A VERSATILE SE-UHPLC METHOD FOR DETERMINATION OF SIZE-VARIANTS IN BISPECIFIC ANTIBODIES

Lloyd R. Snyder (1931–2018): A Personal Tribute

A Statistics Tutorial: Sum of Ranking Differences
Feel good in your method development?

feel better
with us

Trust our analytical products in every stage of your drug product development. Whether you’re working on bioanalytical LC-MS, formulation, or final quality control and release, we have the expertise you need.

Feel better with full workflow solutions at SigmaAldrich.com/feel-better
“Don’t you know of any other separation techniques?”

The unique combination of FFF-MALS-DLS

Moses made separating the Red Sea look easy enough, but if you’re trying to separate your macromolecules or nanoparticles with conventional chromatography — and it isn’t working, maybe it’s time to look at field-flow fractionation (FFF) — an alternative separation technology that has no stationary phase. We’ll show you how FFF combined with multi-angle and dynamic light scattering (MALS-DLS) can determine absolute molecular weights and particle sizes with no column calibration or reference standards.

Learn more at www.wyatt.com/separation
Something you can rely on

The GERSTEL MPS handles Your Sample Preparation and Introduction efficiently and reliably. Our solutions are intelligently automated to your specifications.

No programming, just Setup and Start by mouse-click. Your MPS works day and night, using less solvent and without anyone watching over it.

GERSTEL Solutions for GC/MS and LC/MS with Application Support at your Service.

What can we do for you?

www.gerstel.com

(800) 413-8160
sales@gerstelus.com
COLUMNS

850 COLUMN WATCH
Drawing A Better Map: Recent Advances in Protein Digestion and Peptide Mapping
Cory E. Muraco
New strategies for “bottom-up” analysis of therapeutic proteins, using faster enzymes, new buffer systems, and optimal column chemistries, enable analysts to perform these studies much faster and with fewer artifacts.

860 LC TROUBLESHOOTING
Letting the Chromatograph Talk, Part I: Looking for Troubleshooting Clues in Unexpected Places
Dwight R. Stoll
An ultraviolet (UV) detector signal can be an excellent source of clues for troubleshooting problems with your LC—even if you are using a mass spectrometer. We also share another good source of clues.

894 THE ESSENTIALS
How It Works: UV Detection for HPLC
The fundamental principles of UV detection are explained, in a single page.

PEER-REVIEVED ARTICLES

870 Development of a Multi-Product SE-UHPLC Method for the Determination of Size-Variants in Bispecific Antibody Formats
Tobias Graf, Raphael Ruppert, Alexander Knaupp, Georg Hafenmair, Sebastien Violini, Steffen Kiesig, Markus Haindl, Harald Wegele, and Michael Leiss
The versatile size-exclusion ultrahigh-performance liquid chromatography (SE-UHPLC) platform method described here provides superior separation for bispecific monoclonal antibody formats compared to a previous method.

882 Statistics for Analysts Who Hate Statistics, Part VII: Sum of Ranking Differences (SRD)
Caroline West
The sum of ranking differences (SRD) is a useful statistical tool for comparing methods, models, columns, or samples. It is also simple and straightforward.

FEATURES

888 Lloyd R. Snyder (1931-2018)–A Personal Tribute
John W. Dolan
Lloyd Snyder was one the “founding fathers” of high performance liquid chromatography (HPLC). He was one of the most widely cited chromatographers with many important contributions to the field of separation science.

891 Deep in the Heart of Texas, Chromatography Advances
Kevin A. Schug
The 2019 ISCC and GCxGC Symposia, May 12–17, 2019, in Fort Worth, Texas, promise to provide an exciting and relevant venue for discussions of the latest developments in chromatography.
Ultra smooth bores, low dead volume, clear graduations, and precision calibration.

VICI® Precision Sampling Pressure Lok® Syringes

Syringe options
HPLC syringes, liquid syringes, gas/liquid syringes and autosampler syringes.

PTFE plunger tips
Stress-formed to ensure leak-tight seals.

Self-lubricating plunger tips
Designed to remain smooth, without the seizing or residue of conventional metal plungers.

Needles sealed by PTFE sleeves
Isolates samples and prevents adhesive dissolution and contamination.

Easily replaceable parts
Depending on the specific model, some or all of the wear parts can be easily replaced.

Visit www.vici.com for more information.
Editorial Advisory Board

- Kevin D. Altria – GlaxoSmithKline, Ware, United Kingdom
- Jared L. Anderson – Iowa State University, Ames, Iowa
- Daniel W. Armstrong – University of Texas, Arlington, Texas
- David S. Bell – Restek, Bellefonte, Pennsylvania
- Dennis D. Blevins – Agilent Technologies, Wilmington, Delaware
- Zachary S. Breitbach – AbbVie Inc., North Chicago, Illinois
- Deirdre Cabooter – Department of Pharmaceutical and Pharmacological Sciences, KU Leuven (University of Leuven), Belgium
- Peter Carr – Department of Chemistry, University of Minnesota, Minneapolis, Minnesota
- Jean-Pierre Chervet – Antec Scientific, Zoeterwoude, The Netherlands
- André de Villiers – University of South Australia, Adelaide, Australia
- Peter Carr – Department of Pharmaceutical and Pharmacological Sciences, KU Leuven (University of Leuven), Belgium
- John W. Dolan – LC Resources, McMinnville, Oregon
- Michael W. Dong – MWD Consulting, Norwalk, Connecticut
- Anthony F. Fell – School of Pharmacy, University of Bradford, Bradford, United Kingdom
- Richard Hartwick – PharmAssist Analytical Laboratory, Inc., South New Berlin, New York
- Milton T.W. Hearn – Center for Bioprocess Technology, Monash University, Clayton, Victoria, Australia
- Emily Hilder – University of South Australia, Adelaide, Australia
- John V. Hinshaw – Serveron Corporation, Beaverton, Oregon
- Kiyokatsu Jinno – Department of Chemistry, Toyohashi University of Technology, Toyohashi, Japan
- Ira S. Kull – Professor Emeritus, Department of Chemistry and Chemical Biology, Northeastern University, Boston, Massachusetts
- Ronald E. Majors – Analytical consultant, West Chester, Pennsylvania
- Debby Mangelings – Department of Analytical Chemistry and Pharmaceutical Technology, Vrije Universiteit Brussel, Brussels, Belgium
- R.D. McDowall – McDowall Consulting, Bromley, United Kingdom
- Michael D. McGinley – Phenomenex, Inc., Torrance, California
- Victoria A. McEuffin – Department of Chemistry, Michigan State University, East Lansing, Michigan
- Mary Ellen McNally – FMC Agricultural Solutions, Newark, Delaware
- Inne Molnár – Molnar Research Institute, Berlin, Germany
- Glenn I. Ouchi – Brego Research, San Jose, California
- Colin Poole – Department of Chemistry, Wayne State University, Detroit, Michigan
- Douglas E. Raynie – Department of Chemistry and Biochemistry, South Dakota State University, Brookings, South Dakota
- Fred E. Regnier – Department of Chemistry, Purdue University, West Lafayette, Indiana
- Koen Sandra – Research Institute for Chromatography, Kortrijk, Belgium
- Pat Sandra – Research Institute for Chromatography, Kortrijk, Belgium
- Peter Schoenmakers – Department of Chemical Engineering, University of Amsterdam, Amsterdam, The Netherlands
- Kevin Schug – University of Texas, Arlington, Texas
- Dwight Stoll – Gustavus Adolphus College, St. Peter, Minnesota
- Michael E. Swartz – Stealth Biotherapeutics, Newton, Massachusetts
- Caroline West – University of Orléans, France
- Thomas Wheat – Chromatography Consulting, LLC, Hopedale, Massachusetts
- Taylor Zhang – Genentech, South San Francisco, California

CONSULTING EDITORS:
- Jason Anspach – Phenomenex, Inc.; David Henderson – Trinity College;
- Tom Jupille – LC Resources; Sam Margolis – The National Institute of Standards and Technology; Joy R. Miksic – Bioanalytical Solutions LLC

LC GC’s CHROMacademy
powered by crawford scientific

When it all goes wrong... you can “Ask the Expert”

Premier members can ask our panel of experts and get a reply within 24 hours.

Find out more about CHROMacademy Premier membership contact:

Glen Murry on +1 732.346.3056 | e-mail: Glen.Murry@ubm.com
Peter Romillo: +1 732.346.3074 | e-mail: Peter.Romillo@ubm.com

www.chromacademy.com
Chris Pohl Receives EAS Award for Outstanding Achievements in Separation Science

The EAS Award for Outstanding Achievements in Separation Science was awarded to Christopher Pohl at the Eastern Analytical Symposium (EAS) in Princeton, New Jersey, on November 12. Pohl is the vice president of Chromatography Chemistry in the Chromatography and Mass Spectrometry division of Thermo Fisher Scientific.

Prior to the acquisition of Dionex Corporation by Thermo Fisher Scientific in 2011, Pohl was the senior vice president of Research and Development and the chief science officer at Dionex. Prior to joining Dionex, Pohl worked at the Clorox Technical Center and at Chevron Chemical.

Pohl is the author or co-author of five book chapters and more than 100 scientific papers published in peer-reviewed scientific journals. He is an author or co-author of 85 U.S. patents with numerous foreign equivalents in a number of areas including separation methods, stationary phase design, suppressor technology, solid-phase extraction, capillary electrophoresis techniques, and accelerated solvent extraction (ASE).

Pohl’s most significant accomplishments include the initial development of high pH anion-exchange chromatography for the separation of carbohydrates via anion exchange, the development of hydroxide-selective phases suitable for gradient ion chromatography applications, the development of membrane suppressor technology, the development of ASE, where he was the primary inventor, and the development of hyperbranched anion-exchange condensation polymer synthesis technology.

Linda McGown Receives EAS Award for Outstanding Achievements in the Fields of Analytical Chemistry

The EAS Award for Outstanding Achievements in the Fields of Analytical Chemistry was presented to Linda B. McGown at the Eastern Analytical Symposium (EAS) on November 14 in Princeton, New Jersey. McGown is the William Weightman Walker professor of the Department of Chemistry and Chemical Biology at Rensselaer Polytechnic Institute (Troy, New York).

In her early career, McGown exploited the capabilities of frequency-domain fluorescence lifetime spectroscopy to create multidimensional data formats to characterize and classify complex samples such as human serum, humic substances, and petrolatums. She then integrated fluorescence lifetime detection into separation techniques. In the 1990s, McGown took an interest in aptamers. Her current work focuses on genome-inspired approaches to aptamer discovery to complement combinatorial methods, to explore a naturally evolved sequence space often underrepresented in combinatorial libraries.

McGown has been a Fellow of the American Association for the Advancement of Science since 2001 and received the New York Section of the Society for Applied Spectroscopy Gold Medal Award in 2004. She was included in The Future of Women in Chemistry and Science program in honor of UNES-GO’s declaring 2011 the International Year of Chemistry. She was one of “60 exemplary thinkers” who spoke about how to expand women’s leadership in the sciences, across all disciplines and sectors.

Kerri Pratt Receives EAS Young Investigator Award

The EAS Young Investigator Award was presented to Kerri Pratt at the Eastern Analytical Symposium (EAS), in Princeton, New Jersey, on November 12. Pratt is the Seyhan N. Ege assistant professor of the Department of Chemistry and Department of Earth and Environmental Sciences at the University of Michigan in Ann Arbor, Michigan.

Pratt’s analytical and environmental chemistry research focuses on the application of novel mass spectrometry methods to the study of the chemical interactions of atmospheric trace gases, particles, clouds, and snow to improve understanding and prediction of air quality and climate change. Using a chemical ionization mass spectrometer, she has made significant advances in understanding Arctic snowpack photochemical reactions that result in the production of molecular halogen trace gases at sub-ppt to ppt levels.
Drawing A Better Map: Recent Advances in Protein Digestion and Peptide Mapping

Biotherapeutics have become the hottest topic in pharmaceutical research over the past decade. With the increased interest in biotherapeutics, there has been a concomitant increase in new analytical methods for characterizing these large, complex molecules. This installment of “Column Watch” discusses advances in “bottom-up” analysis of monoclonal antibodies, while highlighting the role and importance column chemistry still plays in developing highly selective high-performance liquid chromatography (HPLC) methods for peptides.

Cory E. Muraco

Monoclonal antibodies (mAbs) are a type of biotherapeutic that is seeing a high degree of interest from academia and industry. Since the first approval in 1986, nearly 60 antibodies have been manufactured and commercialized up until 2015. 2016 alone was a banner year for antibody-based therapeutics, as the FDA approved seven therapeutic antibodies as new molecular entities: monoclonal antibodies are used to fight a plethora of diseases ranging from inflammatory conditions (1), autoimmune diseases (2), various types of cancers (3), and other infectious and neurological diseases (4).

Monoclonal antibodies are complex molecular entities with molecular weights of approximately 150 kDa. These molecules are composed of amino acids that are connected through amide (peptide) bonds. Monoclonal antibodies are expressed in cell culture systems, meaning that these molecules are susceptible to an entire suite of post-translational modifications (PTMs) of an enzymatic or non-enzymatic nature. The most common PTMs to a mAb include N-terminal pyroglutamate formation, methionine oxidation, asparagine deamidation, aspartic acid isomerization, C-terminal lysine truncation, and glycosylation (5). Due to the susceptibility of mAbs to be modified, the resultant product, directly from the expression system, is often a heterogeneous mixture of variants of the mAb. This high degree of complexity of the resultant product necessitates the need for analytical methods for characterizing mAbs.

One of the most useful techniques for characterizing post-translational modifications is to perform “bottom-up” analyses of proteins or mAbs, also called peptide mapping. In peptide mapping, the mAb is digested by a protease, like trypsin, into peptides. These peptides are subsequently separated and identified by liquid chromatography–mass spectrometry (LC-MS). However, due to the complex folding and disulfide bonding present with mAbs, before digestion occurs, these proteins are typically denatured, or unfolded, to allow full access of the protease to the consensus sequence on the protein. In addition, disulfide bonds are reduced and alkylated to prevent spontaneous refolding of the protein. Once these steps are completed, the protease is added to the sample, and the digestion reaction can proceed from anywhere between four

FIGURE 1: Plot of number of peaks identified versus reaction time between recombinant trypsin (SOLu-trypsin) and a standard, animal derived trypsin.
See What It Can Do for You and Your Lab

- Technical Articles & Applications
- Videos & ChromaBLOGraphy
- FAQs & Troubleshooting
- Education & Instruction
- Online Tools & Calculators
- Product Selection Assistance

Sign up today to access Restek’s years of chromatography knowledge at www.restek.com/advantage
to twenty-four hours. After this pre-determined amount of time, the reaction is quenched by addition of formic acid, and the resultant sample is diluted and prepared for reversed-phase liquid chromatography (RPLC) analysis.

The process of digesting a protein, though, can lend itself to artifacts in the peptide mapping results. Usually, this is observed through additional methionine oxidation and asparagine deamidation that is not due to the inherent cellular machinery used to express the mAb. Heating of the digested sample over an extended period, over digestion by the protease, and reactive intermediates generated during the digestion reacting with peptides may cause artefactual modifications of the peptides (6).

Peptide maps of proteins can have anywhere from tens to hundreds of peaks. Therefore, chromatographic methods with high resolving ability or peak capacity are a necessity. One way to calculate resolution is through equation 1, where $R_s$ is resolution, $\Delta t_R$ is differential migration, and $W_A$ and $W_B$ are the peak widths of analyte A and B, respectively.

$$R_s = \frac{\Delta t_R}{0.5(W_A + W_B)} \quad [1]$$

Resolution of peaks in a chromatographic separation comes from two factors: a thermodynamic (chemical) factor, and a kinetic (physical) factor. The kinetic factor (the denominator of equation 1) is due mostly to the particle architecture and how well the column is packed. For proteins, the advent of superficially porous particles (SPPs) has drastically increased the efficiency of methods that were originally developed on fully porous particles (FPPs). For more information on why such advantages are present with SPP-packed columns, the interested reader may consult the following review articles (7, 8).

The thermodynamic factor that mostly affects resolution is the type of ligand that is used in reversed-phase liquid chromatography. Chromatographic generation of a peptide map has typically been done on
a C18 column. Just like with small molecule chromatography, the types of molecular interactions that a chromatographer can take advantage of with a C18 column are mostly dispersive interactions and, to a lesser controlled extent, ionic interactions from silanols on the base silica (9). However, peptides are amphipathic molecules, having both a hydrophobic and hydrophilic domain. It is hypothesized that the hydrophilic domains of peptides could interact with more polar reversed-phase columns through dipole-dipole interactions or hydrogen bonding. With more interactions at the chromatographer's disposal, more selective methods could be developed that permit better retention of polar peptides or orthogonal selectivity to conventional C18 chromatography. For the rest of this installment of “Column Watch,” experiments will be described with the goal of obtaining better peptide maps. First, different digestion protocols are assessed to optimize tryptic digest conditions. Then, the role of phase chemistry will be examined to better resolve critical pairs of peptides that may be present in a digested protein sample.

**Experimental Procedure**

**Tryptic Digest with SOLu-Trypsin**

Since SigmaMAb is a glycosylated immunoglobulin G1 (IgG1) antibody, the antibody needed to be deglycosylated prior to digestion. SigmaMAb was resuspended by adding 1 mL of “reaction buffer” (10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4). Glycinator (Genovis), an endoglycosidase, was resuspended by adding 50 μL of liquid chromatography–mass spectrometry grade water. Fifty microliters of Glycinator was mixed with SigmaMAb. The mAb-endoglycosidase mixture was heated at 37 °C for 35 min. Afterwards, using a HisTrap spin column, Glycinator was removed from the deglycosylated mAb. Prior to digestion, the deglycosylated mAb was dried by vacuum centrifugation. A denaturing solution consisting of a 1:1 mixture of 40% trifluoroethanol and 20 mM dithiothreitol (DTT) in 50 mM ammonium bicarbonate was prepared, and 20 μL of this solution was added to the dried mAb. The mAb plus denaturant mixture was incubated at 57 °C for one hour. Upon completion of the denaturation step, the reduced mAb was alkylated by mixing 5.0 μL of 200 mM iodoacetamide in 50 mM ammonium bicarbonate. The alkylation reaction occurred for one hour in the dark at room temperature. Upon completion of the alkylation reaction, 220 μL of 50 mM ammonium bicarbonate was added to dilute the denaturants. SOLu-Trypsin was added to the reduced and alkylated mAb such that the ratio of trypsin–mAb was 1:20 by mass. The trypsin-mAb solution was incubated at 37 °C at various time points (per Figure 1). After each time point, the digestion reaction was quenched by
adding 2.0 μL of formic acid, the sample was centrifuged at 5,000 x g for 30 s, and the supernatant was collected. Prior to analysis, the sample was diluted 1:1 with water containing 0.1% (v/v) difluoroacetic acid (DFA) prior to LC/MS analysis.

Tryptic Digest with SOLu-Trypsin and Rapid Digest Buffer

Deglycosylation, reduction, and alkylation was performed using the same procedure as in the previous two paragraphs. To the denatured mAb, 500 μL of Rapid Digest buffer was added to the sample. SOLu-Trypsin was added such that the ratio of trypsin: mAb was 1:20. After addition of SOLu-trypsin, the trypsin-mAb sample was incubated at 60 °C for one hour. After the one-hour digest, the reaction was quenched by mixing 2.0 μL of formic acid to the sample, centrifuging the sample at 5,000 x g for 30 s, and collecting the supernatant. The sample was then diluted 1:1 with water containing 0.1% (v/v) difluoroacetic acid (DFA) prior to LC/MS analysis.

Peptide Selectivity Study ("Kappa-Kappa Study")

A standard peptide mix, containing 14 peptides, was assayed using the HPLC conditions listed in Figure 4. Table I lists the identity of the 12 peptides used in the study (two of the peptides in the 14-component mix were not observed in the study).

Results

Rapid Digestion for High-Throughput Peptide Mapping

Since one of the ways that artifacts can present themselves during a peptide map experiment is through over-digestion of the protein, steps were taken to decrease the digestion time as much as possible. At first, the use of a recombinant trypsin was used, over a conventional, animal derived trypsin. Figure 1 displays the results of a time course study where aliquots of the digested mAb were taken, the aliquot was assayed by RPC, and the number of peptide peaks were determined. Note that with the recombinant trypsin, only four hours were required to attain complete digestion of the mAb whereas the standard trypsin required 18 hours.

Even though the reaction time has dropped to four hours, oxidation may still occur on some of the peptide fragments due to the fast reaction kinetics of oxidizing surface amino acids like methionine. Oxidation caused by the digestion protocol could, in a biopharmaceutical setting, cause the rejection of a qualified lot of therapeutic drug. In recent years, some vendors have developed "Rapid Digest Kits" for performing peptide mapping experiments, with an eye for high-throughput. Therefore, a recombinant trypsin coupled with a "Rapid Digest Kit" was employed, allowing for the complete digestion and analysis of the mAb in approximately two hours with only a one-hour digestion time. Figure 2 displays a comparison of the workflow of the "rapid" method versus conventional methods (10).

Upon completion of the Rapid Digestion technique, the digested sample was subjected to LC/MS analysis. Figure 3 displays the results of the analysis. After data analysis of the sample prepared with the Rapid Digest buffer, the sequence coverage of the heavy and light chain was 99% and 97%, respectively. Standard digestion protocols, without the Rapid Digest buffer, yielded sequence coverage of the heavy and light chains of 88% and 94%, respectively.

Peptide Selectivity–Do We Need More than C18?

As noted earlier in the article, most peptide mapping experiments utilize C18 columns for the LC separation. However, peptides have many polar functional groups that, theoretically, could interact...
DECADE™ Elite
Electrochemical Detection for any (U)HPLC System

- Neuroscience: Monoamines and metabolites analysis
- Food/Beverage: Sugars, Lactose-free products analysis
- USP/EP Pharmacopoeias: Antibiotics analysis
- Clinical: Catecholamines, Metanephrines and FDG analysis

www.AntecScientific.com

DECADE™ Elite

SenCell™
TABLE II: Properties and identities of signature peptides from Trastuzumab tryptic digest

<table>
<thead>
<tr>
<th>Elution Order</th>
<th>Peptide</th>
<th>Molecular Weight</th>
<th>pI</th>
<th>Number Aromatic Residues</th>
<th>Number Charged Residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FTISADTSKNTAYLQMNSLR</td>
<td>2261.53</td>
<td>8.59</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>LSAASGFNJKTDYIHWVR</td>
<td>2078.36</td>
<td>8.60</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>GFYPSDIAEWSGOPENNYK</td>
<td>2544.67</td>
<td>4.00</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>LLIYSASFLYSGVPSR</td>
<td>1773.06</td>
<td>8.59</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>SGTASVVLNFFYPR</td>
<td>1637.86</td>
<td>8.46</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>SDKHTTPAPELLGPSPVLFPKP</td>
<td>2855.33</td>
<td>8.25</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>VSVLTVLHQDWLNGKEYK</td>
<td>2228.58</td>
<td>6.72</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

FIGURE 5: Comparison of chromatographic results of a Trastuzumab tryptic digest using different column chemistries. Conditions: column: 160 Å SPP, chemistry as indicated, 10 cm x 2.1 mm I.D., 2.7 μm; mobile phase: [A] 98:2 water (0.1% DDA): acetonitrile (0.1% DDA); [B] 50:50 water (0.1% DDA): acetonitrile (0.1% DDA); gradient: 0% B to 63% B in 60 min; flow rate: 0.3 mL/min; column temp.: 60 °C; detector: mass selective detector–electrospray ionization (MSD–ESI)+(+), 100 – 3000 m/z; injection: 1.0 μL; sample: Trastuzumab tryptic digest, 10 μg/mL, water (0.1% DDA). Data courtesy of Advanced Materials Technology (AMT).

with more polar ligands (i.e. RP-Amide, F5, Phenyl-hexyl, etc.). Previous work (11) has hinted at the possibility for orthogonal selectivity of peptides on different phase chemistries to conventional C18 phase chemistries. Figure 4 illustrates this concept with plots of the logarithm of the retention factors for a set of peptide probes assayed on a C18 column versus a phenyl-hexyl (PH) and a cyano (CN) column (so-called kappa-kappa plots). From examination of Figure 4, one can see that there are differences in retention on the different phase chemistries. The phenyl-hexyl column, with π – π interactions and shape selectivity from the rigid, aromatic ring, brings in additional molecular interactions not present on a C18 column. In addition, the dipole-dipole interactions and π – π interactions of the CN phase also contribute to selectivity that is orthogonal to a C18 column. With peptides being composed of amino acids with varied side chains, these polar interactions may contribute to differential selectivity.

Based on this previous work, columns with different phase chemistries were used to improve the resolution of peptides resulting from a tryptic digest using the Rapid Digest technique. Figure 5 compares chromatograms generated from a tryptic digest of Trastuzumab, a monoclonal antibody used to treat various head, neck, and breast cancers. Table 2 provides the identity of the “signature” peptides that are numbered in Figure 5. As can be noted from the chromatographic data, each column yielded a different elution profile for the signature peptides. In fact, only the phenyl-hexyl column could resolve all seven signature peptides; the other two columns resulted in co-elutions of two or more peptides. These results, again, imply the advantage of using additional column chemistries compared to just C18.

Conclusion
Peptide mapping is an analytical technique where the primary structure of a protein is assessed through the digestion of the protein. Recent strategies have been developed, using faster enzymes and new buffer systems, that enable the analyst to perform peptide mapping experiments and the resultant LC/MS analysis in an afternoon whereas older techniques would take at least one to two days. These older techniques were also prone to under or over digestion as well as oxidation and deamidation due to the digestion protocol. Limiting digestion time to one to two hours minimizes these artifacts without compromising sequence coverage.

SSP-packed HPLC columns have allowed for an increase in efficiency and throughput of methods devoted to the analysis of proteins and peptides. In peptide mapping, a C18 column has mostly been used for such analyses. Using different phase chemistries can allow for orthogonal separations of peptide peaks to that obtained with a C18 column. Future research will involve screening different mobile phase systems and examining how the mobile phase can further improve resolution of peptide peaks and improve the sensitivity of methods.

References


ABOUT THE EDITOR

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

ABOUT THE AUTHOR

Cory E. Muraco is a Senior R&D Scientist in the Liquid Separations R&D group at MilliporeSigma, in Bellefonte, Pennsylvania. Cory completed his graduate studies at Youngstown State University in 2013, focusing on the analysis and characterization of oxidized proteins. Upon graduation, Cory joined MilliporeSigma, first joining the chemical standards R&D group, then transferring to the liquid separations R&D group. Cory’s current role at MilliporeSigma is to research, develop, and present on new particle technology for improved chromatographic separations of both small and large molecules and to develop new methodologies for characterizing biomacromolecules by several modes of chromatography. Cory has written about his research in several trade magazines and presented oral and poster presentations on his research at numerous conferences.

InfinityLab Stay Safe Caps | Solvent Bottles Fittings & Flex Bench Racks | And More!

For more information, contact Neta Scientific today.

Call (800) 343-6015, email sales@netascientific.com or visit NetaScientific.com for all of your lab research needs.

We know only the most accurate analytical products will do. That’s why we offer the Supelco® portfolio of analytical products. Whatever your needs, our portfolio is always fit for purpose, ensuring your results are accurate, precise and reproducible, and your systems fully certified. Our comprehensive portfolio, developed by analytical chemists for analytical chemists, covers a broad range of analytical solutions, and every product is meticulously quality-controlled to maintain the integrity of your testing protocols. And with Supelco® scientists dedicated to your analytical applications, the expertise you need is always on hand.

SigmaAldrich.com/Supelco

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, the vibrant M, Sigma-Aldrich, Sigma, Millipore, Cerilliant, Carbosieve, Certipur, Supelco and TraceCERT are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources. © 2018 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

Lit. No. MS_AD2917EN 10/2018
Footprint of Supelco

1966: Enters GC business with adsorbents and packed GC columns
1971: Enters carbon adsorbent business with Carbosieve®
1979: Enters HPLC with SUPELCOSIL™ line of stable 5 µm spherical particles with true monolayer bonding
1983: Enters the air sampling market by introducing a line of solvent desorption tubes for industrial hygienists to help protect workers from being exposed to toxic chemicals
1985: Enters sample preparation business with Supelclean™ SPE tubes
1993: Launches SPME fibers
2006: Sigma-Aldrich acquires Astec®: leader in chiral chromatography
2007: First to market globally Fused-Core® particles jointly with Advanced Materials Technology, introduced Ascentis® Express
2018: Supelco® expanded to include all analytical products from Merck KGaA for a comprehensive range of analytical techniques
Letting the Chromatograph Talk, Part I: Looking for Troubleshooting Clues in Unexpected Places

Is an ultraviolet (UV) detector signal good for anything if I am using a mass spectrometer?

Dwight R. Stoll

Although I continue to be impressed with the capabilities of modern instrumentation for chromatography, my experience over many years has been that, if I put enough instruments in a lab and mix them with students, I am confronted daily with opportunities to hone my troubleshooting skills. In other words, things can and do go wrong even when working with the latest, greatest instruments.

“The peaks are not coming out.” “The pressure is too high.” “The pressure is too low.” “The UV detector signal looks weird.” This is an assortment of the kinds of observations I hear from researchers in my lab across all experience levels. What I have come to learn is that in almost every case of something not working correctly, there are good clues lying around that can lead us quickly to the cause of the problem. The trick then is more about finding or paying attention to these clues—letting the chromatograph talk—than it is about anything else. Sometimes the clue is a colored residue under a pump head. Other times it is a bottle of solvent on the instrument that does not look well mixed (ask me about the time a pump ran backwards—literally). This month I will discuss two places to find clues that are easily overlooked—the pressure trace recorded by the LC pump, and UV detector signals at low wavelengths.

I vividly recall a conversation I had as a graduate student with a field specialist for a major instrument vendor who had been working with chromatography and mass spectrometry equipment in labs all over the world for three decades. I was describing to him that I was having trouble with an LC instrument that was not delivering flow to the column at the expected flow rate. I had looked carefully at the pump and concluded that the pump was delivering the correct flow at the pump outlet. And yet, I did not observe the expected flow rate at the column exit. He said to me pointedly, “If the pump is working correctly, then you must have a leak somewhere.” I was baffled by this, because there was no obvious leak anywhere in the system. But, taking his point to heart, I began digging deeper and found that the rotor seal of the injector valve was so badly worn that some mobile phase was leaking out to the waste port of the valve. This was not immediately evident, because we had the waste port connected to a waste container instead of running it to a tray with a leak sensor. The point that I took away from the interaction with the specialist is that chromatographs are not mysterious black boxes that have magic happen inside of them. They are instruments built by humans, that abide by classical physical laws (such as conservation of mass, for example). And so, if we take the time to understand how they work, we will have a better chance of interpreting the many clues in and around the instrument and data system that can help us solve the problem at hand, and get back to acquiring good data.

The Pump Pressure Trace as a Source of Clues

Most modern LC systems record the system pressure measured at the pump outlet as part of the data file associated with an analysis. Obviously, the pump has to be functioning reliably for the recorded pressure data to be a useful source of clues to support troubleshooting. If the pump itself is not working properly, that raises a different set of questions that John Dolan has written about extensively in the past (1). If the pump does appear to be working properly, then there are at least two important ways that the pressure trace can be useful. For liquid flow through an open tube (such as a connecting tube in an LC system), the pressure drop across the tube (that is, the difference between the inlet and outlet pressures) is given by the Hagen-Poiseuille law:

$$\Delta P = \frac{8\mu LF}{\pi r^4}$$

where $\mu$ is the dynamic viscosity of the fluid moving through the tube, $L$ is the tube length, $r$ is the tube radius, and $F$ is the flow rate of the liquid. In this equa-
HPLC Polymers 101
The power of polymeric columns

Polymer HPLC columns have a lot of benefits. They don’t require any functionalization for reversed-phase separations, and rigid polymeric supports intrinsically resist chemical and pH degradation, a fundamental problem with silica columns. Plus, polymer’s inertness to most chemical environments makes it a robust and economical solution.

Hamilton offers a line of pH stable polymer HPLC columns for reversed phase, anion exchange, cation exchange, and ion exclusion separations perfect for pharmaceuticals, small molecules, proteins, peptides, DNA, organic, and inorganic ions, and more.

Advantages of polymer over silica

- pH range of 1 – 13
- Widest chemical compatibility
- Temperatures higher than 60 °C
- Maximum sample recovery
- Longest average life span

To learn more about how polymer columns can perform for you, visit www.hamiltoncompany.com/polymers-101 or call toll free 1-888-525-2123
tion, we see two things of practical signif-
ificance to the discussion here. First,
any increase in the length of the tube,
or decrease in the radius of the tube
(such as when a tube is partially blocked,
reducing the effective radius), will result
in an increase in the pressure drop mea-
sured at the pump. Second, any change
in the viscosity of the mobile phase will
result in a corresponding change in the
pressure drop. The dependence of the
viscosity of acetonitrile and water mix-
tures on the fraction of acetonitrile in the
mixture is shown in Figure 1. If we were
to execute a solvent gradient running
from 0 (100% water) to 1 (100% aceto-
nitrile), then we would expect the pressure
trace measured over the course of the
gradient to have the same shape.

Case Study #1:
Are You Sure the Gradient
Was 90% to 50% Acetonitrile?
This case study comes straight out of
some work in my laboratory that we
have been doing to develop a method
for a hydrophilic interaction liquid chro-
matography (HILIC) separation. Early
on in the work, I asked a student to do
some scouting for gradients starting at
90:10 acetonitrile:aqueous buffer and
running to 50:50 acetonitrile:buffer at
the end of the gradient. After some
time, the student reported to me that
the peak for acenaphthene, which can
be used as a dead time marker for
HILIC separations, looked unusually
small. One of the first things I did in
troubleshooting this unexpected result
was to look at the pressure trace, which
is shown in Figure 2.

Given the shape of the viscosity curve
in Figure 1, for a gradient running from
90% to 50% acetonitrile, we would
expect the pressure trace to start low
and steadily increase toward the end
of the gradient. This is clearly not what
we see in this case. Figure 3 shows the
pressure trace for a separation where
the gradient was actually 90% to 50%
acetonitrile. Upon looking further into
the method that was used for this analy-
sis, it became evident that indeed the
gradient that was used was set to 10%
to 50% acetonitrile by mistake. The rea-
son the acenaphthene peak was small
is because it was actually never eluting
in the relatively water-rich mobile phase
(the observed peak was probably an
impurity). In this case, simply looking at
the pressure trace provided a good clue
that something probably was not right
about the method, and that we should
look into this more closely rather than
pursuing other possible problems, such
as the sample itself.

Case Study #2: Something Does
Not Look Right in this Picture
The data for this second case study
comes from work in my laboratory a
few months ago that was focused on
two-dimensional liquid chromatogra-
phy (2D-LC) separations of peptides.
Upon review of chromatograms from
the previous night’s analyses, the data
did not look right at all. Turning to the
pressure trace for the second dimen-
sion of the 2D-LC system, it was obvious
that something was not right with this

*FIGURE 1:* Viscosity (centipoise, cP) vs. volume fraction of acetonitrile in mixtures of acetonitrile and water. Viscosities were calculated for 20 °C and ambient pressure using the correlation reported by Halvorson, et al. in ref. (2).

*FIGURE 2:* Pressure recorded during a solvent gradient that was supposed to run from 90:10 (v/v) acetonitrile and aqueous buffer to 50:50 (v/v) acetonitrile and buffer.
The Rocket TLC Development Chamber Has Launched

Introducing the Sorbtech Rocket, an innovative, robust TLC small-scale development chamber. The Rocket delivers more efficient, effective, and safer handling of TLC. Simply remove the lid, dispense a small amount of solvent, place your TLC plate in the solvent trough, cover and blast off. No need to wait around; review your results when you’re ready.

Sorbtech Rocket saves time and money:

• It’s a fire-and-forget chamber
• Set up your plate and solvent, then walk away
• Solvent consumption is reduced by 80-85%
• Rf overruns are eliminated using reduced solvent
• Overall operational time is faster
• Results are far more consistent test to test
• The TLC plate is always kept in the same orientation
• Rocket shape and white background provide easy visualization
• Designed for 2.5 x 7.5 cm (1in. X 3in.) TLC plates
• Used with glass, plastic and aluminum backed plates

Call us at 770-936-0323 to order your Sorbtech Rocket. Mention promo code BLAST to receive our special introductory price. Or visit our website at www.sorbtech.com/Blast.

Sorbtech values its customers and respects their privacy. When you do business with us, your information is secure and protected.
Using a UV Detector Signal as a Source of Clues (Even When MS Is the Detector You Care About)

One of the interesting dilemmas we face in coupling liquid chromatography (LC) separations to ultraviolet (UV) and mass spectrometry (MS) detection is that the aqueous buffers most suitable to UV detection (for example, phosphate buffers for their transparency) are terrible for MS detection, and vice versa. For example, formic acid is volatile and great for MS, but terrible for UV because it absorbs strongly at low wavelengths. This tendency of formic acid and other volatile buffering agents to absorb at low wavelengths is indeed annoying, and it is tempting to avoid using the UV detector in cases where we are most interested in data from an MS detector. Nevertheless, in my laboratory we routinely use both UV and MS detectors at the same time, because our experience has been that the UV data have been very useful for troubleshooting purposes on many, many occasions.

Using the two detectors together can be done either by running LC column effluent through the UV detector first and on to the MS detector, or by splitting the column effluent so that part of it goes to the UV detector and part of it goes to the MS. There are advantages and disadvantages associated with each of these approaches, but we’ll leave the substance of that comparison for a different day.

In any situation where we are mixing two or more solvents to prepare the mobile phase, and one of the components absorbs more strongly at a particular wavelength, then we will see that the UV detector baseline changes during a solvent gradient elution program. A common example of this is illustrated in Figure 5, where I have recorded the absorbance at 210 nm during a gradient from 10% to 90% B over 4 min, where A is 0.1% formic acid in water and B is acetonitrile. Here, the A solvent absorbs much more strongly than the B solvent (acetonitrile is very transparent at 210 nm), so we see that the measured absorbance decreases as
The FFF - MALS Platform
Next Level Nano, Bio and Polymer Analysis

NEW
With SEC Option!

Contact us for more information: www.postnova.com
the fraction of A solvent in the mobile phases decreases.

Case Study #1 (Revisited): Are You Sure the Gradient Was 90% to 50% Acetonitrile?

Going back to our first case study, the pressure trace provided a good troubleshooting clue, as discussed above. But, if this were inconclusive, we could also have looked at the UV detector signal. Recall that our intended gradient in this work was 90% to 50% B, where A was 0.1% formic acid in water, and B is acetonitrile. The UV absorbance signal at 210 nm from the experiment is shown in Figure 6. Clearly this signal is headed in the wrong direction for a gradient of 90% to 50% B, where we would expect to see an increase in absorbance as the fraction of the absorbing ammonium acetate in the mobile phase increases over the course of the gradient. This again confirms that the gradient had mistakenly been programmed as 10% to 50% B.

Closing Remarks
Modern chromatographs and data systems have the ability to record and store a variety of data streams (for example, column temperature, pump pressure, pump piston position). In some cases, these streams are not recorded by default, but can be selected for recording by the user. My experience has been that these data can be rich sources of information to support troubleshooting. I encourage you to get to know your chromatograph a little better, and listen when it wants to talk to you!

References

Interested in a Troubleshooting Topic?
Is there a troubleshooting topic that you are interested in, but have not seen discussed in this column? I am particularly interested to hear what you, as a regular reader of the column, have to say about topics you would like to see addressed here. Are there topics that are emerging challenges that you have not seen addressed in the past? Are there “old” topics that you would like to see addressed in more depth? I’d love to hear your topic suggestions! Please send them along to LCGCedit@ubm.com.

ABOUT THE COLUMN EDITOR
Dwight R. Stoll is the editor of “LC Troubleshooting.” Stoll is a professor and co-chair of chemistry at Gustavus Adolphus College in St. Peter, Minnesota. His primary research focus is the development of 2D-LC for both targeted and untargeted analyses. He has authored or coauthored more than 50 peer-reviewed publications and three book chapters in separation science and more than 100 conference presentations. He is also a member of LCGC’s editorial advisory board. Direct correspondence to: LCGCedit@ubm.com
"There was an amazing energy level at this year’s show, a coming together of analytical, medical and cannabis experts that felt like an extended cannabis family!"

- Tracy Ryan (CannaKids and SavingSophie.org)
PM$_{2.5}$ measurement faces various challenges and requires critical membrane properties for sample collection.

PM$_{2.5}$, particles that are smaller than 2.5 μm, pose a significant health hazard. Monitoring of ambient air quality helps to reduce this risk. In addition to the reference method set forth by the US Environmental Protection Agency, various equivalent methods are available to quantify PM$_{2.5}$ levels in the air. LCGC recently sat down with Vivek Joshi, PhD, the senior manager for applications development at MilliporeSigma, to discuss the reference method using polytetrafluoroethylene (PTFE) membranes and how it correlates with equivalent methods used to measure PM$_{2.5}$.

**LCGC:** What key pollutants define ambient air quality?

**Joshi:** Six key pollutants are commonly monitored to provide information on ambient air quality. They are ozone, sulfur dioxide, nitrogen oxide (NO$_2$), carbon monoxide, lead, and particulate matter, specifically PM$_{2.5}$, which are particles that are smaller than 2.5 μm.

**LCGC:** What are some common sources of this particulate matter in the air? Why are smaller particles such as PM$_{2.5}$ considered to be a health hazard?

**Joshi:** Common sources of particles in the air are mainly vehicular exhaust as well as burning of fossil fuels for power generation and home heating. Additional sources include industries that generate particles such as mining, cement manufacturing, etc.

The main health issue with these particles is the respiratory and cardiovascular adverse events associated with their inhalation. Because these particles are small, they reach deeper within the respiratory tissues.

In particulate matter testing, both PM$_{10}$ (particles that are smaller than 10 μm) and PM$_{2.5}$ are measured. Since PM$_{2.5}$ are associated with more health issues than PM$_{10}$, they are a bigger concern.

**LCGC:** What does the current regulatory landscape look like for PM$_{2.5}$ analysis from ambient air?

**Joshi:** Ambient air monitoring is conducted using regulated methods, which are generally controlled and enforced by government or regulatory agencies within a given country. The US Environmental Protection Agency (EPA) was one of the first environmental agencies in the world to provide needed guidelines and regulations on how PM$_{2.5}$ are monitored and measured. EPA also sets National Air Quality standards, which provides upper limits for the amount of PM$_{2.5}$ from air that is considered acceptable.
The original PM$_{2.5}$ method was instituted in 1997 and has since been updated multiple times. US EPA regulation number 40 CFR Part 50 provides all of these guidelines. Many other countries around the world have fashioned their regulations and methods of measurement on the basis of the US EPA guidelines. Hence, US EPA 40 CFR is considered the gold standard for PM$_{2.5}$ monitoring.

**LCGC:** What are some common methods of analysis for PM$_{2.5}$?

**Joshi:** There are basically two types of methods used for the analysis of PM$_{2.5}$. There is a reference method (as per US EPA 40 CFR Part 50) that is based on manual sample collection on a membrane followed by gravimetric analysis. Per the US EPA, the membrane used in this method should be a polytetrafluoroethylene (PTFE) membrane with a ring around it.

There are three main types of equivalent methods that are based on beta attenuation monitoring (BAM), or tapered element oscillating microbalance (TEOM) or optical methods. All of these equivalent methods provide automated data on a continuous basis; hence, they are useful for generating real-time data on air quality. Data generated by the reference method is used as quality assurance data or can be used for audit purposes to compare it with equivalent methods.

**LCGC:** What are some key characteristics of PTFE membranes used in PM$_{2.5}$ analyses, according to the US EPA reference method?

**Joshi:** There are several key characteristics that the membrane must meet to be certified for use in the US EPA reference method for PM$_{2.5}$ monitoring. These characteristics are as listed below:

- **Membrane thickness:** 40 ± 10 μm
- **Filter Diameter:** 46.2 ± 0.25 mm
- **Filter pore size:** 2 μm
- **Particle Retention Test (using 0.3 μm dioctyl phthalate aerosol):** ≥99.7% Retention
- **Pressure drop (@ 16.7 LPM):** ≤30 cm of water

In addition tests such as alkalinity, temperature weight loss stability, moisture weight gain stability, and drop test weight loss stability are also performed on this membrane. The US EPA method specifies required values for all these tests. The document also specifies how many filter samples must be tested for each of these tests, based on manufactured lot size.

**LCGC:** How are these particles measured and characterized after the sample is collected on a ring PTFE membrane?

**Joshi:** Post-sample collection, the PTFE filter is typically weighed to obtain the quantitative information on particulate matter. This essentially provides confirmation of data that were generated using automated equivalent methods for PM$_{2.5}$ analysis. As I mentioned, the automated methods are constantly generating data that become the basis for the air quality index, which is available online.

Generally, the reference method data are generated after the fact since the filters are weighed only after they collect sample for 24 hours. Actual sampling from a site can occur at various frequency as well. Data provided by weighing the PTFE membranes confirm that data obtained using automated methods was accurate.

X-ray diffraction is sometimes performed on PTFE membranes after the sample has been collected. It essentially reveals the composition of pollutant particles that have been collected on the filter. This helps to identify sources of pollution (i.e., sources of particles that are collected on a particular filter) and allows for the institution of regulations to control the emission of these specific particulate impurities that are being collected on the PTFE membrane.

**LCGC:** What are some of the challenges commonly faced in PM$_{2.5}$ measurement using PTFE membranes?

**Joshi:** There are two major challenges associated with PTFE membranes with a ring. The first challenge is static electricity, which causes inaccurate measurements. This is especially challenging with measurements of very low levels of PM$_{2.5}$, where the weight difference can be just a few micrograms. To minimize static electricity, most commonly, an antistatic strip is placed within the weighing chamber. In addition, the filter packaging can be designed to minimize static electricity.

The second challenge for a PTFE membrane with a ring is curling. This occurs as a result of stresses that are created during ring formation on a very thin PTFE membrane. Because of these stresses, the flatness of the membrane can be compromised.

This makes weighing the membrane extremely challenging. The difference in weight of the membrane before and after sample collection may only be a few micrograms. Once the membrane is placed on a balance or microbalance, any movement in the membrane can lead to a noisy baseline measurement. This phenomenon is called a pringling (like the potato chips) to indicate the shape of these membranes. Manufacturing process modifications can help to significantly reduce this phenomenon.
Development of a Multi-Product SE-UHPLC Method for the Determination of Size-Variants in Bispecific Antibody Formats

Elevated levels of aggregates in protein therapeutics are associated with an increased risk of triggering adverse immune responses in patients. It is thus of paramount importance to closely monitor these product-related impurities by the most suitable analytical tools. Size exclusion-high performance liquid chromatography (SE-HPLC) is the preferred method applied in quality control laboratories for the quantitative analysis of aggregates (high molecular weight forms), the antibody monomer itself, as well as low molecular weight forms. Size-exclusion ultrahigh-performance liquid chromatography (SE-UHPLC) using new columns with packings of particles ≤3 μm provides significant gains in chromatographic efficiency by a higher resolution, enhanced selectivity, and shorter run times. Here, we present a multi-product SE-UHPLC platform method for the characterization of bispecific monoclonal antibody formats with superior separation characteristics compared to our existing SE-HPLC method, which can be readily applied for process optimization, stability testing, and release analytics.

Tobias Graf, Raphael Ruppert, Alexander Knaupp, Georg Hafenmair, Sebastien Violini, Steffen Kiessig, Markus Haindl, Harald Wegele, and Michael Leiss

Biotherapeutics and, more specifically, monoclonal antibodies (mAbs), rank among the fastest growing and most promising classes of drugs in basic research, as well as in clinical settings for various life-threatening diseases, such as cancer, infectious diseases, and immune-mediated disorders. In 2020, about 70 biotherapeutics are expected to be available for patients, and several hundred have been evaluated in, or are the subject of, ongoing clinical trials (1). Their story of success has boosted the development of more sophisticated, elaborate antibody designs, including a large variety of bispecific antibody formats intended to expand potential areas of application by incorporating multiple antigen-recognizing elements into a single framework (2–4). However, promiscuous pairing of polypeptide chains of two antibodies during expression poses a major challenge for molecular engineering, protein manufacturing, and purification to obtain the desired bispecific product (5). For this purpose, the last two decades have seen a variety of innovative technologies for the construction of bispecific antibodies, including the generation of quadroma cells (6), the heterodimerization of heavy chains by the “knobs-into-holes” technology (7) or electrostatic steering (8), and the correct assembly of light chains by the CrossMab technology (9).

Owing to their complex structure, biotherapeutics generally feature a high degree of intrinsic heterogeneity and susceptibility to diverse chemical modifications, along with an inherent propensity for forming aggregates or decomposing to fragments during production, shipping, and storage (10–12). Particularly, elevated levels of aggregates in protein drug products are closely linked to an increased risk of adverse immune responses in patients, which may affect the safety, potency, and pharmacokinetic parameters (13–15), and are therefore considered critical quality attributes (CQA) by the International Conference on Harmonization (ICH) and the U.S. Food and Drug Administration (FDA) (16,17). Taking the characteristics of bispecific antibodies into account, even more side-products can arise from incorrect association of identical heavy chains and light chains leading to nonfunctional or monovalent molecules (7,9). It is thus mandatory to closely monitor these product-related impurities by robust and sensitive analytical tools.

Size variants are commonly characterized by employing size-exclusion chromatography (SEC), capillary electrophoresis (CE), and light-scattering based methods, such as multi-angle laser light scattering (MALLS) (18–20). For routine testing in quality control (QC) facilities, SE-HPLC is generally the method of choice for quantifying high-molecular weight species (HMWs, such as dimers and other high-molecular aggregates), and for determining the content of low molecular weight species (LMWs, such as low-molecular fragments) (21). The main advantages of this approach include its sensitivity, reproducibility, a relatively high sample throughput, and the mild elution conditions, which are mainly dependent on the mobile phase, temperature, flow rate, and column parameters (such as column length, resin type, or pore size) allowing analysis with minimal impact on the native conformational structure (19). However, conventional SE-HPLC methods are constrained by their comparatively low resolution.

Therefore, recent advances in column design with packings of particles ≤3 μm combined with the application of ultrahigh-pressure liquid chromatography (UHPLC) are instrumental in keeping pace with the rapidly evolving field of complex antibody formats by providing major benefits in terms of peak resolution, peak capacity, and total run time compared to conventional SE-HPLC (22,23). Since routine qual-
Designed by you. Developed for you.

Environmental lab professionals spoke, we listened.

Your input guided our development of the new FastEX-24 rotor to specifically address the challenges of today’s environmental laboratories.

The Milestone Ethos X microwave lab station in combination with our FastEX-24 rotor offers your lab simultaneous extraction of 24 samples in 40 minutes with minimal solvent usage. Our unique Weflon™ material provides homogeneous heating and facilitates accurate contactless temperature monitoring of all vessels. Each vessel holds 100 mL disposable vials allowing you to extract 30 g samples while eliminating memory effects and clean up.

The benefit: Higher throughput and easier handling results in both reduced cost and turnaround time for your lab.

See how the FastEX-24 can help your environmental laboratory. Go to milestonesci.com/extraction
Material and Methods

Material

The mAbs 1+1 CrossMab and 2+1 CrossMab (Roche Diagnostics GmbH) were selected to cover different bispecific mAb formats. Gel filtration standard (molecular weight standard, MWS) was purchased from Bio-Rad Laboratories. USP Monoclonal IgG System Suitability Reference Standard (Cat.# 1445550) was purchased from United States Pharmacopeial Convention (USP Convention).

Equipment

SE-HPLC and SE-UHPLC were performed on an UltiMate 3000 RSLC-system (Thermo Fisher) equipped with a HGP-3400RS Rapid Separation (RS) Binary Pump, an autosampler, a temperature-controlled column compartment, and an ultraviolet–visible (UV–vis) detector with detection at 280 nm. Different Thermo U3000 systems include a 100 or 250 μL syringe, a 100 or 250 μL sample loop, a 2.5, 11 or 13 μL UV flow-cell and a 0.13 x 250 mm capillary from the column to the detector. Data acquisition was controlled by the Chromeleon 7.2 software from Thermo Scientific. For validation, SE-UHPLC was additionally performed on an Acquity UHPLC H-Class Bio System (Waters Corporation) using the Empower 3 software equipped with a 100 μL syringe, a 15 μL sample loop, a photodiode array (PDA) detector including an analytical flow cell with 1.5 Nl illuminated volume and capillaries with 0.127 mm inner diameter. Statistical parameters were calculated using the Validat Software package p1 (03/2014) Version 5.59.1623 (ICD. GmbH & Co. KG).

SE-UHPLC

SE-UHPLC was carried out using a TSKgel UP-SW3000 size exclusion chromatography (SEC) column (4.6 x 300 mm, packed with 2 μm silica-based beads, 250 Å pore size) from Tosoh Bioscience. The method was run with a mobile phase consisting of 200 mM potassium phosphate/250 mM potassium dihydrogen phosphate (pH 7.2) at a flow rate of 0.5 mL/min at 40 °C. The refractive index (RI) detector was used for detection. The column was back-flushed with methanol (100%) at 0.5 mL/min and then re-equilibrated with the mobile phase.

It is clear that this document discusses the development of a multi-product SE-UHPLC method designed for QC testing of monoclonal antibodies (mAbs) and discusses advantages and limitations of commonly used system suitability test (SST) approaches for the application of our new SE-UHPLC platform method.
mM potassium chloride, adjusted to pH 6.2. Standard running conditions were oven temperature 25 °C, eluent flow rate 0.3 mL/min, and overall run time 18 min. Drug material solutions were diluted to a final concentration of 10 mg/mL in bulk buffer. A volume of 5 μL was injected, corresponding to 50 μg total protein amount, onto column. For protein concentrations lower than 10 mg/mL, injection volumes were increased accordingly. Relative quantification was achieved by manual integration of the chromatographic peaks and calculation of the ratio of the relevant peak areas.

**SE-HPLC**
SE-HPLC was performed using a TSKgel G3000SWXL SEC column (7.8 x 300 mm, 5 μm particle size, 250 Å pore size) from Tosoh Bioscience. Running conditions were oven temperature 25 °C, flow rate 0.5 mL/min, total run time 30 min, and mobile phase 200 mM monobasic potassium phosphate (KH₂PO₄)–250 mM KCl, pH 7.0. Sample amounts of 5 μL of a 30 mg/mL protein solution were injected, corresponding to 150 μg on the column.

**Results and Discussion**
An SE-UHPLC Platform Method to Evaluate Size-Variants of Bispecific Antibodies
Herein is described the capabilities of our SE-UHPLC method using the TSKgel UP-SW3000 column initially illustrated in relation to our conventional SE-HPLC release method using the TSKgel G3000SWXL column. Employed are a set of three different monoclonal antibodies (Figure 1A), including USP Monoclonal IgG System Suitability Standard (RS) (24) as a standard immunoglobulin G (IgG) molecule, an asymmetric, bispecific IgG1-like antibody, generated by swapping of the CH1 and CL domains on one arm of the Fab region (1+1 CrossMab), and an asymmetric 2+1 bispecific antibody with head-to-tail fusion of a Fab fragment to the N-terminus of a single heavy chain via a flexible linker (2+1 CrossMab) (9). Visual comparison of the representative chromatograms for the separation of nonstressed and stressed (exposed to 40 °C for 6 weeks) drug solution material (Figure 1B), indicates a significantly improved separation power of our newly developed method at a simultaneous reduction of the total run time by nearly 50%. As described for the application of SE-UHPLC previously (22, 25), notable differences of the chromatograms become primarily evident at the edges of the respective main peak by resolving closely related compounds comprising HMW and LMW species, such as mispaired homodimers (knob-knob and hole-hole species for 2+1 CrossMab formats), and truncated antibody variants lacking one Fab fragment (Fab/c species), respectively, which most often cannot be discerned by conventional SEC.
TABLE I: Comparison of the relative peak areas and the standard deviation (SD) for nonstressed and stressed samples between SE-HPLC and SE-UHPLC (n = 3). Peak assignment was accomplished as indicated in Figure 1.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>SE-HPLC (IgG)</th>
<th>SE-HPLC (1+1 CrossMab)</th>
<th>SE-UHPLC (IgG)</th>
<th>SE-UHPLC (1+1 CrossMab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nonstressed</td>
<td></td>
<td></td>
<td>nonstressed</td>
<td></td>
</tr>
<tr>
<td>1+1 CrossMab</td>
<td>0.01 ±0.01</td>
<td>0.35 ±0.03</td>
<td>0.04 ±0.01</td>
<td>99.53 ±0.06</td>
</tr>
<tr>
<td>stressed</td>
<td>0.11 ±0.01</td>
<td>0.85 ±0.03</td>
<td>-</td>
<td>98.19 ±0.04</td>
</tr>
<tr>
<td>nonstressed</td>
<td>0.02 ±0.01</td>
<td>0.84 ±0.05</td>
<td>0.28 ±0.03</td>
<td>98.73 ±0.06</td>
</tr>
<tr>
<td>stressed</td>
<td>-</td>
<td>0.07 ±0.02</td>
<td>0.95 ±0.04</td>
<td>93.37 ±0.03</td>
</tr>
<tr>
<td>nonstressed</td>
<td>0.01 ±0.00</td>
<td>0.40 ±0.03</td>
<td>0.05 ±0.01</td>
<td>98.95 ±0.05</td>
</tr>
<tr>
<td>stressed</td>
<td>0.08 ±0.01</td>
<td>0.24 ±0.02</td>
<td>0.63 ±0.03</td>
<td>91.38 ±0.04</td>
</tr>
<tr>
<td>nonstressed</td>
<td>0.02 ±0.01</td>
<td>0.86 ±0.04</td>
<td>0.36 ±0.02</td>
<td>98.63 ±0.04</td>
</tr>
<tr>
<td>stressed</td>
<td>0.06 ±0.01</td>
<td>1.00 ±0.02</td>
<td>0.35 ±0.02</td>
<td>92.98 ±0.04</td>
</tr>
</tbody>
</table>

In detail, the enhanced performance of SE-UHPLC is reflected by the occurrence of an additional peak in the HMW region (for IgG and 1+1 CrossMab), which can even be baseline separated for the 2+1 CrossMab (marked with blue asterisks), as well as clearly separable peaks next to the monomer (LMW region, green asterisks) for all tested antibodies. Furthermore, the comparability of SE-HPLC and SE-UHPLC performance with respect to the relative ratio of the single peaks of the HMW/LMW region and the antibody monomer was confirmed by side-by-side comparison of quantitative data for triplicates (n = 3) of nonstressed as well as stressed samples (Table I), showing that both methods yield consistent results apart from unresolved peaks in SE-HPLC.

Conclusively, application of our SE-UHPLC approach results in a greatly enhanced capacity to evaluate size-variants representing product-related impurities in bispecific antibody formats, whose close monitoring is urgently required during process optimization, stability assessment, and release analytics.
Method Development and Robustness Testing

Given that suitability for QC purposes relies heavily on the accurate and consistent quantification of underrepresented process-related impurities in drug solution material, method development steps were designed in a way to achieve high sensitivity along with sufficient separation efficacy, while confounding factors associated with SE-UHPLC application potentially affecting protein recovery and sample composition (on column aggregate formation) (26) were reduced as much as possible. Therefore, optimization steps involved assessment of flow-rate, ionic strength of the eluent, column temperature, amount of protein on column, injection volume, and, most importantly, column lot-to-lot variation by testing different manufacturers (data not shown), whereas eluent composition was largely adopted from the conventional HPLC method. In this study, we provide a brief insight into method development by outlining the results of four key factors comprising a) ionic strength, b) injection amount, c) injection volume, and d) lot-to-lot variability of the TSKgel UP-SW3000 column for the analysis of 2+1 CrossMab (Figure 2), allowing the justification of our designated parameters.

First, secondary electrostatic interactions between the protein and the stationary phase can lead to protein adsorption, peak distortion, retention time shifts, or conformational changes of the protein in dependence on the molecular properties, and, consequently, have a potential impact on the method’s reliability (26,27). For this purpose, a common mitigation strategy involves the application of high salt concentrations to the mobile phase. As described by Goyon and associates previously (28), potassium-based salts are given preference over sodium salts, due to their superior features to a) minimize undesirable interactions of the protein with silica surfaces more efficiently, and b) contribute to an improved stabilization of the protein structure according to their position in the Hofmeister series, resulting in a lower susceptibility to measurement artifacts. The compatibility of the TSKgel UP-SW3000 column with the eluent conditions used for our legacy SE-HPLC method was assessed for different antibody formats by employing salt concentrations from 50 to 350 mM potassium chloride (KCl). As expected, the best separation performance was achieved at high salt concentrations, while reduction of the ionic strength causes peak broadening in the HMW region and less definition of the peak shoulder eluting right after the monomer (Figure 2A). Despite the slightly enhanced chromatographic characteristics at an even higher ionic strength, the KCl concentration was set to 250 mM in order to ensure a maximum longevity of the column by keeping the total salt concentration including the buffer system (200 mM potassium phosphate) in compliance with the operating conditions of the manufacturer (below 500 mM).

Another point concerning the separation performance of SE-HPLC is sample loading, which involves both the protein amount loaded onto the column (mass load) and the injection volume. In general, a higher resolution is achieved at low protein amounts, but the sensitivity may not be sufficient in this case for the reliable quantification of low-abundant product-related impurities and vice versa, which we consider to be essential requirements of a QC method (22). Therefore, we first investigated appropriately balanced mass loading by varying sample concentration in a range from 0.2 to 30 mg/mL while keeping the injection volume constant, with the aim to achieve a high signal-to-noise ratio and adequate separation performance at the same time (Figure 2B). In accordance with the above-mentioned demands,
To examine potential sources of variability for SE-UHPLC analysis, a number of selected method parameters, including (A) flow rate, (B) pH value, and (C) and column temperature were tested for 2+1 CrossMab at defined lower (red) and upper (green) limits of the designated conditions (black). In addition, in (D) the separation performance was evaluated for HPLC instrumentations from two different manufacturers. Note: The signal marked with an asterisk (*) is caused by the sample matrix.

50 μg was selected as the preferred sample amount, since the injection of low protein amounts (1, 5, and 10 μg) results in very sharp and symmetrical monomer peaks, but the accurate determination of the present size-variants is restricted by a poor signal intensity of the respective peaks. On the contrary, by injecting excessively high protein amounts onto the column (100 and 150 μg), an increasing tendency for peak broadening can be observed, which impairs peak resolution and particularly hampers the separation of the peak shoulder in front of the monomer. Second, the effect of the sample volume on the separation performance was studied by injecting constant protein amounts (50 μg), but varying injection volume between 2 and 50 μL. As shown in Figure 2C, with the smallest injection volumes demonstrating the best peak resolution (2 and 5 μL), whereas volume overload causes peak broadening, retention time shifts and, especially at very high volumes, increasing peak tailing. By taking the precision of the autosampler and the applicability for low-concentration protein samples (during process development) into account, the standard injection volume for the method was set to 5 μL.

Finally, given that method robustness and reproducibility strongly depend on the consistent performance of the column employed, lot-to-lot variation was tested for different column lots of the TSKgel UP-SW3000 column (Figure 2D). Chromatograms of lot 1–4 feature very similar peak shapes and comparable results for the relative areas of the monomer, HMWs, and LMWs (data not shown), indicating a nearly constant separation efficacy and, conclusively, the column’s suitability for the developed method.

Robustness testing of our SE-UHPLC method was performed utilizing the one-variable-at-a-time (OVAT) approach, at the end of method development for multiple products, to provide an indication of its reliability during inter-laboratory usage and the subsequent validation process (29). Therefore, the impact of small, but deliberate variations in seven method parameters, including salt concentration of the eluent, injection amount, injection volume (see Figure 2), flow rate (Figure 3A), pH value (Figure 3B), column temperature (Figure 3C), and, finally, by using HPLC instrumentations from different companies (Figure 3D), was assessed by comparing the chromatographic responses with the nominal conditions. The variations applied for robustness testing are summarized in Table II. According to the overlays in Figures 2 and 3, chromatographic characteristics remain mostly unaffected by the variations induced for robustness testing, emphasizing the applicability of our method for routine use. Even though adaption of the flow rate (Figure 3A) results in a retention time shift and a higher number of theoretical plates accompanied by a higher resolution with decreasing flow rate, all expectations set on the separation performance were fulfilled within the tested range. In slight contrast to the definition of robustness (focusing on the impact of altered internal parameters), the term ruggedness, as it is delineated in the USP
guidelines, determines the stability of the method against analyst-, instrument- and laboratory-induced variations, and is, therefore, addressed by the evaluation of reproducibility in the next section.

Because of column aging, the applied method is inevitably susceptible to drifting responses (21). For this purpose, a robustness test evaluating the lifetime stability of a column is very helpful to track deteriorations of the column performance and consequently facilitate the definition of adequate system suitability test (SST) parameters. In this study, a TSKgel UP-SW3000 column was subject to a run-down experiment under idealized, continuous operation conditions, by sequentially injecting 1200 samples onto the column and monitoring changes in the peak shape and resolution. Figure 4 shows representative chromatograms of nonstressed (A) and stressed material (B) from 2+1 CrossMab as well as the behavior of the molecular weight standard (MWS) with increasing injection numbers (C). The quality of separation remains very similar for more than 600 injections, then a gradual performance loss can be observed during the further procedure, which is primarily noticeable by the declining separation of the peak shoulder eluting next to the monomer in stressed material, whereas the resolution factor of the MWS decreases during the entire experiment. A significant drop of the column performance is apparent for both samples and the MWS between injection number 1000 and 1200. Therefore, sufficient separation performance could be achieved under the tested conditions for approximately 1000 successive injections. However, it should be noted that the average column lifetime is significantly reduced to roughly 400 sample injections in routine use, which is considered acceptable under pharmaceutical or industrial conditions (30). In our experience, the described difference can be attributed to multiple connecting/disconnecting (installation) steps, storage conditions, and frequently occurring variations of flow rate during the lifetime of a column in daily use.

Method Validation

All validation studies were carried out per International Conference on Harmonization (ICH) Q2(R1) for specificity (data not shown), linearity, accuracy, repeatability, reproducibility, detection limit (DL), quantification limit (QL), and robustness (32). In brief, statistical parameters were calculated using a validated software package according to corporate guidelines on statistical interpretation of validation results. Evaluation of linearity, accuracy, and repeatability were performed in a combined experiment by injecting triplicates (n = 3) of nine amount levels ranging from 40% to 150% of the designated protein amount (50 μg). For linearity assessment, the averaged main peak area per level (in counts) was plotted against the expected protein amount to determine the correlation coefficient. Accuracy of the method was checked by calculating the mean recovery of the main peak (found area percent versus expected area percent) relative to the standard values. Repeatability of the method was evaluated by determining the maximal relative standard deviation (RSD) of the main peak. For reproducibility, three independent determinations of nonstressed and stressed material were performed in three different laboratories (n = 9) using different HPLC instrumen-

tations. The evaluation of reproducibility was accomplished by calculation of the relative standard deviation over all values and of the relative difference between the nine results based on the difference between the maximum and minimum obtained value relative to the mean result from all the results obtained (in area percent (area %)) for the main peak. The assessment of the limit of detection (DL) and limit of quantification (QL) was performed similarly to the combined experiment using an adapted concentration range instead. DL and QL of the method were established in triplicates (n = 3) based on the standard deviation of the response and the slope of the regression line. Robustness testing was conducted as described above by introducing small variations in diverse crucial parameters followed by visual inspection of the respective chromatograms (see Figures 2 and 3), and by determination of the relative changes in main peak area percent compared to the nominal value (data not shown).

The results from the analytical validation of 1+1 CrossMab and 2+1 CrossMab are summarized in Table III by displaying the consolidated maximum deviations or values for the DL and QL determinations, respectively.

Evaluation of System Suitability

Test (SST) Parameters Development

Based on the results from robustness testing, the capabilities of two different SST approaches for monitoring test performance of our
A commonly applied tool for controlling the separation efficiency involves the injection of commercially available protein standard solutions or MWSs as SSTs at regular intervals. By determining a threshold level for the retardation factor (RF) according to European Pharmacopoeia (EP) (31), the deterioration of column performance should be detected reliably in due time to reduce the risk of insufficient measurements, but also to exhaust the maximum capacity of the column. However, by aligning the decrease of the RF with the changes in relative area of the HMW1 (A), HMW2 (B), sum of HMWs (C) or the monomer (D) for the standard IgG molecule and 2+1 CrossMab (Figure 5), we observed that the data correlate inadequately, making it difficult to define minimum requirements on a sufficient separation efficacy of the column (compare slope of regression lines from MWS and relative area percent of both mAbs). On the contrary, a significantly better correlation was achieved, by directly comparing the relative areas for the monomer, HMW1, HMW2, or sum of HMWs between the Standard IgG and 2+1 Crossmab (compare slope of regression lines from relative area percent of both mAbs). These observations imply that the application of USP Monoclonal IgG System Suitability RS as a SST (according to USP <129> [24]) enables more precise monitoring of column performance for the analysis of mAbs with our SE-UHPLC platform method, which may originate from a comparable behavior of the antibodies on the column, whereas the proteins of the MWS used for the determination of the RF differ in their molecular properties.

One drawback of this SST applied for our SE-UHPLC platform method comprises
the noncompliance with the quantitative criteria defined by USP<129> (24), since, for example, the relative peak area of the LMW region (0.60 area %, see Table I) exceeds the specification limit of 0.2 area %. However, the deviation can be justified with the improved resolution of our method, enabling the identification of an additional peak at the rear of the monomer, which is not visible in the chromatogram provided by USP <129> (24), but needs to be considered for integration (see Figure 1B). We, therefore see a certain need for re-defining USP <129> acceptance criteria, when high-resolution methods are applied.

**Conclusions**

Although SEC is the predominantly used technique for mAb size-variants analysis, it must be stated that it is a chromatographic mode with considerable restrictions in resolution and selectivity. The introduction of a new generation of SE-UHPLC columns with significantly improved separation characteristics sparked great interest in the biopharmaceutical industry, since the increasing importance of mAbs as a therapeutic modality and more recent advances in antibody engineering promote the evolution of more complex antibody formats, which in turn poses a major characterization challenge for analytical laboratories. In this study we present the development steps, robustness assessment, validation results and the evaluation of appropriate system suitability test parameters for a multi-product SE-UHPLC approach using the TSKgel UP-SW3000 SEC column. The consistency of the results shown for both stressed and nonstressed antibody drug samples across various antibody formats with our legacy SE-HPLC method has been shown. In conclusion, our method provides a powerful and cost-effective platform technique for the characterization of mAbs with superior separation power, which has meanwhile been successfully implemented in our commercial QC facilities.

**Acknowledgements**

We would like to acknowledge Jennifer Rea, Wen-Li Chung, Alejandro Carpy, and all colleagues from the global Roche/Genentech SEC Analytical Expert Team (AET) for fruitful discussions, Carmen Heitler and Gabriel Hoffmann for their contributions to the study and Patrick Bulau for carefully reviewing this manuscript.

**References**


Tobias Graf, Raphael Ruppert, Georg Hafemnair, Markus Haindl, Harald Wegele, and Michael Leiss are with Pharma Technical Development Analytics at Roche Diagnostics in Penzberg, Germany. Alexander Knaupp is with Pharma Research and Early Development at Roche Diagnostics in Penzberg, Germany. Sebastien Violini and Steffen Kiessig are with Pharma Technical Development Analytics at F. Hoffmann-La Roche in Basel, Switzerland. Direct correspondence to: michael.leiss@roche.com
TOF has economic advantages and requires 98% less sample than quadrupole to obtain the same result.

A researcher challenged a gas chromatography–time-of-flight mass spectrometry (GC–MS-TOF) instrument (the Pegasus BT, LECO) to its limit of quantification by injecting very dilute solutions of compounds beyond their odor threshold concentration. With Pegasus BT, a quantitation limit of 2 pg per liter was required to obtain the full mass spectrum, whereas a GC–MS quadrupole instrument required 100 pg per liter to obtain the same result. LCGC recently sat down with the researcher, David Benanou, an expert of analytical chemistry at Veolia Research and Innovation, to further discuss the advantages of TOF analysis.

**LCGC:** We recently saw your July 2018 LinkedIn post in which you discuss how “2…is better than 100” in the quantification of solutions beyond their odor threshold concentration. Can you give us an overview of the experiments and further support your conclusions?

**Benanou:** The title of the article has several meanings. The first was a joke, meaning that two Pegasus BT instruments in your laboratory is better than having 100 other instruments. But from a scientific point of view, the meaning was that with only 2 pg injected in the Pegasus BT instrument, it was possible to obtain the full mass spectrum. However, performing the same experiment with a simple quadrupole, it is possible to see the full mass spectrum when a minimum of 100 pg is injected.

The first experiment was based on taste and odor remediation. For example, we injected targeted compounds such as trichloroanisole and tribromoanisoloes. For seven to 10 days, we compared a conventional single-quadrupole system with a Pegasus BT GC–MS-TOF instrument. The result was incredible.

Many people from all over the world visit our laboratory, which is globally well-known, to talk about experiments. We feel that today, it is not necessary to buy a triple-quadrupole system. It is better to buy a TOF instrument such as the Pegasus BT instrument. With this system, we can obtain the same result and the same sensitivity as we can with a triple-quadrupole system. With triple-quadrupole equipment, only targeted compounds can be analyzed. With the Pegasus BT instrument, it is possible to perform analysis with a very low limit of quantification of targeted compound; moreover, screening can be conducted. The Pegasus BT system is really a dual instrument.
From an economic point of view, the Pegasus BT system is very interesting. We spoke with several producers and found that Pegasus BT instrument is approximately 45% cheaper than a triple-quadrupole instrument, regardless of the brand considered.

**LCGC:** On typical (i.e., established) technologies, it is possible to smell a chemical, but it is not possible to analyze it. Why is that?

**Benanou:** The odor threshold concentration of a compound is very low. For example, the odor threshold of trichloroanisole is only 100 pg per liter. It seems that, at this time, no detector is sensitive enough to challenge the human nose.

However, for the last five or 10 years, the sensitivity in sample preparation and green sample preparation has offered the opportunity to challenge the human nose with the same sensitivity as Pegasus BT or a particular treatment on the market. But for the majority of systems, it is very difficult to challenge the human nose.

**LCGC:** Can you tell us more about the matrix you used and how you prepared to analyze the samples?

**Benanou:** Veolia is a worldwide leader in water and wastewater treatment. We are also a leader in the circular economy. Thus, everything that is put into the trash is a matrix of interest for my team. In addition to water and wastewater, it is also necessary to work on plastic, lipstick, fish, yogurt, milk, and so on.

For 17 years, we have pushed for a green sample preparation technique. A challenge arose when Veolia asked me to set up a new team dedicated to treatment and to use a different research center for green analytical chemistry.

For 19 years, we have reduced the consumption of solvent by approximately 600%. We do not use solvent at all. We replaced this old-fashioned technique (even though the technique is standardized in France, Europe, and the United States) with the technical stir bar sorptive extraction (Twister). This is a piece of silicone that can be put in contact with the matrix after performing a thermal desorption. In this case, from an economic point of view, it is neither necessary to buy the solvent, nor is it necessary to pay for destroying the solvent. It is also very important for the safety of the technician or engineer who performs the sample preparation; this is a 100% benefit.

**LCGC:** Why is this research so important?

**Benanou:** Veolia is a private company, and analytical chemistry is interesting. Above all, it is one of my passions. With regard to Veolia, for the treatment that is being considered, we need to give an analytical answer for whatever problem we are facing. For example, a problem may arise if trouble occurs in the water or wastewater treatment plant or in a plastic or polymer process.

Analytical chemistry is also very important when we try to imagine the treatment of the future. When we wish to perform a new membrane treatment or a new biological treatment, we need an analytical answer. For every piece of research at Veolia, analytical chemistry is present at every step, from the first experiment in the laboratory to the time when we are ready to set up a water or wastewater treatment plant.

**LCGC:** What is the background of your company, and what are its research directives?

**Benanou:** I cannot fully answer the question because many things are confidential. Veolia is present in 45 countries around the world, each with its own problems and political issues. Regardless, everybody needs to obtain an analytical answer.

Regarding my goal, I need to be 10 years ahead of the innovation in separation science. Thus, my job includes thinking about what will be the future of analytical chemistry, and how Veolia can help shape it.

“We feel that today, it is not necessary to buy a triple-quadrupole system. It is better to buy a TOF instrument...we can obtain the same result and the same sensitivity as we can with a triple-quadrupole system.”
Statistics for Analysts Who Hate Statistics, Part VII: Sum of Ranking Differences (SRD)

Comparing methods, models, chromatographic columns, or samples can be achieved with several data analysis methods. We have already explored some of them (clustering, principal component analysis, and desirability functions, for instance). In this seventh installment, we explain a simple and straightforward method: sum of ranking differences.

Caroline West

Comparing methods, models, chromatographic columns, or samples can be achieved with several data analysis methods. We have already explored some of them (clustering, principal component analysis, and desirability functions, for instance). In this seventh installment, we will learn about a simple and straightforward method: sum of ranking differences.

Sum of ranking differences (SRD) (1–3) compares methods or models not based on raw data, but based on ranks. To explain the principle with a basic illustration, one simple example where ranking is applied is sports. For instance, the results of decathlon from the London 2012 Olympics are presented in Table IA. The results for the 20 best athletes in the 10 sporting disciplines, with their scores and the final ranks, are shown.

Instead of classifying the athletes, we classified the sports, to see if any trends were visible in the data, relating some sports to others. For instance, it would seem logical that those athletes that are good in the 100 meters should also be good in the 400 meters or that throwing a discus and throwing a javelin should not be unrelated.

The first step is then to assign a rank to each athlete for each of the 10 sports. The ranks can be seen in Table IB. For instance, the man who ranked first overall (Ashton Eaton) ranked 1st in the 100 meters, the 400 meters, and the long jump. He ranked 2nd in the 110 meter hurdles and high jump, 3rd in pole vault, 7th in the 1500 meters, 8th in shot put, 9th in the javelin throw, and 16th (clearly not his favorite) in the discus throw.

The second step is to choose a reference sport. In our example, we will use the 100 meters as the reference sport. Then the rank obtained by each athlete in every other sport will be compared to the reference sport. As you can see from Table IC, for the gold winner, Ashton Eaton, the "ranking difference" (RD) for the 400 meters and the long jump will be 0 (he won those two events), the RD for the 110 meter hurdles and high jump will be 1, the RD for pole vault will be 2, the RD for the 1500 meters will be 6, and so on. Absolute values are used, because only the distance from the reference is considered. Thus, for the athlete who was the 20th in 100 meter race, the ranking difference in long jump, where he was the 18th, should be RD 2.

Finally, for each sport the "ranking differences" obtained by the twenty athletes are added to obtain the "sum of ranking differences" or SRD. The SRD values, at the bottom of Table IC, confirm that an athlete good at the 100 meters should also be good at the 400 meters, because the 400 meters has the smallest SRD value (55) of all nine sports. It is impossible to win those two events, but having the same speed is a logical performance (next closest SRD value is 150). Thus, performance in the long jump is generally a good indicator of overall performance in the decathlon. While the correspondence between the decathlon overall and the long jump is not perfect, there is indeed a tendency showing some agreement (as opposed to the pole vault, where the ranks are completely scrambled). The results are best seen with a figure (Figure 1); the sports appearing on the left side of the Gaussian curve are most similar to the "average sport"; thus the performance of the athletes in these sports should be most representative of the final decathlon ranks. In some cases, the classified objects may appear on the right-hand side of the Gaussian curve, exhibiting "reversed SRD ranking," indi-
### TABLE I: Decathlon results from the London 2012 Olympics

Table IA. Raw data (races are in seconds, distances are in meters)

<table>
<thead>
<tr>
<th>Final Rank</th>
<th>Athlete</th>
<th>100 m run</th>
<th>Long jump</th>
<th>Shot put</th>
<th>High jump</th>
<th>400 m run</th>
<th>110 m hurdles</th>
<th>Discus throw</th>
<th>Pole vault</th>
<th>Javelin throw</th>
<th>1500 m run</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ashton Eaton (US)</td>
<td>10.35</td>
<td>8.03</td>
<td>14.66</td>
<td>2.05</td>
<td>46.90</td>
<td>13.56</td>
<td>42.53</td>
<td>5.2</td>
<td>61.96</td>
<td>273.59</td>
</tr>
<tr>
<td>2</td>
<td>Trey Hardee (US)</td>
<td>10.42</td>
<td>7.53</td>
<td>15.28</td>
<td>1.99</td>
<td>48.11</td>
<td>13.54</td>
<td>48.26</td>
<td>4.8</td>
<td>66.65</td>
<td>280.94</td>
</tr>
<tr>
<td>3</td>
<td>Leonel Suarez (CUB)</td>
<td>11.27</td>
<td>7.52</td>
<td>14.50</td>
<td>2.11</td>
<td>49.04</td>
<td>14.45</td>
<td>45.75</td>
<td>4.7</td>
<td>76.94</td>
<td>270.08</td>
</tr>
<tr>
<td>4</td>
<td>Hans Van Alphen (BEL)</td>
<td>11.05</td>
<td>7.64</td>
<td>15.48</td>
<td>2.05</td>
<td>49.18</td>
<td>14.89</td>
<td>48.28</td>
<td>4.8</td>
<td>61.69</td>
<td>262.50</td>
</tr>
<tr>
<td>5</td>
<td>Damian Warner (CAN)</td>
<td>10.48</td>
<td>7.54</td>
<td>13.73</td>
<td>2.05</td>
<td>48.20</td>
<td>14.38</td>
<td>45.90</td>
<td>4.7</td>
<td>62.77</td>
<td>269.85</td>
</tr>
<tr>
<td>6</td>
<td>Rico Freimuth (GER)</td>
<td>10.65</td>
<td>7.21</td>
<td>14.87</td>
<td>1.90</td>
<td>48.06</td>
<td>13.89</td>
<td>49.11</td>
<td>4.9</td>
<td>57.37</td>
<td>277.62</td>
</tr>
<tr>
<td>7</td>
<td>Oleksiy Kasyanov (UKR)</td>
<td>10.56</td>
<td>7.55</td>
<td>14.45</td>
<td>1.99</td>
<td>48.44</td>
<td>14.09</td>
<td>46.72</td>
<td>4.6</td>
<td>54.87</td>
<td>273.68</td>
</tr>
<tr>
<td>8</td>
<td>Sergey Sviridov (RUS)</td>
<td>10.78</td>
<td>7.45</td>
<td>14.42</td>
<td>1.99</td>
<td>48.91</td>
<td>15.42</td>
<td>47.43</td>
<td>4.6</td>
<td>68.42</td>
<td>276.63</td>
</tr>
<tr>
<td>9</td>
<td>Willem Coertzen (RSA)</td>
<td>11.09</td>
<td>7.17</td>
<td>13.79</td>
<td>2.05</td>
<td>48.56</td>
<td>14.15</td>
<td>43.58</td>
<td>4.5</td>
<td>64.79</td>
<td>266.52</td>
</tr>
<tr>
<td>10</td>
<td>Pascal Behenbruch (GER)</td>
<td>11.06</td>
<td>7.15</td>
<td>15.67</td>
<td>1.96</td>
<td>50.04</td>
<td>14.33</td>
<td>44.71</td>
<td>4.7</td>
<td>64.80</td>
<td>277.46</td>
</tr>
<tr>
<td>11</td>
<td>Eelco Sintricolaas (NED)</td>
<td>10.85</td>
<td>7.37</td>
<td>14.18</td>
<td>1.93</td>
<td>48.85</td>
<td>14.43</td>
<td>32.26</td>
<td>5.3</td>
<td>58.82</td>
<td>271.17</td>
</tr>
<tr>
<td>12</td>
<td>Brent Nedwick (NZL)</td>
<td>11.10</td>
<td>7.36</td>
<td>15.09</td>
<td>1.96</td>
<td>50.22</td>
<td>15.02</td>
<td>46.15</td>
<td>4.7</td>
<td>59.82</td>
<td>278.20</td>
</tr>
<tr>
<td>13</td>
<td>Gonzalo Barroilhet (CHI)</td>
<td>11.18</td>
<td>6.80</td>
<td>14.49</td>
<td>2.05</td>
<td>51.07</td>
<td>14.12</td>
<td>41.27</td>
<td>5.4</td>
<td>57.25</td>
<td>288.23</td>
</tr>
<tr>
<td>14</td>
<td>Yordani Garcia (CUB)</td>
<td>10.80</td>
<td>6.75</td>
<td>14.48</td>
<td>1.99</td>
<td>48.76</td>
<td>14.24</td>
<td>42.27</td>
<td>4.6</td>
<td>59.85</td>
<td>278.57</td>
</tr>
<tr>
<td>15</td>
<td>Kevin Mayer (FRA)</td>
<td>11.32</td>
<td>7.17</td>
<td>14.05</td>
<td>2.05</td>
<td>48.76</td>
<td>15.59</td>
<td>41.20</td>
<td>4.7</td>
<td>62.41</td>
<td>263.02</td>
</tr>
<tr>
<td>16</td>
<td>Ilya Shkurenev (RUS)</td>
<td>11.01</td>
<td>7.25</td>
<td>12.89</td>
<td>2.02</td>
<td>49.81</td>
<td>14.39</td>
<td>43.51</td>
<td>5.1</td>
<td>53.81</td>
<td>282.80</td>
</tr>
<tr>
<td>17</td>
<td>Eduard Mikhan (BLR)</td>
<td>10.74</td>
<td>6.94</td>
<td>14.75</td>
<td>1.93</td>
<td>48.42</td>
<td>14.15</td>
<td>44.42</td>
<td>4.4</td>
<td>55.69</td>
<td>278.06</td>
</tr>
<tr>
<td>18</td>
<td>Dmitriy Karpov (KAZ)</td>
<td>10.91</td>
<td>7.21</td>
<td>16.47</td>
<td>1.99</td>
<td>49.83</td>
<td>14.40</td>
<td>44.93</td>
<td>5.1</td>
<td>49.93</td>
<td>316.83</td>
</tr>
<tr>
<td>19</td>
<td>Luis Alberto de Araujo (BRA)</td>
<td>10.70</td>
<td>7.16</td>
<td>13.52</td>
<td>1.93</td>
<td>48.25</td>
<td>14.79</td>
<td>44.76</td>
<td>4.6</td>
<td>51.59</td>
<td>278.04</td>
</tr>
<tr>
<td>20</td>
<td>Keisuke Ushiro (JPN)</td>
<td>11.32</td>
<td>6.86</td>
<td>13.59</td>
<td>1.99</td>
<td>50.78</td>
<td>15.47</td>
<td>46.66</td>
<td>4.9</td>
<td>66.38</td>
<td>279.33</td>
</tr>
</tbody>
</table>

### TABLE IB: Ranks for the 20 athletes in each discipline

<table>
<thead>
<tr>
<th>Final Rank</th>
<th>Athlete</th>
<th>100 m run</th>
<th>Long jump</th>
<th>Shot put</th>
<th>High jump</th>
<th>400 m run</th>
<th>110 m hurdles</th>
<th>Discus throw</th>
<th>Pole vault</th>
<th>Javelin throw</th>
<th>1500 m run</th>
<th>“Average sport”</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ashton Eaton (US)</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>16</td>
<td>3</td>
<td>9</td>
<td>7</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>Trey Hardee (US)</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>3</td>
<td>17</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>Leonel Suarez (CUB)</td>
<td>18</td>
<td>6</td>
<td>9</td>
<td>1</td>
<td>13</td>
<td>14</td>
<td>9</td>
<td>10</td>
<td>1</td>
<td>5</td>
<td>8.6</td>
</tr>
<tr>
<td>4</td>
<td>Hans Van Alphen (BEL)</td>
<td>13</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>14</td>
<td>16</td>
<td>2</td>
<td>8</td>
<td>10</td>
<td>1</td>
<td>7.1</td>
</tr>
<tr>
<td>5</td>
<td>Damian Warner (CAN)</td>
<td>3</td>
<td>4</td>
<td>17</td>
<td>2</td>
<td>4</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>7</td>
<td>4</td>
<td>6.9</td>
</tr>
<tr>
<td>6</td>
<td>Rico Freimuth (GER)</td>
<td>5</td>
<td>11</td>
<td>6</td>
<td>20</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>14</td>
<td>11</td>
<td>7.9</td>
</tr>
<tr>
<td>7</td>
<td>Oleksiy Kasyanov (UKR)</td>
<td>4</td>
<td>3</td>
<td>12</td>
<td>9</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>15</td>
<td>17</td>
<td>8</td>
<td>8.4</td>
</tr>
<tr>
<td>8</td>
<td>Sergey Sviridov (RUS)</td>
<td>8</td>
<td>7</td>
<td>13</td>
<td>9</td>
<td>12</td>
<td>18</td>
<td>4</td>
<td>15</td>
<td>2</td>
<td>9</td>
<td>9.7</td>
</tr>
<tr>
<td>9</td>
<td>Willem Coertzen (RSA)</td>
<td>15</td>
<td>13</td>
<td>16</td>
<td>2</td>
<td>8</td>
<td>6</td>
<td>14</td>
<td>19</td>
<td>6</td>
<td>3</td>
<td>10.2</td>
</tr>
<tr>
<td>10</td>
<td>Pascal Behenbruch (GER)</td>
<td>14</td>
<td>16</td>
<td>2</td>
<td>15</td>
<td>17</td>
<td>9</td>
<td>12</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>11.0</td>
</tr>
<tr>
<td>11</td>
<td>Eelco Sintricolaas (NED)</td>
<td>10</td>
<td>8</td>
<td>14</td>
<td>17</td>
<td>11</td>
<td>13</td>
<td>20</td>
<td>2</td>
<td>12</td>
<td>6</td>
<td>11.3</td>
</tr>
</tbody>
</table>
TABLE IB (CONTINUED): Ranks for the 20 athletes in each discipline

<table>
<thead>
<tr>
<th>Rank</th>
<th>Athlete</th>
<th>Long jump</th>
<th>Shot put</th>
<th>High jump</th>
<th>400 m run</th>
<th>110 m hurdles</th>
<th>Discus throw</th>
<th>Pole vault</th>
<th>Javelin throw</th>
<th>1500 m run</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Brent Nedwick (NZL)</td>
<td>16</td>
<td>9</td>
<td>5</td>
<td>15</td>
<td>18</td>
<td>17</td>
<td>7</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>13</td>
<td>Gonzalo Barroilhet (CHI)</td>
<td>17</td>
<td>19</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>5</td>
<td>17</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>14</td>
<td>Yordani Garcia (CUB)</td>
<td>9</td>
<td>20</td>
<td>11</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>17</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>15</td>
<td>Kevin Mayer (FRA)</td>
<td>19</td>
<td>13</td>
<td>15</td>
<td>2</td>
<td>9</td>
<td>20</td>
<td>19</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>Ilya Shkurenev (RUS)</td>
<td>12</td>
<td>10</td>
<td>20</td>
<td>8</td>
<td>15</td>
<td>11</td>
<td>15</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>17</td>
<td>Eduard Mikhan (BLR)</td>
<td>7</td>
<td>17</td>
<td>7</td>
<td>17</td>
<td>6</td>
<td>6</td>
<td>13</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>18</td>
<td>Dmitriy Karpov (KAZ)</td>
<td>11</td>
<td>11</td>
<td>1</td>
<td>9</td>
<td>16</td>
<td>12</td>
<td>10</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>19</td>
<td>Luis Alberto de Araujo (BRA)</td>
<td>6</td>
<td>15</td>
<td>19</td>
<td>17</td>
<td>5</td>
<td>15</td>
<td>11</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>20</td>
<td>Keisuke Ushiro (JPN)</td>
<td>20</td>
<td>18</td>
<td>18</td>
<td>9</td>
<td>19</td>
<td>19</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

TABLE IC: Ranking differences for each athlete in each sport, relative to the 100-meter run

<table>
<thead>
<tr>
<th>Final Rank</th>
<th>Athlete</th>
<th>Long jump</th>
<th>Shot put</th>
<th>High jump</th>
<th>400 m run</th>
<th>110 m hurdles</th>
<th>Discus throw</th>
<th>Pole vault</th>
<th>Javelin throw</th>
<th>1500 m run</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ashton Eaton (US)</td>
<td>0</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>15</td>
<td>2</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Trey Hardee (US)</td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>18</td>
<td>Leonel Suarez (CUB)</td>
<td>12</td>
<td>9</td>
<td>17</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td>8</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>13</td>
<td>Hans Van Alphen (BEL)</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>1</td>
<td>3</td>
<td>11</td>
<td>5</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Damian Warner (CAN)</td>
<td>1</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Rico Freimuth (GER)</td>
<td>6</td>
<td>1</td>
<td>15</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Oleksiy Kasyanov (UKR)</td>
<td>1</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>11</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Sergey Sviridov (RUS)</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>10</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>Willem Coertzen (RSA)</td>
<td>2</td>
<td>1</td>
<td>13</td>
<td>7</td>
<td>9</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>14</td>
<td>Pascal Behrenbruch (GER)</td>
<td>2</td>
<td>12</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>Eeelco Sintnicolaas (NED)</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>16</td>
<td>Brent Nedwick (NZL)</td>
<td>7</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>Gonzalo Barroilhet (CHI)</td>
<td>2</td>
<td>7</td>
<td>15</td>
<td>3</td>
<td>12</td>
<td>0</td>
<td>16</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>Yordani Garcia (CUB)</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>19</td>
<td>Kevin Mayer (FRA)</td>
<td>6</td>
<td>4</td>
<td>17</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>12</td>
<td>Ilya Shkurenev (RUS)</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Eduard Mikhan (BLR)</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>13</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>Dmitriy Karpov (KAZ)</td>
<td>0</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>Luis Alberto de Araujo (BRA)</td>
<td>9</td>
<td>13</td>
<td>11</td>
<td>1</td>
<td>9</td>
<td>5</td>
<td>9</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>Keisuke Ushiro (JPN)</td>
<td>2</td>
<td>2</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>14</td>
<td>14</td>
<td>16</td>
<td>4</td>
</tr>
</tbody>
</table>

Sum of ranking differences: 90, 130, 150, 55, 73, 109, 151, 153, 136
Another interesting observation is the clustering of classified objects in this figure: the ranking is not perfectly continuous; rather, groups of sports appear clustered, relative to the reference.

Now that you understand the principle, let us see one example from analytical chemistry. For instance, SRD was applied in the past (4) to compare chromatographic columns based on the retention of a set of analytes. This could be useful for particular applications where the reference column is inadequate, for instance (a) because one target analyte was eluted with a poor peak shape or (b) because of co-elutions. In the first case, finding a chromatographic column that would provide the most similar retention and separation behavior (but hopefully better peak shapes) is desirable. In the second case, on the contrary, finding a dissimilar stationary phase is desirable. In the report referenced above, 70 columns were compared to the reference column. The first three ranked according to SRD provided the most similar elution profiles to the reference column but with improved peak shapes. Other columns with larger SRD values were shown to provide different elution orders from the reference column and thus would be adequate choices when a complementary method is desired.

A second example is the comparison of methods (chromatographic and computational methods) employed to determine lipophilicity measures (5). Chromatographic retention in the reversed-phase or hydrophilic interaction liquid chromatographic modes is often employed to obtain a measurement of lipophilicity (log P). In a 2015 paper, Andrić and Héberger reported a comparison of 28 different measures of lipophilicity. The results indicated that, although the computationally estimated lipophilicity measures were the best, some chromatographic lipophilicity descriptors approached them. The SRD methodology also allowed discriminating between acceptable and non-recommended lipophilicity descriptors.

Another interesting way to use SRD is to identify outliers (6), which will be the topic of a future article in this series.

For those interested, there are of course many more subtleties to SRD (1–3), as I have only drawn a rough picture of it. Freeware designed by the authors and running on Microsoft Excel or MATLAB is available at http://aki.ttk.mta.hu/srd/.

Acknowledgment
Károly Héberger is warmly thanked for assistance with SRD calculations and helpful comments.

References

Past Articles in This Series
Read past articles in this series at www.chromatographyonline.com/caroline-west. Topics include:
• Collect and Examine Your Data
• Linear Regression and Quantitative Structure–Retention Relationships
• Principal Component Analysis
• Clustering
• Discriminant Analysis
• Derringer Desirability Functions.

Caroline West
is an Associate Professor of analytical chemistry at the University of Orleans. Her scientific interests lie in the fundamentals of chromatographic selectivity, both in the achiral and chiral modes, mainly in SFC but also in HPLC. In 2015, she received the LCGC award for “Emerging Leader in Chromatography”. Direct correspondence to caroline.west@univ-orleans.fr.
Meeting the challenges of ever stricter regulations while improving efficiency and reproducibility.

Measurement of the olefin content and impurities in fuel presents challenges for quality control as well as for ensuring compliance with ever stricter government regulations. In this LCGC interview, Jean-Francois Borny, analytical services manager of McDermott Technology, discusses the use of gas chromatography with vacuum ultraviolet absorption spectroscopy detection (GC–VUV) for the analysis of finished gasoline by ASTM D8071 method. He also discusses potential future applications for GC–VUV systems.

LCGC: What do you feel are the most significant challenges faced by analytical scientists in the petrochemical industry today?

Borny: The regulations continuously drive down the accepted levels of impurities in gasoline. Thus, as analytical chemists, we are challenged to find better ways to detect and quantify lower levels of sulfurs and other impurities. At the same time, we are challenged to do more with less. We are always looking for ways to improve both performance and efficiency.

LCGC: Can you describe the importance of accurately monitoring the levels of different hydrocarbon classes in your field of work?

Borny: McDermott Technology provides technologies such as hydrodesulfurization. As we remove ppm levels of sulfur, we need to retain and characterize olefins, aromatics, naphthenes, and the other hydrocarbon classes, to maintain the octane value of the system. We need to be able to see exactly what is going into the unit and what is coming out of the unit.

LCGC: What are the limitations of methodologies that have been used in the past to analyze complex hydrocarbon mixtures and what benefits does gas chromatography vacuum ultraviolet spectroscopy offer in comparison?

Borny: Many of the legacy methodologies were developed during World War II. Bromine number by titration was first developed in 1941 to measure double bonds. While newer technologies have been developed with improved accuracy and precision, the old titration method, ASTM D1159, has a very broad acceptance criteria. It might produce passing results in two different laboratories on the same sample that differ by as much as 20–30%. A bromine number of 80 indicates approximately 40% olefin. If I go to another laboratory and get a bromine number of 65, still meeting the method criteria, it indicates only 32.5% olefin. Our engineers need to know which number is right.
Another method is the detailed hydrocarbon analysis (DHA) GC method. With this method, if a compound is an aromatic, it is classified as an aromatic. If the compound is a styrene, which is predominantly an aromatic, but also has an olefinic component to it, then we have a problem. With GC–VUV analysis, we can actually identify the olefin component of styrene. We are able to look at classes of compounds and even subclasses of compounds in a single measurement.

To summarize, the legacy methods cannot accurately categorize the double-bond components. This is very important when our engineers are trying to design an efficient and reliable hydrodesulphurization unit. Those double bonds make a difference in the hydrogen consumption and heat release and affect our ability to retain our hydrocarbon classes while removing ppm levels of sulfur.

“McDermott Technology provides technologies such as hydrodesulphurization. As we remove ppm levels of sulfur, we need to retain and characterize olefins, aromatics, naphthenes, and the other hydrocarbon classes, to maintain the octane value of the system. We need to be able to see exactly what is going into the unit and what is coming out of the unit.”

To LCGC: How would you compare the selectivity of the GC–VUV analyzers to other GC methodologies?

Borny: To further compare the selectivity of the methodologies, the DHA GC method does really well with the detailed hydrocarbon analysis up to about C8 or even C9. With more complex components, we are left guessing. The flame ionization detector (FID) of the DHA GC can only see carbon and does not have the selectivity to measure the olefinic portion of the styrene, for example. Using the spectral detection of the VUV, we can look at styrene and see the aromatic component as well as the olefinic component. This selectivity is critical for our hydrodesulphurization work.

To LCGC: With the advantages that GC–VUV analysis provides over alternative methods, what were you able to accomplish in your research?

Borny: Well, one of the advantages I see with the VUV method is that we have been able to cut through a lot of the background noise or “junk” that we see. Our analytical work needs to be precise even with variations in quality and sourness of the incoming gasoline streams. The VUV spectra allows us to cut through the carbon “junk” and zoom in to determine the carbon classes even with high background.

To LCGC: Where do you see the greatest potential for successful applications using GC–VUV systems?

Borny: As mentioned, one application is looking at the olefinic components. The diolefin application is another ancient titration method determining maleic anhydride value and that takes four to six hours to run. By providing spectra, the VUV can determine diolefin rapidly. Next, while the legacy DHA analysis takes up to two hours, we can accomplish the same thing with the VUV instrument in less than 45 minutes because we don’t need chromatographic resolution for all of the components. We may even be able to enhance the oxygenate applications. In the future, I am looking at adding the VUV instrument to the GCxGC system, multidimensional GC, for the advantages of the spectra.

Finally, the speed of the GC–VUV analysis can really be an advantage. Some excellent analytical work is done in academia, where they have the time and resources to optimize multiple legacy methods. However, when I run a 24/7 laboratory with an analysis every four minutes, I need the GC–VUV system to go through a lot of samples using one rapid method. It is simple to run and has no routine maintenance or calibration, a dependable resource for analytical chemists in everyday real-world laboratories.
Lloyd R. Snyder (1931-2018) – A Personal Tribute

Lloyd Snyder was one of perhaps ten “founding fathers” of high performance liquid chromatography (HPLC), with seminal publications in most areas, including adsorption (normal phase), reversed phase, isocratic, gradient, and preparative chromatography, plus solvent, temperature, and column selectivity. With nine books, several hundred publications, and an h-index of 83, he was one of the most widely cited chromatographers and received many of the most prestigious awards in separation science.

John W. Dolan

When I spread the word of Lloyd’s passing to friends and colleagues, although his scientific achievements were noted, most replies focused on Lloyd’s character, mentorship, and friendship. So when LCGC North America Editorial Director Laura Bush asked me to write a tribute for Lloyd, I decided to share a more personal look into Lloyd Snyder, the man. Here are a few comments from some people who worked closely with Lloyd at one time or another.

Ron Majors: “My association with Lloyd goes back to my graduate school days in the 1960s. My PhD research involved adsorption chromatography; his pivotal papers, and, later, his book ‘Principles of Adsorption Chromatography,’ were my bibles in terms of adsorption theory and application.

I met my chromatographic ‘hero’ when I interviewed for a job at Union Oil Company, followed by an enjoyable chromatography-filled evening, and a great dinner, at Lloyd and his wife Barbara’s home. Lloyd became a friend and mentor, and was influential in my career development, giving me ‘tips’ along the way. Not only was he a superb scientist, but also a wonderful person who was very reverent and kind, and rarely raised his voice at others in the early days of HPLC, when it was more fashionable to have heated discussion sessions at international meetings. He was a devoted researcher, and recently he still managed to work with colleagues on the latest chromatography theory and leading-edge developments.”

David McCalley: “If I had only two words to describe Lloyd Snyder, they would be ‘modest’ and ‘genius.’ Lloyd was a true polymath of chromatography, being at the very forefront of research in many different areas. Yet Lloyd was never one to boast, or claim superiority over lesser mortals. Indeed, he showed a great personal interest in lending a helping hand to those starting out in their own research careers.

In the mid-’90s, I was working in relative isolation, with few opportunities to interact with other chromatographers. Lloyd first noticed my work in 1995, at the HPLC symposium in Innsbruck, and I remember timorously sending him a fax around UK lunchtime one day, with some tentative interpretations of results I had obtained for the analysis of basic solutes by reverse-phase liquid chromatography (RPLC). I hardly expected a response; imagine my surprise before leaving work on the same day to get a full one page fax commentary on my findings. This pattern of discussion continued for more than 20 years. Lloyd was like the Oracle at Delphi. He could always come up with an answer to any question, or suggest an experiment that might solve the problem.”

Pete Carr: “There are only one or two people, outside my family, who have had as much impact on me as did Lloyd Snyder. His published work was my first connection with him; it informed and inspired some of my first papers in chromatography. Soon after I became active in HPLC, Lloyd asked me to participate in a symposium on RPLC in the early ’80s; after that, we began to collaborate. Not only did I learn a lot of science from Lloyd, but also, and more importantly, I learned a great deal about how to do good science, and how to be a good member of the community. Lloyd was certainly the most open and by far the most generous scientist in sharing credit I have ever known. One of the principles I learned from Lloyd was: ‘On a paper with five co-authors, everyone gets one third of the credit.’

As a newcomer to separation science, I often received Lloyd’s outreach and guidance, especially when he was an editor of the Journal of Chromatography. He was a natural born mentor, and encouraged many who he thought deserving of some help. As a scientist and teacher, he was an outstanding role model. He taught me that we can, and should, staunchly defend our positions, but could still be friends after a technical argument. Not only did he often convince me to change my ideas, but I have seen him completely drop or reverse his position after a rigorous discussion.”

Peter Schoenmakers: “I met Lloyd first when I was a young PhD student at the HPLC meeting in Salzburg in 1977. Lloyd spent an hour at my poster, which was one of a dozen in the first-ever poster session in the series. He asked a long string of ques-
Issue 3 Nov 2018

Featured articles:

- Allergenic Fragrance Testing - New Certified Reference Materials and GC-FID/GC-MS Application
- HPTLC Application & Standards for Passiflora Incarnata
- Headspace SPME-GC/MS Analysis of Terpenes in Hops and Cannabis
- Series of new Terpenoid CRM Solutions
- New CRMs for fully intact Milk proteins
- Analysis of a Bispecific Monoclonal Antibody Using SEC-MS
- VOCs in Water by SPME-GC/MS: ISO Standard 17943
- Ultrasensitive Determination of Silicate in Water by Rapid Tests

Subscribe for free
SigmaAldrich.com/Analytix
tions and gave me great advice. He was a hero for me as a young scientist— and a very helpful and approachable one.”

Imre Molnar: “In 1977, I started teaching HPLC courses in Berlin, similar to those Lloyd and Jack Kirkland were teaching in the US. In 1984, Csaba Horváth suggested that Lloyd and I work together. Soon, Lloyd and I were teaching HPLC courses together in Berlin. Later, he invited me to work with the team writing the DryLab method development software at LC Resources. I became the European distributor, and later acquired sole ownership of DryLab. In all my interactions with Lloyd, he always made me feel that my contributions were highly valued. He wrote in his autobiography, ‘Imre and I have enjoyed a close relationship over the years.’ I will always be grateful for the many ways that Lloyd helped me grow professionally. Thank you, Lloyd, for your years with us, your friendship, and your good spirit to make us successful.”

Mary Ellen McNally: “In my early career as a chromatographer, the Snyder and Kirkland duo led the knowledge base in HPLC. As my career developed, so did my knowledge of Lloyd and Jack on a personal level. Every time I saw Lloyd at a conference or a meeting, he stood out as a driven man, with a thirst for knowledge on whatever topic was at the top of his list at the moment, and if he was listening to your presentation, it was at the top. Lloyd typically made it a point to ask, ‘Well, did you consider this?’ which would set the wheels turning for the next set of experiments. His thoroughness in the details and interest in the big picture of how things were operating at a molecular level were inspiring.”

Dwight Stoll: “I was fortunate to get to know Lloyd Snyder and his work in two quite different ways. The first was from a distance, as a graduate student, aspiring to someday be knowledgeable enough to call myself a chromatographer. There is an entire generation of chromatographers that ‘grew up’ academically learning from Lloyd through his ‘Introduction to Modern Liquid Chromatography,’ now in its 3rd edition.

The second way I came to know Lloyd was through closer and more personal interactions around the topic of selectivity in RPLC. We had many conversations, primarily discussing the Hydrophobic Subtraction Model of reversed-phase selectivity that he developed alongside several of his close collaborators. In these interactions, I greatly appreciated Lloyd’s generosity and collaborative spirit, as well as the depth and breadth of his knowledge of separations science. I don’t think we can overestimate the value of his pragmatic approach to method development, always keeping in mind the needs of people solving real problems. He was incredibly supportive of me and my work throughout my young career, and for that I will be forever grateful.”

John Dolan: “I first met Lloyd during my postdoctoral studies, and then he was my supervisor at Technicon Instruments. He must have seen potential in this young, wet-behind-the-ears PhD, but I think he realized quickly that my chromatography knowledge was weak. So he handed me a hand-typed manuscript of the second edition of the 800 page ‘Introduction to Modern Liquid Chromatography,’ with the instructions, ‘Here, proof this.’ I learned more about chromatography in the two weeks I took to wade through the text, proofreading the equations as I read, than in any other such time in my life. I must have improved, because Lloyd was the one who recommended me in 1983 to be editor of the ‘LC Troubleshooting’ column in LCGC.

In 1984, Lloyd and I started LC Resources, soon to be joined by Tom Jupille. That 34-year run covered nearly half my life as I learned how to be a good human being first, and then apply that to a scientific career. Lloyd, Tom, and I, along with our wives (without whose support none of our careers would have succeeded) met several times a year for dinner and to enjoy each other’s company.

Lloyd taught me to write by example, and often using his dry sense of humor. When he returned my first draft on one book project, paper-clipped to the first page was a sticky label completely covered with commas and a note: ‘I think the comma key isn’t working on your computer.’

At times, working with Lloyd was exhausting. I’d send him the revision of a paper we were working on, and, before the end of the day, he’d have sent his reply. He never missed a deadline, and his writing was amazing; he must have been an editor’s dream. Other than my father, Lloyd was the most influential man in my life.”

I hope these vignettes give a glimpse at the contributions of Lloyd Snyder. Nearly every communication ended with a statement expressed for us all by Pete Carr, “I will miss him very greatly in many ways…. as a colleague and mentor, but most of all, as a friend.”

If you’d like to read more about this great man, I suggest three articles that appeared earlier in LCGC (1-3), as well as Lloyd’s self-deprecating autobiography published by CASSS (4).

References:
Understanding chromatography, microscale separations, sample preparation, and the latest supporting technologies, oh my! When we say that the 43rd International Symposium on Capillary Chromatography (ISCC) and the 16th GCxGC Symposium (www.iscc-gcxgc.com) will bring people together to share the latest developments, we mean it. Starting Sunday, May 12, 2019, we’ll show you how it’s done. The ISCC & GCxGC event truly has it all, and whether it’s the latest developments by industry, professional development and education, interaction with key opinion leaders, or the latest cutting edge research you seek, you will find it in Fort Worth, Texas, at the historical Hilton Ft. Worth in the heart of downtown from May 12 to 17, 2019.

Five days of state-of-the-art science, practical education, social events, and unparalleled cultural experiences make for a great way to spend a week in May in the heart of the Dallas–Fort Worth metroplex. This event is the U.S. version of the highly successful ISCC & GCxGC conferences that are held in even years in Riva del Garda, Italy. By popular demand, 2019 will mark the third successive time the conference will be held in the “Gateway to the West”— Ft. Worth, Texas. Whether it is your first time or your third time attending, you will find that our conference organizers have really stepped up their game this time around. For sure, time spent by industry, academic, and government scientists alike, from near and abroad, will be productive, enjoyable, and informative.

The Latest Developments
Want to know the most recent developments in gas and liquid chromatography columns and detection systems? We have that. Want to know the state of the art in microscale liquid chromatography? We’ll have experts on hand to discuss it. Want to see the latest in pumped and electrically-driven separations, including microfluidics? We have it covered. Are you interested in comprehensive chromatography techniques, and how they can be used to improve resolution and determinations from complex mixtures? Look no further! ISCC and GCxGC is the premier venue for the latest development in comprehensive chromatography, especially two-dimensional gas chromatography (GCxGC), but also in two-dimensional liquid chromatography (LCxLC). Want to see what industry has on hand to support your research efforts? We will have the leading manufacturers and solution providers in the business ready to tell you what’s new and to answer your questions.

A Wide Range of Applications
Are you interested in a particular application space? ISCC & GCxGC puts the most recent and relevant analytical technology in the context of so many areas of interest that there is hardly enough space to convey it here: pharmaceutical, forensic, and process analysis; contaminants in food and the environment; biomarker discovery, and protein analysis; petroleum, energy, and resource development; ionic liquids in chemical analysis; novel stationary-phase chemistry; software and data analysis; atmospheric, air, and breath analysis; lab-on-a-chip and microfluidics; novel detection techniques; multidimensional separations; new materials for sample preparation; and miniaturized and portable systems. The world’s top researchers and thought leaders will be there, and so should you.

Top Plenary Speakers, Award Winners, and Panel Discussions
The plenary speakers will knock your socks off. Fred Regnier from Purdue
Separation system
The Eclipse DualTech separation system from Wyatt Technology is designed for both hollow-fiber flow field-flow fractionation (HF5) and asymmetric-flow field-flow fractionation (AF4) techniques. According to the company, both techniques may be integrated into one instrument and coupled to the company’s DAWN HELEOS II detector.
Wyatt Technology Corp., Santa Barbara, CA. www.wyatt.com/separation

autosampler syringes
Autosampler syringes for liquid and gas chromatography from Hamilton are designed specifically for CTC PAL liquid chromatography autosampler systems. According to the company, the S-Line syringes complement its existing C-Line and X-Type syringes, and are suitable for everyday use.
Hamilton Company, Reno, NV. www.hamiltoncompany.com

GCxGC flow modulator
The INSIGHT flow modulator from SepSolve Analytical is designed for routine comprehensive two-dimensional gas chromatography. According to the company, the modulator provides control of gas flows to fill and flush a sample loop to fractionate the first-column effluent, and deliver it to the second column.
SepSolve Analytical Ltd. Peterborough, UK. www.sepsolve.com/separation

GC mass spectrometers
Shimadzu’s GCMS NX series of gas chromatograph (GC)–mass spectrometers is designed using the company’s latest Nexis GC-2030 as the GC unit, which is equipped with a next-generation flow controller that enables control and accommodates a variety of applications. According to the company, the systems include features to facilitate maintenance and to improve operating efficiency by reducing standby time.
Shimadzu Scientific Instruments, Columbia, MD. www.ssi.shimadzu.com

Carbohydrates analyzer
Antec Scientific’s Alexys carbohydrates analyzer is designed with pulsed amperometric detection for the analysis of carbohydrates, mono-, di-, and polysaccharides. According to the company, the analyzer is based on UHPLC and is suitable for samples such as food and beverages, plants, lactose-free products, and glycoproteins.
Antec Scientific, Boston, MA. www.AntecScientific.com

Thermal field-flow fractionation detector
Postnova’s Triple Detection for thermal field-flow fractionation, gel permeation chromatography, or size-exclusion chromatography detection is designed with a combination of multi-angle light scattering, viscosity detection, refractive index detection, and UV detection. According to the company, the detector provides molar mass distribution, molecular size distribution, and molecular structure of polymers, biopolymers, polysaccharides, proteins, and antibodies.
Postnova, Landsberg, Germany. www.postnova.com

Variable speed rotor mill
The Variable Speed Rotor Mill Pulverisette 14 Premium from Fritsch offers impact, shearing, and cutting comminution in one instrument, with sample throughput of 15 liters or more per h. According to the company, the Ultra Centrifugal Mill enables quiet grinding with highest rotational speeds up to 22,000 rpm.
Fritsch GMBH, Idar-Oberstein, Germany. www.fritsch.de

Captiva syringe filter
Agilent’s Captiva syringe filter, available through Neta Scientific, is designed with high-grade housing and membrane materials. According to the company, these materials provide flow and sample loading capacity, and ensure sample integrity.
Neta Scientific, Hainesport, NJ. www.netascientific.com

Carbohydrates analyzer
Antec Scientific’s Alexys carbohydrates analyzer is designed with pulsed amperometric detection for the analysis of carbohydrates, mono-, di-, and polysaccharides. According to the company, the analyzer is based on UHPLC and is suitable for samples such as food and beverages, plants, lactose-free products, and glycoproteins.
Antec Scientific, Boston, MA. www.AntecScientific.com

GC mass spectrometers
Shimadzu’s GCMS NX series of gas chromatograph (GC)–mass spectrometers is designed using the company’s latest Nexis GC-2030 as the GC unit, which is equipped with a next-generation flow controller that enables control and accommodates a variety of applications. According to the company, the systems include features to facilitate maintenance and to improve operating efficiency by reducing standby time.
Shimadzu Scientific Instruments, Columbia, MD. www.ssi.shimadzu.com

Captiva syringe filter
Agilent’s Captiva syringe filter, available through Neta Scientific, is designed with high-grade housing and membrane materials. According to the company, these materials provide flow and sample loading capacity, and ensure sample integrity.
Neta Scientific, Hainesport, NJ. www.netascientific.com

Thermal field-flow fractionation detector
Postnova’s Triple Detection for thermal field-flow fractionation, gel permeation chromatography, or size-exclusion chromatography detection is designed with a combination of multi-angle light scattering, viscosity detection, refractive index detection, and UV detection. According to the company, the detector provides molar mass distribution, molecular size distribution, and molecular structure of polymers, biopolymers, polysaccharides, proteins, and antibodies.
Postnova, Landsberg, Germany. www.postnova.com

GCxGC flow modulator
The INSIGHT flow modulator from SepSolve Analytical is designed for routine comprehensive two-dimensional gas chromatography. According to the company, the modulator provides control of gas flows to fill and flush a sample loop to fractionate the first-column effluent, and deliver it to the second column.
SepSolve Analytical Ltd. Peterborough, UK. www.sepsolve.com/separation

GC mass spectrometers
Shimadzu’s GCMS NX series of gas chromatograph (GC)–mass spectrometers is designed using the company’s latest Nexis GC-2030 as the GC unit, which is equipped with a next-generation flow controller that enables control and accommodates a variety of applications. According to the company, the systems include features to facilitate maintenance and to improve operating efficiency by reducing standby time.
Shimadzu Scientific Instruments, Columbia, MD. www.ssi.shimadzu.com

Carbohydrates analyzer
Antec Scientific’s Alexys carbohydrates analyzer is designed with pulsed amperometric detection for the analysis of carbohydrates, mono-, di-, and polysaccharides. According to the company, the analyzer is based on UHPLC and is suitable for samples such as food and beverages, plants, lactose-free products, and glycoproteins.
Antec Scientific, Boston, MA. www.AntecScientific.com

GC mass spectrometers
Shimadzu’s GCMS NX series of gas chromatograph (GC)–mass spectrometers is designed using the company’s latest Nexis GC-2030 as the GC unit, which is equipped with a next-generation flow controller that enables control and accommodates a variety of applications. According to the company, the systems include features to facilitate maintenance and to improve operating efficiency by reducing standby time.
Shimadzu Scientific Instruments, Columbia, MD. www.ssi.shimadzu.com

Variable speed rotor mill
The Variable Speed Rotor Mill Pulverisette 14 Premium from Fritsch offers impact, shearing, and cutting comminution in one instrument, with sample throughput of 15 liters or more per h. According to the company, the Ultra Centrifugal Mill enables quiet grinding with highest rotational speeds up to 22,000 rpm.
Fritsch GMBH, Idar-Oberstein, Germany. www.fritsch.de
University is an unparalleled innovator. He will describe analyte-sequestering transport phases, an exciting new sample preparation technology that he and his team have developed to isolate small molecules from complex matrices. Imagine what you can do with a third phase in chromatography-based separations. Jonathan Sweedler from the University of Illinois–Urbana Champaign uses a variety of separation and detection techniques to characterize the role of D-amino acids in a wide array of disease processes. It does not get more cutting edge than that. Chris Reddy from the Woods Hole Oceanographic Institute is the primary researcher studying the impact of the Deepwater Horizon oil spill in the Gulf of Mexico. GCxGC technology features heavily in his work, but he will talk about where there are still gaps and needs in the handling and mining of the massive data sets generated using comprehensive chromatography.

The fireworks don’t stop there. Winners of the prestigious Golay Award, Ettre Award, Jon Phillips Award, Lifetime Achievement in GCxGC Award, and other esteemed leaders will share their work. Learn from the best educators in the country through a series of top-notch short courses on fundamental GCxGC, basic HPLC, capillary LC, and sample preparation for capillary chromatography. As a completely new offering, daily panel discussions will put leading experts from the commercial sector in front of you to discuss practical and emerging topics in analytical chemistry.

Present Your Work
Join us! ISCC & GCxGC offers a perfect setting for you to present your own breakthrough science, orally or in one of our engaging poster sessions. Posters will be judged by experts in the field to award a plethora of cash prizes to the best of the best. Registration and abstract submission open December 7, 2018. Need help getting there? Students and post-docs can apply for a large number of travel awards. Check our website for details.

Social and Networking Events
What more could you ask for? We’ll supplement the presentation of leading science and fundamental education with a series of social events designed for inclusiveness. No nickel-and-diming here. Rub elbows with old and new friends alike at an authentic Tex-Mex conference banquet with open bar, or during happy hour at the local pub. Fort Worth and the Dallas–Fort Worth metroplex have plenty to offer the art enthusiast, foodie, socialite, or sports fan alike, so you might as well bring your family along. Getting there from virtually anywhere in the world could not be easier. We stand ready to field your inquiries at info@isccgcxgc.com. Don’t miss out on an experience like no other!
How It Works: UV Detection for HPLC

The fundamental principles of UV detection

Many organic molecules absorb ultraviolet (UV) radiation over a range of wavelengths, from around 200 nm up to around 400 nm. When using monochromatic light (light of a single wavelength or small range of wavelengths) radiated onto a dilute solution of analyte, the Beer-Lambert law (equation 1) can be applied, which relates absorbance (A) to analyte concentration:

\[ A = \varepsilon lc \]  

where A is absorbance, \( \varepsilon \) is the molar absorption coefficient (dm\(^3\) mol\(^{-1}\) cm\(^{-1}\)), \( l \) is the flow cell path length (cm), and \( c \) is the concentration of the solution (mol dm\(^{-3}\)).

Typically, the wavelength chosen comes from the most intense region of the analyte absorbance spectrum, in order to achieve maximum sensitivity for quantitative measurement. In high performance liquid chromatography with ultraviolet detection (HPLC–UV) applications, it is not necessary to know the value for the molar absorptivity coefficient or flow cell path length, as the instrument response is typically calibrated with one or more solutions of known concentration, and a linear regression model is used to interpolate the concentration of unknowns.

UV detectors with specially designed deuterium lamps are used, because of their almost constant light intensity and spectral reproducibility.

In variable wavelength detectors, the light is collimated using a slit which allows the light beam to fall upon a diffraction grating, which splits the white light into its component wavelengths (Figure 1). The grating turns upon an electromechanical stage, which directs the desired part of the spectrum onto a further slit through which a small number of wavelengths (typically around 5 nm) will be allowed to pass. This light falls upon a beam splitter, which directs the light either to a reference photodiode or through a flow cell in which the HPLC eluent is flowing and subsequently onto a second photodiode (the measuring photodiode). As the analyte elutes into the flow cell, it absorbs light and the changes in the light transmittance (measured in milliabsorbance units, mAU), are registered via the measuring photodiode. The reference photodiode is used to make corrections of fluctuations in the lamp intensity and thus improves the optical stability and performance of the instrument. As the diffraction grating is mounted on a turntable, various wavelengths may be measured throughout the course of an analysis to optimize the sensitivity for each of the analyte components; however, the response time of the grating movement and change in analyte concentration with time in the flow cell means that spectral scanning of a single analyte as it passes through the flow cell is not possible.

In diode array detectors (sometimes known as reverse optics detectors), the white light is passed through the detector flow cell prior to being split into its component wavelengths using a fixed diffraction grating (Figure 2). In this way the change in light transmittance for each constituent wavelength may be measured using a photodiode array (typically 1024 individual diodes), and the signals from each diode are summed to obtain a total intensity, which is plotted against time to generate the "chromatogram." Now, any point on the chromatogram may be selected from within the data system and the data deconvoluted in order to obtain the UV spectrum for that component (or the eluent system if a baseline point is chosen). Diode array detectors can be used for qualitative measurements such as peak identification against a user generated spectral library, or to investigate peak purity using wavelength ratio measurements across the peak. The slit in the diode array detector is usually of variable width and can be used to ‘tune’ the light falling onto the diode to optimize either sensitivity (signal-to-noise) or spectral resolution.
Join thousands of chemists and scientists in your specialty and beyond at Pittcon, the leading annual conference and exposition for laboratory science. This all-in-one event offers a high-caliber technical program and skill-building short courses featuring topics such as atomic spectroscopy, LIBS, UV/Vis, NMR, Raman, vibrational spectroscopy and more. Plus, explore a dynamic marketplace of the latest instrumentation and services. Start collaborating with your colleagues from around the world to find solutions to your greatest laboratory challenges at Pittcon 2019.

Pennsylvania Convention Center | Philadelphia, PA | March 17 - 21 | www.pittcon.org
Effortless Performance
Conduct Critical Qualitative and Quantitative Analysis with Genuine Confidence and Ease

Shimadzu’s research-grade LCMS-9030 quadrupole time-of-flight (Q-TOF) mass spectrometer combines the engineering DNA from our proven triple quadrupole (LC-MS/MS) platform with powerful, new TOF architecture to transform high mass accuracy workflows. The result is a system that delivers high-resolution, accurate-mass detection with incredibly fast data acquisition rates.

Learn more about Shimadzu’s Q-TOF LCMS-9030. Call (800) 477-1227 or visit us online at www.ssi.shimadzu.com
Shimadzu Scientific Instruments Inc., 7102 Riverwood Dr., Columbia, MD 21046, USA