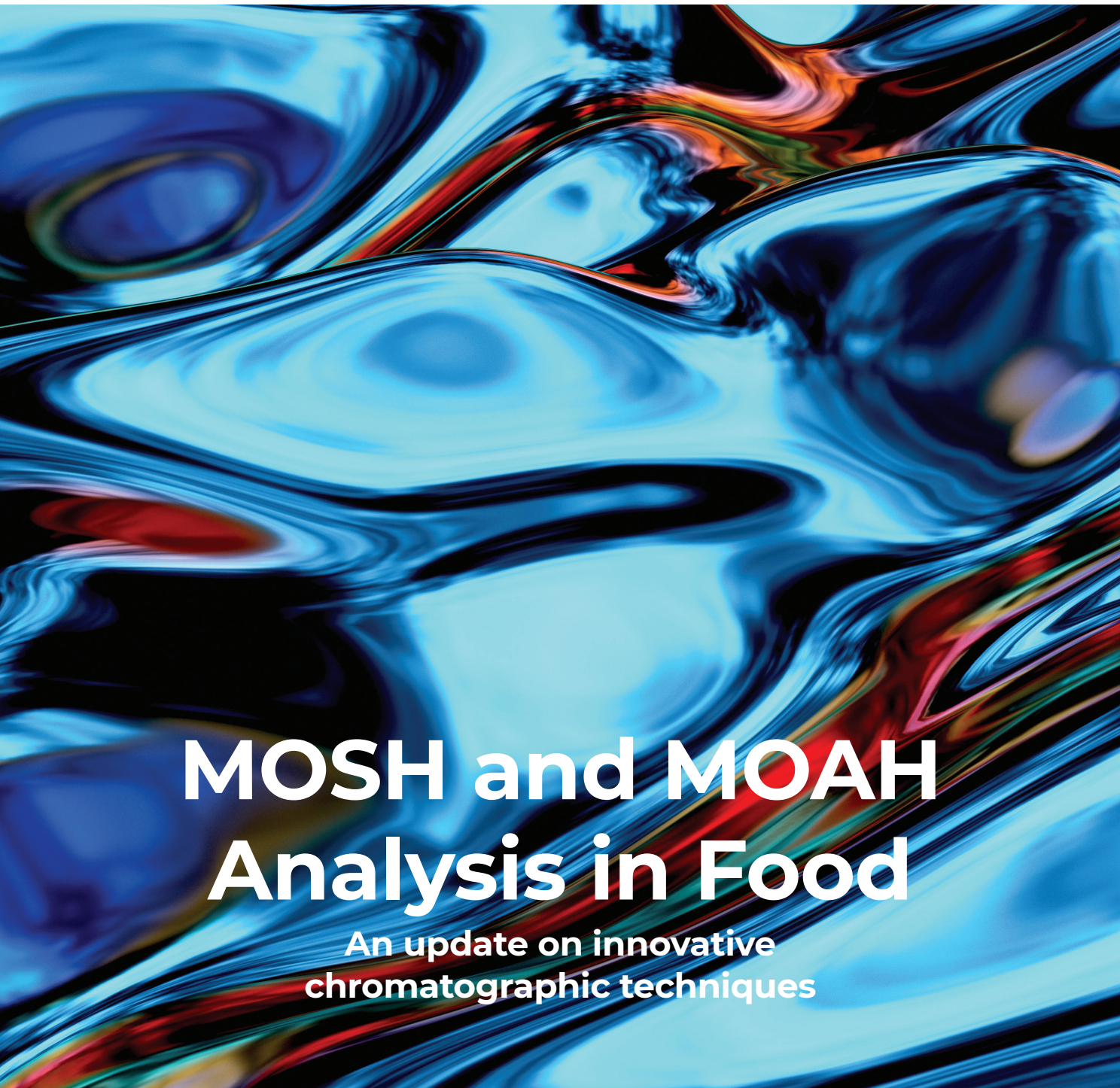
LC GC europe
solutions for separation scientists
February 2021 | Volume 34 Number 2
www.chromatographyonline.com



MOSH and MOAH Analysis in Food

An update on innovative chromatographic techniques

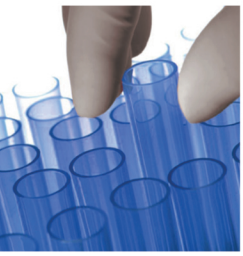
Decade Elite

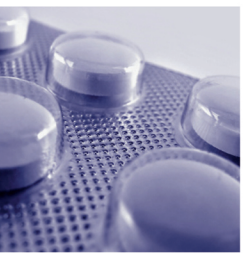
Electrochemical detector for any (U)HPLC system with the software of your choice

DataApex Clarity™

CHROMELEON 7.2

Agilent OpenLAB CDS

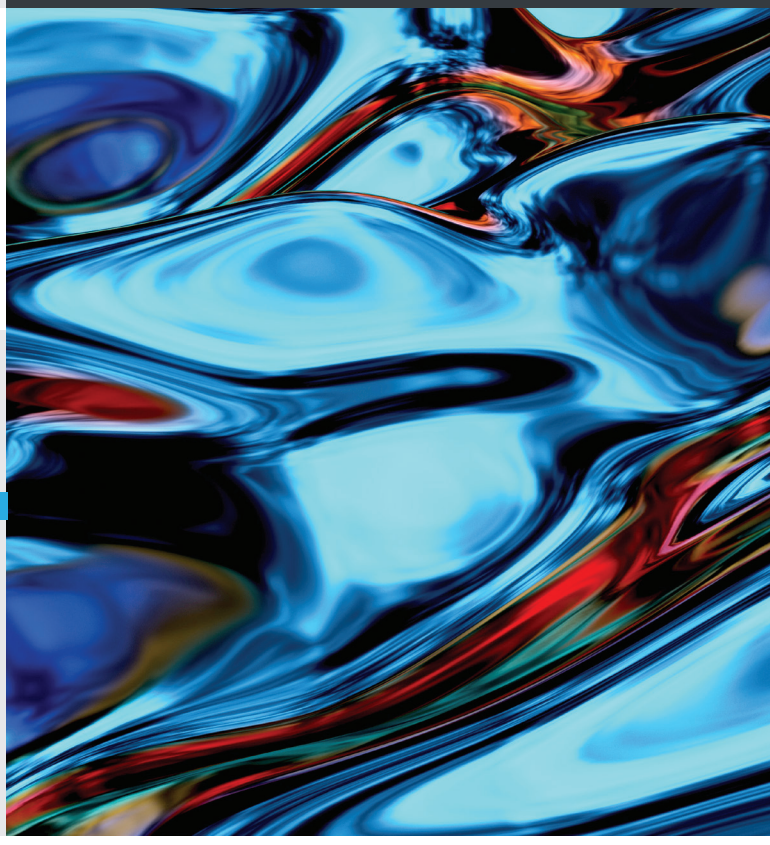




www.AntecScientific.com

LCGC europe
solutions for separation scientists

February | 2021
Volume 34 Number 2



COVER STORY

COLUMNS

Image Credit: SmirkDingo/stock.adobe.com

DEPARTMENTS

LCGC europe
solutions for separation scientists

AN MJH life sciences™ BRAND

Senior 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

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

Senior Art Director
Gwendolyn Salas
gsalas@mjhlifesciences.com

Senior 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

Chief Marketing Officer
Michael Baer

Executive Vice President, Global Medical Affairs & Corporate Development
Joe Petroziello

Senior Vice President, Content
Silas Inman

Senior Vice President, Operations
Michael Ball

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

Vice President, Human Resources & Administration
Shari Lundenberg

Vice President, Mergers & Acquisitions
Chris Hennessy

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 online

Selected highlights of multimedia content from *LCGC Europe* and *The Column*.

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!

LCGC BLOG

10 Great Tips for Electrospray Ionization LC–MS

Here are 10 great tips for optimizing LC-Electrospray Ionization (ESI)-MS so that you achieve the best possible results every time. These tips and tricks are from leading practitioners to form a beginner's guide to LC–(ESI)MS and as a primer for those who are already using the technique.

Read more: *https://bit.ly/2Ulgdg8*

PODCAST

The Importance of Mitigating Non-Specific Adsorption in Chromatographic Separations

This podcast asks the questions: what are the challenges of non-specific adsorption and binding? How does non-specific adsorption affect analytical results?

Read more: *http://bit.ly/3tcEbAe*

SAMPLE PREPARATION PERSPECTIVES

Turning Up the Heat – The Effect of Temperature on Analytical Extractions

Temperature impacts solubility, diffusion, surface tension, and more. It can potentially lead to solute decomposition. This month we will explore the role of temperature in analytical extractions.

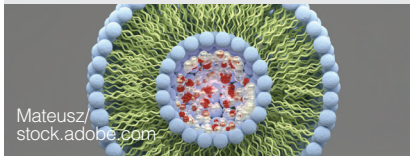
Read more: *http://bit.ly/36fCloo*

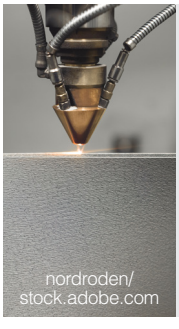
PEER REVIEWED

Untargeted LC–MS Lipidomics with Data Independent Acquisition using Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra

This article explores the wide-ranging potential and peculiarities of SWATH–MS hyphenated with UHPLC for the profiling of lipidomes of various biological samples.

Read more: *https://bit.ly/3t4EBZp*





NEWS

3D-Printed Metal Column Developed for Micro-GC

Researchers have produced a metal column for use in micro-gas chromatography (GC) using 3D-printing technology, and examined its properties and ability to perform gas separations.

Read more: *http://bit.ly/3ciuOIX*

E-BOOK

Optimizing LCs During a Pandemic

This e-book focuses on technologies which are designed to free up experts for more challenging tasks and to provide versatility while still delivering results.

Read more: *http://bit.ly/3t5bgxE*

PEER REVIEW

A Review of MOSH and MOAH Analysis in Food

Nicola Sdrigotti[1,2], Gregory Bauwens[1], and Giorgia Purcaro[1], [1]Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium, [2]University of Udine, Udine, Italy

Mineral oil hydrocarbons (MOH) are a very complex mixture of isomers classified into mineral oil *saturated* hydrocarbons (MOSH) and mineral oil *aromatic* hydrocarbons (MOAH). The analysis of such contaminants in food is a challenging task and requires a comprehensive approach to tackle the cumbersome issues related to their determination. Additionally, their toxicity is still under investigation and requires further studies supported by more detailed analytical data. This review aims to give an overview of the different analytical approaches proposed, from sample preparation to the final chromatographic determination, to respond to the request of consumers and institutions, such as the European Food Safety Authority and European Commission, for a reliable risk assessment. Emphasis is given to hyphenated chromatographic techniques as powerful tools to gain deeper insights into the MOSH and MOAH problem.

KEY POINTS

- **MOSH and MOAH are highly challenging food contaminants.**
- **MOSH and MOAH problems in relation to European policy are discussed.**
- **Modern analytical approaches and the role of hyphenated techniques are discussed.**

The International Agency for Research on Cancer (IARC) defined mineral oils (MO) as follows: "Mineral oils, which are also known as base oils, mineral base oils or lubricant base oils, are chemical substances prepared from naturally occurring crude petroleum oil. Crude oil is distilled first at atmospheric pressure and then under high vacuum to yield vacuum distillates and residual fractions that can be further refined to mineral oils" (1). The strict definition of contamination by mineral oil hydrocarbons (MOH) refers to the accidental presence in food of crude petroleum and other products derived from it by distillation and refining process, for example, diesel fuel, jet fuel, white oils, lubricants, and solvents. Despite that, the quantification of MOH can, sometimes, erroneously include analogue mixtures—the use of which is permitted in food contact material (FCM) and in the food industry—such as polyolefins and white oils (highly refined mineral oil where the presence of aromatic compounds is minimized). Therefore, a careful interpretation of the results is needed.

From an analytical viewpoint MOH are divided into two main fractions, namely MOSH (MO saturated hydrocarbons, composed by linear, branched, and alkyl-substituted cycloalkanes) and MOAH (MO aromatic hydrocarbons, which include mainly alkyl-substituted [poly] aromatic hydrocarbons with a different number of fused rings).

Food is the main source of MOH intake (2) and various studies were conducted to evaluate the exposure to mineral oils over time, both on animal tissues (3–9) and human tissues (10–13), highlighting accumulation of MOSH in the liver, spleen, lymph nodes, and adipose tissue. The end point was identified as hepatic micro-granulomas based on studies on Fischer 344 rats, but later it was considered irrelevant

to humans due to inter-species differences in terms of MOSH absorption, catabolism, and sensitivity (12). MOAH are classified as potentially mutagenic, referring in particular to 3-7 ring compounds (2). A high degree of alkylation is thought to reduce their metabolic activation, leading to the formation of non-mutagenic intermediates (13).

In 2012 the European Food Safety Authority (EFSA) published its first opinion about MOH in food (2) but no definitive conclusions were drawn because of the limited information available.

In 2017, the EFSA and the European Union (EU) (14) required the collection of more data to characterize the sub-classes present in the MOH fractions and so evaluate better the risk, but guidance for harmonized data collection and report were only published in February 2019 (15). In this guidance, the carbon range of MOH was analyzed. This guidance recommended to quantify the C_{10}–C_{50} carbon range. In 2019, the EFSA released a new opinion, following the Foodwatch report on the presence of MOAH in infant and follow-on formulas (16), still concluding that characterization of hazards is not possible in the absence of relevant dose-response data and information regarding the presence of the more health-concerning 3-7 ring MOAH (17).

FIGURE 1: GC-FID profile of the MOAH fraction of a palm oil sample before (orange trace) and after (green trace) epoxidation.

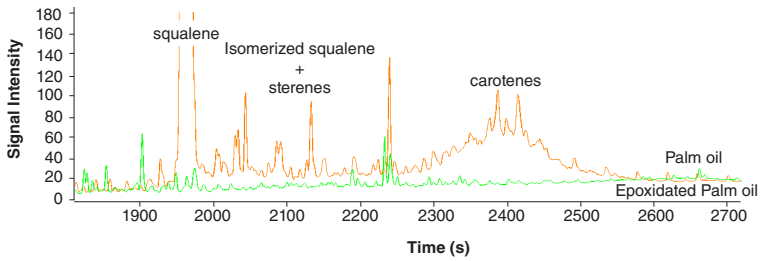
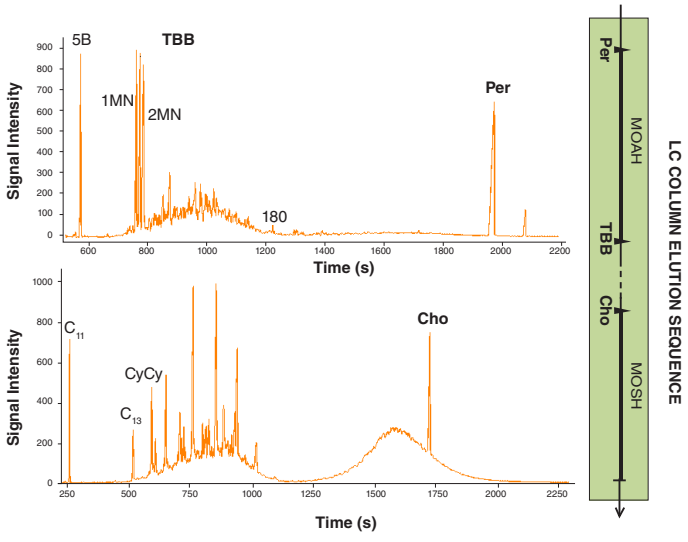


FIGURE 2: Representation of the MOSH and MOAH fractions eluting from the LC silica column, and their corresponding GC–FID analysis. Cholestane (Cho) marks the end of the MOSH fraction. Tertbutylbenzene (TBB) and perylene (Per) mark the beginning and the end of the MOAH fraction, respectively. CyCy is used for MOSH quantification. 1MN and 2MN are used for MOAH quantification.



The situation is also complicated by the permitted use of white mineral oils as food additives (microcrystalline wax and hydrogenated poly-1-decene) (18), food processing aids (mold releasing and anti-caking agents), and FCM additives (19). Moreover, some paraffin oils and mineral oils are allowed to be used as pesticides in the production of organic food (20,21).

While a common regulation about migration in food does not exist yet, some national law can be considered. Specific limits were set for the migration of MOH from inks, by the Swiss Printing Ink Ordinance (RS 817.023.21), and from FCM, by the 22nd German Federal Ministry of Food and Agriculture (BMEL) ordinance. In Belgium, the Scientific Committee of the Federal Agency for the Safety of the Food Chain (FASFC), published "Advice 19-2017", which sets action thresholds between 5–150 mg/kg for the MOSH fraction (C16-C35), depending on the food category (22).

Except for the method EN 16995 (23) for MOSH and MOAH determination in vegetable oil, no official methods exist.

In this context, the goal of this review is to provide a current overview on the topic, with particular focus on the analytical perspective. Emphasis will be given to the most innovative and recent solutions proposed to tackle this issue. Regarding the sample enrichment/purification and MOSH/MOAH separation, a premise has to be made since they are often achieved simultaneously by a single step. For the purposes of logic, they will be separated in two different sections, and discussed as individual treatments. Moreover, a further subdivision in offline and online methods will be done.

Extraction of Mineral Oil Hydrocarbons

Since MOH are ubiquitous contaminants, the possible contribution of additional contamination should be minimized through a conscious choice of solvents (high purity grade), a proper cleaning of the glassware, and high care and possible minimization of the sample handling.

In the extraction step, hexane is by far the most commonly used solvent. It can be utilized alone, in sequence, or mixed with other solvents (such as ethanol), depending on the fat content, moisture of the matrix, and on the type of contamination investigated (superficial migration from FCM or inner contamination). It is used in MOH extraction from many matrices, including cereal or cereal-based dry products, wet foods, oils, fats, and FCM (24,25).

In the case of dry foods, superficial contamination can be easily extracted by direct immersion in hexane, whereas for the inner contamination, prior rehydration of the matrix is required (for example, overnight at 80 °C). Then, for wet or rehydrated matrices, a conditioning step in ethanol (1 h) is fundamental to replace the immobilized water and thus mediate the hexane entrance to the pores. Another positive effect of the ethanol is its swelling ability towards starch and denaturing power towards proteins, thus favouring the release of entrapped hydrocarbons (26,27).

For edible oils and fats, a simple dilution in hexane is performed, followed by online or offline purification steps. Sample enrichment is often performed to increase sensitivity, and auxiliary

purification steps can be required to remove interference such as natural alkanes and olefins.

Another scenario is represented by the extraction of MOH from FCM. Recycled papers and paperboards are generally left to soak in a solution of hexane/ethanol (1:1 v/v) (28,29). The conditions are opportunely chosen to reduce the extraction of high molecular weight hydrocarbons that could remain in the retention gap, causing a carryover effect and appearing randomly as broad unresolved peaks (30). In the case of plastic films, the extraction time with pure hexane must be reduced to the minimum, according to film thickness and permeability, in order not to extract excessive amounts of plastic oligomers.

Rapid, alternative, and efficient MOSH and MOAH extraction can be obtained by microwave assisted saponification (MAS) or pressurized liquid extraction (PLE). MAS with simultaneous solvent extraction was proposed instead of the classical saponification followed by solvent partitioning (31). Different cereal-based foodstuffs were saponified with methanolic potassium hydroxide (KOH) (120 °C × 20 min) in the presence of hexane. The hexane supernatant was directly collected for analysis.

PLE has been proposed as an alternative in dry foods with low fat content (27). The external contamination, deriving from packaging migration, was differentiated from the internal one. Hexane was used to recover the external contamination, while the total contamination was determined by a two-cycle PLE in hexane/ethanol (1:1 v/v) at 100 °C for 5 min, obtaining comparable results to the overnight extraction previously proposed (26). In the case of cardboard (32), MOH were extracted at 60 °C for 5 min, by a two-cycle PLE in hexane, reducing the extraction time compared to the previous method (28).

Sample Enrichment and Purification

Often additional purification steps are required, but they may lead to cross-contamination, thus reducing the accuracy. They should be appropriately applied and only when strictly necessary.

A Q&A

Part 2: Advances in Field-Flow Fractionation: Supporting Development of Novel Nanomedicines

Christoph Johann
Global Product Manager
Wyatt Technology

In the second part of this three-part series, *LCGC* continues its conversation with Christoph Johann, global product manager at Wyatt Technology, as he discusses the benefits of coupling light scattering online, field-flow fractionation's (FFF) role in the pharmaceutical industry, its acceptance by regulatory agencies, and more.

LCGC: Can you explain the benefit of coupling light scattering online with FFF?

Christoph: While FFF generally separates particles according to hydrodynamic size, following strict fluid dynamic equations, there are sufficient uncertainties in channel and membrane properties that you cannot rely on retention time only to determine size accurately. In this sense, it is very similar to size-exclusion chromatography (SEC): online multi-angle light scattering (MALS) and dynamic light scattering (DLS) detectors are crucial for reliable and accurate characterization of the particle size, concentration, conformation, and the molar mass.

As in chromatographic methods, additional detectors can be added for deeper characterization. Typically, refractive index, UV/Vis, and fluorescence HPLC detectors may be included, enabling quantification of drug or nucleic acid loading, encapsulation, and similar properties that relate to particle composition and conjugation.

So, it is the complete system of robust FFF separation, with powerful light-scattering and spectroscopic detection, that provides comprehensive characterization capabilities.

LCGC: Where do you see the increasing need for FFF in the pharmaceutical industry?

Christoph: There are two parallel and similar paradigm shifts going on at full speed. In traditional small-molecule pharmaceuticals, more drugs are formulated as nanoparticles, whether as emulsions, nanosolids, or encapsulated in liposomes or other nanocarriers, including lipid nanoparticles, polymer micelles, polymersomes, albumin particles, polyplexes, etc.

In biopharmaceuticals, commercialization of gene therapies—the delivery of DNA or RNA by viral or non-viral gene vectors—is in high gear. SEC-MALS is suitable for small vectors like adeno-associated virus, but larger vectors such as lentivirus or adenovirus require separation by FFF. Non-viral vectors are very similar to small-molecule nanodrug-delivery systems, e.g., lipid nanoparticles or polymersomes. In both cases, the trend is to deliver therapeutic payloads in delivery vehicles that are in the size range of 30 to 300 nanometers, which is very different from the size of current drugs based on small molecules, peptides, proteins, or microparticles.

The standard tools in place for characterizing these new modalities—whether batch DLS or nanoparticle tracking analysis for nano drug delivery systems (DDS) or qPCR and ELISA for gene vectors—are simply

SPONSORED BY

WYATT TECHNOLOGY

LCGC europe

insufficient to meet the challenges and analytical needs presented by these complex therapeutics. FFF with MALS, DLS, and spectroscopic detectors provide a powerful and versatile characterization platform that is perfectly matched to these products.

> The need for FFF-MALS-DLS in characterization for regulatory fillings of drugs, and eventually quality control of nanomedicines, is fully recognized by regulatory agencies and the institutions and organizations developing standards for the pharmaceutical industry.

LCGC: How complicated are these analyses?

Christoph: Setting up the separation method is pretty straightforward, especially with our SCOUT method simulation software. The actual operation is very similar to HPLC—the samples are loaded into vials, placed in the autosampler, and run through fully automated sequences. Basic analyses like average size, size distribution, shape, and particle concentration for each fraction are also straightforward and can be fully automated. Analyses of composition and encapsulation require a combination of multiple detector signals, which may require method development and calibration steps, but once those are done, the rest is automated and easy. Best of all, all of these characterizations are completed in a single injection. Replicates, of course, are elementary.

LCGC: It sounds like the technology is great for R&D, but where is FFF-DLS-MALS in terms of acceptance by chemistry, manufacturing, and controls (CMC) departments and regulatory agencies?

Christoph: The need for FFF-MALS-DLS in characterization for regulatory filings of drugs, and eventually quality control of nanomedicines, is fully recognized by regulatory agencies and the institutions and organizations developing standards for the pharmaceutical industry. These organizations have been developing protocols and technical documents, as well as publishing reviews and introspection papers, to support the adoption of this method across the pharmaceutical industry. I can say with confidence that it will become an essential characterization tool for nano DDS and gene vectors. The improvements we are making to performance, simplification, robustness, and GMP compliance should meet the needs and expectations of CMC departments, regulatory agencies, as well as quality-control departments.

FIGURE 3: Comparison of the interpretation process of the LC–GC and GC×GC–FID/MS traces of the MOSH fraction of a spice sample. In the 2D plot the POSH are eluted above the MOSH fraction and can be easily removed from the quantification of MOSH.

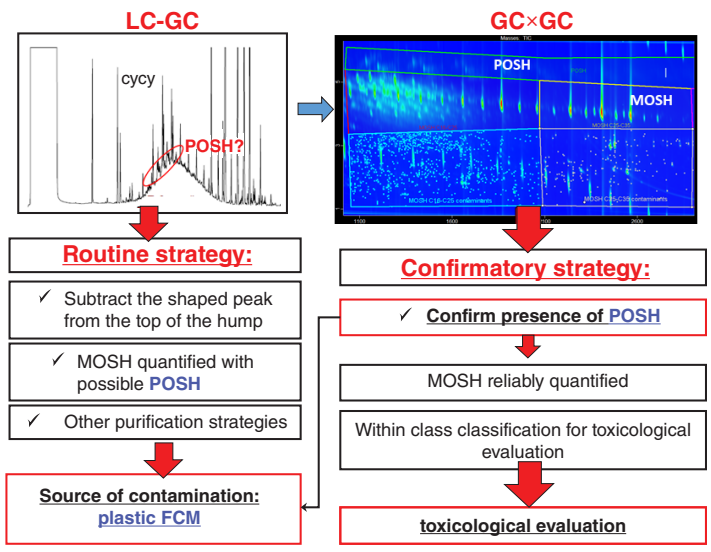
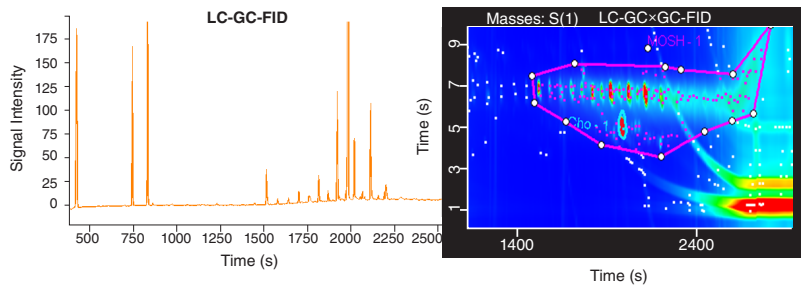


FIGURE 4: Comparison of LC–GC–FID and LC–GC×GC–FID trace chromatograms of the MOSH fraction of a palm oil obtained using the same LC-GC×GC platform in the 1D and 2D mode.



Support in this regard is given by the "decision tree" reported in the JRC Guidance (15). The enrichment and auxiliary techniques are herein briefly summarized.

Offline Enrichment and Purification:

Enrichment: It is required to increase the sensitivity by removing the bulk of the triglycerides. Saponification followed by partitioning in hexane is usually applied (31). Alternatively, offline solid-phase extraction (SPE) with activated silica has been proposed (33,34). Performance of the activated silica is higher compared to the non-activated one and comparable to that of silver silica in terms of fat retaining capacity, the latter also improving olefins retention, thus reducing the need for an additional epoxidation step (33).

Natural alkanes removal: Naturally-occurring alkanes, generally ranging from C_{21} to C_{33} (with odd carbon numbers prevailing over the even ones), are found mostly in vegetable matrices. They are usually subtracted *a posteriori* from the integration of the MOSH hump. However, activated aluminum oxide must be used to remove *n*-alkanes greater than C_{20}, without affecting the iso-alkanes fraction, when they overload the chromatogram (35).

Olefins removal: This includes highly unsaturated natural olefins, for example, squalene, carotene, and sterenes, which interfere with MOAH, and unsaturated molecules released by polyolefin packaging, such as polyolefin mono-unsaturated hydrocarbons (POMH) and poly alpha olefins (PAO), coeluting with the MOSH fraction. It has to be specified that polyolefin packaging also releases polyolefin saturated hydrocarbons (POSH).

The removal of interfering olefins was initially performed, increasing their polarity by bromination of the double bonds. This reaction also significantly affected the MOAH fraction, causing loss of analytes (36), so epoxidation with *meta*-chloroperbenzoic acid (*m*CPBA) was proposed to assure a better selectivity toward aliphatic double bonds, yet a possible loss of 20–35% of MOAH may occur during the reaction (37–39). Figure 1 shows the profile of a palm oil sample before and after epoxidation.

Online Enrichment and Purification:

Enrichment: Applying the routine liquid chromatography–gas

chromatography (LC–GC) method (described in detail later), which exploits the fat retention of an LC silica column (25 cm × 2.1 mm, 5-µm), food extracts can be directly injected into the LC column as long as the fat content is lower than the column capacity (~20 mg) and the limit of quantification (LOQ) for MOH is reached (~ 50–100 ng on column with the flame ionization detector) (30,37,40). When this condition is not fulfilled, enrichment procedures are necessary. The use of a large LC silica column (25 cm × 4.6 mm, 5-µm) to increase the fat retaining capacity (from ~20 to 150–200 mg) was proposed (41), but the eluted hydrocarbon fraction had a volume of 6 mL, which is difficult to handle in the online coupling with gas chromatography (GC). Thus, offline methods are usually, preferred for enrichment purposes.

Natural alkanes removal: Fiselier et al. proposed the use of an online alumina oxide LC column for removal of *n*-alkanes from the MOSH fraction (42). In this case, a primary LC silica column (25 cm × 2 mm, 5-µm) was designed to retain fats and pre-separate MOSH from MOAH. Only the saturated fraction was sent to the secondary aluminum oxide column (10 cm × 2 mm, 63–200-µm) activated at ~400 °C, where the long-chain *n*-alkanes were retained. Compared to the offline method, a great advantage is the possibility to restore the alumina column by a backflush step in iso-octane, allowing operation for many cycles and, of course, reducing the potential contamination.

Olefins removal: An LC–LC–GC method (43) for the analysis of POMH in food and FCM was proposed. After the separation of MOSH and MOAH in a silica column, the MOSH fraction was sent to a secondary silver silica column, that allowed the isolation of the POMH. The MOAH fraction instead, bypassed the silver silica path being directly sent to the GC in order to avoid the possible coelution of the POMH with the following MOAH fraction.

A similar approach was exploited for the removal of natural olefins interfering with the MOAH fraction. In this case, the MOAH fraction was diverted to a secondary silver silica column to remove olefin, while the MOSH fraction was directly sent to the GC–flame ionization detection (GC–FID) system for quantification (44).

Conventional MOSH and MOAH Separation

Offline SPE: Offline SPE methods followed by injection in GC have been developed to meet the instrumental availability of many laboratories that may not afford an online system.

In 2011 Moret et al. (33) published an offline SPE method for MOSH determination in vegetable oils, using a laboratory-made glass cartridge filled with 1 g of silver silica sorbents. The method was later extended to the separation of MOSH and MOAH, eluting the MOSH fraction (1.5 mL) with hexane, and the MOAH fraction (7 mL) with hexane/dichloromethane 1:1 (45). However, for some samples, the method might fail in retaining esterified fatty acid or waxes, limiting its applicability (46).

Fiselier and co-workers (34) overcame the problem by using 3 g of 0.3% silver silica, allowing the analysis of samples containing up to 20% fat, using a mixture of toluene:dichloromethane:hexane (0.5:2:7.5 v/v) as eluent.

Online LC–GC: Online LC to isolate MOSH and MOAH fractions, followed by GC–FID, is the method of choice for quantification (30,37,40). MOSH are the eluted first, followed by the MOAH, while triglycerides and more polar compounds are retained and backflushed with dichloromethane. Internal Standards (ISs) are used to identify the boundaries of the MOSH and MOAH fractions from the LC (Figure 2): cholestane (Cho) is used to mark the end of the MOSH fraction. Tertbutylbenzene (TBB) and perylene (Per) are used as markers of the beginning and the end of the MOAH fraction, respectively (37). Additional ISs are used for quantification purposes and quality control of the entire LC–GC transfer process, based on the assessment that specific quantity ratios are verified at each analysis. Undecane (C_{11}) and bicyclohexyl (CyCy) are used for quantification of the MOSH hump while 1-methylnaphthalene (1MN) and 2-methylnaphthalene (2MN) for the MOAH. C_{11} and 5B (n-pentylbenzene) are used for the MOSH and MOAH fraction, respectively, as watchdogs for volatile losses. Recently it has been reported that CyCy is a more suitable marker for the end of the LC MOSH fraction than Cho, as it elutes slightly later (40). DEHB (1,4-di(2-ethylhexyl)benzene) substituted the use of TBB in order to include the small amount of highly alkylated MOAH that elute right after the latest MOSH fractions.

The critical step in the hyphenation of LC with GC is the transfer of a large volume of

solvent eluted from the LC into the GC column. "On-column" and "Y" interfaces emerge among the other interfaces since they allow the coverage of a broader range of volatility without discrimination or losses of volatiles (30,47).

The GC separation column is preferably not polar (for example, 100% PDMS or 95% methyl, 5% phenyl), ranging from 10 to 30 m in length with thin-film thickness (for example, 0.15 µm) to reduce column bleed and facilitate the elution of high boiling components.

Since both MOSH and MOAH form a hump of unresolved peaks, a fast temperature gradient (~ 20–40 °C/min) is set to obtain the maximum sensitivity and avoid hump broadening. A high gas pressure helps to elute high boiling hydrocarbons.

The FID detector is used for quantification purposes because it gives virtually the same response factor for all the hydrocarbons of interest, rendering the calibration simplified by the use of appropriate standards.

On the other side, mass spectrometry (MS) is required for confirmatory purposes (in accordance with EU Recommendation 657/2002) (48), however similar compounds can present very different response factors (for example, hexane and cyclohexane, or MOAH with different alkylation, such as dimethyl or ethyl-), thus limiting the use of MS for quantification of the complex mixture deriving from the MOSH and MOAH contamination. Nevertheless, MS it is used to confirm the presence of specific markers such as pristane, phytane, diisopropyl naphthalene (DIPN), dibenzothyophene (DBT), and hopanes.

Comprehensive 2D GC based methods: In 2009, Biedermann and Grob introduced GC×GC in the field of MOH analysis to investigate in detail the MOAH fraction of a highly contaminated Ukrainian sunflower oil (49). Offline GC×GC after HPLC pre-separation allowed classifying the unresolved mixture according to the number of aromatic rings and the degree of alkylation with an apolar column in the first dimension (1% vinyl, 99% dimethyl polysiloxane) and a mid-polar column in the second dimension (50% dimethl polysiloxane, 50% biphenyl).

GC×GC provides an enhanced resolution, allowing the separation of all the sub-classes within the MOSH and the MOAH fraction. However, the two fractions need to be analyzed separately due to the different concentration factor (usually in the 4:1 ratio) and to avoid the coelution of four- and five-ring saturated hydrocarbons, such as steranes, hopanes, and bicyclic sesquiterpenes with the highly alkylated two- and three-ring aromatics (50). Differently from the first application, the preferred GC×GC columns combination is medium polar × apolar to maximize the resolution in the MOSH fraction and easily differentiate between MOSH and synthetic hydrocarbons (POSH and PAO) (50).

A GC×GC system, coupled with simultaneous dual detection—namely MS and FID—has recently been proposed (51). The two 2D plots obtained were examined in combination to provide complementary and confirmatory information. A flowchart of the procedure is shown in Figure 3 for the MOSH fraction. The 2D characteristic profile of both the MOSH and the MOAH fraction supports the determination of the possible sources of contamination in food, providing indications about the kind of MOH present and the type of refining they underwent (50), as well as giving additional information on the sub-classes of the MOSH and MOAH fractions for toxicological purposes. In the specific example of Figure 3, the additional class separation obtained in the 2D space allowed easy separation of POSH from the MOSH and provided information on the distribution between *n*-, *iso*-, and cyclic alkanes.

For volatile contamination coming from cardboard, quantification in GC×GC–FID was proposed, obtaining results comparable to the LC–GC–FID (46). More recently, a comprehensive platform—namely LC–GC×GC–TOFMS/FID—has been developed to face the analytical challenge of MOSH and MOAH fractionation, characterization, and quantification in a single analysis (51). Works towards the validation of the quantitative approach are ongoing with promising preliminary results. Figure 4 shows the comparison of an LC–GC–FID trace and an LC–GC×GC–FID trace obtained from the same instrument: quantification of the MOSH hump, subtracted by the alkanes on top of the hump, provided comparable results, namely 9.6 and 9.1 µg/g, respectively.

Alternative Techniques

As previously mentioned, completely automated LC–LC–GC methods using a secondary silver-silica column were proposed to efficiently remove interferents from the MOSH fraction (such as POMH) (43)

and the MOAH fraction (such as olefin) (44) for the analysis of food. Recently, the retention mechanism of the silver silica column was investigated as a first dimension of a comprehensive LC×GC system, alternatively coupled to a flame ionization detector or vacuum ultraviolet (VUV) detector. Small fractions (167 µL) of the eluate from the LC were collected and, offline, transferred to the GC system (1 µL injection in splitless mode). The 2D plot was generated using a programming platform for computational mathematics (52). The silver silica column allowed a group-type separation based on the degree of aromaticity; moreover, the use of the VUV added an extra level of information to the FID.

Later, the same silver silica stationary phase was employed to separate MOSH and MOAH using a supercritical fluid chromatographic (SFC) system coupled with FID and UV (53). Despite a less efficient separation of the MOSH and MOAH fractions, the use of the dual FID/UV detection allowed a deconvolution procedure by subtracting the UV signal from the FID signal, to eliminate the contribution of the aromatic fraction coeluted in the MOSH fraction. Both methods were explored in pure MOH for food and cosmetic applications.

Of high importance, in accordance with the request of the EFSA and the EU, is the possibility to reliably quantify MOAH in sub-classes, with particular emphasis on the 3-7 rings family. Moret et al. proposed an online LC–LC–GC method (41). A first large silica column (250 × 4.6 mm i.d.) retained triglycerides eluting MOH in 6 mL of pentane. An online solvent evaporator (SE) packed with silica gel guaranteed the evaporation of the solvent without loss of the most volatile components. The concentrate fraction was then transferred to an amino LC column, for further separation according to the ring number, and then online transferred to the GC–FID system. Koch et al. (54) proposed the separation of MOAH into mono-/di-aromatic fraction (MDAF) and three/poly aromatic fraction (TPAF) by previously separating the MOSH and MOAH offline, using a silver nitrate loaded silica gel column. The MOAH fraction was then further separated in a donor-acceptor-based HPLC column. Five fractions were pooled together to reach the sensitivity required and to further characterize the TPAF by GC×GC-MS.

Conclusion

The analysis of MOH in food is a challenging task, which requires careful and thoughtful optimization at every step of the analytical procedure, from sample preparation to the analytical determination, and data interpretation. Hyphenated techniques, such as LC–GC, LC–LC–GC, GC×GC, and LC–GC×GC, play a fundamental role in the advances and automation of the analysis. It is the authors' opinion that MOH determination is among the few applications that benefit from more than two chromatographic dimensions.

Further studies are needed to provide reliable and more detailed data for a full risk-assessment, and it is predicted that hyphenated techniques will once again be the key players in achieving the level of information required.

Acknowledgements

This work is supported by Fonds de la Recherche Scientifique Belgique (FNRS) (CDR projects-MOHPlatform, J.0170.20). This article is based upon work from the Sample Preparation Task Force and Network, supported by the Division of Analytical Chemistry of the European Chemical Society.

References

1) International Agency for Research on Cancer (IARC), IARC Monogr. Eval. Carcinog. Risks to Humans 100 F (2012).
2) European Food Safety Authorithy (EFSA), *EFSA J.* **10**(6) (2012).
3) M.J. Miller, E.C. Lonardo, R.D. Greer, C. Bevan, D.A. Edwards, J.H. Smith, and J.J Freeman, *Regul. Toxicol. Pharmacol.* **23**(1), 55–68 (1996).
4) L.C. Griffis, L.E. Twerdok, S. Francke-Carroll, R.W. Biles, R.E. Schroeder, H. Bolte, H. Faust, W.C. Hall, and J. Rojko, *Food Chem. Toxicol.* **48**(1), 363–372 (2010).
5) P.J. Boogaard, K.O. Goyak, R.W. Biles, L.L.P. van Stee, M.S. Miller, and M.J. Miller, *Regul. Toxicol. Pharmacol.* **63**(1), 69–77 (2012).
6) J. Cravedi, K. Grob, U.C. Nygaard, and J. Alexander, *EFSA Support. Publ.* **14**(2) (2017).
7) L. Barp, M. Biedermann, K. Grob, F. Blas-Y-Estrada, U.C. Nygaard, J. Alexander, and J.-P. Cravedi, *Sci. Total Environ.* **583**, 319–333 (2017).
8) L. Barp, M. Biedermann, K. Grob, F. Blas-Y-Estrada, U.C. Nygaard, J. Alexander, and J.-P. Cravedi, *Sci. Total Environ.* **575**, 1263–1278 (2017).
9) G.W. Trimmer, J.J. Freeman, R.A.J. Priston, and J. Urbanus, *Toxicol. Pathol.* **32**(4), 439–447 (2004).
10) L. Barp, C. Kornauth, T. Wuerger, M. Rudas, M. Biedermann, A. Reiner, N. Concin, and K.Grob, *Food Chem. Toxicol.* **72**, 312–321 (2014).
11) M. Biedermann, L. Barp, C. Kornauth, T. Würger, M. Rudas, A. Reiner, N. Concin, and K. Grob, *Sci. Total Environ.* 506–507:644–655 (2015).
12) D. Adenuga, K. Goyak, and R.J. Lewis, *Crit. Rev. Toxicol.* **47**(9), 754–770 (2017).
13) J.-C. Carrillo, A. van der Wiel, D. Danneels, O. Kral, and P.J. Boogaard, *Regul. Toxicol. Pharmacol.* **106**, 316–333 (2019).
14) European Commission, *Off. J. Eur. Union* **L12**(84), 95–96 (2017).
15) S. Bratinova and E. Hoekstra, Joint Research

Centre (JRC), *Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials. In the frame of Commission Recommendation* (EU) 2017/84 (2019). doi:10.2760/208879

16) Foodwatch International, project report *International test of various canned baby milk products for their content of mineral oil hydrocarbons (MOSH/MOAH)*, (October 2019). https://www.foodwatch.org/fileadmin/-INT/mineral_oil/documents/2019-10-24_Projectreport_babymilk_FINAL.pdf (accessed Feb 3, 2021)
17) D. Arcella, K. Baert, and M. Binaglia, *EFSA Support. Publ.* **16**(11) (2019).
18) European Commission, *Off. J. Eur. Union* **L354**(16) (2008).
19) European Commission, *Off. J. Eur. Union* **L12**, 1–89 (2011).
20) European Commission, *Off. J. Eur. Union* **L250**, 1–84 (2008).
21) European Commission, *Off. J. Eur. Union* **L153**(1), 1–186 (2011).
22) Scientific Committee of the Federal Agency for the Safety of the Food Chain, Advice 19-2017 Action thresholds for mineral oil hydrocarbons in food (2017). at http://www.afsca.be/scientificcommittee/opinions/2017/_documents/Advice19-2017.pdf (accessed Feb 3, 2021).
23) EN European Standard 16995:2017, *Foodstuffs - Vegetable oils and foodstuff on basis of vegetable oils - Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis* (2017).
24) S. Weber, K. Schrag, G. Mildau, T. Kuballa, S.G. Walch, and D.W. Lachenmeier, *Anal. Chem. Insights* **13**, 1–16 (2018).
25) G. Purcaro, L. Barp, and S. Moret, *Anal. Methods* **8**(29), 5755–5772 (2016).
26) S. Biedermann-Bremm, K. Grob, *Eur. Food Res. Technol.* **232**(6), 1035–1041 (2011).
27) S. Moret, M. Scolaro, L. Barp, G. Purcaro, M. Sander, and L.S. Conte, *Food Chem.* **157**, 470–475 (2014).
28) R. Lorenzini, K. Fiselier, M. Biedermann, M. Barbanera, I. Braschi, and K. Grob, *Food Addit. Contam. – Part A Chem. Anal. Control. Expo. Risk Assess.* **27**(12), 1765–1774 (2010).
29) M. Biedermann and K. Grob, *Eur. Food Res. Technol.* **230**(5), 785–796 (2010).
30) M. Biedermann and K. Grob, *J. Chromatogr. A.* **1255**, 56–75 (2012).
31) S. Moret, M. Scolaro, L. Barp, G. Purcaro, and L.S. Conte, *Food Chem.* **196**, 50–57 (2016).
32) S. Moret, M. Sander, HG. Purcaro, M. Scolaro, L. Barp, and L.S. Conte, *Talanta* **115**, 246–252 (2013).
33) S. Moret, L. Barp, K. Grob, and L.S. Conte, *Food Chem.* **129**(4), 1898–1903 (2011).
34) K. Fiselier, F. Grundböck, K. Schön, O. Kappenstein, K. Pfaff, C. Hutzler, A. Luch, and K. Grob, *J. Chromatogr. A.* **1271**(1), 192–200 (2013).
35) K. Fiselier, D. Fiorini, and K. Grob, *Anal. Chim. Acta* **634**, 96–101 (2009).
36) C. Wagner, H.P. Neukom, V. Galetti, and K. Grob, *Mitt Leb. Hyg* **92**, 231–249 (2001).
37) B. Maurus, F. Katell, and G. Koni, *J. Agric. Food Chem.* **57**(19), 8711–8721 (2009).
38) M. Nestola and T.C. Schmidt, *J. Chromatogr. A.* **1505**, 69–76 (2017).
39) M. Biedermann, C. Munoz, and K. Grob, *J. Chromatogr. A.* **1624**, 461236 (2020).
40) M. Biedermann, C. Munoz, and K. Grob, *J. Chromatogr. A.* **1521**, 140–149 (2017).
41) S. Moret, K. Grob, and L.S. Conte, *J. Chromatogr. A.* **750**(1–2), 361–368 (1996).
42) K. Fiselier, D. Fiorini, and K. Grob, *Anal. Chim. Acta* **634**(1), 102–109 (2009).
43) M. Lommatzsch, M. Biedermann, T.J. Simat, and K. Grob, *J. Chromatogr. A.* **1402**, 94–101 (2015).
44) M. Zoccali, L. Barp, M. Beccaria, D. Sciarrone, G. Purcaro, and L. Mondello, *J. Sep. Sci.* **39**(3), 623–631 (2016).
45) S. Moret, L. Barp, G. Purcaro, and L.S. Conte, *J. Chromatogr. A.* **1243**, 1–5 (2012).
46) G. Purcaro, P.Q. Tranchida, L. Barp, and S. Moret, *Anal. Chim. Acta* **773**, 97–104 (2013).
47) G. Purcaro, S. Moret, and L. Conte, *J. Chromatogr. A.* **1255**, 100–111 (2012).
48) European Parliament and the Council of the European Union, *Off. J. Eur. Communities* (L 221/8), 8–36 (2002). doi:10.1017/CBO9781107415324.004
49) M. Biedermann and K. Grob, *J. Sep. Sci.* **32**(21), 3726–3737 (2009).
50) M. Biedermann and K. Grob, *J. Chromatogr. A.* **1375**, 146–153 (2015).
51) S. Pantó, M. Collard, and G. Purcaro, *Curr. Trend Mass Spectrom.* **18**(3), 1–6 (2020).
52) A.R. García-Cicourel, B. van de Velde, J. Verduin, and H.G. Janssen, *J. Chromatogr. A.* **1607**, 460391 (2019).
53) A. R. García-Cicourel, B. van de Velde, G. Roskam, and H. G. Janssen, *J. Chromatogr. A.* **1614**, 460713 (2020).
54) M. Koch, E. Becker, M. Päch, S. Kühn, and E. Kirchhoff, *J. Sep. Sci.* **43**(6), 1089–1099 (2020).

Nicola Sdrigotti graduated with honours from the University of Udine, Italy, with an MSc in food quality control. The first approach with analytical chemistry started in the field of electrochemistry, at his home University. Motivated by the desire for knowledge, he took part in two Erasmus experiences abroad, first in the field of proteomics at the Danish National Food Institute, and then in the field of advanced chromatography for mineral oil analysis at the Gembloux Agro-Bio Tech institute, Belgium.
Gregory Bauwens graduated in bioengineering (chemistry orientation) from the University of Liege, Belgium. His master thesis focused on the optimization of a completely hyphenated LC–GC×GC–TOF-MS/FID platform for the analysis of mineral oil. Being very interested in this topic, he has recently started a PhD on the application of advanced analytical techniques for the determination of mineral oil in foods.
Giorgia Purcaro has been analytical chemistry professor at the Gembloux Agro Bio Tech Department of the University of Liége (Belgium) since 2018. Her research interests include the development of advanced multidimensional and comprehensive chromatography techniques (GC×GC, LC–GC, LC-GC×GC) and miniaturized sample preparation approaches for food quality and safety applications. She was awarded the Leslie S. Ettre Award in 2010, and, in 2015, received the J. Philipps award for her contribution to the GC×GC field. She has authored or co-authored over 80 peer-reviewed publications, 10 book chapters, and more than 150 conference presentations.
Direct correspondence to: amatheson@mjhlifesciences.com

Surfing on Mobile Phase, Part 1: Origins of Mobile-Phase Composition Waves and their Effects on Detector Baselines

Dwight R. Stoll, LC Troubleshooting Editor

The most commonly used designs for modern liquid chromatography (LC) pumps produce mobile-phase streams with small short-term variations in mobile phase composition. Understanding the origin of these variations and their effects on chromatographic performance can help us develop high-performing methods. In this instalment, I focus on the effect of these mobile-phase composition "waves" on detector baselines.

This month's instalment of "LC Troubleshooting" is motivated by communication I had with readers in recent months about mobile phase composition, pumps, and mixing. Although these topics have been discussed in this column frequently in the past, they are moving targets to some extent because pump technology for liquid chromatography (LC) continues to evolve. Therefore, user expectations for pump performance in demanding applications also change. As an example of the ongoing importance of these issues, below is an excerpt of an email I received recently from an LC user:

> *"We have two [brand name LCs] with quaternary pumps that are acting up. If we try to run the system using 50% A and 50% B, we get retention time shifts. If we premix the solvent and just run channel A, all is fine. Gradients make the problem worse...so obviously there is a problem with the mixing."*

In this instalment, I review the operating principles of LC pumps that rely on low- or high-pressure mixing approaches, describe how waves of solvent composition can develop in the mobile phase, and evaluate the potential impacts of these waves on detector baselines. In next month's instalment, I will discuss the results of simulations that show how these waves can impact variability in retention time along with some solutions to these problems. One could devote an entire book chapter to these topics, so I discuss the highlights here, providing a concise overview of LC pump technology. For readers that are interested in learning more, I encourage considering the following resources: the books by Kromidas (1) and Snyder and Dolan (2) have chapters dedicated to modern LC pump technology, details about performance specifications, and descriptions of tests that can be used to evaluate pump performance; two "LC Troubleshooting" articles by John Dolan in 2006 (3)and 2014 (4) describe case studies that illustrate what can happen when things go wrong in the pump; and Choikhet and co-workers (5) and Gritti (6) discuss in great detail the impact of imperfect pump performance on LC applications involving trifluoroacetic acid (TFA) in the mobile phase. These are all excellent resources for those looking to add to their LC troubleshooting knowledge.

Review of LC Pumping Principles—Low- and High-Pressure Mixing Designs

Figure 1 illustrates the basic principles of the two most commonly used designs for pumping systems used in LC. In the case of the high-pressure mixing approach, two independent pump heads, each capable of producing a high-pressure stream of a mobile-phase component, draw in and discharge solvent at a consistent flow rate (for isocratic operation). For example,

FIGURE 1: Block diagrams for the two most commonly used designs of high performance liquid chromatography (HPLC) pumps in use today: (a) Binary pump with high pressure mixing; and (b) Quaternary pump with low pressure mixing.

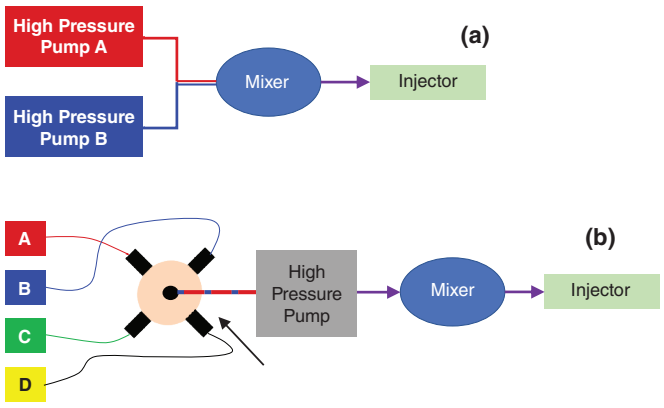
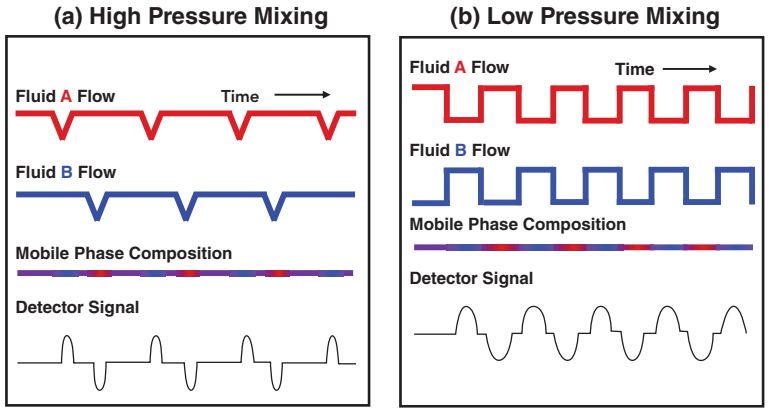


FIGURE 2: Conceptual illustration of the origin of mobile phase composition waves in the case of (a) high pressure mixing and (b) low pressure mixing designs used in modern pumps.



if the total flow rate through the column is 1.0 mL/min, and the desired mobile phase composition is 40:60 acetonitrile:water, then one pump head discharges acetonitrile continuously at 0.4 mL/min, and the other pump head discharges water continuously at 0.6 mL/min. The two streams discharged from the pump heads then converge and pass through a mixer, and the mixed mobile phase proceeds to the sampler, and eventually the column. In the case of the low-pressure mixing approach, there is only one pump head that pressurizes the mobile phase to drive it through the column. The mobile phase composition is determined by assembling small "packets" of individual solvents in a serial fashion into a mobile-phase stream that is drawn into the high pressure pump. In most modern pumps of this design, the volume of each solvent packet is determined by the length of time a solenoid-type valve is open between the solvent bottle and the proportioning valve unit. Furthermore, these times are also related to the volume of each stroke of the high pressure pump and the mobile-phase flow rate. For example, suppose the stroke volume is 100 µL, the desired mobile phase composition is 40:60 acetonitrile:water, and the flow rate is 1 mL/min. The period of each pump stroke will be 6 s; the solenoid for the acetonitrile line will be open for 2.4 s, drawing pure acetonitrile into the tubing leading from the proportioning valve to the pump head. Then, this valve closes, and the solenoid for the water line opens for 3.6 s and pure water is drawn into the tubing. This completes one cycle of mobile phase composition proportioning. The solvent composed in this way is mixed extensively as it travels through the high-pressure pump head itself, and an additional mixer is positioned between the pump and the sampler.

Origins of Solvent Waves and Their Impacts on Detector Baselines

Each of the pump designs discussed above has several strengths and weaknesses. In both cases, however, the mobile-phase composition at the pump outlet will not be perfectly smooth (that is, no variation in composition during isocratic operation; in gradient elution, the change in composition over time would ideally be smooth without any short-term noise). The primary causes of the deviations of the actual mobile phase composition from what is programmed are different for the two designs. In the case of the low-pressure mixing approach, it is intuitive that there would be short-term variations in composition on the timescale of one pump stroke. During the pump stroke, there are times when the fluid entering the high-pressure pump is literally all A or all B. This is illustrated in

FIGURE 3: Comparison of ultraviolet (UV) absorbance signals (214 nm) obtained with different mixers in use. The pump was a high-pressure binary mixing system (Agilent 1290, Infinity II). The column was a 30 mm × 2.1 mm i.d. Agilent SB-C18, and gradient elution was used. Solvent A was 0.1% trifluoroacetic acid (TFA) in water, solvent B was 0.1% TFA in acetonitrile (ACN), and the gradient ran from 2–40% B in 4 min.

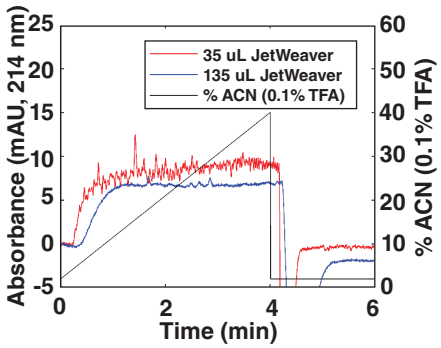


Figure 2b. The resulting variation in mobile phase composition can be smoothed to a large degree with effective mixing downstream from the high pressure pump, but completely eliminating the variation would require a large volume mixer. If the detector is capable of detecting small variations in composition (for example, refractive index detection, or ultraviolet [UV] detection in the case where mobile phase additives absorb UV light, such as TFA), then they will be observable in the detector signal as "waves", or short-term noise. Adding a large mixer introduces other problems, such as a large delay between the time of a programmed change in composition and when that change arrives at the column (in gradient elution this appears as the "gradient delay" or "dwell" time). Thus, the configurations of these pumps, as received from manufacturers, reflect a compromise between doing enough mixing to smooth out these waves to a large extent and not adding a mixer that is so large that it causes other problems. Indeed, pump manufacturers offer mixers of different volumes that allows the user to choose a larger volume mixer for applications that are expected to be especially sensitive to short-term variations in mobile phase composition (for example, applications involving TFA).

The primary origin of solvent waves in the case of high pressure mixing is fundamentally different. In this case, if the flow rate from each pump head were perfectly consistent over time, the composition of the mixed mobile phase would be perfectly consistent over time. But, in a reciprocating piston design (which is the dominant design in use today), there are small changes in the flow from each pump head at the end of a piston stroke due to the imperfect operation of check valves. These small changes in flow are illustrated in Figure 2a. If these flow rate changes are different for channels A and B, and they are not perfectly synchronized in time, then there will be a small, short-term variation in the composition of the mixed mobile phase. The lengths of the resulting waves in this case tend to be shorter in comparison to the low pressure mixing case, as illustrated in Figure 2. These waves

can also be greatly minimized by introducing a mixer between the solvent convergence point in the pump and the LC column. However, the same challenge exists here as with the low-pressure mixing design: Completely eliminating the waves requires a large mixer, so what we use in practice represents a compromise between smoothing the mobile phase composition and having a low gradient delay volume (which is essential for fast gradient elution separations, for example).

As mentioned above, the mobile-phase composition waves that flow from the pump can impact the quality of detector baselines (as measured by noise and drift) if the detector signal is dependent on the composition of the mobile phase itself. Most of the time, this can be avoided or minimized through judiciously choosing the conditions and instrument parameters. For example, acetonitrile is attractive as a mobile-phase organic solvent modifier for reversed-phase liquid chromatography (RPLC) when using UV detection at low wavelengths (< 230 nm). If the mobile-phase components are effectively transparent to the detector, then small variations like waves in the composition will not impact the quality of the detector baseline signal. Sometimes, though, this is impossible, or at least very difficult to avoid. One well known and studied example is the case where TFA is used as a mobile phase modifier for RPLC separations of peptides involving UV detection. TFA is attractive because it tends to improve peak shapes for peptides and increase retention for hydrophilic peptides. However, a disadvantage of TFA in this context is that it absorbs a significant amount of UV light at 214 nm, which is the wavelength typically used for peptide mapping applications. Furthermore, the TFA itself is somewhat retained by RPLC stationary phases. When a mobile phase composition wave travels through the column, the acetonitrile-rich part of the wave will decrease the local retention of TFA, dumping more of it into the mobile phase where it will absorb more UV light. This is a very complex situation that cannot be thoroughly discussed here, but there are at least two excellent papers (5,6) that describe all of the factors involved and demonstrate the impact of different chromatographic variables on baseline quality when using TFA in the mobile phase with UV detection. I strongly encourage readers interested in learning about this situation in more detail to consult these papers.

In a previous instalment of "LC Troubleshooting", I discussed why mobile phase mixers are needed following LC pumps, and the types of situations when a change in the type of mixer might be needed (7). Figure 3 shows the effect of increased mixer volume on the noise level in UV detector baselines when using TFA in the mobile phase delivered by a binary pump. Although the 35 µL mixer might be adequate for other applications, a larger volume mixer is helpful for reducing baseline noise in this case because of the increased sensitivity of the baseline noise to mobile phase composition waves when using TFA as an additive.

Summary

In this column, I have reviewed the operating principles of modern LC pumps based on low- and high-pressure mixing designs and explained how these pumps produce mobile phase streams with small short-term variations in mobile phase composition. These composition "waves" can negatively affect detector baseline quality and also retention time variability. In next month's instalment of "LC Troubleshooting", I will continue exploring this topic by discussing the results of simulations that illustrate the effect of method parameters including the amplitude of the composition waves, flow rate, and pump stroke volume on retention time precision.

References

1) S. Kromidas, ed., *The HPLC Expert II: Find and Optimize the Benefits of your HPLC/UHPLC* (Wiley-VCH, Weinheim, 2017).
2) L.R. Snyder and J.W. Dolan, *High-Performance Gradient Elution: The Practical Application of the Linear-Solvent-Strength Model* (John Wiley, Hoboken, NJ, 2007).
3) J.J. Gilroy and J.W. Dolan, *LCGC North America* **24**(7), 662–667 (2006).
4) J. Chow and J.W. Dolan, *LCGC North America* **30**(4), 312–318 (2012).
5) K. Choikhet, B. Glatz, and G. Rozing, *LCGC Europe* **16**(2), 2–9 (2003).
6) F. Gritti, *J. Chromatogr. A.* **1633**, 461605 (2020). https://doi.org/10.1016/j.chroma.2020.461605.
7) D. Stoll, *LCGC Europe* **31**(11), 608–613 (2018).

COLUMN EDITOR

Dwight R. Stoll is the editor of "LC Troubleshooting." Stoll is a professor and the 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 70 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: amatheson@mjhlifesciences.com

Flying High with Sensitivity and Selectivity: GC–MS to GC–MS/MS

Nicholas H. Snow, Department of Chemistry and Biochemistry, Seton Hall University, New Jersey, USA

Mass spectrometry (MS), often termed mass selective detection, is the most powerful detector available for gas chromatography (GC). Multidimensional mass spectrometry (MS/MS) takes mass selective detection to another level on benchtop systems, offering both universal and selective detection along with low detection limits. In this instalment of "GC Connections," we review the fundamentals of MS/MS and how they relate to MS as a detector for GC. We see how using full-scan analyses can make the detector universal and how by using selected ion monitoring and multiple reaction monitoring the detector can be so selective and noise-free that femtogram quantitative analysis is commonplace. We then examine some scenarios that should lead analysts to consider using GC–MS/MS to solve complex problems.

High sensitivity and selectivity are among the most important goals of any chromatographic method development or optimization process. Instruments, stationary phases, and detectors are usually chosen with one or both of these goals in mind. In gas chromatography, mass selective detectors (MSDs or mass spectrometers) have been used for decades to provide both high selectivity and high sensitivity. Capillary gas chromatography coupled to mass spectrometry (GC–MS) is a straightforward, yet powerful coupling of the selectivity of GC with the high sensitivity and option of universal or selective detection of MS. Traditional GC–MS provides multiple dimensions of separations and low detection limits in benchtop or smaller instruments.

Before flying into the details of MS/MS, we should briefly review some of the terminology specific to MS as a detector for GC. Mass selective detectors operate in two modes. The first mode is full-scan, in which spectra are continuously collected in quadrupole systems at rates usually up to 10–20 spectra per second depending on the mass range selected. The second mode is selected ion monitoring (SIM), in which one or more individual ions are monitored. The data can be obtained in three forms:

- A total ion chromatogram (TIC) is the sum of all signals that reach the detector and is a demonstration of nearly universal detection. The full mass spectrum can be obtained at any point on the chromatogram.
- An extracted ion chromatogram (EIC) is obtained from the TIC by choosing one or more individual masses and extracting these from the full data set. This allows both universal and selective detection in a single experiment, since the ion chosen for analysis can be characteristic of a single compound or compound class.
- Selected ion monitoring (SIM) is obtaining a TIC in which the detector is set to monitor only one or a few ions. If a spectrum is selected from the TIC, it will only show the few ions that were selected when the experiment was set up.

There are several common GC–MS and GC–MS/MS instruments. Single dimension, classical GC–MS is mainly performed using quadrupole mass analyzers. Ion trap, a derivative of quadrupole instruments and time-of-flight (TOF) are also used for specific analyses. Quadrupole-based systems are simpler and less expensive; GC–TOF-based systems offer the highest sensitivity and much greater mass precision and accuracy.

GC–MS/MS can be achieved through several configurations, with a wide range of capability and complexity. The most common of these is GC–triple quadrupole-MS (GC–TQMS), while GC–ion trap-MS (GC–ITMS) and GC-quadrupole time-of-flight MS (GC–QTOF-MS) are also available. A brief discussion of the evolution of ion trap and triple quadrupole mass analyzers over the years is provided in the brochure by Huebschmann

FIGURE 1: Comparison of GC–MS and GC–MS/MS instrument configurations. (a) GC–MS and (b) GC–TQMS.

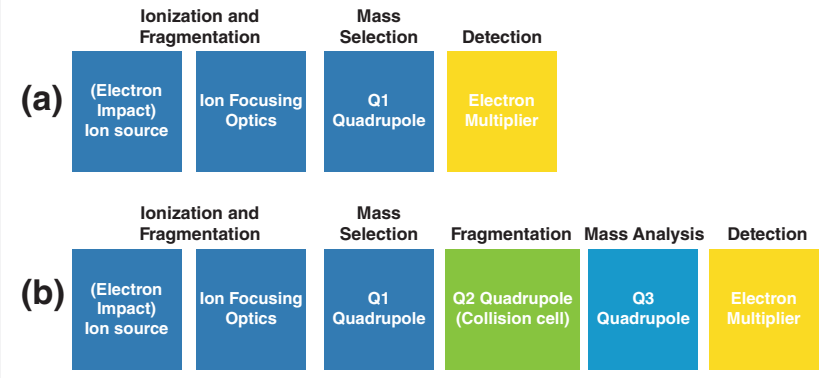
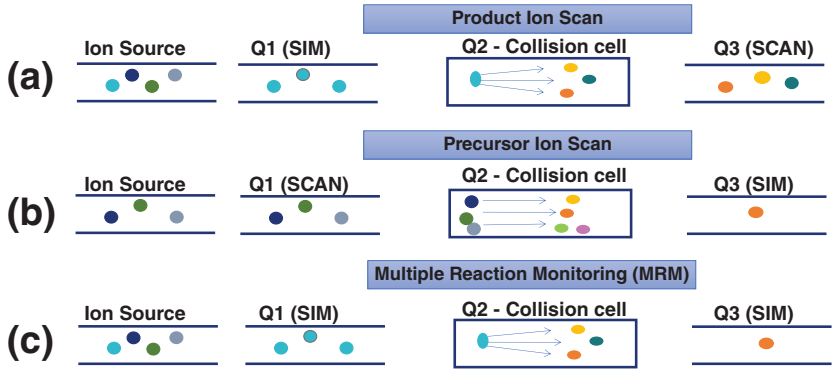


FIGURE 2: Modes of GC–MS/MS Operation. (a) product ion scan; (b) precursor ion scan; and (c) multiple reaction monitoring.



(1). Professors Chris Enke and Rick Yost, inventors of the TQMS, have provided two excellent video interviews discussing the development of the technique in detail (2,3).

Figure 1 shows a block diagram of the detector on a GC–TQMS system compared to a traditional GC–MS system. Both are available in benchtop configurations. The main difference between the two systems is the presence of three quadrupole mass filters on the GC–TQMS system and one on the GC–MS system. Both systems use a transfer line with a capillary direct interface into the ion source and classical electron ionization ion source between the GC and the mass analyzer. Both operate with the ion source and mass analyzer at high vacuum and use a classical electron multiplier to detect ions that pass through the mass analyzer. As described in more detail below, the first quadrupole (Q1) performs in the same manner as the single quadrupole in traditional GC–MS, selecting the ions that are ultimately passed to the electron multiplier detector. It can operate in either full-scan or selected ion monitoring modes. The second quadrupole (Q2) is used as a medium for collision induced fragmentation of ions passed through the first quadrupole to produce new fragments and the third quadrupole (Q3) is used to select and analyze these new fragments.

MS/MS is among the most flexible of all detectors as it operates in several modes. In traditional GC–MS, full-scan MS provides a nearly universal detector; any analyte that can be ionized within the ion source can be detected. SIM-MS is a highly selective detector; the signals for the chosen ions are the only ones recorded. SIM is used for quantitation as the reduction in the signals being monitored versus full scan also reduces the noise, increasing the signal-to-noise (S/N) ratio and therefore lowering the detection limit.

Figure 2 shows how the most common modes of quadrupole MS and MS/MS detection work. Full-scan and SIM single quadrupole detection are seen in the left side of the figure, in the ion source and Q1 images. The ion source generates ions including many masses; the quadrupole can either pass all of them (full scan) or selected ions (SIM). Triple quadrupole MS offers even more flexibility, since two additional quadrupoles are employed, as seen in Figure 2. Note that in both GC–MS and GC–MS/MS, classical electron ionization (EI) is by far the most commonly used ion source mechanism, so this is assumed in the following discussion.

A GC–MS/MS system can be operated exactly as a single quadrupole system. The second and third quadrupoles can be set to pass ions through without any further separation or reaction. This is often the first step in developing a method or transferring one to GC–MS/MS as it provides a traditional total ion chromatogram and traditional mass spectra of the analytes as a starting point.

INTRODUCING THE NEW VICI®
D-3 PULSED DISHCARGE HELIUM IONIZATION DETECTOR

DESIGNED FOR THE AGILENT 8890 GC

VICI model D-3-I-8890 for the Agilent 8890 joins the lineup of Pulse Discharge Detectors already available for plug-and-play installation on the Agilent 6890 and 7890, and is optimized for trace level work in the helium ionization mode. Each kit includes all components required for installation:

- Detector
- Pulser module
- Tubing
- Helium purifiers
- Power supply
- Fittings

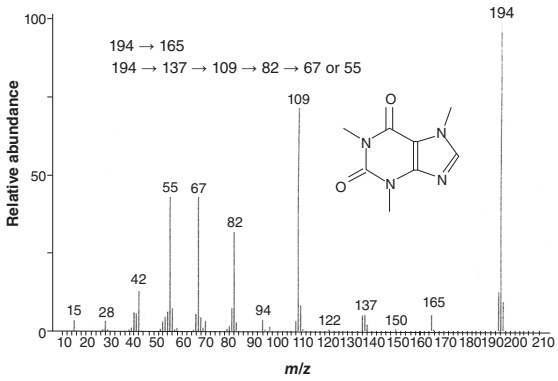
FEATURES

- Universal detector
- Wide linear dynamic range (>5 orders LDR, from low ppb to high ppm)
- Concentration Sensitive
- Easily added and configured on new or existing 8890 GC, using Agilent electrometer and interface boards

www.vici.com +41 (41) 925 62 00 info@vici.ch

VICI

FIGURE 3: Full-scan mass spectrum and mass fragment transitions for caffeine.



In a product ion scan, the first quadrupole (Q1) can be set for selected ion monitoring, as in traditional GC–MS. Collision-induced ionization then occurs in the second quadrupole (Q2) to generate further fragmentation of the chosen ion, providing additional fragmentation that is analyzed by scanning the third quadrupole (Q3). This is a powerful tool for qualitative analysis since larger fragments from a traditional single dimension mass spectrum can be further fragmented to aid in confirming the correct structure. A product scan is also used for choosing transitions in initial method development for multiple reaction monitoring.

In a precursor ion scan, Q1 can be operated full scan, which passes all of the fragments generated in the ion source; think of traditional MS without the detector, with all of the resulting fragments reionized in Q2 and a single fragment chosen for monitoring in Q3. This is very similar to SIM in traditional GC–MS and is useful for quick quantitative analysis method development.

In multiple reaction monitoring (MRM), the most sensitive mode for quantitative TQMS, both Q1 and Q3 are set for single ion analysis. Based on the full scan mass spectrum or a product ion scan, a fragment from Q1 is chosen and then based on the further fragmentation in Q2, as seen by scanning Q3, a single fragment from Q3 is chosen. This provides possibly the ultimate selectivity as the possibility of two compounds, even closely related isomers, having the same transitions from precursor ion fragment to product ion fragment and the same (or close) retention time in the column decreases greatly. MRM also provides very low detection limits by significantly reducing noise in both dimensions.

Figure 3 shows the well-known mass spectrum for caffeine which provides an example for the utility and power of MRM. The mass transitions that generate the signals seen in the mass spectrum are also provided. In MRM, this full-scan spectrum is the starting point for method development, either determined experimentally or obtained from the literature. As this is an election ionization spectrum, it is good practice to interpret the spectrum, using traditional spectral interpretation rules at this point for a full understanding of the structural elements and decomposition reactions that generate each of the fragments. There are multiple excellent tutorial books relating to election ionization mass spectral interpretation available (4,5). Should there be additional spurious peaks, these can be identified at this point so they do not cause confusion later.

In traditional single dimension GC–MS, the full-mass spectrum is used to identify the analyte. Quantitation is performed using either extracted ion chromatograms obtained by selecting one or more individual masses from the total ion chromatogram or by selected ion monitoring. SIM offers lower detection limits by reducing noise as most of the mass signals seen in the full-scan spectrum and their accompanying noise are eliminated.

In MRM, further advantage is taken of this noise reduction. One or more peaks from this initial mass spectrum is then chosen for further fragmentation. Usually this is the largest (base) peak, or it can be another strong signal that may be more characteristic of that compound. With this mass chosen, a second experiment is performed with Q1 operating in SIM mode to pass the one ion, termed a Precursor ion, with that ion being reionized in Q2, with the resulting new fragments, termed product ions, passed to Q3 operating in scan mode as seen in the Figure 2 product scan, to generate a mass spectrum of the fragment. One or more ions from this product spectrum can then be chosen for the final quantitative method. Any of the transitions seen in Figure 3, such as those from mass 194 to mass 109 are termed MRM transitions and are often reported in the literature for completed methods. Any of the transitions can be used with one for quantitation and additional transitions for confirmation. Selectivity is generated because instead of looking at individual masses which may not be unique to a compound, this is looking at transitions, which

are very unique, especially when multiple transitions are used.

The ability to obtain spectra from precursor ions and to perform MRM leads to three situations in which GC–MS/MS is especially useful:

1. Targeted analysis of a few analytes, in which you know the identity of the analyte or analytes, for which extreme sensitivity or low detection limits are required and/or the sample matrix is highly complex.
2. Simultaneous targeted analysis of many analytes whose chromatographic peaks are not fully resolved.
3. Untargeted analysis in which the matrix is complex, analyte chromatographic peaks are overlapped, and the additional qualitative information about fragmentation is needed.

Analysis of extremely low levels of emerging contaminants in environmental water samples is an example of the first case. These appear at very low levels and are the result of human activity. One application, freely available online, shows analysis of several steroids in water at low parts per billion (ppb) and parts per trillion (ppt) levels using solid-phase micro-extraction coupled to GC–MS/MS (6). In this work, the sample preparation and detection were optimized but the chromatography was not, so it also illustrates the second problem: the analyte peaks are not resolved by the chromatography but are easily resolved using their differing MRM transitions. In a more extreme example, over 300 pesticides were extracted from apples and determined simultaneously in a single run using GC–MS/MS (7). This work illustrates the second and third cases: there are many analytes, the analysis can be either targeted or untargeted, and the sample matrix is a complex food sample.

MS/MS provides the ultimate in detection for capillary gas chromatography. It can be both universal (full scan) or selective (SIM or MRM) and it is highly sensitive with detection limits of femtograms readily available. MS/MS is especially suited to the most difficult separation and detection problems. The trade-off of this capability is capital and ongoing expense. MS/MS detectors are expensive, with fully loaded systems costing hundreds of thousands of U.S. dollars and have higher ongoing costs than traditional GC–MS systems, requiring special training to operate and needing additional maintenance compared to GC and GC–MS systems. The high sensitivity and low detection limit of MS/MS makes it especially sensitive to laboratory conditions such as clean carrier gases and careful sample preparation. Errors in sample preparation and contamination issues are often amplified when instrumental noise from the detector is lowered, so special care in sample preparation and laboratory management of GC–MS/MS is required. The unmatched combination of sensitivity with the ability to be both selective and universal makes MS/MS the most powerful detection tool available for capillary gas chromatography.

References

1) H.J. Huebschmann, "Workhorses of the Chromatography Lab: Quadrupoles and Ion Traps," Thermo-Fisher (Waltham, Massachusetts, 2014). http://tools.thermofisher.com/content/sfs/brochures/WP-10427-GC-LC-MS-Workhorses-Quadrupoles-Ion-Traps-WP10427-EN.pdf.
2) *A Look Back at the Birth of the Triple Quadrupole Mass Spectrometer* (video), https://www.youtube.com/watch?v=whEO8kspM_g&vl=en Agilent Technologies (Santa Clara, California, 2017).
3) *One Man's Noise: Christie G. Enke remembers development of the Triple Quad Mass Spectrometer* (video) https://vimeo.com/102356325 Science History Institute (Philadelphia, Pennsylvania, 2014).
4) F.W. McLafferty and F. Turecek, Interpretation of Mass Spectra, *4th Edition* (University Science Books, Sausalito, California, 1993).
5) R. Martin Smith, *Understanding Mass Spectra, A Basic Approach* (John Wiley and Sons, New York, New York, 2004).
6) S. Chopra, P.F.C.L. Gomes, R. Dhandapani, and N.H. Snow, *Scientia Chromatographica.* **6**(2), 105–116. (2014). https://www.iicweb.org/scientiachromatographica.com/files/v6n2a02.pdf
7) H.J. Schulte, H-U. Baier, S. Moreau, and K. Bollig, "Fast GC-MS/MS Analysis Of Multicomponent Pesticide Residues (360) In Food Matrix," Shimadzu (Columbia, Maryland, 2015). https://www.shimadzu.com/an/sites/shimadzu.com.an/files/pim/pim_document_file/technical/white_papers/12389/ego215070.pdf

COLUMN EDITOR

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

What's Good About the WHO Good Chromatography Practices Guidance? Part 2

R.D. McDowall, R.D. McDowall Ltd, Bromley, Kent, UK

In September the World Health Organization (WHO) issued a new guidance document on Good Chromatography Practices. What guidance does it contain and is it useful? Has the document failed its system suitability test (SST) acceptance criteria?

In September 2020 the WHO issued a guidance document on Good Chromatographic Practices (1). The scope, content, and sections of the document dealing with the system and its set up were discussed in Part 1 (2). In this part we'll take a close look at the sections dealing with chromatography analysis and see if any additional bouts of lalochezia occur.

Chromatographic Analysis

Let's get down to some chromatography, as the remainder of the guidance document is focused on analysis of samples, as shown in Figure 1, and has the following sections:

- Solvents, buffer solutions and mobile phases (Section 9)
- Column management (Section 10)
- Sample management and sample set (Section 11)
- Chromatographic methods (acquisition and processing) (Section 12)
- Peak integration (Section 13)
- Data management (Section 14)

I am at a loss to understand the difference between processing and peak integration in Sections 12 and 13 respectively? Especially as there is no mention of processing in Section 12!

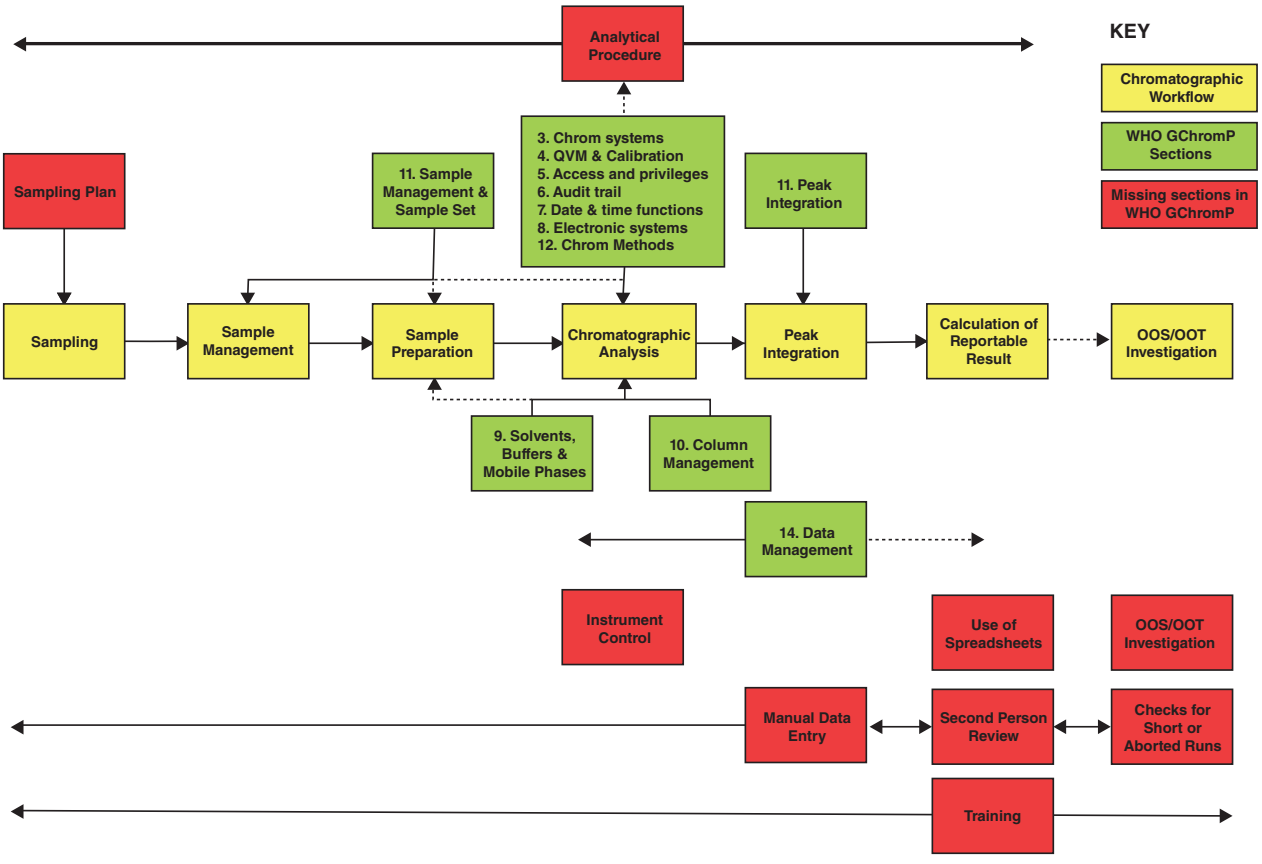
OK, competition time! What's missing from the list above? Give up? Quite a lot! Figure 2 shows the contents of the GChromP document (green boxes) mapped against a generic analytical process (yellow boxes) with the main missing tasks (red boxes). Presented in this way you can see the areas that the WHO guidance omits. The dashed lines indicate partial coverage in the WHO guidance document. There is no mention of the following that I believe should be part of a chromatography guidance to ensure data integrity:

- **Analytical Procedure:** The overall analytical procedure is the controlling document for the work and this is not mentioned here as such. This is not to be confused with the chromatographic method that is discussed partly in Section 11 and in more detail in Section 12 of this guidance as this only focuses on column management then the set up and processing within the chromatography data system (CDS). The difference between a procedure and method was discussed in a stimulus paper published in Pharmacopoeial Forum by Schofield et al. (3). Here the difference between procedure and method was stated as: *the term analytical method refers to operational components comprised of instrumentation, reagents, standards, sample preparations, calibrations, controls, and suitability criteria on the system. … the method is the "ruler" used to make measurements. A measurement is an output from a single implementation of the method on a sample of a test article. By contrast the term analytical procedure will refer to a use of the method to make a decision…*for example, batch release. If the WHO guidance only wanted to focus on the method why not say so?
- **Sampling Plan:** A sampling plan as the guidance only starts

when the sample arrives in the laboratory, but if the sample is not taken correctly then all the work in the laboratory is wasted. WHO have published detailed guidance for sampling with three suggested plans (4) but this is not referenced here.

- **Sample Preparation:** The phase where samples are prepared for injection into a chromatograph is not mentioned apart from 11.4. This is the most critical part of the analytical process after sampling and can be associated with the most experimental error. It is also mostly manual with paper records and is a key point for ensuring ALCOA+ criteria (attributable, legible, contemporaneous, original, and accurate, plus complete, consistent, enduring, and available) are met plus assessing if any data integrity lapses or falsification have occurred.
- **Manual Data Entry:** Manual entry of data into the CDS (for example, sample identities, weights, dilutions, and so on) are not mentioned. This a key area to check for transcription errors.
- **Instrument Control:** There is no mention of instrument control of the chromatograph, that is a feature of most chromatography data systems.
- **Comparative Technique:** There is no mention of the fact that chromatography is a comparative technique and that standards and samples should be integrated similarly to avoid biasing the results. It is essential that this is included in Section 13 on peak integration but is not.
- **No Spreadsheets:** Calculations should be performed within the CDS, not outside of it, to avoid transcribing and checking manual data entries.
- **Second Person Review:** No second person review of data generated is mentioned apart from one word: reviewed. This is a key requirement for ensuring the integrity of any laboratory result and the underlying records and data, and it is missing in action. Given its importance, there should be a separate section to detail the review tasks by a second person.
- **Short or Aborted Runs:** Injecting one or two sample injections is a current practice for unofficial testing, to see if the batch passes

FIGURE 1: Mapping the WHO Guidance on Good Chromatography Practices to an Analytical Process.



or not. This is an area that needs to be checked by the second person review as a minimum and as part of a quality assurance (QA) data integrity audit.

- **OOS Results:** Missing in action, but an investigation should follow the out of specification (OOS) results guidance from the FDA (5) this is a current area of testing into compliance by invalidating these results (6).
- **Training:** There is no mention of training in either data integrity or ethics, as well as chromatographic analysis, especially integration, anywhere in this document.

How much a guidance document goes into detail is at the discretion of the authors. However, if items are to be omitted from the scope of a guidance document then it is the responsibility of the authors to state this. In this respect the authors and quality oversight for the document have failed. Please excuse me while I have another bout of lalochezia.

Reagents and Column Management

Section 9 deals with solvents, buffers, and mobile phases and is relatively straightforward except for one phrase. Clause 9.1 covering the use of these items states in the last sentence: *These should be used within appropriate, scientifically justifiable timelines.* Why not simply say that expiry dates should be justified scientifically?

Section 10 covers column management and provides general

advice about column management, from purchase to how to handle the column in the laboratory and keeping records of each one's use. However, there should be a warning that if a column is used for different analytical methods, it may change the chromatographic properties over time. Ideally it is better to have dedicated columns, though cost may be a factor in some laboratories.

Clause 10.4, requiring chromatograph tubing and fittings to be appropriate (but you are left to define that!), is in the wrong place and should be in Section 3 on chromatographs. Given the organization of the document…

Clause 10.5 suggests that the only method of monitoring a column performance is to calculate the theoretical plates. In my experience, very few laboratories use plate count to measure column performance—it is an academic exercise because it depends on the separation in question. For example, if separation of two closely running peaks is required, then peak resolution is a better criterion. You could have a situation where the plate count is acceptable but peaks are not resolved. Perhaps a better way of expressing this is that the criteria for monitoring any column's performance should be scientifically sound. This leaves the laboratory to select and monitor appropriate criteria through system suitability tests (SSTs). On the topic of SSTs, clause 4.8 mentions that on SST failure there should be a (corrective action and preventative action) CAPA generated. This is stupid. What must happen is that the failure must be documented, investigated, and appropriate action agreed which may include a CAPA plan but not necessarily so. Consider a pump leak as the source of SST failure: document, replace the seal, requalify, and get on with the analysis, there is no need for a CAPA. It raises the question: how many of the authors have actually worked in an analytical laboratory?

Sample Management and Preparation

Sample management is presented in overview but sampling and the sample plan is omitted, and so is reference to the overall analytical procedure. Notwithstanding, the outline of sample preparation and placing the vials in the correct order to match the sequence file order is presented well. However, from 11.9 to 11.13 we move into SST injections and analysis of the samples before discussing the chromatographic method in Section 12 and therefore these are in the wrong sequence (sorry!). Unfortunately, manual entry of sample information such as identity, lot number, weight, dilution factor, and standard purity, which can be a source of transcription errors, is not even considered.

Trial Injections

Clause 11.9 is badly phrased and states that *trial or system check injections that are not specified as an injection sequence is not recommended. (Normally, only standard solutions may be used for this purpose, unless otherwise needed and justified (e.g. biologics).* This is similar to the approach that Heather Longden and I discussed in a recent Questions of Quality on peak integration (7) but is written more clearly. There is a Level 2 guidance question and answer on the FDA website that goes into more detail about this topic by asking question 17: *Is it ever appropriate to perform a "trial injection" of samples?*

No. ….. This is in ***contrast to the appropriate practice where an injection of a standard is performed with the sole intention of determining***

if the chromatographic system is fit for purpose. *The injection of trial samples is not acceptable, in part, because all data from analysis of product samples must be retained and reviewed…*

Column conditioning does not involve injecting a sample from a lot *and is not considered a trial injection.* ***When its use is scientifically justified, column conditioning should be fully described in the method validation package as to the conditions needed to make the measurement (i.e., based on data from the method validation) and should be clearly defined in an approved and appropriate procedure.*** *Only validated test methods that demonstrate accuracy, sensitivity, specificity, and reproducibility may be used to test drugs (21 CFR 211.165[e]). Consistent and unambiguous injection nomenclature should be used, and* ***all data from the column conditioning, including audit trail data, should be maintained and subject to review*** (8).

Using standard injections to confirm that the column is conditioned is an acceptable practice BUT it needs to be scientifically justified, included in the method validation report and MUST be part of complete data for the second person review under 21 CFR 211.194(a), clause 8 (9). There are no excuses or justifications for taking any different approach.

Chromatographic Methods

Section 12 is titled Chromatographic Methods (acquisition and processing) but there is not much in this section about acquisition and nothing about data processing. The order of clauses is random and confusing (lucky dip time or a chat after a session at the bar?):

- 12.1: Chromatographic methods should be suitable for intended use with acceptance criteria, for example, selectivity, peak symmetry, repeatability, and integration conditions.
- 12.2: Non-pharmacopoeial methods should be validated.
- 12.3: Methods should be saved in the CDS by authorized personnel and should only be changed where justified.
- 12.4: CDS software should be validated! What!? This clause does not belong here it should be in Section 4! This clause is also shared with the statement that methods selected for acquisition and processing should be traceable and reflected in the audit trail—how about moving this to the audit trail section?

At this point in the document, even an extended bout of lalochezia fails to work and I'm getting depressed as I've still got two more sections to review.

Peak Integration

Hope springs eternal and I'm hopeful that I'll find something positive to say about this part of the document. Clause 13.1 with its scientifically sound approach to integration raises my hopes, but they are dashed when I get to clause 13.2. Here I find that I should connect the chromatograph to a CDS—sorry—to *computerized chromatographic data capturing and processing systems.* What is this and why do we need to connect a chromatograph to two of them? Again, the document organization is appalling with an abject failure in technical understanding as well as editorial and quality oversight.

After discussing some of the types of integration and the ideal peak shape for integration (symmetrical) we come to manual integration. Here procedures for manual integration should be followed and records, including the authorisation and justification for manual integration, should be maintained. The guidance fails to define what is manual integration: manual positioning of the baselines by an analyst (7,10,11).

From the wording in the guidance it appears that authorisation is required each time that manual integration is needed. This is too draconian and manual integration must be justified in the validation report and the analytical procedure. Manual integration can occur daily if a method is analysing biologicals, contrast media, or impurities. **Scientific justification not regulatory diktat must be applied for manual integration.** The PDA TR80 guidance (10) is the best guidance for integration as it has figures illustrating both good and bad integration practices, coupled with the differentiation between manual intervention and manual integration (11,12).

Data Management

Most of the section covers managing the administration of the *computerized chromatographic data capturing and processing system* (singular, this time!) better known as a CDS. The guidance does not say who is responsible

but ideally this should be an IT function. True to form, there is another clause out of place with 14.2 requiring that data should be timely processed and reviewed, posing the question of why is this clause here and not under peak integration? If compliance with ALCOA+ requirements is required, why is there not a separate section for second person review, as this is where mistakes and errors should be caught in the laboratory. Clause 14.5 notes that printed records may be retained. May? May?? What planet are these writers on??? Strike three and out for quality and technical oversight and cue an extended bout of lalochezia.

References Section

There are three WHO references listed at the end of the document but these are only referenced to clause 14.1, which is most unhelpful. References to the United States and European Pharmacopoeias are equally unhelpful especially the specific chromatography and instrument qualification general chapters are not mentioned explicitly, for example, USP <621> (13), USP <1058> (14) and EP <2.2.46> (15).

Summary

The question posed by the title of these two columns is, "What is good about the WHO Good Chromatography Practices guidance?" Very little is my answer, and the document fails its SST criteria (if it had any). From the organization of the document, clauses in the wrong place, missing topics, wrong approaches to computerized system validation (CSV), to the inability to call a chromatography data system a CDS, it is a document that has been cobbled together without knowledge of the subject coupled with a total lack of technical and quality oversight. I'm not sure who this is written for and by whom.

This is a dangerous document if you were relying on it as your sole guide for good chromatography practices in a regulated laboratory. The writing is just too vague, generic and imprecise to be of real value: there is just one *must* and over 120 *should* statements in the document.

There is a need for a guidance document for Good Chromatographic Practice. However, with the problems and omissions presented here, the WHO guidance is not it. The current guide needs to be extensively rewritten, reorganized and expanded, ideally to highlight best practices that a laboratory should aim towards, if they do not work this way now.

Acknowledgements

I would like to thank Chris Burgess and Paul Smith for helpful review comments on the two parts of this column.

References

1) *WHO Technical Report Series No.1025, Annex 4 Good Chromatography Practices* (World Health Organization, Geneva, 2020).
2) R.D. McDowall, *LCGC Eur.* **33**(11), 579–584 (2020).
3) T. Schofield, et al., "Distinguishing the Analytical Method from the Analytical Procedure to Support the USP Analytical Procedure Life Cycle Paradigm", *Pharmacopeial Forum*, **45**(6) (2019). Online only.
4) *WHO Technical Report Series No.929, WHO Guidelines for Sampling of Pharmaceutical Products and Related Materials* (World Health Organization, Geneva, 2005).
5) US Food and Drug Administration, *Guidance for Industry: Out of Specification Results* (FDA, Rockville, Maryland, USA, 2006).
6) R.D. McDowall, *LCGC N. Am.* **38**(7), 411–419 (2020).
7) H. Longden and R.D. McDowall, *LCGC Eur.* **21**(12), 641–651 (2019).
8) US Food and Drug Administration, *Questions and Answers on Current Good Manufacturing Practices—Laboratory Controls* (FDA, USA, 2019). Available from: https://www.fda.gov/drugs/guidances-drugs/questions-and-answers-current-good-manufacturing-practices-laboratory-controls.
9) US Food and Drug Administration, *21 CFR 211 Current Good Manufacturing Practice for Finished Pharmaceutical Products* (FDA, Silver Spring, Maryland, USA, 2008).
10) Parenteral Drug Association, *Technical Report 80: Data Integrity Management System for Pharmaceutical Laboratories* (PDA, Bethesda, Maryland, USA, 2018).
11) R.D. McDowall, *LCGC Eur.* **28**(6), 336–342 (2015).
12) R.D. McDowall, *Validation of Chromatography Data Systems: Ensuring Data Integrity, Meeting Business and Regulatory Requirements* (Royal Society of Chemistry, Cambridge, UK, 2nd ed., 2017).
13) United States Pharmacopoeia General Chapter <621> "Chromatography" (United States Pharmacopoeia Convention, Rockville, Maryland, USA).
14) United States Pharmacopoeia General Chapter <1058> "Analytical Instrument Qualification" (United States Pharmacopoeia Convention, Rockville, Maryland, USA).
15) General Text 2.2.46 "Chromatographic Separation Techniques," *European Pharmacopoeia* (Strasbourg, France, 2005), pp. 69–73.

"Questions of Quality" Column Editor **Bob McDowall** is Director of R.D. McDowall Limited, Bromley, Kent, UK. He is also a member of *LCGC Europe*'s editorial advisory board. Direct correspondence about this column to the Editor-in-Chief, Alasdair Matheson: amatheson@mjhlifesciences.com

Modern Trends in Mixed-Mode Liquid Chromatography (LC) Columns

David S. Bell, Column Watch Editor

Commercialization of columns that provide multiple modes of chromatographic separations have recently been on the rise. For example, combinations of retention modes such as ion-exchange and reversed-phase, often enable the separation of complex mixtures of analytes not possible using single-mode columns. In this work, recent trends in what is often referred to as "mixed-mode" phase are investigated. Particular attention is made to recent fundamental research, stationary phase development and design, and areas of application.

In recent *LCGC* reviews of newly launched high-performance liquid chromatography (HPLC) columns, it was observed that several stationary phase developments fell into a category that can be characterized as "mixed-mode" (1,2). The idea of mixed-mode chromatography is not new. However, the observation prompted the question, "What is the current status of mixed-mode chromatography?"

An initial search through the literature and various websites revealed that there have been a multitude of publications, including reviews, that have written about mixed-mode chromatography in recent years. For example, West and others published a review of mixed-mode chromatography that covered many important developments, including combinations of reversed-phase liquid chromatography (RPLC) and ion-exchange chromatography (IEC) as well as hydrophilic interaction liquid chromatography (HILIC) and ion-exchange (3). Zhang and Liu published another notable review that focused on mixed-mode chromatography applications in pharmaceutical and biopharmaceutical analyses (4). The reader is referred to these reviews and references within for a more in-depth discussion of the technology.

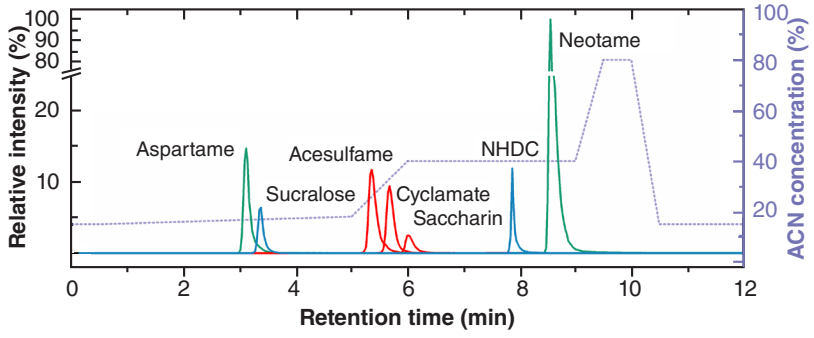
Mixed-mode chromatography remains somewhat ill-defined. West and co-workers defined the term as the combined use of two (or more) retention mechanisms in a single chromatographic system. Adding to this, Gilar and co-workers further define the term as the use of multiple dominant retention mechanisms (5). The dominant designation is an important one as all chromatographic systems involve at least some minor contributions from retention mechanisms other than the primary ones. These minor, but usually important, contributions are often referred to as *secondary interactions*. For the purposes of this column, we will refer to mixed-mode chromatography where multiple retention mechanisms play a dominant role in the overall chromatographic performance. Mixed-mode systems may be achieved via a number of different routes. However, the combination of partition (RPLC or HILIC) chromatography and ion-exchange seems to be associated most closely with the term.

In their description of new phases that combined partitioning and anion-exchange chromatography, Pohl and Liu noted that mixed-mode columns address a number of key challenges in chromatographic method development (6). Using reversed-phase columns only can result in poor polar analyte retention, basic analyte peak tailing, and column "dewetting" under high aqueous mobile phase content. They go on to note that ion-exchange can be used to tackle some of these issues, but ion-exchange alone is not suitable to separate molecules based on their differences in hydrophobicity. The combination of the two modes of chromatography

often provides the potential to address a number of analytical challenges. Mixed-mode chromatography has played a significant role in modern chromatographic practices and has been applied to areas such as oligonucleotides, peptides, proteins, metabolomics, pharmaceutical analysis, and natural product studies, among many others (7).

In an attempt to take an even more recent pulse on the level of interest and utilization of mixed-mode chromatography, a non-exhaustive search of publications in the past year along with a perusal of well-known column vendor websites was conducted. The limited search revealed a significant number of papers and technical notes in this realm, indicating continued interest. What follows is a brief synopsis of what was found, broken down into fundamental studies, new stationary phase designs, and applications of the technology.

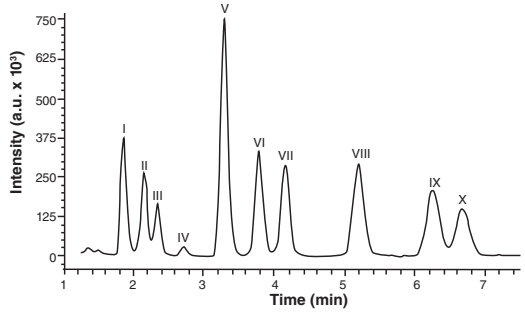
FIGURE 1: Optimized separation of seven artificial sweeteners on a mixed-mode stationary phase. Reproduced with permission from reference (17). ACN is acetonitrile.



Fundamental Research

The application of mixed-mode chromatography is often limited because of the complexity of the

FIGURE 2: Optimized separation of pentacyclic triterpenoids on a mixed-mode stationary phase. I is betulin, II is erythrodiol, III is uvaol, IV is friedelin, V is lupeol, VI is β-amyrin, VII is α-amyrin, VIII is betulinic acid, IX is oleanolic acid and X is ursolic acid. Note a.u. is arbitrary units. Reproduced with permission from reference (22).



resulting chromatography. With more mechanisms playing a significant role, additional parameters that control these mechanisms need to be taken into account during method development and practice of the resulting procedures. In addition, it is often difficult to find a full description of surface chemistry from vendors or detailed characterization results (8). Fundamental studies that provide the user with information to help guide method development and understand both the potential and limitations of stationary phases is of utmost importance.

Lämmerhofer and others used chromatographic, molecular modelling and electrochemical techniques to characterize chiral zwitterionic materials (7). Although the target phases were commercialized for use in chiral separations, the group notes significant achiral utility. The columns studied were described as cinchona carbamate stationary phases decorated with cyclohexylsulfonic acid and carbamate residues and are thus likely to provide multiple dominant retention mechanisms. Indeed, the group found the phases to exhibit moderate hydrophobicity, the potential for HILIC operation, and ion-exchange character. A unique aspect of this research was that investigators used a sequential building and analysis of intermediary versions of the stationary phases to better understand the individual structural contributors to retention mechanisms.

Gilar and others investigated the chromatographic attributes of several commercially available columns, including single-mode as well as a recently launched mixed-mode column combining C18 and anion-exchange chemistry (5). The group focused their efforts on understanding the ion-exchange interaction potential of the columns across a wide pH range. Fundamental information regarding the interactions available across a number of different conditions is important to method developers for both selecting and utilizing various stationary phases. For instance, the researchers were surprised to find that the ion-exchange interactions for the mixed-mode phase dropped off at a pH value much lower than the ligand pKa value. The observation was attributed to the impact of ionized surface silanols on the overall surface charge and demonstrates both the complexity and the need to carefully study mixed-mode systems.

New Stationary Phase Research

The majority of the published research over the past year has focused on the development of new stationary phases and their characterization. What follows is not intended to be an exhaustive list, but it should provide an overview of the types and breadth of stationary phases being investigated across the globe in this field.

Shields and Webber reported on the development of a mixed-mode, reversed-phase cation-exchange system based on a thiol-yne reaction. The intent was to develop a mixed-mode column with low pH stability. The authors used the separation of monoamine neurotransmitters to demonstrate both the ion-exchange and partition properties of the phase (9). Guo and others published a paper describing the interesting combination of MOF-235, polyethylene glycol, and silica, in a core–shell-based format. The authors indicate a combination of HILIC and ion-exchange is possible with the phase composition (10).

Li and others described the use of modified dialdehyde cellulose as a substrate for developing stationary phases that exhibit both HILIC and ion-exchange properties. The authors note facile functionalization and suggest this approach as the basis for further stationary phase development (11).

Several stationary phase developments have been recently published by Wang and others. The authors describe a poly(ethyleneimine) embedded N-acetyl-L-phenylalanine stationary phase that is shown to exhibit RPLC, HILIC, and ion-

exchange characteristics (12). In a second paper from the group, a stationary phase co-modified with N-isopropylacrylamide and aminophenylboronic acid is described. The resulting phase was also characterized as providing both hydrophobic and hydrophilic retention capabilities along with anion-exchange properties (13). Wang and others also reported on the incorporation of ionic liquids with C18 and cyclodextrin moieties bonded to silica supports as potential stationary phases for liquid chromatography (LC) (14). The group characterized the phases as exhibiting hydrophobic and ion-exchange character as well as the potential for use in the HILIC domain. A positive comparison with commercially available, single-mode columns was provided.

Heydar and Hosseini published a paper describing the preparation of a novel, silica-based stationary phase using 9-methylacridine and 9-undecylacridine (15). The phases were shown to exhibit hydrophobic and hydrophilic partitioning as well as anion-exchange characteristics. Finally, Wolrab and others investigated a series of zwitterionic and strong cation-exchange based mixed-mode phases under RPLC, HILIC, and supercritical fluid chromatography (SFC) conditions (16). The authors noted that ion-exchange interactions appear to prevail over others for the phases studied. However, other interactions, such as partitioning, can be enhanced or attenuated using various mobile phase conditions.

It is clear from the number of reports over a short period of time, as well as from the diversity of approaches, that interest in developing mixed-mode chromatography stationary phases continues.

Applications

Mixed-mode chromatography is powerful, but complex. As noted above, when multiple dominant retention mechanisms are invoked, many variables must be controlled to produce a robust and repeatable method. Mixed-mode chromatography is therefore most often applied only to complex systems. The following are examples of mixed-mode applications found in recent literature.

Artificial Sweeteners in Surface Waters

The combination of anion-exchange and RPLC was used to analyze artificial sweeteners in surface waters. Anionic sulfamates (acesulfame, cyclamate, saccharin), zwitterionic dipeptides (aspartame, neotame), and polar derivatives of natural products (sucralose, neohesperidin dihydrochalcone [NHDC]) were all efficiently separated on an octadecylsilane (C18)-strong anion exchanger (SAX) combination phase, as shown in Figure 1. One of the major advantages of the mixed-mode approach noted by the authors was the ability to inject large volumes of sample, presumably because of the accumulation effect of the ion-exchange character (17).

Separation of Oligonucleotides

Zhang and others recently reported on the use of mixed-mode chromatography for the analysis of oligonucleotides. The use of ion-pair reversed-phase chromatography (IP-RPLC) appears to be the most heavily employed mode of separation for oligonucleotides to date. However, anion-exchange, HILIC, and mixed-mode methods have also been successful. According to the authors, the use of mixed-mode separations for oligonucleotides dates back to the early 1980s. The authors also note that RPLC and ion-exchange mixed-mode approaches have displayed improved separations over either mode alone. In addition, it is speculated that multidimensional liquid chromatography (mLC) is poised to deliver much of what mixed-mode chromatography can do and is expected to continue to grow in this space (18). Lämmerhofer and co-workers also proposed the use of a chiral zwitterionic phase, which exhibits both partition and anion-exchange properties, for the first dimension separation of oligonucleotides using RPLC as the second dimension (19).

Underivatized Amino Acids

The application of mixed-mode chromatography for the separation of underivatized amino acids was demonstrated by Moussa and others (20). The traditional amino acid analysis routine consists of an ion-exchange based separation followed by reaction with ninhydrin or similar reagent. The traditional approach is noted as being time-consuming, nonspecific, and requires a dedicated analyzer. The authors used a “trimodal” column consisting of anion-exchange, cation-exchange, and hydrophobic properties to separate 52 amino acid-related compounds in 18 min with minimal sample preparation. The authors noted that the bimodal systems investigated did not provide the necessary separation of critical pairs.

Deamidation of Proteins

Sze and co-workers reported on the utility of employing ion-exchange in combination with reversed-phase and HILIC separations for the

improved analysis of deamidation in proteins (21). The authors note that deamidation of proteins results in several species of very similar hydrophobicities that are thus difficult to separate using partition chromatography alone. The authors suggest that the use of electrostatic interactions—coupled with HILIC chromatography along with improved sample preparation and advanced mass spectrometric techniques—will help serve to fill deficiencies in this important area of research.

Pentacyclic Triterpenoids

Another application of mixed-mode chromatography published in 2020 tackled the separation of pentacyclic triterpenoids using a mixed-mode, weak-anion-exchange stationary phase (22). The critical pairs of erythrodiol and uvaol, as well as oleanolic acid and ursolic acid, were only resolved with a combination of reversed-phase or HILIC with ion-exchange mechanisms, whereas both RPLC and HILIC alone were found to be insufficient. As shown in Figure 2, the mixed-mode column utilized provided the separation of 10 analytes in approximately a 7-min run time.

It should also be noted that many vendors provide application data in support of the use of mixed-mode phases. A cursory search through websites of many prominent vendors showed recent activity in this realm. The reader is encouraged to visit column vendor sites for more information.

Conclusions

A non-exhaustive literature search and perusal of web-based information revealed that interest in mixed-mode chromatography is alive and well. Recent applications in environmental, pharmaceutical, and biopharmaceutical areas demonstrate that the use of multiple dominant retention mechanisms continues to assist researchers in meeting the need for complex separations. The breadth of research toward the development of new mixed-mode stationary phases and their subsequent characterization over the past year indicates there is significant space for, and interest in, new discoveries. Finally, fundamental research and characterization of commercially available columns is paramount and is expected to greatly facilitate the intelligent use of these powerful stationary phases.

References

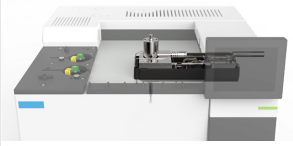
1) D.S. Bell, *LCGC Eur.* **33**(4), 185–192 (2020).
2) D.S. Bell, *LCGC Eur.* **32**(4), 206–214 (2019).
3) C. West, et al., *LCGC North Am.* **30**(6s), 22–33 (2017).
4) K. Zhang and X. Liu, *J. Pharm. Biomed.* **128**, 73–88 (2016).
5) Z. Kadlecová, et al., *J. Chromatogr. A.* **1625**, 461301 (2020).
6) X. Liu and C. Pohl, *A Weak Anion-Exchange/ Reversed-Phase Mixed-Mode HPLC column and its Applications.* Am. Lab. 2007; Available from: https://www.americanlaboratory.com/913-Technical-Articles/1454-A-Weak-Anion-Exchange-Reversed-Phase-Mixed-Mode-HPLC-Column-and-its-Applications/.
7) M. Ferri, et al., *J. Chromatogr. A.* **1621**, 461075 (2020).
8) R. Karongo, et al., *J. Chromatogr. A.* **1627**, 461430 (2020).
9) E.P. Shields and S.G. Weber, *J. Chromatogr. A.* **1618**, 460851 (2020).
10) T. Si, et al., *Anal. Chim. Acta.* **1116**, 62–69 (2020).
11) J. Gao, et al., *J. Chromatogr. A.* **1618**, 460885 (2020).
12) M. Wan, et al., *Microchem J.* **157**, 105021 (2020).
13) D. Zhou, et al., *J. Chromatogr. A.* **1627,** 461423 (2020).
14) J. Zhou, et al., *J. Chromatogr. A.* **1634**, 461674 (2020).
15) E.S. Hosseini and K.T. Heydar, *Talanta.* **221**, 121445 (2021).
16) D. Wolrab, et al., *J. Chromatogr. A.* **1635**, 461751 (2021).
17) J. Henschel and H. Hayen, *MethodsX.* **7**, 101134 (2020).
18) A. Goyon, P. Yehl, and K. Zhang, *J. Pharm. Biomed.* **182**, 113105 (2020).
19) F. Li, et al., *J. Chromatogr. A.* **1625**, 461338 (2020).
20) A. Desmons, et al., *J. Chromatogr. A.* **1622**, 461135 (2020).
21) S.K. Sze, G. JebaMercy, and S.C. Ngan, *Methods.* **3**, 215–233 (2020).
22) D.I. Falev, et al., *J. Chromatogr. A.* **1609**, 460458 (2020).

COLUMN EDITOR

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 for "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: amatheson@mjhlifesciences.com

GC Detector



VICI's Model D-3-1-8890 is a plug-and-play pulsed discharge detector for easy installation and configuration on the Agilent 8890 GC. This detector is optimized for trace level work in helium photoionization mode, and is a non-radioactive, low maintenance universal detector with a wide linear range. It also utilizes the electronics and power supply of the host GC.

www.vici.com
VICI AG International, Schenkon, Switzerland.

µPAC

According to the company, the 200 cm µPAC is your best choice for comprehensive proteomics, while their 50 cm µPAC column is perfectly suited to perform more throughput analyses with shorter gradient times. In addition, their µPAC Trapping columns were developed with identical morphology as the analytical columns to fulfil your needs for peptide sample enrichment.

www.pharmafluidics.com
PharmaFluidics, Ghent, Belgium.

Field-Flow Fractionation

The Eclipse offers next generation FFF for nanoparticle and macromolecular separation and characterization. Built-in intelligence throughout the FFF workflow, from computer-aided method design to continuous diagnostics and recommendations for maximum productivity, and includes: Mobility EAF4 for zeta potential, dilution control, FFF-SEC switching, and re-engineered channels with temperature control.

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

Polymeric HILIC Columns

iHILIC-Fusion(P) and iHILIC-(P) Classic are two lines of polymeric HILIC columns with different surface chemistries. They provide complementary selectivity, ultra-low column bleeding, and excellent durability at basic conditions. According to the company, the columns are particularly suitable for LC–MS-based analysis of polar compounds in "Omics" studies at pH 1–10.

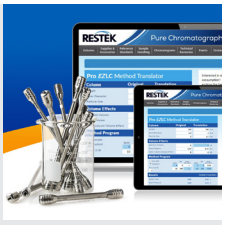
www.hilicon.com
Hilicon AB, Tvistevägen, Umeå, Sweden.

Pharmaceutical Reference Standards

Discover the LGC Mikromol range of more than 5,000 API, impurity and excipient reference standards, each accompanied by a comprehensive CoA detailing characterisation and with a growing portfolio accredited to ISO 17034.

www.lgcstandards.com/GB/en/Mikromol/cat/279844
LGC Group, Teddington, UK.

Method Translator



Pro EZLC method translation software makes it possible to scale down an existing LC method to a smaller column format so that users can speed up run time, increase sample throughput, and reduce solvent use, according to the company. The user can input current column dimensions and method conditions, then specify the dimensions of the new column that they want to try.

www.restek.com/Pages/Pro-EZLC-Method-Translator
Restek Corporation, Bellefonte, Pennsylvania, USA.

SEC Columns

PSS MAB, for size-exclusion chromatography (SEC) of monoclonal antibodies, is the latest addition to the PSS column family. Analytical and semi-micro columns, which cover a wide molar mass range and are pre-equilibrated for light scattering detection, are available. Bio-inert coated hardware is also optional for separations that are required to be metal-free.

www.pss-polymer.com
PSS GmbH, Mainz, Germany.

Ion Chromatography System

Shimadzu's IC system with anion suppressor reportedly reduces band spreading and achieves high sensitivity and reliable performance for the quantitative determination of anions. According to the company, the suppressor provides stable functionality over long periods of operation. The system features a compact design and integrates with Shimadzu's LabSolutions.

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

HIC Column

BioPro HIC HT, YMC's latest HIC column, is designed for biopharmaceuticals like antibody-drug-conjugates. Higher flow rates are applicable as a result of extremely high-pressure stability of the polymer particles allowing very short run times and high throughput. BioPro HIC HT columns are the ideal choice for DAR determination with high resolution.

www.ymc.de
YMC Europe GmbH, Dinslaken, Germany.

Electrochemistry-MS

The Roxy Exceed is a new generation potentiostat dedicated to on-line coupling of electrochemistry with mass spectrometry (MS). The system supports DC, scan, and pulse mode and can be controlled from any LC–MS system. The instrument is suitable for predicting drug metabolism, and for MS proteomics.

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

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.

Process Gas Chromatography

The new Eclipse Process Gas Chromatographs provide real-time, laboratory-quality analysis of high-value process streams. Low ppm–ppb levels of hydrocarbons (C1–C20), sulfurs (H_2S, COS, mercaptans), catalyst poisons (AsH_3, PH_3, CO, CO_2), and other analytes can be analysed. The systems inventively incorporate capillary chromatography and multiplexed detectors.

www.go-jsb.co.uk/assortiment/chromatografie_oplossingen/valving_JSsolutions/eclipse__process_gc_wasson
JSB Group, Eindhoven, The Netherlands.

Build brand awareness with the right content.

In today's heavily branded world, buyers are not lacking for choice, which is why creating a meaningful brand experience is more important than ever. Staying top of mind and in front of your buyers is important, but it is even more essential to communicate your brand's purpose and values through the right marketing channels.

LCGC™ Europe will get your message in front of the right audience across our multiple content platforms that build brand awareness.

- Brand Insights
- Custom Video
- Podcasts
- eBooks
- eNewsletters
- CHROMacademy… and more!

Partner with *LCGC™ Europe*, the content experts in the chromatography market, to create a multimedia strategy that builds thought leadership.

To discuss our multimedia solutions for your brand, contact:

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

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

Follow and tag us on social media

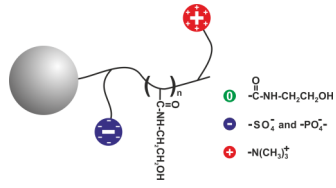
THE ESSENTIAL MULTIMEDIA RESOURCE FOR SEPARATION SCIENCE IN PRACTICE

LCGC europe
AN MJH life sciences BRAND

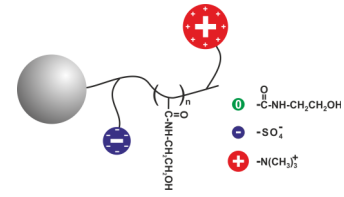
HILICON

iHILIC®

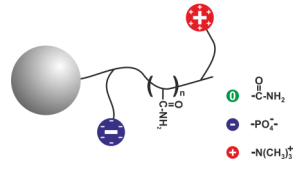
advances HILIC separations in UHPLC and HPLC



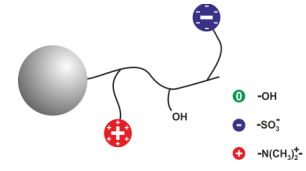
iHILIC®-Fusion
Silica based



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

HILICON AB
Email: info@hilicon.com | Website: www.hilicon.com