Streamline your GC workflow with Precision

Combining convenience and safety in a stackable and modular laboratory gas generator system, Precision is the practical solution to your GC detector and carrier gas needs, providing hydrogen, nitrogen or zero air at the push of a button. Eliminate the need for gas cylinder deliveries, reducing your lab’s carbon footprint whilst minimizing the risks of Covid-19 contact transmissions, with an on-demand gas generator.

Contact us today to discover more! www.peakscientific.com/labgas

www.peakscientific.com  Your local gas generation partner
With a Peak generator in your lab you can forget about your instrument gas supply and focus on your analysis. Your gas flow is our top priority, that’s why we have Peak Service Engineers located in every continent, delivering rapid response on-site technical support wherever your lab may be. What’s more, our unique comprehensive product warranty covers every single part of your generator, giving you complete peace of mind. That’s what we call the Peak brand promise.

A Peak gas generator will bring to your lab:

- **Convenience**: Gas delivered on-demand, no cylinders to change or supply stocks to maintain
- **Consistency**: Consistent gas quality and supply, no impurities or running out of gas
- **Economy**: Eliminate on-going costs of cylinders, manage lifetime running costs
- **Safety**: No pressurised compressed gas cylinders in your lab
- **Sustainability**: No repeated gas deliveries, energy efficient
- **On-site service**: Comprehensive on-site warranty and service plans

[Contact us today](www.peakscientific.com/contact) to discover more!
Pharmaceutical Method Transfer
Tips for transferring methods to manufacturing sites
The FFF - MALS Platform
Next Level Nano, Bio and Polymer Analysis

FFF-Made-Easy!
Complete System Support
Method Development Software
Reliable and flexible Hardware

Contact us for more information: www.postnova.com
Turning Up The Heat: The Effect of Temperature on Analytical Extractions
Douglas E. Raynie

The application of temperature is frequently employed to enhance analytical extractions, especially with solid samples or volatile analytes. This article explores the role of temperature in analytical extractions.

What is on Your HPLC Particle? A Look at Stationary Phase Chemistry Synthesis
Diego A. Lopez, Ahren I. Green, and David S. Bell

This article reviews historical bonding techniques still in use for manufacturing high performance liquid chromatography (HPLC) stationary phases today, and also examines some emerging technologies that may be able to tackle unmet needs in novel platforms and phase construction.

Tips for Liquid Chromatography Coupled with Charged Aerosol Detection
Imad A. Haidar Ahmad

Charged aerosol detection (CAD) is a powerful complement to ultraviolet (UV) absorbance and mass spectrometric (MS) detection for liquid chromatography (LC), particularly for analytes that have no UV chromophore, or do not ionize well by electrospray ionization. This article explores how to successfully use this technique.

Control or Chaos: How Can We Run a Gas Chromatograph from Anywhere?
Nicholas H. Snow

A look at how the data system controls the functions of the instrument. The same fundamental electronic principles used to manually control gas chromatographs in the 1970s are still at the center of today’s modern electronically controlled systems.
An MH Life Sciences Brand

LCGC Asia Pacific solutions for separation scientists

Senior Vice President
Mike Tessalone
mtessalone@mh lifesciences.com

Editorial Director
Laura Blush
lcbush@mh lifesciences.com

Editor-in-Chief
Aladar Matheson
amatheson@mh lifesciences.com

Managing Editor
Katie Jones
kjones@mh lifesciences.com

Associate Editor
Levi Batchley
lbatchley@mh lifesciences.com

Publisher
Oliver Waters
owaters@mh lifesciences.com

Sales Executive
Liz Moss
lmoss@mh lifesciences.com

Sales Operations Executive
Sarah Darby
sdarby@mh lifesciences.com

Senior Director, Digital Media
Michael Kathriner
mkathriner@mh lifesciences.com

Webcast Operations Manager
Kristin Moote
kmoote@mh lifesciences.com

Project Manager
Varita Oliveira
voliveira@mh lifesciences.com

Digital Production Manager
Sabrina Advani
sadavani@mh lifesciences.com

Managing Editor, Special Projects
Kaylinn Chang/Elber
kchangmh@mh livesciences.com

Creative Director, Publishing
Melissa Farnen
mfarnen@mhmag.com

Senior Art Director
Gwendolyn Sales
gsales@mh lifesciences.com

Senior Graphic Designer
Courtney Soden
csoden@mh lifesciences.com

Follow us @ LC_GC
Like our page LCGC
Join the LCGC LinkedIn group

Corporate
Chairman & Founder
Mike Henryssen Jr
Vice Chairman
Jack Lepping
President & CEO
Mike Henryssen Jr
Chief Financial Officer
Neil Glasser, CPA/CFE
Chief Marketing Officer
Michael Baar
Executive Vice President, Global Medical Affairs & Corporate Development
Joe Petrizzi
Senior Vice President, Content
Slas Irmann
Senior Vice President, Operations
Michael Ball
Senior Vice President, I.T. & Enterprise Systems
John Moriconi
Vice President, Human Resources & Administration
Shirinder Randhawa
Vice President, Merchers & Acquisitions
Chris Herwig
Executive Creative Director, Creative Services
Jeff Brown

Subcriber Customer Service
Visit chromatographyonline.com to renoe your or change a subscription or email mmhinfo@mmgroump.com

MULTIMEDIA
Multimedia UK, LLC
Sycamore House, Suite 2 Ground Floor,
Lloyd Drive, Chichester Oasis Business Park,
Eслиssmore Port, Chichester, West Sussex, UK
Tel:+44 (0)151 705 7601

MANUSCRIPTS:
For manuscript preparation guidelines, visit www.chromatographyonline.com or call the Editor. +44 (0151) 705 7601. All submissions will be handled with reasonable care, but the publisher assumes no responsibility for safety of artwork, photographs or manuscripts. Every precaution is taken to ensure accuracy, but the publisher cannot accept responsibility for accuracy of information supplied therein or for any opinion expressed.

DIRECT MAIL LIST:
Telephone: +44 (0151) 705 7601.

Reprints: Reprints of all articles in this issue and past issues of this publication are available (250 minimum). Licensing and Reuse of Content: Contact Mike Tessalone at MJH Life Sciences. Telephone: (732) 346 3016. E-mail: mtessalone@mh lifesciences.com

© 2021 Multimedia Healthcare LLC all rights reserved. No part of the publication may be reproduced in any material form (including photocopying or storing it in any medium by electronic means and whether or not transiently or incidentally to some other use of this publication) without the written permission of the copyright owner except in accordance with the provisions of the Copyright Designs & Patents Act (UK) 1988 or under the terms of the license issued by the Copyright License Agency's 90 Tottenham Court Road, London W1P OLP, UK. Applications for the copyright owner's permission to reproduce any part of this publication outside of the Copyright Designs & Patents Act (UK) 1988 provisions, should be forwarded in writing to Permission Dept. email: ARockerchen@mh lifesciences.com. Warning: the doing of an unauthorized act in relation to a copyright work may result in both a civil claim for damages and criminal prosecution.

EDITORIAL ADVISORY BOARD

Daniel W. Armstrong
University of Texas, Arlington, Texas, USA

Günther K. Born
Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Austria

Deidre Cabooter
Department of Pharmaceutical and Pharmaceutical Sciences, University of Leuven, Belgium

Peter Carr
Department of Chemistry, University of Minnesota, Minneapolis, Minnesota, USA

Jean-Pierre Chervet
Antec Scientific, Zoeterwoude, The Netherlands

Jan H. Christensen
Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark

Adrian Clarke
Novartis, Switzerland

Danilo Corradini
Istituto di Cromatografia del CNR, Rome, Italy

Gert Desmet
Transport Modelling and Analytical Separation Science, Vrije Universiteit, Brussels, Belgium

John W. Dolan
LC Resources, McMinnville, Oregon, USA

Anthony F. Fell
Pharmaceutical Chemistry, University of Bradford, Bradford, UK

Attila Feilinger
Professor of Chemistry, Department of Analytical and Environmental Chemistry, University of Pécs, Pécs, Hungary

Paul Ferguson
AstraZeneca, UK

Francesco Gasparini
Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive, Universita `La Sapienza`, Rome, Italy

Joseph L. Glajch
Momenta Pharmaceuticals, Cambridge, Massachusetts, USA

Davy Guillaume
School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland

Jun Haginaka
School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women`s University, Nishinomiya, Japan

Javier Hernández-Borges
Department of Chemistry (Analytical Chemistry Division), University of La Laguna Canary Islands, Spain

John V. Hinshaw
Severn Corp, Beaverton, Oregon, USA

Tuula Hyvärinen
VTT Technical Research of Finland, Finland

Hans-Gerd Janssen
VTT Institute for the Molecular Sciences, Åmål, Sweden

Kiyokatsu Jinno
School of Materials Sciences, Toyohashi University of Technology, Japan

Huba Kalázs
Semmelweis University of Medicine, Budapest, Hungary

Hank Lee
University of Science and Technology, Singapore

Wolfgang Lindner
Institute of Analytical Chemistry, University of Vienna, Austria

Henk Lingeman
Faculteit der Wetenschappen, Free University, Amsterdam, The Netherlands

Tom Lynch
Analytical consultant, Newbury, UK

Ronald E. Majors
Analytical consultant, West Chester, Pennsylvania, USA

Debby Mangenlies
Department of Analytical Chemistry and Pharmaceutical Technology, Vrije Universiteit, Brussels, Belgium

Phillip Marrott
Monash University, School of Chemistry, Victoria, Australia

David McCalley
Department of Applied Sciences, University of West of England, Bristol, UK

Robert D. McDowell
McDowell Consulting, Bromley, Kent, UK

Mary Ellen McNally
DuPont Crop Protection, Newark, Delaware, USA

Imre Moinár
Mohr Research Institute, Berlin, Germany

Luigi Mondello
Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università di Messina, Messina, Italy

Peter Myers
Department of Chemistry, University of Liverpool, Liverpool, UK

Janusz Pawliszyn
Department of Chemistry, University of Waterloo, Ontario, Canada

Colin Poole
Wayne State University, Detroit, Michigan, USA

Fred E. Regnier
Department of Biochemistry, Purdue University, West Lafayette, Indiana, USA

Harald Ritchie
Advanced Materials Technology, Chester, UK

Koen Sandra
Research Institute for Chromatography, Kortrijk, Belgium

Pat Sandra
Research Institute for Chromatography, Kortrijk, Belgium

Peter Schoenmakers
Department of Chemical Engineering, Universiteit van Amsterdam, Amsterdam, The Netherlands

Robert Shellie
Deakin University, Melbourne, Australia

Yvan Vander Heyden
Vrije Universiteit Brussel, Brussels, Belgium

The Publishers of LCGC Asia Pacific would like to thank the members of the Editorial Advisory Board for their continuing support and expert advice. The high standards and editorial quality associated with LCGC Asia Pacific are maintained largely through the tireless efforts of these individuals. LCGC Asia Pacific provides troubleshooting information and application solutions on all aspects of separation science so that laboratory-based analytical chemists can enhance their practical knowledge to gain competitive advantage. Our scientific quality and commercial objectivity provide readers with the tools necessary to deal with real-world analysis issues, thereby increasing their efficiency, productivity and value to their employer.
Our Mikromol portfolio of more than 4,000 high-quality impurity reference standards is here to support your analytical needs in detecting, identifying, qualifying and quantifying impurities in accordance with legislation and ICH guidelines.

Each Mikromol impurity standard comes with a comprehensive Certificate of Analysis containing the assay value and detailed identity information, in order to provide you with greater analytical certainty and confidence in the accuracy of your results.

Find out more: lgcstandards.com/mikromol

Mikromol, together beyond the standard.
Technical Challenges Encountered During Chromatographic Method Transfers to Pharmaceutical Manufacturing Sites

Peter Tattersall, Jia Zang, and Brent Kleintop, Chemical and Synthetic Development, Bristol-Myers Squibb, New Jersey, USA

Drug substance development requires a range of analytical methods to be developed to generate process knowledge and to support in-process and release testing throughout a synthetic sequence. Analytical chemists are challenged to ensure these fit-for-purpose methods are reliably transferred and executed at external manufacturing sites. The performance and robustness will differ based on method complexity, experience, proximity to final drug substance, and by the clinical stage of development. Here, we share experiences with a wide variety of transfer challenges and our remediation strategy.

In support of drug substance development, analytical methods are developed to generate process knowledge, monitor reactions, and assess quality of input materials, intermediates, and drug substances. During clinical stages of development, we are challenged to ensure these fit-for-purpose methods are reliably executed at both external manufacturing sites as well as at internal process plants. In many cases, the methods are transferred to several analytical laboratories as a compound progresses through development (1). The needs and requirements for method transfers vary, ranging from compendial methods, which require familiarization, to project-specific methods, where the transfer is often driven by a formal process (2). The elements and criteria in the transfer protocols will differ, based on proximity to final drug substance or drug product, and by the clinical stage of development. During early stages of development, we typically have the receiving site qualify the method in their laboratories using requirements within their own quality systems. In later stages, methods are extensively evaluated for feasibility and robustness. For transfer of methods that will support critical regulatory requirements, such as support of registrational stability studies or process validation, many more details and formal protocols or co-validation strategies are employed (3). Some typical criteria applied at the clinical (Investigational New Drug [IND]) vs. registrational (New Drug Application [NDA]) stages to ensure appropriate method performance and data quality are shown in Table 1. In addition to fulfilling regulatory requirements (4–7), these more detailed protocols also help reduce the likelihood that method performance investigations will be encountered during the commercial product lifetime, which could jeopardize the supply chain and the availability of marketed products to patients.

Ultimately, the goal of all method transfers should be to ensure the methods generate equivalent data, regardless of which laboratory they are executed in. The risk to achieving successful transfers, as well as subsequent routine execution,
can be linked to many factors, including method complexity, method robustness, instrumentation differences, and scientist experience or skill sets (8).

In recent years, we have observed an increase in the structural complexity of small-molecule drug candidates, which has necessitated developing more complex and challenging analytical methods to gather vital process knowledge and ensure the quality of clinical materials (9,10). This, combined with the need for low-level monitoring (ppm) of potentially genotoxic impurities (11), has resulted in analytical control strategies increasingly utilizing analytical detection techniques outside of traditional quality control (QC)-friendly liquid chromatography–ultraviolet (LC-UV) and gas chromatography–flame ionization detector (GC-FID) instruments. Advances in commercially available instrumentation such as ultrahigh-pressure liquid chromatography (UHPLC), liquid chromatography–mass spectrometry (LC–MS), and specialty detectors, such as charged aerosol (CAD) and vacuum ultraviolet (VUV), have made more efficient, extensive characterization both a capability and an expectation amongst our collaborators and worldwide health authorities. As a result, transferring and executing these more challenging techniques is now routinely required in both process development laboratories and QC settings.

In Figure 1, we characterize our general expectations regarding the acceptable level of risk tolerance for methods as it relates to method complexity and stage of clinical development. Early in a project’s lifecycle, we expect the process to change, which often requires methods to change as well. At later stages of development, we want to perform more extensive robustness studies to ensure that the risks to transfer, validation, and execution are low, as we expect the process and methods to be close to those that will be used throughout the commercial lifecycle. In practice, we perform fewer robustness studies on early-stage methods, and, as a result, expect a higher level of risk to be associated with transferring these methods. General techniques such as Karl Fischer (KF), loss on drying (LOD), colour, and appearance are often conducted under compendial conditions, and are not expected to provide significant risk during method transfer and execution. However, more challenging techniques, such as LC–MS or techniques using other specialty detectors, are expected to provide greater risk, largely due to instrumentation differences between sites and levels of experience at the vendor with these techniques.

Many other factors that can influence whether the expected risks are realized during method transfer and execution in support of releasing clinical batches. Lack of process knowledge in early stages can result in new impurities being detected, potentially necessitating method modifications. Lack of full understanding of sample
preparation techniques, stability, and chromatographic column robustness can cause issues. Later in development, full understanding of robustness ranges of critical method parameters can challenge the successful validation, transfer, or execution of methods as well. In this article, we share some of the hurdles we have encountered, and the lessons learned that we now apply to help reduce the risk of success for future method transfers and execution.

Detecting Changes in Impurity Profiles

In early stages of development, we have limited experience with our synthetic processes, especially at larger scales of manufacture. We have also limited experience with our analytical methods, especially across a restricted number of on-scale drug substance batches. At these early stages, methods need to be objective, and therefore capable of detecting new impurities that would need to be characterized to ensure patient safety. Related, diligence in investigating the origin of these impurities is also needed, to provide critical knowledge regarding the process being developed.

In this example, a GC method was initially developed in our laboratory to characterize a key reagent (material A) in a synthetic step. The method was successfully transferred to a vendor QC laboratory, and utilized to characterize the key reagent as it was received at their site. New impurities were found when analyzing a trial batch of the intermediate, which was produced using a new batch of the key reagent characterized by the GC method. Since these impurities were previously not seen, we launched an investigation to determine the origin of the unknown impurities. We first identified the impurities in the trial batch as urea monomers and dimer related substances. Nuclear magnetic resonance (NMR) analysis detected low levels of urea in the new lot of material A. However, urea could not be detected by the original GC method, because it was found to decompose in the injector. To address this, a low
A wavelength high performance liquid chromatography (HPLC) method was developed monitoring at 200 nm. As shown in the Figure 2, using the new HPLC method, urea, imide, and other unknown impurities were successfully detected in the various lots of material A. Analysis of several batches of material A demonstrated that the quality of this key reagent from various potential vendor lots was significantly different, and needed better analytical controls than what our original GC method provided. Therefore, the HPLC method was transferred and used for subsequent material release testing.

For methods where we have limited experience, we need to look at data critically to ensure the data are appropriate. Investigation into data accuracy and peak purity can involve using orthogonal techniques to determine whether undetected impurities are sufficiently evaluated and characterized. In this way, patient safety is not compromised using a fit-for-purpose approach.

Sample Preparation and Handling Challenges

In our experience, subtle differences in common analytical practices (such as weighing, mixing, and pipetting) at different testing sites can provide significant transfer difficulties. For example, we have encountered issues with aluminum weighing boats used at a receiving laboratory to weigh small sample amounts when the original method used plastic weigh boats. When the receiving laboratory subsequently sonicated the solution with the weighing boat in the volumetric flask, low recoveries were seen for analytes that could chelate with aluminum.

Another challenge that is often encountered with methods in early stages is inconsistent levels of known impurities reported by a receiving laboratory. In Figure 3a, the results table illustrates significantly different levels of a known impurity (labelled as impurity X in the chromatogram) being detected in intermediate material B during an LC method transfer. In this case, the high levels (0.35%) of impurity X in the first preparation and analysis are of significant concern, as...
ABALONASE™ ULTRA
3X STRONGER THAN THE INDUSTRY STANDARD

Stop paying extra money to waste enzyme.
Achieve maximum activity thresholds with less volume per sample.

Contact us at info@unitedchem.com or 800-385-3153 to try a sample today!
they would have triggered the need for studies to understand the quality impact to drug substance upon release. During our initial investigation, we did not discover any issues that would suggest instrument variability was the root cause. Similar results were obtained on different instruments in the laboratory, different sample batches, new columns, and freshly prepared mobile phases and diluent.

In the course of investigating the sample preparation procedure through the use of video, we observed that it took a much longer time for the samples to dissolve in the receiving laboratory. The mixing practice employed in our laboratory involved dissolving the sample with a reasonable amount of diluent and agitation prior to bringing the volumetric flask to volume. In the receiving laboratory, after the sample weight measurement, the solids were rinsed into a volumetric flask with a minimal volume of diluent, and left for several minutes without further dilution or mixing. A significant amount of undissolved solids remained on the bottom of the volumetric flask until the second addition of diluent was added. During method development, a 50 mM sodium hydroxide (NaOH) in 50:50 acetonitrile:water diluent was chosen to stabilize the sample solution, which we knew degraded to form impurity X in neutral or acidic diluents. We believe inconsistent levels of impurity X were caused by degradation occurring prior to mixing completion, due to undissolved material B (a disulfuric acid salt), creating a localized environment which was not basic enough to prevent degradation.

In a concentrated and non-agitated solution, the base in the diluent near the solids were consumed by the sulfuric acid counter ion, and therefore unable to prevent the formation of impurity X. This was remedied by ensuring consistent dilution, and mixing methods were used across the laboratories to prepare the sample. Training videos, clearly describing the sample preparation steps, were created, which were effective at achieving consistent and accurate results from the receiving laboratory, as shown in Figure 3b.

Another sample preparation related example involved ensuring an in-process control (IPC) method was providing results representative of when the sample was drawn from a reaction. IPC samples are often slurries or suspensions, which provide unique challenges related to sample homogeneity and stability when compared to isolated synthetic intermediates, which are often crystalline materials. In order to provide results that are representative of the batch, sample quenching frequently needs to be employed as part of the sample preparation procedure for unstable samples. In these cases, it is critical to understand the details regarding sample-handling logistics, to ensure that accurate results can be achieved at different manufacturing sites.

In this example, we developed a method to analyze a reaction completion sample for the synthesis of material C that was unstable at room temperature. This required the sample to be delivered on ice to the quality control laboratory after it was drawn from the reaction vessel. Adding to the challenge, the sample was also water sensitive, and would convert back to the starting material of the reaction. As a result, failure to prevent the degradation of the sample could cause the IPC to not pass, due to a false negative result. During in-house small-scale production runs, the IPC sample was delivered cold from the manufacture laboratory, and the sample was immediately quenched with anhydrous methanol in the analysis laboratory. Quenching the sample with methanol provided adequate stability, and low temperature was not required during the remainder of the sample preparation and subsequent analysis. When utilizing a contract manufacturing organization to provide increased scale of production, delivering the sample on ice proved challenging, because the analytical laboratory was not in close proximity.
to the production plant. Therefore, to reduce the risk of a false IPC result, a protocol was instated to quench the IPC sample on the production floor. This required providing a detailed sample quenching procedure, and training for the unit operation workers in the plant. This enabled a stable IPC sample to be delivered to the quality control laboratory for further sample preparation and testing. At commercial vendors, this is a more common practice for the preparation of unstable IPC samples for analytical testing and can be effective with clear and simple instructions and plant operator training.

The above examples provide real-world experience where sample preparation can provide challenges to ensure successful transfer and execution of methods. Small differences in sample preparation techniques related to weighing, dilution, and mixing can create variability in analytical results. Sharing important knowledge on sample stability and clear, detailed sample preparation details with appropriate limitations will lower the risk of test problems. Additionally, close evaluation of receiving laboratories’ unit operation techniques should be an early focus when investigating unexpected results, particularly for intricate and sensitive sample preparations. Clear instructions within the method and developing training videos are additional best practices we have employed to minimize the risk of sample preparation differences causing inaccurate results.

**Long-Term Column Robustness**

Chromatographic column-to-column evaluation is important to ensure robustness for long-term commercial support. During method robustness studies, LC columns packed from three different production batches of stationary phase are tested to ensure adequate reproducibility. Another practical consideration to consider is the long-term availability of the column chemistry and geometry. This risk can be mitigated by selecting established column chemistries and formats from global vendors where one should expect commercial (and not custom production) availability over many years. In our experience, column manufacturers have welcomed establishing meaningful collaborations to mitigate this risk. While these collaborations are extremely beneficial, they do not eliminate the risk that future batches of stationary phase synthesized by the column vendors will perform precisely the same as columns that have been utilized during our method robustness studies. New batches of stationary phases are verified to be equivalent using standard test mixtures; however, this does not always ensure the columns will perform equivalently for our more difficult separations as well.

One example we have encountered involved the use of a highly end-capped C18 column to monitor the impurity profile of a compound we expected to be commercialized. During our method robustness, we observed satisfactory column-to-column reproducibility on several batches of columns that were packed with different stationary phase lots. All columns tested exhibited a resolution (Rs) > 3.0 between a critical impurity and the main peak (Figure 4a). However, when transferring the method to one of the anticipated commercial vendors, we observed a much lower resolution (Rs = 1.3) for the same critical pair (Figure 4b). During our investigation,

<table>
<thead>
<tr>
<th>Robustness Parameter</th>
<th>Clinical Stage 24 hours (one condition):</th>
<th>Registral Stage 3 to 7 days (multiple conditions):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample and standard solution stability</td>
<td>• No individual impurity &lt;0.10% changes by more than 0.05 (absolute) • No individual impurity ≥0.10% changes by more than 30% (relative)</td>
<td>• No individual impurity &lt;0.10% changes by more than 0.03 (absolute) • No individual impurity ≥0.10% changes by more than 30% (relative)</td>
</tr>
<tr>
<td>Chromatographic conditions</td>
<td>Mobile phase ± 0.5 pH units</td>
<td>Not required</td>
</tr>
<tr>
<td></td>
<td>Molarity of mobile phase ± 5 mM</td>
<td>• No individual impurity &lt;0.10% changes by more than 0.03 (absolute) • No individual impurity ≥0.10% changes by more than 30% (relative)</td>
</tr>
<tr>
<td></td>
<td>Solvent content of mobile phase at time zero of the gradient ± 5% (absolute)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Column temperature ± 10 °C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flow rate ± 20% (relative)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wavelength ± 2 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Column packing material 3 lots</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1: Example of robustness parameter evaluation and acceptance criteria for a LC impurity method at a clinical stage and a registrational stage**

www.chromatographyonline.com
we discovered the column was packed with a new batch of stationary phase synthesized by the vendor. Ultimately, we addressed this challenge in two manners that solved the reproducibility issue. First, we worked with the vendor to ensure an adequate stock of columns packed with previous batches of stationary phase were either purchased or remained in the vendor’s inventory for future use. Second, we developed a column conditioning procedure using a methanol, water, and trifluoroacetic acid solution, which produced reproducible $R_s > 2.0$ for the critical pair (Figure 4c). The instructions for the column conditioning were incorporated into the method to achieve robust column performance for long-term commercial manufacturing support. This, along with appropriate system suitability requirements for the $R_s$ of the critical pair, have ensured the method performs as expected.

**Laboratory Instrument Set Up Differences**

Method robustness studies typically ensure that methods can produce equivalent results on different brands of the same instrument that are to be utilized across the vendor network. Many development laboratories maintain a diverse fleet of instrumentation, so this can be tested prior to transferring methods, as well as troubleshoot on the same type of instrument used at the receiving laboratory. When transferring methods to different laboratories, we also have encountered unanticipated problems caused by other laboratories using different default parameters in their instrument setup than we utilize in our laboratories. As an example, a GC residual solvent method was developed for trace $n$-methyl pyrrolidone (NMP) in an isolated intermediate, material E. Due to the low solubility and stability of material E in many solvents, we chose a diluent of ethylene glycol for sample preparation. Although we considered this a “non-standard” diluent, the method showed good reproducibility in our laboratories, as shown in Figure 5a, and was successfully validated. During method transfer to a vendor quality control laboratory, we observed significantly worse tailing of the ethylene glycol diluent peak after several sample solution injections, such that it interfered with the analyte of interest as shown in Figure 5b. During our investigation, we discovered the receiving laboratory had the “gas saver” option turned on, which differed from our laboratory’s instrument setting but crucially was not part of our analytical method instructions. Although this setting enables saving helium gas, it also enabled a greater degree of sample degradation in the injector port that produced tailing of the ethylene glycol after multiple injections. This was not observed when the gas saver option was off. To address this, we ensured the method description was updated to explain clearly that the gas saver setting should be turned off.

**Ensure Performance with Well-Defined System Suitability**

A challenge associated with developing LC-UV impurity profiling methods is ensuring accurate quantitation of all the critical impurities, especially when impurities have significantly different UV absorbance spectra. Different impurities may have different UV maxima and, as a result, the choice of detection wavelength for a method can limit both sensitivity for one or more components and vary the relative response factor for quantitation. It is impractical to develop methods where many different wavelengths are utilized and, ideally, a single method wavelength can be employed. As a result, choosing a wavelength that happens to fall on a slope in the UV spectrum of an impurity cannot always be avoided. When this occurs, variability in the data accuracy with the UV detector needs to be considered due to small differences in detector calibration. An example of this is provided in Figure 6, where the chosen UV wavelength for an intermediate material F falls on a part of the absorbance spectra that has a large slope for a specific impurity. In this example, variation in detection wavelength of ±2 nm (the typical robustness range evaluated) provided a 55% difference in the area percent of the impurity, as shown in Table 2. In this case, all other elements of the validation met acceptance criteria, and this specific impurity was the only robustness concern the method provided. The impurity was highly purged in the downstream chemistry, and was not expected to provide a significant risk to the overall quality of the final drug substance. After verifying typical UV detector calibration and

---

**Table 2: The level and % change of impurity in material F obtained during detection wavelength robustness assessment**

<table>
<thead>
<tr>
<th>Detection wavelength (UV)</th>
<th>218 nm</th>
<th>220 nm</th>
<th>222 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impurity (area % versus material F)</td>
<td>0.30</td>
<td>0.19</td>
<td>0.11</td>
</tr>
<tr>
<td>% Change relative to the level in control</td>
<td>55</td>
<td>NA</td>
<td>41</td>
</tr>
</tbody>
</table>

Acceptance criteria: ≤ 30%
verification specifications were ±1 nm, and, confirming this performance on a large number of LC-UV instruments, we decided to validate the method at the vendor using a detector robustness range of ±1.0 nm for this impurity. To ensure instrument-to-instrument reproducibility throughout the commercial lifecycle of this project, we also employed an additional system suitability criterion using an impurity cocktail to confirm the results for this impurity were within ±30% of the area percent listed in the cocktail certificate of analysis. With these precautions, the detection wavelength variation is well monitored with controls in place to ensure accurate results are reported.

### Human Errors Found During Transfers

A risk to successful method transfer and execution that can likely never be fully mitigated is that of human error. Unfortunately, these errors are often difficult to identify and assign conclusively as a root cause, especially when investigating a remote laboratory location. The chromatograms in Figure 7 illustrate significant retention time differences (>2 min) for the toluene peak obtained between two laboratories conducting a reaction completion IPC analysis for the synthesis of intermediate material G. Significant retention differences were not observed for the other peaks in the chromatogram. A thorough investigation was performed, and no evidence was found that the method parameters could affect the retention time on toluene only. Eventually the root cause was identified as the analyst using a C8 column, instead of the method’s specified C18. A typical separation was obtained at the receiving laboratory once the correct column was used.

### Conclusion

Method development, validation, and technology transfer requires appropriate planning to help minimize incidences and risks. However, as illustrated above, even when good plans with control strategies are implemented, seemingly simple routine transfers can sometimes be challenging. These challenges can come from a variety of sources. An open mind and thorough investigation may be needed to ensure the method will perform well in the future. How to manage the amount of time, resources, and effort expended in a root cause determination when methods fail depends on long-term needs. With a combination of risk planning prior to transfers and sharing all appropriate available knowledge between sending and receiving laboratories, the number of issues can be reduced. However, it is hard to cover all risks, as some are inevitably unpredictable. Method transfers provide a challenge for any method developer, however, they also enable a significant amount of method performance knowledge to be gathered in a short period of time. This transfer experience helps build skills and insight that feed back into developing appropriate methods for early and late stage drug development support.

### Acknowledgements

The authors wish to acknowledge our colleagues with whom we have collaborated to perform method transfers and gain the experience summarized within this paper. Xuejun Xu, Kieran O’Conner, Rachel Wall, Jarlath Groarke, Vincent Gavin, Rafał Stachura, Junbæ Oh, Shinyoung Yune, Sandrine Cudre, Romain Curti, Virginie Colombel, Li Li, Phillip Houde, Qinggang Wang, Weiqing Fu, Elizabeth Yuill, Jonathan Shackman, and Morgan O’Sullivan have all worked diligently to execute the method transfers described in this manuscript. We also want to recognize our synthetic chemistry and chemical engineering colleagues such as Albert Delmonte, Kenneth Fraunhofer, Amanda Rogers, Ke Chen, Daniel Treitler, Dong Lin, Bahar Inankur, Michaël Fenster, Collin Chan, Matthew Hickey, Patrick Sipple, Jose Tabora, Thomas La Cruz, Eric Huang, Robert Forest, Steve Tymonko, Shawn Pack, Jason Sweeney, and Benjamin Cohen who provided important expertise in their areas of specialty in solving some of the problems encountered during the transfers described.

### References

5. USP 41, General Information Chapter <1224>: Transfer of Analytical Procedures (Rockville, Maryland, USA).
6. USP 41, General Information Chapter <1225>: Validation of Compendial Procedures (Rockville, Maryland, USA).
7. USP 41, General Information Chapter <1226>: Verification of Compendial Procedures (Rockville, Maryland, USA).

### AUTHORS

Peter Tattersall, Jia Zang, and Brent Kleintop work in Chemical and Synthetic Development at Bristol-Myers Squibb, in New Brunswick, New Jersey, USA. Direct correspondence to: amatheson@mjhlifesciences.com
Tips for Liquid Chromatography Coupled with Charged Aerosol Detection

Imad A. Haidar Ahmad, Associate Principal Scientist and Supervisor, the Analytical Chemistry Enabling Technology Group, Merck & Co., Rahway, New Jersey, USA

Charged aerosol detection (CAD) is a powerful complement to ultraviolet (UV) absorbance and mass spectrometric (MS) detection for liquid chromatography (LC), particularly for analytes that have no UV chromophore, or do not ionize well by electrospray ionization. As with all detector types, successful implementation requires an understanding of behaviour and characteristics of the detector. In this instalment, we discuss the characteristic nonlinear response of CAD, and approaches to deal with this, as well as mobile-phase requirements to consider for successful separations involving CAD.

Ultraviolet (UV) absorbance and mass spectrometry (MS) detectors are by far the most commonly used detectors for liquid chromatography (LC). They work exceptionally well for both qualitative and quantitative methods in many application areas. However, a significant weakness of UV-absorbance detection is that not all molecules of interest have chromophores (that is, a part of a molecule that absorbs UV light) that absorb strongly enough to make UV detection a viable option, especially in applications where detection sensitivity is an important performance metric. Over the years this weakness of UV detection has motivated research and development aimed at more “universal” detection schemes for LC (1), including refractive index detection (RID), evaporative light scattering detection (ELSD), and charged aerosol detection (CAD). Although these detection schemes have the tremendous upside that they are less dependent on the presence of a chromophore in our analytes compared to UV detection, they have their own weaknesses, including significant response dependencies on temperature, nebulization process, analyte volatility, and mobile-phase composition, as well as occasional nonlinear dependence of the detector response on analyte concentration. For users who were trained using UV or MS detection, these characteristics of RID, ELSD, and CAD can be challenging. For this month’s instalment of “LC Troubleshooting,” I have invited Dr. Imad Haidar Ahmad, Associate Principal Scientist at Merck & Co., Inc., to join me in sharing some tips and tricks he has found useful for the routine use of CAD in the research and development laboratories where he has worked.

Dwight Stoll

Charged aerosol detection (CAD) is a detection method that is widely and routinely used in the pharmaceutical and biopharmaceutical industries for analyses involving analytes with weak or no UV chromophore. Some well-known applications of CAD in these contexts are analyses for organic and inorganic counterions (2), sugars (3), and lipids (4). In addition to the characteristic of responding more uniformly to a broad range of analytes, CAD has other strengths, including ease of use, reliability, and wide dynamic range. These characteristics have earned CAD a place on the benches of many research and development laboratories. Moreover, CAD-based methods are also used in regulated quality control laboratories where methods are validated according to demanding ICH guidelines. Success in this environment requires good performance as measured by metrics—including accuracy, repeatability, sensitivity (as measured by limits of detection [LOD] and quantitation [LOQ]), and response linearity. However, as stated earlier, there are some characteristics of CAD in particular that require familiarization for those of us who were trained on UV and MS detectors. An excellent source of information on CAD is the recently published book edited by Gamache (5).
Coping with the Nonlinear Response of CAD

Modern UV-absorbance detectors used for LC exhibit a linear response over an analyte concentration range of more than four orders of magnitude (powers of ten). This makes quantitation very straightforward, as simple two-parameter equations (the slope and intercept of a line) can be used to describe the calibration curve. With CAD, however, the detector response to analyte concentration is not linear, and more complex relationships must be used to describe calibration curves. Several approaches have been used to overcome this limitation for quantitative applications. These include using localized linear fitting over a narrow concentration range, and plotting the response data on a logarithmic scale (log[response] vs. log[concentration]).

FIGURE 1: Plot for determining the optimum power function value (PFV). The left y-axis refers to the slope of a plot of response factor (RF) vs. analyte concentration (black circles), while the right y-axis refers to the relative standard deviation of the response factors calculated at different concentrations (blue circles). Waters Cortecs C18 (150 × 4.6 mm, 2.7 µm). Eluent A, 0.1% trifluoroacetic acid in H$_2$O; Eluent B, 0.1% trifluoroacetic acid in acetonitrile; Gradient: 5–100%B (0–15 min), 100%B (15–20 min), 100-5%B (20–20.1), 5%B (20.1–25 min); $F = 1.5$ mL/min; Injection volume = 20 µL; Column temperature = 40 °C; CAD evaporative temperature = 35 °C.
followed by a linear fit of the data over the entire concentration range. Neither of these are particularly attractive from a practical point of view, especially in regulated environments. The former approach is limited to a narrow concentration range, or establishing multiple calibration functions and choosing one that is most appropriate to the analyte concentration in each sample that is analyzed. The latter approach involves electronic data processing and transformation in spreadsheets, and these processes need to be reviewed, validated, and approved.

An alternative approach to expand the linear dynamic range of CAD is to transform the signal using a power function. Typically, a power function is implemented by raising the signal stream of the detector to a power $n$. This approach can yield highly linear plots of peak area vs. analyte concentration; however, in practice, it has been a challenge to determine the value of $n$ (referred to hereafter as the power function value [PFV]) that yields the best quantitative performance in terms of accuracy and precision. The optimum PFV can be obtained by studying the variation in the detector response factor ($RF = \text{peak area}/\text{analyte concentration}$) with analyte concentration. Specifically, we can look at the slope of $RF$ vs. analyte concentration, and the relative standard deviation (RSD) of $RF$ values over the range of concentrations studied. If the PFV were to perfectly linearize the detector response, we would expect both the slope and RSD to be zero.

Figure 1 shows experimental results from a study of the dependence of this slope and RSD on PFVs. We see that the optimum PFV in this case is about 1.4, corresponding to the lowest RSD value and a slope that is closest to zero. Figure 2 shows that, at the optimum PFV of 1.4, the relationship between peak area and analyte concentration is clearly more linear than when a PFV of 1.0 is used (that is, the default value in the software).

The conventional approach to obtain the optimum PFV involves acquiring peak areas for a standard at several concentrations, using several different PFVs, as shown in Figure 1. In our own work we have demonstrated the ability to predict the optimum PFV using only the CAD signal acquired with PFV = 1.0 (6). Recently, changes to commercially-available software for use with CAD have made determination of the optimum PFV much more user friendly.

**Mobile Phase Considerations that Promote Success with CAD**

As discussed above, a significant virtue of CAD is that it can “see” some compounds that are practically invisible to UV-absorbance detectors. The flipside of this characteristic of CAD, though, is that it will also “see” impurities in the mobile phase, or interferences in the sample, that would otherwise not be detected by UV-absorbance detectors if they do not contain chromophores. This is similar to the dilemma we often face with MS detection with electrospray ionization for LC separations—that it is exquisitely sensitive for detection of low concentration ionogenic compounds, but it is also very prone to detection of mobile-phase impurities that can give rise to high levels of detector background. Given that CAD is most sensitive to non-volatile compounds, the background signal in CAD is strongly affected.
by the concentration of non-volatile impurities in the mobile phase. Therefore, many of the recommendations made regarding use of LC systems with MS detection and preparation of mobile phases for LC–MS also apply when using CAD. These are listed below:

1. When switching from the use of non-volatile buffers with UV-absorbance detection to volatile buffers and CAD, be sure to thoroughly flush (30–60 min) the LC system with the new, volatile buffers before connecting the CAD to the column outlet.

2. Use ultrapure water with low conductivity (18.2 MΩ cm and less than 5 ppb total organic carbon [TOC]) for mobile-phase preparation to minimize the concentration of non-volatile impurities. It is recommended to use fresh water rather than water stored in bottles because of the possibility of sodium leaching from glass.

3. Use high purity organic solvents to prepare the mobile phase to minimize the concentration of non-volatile impurities. We find that LC–MS-grade solvents are generally adequate.

4. Use volatile, low molecular weight acids and bases for buffer constituents (examples include, but are not limited to, formic acid, acetic acid, ammonium formate, and ammonium acetate).

5. Filter samples prior to analysis whenever large particulate matter may be present in the sample.

6. Use flow rates within the recommended range of the detector.

7. Adjust the evaporation temperature of the detector to ensure efficient and consistent nebulization for different mobile-phase conditions.

8. Choose chromatographic conditions (such as temperature, mobile phase pH, or organic solvent) that minimize the likelihood of stationary phase bleed from the column.

9. Avoid sources of contamination from glassware, filters, stir-bars, pH electrodes, and laboratory equipment.

If a high background signal is observed, we recommend to start troubleshooting by removing the column while running LC–MS-grade mobile phase without additives. Once the background has returned to normal, the other components (buffer and column) can be added back one at a time while tracking the signal until the source of the higher-than-expected background is discovered.

Figure 3 shows the impact on the CAD background signal of flushing the LC system and detector with high purity methanol and water after a period of use with buffered mobile phase. Comparing the background after flushing (black trace) to that before flushing (red trace), we see that there are decreases in short-term noise, magnitude of the background signal, and long-term drift of the signal.

Summary

Many LC practitioners were trained using UV absorbance or MS detectors. Other detectors, including CAD and ELSD, which are attractive for the analysis of semi- and non-volatile compounds because they do not require that analytes have a light-absorbing chromophore, have increased in prominence in recent years. In this instalment of “LC Troubleshooting,” we have highlighted some of the ways that CAD in particular is different from UV-absorbance detection, including nonlinear response to analyte concentration, and sensitivity to mobile-phase impurities. We hope that these tips are especially helpful for those new to CAD, and lead to development and implementation of successful methods involving this powerful detection technique.

References


AUTHOR

Imad A. Haidar Ahmad is Associate Principal Scientist and Supervisor in the Analytical Chemistry Enabling Technology group at Merck & Co., in Rahway, New Jersey, USA.

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 60 peer-reviewed publications and four book chapters in separation science and more than 100 conference presentations. He is also a member of LCGC’s editorial advisory board.

Direct correspondence about this column to the Editor-in-Chief, Alasdair Matheson: amatheson@mjlifesciences.com
Control or Chaos: How Can We Run a Gas Chromatograph from Anywhere?

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

This instalment of “GC Connections” is a follow up to November/December 2020’s column “From Detector to Decision: How Does the GC Instrument Generate Your Data?” This time, we explore the other side of the instrument-data system relationship—how the data system controls the functions of the instrument. Drawing on classical electronics and instrument designs, we see the evolution of instrument controls from knobs and gauges on the front panel of the instrument to computer control and, finally, to today’s web-based systems that allow instrument control and monitoring from anywhere. We see that the same fundamental electronic principles used to manually control gas chromatographs in the 1970s are still at the centre of today’s modern electronically controlled systems.

In our previous column, we explained that the controls for today’s gas chromatographs include an on-board computer that provides the low voltage signals that actuate and direct analog devices that control functions such as starting, stopping, and data collecting (1). Taking this idea one step further, we see that this computer controls all aspects of instrument operation, including temperature and flow control and valve or switch actuation. As seen in the November/December column, the basic controls (valves opening and closing, heaters heating, and switches moving) for data collection and analysis are still analog operations that are performed by mechanical devices even though these devices have been miniaturized. Digital control of these analog devices is what allows a gas chromatograph (GC) instrument to be operated from an external data system or even from off-site as long as the requisite software and networking are available.

Figure 1 shows the flow of data or instructions between an external control system and an electronic component on a GC instrument, such as the inlet heater. As the user, you want to instruct the instrument to heat the inlet to 250 °C. First, you enter the value into the data system (or on the front panel of the instrument if it has a keypad or touch screen). Second, the data system transfers the request to the instrument using one of the communication protocols (typically USB or ethernet) where the microprocessor on the instrument receives the signal and, through its on-board software, sends a low-voltage signal to a relay, which closes a mechanical switch to activate the heater. A second circuit within the inlet block includes a temperature sensor (usually a temperature-sensitive resistor) that provides a signal back to the on-board software when the desired temperature is reached so that the heater circuit can be opened (in other words, turned off).

With digital electronics and software providing the interface between the operator and the device (in this case the inlet heater), we can see that, if the software can be operated through a data system separated from the GC instrument over a company intranet or over the internet, then the instrument can as well.

Even though software provides the user with convenient and powerful controls, the instrument itself still relies on mechanical devices to operate. Although these devices have been miniaturized, they still fundamentally perform the same tasks that the first GC instruments did. In our previous instalment, we discussed the basic principles of digital and analog electronics in gas chromatographs. We saw that, although instrument control and data collection are now digital, the underlying chemistry that occurs at the detector to generate signals and the underlying mechanics that allow control of temperatures,
INTRODUCING THE NEW VICI®
D-3 PULSED DISCHARGE HELIUM
IONIZATION DETECTOR

DESIGNED FOR THE AGILENT 8890 GC

VICI model D-3-I-8890 for the Agilent 8890 GC 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
- Helium purifiers
- Pulser module
- Power supply
- Tubing
- Fittings

FEATURES

- Universal detector
- Wide linear dynamic range (5 orders LDR, from low ppb to high ppm)
- Lower detection limits (<1pbb for most compounds)
- Easily added and configured on new or existing 8890 GC, using Agilent electrometer and interface boards
- Low maintenance

www.vici.com  +41 (41) 925 62 00  info@vici.ch
gas flows, and instrument functions, are analog. We noted that analog and digital signals can be interconverted and that digital signals also provide the capability for instruments to have friendly and easy-to-use controls and user interfaces.

In this instalment, we look more closely at the processes by which modern GC instruments are controlled on the front end, with functions such as starting and stopping, actuating valves, such as the split purge vent, controlling temperature zones, and gas flows. As we did in November/December, we see how these operations work in a modern GC instrument by looking back at some older systems in which these operations were performed manually or with mechanical controls.

All of the control functions on early GC instruments were manually operated, as seen in many pictures of early systems available on the Internet, such as a photograph of an F&M Scientific Model 700 dual column gas chromatograph of the early 1960s, available from the Science History Institute website (2). Several knobs and switches and gas outlet ports can be seen. Needle valves and manual regulators controlled the gas flows. The column oven was accessed from the top. Clearly, this instrument required the operator to be present to perform every function, including injecting the sample; there was no auto-sampler.

The several knobs on the front of this and other early and simple gas chromatographs provided the familiar temperature controls for the column oven, detector, and injector port. Additional knobs provided control functions for a thermal conductivity detector, including filament current, with coarse and fine adjustments and signal attenuation. A chart recorder (not shown) would have plotted the analog detector signal vs. time on a roll of paper. Compared with today’s instruments, this system was also very bulky, had high power consumption, and required the user to be present for every analysis. Following the analysis, data processing was done by hand, with the chromatogram being printed out on a paper roll. Instrumental conditions were entered by hand directly on the paper or entered into a lab notebook.

The knobs for the temperature controllers are called potentiometers, which provide a convenient means for regulating the voltage by changing the resistance with constant current and applying Ohm’s Law, which states:

$$V = IR$$  \[1\]

where $V$ represents the voltage (V), $I$ the current (A), and $R$ the resistance (Ohms). A simplified circuit diagram of a potentiometer is shown in Figure 2. You can think of a potentiometer as performing the same function as the knobs on your electric stove or oven, providing variable resistance to the heating coils. At the point where the arrow connects to the resistor, the resistance changes and therefore the voltage. Dials, like those seen on the front of old GC instruments, are simply circular resistors that allow for changes in the resistance and therefore the voltage, as the knob is turned. In Figure 2, the arrow represents the dial, showing the variability of the resistance. One disadvantage of this kind of
variable resistor is that the excess current must be dissipated as heat, so this function today is often performed using semiconductor-based devices that have much less heat dissipation and can be much smaller than knobs designed to be turned by hand.

Even in today’s fully digital GC instruments, the digital controls, which are convenient for users to operate, are still operating analog devices. As examples, we will examine three of the important control functions on any gas chromatograph: the remote start–stop and valve actuators, the inlet (and detector) heaters, and the split vent (purge) valve. Although we now control these through the keypad, software, or an app, and even though some of the devices themselves have been miniaturized, they still perform the same functions as on past instruments.

In November/December, we examined the remote-control port on a 1990s-era GC instrument and saw several control functions. Each of these functions works using a simple relay called a contact closure, essentially an electrically operated switch that can leave the circuit open (no current is flowing) or closed (current flows). The flowing current in a closed contact closure can direct the actuation of a switch or valve or perform other tasks. The contact closure is directed to open or close by the application of a small voltage to the switch.

**FIGURE 3:** Circuit diagram for a simple relay with a contact closure. When the circuit is closed, the current flows. The closure is controlled by the relay in the middle, which receives a low-voltage signal from the controller.

---

**Streamline your GC workflow with a Precision gas generator**

Combining convenience and safety in a stackable and modular **laboratory gas generator system**, Precision is the practical solution to your **GC detector and carrier gas** needs, providing hydrogen, nitrogen or zero air at the push of a button. **Eliminate the need for gas cylinder deliveries**, reducing your lab’s carbon footprint whilst **minimizing the risks of Covid-19 contact transmissions**, with an on-demand gas generator.

**Contact us today to discover more!**
**www.peakscientific.com/labgas**

---

Your local **gas generation partner**
Figure 3 shows a simplified circuit diagram including a contact closure relay. A common relay for a single switch includes four contacts: Two are for the circuit being opened or closed, and the other two are for controlling the relay itself, as seen by the four leads shown in Figure 3. In the case of the start-stop function, when the GC instrument is not performing a run, the circuit is open (the switch is off). A low-voltage signal from a data system is sent to the relay, which mechanically closes the circuit, causing current to flow and starting the GC instrument. The relay is much like turning on the switch for a light bulb. When the instrument starts, the relay usually switches “on”, then resets quickly to the “off” position, generating a start “pulse” that instructs the instrument to begin the run. A similar relay instruction is then used at the end of the run for the GC instrument to signal to the data system and other devices that the run is over.

The zone heaters are a second set of analog devices that are now controlled digitally. All gas chromatographs have three heated zones: the inlet, the column oven, and the detector. The inlet is heated to assist in vaporizing the injected sample, the column oven is heated to control the separation, and the detector is heated to keep it clean. Each of these heaters is a direct current device that operates according to Ohm’s Law and Joule heating, often called ohmic or resistive heating. The relationships between voltage \( V \), current \( I \), resistance \( R \), and heat \( J \) are given by:

\[ V = IR \]  
\[ P = \alpha FR, \text{ where } P \text{ (watts)} = J/s \]  

In short, heat is generated in a resistor proportionally to the square of the input current and proportionally to the resistance. The heaters in the oven, inlet, and detector are simple resistors to which current is applied to produce heat, much in the same way as the oven in your kitchen. On older GC instruments, these heaters were controlled using the potentiometers on the front of the instrument. On newer, digitally controlled GC instruments, the heaters are turned on and off by low voltage signals sent to relays, as described above and the temperature is monitored by a second circuit using a temperature-sensitive resistor.

Gas flow control valves are a third necessary control in a GC instrument. A typical solenoid valve classically used in split–splitless inlets to switch between split and splitless mode is shown in Figure 4. The fundamentals of split and splitless injection modes were discussed in a recent “GC Connections” installment (3). In short, the solenoid valve is used to redirect most of the carrier gas flow from the glass sleeve to the split purge vent during splitless operation, without changing the overall flow into the column.

The valve is actuated by a simple contact closure that directs the valve to be either “open” in split mode or “closed” in splitless mode. When the valve is open in a split injection, flow enters through both the septum purge and split vent lines and exits to the septum purge vent and to the total flow controller and back pressure regulator ultimately to the split vent. When the valve is closed for a splitless injection, the split vent inlet line from the inlet is closed, forcing all of the flow to enter the valve through the septum purge line and still exit through both exit lines. With the inlet
still pressurized this has the effect in the inlet of reducing the flow through the inlet liner for the splitless injection. With these older valves, an audible “click” could be heard whenever the valve was opened or closed. For many old-school chromatographers, this sound was routine and comforting.

Figure 4 shows several additional gas lines and wires seen in and around those for the split–splitless inlet, which serves as a second inlet in the pictured system. Today’s gas chromatographs include all of the pneumatic components and the associated electronics for operating the inlets in single pneumatic packs that can be easily inserted or removed from the main instrument. Although this provides easy installation and “plug and play” convenience, it comes at the cost that none of the components are user serviceable. In the 1990s, if the solenoid valve wore out (because it is mechanical; it eventually would), it was a simple and inexpensive matter to order a new one and replace it. If the solenoid valve equivalent or another single component in the pneumatic pack has to be replaced, the entire unit usually has to be replaced, often at a significant cost.

Within the GC instrument, all of these electronic components are controlled through the main board, which is shown in Figure 5. Most interesting to note is the Z-80 microprocessor for a 1990s vintage GC instrument, which was discussed in detail in our previous article (1). This simple 8-bit microprocessor developed in the 1970s still finds its use today in a variety of devices in the “Internet of things”. The chip with the white label is the instrument’s own firmware; the actual programming that runs the GC instrument. Between them is a static memory chip. Also note the accumulated dust from 30 years of service with never removing the panel covering the electronics. Like the interface boxes we discussed in November/December, the GC instrument is a computer in and of itself. The main board also includes connections for separate boards that control detectors (to the left in the picture), inlets (to the right) and network interfaces (to the far right). The keypad on the front panel of the instrument is connected by a ribbon cable to the far left of the motherboard, and the communications cables, which connect to the rear panel of the instrument, are on the far right.

Having seen how the various mechanical devices that are needed to operate a gas chromatograph work, we can now put these together with the possibilities for remote operation and data collection. External control of an instrument requires a system modern enough to allow a digital data system to control the functions of the instrument, either internally or externally, an interface to the Internet and control software that can operate over the Internet.

All of the devices we have discussed so far—potentiometers, relays, valve actuators, flow controllers, and regulators—have been miniaturized and placed on chips or in solid-state cartridges in most modern gas chromatographs. They can be easily actuated using the same low-voltage pulses (binary, on and off) involved with the transistor–transistor logic (TTL) and digital communications we discussed in November/December.

The development of solid-state electronic controls and devices for the various valves, switches, and controllers needed on a modern GC instrument have revolutionized instrument design and control. Miniaturized GC instruments designed from the “ground up” for capillary GC and ease of operation are now commonplace and have progressed greatly from the large, fully manual systems used in the 1970s. Connecting these microprocessor-controlled instruments directly to the Internet or through a company intranet provides the possibility of operating the instrument and analyzing the data remotely. Even as the instruments have become smaller and the once bulky control devices are now contained in solid-state electronic or pneumatic packs, the same basic electronic and mechanical controls still underlie instrument operation. By looking back at these more bulky components and how they operate, we can better understand what these automated devices are doing and how they are doing it.

References
1) N.H. Snow, LCGC Asia Pacific 23(4), 17–21 (2020).
2) https://www.sciencehistory.org/model-700-dual-column-gas-chromatograph

AUTHOR
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. Direct correspondence to: amatheson@mjh lifesciences.com
Turning Up The Heat: The Effect of Temperature on Analytical Extractions

Douglas E. Raynie, Sample Preparation Perspectives Editor

The application of temperature is frequently employed to enhance analytical extractions, especially with solid samples or volatile analytes. Dating back to the earliest development of modern chemical analysis, such as with Soxhlet extraction, using temperature to enhance analytical extractions continues today with more recently developed techniques. Too often, chemists rely on the rule of thumb from chemical kinetics, which states that reaction rates double for every 10 degree temperature change. However, extractions are not reactions. Temperature impacts solubility, diffusion, surface tension, and other properties of the sample, potentially leading to solute decomposition. This month, we explore the role of temperature in analytical extractions.

Who am I to disagree with Bill Gates? Like the Microsoft founder, I found the book *Range: How Generalists Triumph in a Specialized World*, by David Epstein (1) to be one of my recommended, thought-provoking reads during the COVID-19 pandemic. Epstein argues that, with exceptions like golf which require rote repetition (such as muscle memory), a breadth of knowledge and experiences are essential for personal and professional development. He claims that innovators and “systems thinkers” cultivate an “ability to connect disparate pieces of information from many different sources” (1). This philosophy is embraced in the American Chemical Society’s push to include systems thinking of addressing the United Nations Sustainable Development Goals (see, for example, a special issue of *Journal of Chemical Education* [2]).

When it comes to analytical problem solving, the hyperspecialization of our discipline into high-resolution mass spectrometrists, vibrational spectroscopists, open-tubular chromatography gurus, and others can be to our detriment because the analyst must understand what the nature is of the problem at hand, how might the samples of interest be similar to or different from previous samples, and what tools are available in the analytical arsenal to solve the problem. Fundamental knowledge of our techniques, and their limitations, is essential to applying them in the most appropriate manner.

Many of our analytical extraction methods manipulate temperature to enhance extraction yields. In Soxhlet extraction and its derivatives, accelerated solvent extraction (ASE), also called pressurized solvent extraction (PSE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (USAE), and related methods all use increased operating temperatures to improve extraction yields and accelerate the rate of extraction. As a consequence of the increased solute solubility, solvent use can be dramatically decreased compared to room temperature methods. For volatile analytes, temperature is often elevated with purge and trap, static or dynamic headspace, or thermal desorption systems to drive compounds of interest into the gas phase for trapping and analysis. However, whenever increased temperature is applied to an analytical extraction, it is not as simple as turning up the heat, and more heat (higher temperatures) is not necessarily better. Thermal degradation of sample components is always a concern, but it is also important to note that while there is a kinetic effect to elevated temperatures, it is thermodynamics that drives the extraction in many cases. In subsequent sections, we provide a broad overview of these temperature effects on analytical extractions.

**Solubility and Partitioning**

Generally speaking, increasing temperature increases the solubility of solid solutes in liquid solvents. However, the extent to which this occurs may
vary dramatically between solutes. For example, we can see in Figure 1 that the solubility of sugar and potassium nitrate in water changes dramatically as a function of temperature. For sugar, the aqueous solubility of sugar approximately doubles with a temperature increase of 40 °C, and the effect is even greater for potassium nitrate. On the other hand, the water solubility of salt (sodium chloride) is essentially unchanged over a broad temperature range, while for cesium (III) sulfate ($\text{Ce}_2\left(\text{SO}_4\right)_3$), there is even a solubility decrease as the system rises above room temperature. Now imagine the most probable scenario when our system of analytical interest is a mixture. We cannot be assured extraction enhancement because increased solubility as a function of temperature is not equivalent for all analytes of interest. Luckily, it is exceedingly rare where we work close to the solubility limits of a chemical system.

A special case of applying increased temperature to liquid solvents is with so-called subcritical or superheated water. At temperatures above 200 °C up to the critical temperature (374 °C), the dielectric constant of water is lowered until it is similar to some common organic solvents (note that increased pressure is also applied to keep water in its liquid state). In this case, the added thermal energy is sufficient to begin to disrupt the hydrogen bonding between water molecules, allowing the “hot water” to begin to solubilize nonpolar solutes, which may be completely insoluble in conventional liquid water. This is the basis for “hot water extraction”, which has shown some promise in the literature but has yet to be exploited for widespread use.

In supercritical fluid extraction (SFE), the situation is even more complicated. The solvating power of a supercritical fluid is directly related to the fluid density, which is a function of both temperature and pressure. Within a given extraction, pressure is most easily controlled, so temperature is held at a selected value and pressure is used to adjust density (change solubility) if selectivity during extraction is desired. At constant pressure (often the case during an analytical extraction), increasing temperature will decrease density, and, hence, solubility. However, this is not always the case, and, when it is, there is not a clear relationship between temperature, pressure, and solubility. For example, Figure 2 shows the solubility of soybean oil in supercritical carbon dioxide. In this diagram, solubility of the triglycerides is fairly low until a certain temperature (60–70 °C in this case) is reached, at which point solubility increases to a great extent. We can infer that solute fugacity may play some role and numerous equations of state have been developed to predict density and corresponding solubility. Adding an additional level of complexity, and not addressed in this column, is

---

**Figure 1**: Solubility of sugar and selected inorganic salts as a function of temperature from 0 °C to 100 °C.

**Figure 2**: Solubility of soybean oil in supercritical carbon dioxide over the temperature range of 40–80 °C and pressures up to 1300 bar. Reproduced from reference (3).
the common approach of adding small amounts (5–20%) of organic co-solvent (modifier) to increase solute solubility in supercritical fluids.

We often use equilibria to describe analytical extractions, though in many (if not most) cases we do not operate at equilibrium conditions. Commonly the octanol-water partition coefficient, $K_{ow}$, is used to estimate relative solubility in extraction solvents:

$$K_{ow} = \frac{[\text{solute}]_{octanol}}{[\text{solute}]_{aq}}$$  \[1\]

as the octanol solubility may, for example, mimic solute partitioning through a nonpolar lipid cellular membrane. Given that solute solubility as a function of temperature varies greatly from solute to solute, can we predict what may happen to $K_{ow}$ values? While there are no simple generalizations, we can look at the heat of solution and apply Le Chatelier’s principle. That is, if the dissolution process is exothermic (releases heat), the partition coefficient should decrease with increasing temperature, while for endothermic systems, which absorb heat, the corresponding partition coefficients will increase. The magnitude of the change in partition coefficient will be proportional to the magnitude of the change in the molar heat of solution.

### Volatility

Like solubility, relative volatility can vary to a large extent with temperature and is dependent on operating pressure and the contribution of other components of the sample mixture. This has even led some to claim that headspace extractions may not be quantitative and was the subject of a previous “Sample Prep Perspectives” column about two years ago (4). In that column, we claimed, “It makes sense that heating will increase the rate of evaporation, enhancing the mass transfer, and driving analytes into the vapour phase, thus increasing their concentration for subsequent collection. The problem lies in the fact that different molecules possess different volatilities and, thus, differing amounts extracted” (4). This is demonstrated in Figure 3 where aromatic hydrocarbons are sampled, using solid-phase microextraction (SPME), in the headspace above samples of olive oil. The results show that, as the sample is heated, the behaviour of different sets of compounds change in a less than straightforward manner. This resulting change in headspace composition as a function of temperature not only influences the extraction yield of an individual analyte, but it could also potentially be exploited to gain selectivity or the ability to isolate one compound in preference to others in the sample. As a result, we admonished that the extraction temperature during headspace analysis must be well-documented.

### Thermodynamic Parameters

It is well-established that chemical extractions do not occur solely via solubility concerns, but also due to the system thermodynamics. Most importantly, especially when isolating analytes from solid samples, is the role of solute diffusion through the solid. Diffusion is often described using the Stokes-Einstein equation,

$$D = \frac{kT}{6\pi\eta a}$$

where $D$ is diffusivity, $k$ is a proportionality constant, $T$ is temperature, $\eta$ is viscosity, and $a$ is activity. At first glance, there is a direct proportionality between diffusion and temperature, which means higher temperatures result in fast diffusion. Diffusion, though, is also a characteristic of other solvent properties like viscosity and surface temperature. Starting with viscosity, we can describe the temperature dependence with the Andrade equation,

$$\mu = Ae^{B/T}$$

where dynamic viscosity is related to temperature ($T$) through an exponential function and

---

**FIGURE 3:** Thirty-minute SPME extractions of aromatic hydrocarbon standards in olive oil as a function of temperature, specific standards include (■) C$_1$-benzene, (▲) C$_2$-benzene, (●) C$_3$-benzene, (○) napthalene, (□) acenapthene and acenaphthyene, (+) phenanthrene, anthracene, and fluorine, and (×) fluoranthene and pyrene. Reproduced from reference (4).
the constants A and B are available in comprehensive thermodynamic tables. The relationship between surface tension and temperature is an even more complex function also involving the solvent critical temperature, as surface tension decreases to zero at the critical temperature. Thus, increasing temperature will increase solute diffusion, speeding extractions, but the relationship with extraction rate is not necessarily linear. We have previously discussed that the effect of diffusion is independent of extraction type (ASE/PSE, MAE, USAE) at constant temperature (6).

The case of supercritical fluids is a bit more confounding. Solute diffusion in supercritical fluids is indirectly related to density via the fluid viscosity. The diffusivity in these fluids decreases with increasing pressure at constant temperature and increases with increasing temperature at constant pressure.

Kinetics or Rates of Extraction
Extractions generally follow a first-order rate model, similar to the kinetics of chemical reactions. However, extractions are due to a combination of solubility and analyte diffusion. Neither alone dictates speed of a reaction and, as we have seen, neither is necessarily a linear function of temperature. So, while adages like “doubling the rate with every ten degree temperature change” may have merit to estimate reaction kinetics, this does not hold for analytical extractions.

Sample Thermal Degradation
Any time we heat a sample, breakdown, usually by oxidation, is a concern. A good analytical practice is to run suitable standards or well-characterized samples and check for degradation products. Is the degradation concern exacerbated with the temperatures used in modern analytical extractions? This was a concern in the early development of ASE and was addressed in research by Ezzell (7). He looked at the ASE of dicumyl peroxide (DCP), a known free radical generator. Half-life calculations predicted that up to 30% decomposition would be expected in at 7.5 min extraction at 150 °C. Next, DCP was spiked onto sand and extracted via ASE with hexane, first at 100 °C, then at 150 °C, with a 10 min heat and extract time. At 100 °C, no sample degradation was observed, as 101% recovery with a 5.9% relative standard deviation (RSD) was obtained. With an extraction at 150 °C, extraction recovery dropped to 77%, close (within 75–80%) to the estimated 30% decomposition. In both cases, the solvent bottle was pressurized with air and was not degassed. When the hexane was degassed and pressurized with nitrogen, the extraction yield at 150 °C increased to 91% with a 0.83% RSD, demonstrating that when proper precautions are taken, thermal decomposition can be avoided in many cases. It is assumed that these findings, with similar precautions, are transferrable to other techniques. For example, Ezzell also noted that degradation of unsaturated fatty acids during extraction of triglycerides from oilseeds with ASE at 130 °C was similar to that observed during Soxhlet extraction. However, free-radical generation with ultrasound extractions is well-known among practitioners, in this case because of the high sonic energy applied during the extraction.

Conclusions
The development of modern analytical extractions has progressed through the application of increased extraction temperature. In fact, aside from solute solubility in the chosen extracting solvent, applied temperature is perhaps the single most important parameter (along with particle size, for solid samples) driving sample extractability and, to a lesser extent, selectivity. However, to most effectively exploit the role of temperature in extraction, the analyst must have the suitable combination of knowledge and experience with their sample type and their extraction techniques.

References
What is on Your HPLC Particle? A Look at Stationary Phase Chemistry Synthesis

Diego A. Lopez¹, Ahren I. Green¹, and David S. Bell², ¹Scientist II in the LC-R&D group at Restek Corporation, Pennsylvania, USA, ²Column Watch Editor

In 1973, Waters Corporation launched the first commercial 10-μm particle C18 column using a bonded monofunctional silane (μBondapak C18). Almost 50 years later, the C18 phase is still the reigning champion in the reversed-phase liquid chromatography (RPLC) arena, and many chromatography companies are still bonding using the same or a very similar synthetic approach. Several innovative bonding chemistries have been developed over the years to mitigate some of the common problems associated with the traditional C18 bonding approaches. These may include low and high pH stability, undesirable silanol activity, and a lack of polar retention. Nevertheless, particle technologies based on silica have received the most attention because of their undisputed chromatographic benefits. Silica supports offer high mechanical strength, allowing the formation of packed beds that are stable for long periods under high operating pressures. Controllable surface area, diversity in particle morphology, and higher efficiency values when compared to other support materials are just some of the advantages of silica-based columns. Advances in platforms that drift away from legacy products in terms of both the solid supports and in device architecture are trending in the literature. Some of these areas of interest include monoliths, open tubular columns (OTCs), microchip based columns, metal-organic frameworks (MOFs), and overall column and instrument miniaturization. Such developments will challenge traditional approaches toward surface chemistry modification. This article will review historical bonding techniques still in use for manufacturing high performance liquid chromatography (HPLC) stationary phases today, and also examine some emerging technologies that may be able to tackle unmet needs in novel platforms and phase construction.

Various advances in silica manufacturing have paved the way for modern chromatography. Since their inception in the 1970s, superficially porous particles (SPPs) have offered good performance and are indispensable in high-speed high performance liquid chromatography (HPLC) (1). Recent trends show the preference towards using SPP, especially in emerging markets such as cannabis (2); nevertheless, conventional particle sizes are still beneficial due to their higher surface area and their high loadability required for preparative scale applications. These advancements on solid supports have contributed great benefits to every industry where chromatography takes place, but novel approaches to functionalization of such platforms has remained stagnant for decades. According to the United States Pharmacopeia (USP), there are 858 C18 liquid chromatography phases registered under code L1 (octadecylsilane chemically bonded to porous silica or ceramic microplates, 1.5 to 10 μm in diameter, or a monolithic rod) (3). The vast amount of commercially available C18 columns are constructed on a wide range of solid supports, and may exhibit ancillary options such as endcapping and aqueous compatibility (AQ). They can also be mixed with other reversed-phase liquid chromatography (RPLC) phases. Although silica particle manufacturing has become more normalized in the last few decades, these subtle...
differences in commercial C18 make it extremely difficult for the novice, and even the seasoned chromatographer, to “grab a column and go” for a given application.

Silica is an amorphous polymer of silicon and oxygen. This polymer’s surface contains reactive silanols (Si-OH) that offer a number of possibilities for the synthesis of chemically-bonded phases. Organosilanes have been used as early as the 1950s to functionalize filter paper for the separation of steroids (4). In 1973, Locke correctly predicted that organosilanes would transform HPLC columns via chemically-bonded phases, mentioning that polymerization of silanes would be the synthetic route taken in general with chemical reactions being carried out to produce a primary organosilane layer (5). At the same time, he hoped for new developments in bonding stationary phases through the introduction of specific groups onto the organosilane bonding reagents; however, almost half a century later, organosilanes are still being grafted onto silica particles via the same chemistry described back then. Although new synthetic approaches have been applied to reversed-phase ligands, the bulk of the commercially available phases are still manufactured via conventional methods (6).

C18 Ligand Chemistries
Using reactions developed by John Speier at Dow Corning, USA, Waters’ scientists successfully synthesized octadecyldimethylchlorosilane (ODS), leading to the first commercial monomeric-bonded C18 column in 1973 (7). Since then, traditional bondings of reversed phases use a monofunctional silane in order to maximize ligand coverage and avoid unwanted polymerization that could affect batch-to-batch reproducibility. A typical monofunctional silane will yield a ligand density of around 3–4 µmol/m² under optimal bonding conditions, leaving behind up to 50% of the original amount of silanols based on an average of 8 µmol/m² on a typical silica surface. HPLC phases labelled as “end-capped”, “maximum coverage”, or “high density” still abide by this maxima, and any residual silanol would still be able to interact with analytes, contributing to the overall adsorptive properties of the bonded phase.

Over the years, traditionally-bonded phases have suffered from disadvantages tied to the use of silica as a solid platform. The tethering of the ligand to the silica surface is subject to hydrolytic cleavage at pH < 2, leading to loss of bonded ligand while silica particles are prone to dissolution at pH > 8. However, several bonding chemistries have been developed to circumvent such disadvantages, and numerous commercially-available phases are able to mitigate these problem areas.

Figure 1 shows some of the innovative bonding constructs
for several C18 phases. Element (a) shows a conventional monofunctional octadecylsilane and the most common phase available on the market. Element (b) shows a difunctional silane that is formed when a dichloro- or a dialkoxysilane is chemically bonded to silica; this double attachment is thought to increase the stability of the ligand at lower pH values and decrease phase bleed. Some manufacturers claim to use trifunctional silanes (not shown), but the exact control of tridentate reactions with the silica surface is still open to debate, and may lead to lower reproducibility of manufacturing. Element (c) shows a “bulky” silane where an isopropyl or isobutyl group hinders the siloxane linkage from hydrolytic cleavage at low pH values (8). Element (d) shows a “bridged” phase, where a bidentate organosilane is grafted on a silica particle, better shielding the surface from dissolution at a high pH. This latter bridging (crosslinking) technology can extend from one to several protective organic and inorganic layers, rendering high stability to the phase overall (9). This is not to be confused with hybrid silica particles where the organic moiety is a main component of the particle construction, and not just surface functionalization (10).

Element (e) in Figure 1 shows an alkyl chain much like its C18 counterparts, but contains a polar group intrinsic to the chain (amide, urea, and carbamate). These polar-embedded groups (PEG) have led to a new class of phases that offer some surface silanol shielding and additional polar retention (11). The polar-embedded moiety yields good peak shape towards basic analytes while making the phase compatible in 100% aqueous mobile phases without the “dewetting” effect. These functional groups can be obtained via a single step, using a pre-formed PEG containing silylating ligand directly onto silica, or a multi-step process, as outlined in Figure 2 (12). Amines and hydroxyl groups react with acid chlorides to yield amides and esters respectively; however, since ester groups are more unstable at low pH, amide linkages have been the preferred one among HPLC phases, along with sulfonamides (13). However, due to their toxicities, high reactivities, and non-selective behaviours, acid chlorides are rarely used in amide coupling reactions currently. Instead, several peptide-coupling reagents have been developed over the years to fulfil the safe and efficient processes required in drug development, and eventually applied towards manufacturing HPLC phases. Some of these popular reagents are highlighted in the following manuscripts for the construction of chiral and achiral ligands: 1,1’-carbonyldiimidazole (CDI) (14), hexafluorophosphate azabenztiazole tetramethyl uronium (HATU) (15), 1-hydroxybenzotrazole (HOBT) (16), and N-Ethyl-N’-(3-dimethylaminopropyl) carbodiimide (EDC) (17).

The other major categories of polar-embedded groups are ureas and carbamates. They are obtained by reacting isocyanates with primary amines and alcohols respectively. These functional groups provide additional polar interactions as hydrogen bond acceptors and less ionic interactions when compared to alkyl phases, leading to selectivity differences for polarizable compounds while improving peak shape of basic analytes (18). Additionally, carbamate linkages have been used specifically in the derivatization of chiral selectors such as Pirkle type (19) and polysaccharides due to the abundance of hydroxyl groups while enhancing their chiral recognition (20).

Silanization Chemistries
While the diversity of column chemistries for all types of separation modes is never ending, the chemistry of ligand attachment
or grafting is not nearly as varied. Chlorosilanes, alkoxysilanes, and silazanes have been the workhorses of silica functionalization. They react through hydrolysis, condensation, and polymerization reactions, where a new siloxane bond (Si-O-Si) forms while yielding a small molecule, typically hydrochloride gas, diethylamine, methanol, ethanol, or water. The result of reacting an organosilane with silica's surface is not only the bridging between organic and inorganic materials, but also is what imparts the main mode of separation to each stationary phase.

The advance of silicon-related technologies in material science has driven silane ligand synthesis. However, recent acquisitions of two major silane manufacturing companies leads to uncertainty of the current silane portfolio (21,22). Silane synthesis, although straightforward, suffers from one caveat: purification. Flash column chromatography is the method of choice when purifying a newly-synthesized compound from a mixture. However, a desired chlorosilane or alkoxysilane may permanently bind to the silica gel, leading to poor recoveries. Although some protocols exist to passivate silica gel from interacting with the silane ligand (23), the bulk of the reactive organosilane purification have relied on simple distillation. Such an approach leads to the confinement of commercially available ligands to a specific molecular weight range and containing functional groups that are thermally stable enough to endure purification by this technique.

An evolutionary bonding technology uses hydrosilanes, which Pesek developed through the development and the application of hydrosilylation chemistry in the production of HPLC stationary phases (24). The “Type C” silica possesses silica hydride (Si-H) at the surface of the particle and lacks the negative effects of silanols found on Type A and Type B silica. In order to functionalize this surface, a terminal alkyne or alkene will undergo hydrosilylation with Si-H in the presence of a platinum metal catalyst, leading to a phase with improved resistance to conditions that may cause hydrolysis in Type B silica columns. This is partially due to the much more hydrophobic surface offered by the silicon hydride moieties versus the usual hydrophilic silanol (25).

Figure 3 displays the reaction outcomes of alkene and alkyne hydrosilylation of a hydride-terminated particle. Alkynes may undergo a double hydrosilylation resulting in a bidentate attachment onto the surface, although most of the ligand will be singly attached via silicon-carbon double bond (Si=C) due to steric hindrance (26). The presence of hydrides, or the lack of silanols (<5%), and the strong Si-C or Si=C resulting from hydrosilylation is what gives these phases their chemically stability and interesting selectivity for chromatographic applications. Several metal catalysts have been used throughout the years but platinum remains the gold standard. Hexachloroplatinic acid (H\(\text{PtCl}_6\)), also known as Speier’s catalyst, and later the silicone-soluble Karstedt’s catalyst was adopted for this type of chemistry (27).

A look into the hydrosilylation platform synthesis leads to some unanswered questions and challenges. The first challenge is regarding the complete removal of the platinum reagent after the bonding reaction. The catalyst may form colloidal platinum that could deposit deep inside the particle making it almost impossible to remove; therefore, defeating the purpose of using Type B silica as the very first solid support (28). The second challenge is the catalyst’s selective functional group tolerance, leaving the weak anion-exchange and many mixed-mode phases out of the hydrosilylation platform (29). The third challenge, which is still subject to debate, is whether the coverage of Si-H is enough (~95%), hindering as many surface silanol and the inertness of such moieties during the lifetime of the column (30,31). The last challenge is phase availability, and the difficulty of finding equivalent columns by other manufacturers (32).

**Functionalization of Novel Solid Supports**

The most common formats for analytical columns remain 4.6 mm and 2.1 mm i.d. dimensions, although smaller, capillary-size columns have found a place within the HPLC arena, especially for complex biological applications. Capillary (0.3–0.5 mm i.d.) and nano formats (0.075–0.1 mm i.d.) have become more popular in the last few years, and many vendors offer a decent variety of stationary phases in these dimensions. The sub-millimeter internal diameter columns are packed with functionalized silica particles via slurry methods similar to their larger dimension counterparts; nevertheless, this process has
shown to be challenging, and much research has been devoted into this field (33,34). As an alternative, other phase supports, including monoliths and pillar arrays, have gained traction in recent years, and although both of them promise either lower backpressure or higher efficiencies than the particle packed beds (35), very little has been mentioned in regards to the pragmatic functionalization of such formats.

Monolithic beds are usually created in situ by free radical polymerization of monomers in the presence of porogens. Although thermal polymerization is a viable option, temperature fluctuations can occur in the confined spaces of the fluidic path, altering bed homogeneity along the column (36). Monomers can be organic (styrene, or acrylate-based) or siliceous in nature and offer the right amount of synthetic handles to permit surface functionalization. However, due to the nature of the inorganic monolith requiring a high temperature calcination step during its manufacturing, the appropriate functionalization must take place in situ as well. Hilder and co-workers developed a flow method for the ODS grafting on a 100 x 4.6 mm i.d. silica monolith, and its performance benchmarked against commercial C18 monolithic and a particle packed column (37). Since then, similar flow-like protocols have been applied to other column dimensions; however, slow reagent flow rates (mL/min) and high temperatures are still needed to graft a common C18 ligand, leading to bonding times of up to 24 h for a single analytical size column (38). Organic-based monoliths offer a wider variety of synthetic functionalization that are out of the scope of this article; however, their pervious behaviour towards organic solvents and their hindered mass transfer kinetics for small molecules result in the chromatographic preference towards silica-based monoliths. For more information, El Rassi and co-workers recently published a review discussing several post-polymerization functionalization strategies (39).

Another column format involves microfabricated devices with microfluidic channels. Sepaniak and co-workers describe the functionalization of a pillar array architecture, which includes submerging the silicon oxide layers of the pillars in pure octadecyltrichlorosilane (OTS) and heating it to 170 °C for 2 h (40). On the other hand, De Malsche describes a flow method to functionalize the silica porous layer of radially elongated pillars (REP) where a solution of ODS is infused under 40 bar of pressure overnight. Although this method successfully grafted a C18 phase, the process only allows for a single “column” functionalization in about a 24-h window (41).

As novel formats show promising chromatographic benefits, whether they are open tubular columns, chip-based platforms, or overall miniaturization of the column compartment, the grafting protocols must deviate from legacy silanization chemistry to facilitate their large scale manufacturing while securing low cost and high batch-to-batch reproducibility. Vendors are currently able to manufacture functionalized silica in kilogram scale within 24 h, which can be used to pack hundreds or maybe even thousands of conventional analytical or capillary size columns. New solid supports and platforms such as metal-organic frameworks will obligate scientists to figure out interesting ways to functionalize them, given their lack of attachment points while at the same time widening their applicability in separation science (42).

**Conclusion**

Neue wrote that, when compared to gas chromatography (GC), variety in HPLC stationary phases is not necessary since scientists have control over the mobile phase composition, which is a powerful tool over the selectivity of the separation (43). However, we are seeing an influx of novel stationary phases in the literature and the market, especially in the mixed-mode arena, to fulfill wider customer demand for solutions. Many innovative chemistries have been designed to tackle the common problems of reversed-phase associated with silica as solid support from bulky silanes to polar embedded ones; nevertheless, with the exception of hydrosilylation platform, the actual silanization technique has remained largely the same for almost half a century. Miniaturized chromatography systems are already on the market, but the bulk of surface modification processes cannot be adapted into these new formats, especially at the rate of customer needs. Flow functionalization has shown to be an alternative to conventional particle bonding methods but reactions times per column bonding remain excessively...
high. Historically, silanization techniques have been developed by both academia and the silicon industry; furthermore, these new methods are readily available in the literature and require metal-free conditions, room temperature, and faster kinetics. It is time for chromatography firms to implement such innovative grafting protocols.

**References**


**AUTHORS**

Ahren I. Green is a Scientist II in the LC-R&D group at Restek Corporation, USA, where he splits his time between synthetic work related to stationary phase construction and fine chemical synthesis for reference standard production. Diego A. Lopez is a Scientist II in the LC-R&D group at Restek Corporation, USA, where his role is to research, develop, and present on novel separation technologies, including new functionalization strategies for chromatography, and direct-to-MS applications. David S. Bell is a director of Research and Development at Restek, USA. 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 liquid chromatography, gas 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
Enzyme for High-throughput Applications

UCT’s highly concentrated Abalonase Ultra features enzymatic activity levels upwards of 150,000 fishman units/mL. According to the company, this three-times concentrated β-glucuronidase can be used for real time and room temperature hydrolysis for high-throughput drug-screening applications.

www.unitedchem.com/product/abalonase-ultra
UCT Inc., Bristol, Pennsylvania, USA.

UHPLC HILIC Columns

iHILIC-Fusion and iHILIC-Fusion(+) have two lines of 1.8-µm UHPLC HILIC columns with different surface chemistries. These columns provide customized and complementary selectivity, ultimate separation efficiency, and ultra-low column bleeding. The columns are particularly suitable for LC–MS based applications for the analysis of polar compounds.

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, according to the company.

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

SEC Columns for mAbs

The new PSS MAB columns satisfy the increasing demand for robust columns for the analysis of monoclonal antibodies. High resolution and a wide separation range enable separations of fragment or aggregate analysis on one column, according to the company. MAB SEC columns are available as analytical (8 × 300 mm) or semi-micro (4.6 × 250 mm) columns and are designed and ready to use for light scattering detection.

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

GC Generators

The Precision Hydrogen Trace generators are designed for GC carrier gas use and detectors requiring hydrogen fuel gas, such as FID and FPD. With three flow rate models (250 cc/min, 500 cc/min and 1200 cc/min) one generator is capable of supplying multiple GC instruments and can be stacked with nitrogen and zero-air Precision models for a “space-saving” GC gas solution with no need for cylinders.

www.peakscientific.com
Peak Scientific, Inchinnan, UK.

Nitrogen Generator

The VICI DBS HP Tower Nitrogen Generator produces 24/7 on-demand supply of high-purity nitrogen with flow rates from 500 to 4,000 m/min, purity up to 99.999% and less than 0.1 ppm, and THC pressure up to 5 bar. The generator can be placed close to the instrument, which reportedly eliminates the need for long gas lines from external cylinder supplies.

www.vicidbs.com
VICI AG International, Schenkon, Switzerland.
iHILIC®
Advancing 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 LC-MS based "Omics" studies and other 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
Baltimore, MD

June 28th – 30th

Baltimore Convention Center

The World’s Largest Cannabis Science Event!

Cannabis Science Conference

Analytical, Medical, Cultivation & Hemp Tracks

Come grow with us!

- 125+ Speakers
- Exciting Exhibits
- Canna Boot Camp
- Panel Discussions
- Networking Mixers
- Cannaquarium Experience
  and much more!

The CSC Events team hopes that you are healthy and safe and we are excited to get our community back together in 2021. Now more than ever advancing science and medicine and sharing our research is needed and we look forward to seeing you again soon!

“Cannabis Science Conference has proven to be among the most influential educational events in the movement. They assemble the brightest researchers in the world to teach patients & medical professionals sitting side-by-side, learning together. Cannabis Science Conference is helping to galvanize rigorous scientific data supporting cannabis as a medicine.”

- Sue Sisley, MD

Sponsorship and exhibition opportunities are available. Please contact Andrea at Andrea@CannabisScienceConference.com for more info.

CannabisScienceConference.com  @CannabisScienceConference  @CannabisScienceConference