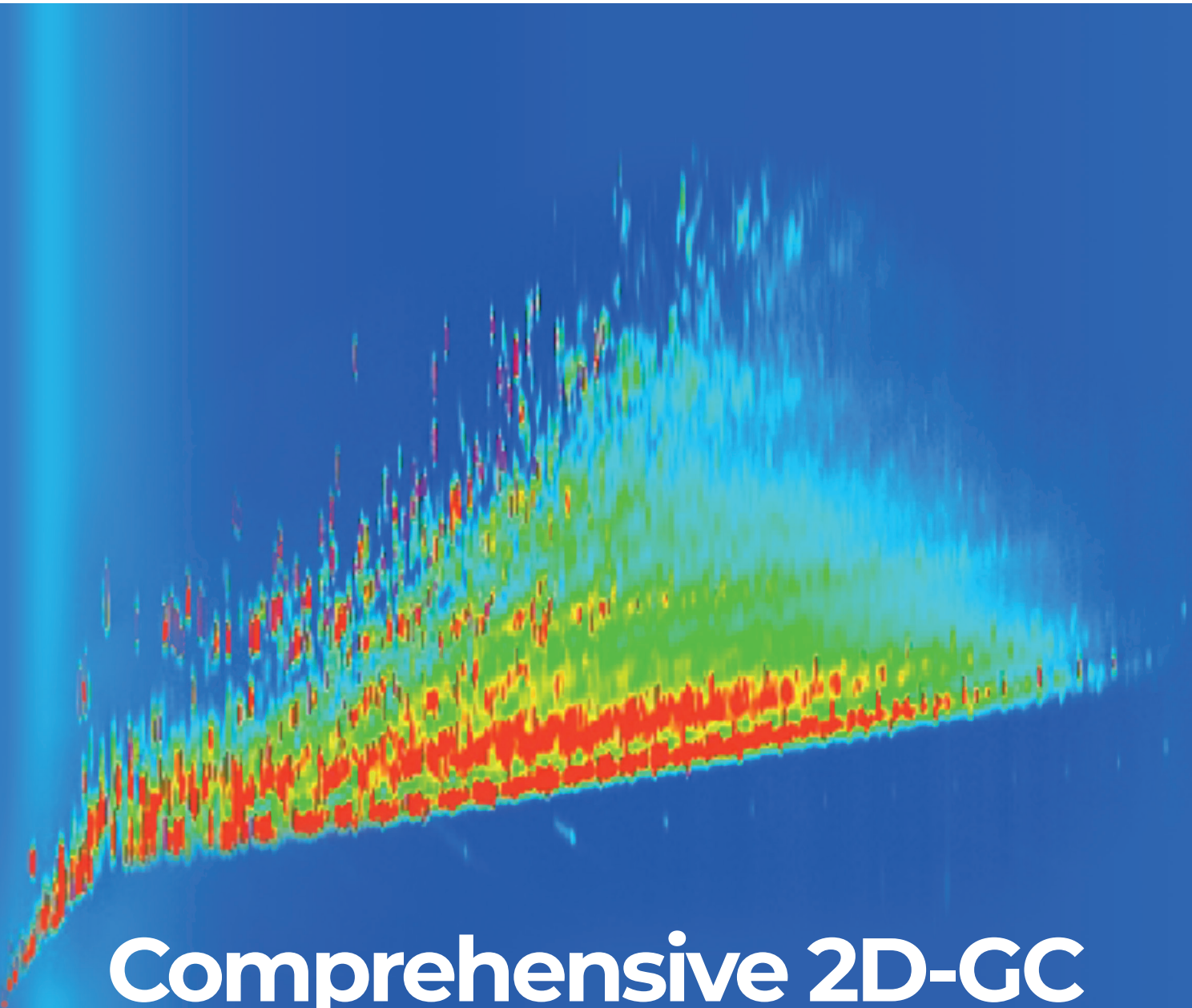
LC GC europe
solutions for separation scientists
April 2020 | Volume 33 Number 4
www.chromatographyonline.com





# Comprehensive 2D-GC

## An update on recent advances

# If your standards and your samples are the same size, they must be the same mass, right?


With DAWN you can determine absolute molecular weights and sizes without using reference standards made in someone else's lab.

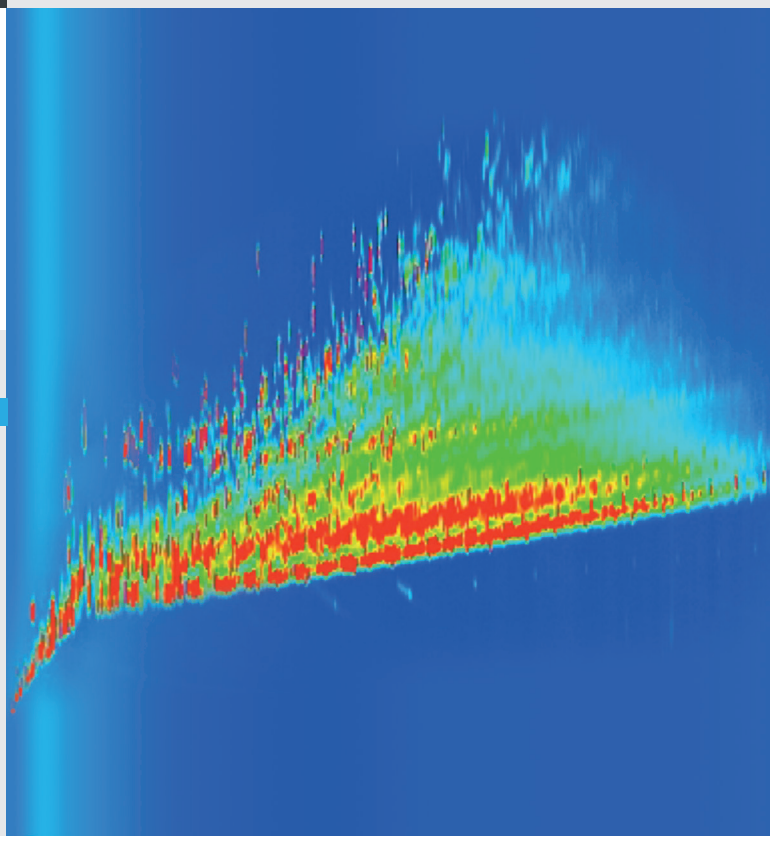
That's the problem with calibration-based measuring techniques. You don't know—you can only assume. Which is why every major pharmaceutical and biotechnology company, as well as most federal regulatory agencies are switching from relative methods to Wyatt Technology's absolute measurements. Our DAWN® multi-angle light scattering (MALS) instruments allow you to determine absolute molecular weights and sizes without relying on so-called standards, or measurements made in someone else's lab. Wyatt instruments measure all of the quantities required for determining absolute molar masses directly.

Learn how to end you dependence on reference standards forever at wyatt.com/NoRef

WYATT TECHNOLOGY
wyatt.com

LCGC europe
solutions for separation scientists

April | 2020
Volume 33 Number 4



## COVER STORY

PEER-REVIEWED ARTICLE



## INTERVIEW

ANALYSIS FOCUS



## COLUMNS

LIQUID CHROMATOGRAPHY



Image credit: Courtesy of Hans-Gerd Janssen, Wageningen University, Agrotechnology and Food Sciences Group, Laboratory of Organic Chemistry. The Netherlands.

CHROMATOGRAPHY TECHNOLOGY



PERSPECTIVES IN HPLC



## DEPARTMENTS

MULTIMEDIA HIGHLIGHTS



PRODUCTS

LCGC europe
solutions for separation scientists

AN MJH life sciences™ BRAND

**Group Vice President**
Mike Tessalone
*mtessalone@mjhlifesciences.com*

**Editorial Director**
Laura Bush
*lbush@mjhlifesciences.com*

**Editor-in-Chief**
Alasdair Matheson
*amatheson@mjhlifesciences.com*

**Managing Editor**
Kate Jones
*kjones@mjhlifesciences.com*

**Associate Editor**
Lewis Botcherby
*lbotcherby@mjhlifesciences.com*

**Associate Publisher**
Oliver Waters
*owaters@mjhlifesciences.com*

**Sales Executive**
Liz Mclean
*lmclean@mjhlifesciences.com*

**Sales Operations Executive**
Sarah Darcy
*sdarcy@mjhlifesciences.com*

**Senior Director, Digital Media**
Michael Kushner
*mkushner@mjhlifesciences.com*

**Webcast Operations Manager**
Kristen Moore
*kmoore@mjhlifesciences.com*

**Project Manager**
Vania Oliveira
*voliveira@mjhlifesciences.com*

**Digital Production Manager**
Sabina Advani
*sadvani@mjhlifesciences.com*

**Managing Editor, Special Projects**
Kaylynn Chiarello-Ebner
*kebner@mjhlifesciences.com*

**Creative Director, Publishing**
Melissa Feinen
*mfeinen@mdmag.com*

**Art Director**
Gwendolyn Salas
*gsalas@mjhlifesciences.com*

**Graphic Designer**
Courtney Soden
*csoden@mjhlifesciences.com*

## Corporate

**Chairman & Founder**
Mike Hennessy Sr

**Vice Chairman**
Jack Lepping

**President & CEO**
Mike Hennessy Jr

**Chief Financial Officer**
Neil Glasser, CPA/CFE

**Executive Vice President, Operations**
Tom Tolvé

**Senior Vice President, Content**
Silas Inman

**Senior Vice President, I.T. & Enterprise Systems**
John Moricone

**Senior Vice President,**
**Audience Generation & Product Fulfillment**
Joy Puzzo

**Vice President,**
**Human Resources & Administration**
Shari Lundenberg

**Vice President, Business Intelligence**
Chris Hennessy

**Vice President, Marketing**
Amy Erdman

**Executive Creative Director, Creative Services**
Jeff Brown

**Subscriber Customer Service**
Visit *chromatographyonline.com*
to request or change a subscription or email
mmhinfo@mmhgroup.com.

Multimedia UK, LLC,
Sycamore House, Suite 2 Ground Floor,
Lloyd Drive, Cheshire Oaks Business Park,
Ellesmere Port, CH65 9HQ, UK
Tel: +44 (0)151 705 7601

**Follow us @ LC_GC**

**'Like' our page LCGC**

**Join the LCGC LinkedIn group**

# *LCGC* Multimedia

**Selected highlights of digital content from *LCGC Europe*:**

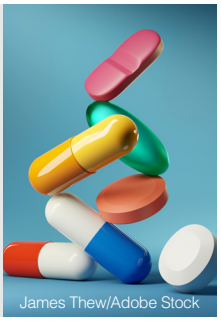
Connect with *LCGC*: Stay in touch with *LCGC* and keep updated with the latest news. Follow us on social media to keep up-to-date with the latest troubleshooting tips and technical peer-reviewed articles featured on our website. Follow @LC_GC on Twitter, join our LCGC Magazine LinkedIn group, or Like our page on Facebook. You are also free to post your questions or discussions for other members to view and comment on!

PHARMACEUTICAL PERSPECTIVES

## The Current Status and Future of Two- and Multidimensional Liquid Chromatography in Pharmaceutical R&D and QC

This article discusses the benefits of 2D-LC and multiple application areas in (bio)pharmaceutical analysis, and highlights the challenges and future of this technique.

***Read more:*** ***https://bit.ly/2UzS1vM***



James Thew/Adobe Stock

NEWS

## Investigating the Environmental Impact of Anticoccidials

Researchers have developed a new LC–MS/MS method for the simultaneous chromatographic separation and detection of anticoccidials, which are used as an agricultural animal treatment, in environmental waters.

***Read more:*** ***https://bit.ly/2JbvmAG***

chayakorn/Adobe Stock


EBOOK

## Data Integrity in Regulated Labs, Part 3

Maintaining data integrity involves a solid understanding of the data life cycle, documentation, and metrics for regulated activities. In this eBook, *Data Integrity in Regulated Labs, Part 3*, *LCGC* presents Parts 7–9 in a nine-part series on data integrity in the regulated chromatography laboratory.

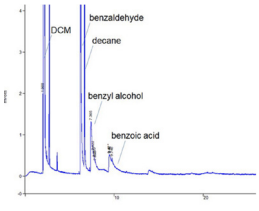
***Read more:*** ***https://bit.ly/3bnZy7Z***

*LCGC* BLOG

## GC Diagnostic Skills 1: Peak Tailing

Peak tailing is a problem that is regularly encountered in capillary GC. In this first of a series on GC diagnostic and troubleshooting, discover how best to identify the source of the issue, and find suggestions on how to prevent or fix the problems that underlie the issue.

***Read more:*** ***https://bit.ly/39aqpCY***





Oleksii Sergieiev/Adobe Stock

SUPPLEMENT

## *Current Trends in Mass Spectrometry* March 2020

The latest issue of *Current Trends In Mass Spectrometry* March 2020 is available in an interactive PDF format.

***Read more:*** ***https://bit.ly/3bcHL3s***

CHROMACADEMY

## Interactive HPLC Troubleshooter

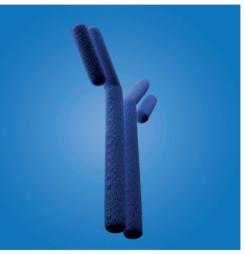
Time-limited free access to our Lite members. In three simple steps we can help you overcome your instrument, separation, and quantitation issues.

***Read more:*** ***https://bit.ly/2PrrHIP***

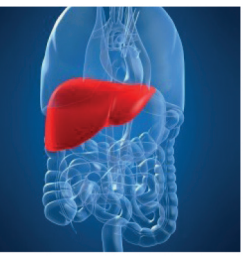
NEW

# Roxy Exceed

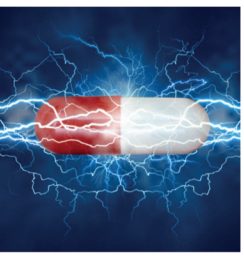
## Electrochemistry System for EC-MS



Proteomics



Metabolism



Drug Stability

Environmental

Synthesis


www.AntecScientific.com

PEER REVIEW

# Recent Application and Instrumental Trends in Comprehensive Two-Dimensional Gas Chromatography

**Peter Q. Tranchida[1], Ivan Aloisi[1], and Luigi Mondello[1,2,3,4],** [1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy, [2]Unit of Food Science and Nutrition, Department of Medicine, University Campus Bio-Medico of Rome, Rome, Italy, [3]Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy, [4]BeSep s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

**This critical review describes recent applications and instrumental trends in comprehensive two-dimensional gas chromatography (GC×GC), with particular (though not exclusive) attention to the period 2018–2019 and that the concept of GC×GC is inherently simple. The maturity of GC×GC and future developments are also discussed.**

## KEY POINTS

- **Recent applications and current instrumental trends are critically reviewed.**
- **Particular focus is devoted to the fact that the concept of GC×GC is simple.**
- **An outlook on the future prospects of GC×GC is provided.**

Comprehensive two-dimensional gas chromatography (GC×GC) is the most powerful GC approach available for the analysis of mixtures of volatile compounds. This multidimensional technique was first reported in 1991, and stemmed from the research work of J.B. Phillips (1). Considering that two-dimensional (2D)-GC has been on the analytical scene for nearly 30 years, it can now be defined as "well known". For this reason, no explanations on the basics of GC×GC are provided in this article. The reader is directed to the many reviews present in the literature, if in-depth details are required (2–5). From consulting such literature, it can be deduced that GC×GC has undergone great hardware and software evolution over the years. For example, during the first 10 years of GC×GC use, flame ionization detection (FID) was, by far, the most common form of detection. After that period, a rapid increase in the use of mass spectrometry (MS) was observed. It was only in 1998 that the first forms of cryogenic modulation (CM) and flow modulation (FM) were introduced (6,7), while in 2003 the first commercial instrument with fully integrated software was proposed (8). Finally, the overall history of GC×GC has been characterized by illustrations of chromatograms highlighting its exceptional separation power. An example was provided by Phillips and Xu (dated 1995) (9), who showed a GC×GC–FID chromatogram of kerosene. The separation was performed on an apolar 10 m × 530 µm, 8-µm $d_f$ column in the first dimension ($^1$D), and a more polar longer one (25 m × 250 µm) in the second dimension ($^2$D). Each column was housed in a separate GC oven, thus recognizing the importance of independent temperature optimization. A dual-stage thermal modulator linked the two analytical dimensions and was located between the two GC ovens. The transfer device was of simple construction, inasmuch that it was prepared by using a 40 cm × 250 µm, 0.25-µm $d_f$ apolar column, coated

with gold paint. The first stage of modulation was performed on the initial 25 cm of the column segment, while the second stage was performed on the remaining 15 cm. A heating pulse was directed to each modulator segment with a 1 s delay at 60 s intervals. More details on the thermal modulator can be found in the literature (1,10). Compared to current published research, both the column combination (a conventional column was used in the $^2$D) and the modulation period (60 s) were unusual. Moreover, the temperature gradient was extremely slow (0.1 °C/min) leading to a very long analysis time (it exceeded 800 min), which ended with the elution of alkane $C_{15}$.

The outstanding potential of GC×GC was clearly evident in these early works (1,9,10), and certainly created great excitement amongst many GC practitioners. In 1996, an article appeared reporting on "sophisticated separation methods and the oil industry", with the authors declaring that the "instrumentation required for GC×GC is beautifully simple and potentially inexpensive" (11). Such a statement, in itself related to the instrumentation initially developed by J.B. Phillips and collaborators, is certainly agreeable (1,9,10).

## Recent Application and Instrumental Trends

As mentioned earlier, recent application and instrumental trends will relate to the combined period 2018 to 2019. To obtain such information, a search process was launched by using the Scopus database and the keywords: "comprehensive two-dimensional gas chromatography". The database provided a list of 136 papers for 2018 and 121 papers for 2019. It is obvious that, even though some published data were most probably missed, a total number of 257 works do give an overall view on the current situation. It is noteworthy that a recent review entitled: "Comprehensive two-dimensional gas chromatography advances in technology and applications: biennial update" has been published (5). Overlap of the information herein provided with respect to that work will try to be avoided, while particular attention will be devoted to the fact that the concept of GC×GC is inherently simple.

With regard to the topics, a main one was defined for each publication, with a clear dominance of application research observed (77%), followed by reviews and perspectives (9%) (Figure 1). Among the applications (Figure 2), food and beverage (29%), energy (24%), environment (21%), and biological (9%) occupy the first four positions, with the first three being by far the most popular. With regard to reviews and perspectives, these involve manuscripts covering both a specific GC×GC aspect or application (for example, metabolomics [12], modulation [13], environment [14]) and those describing GC×GC as an option within a

**FIGURE 1:** Graph reporting the topics of GC×GC research published across the combined period 2018–2019. Refer to the text for the significance of the abbreviation. The % values have been rounded to the nearest integer.

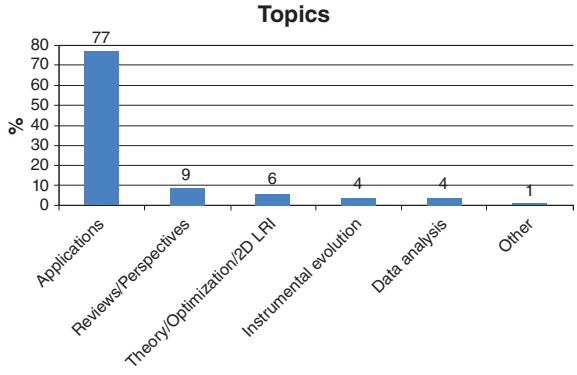
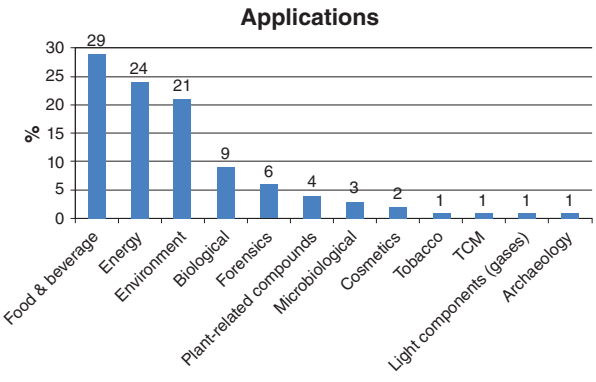



**FIGURE 2:** Graph reporting the specific types of applications of GC×GC research published across the combined period 2018–2019. Abbreviation: TCM: traditional Chinese medicine. The % values have been rounded to the nearest integer.




specific research field (for example, analysis of S-containing compounds in petroleum [15], solid-phase microextraction [SPME] [16]). Considering the sum of application research and reviews and perspectives, these represent 86% of the published work across the period 2018–2019. A third topic relates to theory and optimization (for example, retention time prediction [17], FM model [18], solid-state modulation optimization [19]) and 2D linear retention index (LRI) calculation (20,21), represented by 6% of the published works. With regard to instrumental evolution and data analysis, these were characterized by only 4% each of the published work. Technological advances have involved, among others, a miniaturized GC×GC system (22), the use of a

**FIGURE 3:** Scheme of the MMM transfer system. Refer to the text for the significance of the symbols. Reproduced with permission from J.V. Seeley, N.E. Schimmel, and S.K. Seeley, *J. Chromatogr. A* **1536**, 6–15 (2018). Copyright 2018 (Elsevier).

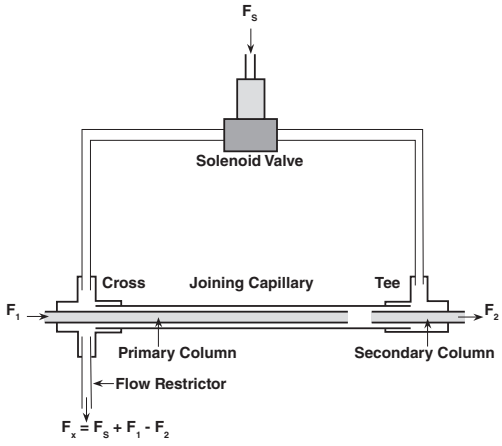
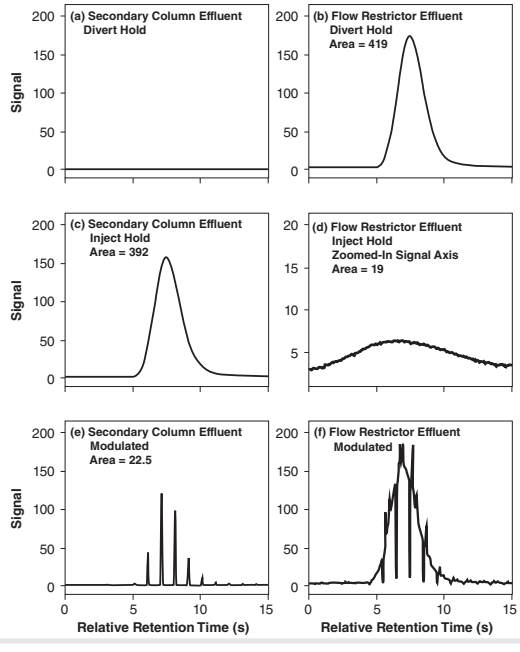



**FIGURE 4:** Low duty cycle MMM GC×GC–FID analysis of n-pentane: (a) $^2$D result when the valve is in the NO position; (b) the restrictor result when the valve is in the NO position; (c) $^2$D result when the valve is in the NC position; (d) the restrictor result when the valve is in the NC position; (e) $^2$D result for modulated n-pentane; (f) FID1 result for modulated n-pentane. Reproduced with permission from J.V. Seeley, N.E. Schimmel, and S.K. Seeley, *J. Chromatogr. A* 1536, 6–15 (2018). Copyright 2018 (Elsevier).




micro-reactor prior to FID detection (23), a silver-based ionic liquid $^2$D column (24), and different FM approaches (25,26).

Proceeding onto the utilization of modulation approaches, specific information was attained from the majority of the 257 publications (for some papers we had access only to the abstract). As expected, CM was by far the most common choice (87%), followed by FM (10%), and solid-state modulation (3%).

Focus is herein devoted to FM, which is an area that has undergone evolution in recent years. For example, in 2018 Seeley *et al.* introduced a novel system based on the Deans switch principle and defined multi-mode modulator (MMM) (25). A scheme of the MMM GC×GC system is shown in Figure 3. As can be observed, the MMM is of simple construction and is characterized by a deactivated metal joining capillary, connected on the $^1$D side to a cross union and to a tee union on the $^2$D side. The tips of the $^1$D and $^2$D columns are fixed near to one another within the joining capillary. Two metal capillaries link the unions to a solenoid valve (the normally opened [NO] port of the solenoid valve is connected to the tee union), which in turn receives a gas flow ($F_S$) from a pneumatic control module. The fourth port of the cross union is linked to an FID-connected restrictor (FID1). There are two flows entering the modulator, $F_S$ and $F_1$ ($^1$D flow), and two flows exiting it, $F_X$ (restrictor flow) and $F_2$ ($^2$D flow). When the valve is in the NO position, the $^1$D flow is directed to the restrictor (low duty cycle) or stored in the joining capillary (high duty cycle); when the valve is in the normally closed (NC) position, effluent from the $^1$D column is directed to the second one, and then subjected to FID monitoring (FID2). Depending on the gas flows involved, and on the proximity of the $^1$D and $^2$D column tips within the joining capillary, the MMM could be operated as a low or high duty cycle modulator (consult reference 25 for more information). Under low duty cycle conditions, a 6% cyanopropyl phenyl + 94% polydimethylsiloxane 30 m × 0.25 mm, 1.4-µm $d_f$ column was used in the first dimension (flow: 1.0 mL/min), and a polyethylene glycol 0.5 m × 0.18 mm, 0.18-µm $d_f$ column in the second (flow: 0.9 mL/min). The modulation period was 1000 ms with a 75 ms injection time, meaning that only a low percentage of the $^1$D effluent reached the $^2$D. A nice example on the various effects of a low duty cycle MMM process on a highly volatile compound (*n*-pentane) can be observed in Figure 4(a–f): the FID2 result when the valve is in the NO position is shown in (a), highlighting the fact that no effluent from the first dimension reaches the second; the FID1 result when the valve is in the NO position is shown in (b); the FID2 result when the valve is in the NC position (transfer mode) is shown in (c); the FID1 result when the valve is in the NC position is shown in (d), highlighting the fact that a small amount of effluent from the first dimension

reaches the restrictor; the effects of modulation on *n*-pentane are shown in (e) (FID2 result) and in (f) (FID1 result). Very narrow peak widths at half height were reported for modulated *n*-octane (53 ms). Such MMM conditions were characterized by a duty cycle of 0.054 (ratio of the total area of the modulated pulses to that of the unmodulated peak), and were suitable for high-speed separations on short segments of micro-bore (≤ 0.18 mm internal diameter [i.d.]) column, and for MS detection (if used).

Under high duty cycle conditions, an apolar 40 m × 0.18 mm, 0.18-µm $d_f$ column was used in the first dimension (flow: 0.50 mL/min), and a polyethylene glycol 5 m × 0.25 mm, 0.25-µm $d_f$ column in the second (flow: 10.0 mL/ min). The modulation period was 1500 ms, with a 150 ms injection time. A fuel sample was subjected to analysis with peak widths at half height of approximately 80 ms. Such MMM conditions were characterized by a duty cycle of 1, and were suitable for high-speed separations on medium-length (5–8 m) segments of columns with an i.d. ≥ 0.25 mm. A flow of 10.0 mL/min can be considered as rather high for mass spectrometry, and possibly requires splitting prior to the ion source depending on the MS system used.

The 100% transfer FM approach described in reference 26 is characterized by increased simplicity (compared to reference 25): a six-port valve modulator (without a sample loop) was used, with connections (in an anticlockwise manner) to the injector, to the inlet and outlet of the $^1$D, to the inlet of the $^2$D, and to an additional gas source (the sixth port was blocked). Different samples were subjected to FM GC×GC–FID analyses under a variety of operational conditions: for example, a sample of diesel was subjected to a $^1$D separation on an apolar 15 m × 0.25 mm, 0.25-µm $d_f$ column, and a $^2$D separation on a polyethylene glycol 5 m × 0.25 mm, 0.25-µm $d_f$ column. The flow rates were 10 mL/min during the transfer process (0.4 s) and 30 mL/min for the remaining part of the separation on the $^2$D (14.6 s). During the analysis, the valve was maintained at a temperature of 300 °C. It is noteworthy that during the main part of the modulation period (14.6 s), there was a condition of stop-flow in the $^1$D, leading to an increase in the analysis time but also to a non-interdependence between the $^1$D and $^2$D separations. In general, the reported $^2$D gas flows were rather high (≥ 10.0 mL/min).

An even more simple FM approach of high interest (defined *dynamic pressure gradient modulation* [DPGM]) was described by Trinklein *et al.* at the beginning of 2020 (27): the $^1$D and $^2$D columns were linked through a tee union and connected to a pulse valve (Figure 5). An auxiliary gas source fed the valve ($P_{aux}$). Under suitable conditions of injector pressure ($P_{inlet}$), $P_{aux}$ (both inlet and auxiliary pressures are ramped

**FIGURE 5:** Scheme of the DPGM transfer system. Refer to the text for the significance of the symbols. Reproduced with permission from T.J. Trinklein, D.V. Gough, C.G. Warren, G.S. Ochoa, and R.E. Synovec, *J. Chromatogr. A* **1609**, Art. 460488 (2020). Copyright 2019 (Elsevier).

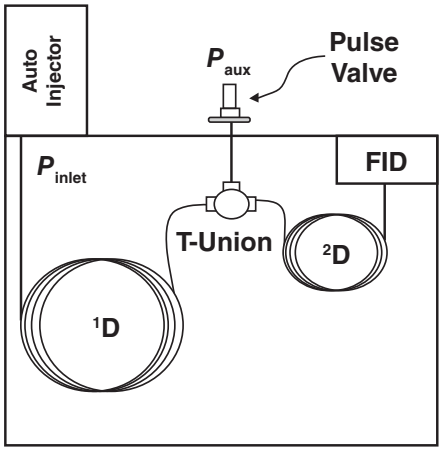
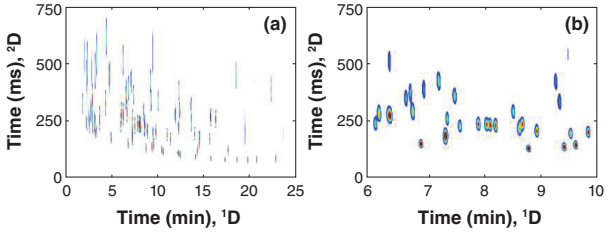



**FIGURE 6:** DPGM GC×GC–FID result for (a) a 90-compound mixture and (b) a chromatogram expansion. Reproduced with permission from T.J. Trinklein, D.V. Gough, C.G. Warren, G.S. Ochoa, and R.E. Synovec, *J. Chromatogr. A* **1609**, Art. 460488 (2020). Copyright 2019 (Elsevier).




during the analysis), and valve open and close times, a 100% transfer GC×GC analysis could be achieved. When the valve was closed a fraction of the $^1$D effluent was transferred onto the $^2$D, while when it was opened, $^1$D elution was interrupted (stop flow) and the $^2$D separation proceeded. For example, a 90-compound mixture was subjected to a DPGM GC×GC–FID analysis by using an apolar 10 m × 0.18 mm, 0.18-µm $d_f$ $^1$D column, and a polar 1 m × 0.18 mm, 0.10-µm $d_f$ $^2$D column. The modulation period was only 750 ms, with a 60 ms valve close time. The applied $P_{aux}$ generated a $^2$D gas flow of 22.9 mL/min at the beginning of the analysis. Peak widths were narrow in time and variable, being in the range 20–180 ms (Figure 6). In a further DPGM GC×GC–FID analysis on diesel fuel, the use of a longer $^2$D column (2 m × 0.18 mm,

**FIGURE 7:** (a) A cryogenic- and (b) flow-modulation GC×GC–MS chromatogram of a sample of coconut-derived bio-oil. Refer to reference 29 for peak identity. Reproduced with permission from I. Aloisi, T. Schena, B. Giocastro, M. Zoccali, P.Q. Tranchida, E. Bastos Caramão, and L. Mondello, *Anal. Chim. Acta* **1105**, 231–236 (2020).

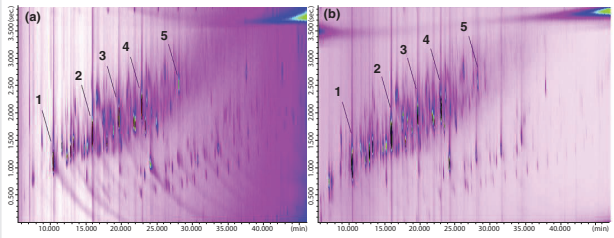
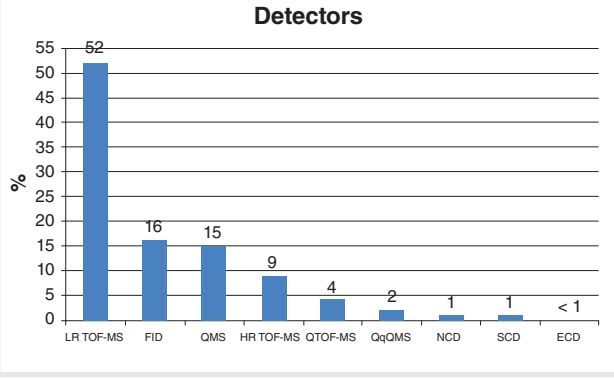



**FIGURE 8:** Graph reporting the specific types of detection in GC×GC research published across the combined period 2018–2019. Refer to the text for the significance of the abbreviations. The % values have been rounded to the nearest integer.




0.18-µm $d_f$) enabled the reduction of the $^2$D flow to 8 mL/min. In this instance, a modulation period of 2000 ms with a 150 ms valve close time was used. Very recently, DPGM GC×GC was combined with low-resolution time-of-flight (LR TOF)-MS, using a $^2$D gas flow of 4 mL/min (28).

A major concern when using FM is the lack of analyte band compression; this is a typical feature of CM leading to very high peak capacities. It was within such a context that Aloisi *et al.* attempted to determine an equivalent standard column set between CM and FM GC×GC–MS (29). Cryogenic modulation was performed by using a loop-type system, while FM was performed by using a seven-port wafer chip equipped with an external sample loop and was a 100% transfer device (29). Very similar chromatography performances were attained when using an apolar 30 m × 0.25 mm, 0.25-µm $d_f$ + polar 1.5 m × 0.25 mm, 0.25-µm $d_f$ CM column set and an apolar 20 m × 0.18 mm, 0.18-µm $d_f$ + polar 5 m × 0.32 mm, 0.25-µm $d_f$ FM set (obviously the same types of stationary phases were used). The CM and FM results, attained on a sample of coconut bio-oil, are shown in Figure 7. This study provided an idea of the potential of the FM approach used compared with CM.

Considering detection (again, specific information was attained from the majority of the publications, not from all), obviously mass spectrometry confirms its dominant role (Figure 8). The first four positions are occupied by LR TOF-MS (52%), FID (16%), single quadrupole (Q)MS (15%), and high-resolution (HR) TOF-MS (9%). The use of both LR TOF-MS and rapid-scanning QMS are now well-established. With regards to HR TOF-MS, the definition of "high resolution" is rather vague. A powerful HR TOF-MS system used in the GC×GC field is capable of exceeding a resolution of 25,000 (full width half maximum [fwhm]), operating with a normal GC mass range and a high acquisition frequency (200 Hz) (30). Even though there has been an increasing use of QTOF-MS, it should be noted that its MS/MS capabilities are usually not exploited. In fact, the quadrupole is normally operated as a fly-through zone, with high-resolution mass spectra generated by the TOF analyzer. For example, Bowman *et al.* used GC×GC–QTOF-MS with atmospheric-pressure chemical ionization (APCI) in an environmental study involving the Athabasca oil sands (31). The mass spectrometer was operated in the TOF mode, at a resolution of 20,000 (fwhm) and at an acquisition frequency of 30 Hz. If one sums up the use of HR TOF-MS and QTOF-MS during the period 2018–2019, then HR MS is used at a similar level with respect to QMS. Considering ionization processes, electron ionization (EI) is by far the most popular approach. The use of soft forms of ionization, such as APCI (31), is reported rather rarely. The use of an LR TOF-MS system with the capability to perform hard (70 eV) and soft (14 eV) EI, in a rapid alternate manner and with a satisfactory analytical response, has started to appear (32). The use of triple quadrupole (QqQ) MS was found in only 2% of the published research. Such a trend can be related to the targeted nature of QqQMS analyses, whereas the power of GC×GC stands out in untargeted analyses. However, the use of QqQMS, with the capability to generate both untargeted (scan) and targeted (multiple reaction monitoring) data in a rapid alternate manner, has been reported (33).

The use of FID has declined greatly and is now comparable to that of QMS. Forms of selective detection, such as nitrogen and sulphur chemiluminescence detection (NCD, SCD), and electron capture detection (ECD), find little current use (Figure 8). In the authors' opinion, among several reasons for such a trend is the fact that GC×GC separations are generally

# INTRODUCING OUR NEW VICI M SERIES PUMP RATED UP TO 1500 PSI

Biocompatible materials:
- Ceramic
- PEFE
- PEEK

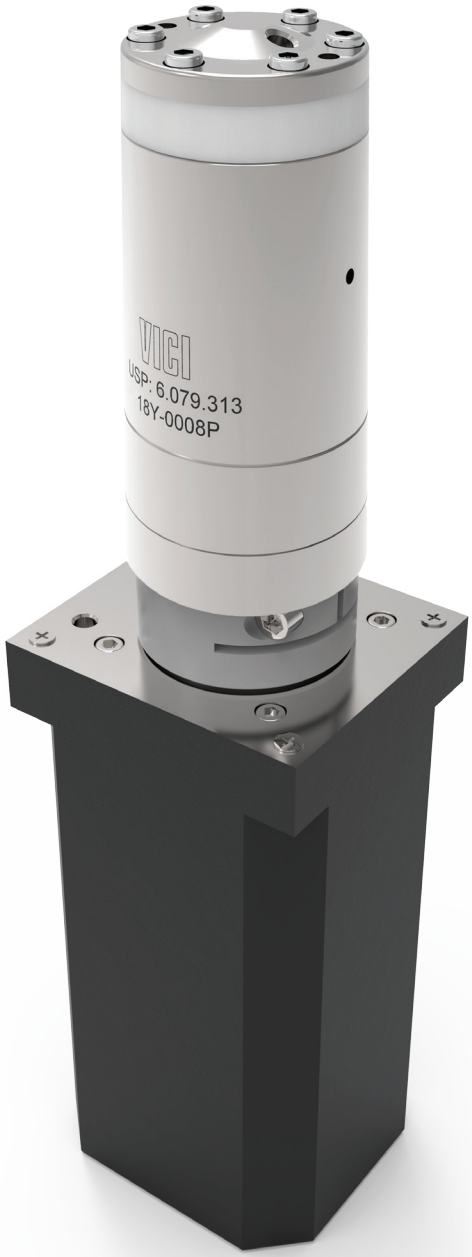
M6 pump:
- 5 nL/min to 5 mL/min

M50 pump:
- 1 µL/min to 25 mL/min

Pulseless, continuous bi-directional flow

Syringe-free

Less than 0.5% error flow rate




VICI®
+ 41 41 925-6200
info@vici.ch
www.vici.com

characterized by many more peaks compared to conventional GC ones, increasing the need for mass spectrometry.

## Future Prospects

Comprehensive 2D-GC is becoming a mature technology, as can be concluded by observing recent literature, which is dominated by application research and with less space devoted to technological evolution. Such a tendency will probably continue, also in part as a result of the continuous evolution of MS. In fact, the availability of (powerful) MS reduces the requirements of an improved separation performance on the GC×GC side.

What are the future prospects of GC×GC? There will probably be an increasing development of smaller, less-energy consuming GC×GC(–MS) devices. It is anticipated that modulation will be part of such a downscaling, involving the use of robust, compact, simple, and effective devices. Modulation approaches that could potentially fit such a description are present both in past (1,10) and in present research (25–29).

## References


1) Z. Liu and J.B. Phillips, *J. Chromatogr. Sci.* **29**, 227–231 (1991).
2) J.B. Phillips and J. Beens, *J. Chromatogr. A* **856**, 331–347 (1999).
3) M. Adahchour, J. Beens, and U.A.Th. Brinkman, *J. Chromatogr. A* **1186**, 67–108 (2008).
4) J.V. Seeley and S.K. Seeley, *Anal. Chem.* **85**, 557–578 (2013).
5) M.S.S. Amaral, Y. Nolvachai, and P.J. Marriott, *Anal. Chem.* **92**, 85–104 (2020).
6) R.M. Kinghorn and P.J. Marriott, *J. High Resol. Chromatogr.* **21**, 620–622 (1998).
7) C.A. Bruckner, B.J. Prazen, and R.E. Synovec, *Anal. Chem.* **70**, 2796–2804 (1998).
8) J-M. Dimandja, *Am. Lab.* **35**, 42–53 (2003).
9) J.B. Phillips and J. Xu, *J. Chromatogr. A* **703**, 327–334 (1995).
10) Z. Liu and J.B. Phillips, *J. Microcol. Sep.* **6**, 229–235 (1994).
11) P. Schoenmakers, J. Blomberg, and S. Kerkvilet, *LCGC International* **14**, 718–724 (1996).
12) E.A. Higgins Keppler, C.L. Jenkins, T.J. Davis, and H.D. Bean, *TrAC, Trends Anal. Chem.* **109**, 275–286 (2018).
13) H.D. Bahaghighat, C.E. Freye, and R.E. Synovec, *TrAC, Trends Anal. Chem.* **113**, 379–391 (2019).
14) A.M. Muscalu and T. Górecki, *TrAC, Trends Anal. Chem.* **106**, 225–245 (2018).
15) Y. Han, Y. Zhang, C. Xu, and C.S. Hsu, *Fuel* **221**, 144–158 (2018).
16) S. Huang, G. Chen, N. Ye, X. Kou, F. Zhu, J. Shen, and G. Ouyang, *Anal. Chim. Acta* **1077**, 67–86 (2019).
17) R. Jaramillo and F.L. Dorman, *J. Chromatogr. A* **1581–1582**, 116–124 (2018).
18) M. Giardina, J.D. McCurry, P. Cardineal, G. Semard-Jousset, C. Cordero, and C. Bicchi, *J. Chromatogr. A* **1577**, 72–81 (2018).
19) M. Zoccali, B. Giocastro, P.Q. Tranchida, and L. Mondello, *J. Sep. Sci.* **42**, 691–697 (2019).
20) M. Jiang, *Anal. Chem.* **91**, 4085–4091 (2019).
21) D.M. Mazur, I.G. Zenkevich, V.B. Artaev, O.V. Polyakova, and A.T. Lebedev, *J. Chromatogr. A* **1569**, 178–185 (2018).
22) J.J. Whiting, E. Myers, R.P. Manginell, M.W. Moorman, J. Anderson, C.S. Fix, C. Washburn, A. Staton, D. Porter, D. Graf, D.R. Wheeler, S. Howell, J. Richards, H. Monteith, K.E. Achyuthan, M. Roukes, and R.J. Simonson, *Lab Chip* **19**, 1633–1643 (2019).
23) J. Luong, Y. Hua, R. Gras, and R.A. Shellie, *Anal. Chem.* **91**, 11223–11230 (2019).
24) I.D. Souza, H. Nan, M.E.C. Queiroz, and J.L. Anderson, *Anal. Chem.* **91**, 4969–4974 (2019).
25) J.V. Seeley, N.E. Schimmel, and S.K. Seeley, *J. Chromatogr. A* **1536**, 6–15 (2018).
26) H. Cai and S.D. Stearns, *J. Chromatogr. A* **1569**, 200–211 (2018).
27) T.J. Trinklein, D.V. Gough, C.G. Warren, G.S. Ochoa, and R.E. Synovec, *J. Chromatogr. A* **1609**, Art. 460488 (2020).
28) S. Schöneich, D.V. Gough, T.J. Trinklein, and R.E. Synovec, *J. Chromatogr. A* In press DOI: 10.1016/j.chroma.2020.460982.
29) I. Aloisi, T. Schena, B. Giocastro, M. Zoccali, P.Q. Tranchida, E. Bastos Caramão, and L. Mondello, *Anal. Chim. Acta* **1105**, 231–236 (2020).
30) B. Savareear, J. Escobar-Arnanz, M. Brokl, M.J. Saxton, C. Wright, C. Liu, and J.-F. Focant, *J. Chromatogr. A* **1581–1582**, 105–115 (2018).
31) D.T. Bowman, K.J. Jobst, X. Ortiz, E.J. Reiner, L.A. Warren, B.E. McCarry, and G.F. Slater, *J. Chromatogr. A* **1536**, 88–95 (2018).
32) E. Omar, E. Bichon, S. Hutinet, A.-L. Royer, F. Monteau, H. Germon, P. Hill, G. Remaud, G. Dervilly-Pinel, R. Cariou, and B. Le Bizec, *J. Chromatogr. A* **1601**, 327–334 (2019).
33) M. Zoccali, S. Cappello, and L. Mondello, *J. Chromatogr. A* **1547**, 99–106 (2018).



**Peter Q. Tranchida** is Associate Professor in food chemistry in the Department of Chemistry, Biological, Pharmaceutical and Environmental Sciences at the University of Messina, in Italy. His research activities are focused mainly on the study of complex food samples by using advanced chromatography processes. In particular, he has performed a great deal of research work in the field of classical multidimensional gas chromatography, multidimensional liquid–gas chromatography, and comprehensive 2D-gas chromatography (GC×GC).

**Ivan Aloisi** received his master's degree in pharmaceutical chemistry and technology in 2017 (University of Messina), discussing a thesis entitled "Qualitative analysis of the unsaponifiable fraction of vegetable oils using comprehensive two-dimensional gas chromatography coupled with high resolution mass spectrometry." He is now Ph.D. student in chemical sciences in the University of Messina.

**Luigi Mondello** is Full Professor of Analytical Chemistry at the University of Messina. His research is focused on the development of multidimensional chromatographic instrumentation and software, coupled to state-of-the-art MS, for the study of complex matrices constituents and contaminants. He is the author of around 500 scientific papers and 1000 conference presentations, with an H-index of 64 (Google Scholar) and a total impact factor of more than 1000. He has been awarded with the HTC Award, COLACRO Medal, Silver Jubilee Medal, Liberti Medal, TASIAs, IFEAT Medal, GC×GC Lifetime Achievement, Golay Award, Robert Kellner Lecture, and the Herbert J. Dutton Award.

# Selectivity in Reversed-Phase Liquid Chromatography: 20 Years of the Hydrophobic Subtraction Model

**Dwight R. Stoll,** LC Troubleshooting Editor

**How can I use the hydrophobic subtraction model of reversed-phase selectivity to help me in method development? A recent Pittcon symposium discussed the history and practical use of the model, as well as insights into recent research that may enable expanded use of the model in the future.**

For the 2020 edition of the Pittsburgh Conference (Pittcon) held just a few weeks ago in Chicago, USA, Professor Joe Foley of Drexel University organized a symposium entitled "To Selectivity and Beyond: Celebrating 18 Years of the Hydrophobic Subtraction Model". It has been roughly 20 years since Lloyd Snyder, John Dolan, Peter Carr, and co-workers began work on what has become known as the hydrophobic subtraction (HS) model of selectivity for reversed-phase liquid chromatography (LC) columns. In my view, this model, and the accompanying public database of parameters for 750 commercially-available columns, has been remarkably successful, and this Pittcon symposium aimed to both discuss the impact of the model on contemporary method development, and provide some insights from recent research on the use of the model going forwards. Choosing a column is an important decision, not only at the beginning of method development but also for the analytical life cycle of a method. In this instalment of "LC Troubleshooting", I will first review the basic premise and features of the HS model and the column database, and then touch on the highlights of the talks presented as part of the Pittcon symposium. From my point of view, and I think the other contributors to the symposium would agree, the HS model database is a column characterization tool that is underutilized by the LC community, in spite of the fact that it is a free resource. Users can leverage the database to supplement information available from suppliers and become more familiar with their columns. Separation performance can improve by locating and evaluating more than one column to optimize selectivity, and quickly locate replacement columns if problems develop. I hope that symposia like the one at Pittcon can both help other users understand how the model can benefit their work, and promote discussion about how we can improve this and other resources as we work into the future.

## Basics of the Hydrophobic Subtraction Model of Reversed-Phase Selectivity

The basic principle of the HS model was first described in a journal article by Snyder and co-workers in 2002 (1). Since then, many articles have been published on the topic, but two resources are particularly noteworthy for readers interested in learning more about the model. First, in 2012, Snyder and co-workers published a book chapter in *Advances in Chromatography* that is still the most comprehensive discussion of the model and its application that has been published to date (2). Second, a more recent article in *LCGC* provides more of an overview

**Choosing a column is an important decision, not only at the beginning of method development but also for the analytical life cycle of a method.**

**FIGURE 1:** Conceptual illustration of the five major solute–stationary phase interactions accounted for by the HS model, reprinted from reference 3.

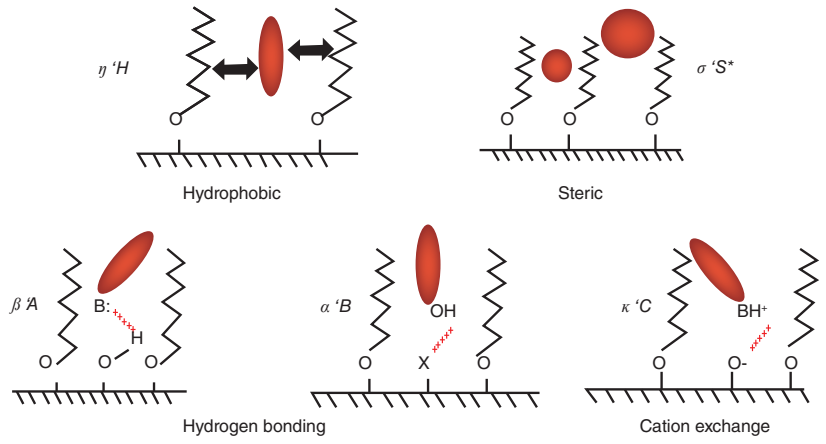
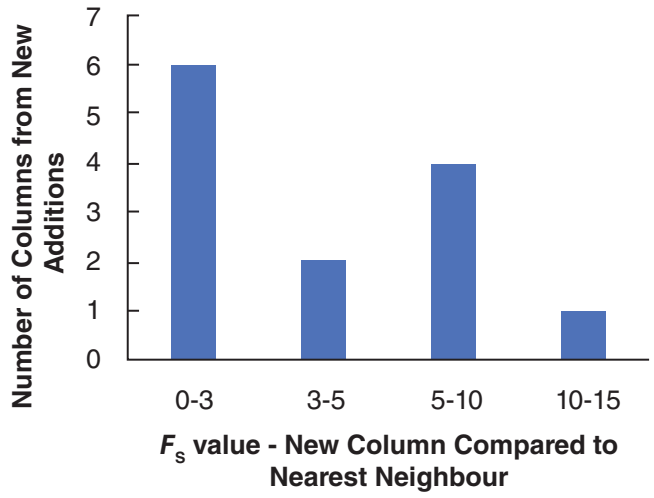



**FIGURE 2:** Distribution of $F_S$ values from comparisons of each new column added to the HS model database and its nearest neighbour in the database prior to the new additions.




of the model and its application that may be an easier place to start for those that are completely new to the idea (3). The model, which was originally developed using retention data from alkyl phases (such as C4, C8, and C18) bonded to high-purity type B silicas, assumes that *reversed-phase selectivity* (defined here as the ratio of retention factors for a compound of interest, and ethylbenzene) can be described using the sum of five pairs of column and solute parameters that are related to different physicochemical interactions between solutes and the reversed-phase stationary phase. A view of the nature of each of these interactions is shown in Figure 1.

The mathematical expression of the model is shown in equation 1, where the capital Roman letters *H*, *S**, *A*, *B*, and *C* are column parameters, and the Greek small letters η, σ, β, α, and κ are solute parameters:

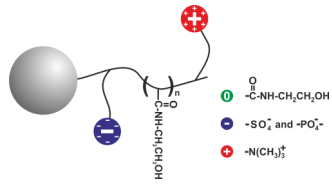
$$\log\left(\frac{k}{k_{EB}}\right) = \eta'H - \sigma'S^* + \beta'A + \alpha'B + \kappa'C \quad [1]$$

The column parameters are determined experimentally by measuring the retention times of 16 carefully chosen probe solutes in a mobile phase composed of acetonitrile and potassium phosphate buffer at pH 2.8, calculating the selectivity value for each compound ($k/k_{EB}$), and regressing those selectivities against the known solute parameters for the probe compounds (2). To date, parameters for 750 commercially-available columns have been determined, and are publicly available for free through two websites: 1) a site maintained by the *United States Pharmacopeia* (https://apps.usp.org/app/USPNF/columnsDB.html); and 2) a site maintained by my research group (www.hplccolumns.org). The two primary uses of this database are finding columns that have similar selectivities (for example, for identifying a backup column during method development), and finding columns that have very different selectivities (for example, for identifying a set of columns to screen during method development). These applications will be discussed in more detail in the next sections.

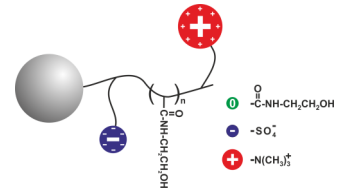
HILICON

# iHILIC®

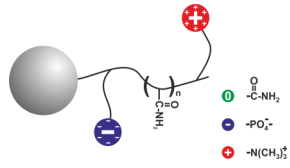
advances HILIC separations in UHPLC and HPLC



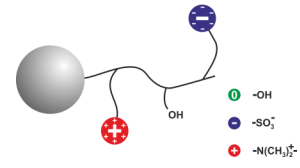

**iHILIC®-Fusion**
Silica based




**iHILIC®-Fusion(+)**
Silica based




**iHILIC®-Fusion(P)**
Polymer based




**iHILIC®-(P) Classic**
Polymer based

- Charge modulated amide and diol HILIC columns
- Complementary selectivities for separation of polar compounds
- Excellent durability and ultra-low bleeding
- Versatile columns for "Omics" studies and other LC-MS applications
- iHILIC®-Fusion and iHILIC®-Fusion(+): 1.8, 3.5, and 5 µm; pH 2-8
- iHILIC®-Fusion(P) and iHILIC®-(P) Classic: 5 µm; pH 1-10

For more information:
Email: info@hilicon.com | Website: www.hilicon.com

**In the early stages of method development, it is common practice to choose a diverse set of stationary phases that can be screened to identify candidate phases that exhibit the selectivity needed to separate the analyte mixture at hand.**

### Overview of the Symposium

The Pittcon symposium was composed of five podium presentations:

- Prof. Dwight Stoll (Gustavus Adolphus College) reviewed the early history of the development of the HS model, its physicochemical basis, and shared some insights from analysis of the selectivities of reversed-phase columns recently introduced to database.
- Dr. Richard Henry (Independent Consultant) discussed use of the column parameters database from a practical perspective, with an emphasis on tools that can leverage the database to identify equivalent columns.
- Dr. John Dolan (LC Resources) discussed recent efforts by himself, Paul Haddad, and co-workers to predict retention times in reversed-phase LC (and other modes) starting from physicochemical descriptors of solutes of interest.
- Dr. Tony Taylor (Arch Sciences Group) discussed a recent study aimed at a retrospective refinement of the HS model, and the potential for a refined model to be used for retention prediction in reversed-phase LC.
- Prof. Joe Foley (Drexel University) described recent efforts by his research group to use the HS model to demonstrate the advantages of serially-coupled columns from the HS model database, and ultimately predict which pairwise combinations of columns might yield the best separations of different mixtures of real or synthetic solutes.

### Use of the HS Model for Column Selection During Method Development

In the early stages of method development, it is common practice to choose a diverse set of stationary phases that can be screened to identify candidate phases that exhibit the selectivity needed to separate the analyte mixture at hand (4). In this case, the HS model database can be used to identify a set of columns with sufficient diversity to be useful in this regard. Later in method development, when a leading candidate column has been identified and the method is refined prior to validation, it is also common to try to identify other columns with very similar, even "equivalent", selectivity such that this column can be used as a drop-in replacement in the event that the manufacturer of the primary column stops making it, or has trouble manufacturing the column reproducibly. Here again, the HS model database can be used for this purpose, by generating a short list of columns to evaluate experimentally and assess their similarity to the primary column. For both of these purposes Snyder and co-workers advocated for the use of a "similarity factor, $F_S$", which is a weighted distance between two columns in five-dimensional column parameter space. While this might sound complicated, calculating $F_S$ is very straightforward, as shown in equation 2, where $H_1$ and $H_2$ are the *H* parameters for the first and second columns in the comparison, and so on:

$$F_S=\sqrt{[\chi_H(H_1-H_2)]^2+[\chi_{S^*}(S^*_1-S^*_2)]^2+[\chi_A(A_1-A_2)]^2+[\chi_B(B_1-B_2)]^2+[\chi_C(C_1-C_2)]^2} \quad [2]$$

The weighting factors $\chi_H$, $\chi_{S^*}$, and so on are user-adjustable parameters in the calculation, but usually taken as 12.5, 100, 30, 143, and 83 for *H, S*, A, B,* and *C,* respectively (2). Both of the web-based tools cited above do this $F_S$ calculation for you, and facilitate sorting the database to identify columns that are similar, or different, compared to a target column you specify.

In his presentation, Henry emphasized the point that the task of identifying columns with similar selectivities has become more difficult over the past two decades, as the stationary phase offerings from manufacturers have become more diverse. The L-code system of the *United States Pharmacopeia* is based on stationary phase types such as "C18" and "phenyl", and compendial methods specify columns from a particular phase type. However, stationary phases with mixed chemistries are becoming more and more common (for example, phenyl-hexyl is a mixed chemistry phase with both aromatic and alkyl components), and increasingly we are coming to understand that the properties of the underlying silica substrate (for example, metal impurities, and specific synthetic methods) can have a significant impact on selectivity, particularly for complex solutes with many different types of functional groups. The net effect is

that, when exploring the HS model database, it is possible to find that the column most similar to a target column of interest as measured by $F_S$ belongs to a different phase type. On one hand, this means that we should be somewhat open-minded when scouting for similar columns, but, on the other hand, this creates challenges when identifying columns that can be used as replacements in a regulated environment.

### Recent Efforts to Predict Reversed-Phase Retention Using the HS Model

In the process of establishing the HS model 20 years ago, it was demonstrated that the model described by equation 1 could accurately reproduce the retention factors of about 90 solutes obtained for 10 different alkyl stationary phases based on high-purity type B silicas with an accuracy of about 2% (1). Since the large database of column parameters already exists, it is logical to think about using the model and the database to predict retention for new analytes that are not represented in the set of solutes used to establish the model initially. However, this requires determination of the solute parameters to use the model (equation 1) for this purpose, and doing so experimentally is currently quite time- and resource-intensive. In his presentation, Dolan gave a summary of work by Paul Haddad, his group at the Australian Centre for Research on Separation Science (ACROSS), and other collaborators in recent years to predict HS model solute parameters from chemical structures that could in turn be used to predict reversed-phase retention via the HS model. Readers interested in learning more about these efforts are referred to recent articles in *LCGC* and other publications where the details of the work are discussed (5,6). Briefly, Haddad and co-workers explored the use of computationally-derived molecular descriptors produced by programs such as VolSurf+, and different approaches to constructing local and global models, and evaluated their effects on reversed-phase retention prediction. Again, the basic premise here is that one would take the chemical structures for analytes they are trying to separate, calculate molecular descriptors based on those structures (for example, log *D*, polar surface area, and so forth), then calculate HS model solute parameters

**I think we can all look forward to further development as research groups continue to refine the model and expand its scope of application.**

based on those descriptors, and finally calculate retention factors for those compounds using equation 1 and the column parameters from the HS model database. In principle, all of this could be done without doing a single laboratory experiment. Dolan reported that using molecular descriptors to calculate the η' parameter alone (equation 1) for each solute was sufficient to make retention time predictions that were accurate to within 30 s for 70% of the 146 solutes evaluated in their study (5). Although this obviously leaves room for improvement in the future, this kind of work should capture the imagination of research groups around the globe to improve these modelling and retention prediction efforts.

## Recent Additions to the Public Database of Reversed-Phase Column Parameters

In March of this year, we added 13 new columns (from three different vendors) to the HS model database, bringing the total number of columns in the dataset to 750. Whenever we make these additions, I am always curious to know what is being added; redundancy in selectivity, or unique selectivities? I don't know the motivations of the manufacturers of these new columns, but, of course, there is value in both types of additions. It is helpful to have the redundancy in the selectivities of column offerings to have a backup column to use, in case there is some problem with the supply of the column of first choice. On the other hand, LC users are always interested in new selectivities that might be able to solve their challenging separation problems. Figure 2 shows the $F_S$ values obtained from a comparison of each new column to its nearest neighbour (in selectivity space) in the database prior to the most recent additions. From these values, we see that 6 of the 13 new additions are equivalent to some other column already in the database ($F_S < 3$). Of the other seven, two of them are very similar to existing phases ($3 < F_S < 5$), and only one of them has a nearest neighbour with an $F_S > 10$, which we might consider somewhat unique. Here again, the HS model is useful for assessing the characteristics of commercial offerings as these continue to grow. New additions to the database have grown at a remarkably consistent pace of about two to three columns per month over the last 10 years!

## Summary

In this instalment of "LC Troubleshooting", I have reviewed the basic concept of the hydrophobic subtraction model of reversed-phase selectivity for LC, and summarized some highlights from a recent Pittcon symposium organized to celebrate the success of the model over the last two decades. I encourage readers who are unfamiliar with the model to consider how it might benefit method development work, and I think we can all look forwards to further development as research groups continue to refine the model and expand its scope of application.

## Acknowledgement

I want to thank Dick Henry, Joe Foley, John Dolan, and Tony Taylor for reviewing the manuscript for this article and providing helpful feedback.

## References

1) N.S. Wilson, M.D. Nelson, J.W. Dolan, L.R. Snyder, R.G. Wolcott, and P.W. Carr, *J. Chromatogr. A* **961**, 171–193 (2002). DOI: 10.1016/S0021-9673(02)00659-3.
2) L.R. Snyder, J.W. Dolan, D.H. Marchand, and P.W. Carr, in *Advances in Chromatography,* E. Grushka and N. Grinberg, Eds. (CRC Press, Boca Raton, Florida, USA, 2012), pp. 297–376.
3) J.W. Dolan and L.R. Snyder, *LCGC North Am.* **34**(9), 730–741 (2016).
4) K.M. Biswas, B.C. Castle, B.A. Olsen, D.S. Risley, M.J. Skibic, and P.B. Wright, *J. Pharm. Biomed. Anal.* **49**, 692–701 (2009). DOI: 10.1016/j.jpba.2008.12.039.
5) Y. Wen, M. Talebi, R.I.J. Amos, R. Szucs, J.W. Dolan, C.A. Pohl, and P.R. Haddad, *J. Chromatogr. A* **1541**, 1–11 (2018). DOI: 10.1016/j.chroma.2018.01.053.
6) P.R. Haddad, *LCGC North Am.* **35**(8), 499–502 (2017).

**Dwight R. Stoll** is the editor of "LC Troubleshooting". Stoll is a professor and co-chair of chemistry at Gustavus Adolphus College in St. Peter, Minnesota, USA. His primary research focus is on the development of 2D-LC for both targeted and untargeted analyses. He has authored or coauthored more than 60 peer-reviewed publications and four book chapters in separation science and more than 100 conference presentations. He is also a member of *LCGC*'s editorial advisory board. Direct correspondence to: LCGCedit@mmhgroup.com

# New Chromatography Columns and Accessories for 2020

**David S. Bell,** Column Watch Editor

**Our annual review of new liquid chromatography (LC) columns and accessories, introduced at Pittcon and other events.**

This article covers liquid chromatography (LC) columns and accessories commercially released after Pittcon 2019 through this year's conference held in Chicago, Illinois, USA. *LCGC* sent out a survey in late 2019 and early 2020, asking vendors to supply information on products launched after Pittcon 2019. Other areas of interest such as gas chromatography (GC), chromatographic instrumentation, and sample preparation will be covered in other articles published in this and next month's issues. Information for this article is obtained over the course of many months, and thus it is possible that some information has been missed or misinterpreted. The reader is encouraged to check with specific vendor sites for additional products, as well as more detailed information on product usage and attributes.

The vendors that responded to the survey with high performance liquid chromatography (HPLC) or ultrahigh-pressure liquid chromatography (UHPLC) columns and LC accessories are listed in Table 1. The products launched over the past year vary in targeted analyte type, as well as mode of operation. The entries can be initially categorized as addressing small molecule or large molecule needs. Within these categories, the products can be further separated based on the specific modes of separation they employ, including reversed-phase, hydrophilic interaction liquid chromatography (HILIC), chiral, and ion-exchange (IEC) chromatography. In addition to new chromatography columns, several vendors have released accessories, as well as new column formats, to address the needs of separation scientists. Trends noted throughout the article are based on comparisons to yearly reports since 2016 (1–4).

Upon examination of Table 1, it is immediately apparent that there was a low number of respondents in 2020 compared to previous years. Table 2 shows this more clearly by providing a listing of the number of companies and the products they launched over the past five years aimed at specific categories. Please note that many products can be categorized in multiple areas, depending on usage. This is meant to be a loose interpretation to highlight trends only. The number of companies that provided new product information dropped from a high of 27 in 2017 to just 11 in 2020. Even more striking is that the number of total new column phases launched dropped from an average of about 60 columns per year over the past four years to just 22 this year. It will be interesting to see if these trends continue in the future.

## Columns for Small Molecules

**Reversed-Phase, HILIC, and IEC:** The product offerings assigned to the small molecule category intended for reversed-phase, HILIC, and IEC are listed in Table 3. A total of 11 new entries are shown. Within this broad category, ChromaNik Technologies has launched the most new products. SunArmor NH2 is described by the company as a highly stable and retentive aminopropyl phase with hydrophilic endcapping. The column is suggested for the analysis of sugars and other polar compounds. The company also released SunShell HILIC-S, described as a bare silica phase built on a superficially porous particle (SPP) that is suitable for polar compound analysis. Agilent Technologies also released a HILIC phase. The company applied their zwitterionic surface chemistry previously built on a 2.6-µm SPP to their 1.9- and 4-µm SPP sizes. These three phases constitute all of the HILIC phases released this past year. As noted in the 2019 edition of this article series, HILIC phase development slowed significantly. This trend continued in 2020.

ChromaNik Technologies also developed two new columns based

**TABLE 1:** Vendors responding to the 2020 *LCGC* new product survey

| Company | Product |
|---|---|
| Agilent Technologies, Inc. | Poroshell 120 SB-C18 |
| | Poroshell 120 SB-Aq |
| | Poroshell 120 HILIC-Z |
| ChromaNik Technologies | SunShell Micro/Nano Columns |
| | SunShell HILIC-S |
| | Sunniest PFP&C18 |
| | SunShell PFP&C18 |
| | SunArmor NH2 |
| ColumnTek | Enantiocel A6 |
| | Enantiocel C9 |
| Develosil USA | FlexFire Series |
| DWK Life Sciences | Chromaflex |
| Optimize | EXP 2 filters, fittings and adapters |
| Phenomenex | LUX-i-Amylose-3 (3 and 5 µm) |
| | Kinetex PS C18 |
| Regis Technologies | Reflect I-Cellulose J Polysaccharide Chiral Column |
| Shodex | SI-36 4D |
| Waters Corporation | Mobile phase additive - IonHance DFA |
| | Atlantis Premier BEH C18 AX |
| YMC | YMC-Triart Bio C18 |

on a mix of octadecyl (C18) and pentafluorophenyl (PFP) ligands. The SunShell PFP&C18 is constructed on a 2.6-µm SPP particle, whereas the Sunniest PFP&C18 column uses a 5-µm fully porous particle (FPP) architecture. The company reports that the combination of ligands provides increased retention and stability, while maintaining much of the selectivity of a PFP phase. In addition to the new phases noted, ChromaNik Technologies also released many of its SunShell line of phases in capillary dimensions. The phases include C18, RP-Aqua, phenyl, C8-30HT, and C4-100 and are available in dimensions from 50 × 0.075 mm to 150 × 0.5 mm. Particle sizes of 2.0-, 2.6-, and 5-µm may be acquired.

Phenomenex launched an alkyl C18 phase with a positive surface charge based on its 2.6-µm SPP particle. The Kinetex PS C18 is suggested for reversed-phase analysis of basic compounds. The company notes that the phase exhibits multi-interaction selectivity and improved peak shape for bases. The phase is also purported to provide unique selectivity. Continuing the trend of modified C18 phases, Waters Corporation released the Atlantis Premier BEH C18 AX phase. The stationary phase is described as a "C18 and alkylamine", and is suggested for separations containing polar acids or where alternative selectivity to other C18 phases is required. The phase exhibits increased retention for polar acidic compounds as a result of a mixed mode (reversed-phase/anion exchange) mechanism. The company also claims high batch-to-batch reproducibility and a wide (2–10) pH stability range.

Agilent Technologies also released two new phases on its 1.9- and 4-µm SPP for reversed-phase operation. The SB-C18, according to the company, possesses a proprietary bonding chemistry that allows for operation down to pH 1 and is stable to temperatures as high as 80 °C. Another phase denoted as SB-Aq is claimed by the organization to provide superior retention and peak shape for polar compounds that are poorly retained on traditional reversed-phase materials. The SB-Aq phase is also touted as being stable at low pH and high temperatures.

To round out the reversed-phase, small-molecule offerings from this past year, Thermo Fisher Scientific introduced a biphenyl chemistry to its Accucore line of stationary phases. The company recommends the biphenyl phase for use in the separation of critical pairs and isomers. The phase is touted as being suitable for the determination of drugs of abuse or steroids, providing unique selectivity for aromatic and moderately polar analytes. The Accucore series is based on SPP technology.

**The products launched over the past year vary in targeted analyte type, as well as mode of operation.**

**TABLE 2:** Trending data in the numbers and types of liquid chromatography columns launched in the period 2016–2020

| Year | Companies (Count) | Small-Molecule Columns (Count) | | | | | Large-Molecule Columns (Count) | | | | | Total (Count) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Reversed-Phase | HILIC | Chiral | Ion-Exchange | SFC | Reversed-Phase | HIC | Ion-Exchange | SEC | Affinity | |
| 2020 | 11 | 7 | 3 | 4 | 1 | 0 | 7 | 0 | 0 | 0 | 0 | 22 |
| 2019 | 17 | 25 | 5 | 5 | 5 | 0 | 5 | 1 | 2 | 2 | 1 | 51 |
| 2018 | 17 | 17 | 8 | 14 | 5 | 6 | 9 | 0 | 1 | 2 | 0 | 62 |
| 2017 | 27 | 17 | 8 | 8 | 4 | 4 | 17 | 0 | 0 | 5 | 0 | 63 |
| 2016 | 18 | 25 | 18 | 3 | 7 | 7 | 7 | 0 | 0 | 0 | 2 | 69 |

IEC exploits strong interactions between opposite charges of a surface and an analyte. For the analysis of small ionic compounds, polymeric supports are often modified to carry permanent (strong cation- or anion-exchange) or variable (weak cation- or anion-exchange) charge that can be used to interact with and separate analytes with the opposite charge. Only a single product was released this year in this category, the Shodex SI-36 4D column. The column is described as a quaternary ammonium functional group attached to a polyvinyl alcohol particle of 3.5-µm, and is suggested for use as an anion suppressor device. Suppressor columns act to lower the conductivity of the eluent in an IEC system to enhance the signal from the conductivity of the analytes reaching the detector.

**Chiral Chromatography:** Table 4 provides information on columns introduced this year intended for chiral separations. As shown in Table 2, the number of chiral stationary phases (CSPs) is similar to those released in the previous year, but substantially less than the number reported in 2018. Similar to last year, all newly released chiral phases were based on polysaccharide stationary phases. In addition, like in 2019, all of the chiral phases released this past year were developed on FPPs, reversing the trend noted in 2018 of CSPs based on SPP architecture.

A company new to the article series, ColumnTek, introduced two new CSPs. Enantiocel A6 is an amylose tris(3-chloro-5-methylphenylcarbamate) phase built on 3-, 5-, and 10-µm FPP particles. The Enantiocel C9 offering is the identical ligand attached to a cellulose structure with the same particle size availability. Both phases are supplied from analytical to preparative dimensions, and are purported to provide unique selectivity, high column efficiency, and excellent peak shapes.

Phenomenex launched Lux i-Amylose-3 CSP. The column is based on amylose tris(3-chloro-5-methylphenylcarbamate) modification and is noted as having strong solvent stability, broad enantioselectivity, and robust reproducibility.

Regis Technologies released a new line of CSPs called Reflect Polysaccharide Chiral columns that was reported in the 2019 article. Adding to that line, the company launched the Reflect I-Cellulose J Polysaccharide Chiral Column this past year. The phase is described as a tris(4-methylbenzoate) ligand attached to a cellulose backbone. The immobilized stationary phase is noted to be a "J" type selector. The columns are available in analytical and preparative dimensions and particle sizes. The company notes that a unique proprietary phase coverage provides excellent peak shape and improved resolution versus leading chiral phases.

## Columns for Large Molecules

New columns introduced since Pittcon 2019 intended for large-molecule separations are provided in Table 5. The categorization was done loosely based on the pore size of the base particles employed. Columns using particles with pore sizes of 300 Å

**TABLE 3:** Small-molecule reversed-phase, ion-exchange (IEC), and hydrophilic interaction liquid chromatography (HILIC) columns

| Company | Product Name | Stationary Phases | Chromatographic Mode | Particle Sizes (µm) | Particle Type* | Dimensions (mm) | Comments** |
|---|---|---|---|---|---|---|---|
| Agilent Technologies, Inc. | Poroshell 120 SB-18 | C18 | Reversed-phase | 1.9, 4 | SPP | 50 × 2.1 to 250 × 4.6 | Proprietary bonding technique allows operation in low pH (1–8) and at high temperatures (80 °C) |
| | Poroshell 120 SB-Aq | Not disclosed | Reversed-phase | 1.9, 4 | SPP | 50 × 2.1 to 150 × 2.1 (1.9 µm) and 50 × 4.6 to 250 × 4.6 (4 µm) | Designed for the retention of polar molecules in reversed-phase |
| | Poroshell 120 HILIC-Z | Zwitter-ionic | HILIC/SFC | 1.9, 4 | SPP | 50 × 2.1 to 150 × 2.1 (1.9 µm) and 50 × 4.6 to 250 × 4.6 (4 µm) | Unique selectivity for HILIC separations, outstanding pH stability range (pH 2–12) |
| ChromaNik Technologies | SunArmor NH2 | Aminopropyl | HILIC/IEC | 3, 5 | FPP | 150 × 2.0 to 250 × 20 | Hydrophilic endcapping. Suggested for sugars and polar compounds. High stability and high retention. |
| | Sunniest PFP&C18 | Pentafluoro phenyl (PFP) and C18 | Reversed-phase | 5 | FPP | 50 × 2.0 to 250 × 20 | Recommended for polar compounds and isomers. Increased stability and hydrophobicity. Long column life. |
| | SunShell HILIC-S | Bare silica | HILIC | 2.6 | SPP | 50 × 2.1 to 150 × 2.1 | Suggested for polar compounds. Suitable for LC–MS. |
| | SunShell PFP&C18 | PFP and C18 | Reversed-phase | 2.6 | SPP | 30 × 2.1 to 150 × 4.6 | Recommended for polar compounds and isomers. Increased stability and hydrophobicity. Long column life. |
| Phenomenex, Inc. | Kinetex PS C18 | C18 with a positive surface charge | Reversed-phase | 2.6 | SPP | Not disclosed | Multi-interaction selectivity and improved peak shape for basic compounds. |
| Shodex | SI-36 4D | Quaternary ammonium | Ion-exchange (IEC) | 3.5 | Polymeric - polyvinyl alcohol | 4.0 × 150 | Anion suppressor column |
| Thermo Fisher Scientific | Accucore Biphenyl | Biphenyl | Reversed-phase | 2.6 | SPP | 10 × 2.1 to 100 × 2.1 | For use in the separation of critical pairs and isomers. |
| Waters Corporation | Atlantis Premier BEH C18 AX | C18 and alkylamine | Mixed mode reversed-phase/ anion-exchange | 1.7, 2.5, 5 | Hybrid | 30 × 2.1 to 250 × 4.6 | Reversed-phase separation of mixtures containing polar acids; alternative selectivity vs. conventional C18 columns; outstanding peak shapes for bases. |

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

**The number of companies that provided new product information dropped from a high of 27 in 2017 to just 11 in 2020.**

and greater are often constructed to enable large molecules to easily access the internal structure of the particles. The data in Table 2 show that only phases in the reversed-phase category of large-molecule separations were released this past year (included in this was one unique 300 Å HILIC phase). In previous reports, columns utilizing different modes of separation such as hydrophobic interaction chromatography (HIC), size-exclusion chromatography (SEC), IEC, and affinity have been noted. The overall yearly number of large molecule offerings is down substantially from a height of 22 reported in 2017.

Develosil released a new line of columns branded as FlexFire. The series includes bonded phases of C1, C4, C8, C18, C30, and HILIC based on fully porous silica particles ranging from 1.6 to 5 µm with 300 Å pores. The company states that its value and performance lies in its high-quality silica and 40 years of manufacturing experience. The new columns are suggested for oligonucleotide, insulin, and antibody separations. Develosil claims scalability from analytical to semipreparative dimensions, as well as high temperature and high pH functionality for its phases.

The other entry for this report is the YMC-Triart Bio C18. The column is based on a C18 polymerically bonded to a hybrid silica with a pore size of 300 Å. The new phase complements a 300 Å C4 surface chemistry already introduced. The company states that the column is designed to separate proteins, peptides, and oligonucleotides, and is particularly suited for biomolecules with a molecular weight >10,000 Da. The unique bonding and surface chemistry provides a wide usable pH range and high temperature durability.

**Accessories**

Accessories are important products that facilitate, and often enable,

**TABLE 4:** Chiral stationary phases

| Company | Product Name | Stationary Phases | Particle Sizes (µm) | Particle Type * | Dimensions (mm) | Comments** |
|---|---|---|---|---|---|---|
| ColumnTek | Enantiocel A6 | Amylose tris(3-chloro-5-methylphenylcarbamate) | 3, 5, 10 | FPP | Analytical and preparative | Unique enantioselectivity, high column efficiency, and excellent peak shape. |
| | Enantiocel C9 | Cellulose tris(3-chloro-5-methylphenylcarbamate) | 3, 5, 10 | FPP | Analytical and preparative | Unique enantioselectivity, high column efficiency, and excellent peak shape. |
| Regis Technologies | Reflec I-Cellulose J | Immobilized tris(4-methylbenzoate) | 3, 5, 10 | FPP | Analytical and preparative | Unique, immobilized "J" type selector, proprietary phase coverage provides excellent peak shape and improved resolution. |
| Phenomenex Inc. | Lux i-Amylose-3 | Amylose tris(3-chloro-5-methylphenylcarbamate) | 3, 5 | FPP | Analytical and preparative | Strong solvent stability, broad enantioselectivity, and robust reproducibility. |

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

**TABLE 5:** Liquid chromatography columns for large-molecule separations

| Company | Product Name | Stationary Phases | Chromatographic Mode | Particle Sizes (µm) | Particle Type* | Dimensions (mm) | Comments** |
|---|---|---|---|---|---|---|---|
| Develosil USA | FlexFire Series | C1, C4, C8, C18, C30, HILIC | Reversed-phase and HILIC | 1.6, 2.6, 5 | FPP | Not disclosed | Suggested for oligonucleotide, insulin and antibody separations. Scalability from analytical to semiprep, HPLC to UHPLC, high temperature, high pH functionality. |
| YMC Co., Ltd. | YMC-Triart Bio C18 | C18 | Reversed-phase | 1.9, 3, 5 | Hybrid, Polymerically bonded | Not disclosed | YMC-Triart Bio C18 is a novel, organic-inorganic hybrid silica dervatized with C18 and based on wide-pore particles. The phase is designed for separations of proteins, peptides, and oligonucleotides. |

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

liquid separations. Products introduced in the past year that fit this category are listed in Table 6. DWK Life Sciences released a line of user-filled gel filtration columns under the brand name Chromflex. The reusable standard or jacketed columns range in volumes from 12 mL to 2 L. The borosilicate glass barrel offers purity and strength with flangeless high-density polyethylene (HDPE) endfittings. Accessories such as flow adapters, bed support frits, valves, fittings, and tubing adapters are available, according to the company.

**The number of total new column phases launched dropped from an average of about 60 columns per year over the past four years to just 22 this year.**

Optimize Technologies launched several additions to its prefilter and connection lines of products. The EXP2 Stem Filter is described as a slim device ideally suited for the protection of expensive UHPLC columns, injectors, autosamplers, and mass spectrometer (MS) electrospray tips, without added extracolumn

**Liquid chromatography columns that were introduced this past year were largely product line extensions or alternatives to offerings existing on the market.**

effects. The device may be hand-tightened, which seals up to 8700 psi, or wrench-tightened, to withstand pressures up to 20,000 psi. The company's new EXP2 Filters provide added protection at back pressures up to 20,000 psi, with a redesign that offers more cost-efficient cartridges. In the category of connection devices, the company released EXP2 Ti-Lok and EXP2 Ti-Lok All-In-One (AIO) fittings. The first mentioned product is noted by the company as ideal for making HPLC and UHPLC connections that easily fit into tight spaces. The fittings feature built-in PEEK ferrules and threaded nuts precision machined from titanium. The device allows for repeated use, adjustment of the tubing to different port depths, and includes a removable, knurled torque driver for hand-tightening and loosening. When more room is available for making connections, the company recommends the all-in-one (AIO) format, where the driver is permanently fixed.

Finally, Waters Corporation released IonHance Difluoroacetic Acid mobile phase additive. The MS-grade mobile phase additive can be used for LC and LC–MS techniques. The product is reported to be purified to achieve low metal content and high-quality standards. The company notes that the product improves sensitivity, retentivity, and peak shapes, and is quality-control tested, to ensure low metal content and high purity. The material is available in approximately 1 mL quantities in chemically-resistant vials to maintain high levels of purity.

## Conclusions

Liquid chromatography columns that were introduced this past year were largely product line extensions or alternatives to offerings existing on the market. One trend of introducing alkyl phases with alternative selectivity that has been noted in the past several yearly reports continued in 2020 (C18/PFP mixed ligands and charge-modified C18 phases). The most striking learning from the effort this year, however, was the drastically reduced number of products released. The decline was observed in the number of companies that

**TABLE 6:** Accessories for liquid chromatogaphy

| Company | Product Name | Product Description | Comments* |
|---|---|---|---|
| DWK Life Sciences | Chromaflex | User-filled gel filtration columns | Reusable standard or jacketed user-packed gel filtration columns for manual or automation-compatible applications. |
| Optimize Technologies, Inc. | EXP 2 Stem Filter, EXP 2 Filter, EXP 2 Ti-Lok fittings and adapters, EXP 2 AIO fittings | Filter systems and connection devices for HPLC and UHPLC equipment | Slim architecture, hand-tight or wrench tight fittings, filters and adapters. |
| Waters Corporation | IonHance Difluoracetic Acid | MS-grade mobile phase additive | MS-grade mobile phase additive that can be used for LC and LC–MS techniques, purified to achieve low metal content and high-quality standards. |

*Comments supplied by vendors

**One trend of introducing alkyl phases with alternative selectivity that has been noted in the past several yearly reports continued in 2020.**

reported product launches, as well as in all categories generally used to parse and discuss interesting aspects of the new releases. It will be interesting to see if the trend continues. What it may take is a breakthrough technology or a new market trend to emerge. The development of SPP architecture, for example, drove LC product development for several years following its introduction.

**The most striking learning from the effort this year, however, was the drastically reduced number of products released.**

Another recent product driver was the migration of the pharmaceutical market towards biotechnology. This resulted in many new product lines based on larger pore particle technology, as well as the resurgence of techniques such as IEC and SEC. Is there another innovation or market driver on the horizon?


## Acknowledgements

Product reviews such as the present work would not be possible without the contributions and cooperation of the manufacturers that have responded to the *LCGC* survey. Your effort is greatly appreciated. Although *LCGC* has made every attempt to include every submission in the series of review articles, it is possible that some have been missed. If there have been omissions, or if you want to be sure to be included in the 2021 review series, please contact Laura Bush, Editorial Director, *LCGC Europe* at lbush@mjhlifesciences.com


## References


1) D.S. Bell, *LCGC Europe* **29**(4), 214–224 (2016).
2) D.S. Bell, *LCGC Europe*-**30**(4), 196–207 (2017).
3) D.S. Bell, *LCGC Europe* **31**(4), 202–211 (2018).
4) D.S. Bell, *LCGC Europe* **32**(4), 206–214 (2019).



**David S. Bell** is a director of research and development at Restek. He also serves on the Editorial Advisory Board for *LCGC* and is the Editor of "Column Watch." Over the past 20 years, he has worked directly in the chromatography industry, focusing his efforts on the design, development, and application of chromatographic stationary phases to advance gas chromatography, liquid chromatography, and related hyphenated techniques. His main objectives have been to create and promote novel separation technologies and to conduct research on molecular interactions that contribute to retention and selectivity in an array of chromatographic processes. His research results have been presented in symposia worldwide, and have resulted in numerous peer-reviewed journal and trade magazine articles. Direct correspondence to: LCGCedit@mmhgroup.com

# New HPLC Systems and Related Products Introduced in 2019–2020: A Brief Review

**Michael W. Dong,** Perspectives in Modern HPLC Editor

**This instalment describes newly introduced high performance liquid chromatography (HPLC), mass spectrometry (MS), chromatography data systems (CDS), and related products at Pittcon 2020 and the year prior, and summarizes significant features and user benefits.**

The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) is one of the world's largest conferences on laboratory science. The 71st Pittcon was held at McCormick Place in Chicago, USA, from 29 February to 5 March 2020, a week before the semi-shutdowns of Europe and North America from the spreading Covid-19. Previous Pittcons in Chicago were in 2014 and 2017—in rotation every three years as the most frequent venue for Pittcon. Chicago is an industrial, agricultural, financial, transportation, and communication centre, as well as the 3rd largest city in the US. The city has 2.7 million residents, and the Greater Chicago area boasts a population of 9.5 million. Chicago is home to 69 of the 1000 most prominent companies in the US, which include Boeing, Abbott Laboratories, Caterpillar, and Kraft Foods. Nevertheless, one potential issue for this location is the unpredictable weather of this midwestern city near the Great Lakes in early March. We were lucky this year with moderate temperatures and no precipitation, though the meeting attendance was reduced due to travel bans.

The technical programme remained strong this year with 200 plus technical sessions, plenary lectures, invited, contributed, or award symposia, workshops, posters, networking sessions, and approximately 90 short courses. The 3-day exposition, however, was visibly smaller, with the number of exhibitors from the United States dropping from 630+ in prior years to about 400. The number of international exhibitors was also dramatically reduced due to the travel bans from China and many European countries (1).

## New High Performance Liquid Chromatography (HPLC), Mass Spectrometry (MS), and Chromatography Data Systems (CDS) Product Introductions in 2019–2020

While introductions of new UHPLC (ultrahigh-pressure liquid chromatography) systems appear to be slowing down (1,2), manufacturers are turning their attention to high performance liquid chromatography (HPLC) system extensions, chromatography data systems (CDS), and mass spectrometers (MS) for LC–MS or direct liquid and solid sampling.

Table 1 lists new product introductions ordered alphabetically by the vendor at Pittcon 2020 or in the prior year, followed by descriptions and commentaries of each product, categorized as systems, modules, CDS, MS, software, or accessories.

### HPLC/UHPLC Systems and Line Extensions

New UHPLC systems introductions have slowed as manufacturers appeared to focus more on HPLC

**Manufacturers are turning their attention to HPLC system extensions, CDS, and mass spectrometers for LC–MS or direct liquid and solid sampling.**

**TABLE 1:** Summary of new HPLC product introductions at Pittcon 2020 or in the year prior

| Exhibitor (Vendor) | HPLC, MS, and CDS | Description |
|---|---|---|
| 908 Devices | Rebel | An integrated online MS analyzer for fresh and spent media analysis in the biopharma industry. |
| ACD/Labs | ACD/method selection suite | The new improvement includes the generation of robust methods *in silico* and capable of predicting the impact of method changes. |
| Agilent | 1290 Infinity II preparative open-bed sampler/collector | An extension to InfinityLab Purification Solutions to allow analytical-scale compound isolation to multiple grams of material. |
| | InfinityLab LC companion | A mobile LC user interface to allow for remote control, monitoring, signal plotting, and diagnostics of Agilent 1260 and 1290 Infinity II LC systems. |
| | InfinityLab LC–MSD iQ | A compact Mass Selective Detector (MSD) for HPLC with auto data acquisition and maintenance to track instrumental health. |
| | 6495C TQ LC–MS | Agilent's most sensitive triple-quad MS with easy maintenance to reduce downtime. |
| | 6546 LC/QTOF | This quadrupole-time-of-flight MS has an accelerated workflow with high resolution and wide dynamic range to deliver data for quick review. |
| Antec Scientific | Decade elite electrochemical detector (ECD) | An ECD is compatible with UHPLC and micro-LC for sensitive detection of neurotransmitters, carbohydrates, and pharmaceutical compounds. |
| ARC (Activated Research Company) | Solvere carbon selective detector | A universal detector using a flame ionization detector that converts carbon to methane for HPLC/UHPLC applications. |
| Clarity | DataApex 8.2 | A new version of web-based and 21 CFR part 11 compliant CDS used by many smaller instrument manufacturers. |
| Cornerstone Scientific | SOLVFil disposable filter degassers | A disposable, polypropylene device with 90-mm membranes for filtering HPLC mobile phases directly into solvent reservoirs. |
| EXUM Instruments | Massbox | The Massbox couples TOF-MS with solid sampling using laser ablation. |
| IDEX | Film degasser | A flat film Teflon AF-based membrane online degasser for removal of dissolved air from typical HPLC mobile phases. |
| ELGA LabWater | PURELAB Quest | The Quest is a water purification unit that produces Type I, II, and III water with a small footprint and low life cycle costs. |
| Knauer | Automated quality control of LC columns | A dedicated HPLC system capable of generating test certificates for LC and FPLC columns at a column manufacturing facility. |
| Optimized Technologies | EXP2 All-in-One TI-LOK | A hand-tight UHPLC fitting with integral ferrule capable of connections up to 18,000 psi. |
| Pickering Laboratories | ONYX PCX | An optimized postcolumn derivatization system with pulseless dual-syringe reagent pumps for many regulated analyses. |
| S-Matrix | Fusion QbD 9.9.0 | This new version offers features in PeakTracker with MS, an enhanced resolution response map, and support for forced degradation studies. |
| Sciex | Triple Q-TOF 6600+ system | A low-flow LC–MS system with ultra-fast scanning acquisition and high-resolution data. |

**TABLE 1:** Summary of new HPLC product introductions at Pittcon 2020 or in the year prior (continued)

| Exhibitor (Vendor) | HPLC, MS, and CDS | Description |
|---|---|---|
| Shimadzu | Nexera series LC-40 | The ultra-compact system incorporates innovative features of many auto functions, mobile phase/maintenance monitoring, and remote user control and service diagnostics. |
| | Anion suppressor ion chromatograph (IC) | A compact 3000-psi PEEK-based IC for quantitative analysis of anions. |
| | LCMS-9030 QTOF | A new high-resolution QTOF-MS with a 3-m internal flight path that supports many ionization sources. |
| | MALDImini-1 digital ion trap (DIT) MS. | The MALDImini-1 is a compact bench-top ion trap MS that allows the user to check MS results right next to the sample preparation area. |
| Thermo Scientific | Vanquish Core HPLC | A new 700-bar HPLC system for routine analysis with many features to enhance productivity, including fully automated solvent monitoring and integrated health checks. |
| | Vanquish UHPLC online 2D-LC | Now supports two independent workflows and several standardized 2D-LC configurations. |
| | An automated peptide mapping system | A smart digest automation system capable of reproducibility of ~3%. |
| | Orbitrap Exploris 480 MS | The Exploris combines technology refined over 20 years with advanced capabilities, intelligence-driven approaches, day-to-day reliability, and a compact footprint for rigorous, high-throughput protein identification, quantitation, and strutural characterization. |
| | Orbitrap Eclipse Tribrid MS | The Eclipse is the latest Orbitrap Tribrid mass spectrometer designed for a wide range of applications from small molecules to intact proteins for performing qualitative and quantitative analyses at low to high flow rates. |
| | Chromeleon 7.3 CDS | This new version of Chromeleon offers enhanced features for the laboratory and the information technology department. |
| Waters | Select series cyclic ion mobility separation (IMS) | This IMS combines innovative cyclic flight path design and a TOF-MS that supports resolution >100,000. |
| | SYNAPT XS | A tribrid (Q-IMS-TOF-MS) with extended pathlength and improved sensitivity and flexibility. |
| Wyatt Technology | DAWN, miniDAWN and microDAWN, multi-angle light scattering systems (MALS) | A new generation of MALS with updated optical, electrical, and mechanical components and enhanced interfacing for ease-of-use. |
| Young In Chromass (YL Instruments) | ChroZen UHPLC | A slimline UHPLC system with a binary pump (18,800 psi), equipped with UV–vis or PDA detector (2.4-µL flow cell), autosampler, and column oven (4 to 90 °C, three 15-cm columns. |

**There is no slowing down of new MS introductions this year as manufacturers continued to upgrade their product offerings.**

line extensions and customized systems for specific applications (1).

**Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector:** Agilent offers a new extension to InfinityLab Purification Solutions to allow analytical-scale compound isolation through preparative-scale purification from a few milligrams to multiple grams of material. This instrument combines analytical and preparative tasks in one instrument. It has the unique capability to sample from any position of the fraction collector and allow purification and fraction reanalysis to be combined and homogenized before reinjections.

**Knauer Automated Quality Control of LC Columns:** Knauer introduced a dedicated HPLC system capable of generating test certificates automatically for HPLC and FPLC (fast protein LC) columns at a column manufacturing facility.

**Shimadzu Nexera Series LC-40:** Shimadzu Nexera Series LC-40 employs concepts of AI and IoT (artificial intelligence and internet of things) to incorporate innovative features such as auto-start, auto-purge, auto-shutdown, flow pilot, mobile phase/maintenance monitoring, multiplexing with dual injectors, and remote user control and service diagnostics. The ultra-compact system consists of these modules: system controller SCL-40, CBM-40; absorbance detector SPD-40/SPD-40V and photodiode detector SPD-M40; solvent delivery unit LC-40 series (XS, XR, or X3, with pressure rating of 80, 105, and 130 MPa, respectively); autosampler SIL-40 series/plate changer; column oven CTO-40 series.

**Shimadzu Anion Suppressor Ion Chromatograph (IC):** Shimadzu's entry into the ion chromatography market is a compact and low-dispersion 3000-psi PEEK IC designed for quantitative analysis of anions. It is controlled by the LabSolutions CDS with auto-shutdown, data processing, and report generation.

**Thermo Scientific Vanquish Core HPLC System:** Thermo Scientific introduced the new 700-bar HPLC system to complement its family of Vanquish HPLCs (Flex, Duo, and Horizon with pressure ratings ranging from 1000 to 1500 bar). The Vanquish Core HPLC system targets routine analysis and quality control laboratories with a selection of quaternary, binary, dual-gradient, or isocratic pumps paired with a full line of detectors including UV–vis, diode array, fluorescence, charged aerosol, and MS detectors. The Vanquish Core HPLC system integrates SmartInject with additional intelligent features such as a fully automated solvent monitor and integrated automatic system health checks. Transitioning to this system from other HPLC systems is made simple with customizable injection programs, a fully tunable gradient delay volume, and enhanced method translation/transfer tools.

**Thermo Scientific Vanquish UHPLC Online 2D-LC:** This customizable 2D-LC system supports two independent workflows and several standardized 2D-LC configurations. It can also function as two independent HPLC systems without manual replumbing.

**Thermo Scientific Automated Peptide Mapping:** Thermo Scientific introduced an automated peptide mapping system capable of performing online digestion of a protein sample with reproducibility of ~3%.

**Young In Chromass ChroZen UHPLC System:** Young In Chromass (YL Instruments from Korea) introduced a slimline ChroZen UHPLC system with a binary pump (18,800 psi), equipped with UV–vis or photodiode array detector (2.4 µL flow cell), autosampler (injection volumes up to 10 µL), and column oven (4–90 °C, which accommodates up to three 15-cm columns). It can be controlled by Clarity's DataApex CDS.

### HPLC Modules

**Antec Scientific Decade Elite Electrochemical Detector (ECD):** Antec Scientific introduced an ECD compatible with UHPLC and micro-LC for the selective and sensitive detection of neurotransmitters, (poly) phenols, carbohydrates, and many pharmaceutical compounds. It is capable of a wide linear dynamic range of six orders of magnitude.

**ARC (Activated Research Company) Solvere Carbon Selective Detector:** The Solvere carbon selective detector is a universal detector using a flame ionization detector that converts

**The opportunities to meet with friends and colleagues, learn new technologies, and see new instrumentation on the exhibition floor are what inspire many analytical chemists to come back to Pittcon year after year.**

compounds with carbon atoms to methane for HPLC and UHPLC applications. It is capable of a linear dynamic range of 5 orders of magnitude from 10 ppm to 100% and allows an absolute calibration using a reference standard such as sucrose. The detector is compatible with most organic or volatile buffer mobile phases at flow rates of 0.3–0.5 mL/min. It is applicable to proteins, polymers, sugars, and other nonvolatile carbon-containing analytes.

**Pickering Laboratories ONYX PCX:** Pickering Laboratories introduced a new optimized postcolumn derivatization system with pulseless dual-syringe reagent pumps for targeted analysis of amino acids, glyphosate, carbamates, toxins, antibiotics, and cannabinoids.

**Wyatt DAWN, miniDAWN, and microDAWN Multi-Angle Light Scattering Detectors (MALS):** Wyatt Technology introduced a new generation of MALS detectors designed with updated optical, electrical, and mechanical components and enhanced interfacing for ease-of-use.

DAWN is the premier size-exclusion-MALS detector for absolute molecular mass and size measurement of 200 Da to 1 GDa with 18 angle measurements and temperature control. This detector is indispensable for use with gel permeation (GPC) and size-exclusion chromatography (SEC) to obtain reliable molecular mass distributions and information on molecular conformation, branching ratio, fragments, and aggregates.

miniDAWN and microDAWN have a 200 Da to 10 MDa range with three angles of measurements that operate at ambient only. microDAWN is equipped with a microflow cell and is compatible with most UHPLC systems and an optional microOptilab refractive index detector. It uses an ultra-stable laser with a sensitivity performance of 1.0 µg/mL of bovine serum albumin or 50 ng/mL 100 kDa polystyrene.

## Mass Spectrometers

The marketing landscape for mass spectrometers has been described elsewhere (1). There is no slowing down of new MS introductions this year as manufacturers continued to upgrade their product offerings in high-resolution, hybrid, and tribrid MS—such as time-of-flight (TOF), quadrupole-TOF (QTOF), and orbital trap. Also, there are more offerings in compact MS with unit resolution such as single-quadrupole (SQ), triple-quadrupole (TQ), and ion-mobility MS (IMS) (1,3). There are also newer startup companies focusing

**The four leading chromatography manufacturers dominate the global CDS market with their current CDS product offerings of workstations and client-server systems.**

on unique application systems such as analyzers for fermentation media or solid sampling.

**908 Devices Rebel:** The Rebel is an integrated online MS analyzer for fresh and spent media analysis in the biopharmaceutical industry. It is capable of a 7-min assay of 32 analytes (amino acids, bioamines, vitamins, dipeptides) with a 10 µL sample volume. The system is good manufacturing practices (GMP) compliant and can include automated sample preparation steps such as filtration and dilution.

**Agilent InfinityLab LC–MSD iQ:** One of three new MS systems introduced by Agilent Technologies in 2019–2020, the iQ is a compact single-quadrupole mass selective detector (MSD) for HPLC with auto data acquisition/reporting in auto acquire mode, which automatically establishes optimal MS parameters with automatic tuning. The instrument can also help with maintenance tasks by its ability to track instrument health. It can be controlled by OpenLab CDS or a simpler open-access software. It is designed for small molecule drug development and quality control, food/materials laboratories, academic, chemical, or food industries. The iQ has a mass range of 2 to 1450 *m/z*, ~1 pg sensitivity, a maximum scan speed of 10,400 Da/s, unit resolution, and mass accuracy of 0.13 Da.

**Agilent 6495C TQ LC/MS:** The 6495C triple quadrupole (TQ) is a third-generation design of Agilent's most sensitive TQ-MS with easy maintenance to reduce downtime. The system is ideally suited for peptide quantitation as well as applications that require part-per-trillion sensitivity. It has a mass range to 3000 *m/z* allowing flexibility to handle any MRM transition.

**Agilent 6546 LC/QTOF:** This QTOF-MS has an accelerated workflow with high mass resolution and wide dynamic range to deliver high-quality data for quick and simplified data review. With a mass resolution over 60,000 (for high masses) and over 30,000 (for low masses), sub-ppm mass accuracy, and isotope fidelity within 5%, it can provide quick answers for complex samples in metabolomics research, food safety, food authenticity, and environmental screening.

**EXUM Instruments Massbox:** EXUM introduced the new Massbox, which couples a TOF-MS with a mass range of 1000 to 14,000 with an innovative laser ablation ionization system for solid sampling.

**Shimadzu LCMS-9030 QTOF:** Shimadzu introduced a high-resolution QTOF-MS with a resolution up to 30,000 and a mass accuracy ~1 ppm. It has an internal 3-m flight path for better thermal stability and can maintain a mass accuracy of 1 ppm with calibration every 48 h. It is controlled by LabSolutions CDS and supports many ionization modes, including a dual source.

**Shimadzu MALDImini-1 Digital Ion Trap (DIT) MS:** The MALDImini-1 is a compact bench-top ion trap MS that allows the user to check MS results right next to the sample preparation area. The system's digital ion trap uses rectangular wave radio frequency to enable ion trapping up to 70,000 Da. Furthermore, the MS/MS and MS3 functionality of the DIT allows researchers to perform comprehensive structural analysis.

**Thermo Scientific Orbitrap Exploris 480:** The soundness of data is assured with high-resolution accurate-mass (HRAM) selectivity (resolution up to 480,000), high scan speed (up to 40 Hz), and best-in-class mass spectral quality, all within a compact footprint. The standard mass range for the instrument is *m/z* 40–6000 and up to *m/z* 8000 with the BioPharma option.

**Thermo Scientific Orbitrap Eclipse Tribrid:** This newest Orbitrap Tribrid MS platform includes advanced ion management technology (AIM+) with the new QR5 segmented quadrupole mass filter, real-time search, enhanced vacuum technology, optional proton transfer charge reduction (PTCR), and optional high mass range MSn (HMRn) mode. Collectively, these features make this instrument uniquely suited for accurate and high-throughput full-proteome quantitation, characterization of complex mixtures of protein or small-molecule drugs, and deciphering higher-order protein structures.

**Sciex Triple TOF 6600+ System:** This TQTOF system supports low-flow applications with ultra-fast scanning acquisition and high-resolution MS data.

**Waters Select Series Cyclic Ion Mobility Separation (IMS):** This IMS combines innovative design with a circular 98-cm flight path capable of multiple recycling, and a TOF-MS that supports resolution >100,000 for lipids, oligosaccharides, and other isobaric compounds.

**Waters SYNAPT XS:** The SYNAPT XS is an extended platform of the SYNAPT line of

**Pittcon 2020 will be remembered as the chemistry conference "squeezed" by the devastating coronavirus Covid-19.**

MS, which a tribrid (Q-IMS-TOF-MS) with an extended pathlength, improved sensitivity, and flexibility in supporting multiple ion sources and acquisition modes for solving challenging analytical problems.

### Chromatography Data System (CDS) and Software Products

The marketing landscape of the chromatography data system (CDS) has been described in a white paper published in L*CGC North America*'s December issue of 2019 (4). The four leading chromatography manufacturers dominate the global CDS market with their current CDS product offerings of workstations and client-server systems (Waters Empower 3 feature release 5, Thermo Fisher Scientific Chromeleon 7.3, Agilent OpenLab versions 2.4, and Shimadzu LabSolutions version 5.3).

**ACD/Method Selection Suite:** The new version for HPLC method development offers improvements such as the generation of robust methods *in silico* and the capability of predicting the impact of method changes. The software is based on principles of quality by design (QbD) with multivariate analysis, and can utilize databases of archived physicochemical properties.

**Agilent InfinityLab LC Companion:** This software platform is a mobile LC user interface that allows for remote control, monitoring, signal plotting, and diagnostics of Agilent 1260 and 1290 Infinity II LC systems. It resides on any mobile device (tablet or smartphone) using a compatible web browser.

**Clarity DataApex 8.2 CDS:** Clarity introduced a new version of its web-based and 21 CFR Part 11 compliant CDS used by many instrument manufacturers. The CDS offers an improved user-interface with many new instrument control drivers (up to 600), MS extension, compound search, and options for good laboratory practices (GLP) environment. It provides control and data handling for Advion MS and speciation analysis by PerkinElmer NexION ICP-MS (inductively coupled plasma).

**S-Matrix Fusion QbD 9.9.0:** S-Matrix introduced a new version of its popular HPLC method development software based on the principles of QbD and design of experiments (DoE) as well as new features of PeakTracker (with Waters QDa SQ-MS), an enhanced resolution response map (versus overlaid graph) for method robustness evaluation, and automation support for forced degradation studies.

**Thermo Fisher Scientific Chromeleon 7.3 CDS:** Thermo Scientific introduced Chromeleon 7.3 CDS, which offers enhanced features for both the laboratory (streamlined user interface with up to 33% higher performance, system health diagnostics for the new Vanquish Core HPLC, superior auditing/review/query/support, and improved e-workflow procedures) and the information technology (IT) department (scalable enterprise solutions for global and in-cloud deployment with improved data security and stability) (4).

### Other Accessories

**Cornerstone Scientific SOLVFil Disposable Filter Degassers:** A disposable, polypropylene device with 90-mm membranes for filtering HPLC mobile phases directly into solvent reservoirs.

**IDEX Film Degasser:** IDEX introduced a flat film Teflon AF-based membrane online degasser for removal of

dissolved air from HPLC mobile phases. It is adaptable to any HPLC system up to 10 mL/min and has either 2 or 4 channels.

**ELGA LabWater PURELAB Quest:** ELGA LabWater introduced PURELAB Quest, a diverse water purification unit that can produce Type I (HPLC), II (reagent preparation), or III (rinsing) water with a small footprint and low life cycle cost.

**Optimized Technologies EXP2 All-in-One TI-LOK:** The EXP2 is a hand-tight UHPLC fitting with an integral ferrule capable of connections up to 18,000 psi. It has a slim-fit nut and a wing nut for hand-tightening.

## Summary and Commentaries

Pittcon 2020 will be remembered as the chemistry conference "squeezed" by the devastating coronavirus Covid-19. Less than a week later, the World Health Organization declared the spreading virus a global pandemic, and President Trump announced a state of national emergency for the United States. The country was in a semi-shutdown with travel bans, school closings, and indefinite cancellation of all major public gatherings. The American Chemical Society (ACS) National Meeting in Philadelphia for late March was cancelled, and the Analytica Conference in Munich was postponed from March to October. Still, Pittcon did suffer significantly from many cancellations of exhibitors and conferees, resulting in lower attendance and fewer exhibitors.

One disturbing trend unrelated to the coronavirus was the skipping of Pittcon this year by two major manufacturers (Waters and Thermo Fisher Scientific). While Pittcon as a premier event for new product introductions has been diminishing in recent years from competing conferences and other communication channels, it remains the largest general analytical chemistry conference in North America with global draws for buyers and sellers alike. It is my opinion that skipping Pittcon is a drastic and unpopular move in the eyes of many conferees, who wish to compare new products on the exhibition floor. The opportunities to meet with friends and colleagues, learn new technologies, and see new instrumentation on the exhibition floor are what inspire many analytical chemists to come back to Pittcon year after year.

This instalment summarizes new HPLC and MS product introductions at Pittcon 2020 and in the prior year and describes the pertinent features of these products. Personally, this is my 19th consecutive year of giving Pittcon HPLC short courses, which had a record attendance of 44 this year. My busy schedule included attending symposia, networking sessions, and the exhibition, interspersed with many meetings and social events, such as the board/dinner meetings of *LCGC* and Chinese American Chromatography Association (CACA), the Separation Community Mixer of the Chrom Forum of Delaware Valley, and the Pittcon Party at the Museum of Science and Technology.

In this unusual time of severe disruptions of conferences and travels, we are all hopeful that the country survives the calamity and return to some normalcy for Pittcon 2021 in New Orleans.

## Acknowledgements

The author thanks the marketing staff of all manufacturers who provided timely responses to the *LCGC* questionnaires. The author is grateful for M. Farooq Wahah of U. Texas Arlington and Adrijana Torbovska of Farmahem DOOEL, Mengling Wong of Genentech, He Meng of Sanofi, and Michael Heidorn, Joel Stradtner, and Susanne Fabel of Thermo Fisher Scientific for providing useful editorial suggestions and comments. The content of this article stemmed from information and opinions gathered from the open literature, websites, and personal networking and observations at Pittcon 2020 and the year prior, and bears no relationship to those from *LCGC*, Pittcon, or any other organizations.

## References

1) M.W. Dong, *LCGC Europe* **32**(4), 196–203 (2019).
2) C.H. Arnaud, *Chem. & Eng. News* **94**(24), 29–35 (2016).
3) M.W. Dong, *HPLC and UHPLC for Practicing Scientists* (Wiley and Sons, Hoboken, New Jersey, USA, 2nd Ed., 2019).
4) R. Mazzarese, S.M. Bird, P.J. Zipfell, and M.W. Dong, *LCGC North Am.* **37**(12), 852–866 (2019).

**Michael W. Dong** is a principal of MWD Consulting, which provides training and consulting services in HPLC and UHPLC, method improvements, pharmaceutical analysis, and drug quality. He was formerly a Senior Scientist at Genentech, Research Fellow at Purdue Pharma, and Senior Staff Scientist at Applied Biosystems/PerkinElmer. He holds a Ph.D. in analytical chemistry from City University of New York, USA. He has more than 130 publications and a best-selling book in chromatography. He is an editorial advisory board member of *LCGC North America* and the Chinese American Chromatography Association. Direct correspondence to: LCGCedit@mmhgroup.com

# 2020 HTC Innovation Award

***LCGC Europe*** **sponsored the 2020 HTC Innovation Award to highlight innovation in separation science. The winner, Ryan Kelly, from Brigham Young University, in Utah, USA, has introduced an impressive array of innovative approaches to advance proteomics research using nano-liquid chromatography–mass spectrometry (nano-LC–MS), LC–MS, and two-dimensional (2D)-LC–MS, including the development and application of nanodroplet processing in one pot for trace samples (nanoPOTS). This platform, when combined with nano-LC–MS/MS, can identify more than 3000 protein groups in 10 cells, a greater level of proteome coverage than was previously possible for samples containing 5000 cells.**

Interview by Alasdair Matheson, Editor-in-Chief, *LCGC Europe*

**Q. Congratulations on winning the 2020 HTC Innovation Award, which was sponsored by *LCGC Europe*. When did you first become involved in hyphenated techniques?**

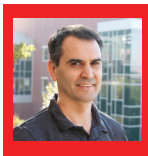
**A:** Thank you very much. It means a lot to see the work of our team being recognized by the separation science community. I first became involved in hyphenated techniques as a postdoc in Dick Smith's group at Pacific Northwest National Laboratory. I initially focused on electrospray emitter technology at the interface between liquid chromatography (LC) and mass spectrometry (MS), developing a chemical etching technique to shape fused-silica capillaries into fine tips rather than standard heating and pulling methods. These etched tips have no internal taper, making them far less prone to clogging than conventional nanospray emitters, and because they are fabricated through a purely chemical means, they are highly reproducible. Perhaps most importantly, they can operate stably at far lower flow rates than conventional nanospray emitters (extending to the picolitre-per-minute range), opening the door to much lower flow separations than were previously possible.

Another area of interest in my early involvement with hyphenated techniques was dividing the flow from an LC separation among an array of emitters. There are tradeoffs in miniaturizing LC–MS analyses for ultralow flow rates; compared to micro-LC–MS operated at flow rates greater than 1 µL/min, it can be difficult to achieve high run-to-run reproducibility, extreme measures need to be taken to minimize dead volumes, and sample loading and column regeneration times can become excessive. Yet, the sensitivity of an LC–MS analysis is dramatically improved at low flow rates as a result of increased ionization efficiencies, driving the need for column miniaturization.

Multielectrospray sources could potentially provide the best of both worlds, allowing for robust micro-LC separations, but then dividing the flow postcolumn among an array of nano-electrospray ionization (ESI) emitters, providing high ionization efficiencies. We were able to demonstrate an approximate 40-fold sensitivity increase for our multispray emitters in combination with a modified MS inlet. A remaining challenge is that the emitter arrays can generate more electrospray current than commercial mass spectrometers are designed to deal with, so this is an area that could definitely benefit from additional R&D.

**Q. What are the biggest challenges facing chromatographers in proteomics research from an analytical perspective?**

**A:** Well, I think that we and others are demonstrating significant sensitivity gains by continuing to miniaturize liquid chromatography with ultranarrow-bore columns that operate at low nanolitre-per-minute flow rates, but commercial LC systems are not well equipped for these columns. The autosamplers can't inject samples smaller than several microlitres and the extracolumn tubing can lead to huge delay volumes with long sample loading times and gradient delays. Also, there are limits to the flow rates that current systems can deliver, and flow splitting is typically necessary to get to these really low flow rates. Besides this, almost all commercial columns for proteomics have an internal diameter of 75 µm and



**Ryan Kelly** is an associate professor in the Department of Chemistry and Biochemistry at Brigham Young University (BYU), in Utah, USA. He received his Ph.D. in 2005 from BYU and then spent 13 years at Pacific Northwest National Laboratory, Washington (USA) before returning to BYU in 2018. A central theme of Kelly's research is the development of new technological solutions in separations, microfluidics, and MS for improved biochemical analyses, including nano-LC–MS-based proteomics.

operate at 300 nL/min, which is not ideal for proteomic analysis of small samples. This leads to relatively few groups who have expertise in customized column preparation and instrumentation being able to work in ultrasensitive nano-LC–MS. Of course, each one of these challenges is also an opportunity for LC instrument and column manufacturers, and as demand for these capabilities continues to grow, the market will respond with new solutions.

**Q. You recently published a paper on improved single-cell proteome coverage hyphenating narrow-bore packed nano-LC columns with ultrasensitive mass spectrometry (1). What is innovative about this research?**

**A:** When you are trying to extract as much chemical information as possible from a tiny biological sample, such as a single human cell, you have to look at every possible way to boost signal and reduce noise. One of the ways we are trying to do that is by continuing to miniaturize LC. Early on in this project we demonstrated substantial improvements in proteome coverage using custom-packed 30-µm internal diameter (i.d.) columns compared to conventional 75-µm-i.d. columns, and we have used the 30-µm columns ever since. In this most recent study, we wanted to see how much more could be gained by going to even smaller columns, and in this case we packed 20-µm-i.d. columns in-house and evaluated their performance in the context of single-cell proteomics. We found that dropping to 20 µm, which resulted in a flow rate reduction from ~50 nL/min to ~20 nL/min, allowed us to identify about 40% more proteins on average from single cells.

**Q. You recently gained recognition for developing nanoPOTS. What is nanoPOTS and what led you to develop this concept?**

**A:** A major challenge in the field of proteomics has been that large amounts of sample comprising thousands or millions of cells were required to achieve sufficient signal for a measurement. This means that we are typically measuring a mixture of different cell types in different states, so we were getting a bit of a blurry picture of the underlying cell biology. Overcoming this limitation has motivated efforts to extend proteomic analyses to the single-cell level.

We saw that the sensitivity of LC–MS analyses for proteomics continued to improve to the point that samples containing about the same protein content as single mammalian cells (around 0.1 ng or 0.2 ng of total protein) could be analyzed, but these demonstrations were typically performed using small aliquots of bulk-prepared samples and there was no way to prepare actual single cells. A biological sample has to undergo a number of processing steps such as cell lysis, protein extraction, reduction of disulphide bonds, digestion with trypsin, etc. to produce ready-to-analyze peptides. Conventional preparation methods are performed in volumes of tens of microlitres, and there are multiple sample transfer steps.

If you take a single cell through a conventional sample preparation workflow, you're probably diluting the cell's proteins by a factor of about $10^8$ (from ~1 pL to ~100 µL). Such dilution results in samples being exposed to a lot of adsorptive surfaces, and the reaction kinetics for processing steps such as trypsin digestion become highly unfavourable at these reduced concentrations.

NanoPOTS, which stands for Nanodroplet Processing in One pot for Trace Samples, essentially preserves the standard form factor used for proteomic sample preparation, but miniaturizes volumes by several orders of magnitude; we are still pipetting into a well plate, but the pipettor is now a robotic nanolitre liquid handler, and the well plate is now a micropatterned glass slide. This allows trace proteomic samples such as single cells to be effectively prepared in a volume of just ~200 nL versus ~100 µL, and the sample is exposed to less than 1 $mm^2$ of surface, so sample recovery is greatly improved. In our initial study (2), we were able to use the nanoPOTS platform in combination with highly sensitive nano-LC–MS/MS to identify more than 3000 protein groups from as few as 10 cells. This is a greater level of proteome coverage than was previously achieved for samples containing 5000 cells, so this is an "orders-of-magnitude" improvement.

**Q. What benefits does this offer the analyst?**

**A:** NanoPOTS enables us to extract more chemical information from smaller samples than was previously possible. As analytical chemists, we are often focused on improving the separation or the mass spectrometry, but sometimes the battle is won or lost before we even get to the analytical platform. If most of the sample is being lost during the preanalytical steps, which was the case for small proteomic samples, that's where we need to focus and that's what drove the development of nanoPOTS. Another benefit is that nanoPOTS preserves the basic form factor of a pipettor interfacing with a well plate. There are a lot of other ways that surface exposure and dilution could be minimized, but, because nanoPOTS is an open platform, it is compatible with widely used sample isolation techniques including fluorescence-activated cell sorting and laser microdissection. This gives us

**Ryan Kelly: 2020 HTC Innovation Award Winner**

**Research Interests**

Professor Ryan Kelly develops innovative hyphenated separation techniques including ultrasensitive nano-liquid chromatography mass spectrometry (nano-LC–MS) using narrow-bore (20 µm) separation columns combined with mass spectrometry (MS) for bioanalysis, comprehensive two-dimensional liquid chromatography–mass spectrometry (2D-LC–MS), and liquid chromatography ion mobility spectrometry (LC–IM-MS) or proteomic analysis of trace samples, including single cells. He also develops microfluidic sample preparation strategies for these trace samples.

**Winning Credentials**

Ryan Kelly was awarded the **2020 HTC Innovation Award** because of his outstanding contributions to the field of microcolumn separations involving hyphenation. A central theme of Kelly's research is the development of new technological solutions in separations, microfluidics, and mass spectrometry (MS) for improved biochemical analyses. To this end, he has developed novel techniques based on one-dimensional (1D)- and 2D-LC, hyphenated LC-ion mobility spectrometry-MS, and novel preconcentration/injection strategies for capillary LC–MS and electrophoresis MS. He is the author of more than 90 publications with more than 3500 total citations and inventor of 11 issued and pending patents, several of which have been licensed and commercialized.

Kelly has an extensive background in microfluidics that allows him to address preanalytical bottlenecks to overcome the inefficiencies associated with sample isolation and processing of biological samples for proteomic analysis. His research group recently developed nanodroplet processing in one pot for trace samples (nanoPOTS) a breakthrough microfluidic platform based on robotic nanopipetting from microfabricated well plates, which greatly reduces sample losses and enhances reaction kinetics. When combined with ultrasensitive LC–MS, nanoPOTS has enabled more than1000 proteins to be quantified from single mammalian cells. This is the first example of in-depth proteome profiling from such trace samples, and it has enormous implications for advancing biomedical research. A summary of Kelly's recent achievements include:

- NanoPOTS in combination with 30-µm-i.d. nano-LC–MS enables highly quantitative profiling of more than 3000 proteins from as few as ten mammalian cells. This level of proteome coverage has not been achieved previously for fewer than 10,000 cells, indicating a three-order-of-magnitude advance. Approximately 700 proteins were subsequently identified from single cells (3), which has since been increased to more than1000 proteins/cells by further miniaturization of LC columns to a 20-µm i.d.
- He developed nanowell-mediated 2D-LC, where high-pH reversed-phase nano-LC separations are fractionated into microfabricated nanowells for subsequent low-pH nano-LC. This method was used to identify more than 6000 proteins from low-nanogram biological samples (4). A similar, fully automated platform was subsequently developed for 2D-LC of phosphopeptides (5).
- In another hyphenated separation technique, he coupled nano-LC with high-resolution ion mobility spectrometry to achieve a total peak capacity of approximately 3600 in 2 h (6).
- Kelly focuses on applying his technologies to biological systems to maximize societal impact. For example, the nanoPOTS/nano-LC platform has been applied to a wide variety of biological tissues and problems, including comparing individual pancreatic islets of type 1 diabetic and non-diabetic donors, brain tissues, hair cells involved in auditory signal transduction, lung, liver, circulating tumour cells, and plants.

**How Does This Research Benefit Society?**

Healthy and diseased tissues contain diverse cell types and subtypes with distinct functions, and understanding the heterogeneity of cellular sub-populations at the single-cell level is of great importance for biomedical research. Kelly's ultrasensitive separations and sample processing enable biological systems to be probed with unprecedented molecular depth and granularity, extending to the single cell level. The biological insights that these analytical tools provide are expected to lead to advances in understanding and therapies for cancer, diabetes, neurological disorders, and other pathologies.

options for targeting specific cells or tissues of interest that, for example, a closed microfluidic platform doesn't have.

**Q. Can you describe an application that demonstrates the inventiveness of nanoPOTS in practice?**

**A:** One example of interest is proteome profiling of circulating tumour cells (CTCs), which originate at a solid tumour but make their way into the blood stream. These cells can potentially inform on disease progression—how an individual is responding to a therapy and when resistance to therapy is developing—all without requiring invasive and risky biopsies. So this is a high value target that could have a real impact on treatment, but CTCs are very rare and only a small number are isolated from a blood draw. Conventional proteomics approaches lack the sensitivity to obtain an in-depth profile of protein expression from such small samples, but nanoPOTS sample preparation in combination with highly sensitive LC–MS offers hope that we can begin to extract protein information from these previously inaccessible samples. In fact, in collaboration with George Thomas at Oregon Health and Science University, we have begun to develop a workflow that begins with enriching CTCs from whole blood and then profiling the cells for biomarkers and an initial demonstration was recently published (7).

This is just one example, and really any sample that was too small for proteome profiling using conventional techniques, even single mammalian cells, can now be addressed.

**Q. Where else has nanoPOTS been beneficial?**

**A:** Another area that we are very interested in is mapping protein expression across tissues. The amount of biochemical information obtained from a thin tissue section on a microscope slide like those studied by pathologists is generally limited to staining for a few molecules of interest. We used laser microdissection to convert a thin section of mouse uterine tissue into tiny pixels just 100 µm × 100 µm across and then subjected each of these pixels to the nanoPOTS workflow. This allowed us to reconstruct an image in which the protein abundance of each of the 2000 proteins (approximately) that were detected were overlaid over an optical image of the same tissue to determine which proteins were up and downregulated across different regions of the tissue. Proteins have been very difficult to detect using other mass spectrometry imaging approaches, and so nanoPOTS enables us to identify more than 100 times more protein groups from each pixel than was previously possible. We think this will provide biomedical researchers with an important tool to determine the contribution of the microenvironment to give rise to a given phenotype, and to get a better understanding of what is happening in different tissues.

**Q. You have also published on multidimensional separations combining nano-LC with structures for lossless ion manipulation ion-mobility mass spectrometry (SLIM IM-MS) (6). What is novel about SLIM IM-MS?**

**A:** Yes, while we were focused on developing nanoPOTS, Dick Smith, Yehia Ibrahim, and colleagues at Pacific Northwest National Laboratory have been developing structures for lossless ion manipulation (SLIM). SLIM devices comprise ion optical components that are typically constructed using printed circuit boards, and these can provide extended pathlengths for ion-mobility (IM) spectrometry separations. SLIM IM makes it possible to achieve high peak capacities akin to those of LC separations, but with a total separation time of ~1 s or less. We wanted to couple LC with SLIM IM to achieve ultrahigh peak capacity separations for trace samples using no more time than a typical LC separation requires. To interface LC with SLIM IM, we used our nanoPOTS robot to fractionate a nano-LC separation into different nanowells in 1-min intervals. The fractions were then dried for storage. To inject each sample, the modified nanoPOTS robot dispensed solvent into each well to reconstitute the sample, then pick up that sample and electrospray it into the SLIM IM-MS platform. Using this multidimensional separation platform, we were able to achieve peak capacities of more than 3600 in about 90 min, which is an order of magnitude increase in peak capacity per unit time compared to LC alone.

**Q. What benefits does nano-LC-SLIM IM-MS offer the analyst?**

**A:** It is difficult to achieve a peak capacity more than 500 for a one-dimensional (1D)-LC separation, even when extended (more than10 h) gradients are employed. Nano-LC–SLIM IM-MS offers ultrahigh peak capacity separations of greater than 3600 in a short timeframe of less than 90 min by coupling two orthogonal techniques. We are in the early days of coupling LC separations with high peak capacity IM-MS such as SLIM provides, but I think this is a very promising avenue of research for analyzing difficult-to-separate compounds and for comprehensive analysis of complex mixtures.

**Q. You have developed picoelectrospray ionization mass spectrometry using narrowbore chemically etched emitters. What was the rationale behind this research?**

**A:** Since the initial development of ESI-MS, it had been observed that

# We will solve your multimedia marketing challenges

***LCGC*™ *Europe*** is the leading multimedia platform for the chromatography market, enhancing productivity, efficiency, and the overall value of separation science globally. With our commitment to editorial excellence, we have pioneered innovation across our broad portfolio of digital and print platforms:

- Digital advertising
- Print advertising
- Custom content creation
- Videos
- Targeted Emails
- Surveys
- Lead Nurturing
- Webcasts
- eBooks
- Podcasts
- Whitepapers
- Infographics
- eNewsletters
- CHROMacademy

## To discuss solutions for your marketing strategy, contact:


Oliver Waters
+44 (0) 794 619 8269
owaters@mmhgroup.com

Liz McLean
+44 (0) 797 023 2975
lmclean@mmhgroup.com


**Follow and tag us on social media**

THE ESSENTIAL MULTIMEDIA RESOURCE FOR SEPARATION SCIENCE IN PRACTICE

LCGC europe
AN MJH life sciences BRAND

a greater portion of solution-phase molecules could be converted to gas-phase ions by operating the electrospray at reduced flow rates, that is, ionization efficiency increases at low flow rates as a result of the formation of smaller, more readily desolvated charged droplets. This led to the development of nano-ESI, where the electrospray emitter is operated at flow rates of less than 20 nanolitres to several hundred nanolitres per minute. Indeed, the vast majority of proteomics separations take place at 300 nL/min to realize some of the benefits of nano-ESI. But we analytical chemists are greedy, and we reasoned that if nano-ESI was good, wouldn't picoESI, where we operate at less than 1 nL/min, be better?

In other words, we had some unanswered questions about the limits of improved ionization efficiency and reduced ionization suppression at low flow rates, but there were no electrospray emitters that could operate reproducibly at such low flow rates.

I had previously developed a chemical-etching technique to shape fused-silica capillaries into very sharp emitters that are ideal for nano-ESI (8). In this study, we applied the same technique to 2-µm-i.d. capillaries and evaluated their spray stability at ultralow flow rates. We were able to spray stably at flow rates as low as 0.4 nL/min, which enabled us to explore the analytical performance of ESI in uncharted territory. We found that ionization efficiency continued to improve in the picoelectrospray range, ionization suppression was not much different than in the nanospray regime, and we observed greatest signal-to-noise ratios at about 1 nL/min.

**Q. Where has this approach been adopted?**

**A:** Until recently, this was more of an academic curiosity rather than of practical utility as it was exceedingly difficult to perform LC separations at approximately 1 nL/min to take advantage of the sensitivity gains that our study indicated should be achieved at such low flow rates. However, in a new study led by Shaorong Liu from the University of Oklahoma with Ying Zhu at Pacific Northwest National Laboratory (currently in review), it has been shown for the first time that picoLC–MS using 2-µm-i.d. open-tubular LC columns can identify ~1000 proteins from just a few pg of protein equivalent to 10% of a typical single cell. While this is not quite ready for prime time, this is definitely an area worth continuing to explore.

**Q. What benefits does nanowell-mediated two-dimensional liquid chromatography (2D-LC) provide in proteomics research (4)?**

**A:** Higher peak capacity separations can greatly increase proteome coverage by reducing the number of peptides entering the mass spectrometer at any given time, reducing ionization suppression, and, with typically longer analysis times, providing more time for MS/MS sequencing. Two-dimensional separations are often employed to dig deeper into the proteome, with the first dimension being distributed into fractions that are then analyzed by standard low-pH reversed-phase nano-LC. For 2D-LC employing high pH followed by low pH reversed-phase LC, a relatively high flow high-pH separation is employed and samples are fractionated into multiwell plates. The fractions are dried and then reconstituted in tens of microlitres of aqueous solution for injection into a low-pH reversed-phase LC–MS/MS analysis platform. The problem we run into is that we are again exposing our samples to a lot of surface area with potential for losses in between these two separation dimensions and so large samples are required up front so that we still have sufficient material to measure after the peptide losses and incomplete injection. We thought that just as microfabricated nanowells could replace well plates for reduced sample losses during sample preparation in the nanoPOTS workflow, the same nanowells could also reduce losses in between separation dimensions. So we went ahead and used a nano-LC separation for the first dimension, fractionated into nanowells, and then reconstituted the samples for injection into our custom ultralow-flow nano-LC. From samples comprising just a few hundred cells, we were able to identify around 6000 proteins, which was unprecedented from such small samples. We are currently miniaturizing this to extend 2D-LC to even smaller samples.

**Q. What are you currently working on at the moment?**

**A:** We are definitely still interested in increasing proteome coverage for single cells and increasing the throughput with which these can be measured. We are very encouraged that there appears to be no end in sight to the achievable gains and have a couple of significant improvements close to submission for publication. At the same time, we are working to automate sample injection from nanoPOTS chips to nano-LC, which should take a lot of the skill and labour intensiveness from the process (and allow my group members to sleep at night rather than tending to the instruments around the clock). We are also working hard to make the nanoPOTS platform accessible to a wide range of researchers interested in single-cell and nanoscale proteomics.

Currently, the robotic platforms that we use are quite expensive and require some expertise to fabricate and use. We are now adapting some low-cost automated liquid handling systems for nanopipetting, which will hopefully broaden access. Finally, it is very important that we not only develop new technologies but that we also put them into practice, and we enjoy working with a number of biomedical researchers to apply our systems to new sample types and help to uncover new biomedical insights.

**Q. Can you name one scientist who represents innovation in separation science?**

**A:** I would like to recognize Yufeng Shen, who I think is a bit of an unsung hero in separation science. Yufeng received his Ph.D. at Brigham Young University under the direction of Milton Lee after publishing something like 35(!) papers (~25 first author) before graduating. He then moved to the Biological Separations and Mass Spectrometry group at Pacific Northwest National Laboratory where he was responsible for many innovations in ultrahigh-pressure liquid chromatography (UHPLC), ultrafast separations, and ultrahigh peak capacity separations that made modern proteomics possible. Much of this groundbreaking work was done in the early 2000s, but I think a number of the "world records" that he set in separation science still stand today, and his work certainly inspires a lot of what we do.

## References


1) Y. Cong, Y. Liang, K. Motamedchaboki, R. Huguet, T. Truong, R. Zhao, Y. Shen, D. Lopez-Ferrer, Y. Zhu, and R.T. Kelly, *Anal. Chem.* **92**, 2665–2671 (2020).
2) Y. Zhu, P.D. Piehowski, R. Zhao, J. Chen, Y. Shen, R.J. Moore, A.K. Shukla, V. Petyuk, M. Campbell-Thompson, C.E. Mathews, R.D. Smith, W.-J. Qian, and R.T. Kelly, *Nat. Commun.* **9**, 882 (2018).
3) Y. Zhu, G. Clair, W.B. Chrisler, Y. Shen, R. Zhao, A.K. Shukla, R.J. Moore, R.S. Misra, G.S. Pryhuber, R.D. Smith, C. Ansong, and R.T. Kelly, *Angewandte Chemie* **57**, 12370–12374 (2018).
4) M. Dou, Y. Zhu, A. Liyu, Y. Liang, J. Chen, P.D. Piehowski, K. Xu, R. Zhao, R.J. Moore, M.A. Atkinson, C.E. Mathews, W.-J. Qian, and R.T. Kelly, *Chem. Sci.* **9**, 6944–6951 (2018).
5) M. Dou, C.-F. Tsai, P.D. Piehowski, Y. Wang, T.L. Fillmore, R. Zhao, R.J. Moore, P. Zhang, W.-J. Qian, R.D. Smith, T. Liu, R.T. Kelly, T. Shi, and Y. Zhu, *Anal. Chem.* **91**, 9707–9715 (2019).
6) M. Dou, C.D. Chouinard, Y. Zhu, G. Nagy, A.V. Liyu, Y.M. Ibrahim, R.D. Smith, and R.T. Kelly, *Anal. Bioanal. Chem.* **411**, 5363–5372 (2019).
7) Y. Zhu, J.Podolak, R. Zhao, A.K. Shukla, R.J. Moore, G.V. Thomas, and R.T. Kelly, *Anal. Chem.* **90**, 11756–11759 (2018).
8) R.T. Kelly, J.S. Page, Q. Luo, R.J. Moore, D.J. Orton, K. Tang, and R.D. Smith, *Anal. Chem.* **78**, 7796–7801 (2006).

## HILIC Columns

Hilicon offers a broad range of HILIC products to separate polar compounds. Three column chemistries in UHPLC and HPLC, iHILIC-Fusion, iHILIC-Fusion(+), and iHILIC-Fusion(P), provide customized and complementary selectivity, excellent durability, and very low column bleeding, according to the company. The columns are suitable for the analysis of polar compounds in "omics" research, food and beverage analysis, pharma discovery, and clinical diagnostics.

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

## Positive Pressure Manifold

UCT's 48-position positive pressure manifold features offers rugged performance but now has a streamlined, compact footprint, according to the company.

**www.unitedchem.com**
**UCT, Inc., Bristol, Pennsylvania, USA.**


## Pulsation Pump

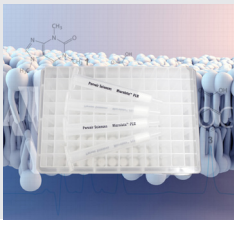
At low flow rates of 0.5 mL/min or lower check valves become a variable factor for flow volume and pulsation. The GL Sciences' MP-22 No-Pulsation pump does not use any check valves, instead it uses a fast switching valve. Together with the linear-driven pistons, this results in stable flows and pressure, even with low flows, according to the company.

**www.glsciences.eu**
**GL Sciences B.V., Eindhoven, The Netherlands.**

## Phospholipid Removal Microplate

Phospholipid-based matrix effects are a major source of variability and inaccuracy in MS and HPLC/UHPLC protocols. Porvair Sciences has introduced Microlute PLR for these applications. According to the company, this 96-well microplate provides removal of more than 99% of phospholipids and proteins with excellent reproducibility from plasma and serum samples while maintaining maximum recovery of target analytes.

**www.microplates.com/microlute-plr**
**Porvair Sciences Ltd, Wrexham, UK.**



## Gas Generator

The NG-Castore XL iQ generator uses a silent technology (from 48 dB only) combining a scroll compressor and an inverter for an on-demand supply of pure nitrogen to the applications. According to the company, it is designed for all LC–MS and evaporation applications on the market because of requirements in terms of gas, flow, and pressure.

**www.lni-swissgas.eu**
**LNI Swissgas Srl, Milan, Italy.**

## Adsorbents

MP Bio's Silica and Alumina Woelm Adsorbents can be used for a range of laboratory and industrial-scale chromatography applications as well as for filtration, MPLC/HPLC, TLC, DCC, low-pressure chromatography, dioxin analysis, industrial-scale separation, selective filtration, and drug production. A range of particle sizes and activities are available.

**www.mpbio.com**
**MP Biomedicals, Eschwege, Germany.**

## Liquid Chromatography

Shimadzu's Nexera Ultra high-performance liquid chromatograph series incorporates artificial intelligence because analytical intelligence allows systems to detect and resolve issues automatically. According to the company, the series makes laboratory management simple by integrating the internet of things and device networking, enabling users to easily review instrument status, optimize resource allocation, and achieve higher throughput.

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

## MALS Detector

The DAWN is a multi-angle static light scattering (MALS) detector for absolute characterization of the molar mass and size of macromolecules and nanoparticles in solution. DAWN offers high sensitivity, a wide range of molecular weights, sizes, and concentrations, and a large selection of configurations and optional modules for enhanced capabilities.

**www.wyatt.com/nextgen**
**Wyatt Technology, Santa Barbara, California, USA.**

## Electrochemical Flow Cell

A new electrochemical flow cell (µ-PrepCell SS) is available for efficient and robust reduction of S-S bonds in proteins, such as mAbs and other biopharmaceuticals. The cell can be used online with LC–MS or HDXMS and is compatible with any HPLC–MS system, according to the company. Increased sequence coverage and excellent HDXMS data are reportedly obtained compared to chemical (DTT, TCEP) reduction.

**www.AntecScientific.com**
**Antec Scientific, Zoeterwoude, The Netherlands.**

## Column Selection App

Columns are the heart of any GPC/SEC system. However, it can be a challenge to find the best matching column from the wide range of options. PSS has therefore developed the Column Selection App to support users. The app offers the following choices: how to replace existing columns, column recommendations, *USP* methods/*EP* methods, and application searches.

**www.psscolumnselector.com**
**PSS GmbH, Mainz, Germany.**


## FID Gas Station

The VICI FID gas station combines the reliability of the VICI DBS hydrogen and zero-air generators into one compact and convenient package, according to the company. Available in high- and ultrahigh purity for all GC detector and carrier gas applications. The generator is available in two styles: flat for placement under a GC, or the Tower. Available in $H_2$ flow ranges up to 1 L/min and 10.5 bar.

**www.vicidbs.com**
**VICI AG International, Schenkon, Switzerland.**

## IEX Columns

If reproducibility in analyzing proteins and charge variants is very important, YMC's BioPro IEX columns reportedly offer excellent lot-to-lot reproducibility. These columns also provide high loadability together with high resolution, according to the company. For fast analysis results, there is a non-porous version.

**https://ymc.de/ymc-biopro-iex-143.html**
**YMC Europe GmbH, Dinslaken, Germany.**

## LC Method Migration

Pro EZLC method translation software simplifies LC method downscaling by taking manual calculations and time-consuming laboratory work out of the way, according to the company. Input current column dimensions and method conditions, then specify the dimensions of the new column to try. The software will generate new injection volumes and isocratic or gradient programme conditions.

**www.restek.com/ezlc**
**Restek Corporation, Bellefonte, USA.**


## Dynamic Headspace System

The Dynamic Headspace System (DHS 3.5) holds up to four times more sorbent, resulting in improved recovery, accuracy, and limits of quantitation, according to the company. Standard 3.5" tubes can be used for trapping. The DHS 3.5, Thermal Desorber TD 3.5+, and MultiPurpose Sampler MPS can process 120 samples in one run.The optional DHS large holds 250, 500, and 1000 mL containers.

**www.gerstel.com**
**Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany.**

## Micro-Pillar Array Columns

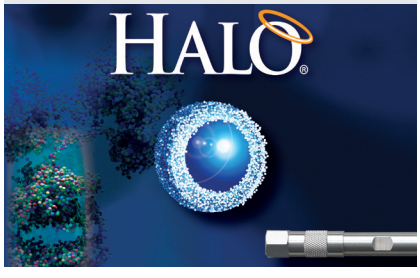
PharmaFluidics has introduced the µPAC capLC column for highly sensitive LC–MS analysis with shorter run times for higher throughput, according to the company. Taking advantage of the micromachined pillar structure, µPAC capLC columns reportedly offer high reproducibility and robustness. The flow rate range of 1–15 µL/min allows for applications such as those in the "omic" fields.

**www.pharmafluidics.com**
**PharmaFluidics, Ghent, Belgium.**

## LC Columns

With three silica-bonded phases on a large pore size of 1000 Å utilizing fused-core technology, the benefits of total pore access and analysis speed can be achieved for large biomolecule reversed-phase HPLC separations, according to the company. HALO HPLC and UHPLC columns reportedly deliver fast separations with high resolution and narrow peak widths.

**www.fused-core.com**
**Advanced Materials Technology, Delaware, USA.**




## SEC-MALS Detector

The LenS$_3$ MALS Detector for GPC, HPLC, and UHPLC is based on an approach to light scattering technology. The proprietary design increases *S/N* and extends the range of size determination (Rg) down to a few nanometres. According to the company, it is a sensitive detector for the characterization of polymers, proteins, or antibodies by SEC-MALS.

**www.tosohbioscience.de**
**Tosoh Bioscience GmbH, Griesheim, Germany.**


## Hydrogen Generator

Designed for GC–FID, Precision SL is a small and easy-to-use laboratory-grade hydrogen generator, according to the company, producing hydrogen gas at the touch of a button. Available in both 100 cc and 200 cc, the hydrogen generator is reportedly simple to use and maintain with advanced technology, to offer a safer solution for flame detectors.

**www.peakscientific.com/precisionSL**
**Peak Scientific, Scotland, UK.**

## GC Columns

Polysiloxane-based GC columns of earlier generations tend to column bleed at increased temperature levels. In order to overcome column bleed, Macherey-Nagel offers silarylene-stabilized polysiloxane columns such as Optima 5 MS Accent, 1301 MS, 1701 MS, 35 MS, and 17 MS. They are commonly used in the fields of food analysis, drug abuse, and environmental analysis.

**www.mn-net.com**
**Macherey-Nagel GmbH & Co. KG, Düren, Germany.**

## Pocket-Sized Detectors

The new range of Runge Mikron detectors recently introduced by Biotech AB unites many attractive features in a tiny package, according to the company. Different modules can be combined to measure absorption, fluorescence, or conductivity in a variety of fluidic systems, including liquid chromatography.

**www.biotechfluidics.com**
**Biotech AB, Onsala, Sweden.**

## CRM Mixtures

Cayman reportedly offers a suite of ISO 17034-produced multi-component CRM mixtures designed and engineered to offer confidence in analytical data. According to the company, pre-made CRM mixtures deliver simplicity of use, provide accurate and precise data, and save time and consumable costs in the preparation of standard curves.

**www.caymanchem.com/phytomix**
**Cayman Chemical, Ann Arbor, Michigan, USA.**

## Sampling Tubes

Markes' industry-standard-sized thermal desorption tubes are manufactured to the highest quality to deliver optimum results, according to the company. The complete range of tube materials and sorbent packings offer flexibility, making them suitable for VOC and SVOC analysis for all TD applications, including environmental air monitoring, fragrance analysis, and breath monitoring.

**http://chem.markes.com/sampling-tubes**
**Markes International Ltd., Llantrisant, UK.**

## Purge Valve

The purge valve complete with an PTFE frit is an appropriate option to use with Agilent HPLC instruments. This purge valve has been engineered and tested to be equivalent to the corresponding OEM product. The valve uses a replaceable gold seal and a PTFE frit to protect the HPLC system.

**www.sciencix.com**
**Sciencix, Burnsville, Minnesota, USA.**

## Chromatography Accessories

Action Europe offers bottles, closures, caps, seals, and syringe filters. Crimping and screwing bottles are offered from 2 mL to 1 L, as are closures and seals for all types of bottles and caps. Caps, aluminium standard caps, flip-off caps, flip tear-off caps, flip tear-up caps, and magnetic caps are available. Electric crimping machines are also available. Samples and a catalogue are available from the company.

**www.sertir.fr**
**Action Europe, Sausheim, France.**

# Tailored Analysis of Phospholipid Classes Using iHILIC-Fusion(+) as First Dimension for Online Two-Dimensional HILIC–Reversed-Phase LC–MS

**Patrick O. Helmer[1], Carina M. Wienken[1], Wen Jiang[2], and Heiko Hayen[1],** [1]Institute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany, [2]HILICON AB

Phospholipids (PLs) are a large lipid subgroup that is involved in important cell functions in all organisms. They are the main components of biological membranes and are essential for many biologic processes within and between cells, for example, signal transduction, cell growth, and various transport processes. The basic building blocks for PLs are fatty acids linked to a glycerol backbone and polar head groups (Figure 1). In addition to the main membrane PLs, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE), other PL classes are of great importance as well. Some PLs have significantly lower concentrations. For example, in eukaryotic organisms, cardiolipin (CL) is exclusively located in the inner mitochondrial membrane (1). Thus, the concentration of CL in total lipid extracts is very low in comparison to the major membrane lipids, such as PLs and cholesterol, but also triacylglycerols (TGs) (2,3). Furthermore, ion suppression effects complicate the mass spectrometric (MS) analysis and a tailored analysis of low abundant PL classes is often required.

In our previous study, we demonstrated the advantage of performing sample preparation by solid-phase extraction (SPE) in hydrophilic interaction liquid chromatography (HILIC) mode (4). The nonpolar fraction of lipids was first separated from polar PLs using an offline HILIC SPE method, and thereafter separation by reversed-phase liquid chromatography (LC) was performed. Offline methods often require solvent evaporation and reconstitute analytes in a suitable solvent for the second dimension, which is a time-consuming approach and risks losing labile PLs. In this application, a novel online heart-cut two-dimensional (2D) HILIC–reversed-phase LC–MS method is presented for the separation of PL classes and nonpolar lipids, where HILIC was utilized in the first dimension ($^1$D) and reversed-phase LC in second dimension ($^2$D).

## Experimental

**Lipid Standards:** Triacylglycerol (TG 48:0), cholesterol (Chol), phosphatidylcholine (PC 32:0), phosphatidylethanolamine (PE 32:0), cardiolipin (CL 64:4), and yeast total lipid extract (*S. cerevisiae*).

**LC–MS/MS Setup:** Thermo Scientific Ultimate 3000 system with dual gradient pump hyphenated to a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer. Ionization was performed utilizing electrospray ionization in negative ionization mode as described by Helmer *et al.* (3).

**$^1$D HILIC Separation:**

*Column:* 20 × 2.1 mm, 5-µm, 100 Å, iHILIC®-Fusion(+) (P/N 100.022.0510, HILICON)

*Eluents:* A) ammonium formate solution (35 mM, pH 3.5) and 95:5 (*v/v*) acetonitrile; B) acetonitrile

*Gradient Elution:* 0–0.2 min, 97% B; 0.2–0.5 min, from 97% to 93% B; 0.5–2.75 min, 93% B; 2.75–7.5 min, from 93% to 60% B; 7.5–11 min, 60% B; 11–11.5 min, from 60% to 97% B; re-equilibration is parallel to reversed-phase LC separation at 97% B.

*Flow Rate:* 0.3 mL/min; 0.05 mL/min (during parallel reversed-phase LC separation)

*Column Temperature:* 40 °C

*Injection Volume:* 2–20 µL

**Heart-Cut Setup for PL Transfer:** An online heart-cut 2D HILIC–reversed-phase LC–MS setup was developed for PL transfer from $^1$D (HILIC) to $^2$D (reversed-phase LC). As described earlier, a six-port valve equipped with a sample loop (300 µL, PEEK) was utilized as the transfer (Figure 2) (3). At

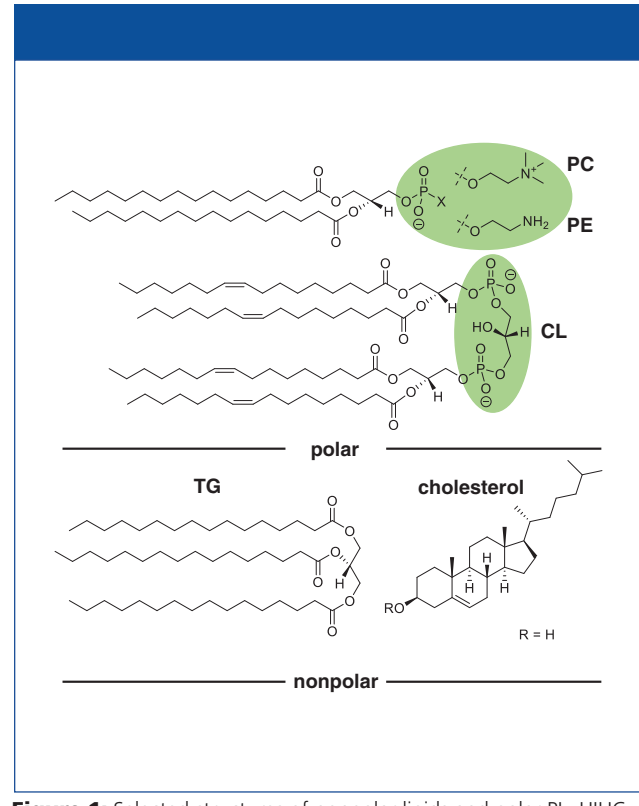



**Figure 1:** Selected structures of nonpolar lipids and polar PL. HILIC separation of PL takes place according to their polar head groups (shown in green).

first, the HILIC eluate was directed to the waste. By switching the valve from A (*2_1*) to B (*1_6*) position, the selected PL fraction was transferred into the loop for the separation in the second dimension. Since we focused on CL in this study, the transfer time window was set at its retention time of between 6.35–6.95 min.

## Results and Conclusion

A tailored analysis of CL species in yeast (*S. cerevisiae*) was achieved utilizing two-dimensional HILIC–reversed-phase LC–MS. In the first dimension, the separation of PL classes was carried out using a HILIC method. In addition to that, nonpolar lipids did not show retention and eluted at the void volume. As shown in Figure 3(a), the lipid classes (TG, PE, CL, and PC) were well separated according to their different head groups with this new method. The application of this method to a yeast total lipid extract (Figure 3[b]) shows the intended coelution of the CL species in yeast, where one typical CL species is CL 66:4 with 66 carbon atoms and four double bonds in the four linked fatty acids.

In the developed 2D HILIC–reversed-phase LC separation method, a transfer window with all CL species from $^{1}$D HILIC was first determined for the transfer via a heart-cut setup to the $^{2}$D reversed-phase LC system. Compared to the conventional one-dimensional (1D) reversed-phase LC (red), $^{2}$D reversed-phase LC (black) shows a significant decrease of background signals (mainly TGs), and low abundant CL species can even be observed in the total ion current (TIC) chromatogram (Figure 3[c]). Furthermore, by the reduction of the background as observed in 1D reversed-phase LC, higher CL signal intensities have been observed (3).

The selective HILIC pre-separation of lipid classes in the first dimension significantly reduced the matrix effects in the second dimension separation of low abundant PL classes.

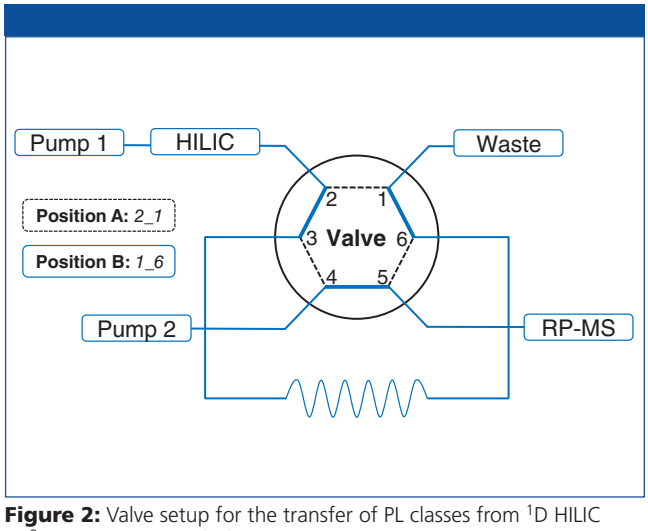



**Figure 2:** Valve setup for the transfer of PL classes from $^{1}$D HILIC to $^{2}$D reversed-phase (RP) LC via heart-cut approach.

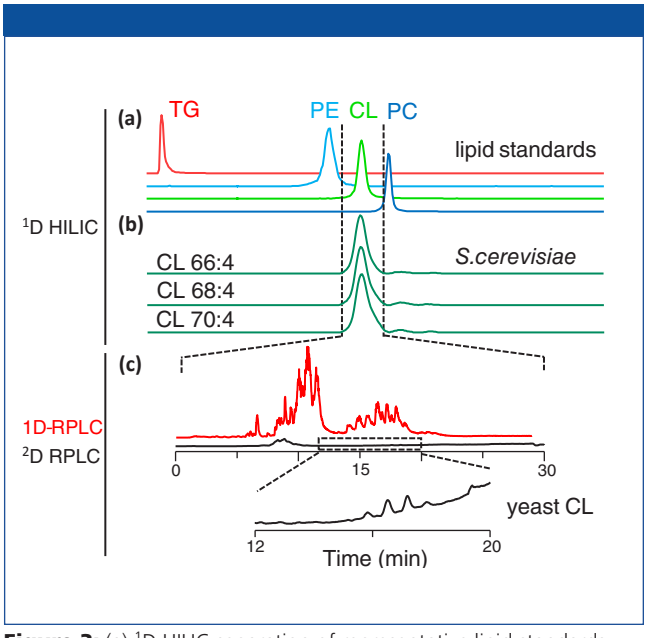



**Figure 3:** (a) $^{1}$D HILIC separation of representative lipid standards; (b) coelution of dominant CL species in yeast (*S. cerevisiae*) within the transfer window; (c) comparison of reversed-phase (RP) LC TICs in 1D (red) and $^{2}$D (black).

Interestingly, low abundant CL species can be sensitively detected and observed, even in the TIC. In summary, the automated online 2D method via heart-cut transfer offers a fast and gentle sample preparation and clean-up procedure that is very useful for analyzing low abundant PL classes as well as labile PLs, such as PL oxidation products (3).

## References

(1) J.M. Berg *et al.*, *Stryer Biochemistry*, Springer (2018).

(2) M. Lange *et al.*, *Chromatographia* **82**, 77–100 (2018).

(3) P.O. Helmer *et al.*, *J. Chromatogr. A*. 460918, in press https://doi.org/10.1016/j.chroma.2020.460918

(4) P.O. Helmer *et al.*, *Rapid Commun. Mass Spectrom.* **34**, e8566 (2020). https://doi.org/10.1002/rcm.8566

**HILICON AB**
Tvistevägen 48A, SE-90736 Umeå, Sweden
Tel.: +46 (90) 193469
E-mail: info@hilicon.com
Website: www.hilicon.com

# Exceptional Recoveries of Planar Pesticides from Spinach and Fresh Oregano

Merck KGaA, Darmstadt, Germany

When analyzing pigmented samples, some planar pesticides may be lost in sample preparation. To address this, a "Quick, Easy, Cheap, Effective, Rugged, and Safe" (QuEChERS) cleanup method has been developed using Supel™ QuE Verde, a sorbent combination containing an improved graphitized carbon black (GCB), Z-Sep+, and primary-secondary amine (PSA) to provide improved recovery of planar pesticides over current QuEChERS sorbents while maintaining sufficient colour removal in high chlorophyll matrices.

## Experimental

QuEChERS extraction and cleanup was similar to that outlined in the AOAC International Official Method 2007.01 (1). Organic spinach and freshly picked oregano were extracted with acetonitrile. The procedure outlined in Figure 1 fully describes the extraction and cleanup procedure for use with 2 mL tubes. The acetonitrile extract was spiked with a mixture predominantly composed of planar pesticides in acetonitrile to a concentration of 50 ng/mL. Extractions were performed in triplicate. Analysis of the final extracts was carried out by GC–MS as listed in Figure 1.

## Results and Discussion

A background spectrum was taken and evaluated by analysis of the final extracts by GC–MS in full scan mode. The analysis of the spiked extracts in selected ion mode (SIM) revealed that the Supel™ QuE Verde cleanup removed enough matrix interferences to easily identify and quantitate all 12 residues in a mixture containing planar pesticides (Figure 1).

Most of the green pigment from the spinach and oregano extracts was removed after cleanup with Supel™ QuE Verde. Total chlorophyll

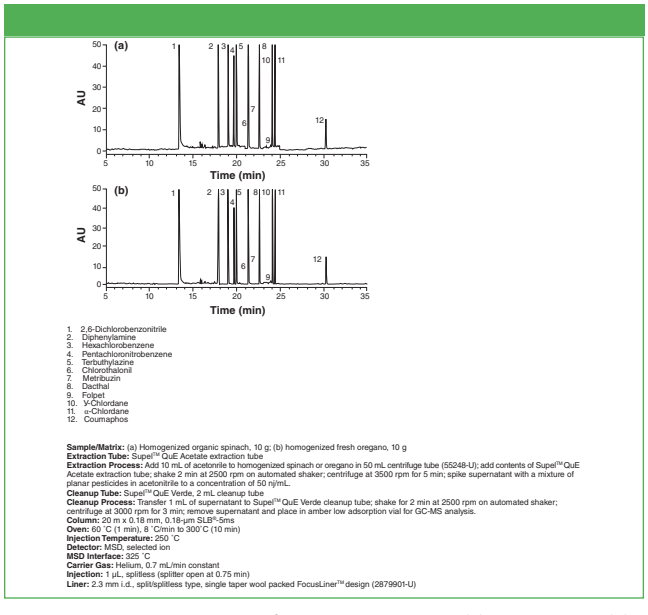



**Figure 1:** Chromatograms of planar pesticides in (a) spinach and (b) fresh oregano after cleanup with Supel™ QuE Verde.

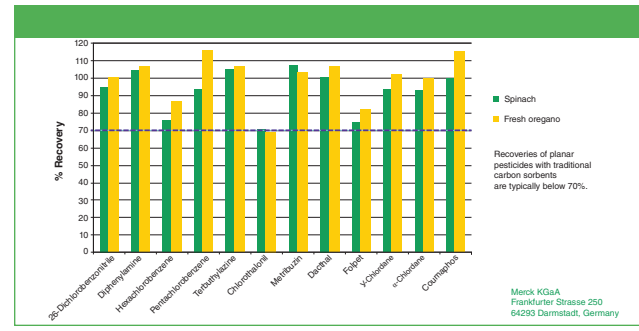



**Figure 2:** Average percent recoveries from spinach and oregano extracts spiked at 50 ng/mL after cleanup with a 2 mL Supel™ QuE Verde Tube (n = 3). Note: Recoveries with traditional carbon sorbents are typically below 70%.

removal was evaluated using a spectrophotometer by measuring absorbance at 664 nm, 647 nm, and 630 nm, and comparing the processed extracts to the initial acetonitrile extract of each plant material. In all cases, chlorophyll removal was 95% or greater.

Average analyte recoveries obtained from 50 ng/mL spiked spinach and oregano extracts using 2 mL Supel™ QuE Verde cleanup tubes are summarized in Figure 2. Recoveries were in the range of 70% to 120% for all of the planar pesticides tested in both matrices. Reproducibility (%RSD for n = 3 spiked replicates) was very good for both matrices. Each pesticide exhibited a %RSD value less than 5% with the exception of chlorothalonil, which had a %RSD value of 20%.

## Conclusion

The consensus among food analysts is that pesticide recovery should be between 70% and 120%, and chlorophyll removal to be 95% or greater.

Because of the strong interaction between planar pesticides and the planar surfaces of GCB, there remains a compromise between colour removal and analyte recovery, especially for the most planar pesticides such as hexachlorobenzene and chlorothalonil. Supel™ QuE Verde combines a novel carbon with zirconia-coated silica (Z-Sep+) to provide an optimum balance between planar pesticide recovery and colour removal, as shown in this application where it was used prior to GC–MS analysis to provide sufficient chlorophyll removal while maintaining excellent recovery of planar pesticides from spinach and oregano matrices.

## Reference

(1) AOAC Official Method 2007.01, Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate

**Merck KGaA, Darmstadt, Germany**
Frankfurter Strasse 250,
64293 Darmstadt, Germany
www.SigmaAldrich.com/Quechers

# 44th International Symposium on Capillary Chromatography and 17th GC×GC Symposium

*...with particular emphasis on all Comprehensive Separation Technologies and MS Hyphenation*

**24 - 29 May, 2020**

***Palazzo dei Congressi***
***Riva del Garda***
***Italy***

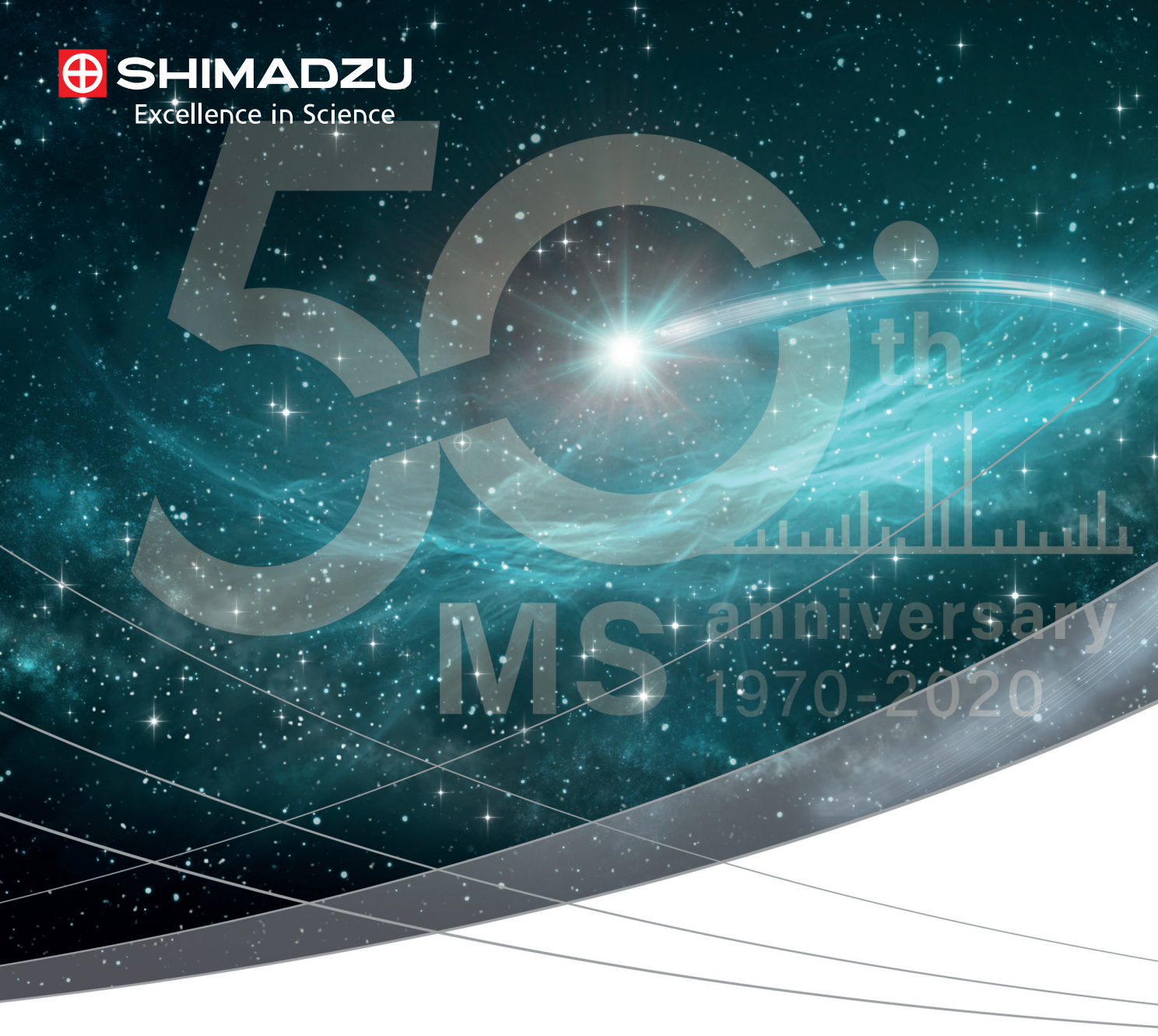
The 44th ISCC and the 17th GC×GC Symposia is a "hyphenated" meeting which will be held again in wonderful Riva del Garda (Italy), from 24 - 29 May, 2020.
Apart from the most recent advances in the fields of pressure and electrodriven microcolumn separations, and comprehensive 2D GC. This year particular emphasis will be directed to all Comprehensive Separation Technologies in combinations of capillary chromatography and 2D GC with various forms of MS... from unit-mass to high resolution, and from single to hybrid analyzers. Consequently, both the importance and complementary nature of chromatographic and MS processes will be given to the sample preparation process, in both oral and poster sessions.
The ISCC/GC×GC scientific program will be a rich one, it being characterized by:
- invited contributions from leading scientists reporting the latest most exciting developments
- keynote lectures from promising young researchers
- very active poster sessions
- discussion sessions
- workshop seminars presenting the most recent novelties in scientific instrumentation
- a world-class GC×GC course
- a world-class LC×LC course

Researchers in all areas relevant to the subjects of the symposia are invited to submit abstracts.
As is traditional for the Riva meetings, the majority of presentations will be in a poster format and the Scientific Committee will select contributions for oral presentations. As always, many awards will be assigned in both the ISCC and GC×GC events, recognizing excellence in both established and young scientists, in oral and poster presentations.
Exhibitors and sponsors are a fundamental part of the meeting (without them...Riva wouldn't be Riva!) and are encouraged to participate by reserving booth space, becoming a sponsor and to promote the ISCC and GC×GC events.
Last, but not least, the traditional "Riva" social program. Please keep visiting our web site (www.chromaleont.it/iscc) for new information as it becomes available.

Looking forward to meeting you in astonishing Riva del Garda!

Organized by
Chromaleont s.r.l.

*The Forum of Microcolumn Separations*




# Milestones to celebrate. Futures to build.

It is an event to celebrate, but even more the chance to shape the future: Shimadzu's 50th anniversary of mass spectrometry solutions. Based on its benchmarks of novelties and milestones in the past, Shimadzu is now driving progressive technological and operational opportunities in the high-sensitivity MS analysis of unknown substances. Users benefit from the company's high-tech capabilities combined with scientific and innovative capacities.

**The full range of solutions for R&D and routine applications labs**
using quadrupole systems and hi-res TOF instruments for GC-MS, LC-MS, MALDI-MS and ICP-MS methods

**Next-generation higher sensitivity, efficiency and accuracy**
based on the combination of miniaturization, automation and coupling technologies

**Ahead-of-the-art and visionary applications**
merging MS and medical technologies, and envisioning currently unimaginable onsite applications, e.g. in food retail and private homes

**www.shimadzu.eu**/celebrating_50_years