

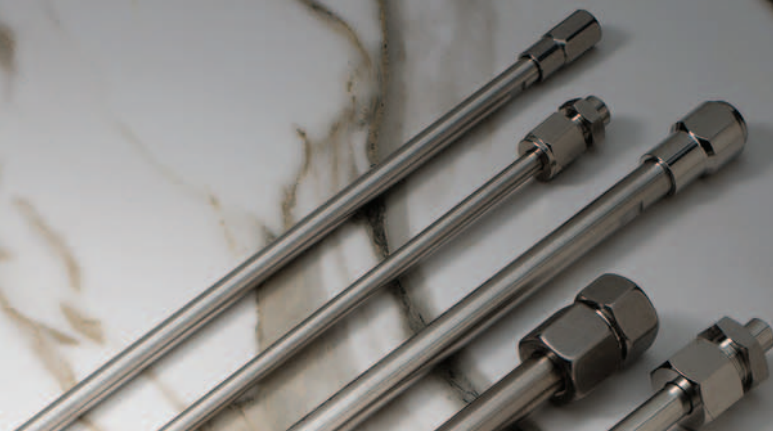


LCGC

north america

solutions for separation scientists

Volume 35 Number 4 April 2017



High Performance Protein A Column:

TSKgel® Protein A-5PW

Designed for fast and accurate
IgG titer determination with the widest
affinity for IgG subclasses

Bridging the gap between



Shares the same recombinant

TOSOH BIOSCIENCE
www.tosohbioscience.com



The High Capacity Protein A Resin
TOYOPEARL® AF-rProtein A HC-650F

Delivers high purity mAbs with
excellent recovery at loading levels
approaching the resin's capacity

analytical & process protein A

protein A ligand base

TSKgel, TOYOPEARL and Tosoh Bioscience are registered trademarks of Tosoh Corporation.

TOSOH BIOSCIENCE
www.tosohbioscience.com



LCGC

north america

solutions for separation scientists

Volume 35 Number 4 April 2017
www.chromatographyonline.com



NEW Chromatography Columns and HPLC Systems for 2017

How Retention Influences Resolution in LC

Hydrophobic Interaction Chromatography for Biopharmaceutical Analysis

HUMAN HEALTH

ENVIRONMENTAL HEALTH



WHOSE TRIPLE QUAD
CAN GIVE YOU
15% MORE TIME?

SIMPLE: PERKINELMER.

Whether you're performing environmental, food, or industrial testing, your lab needs to analyze more and increasingly complex samples under tighter regulatory standards. Because our new QSight™ triple-quad LC/MS/MS needs no routine cleaning and maintenance, you get up to 15% more uptime (that's up to 35 more days per year) to take on your increased testing workload. Plus, our patented technology delivers exceptional sensitivity and reliability along with all that productivity.

QSight: A triple quad that simply knows the value of time.

Learn more at www.perkinelmer.com/QSight



PerkinElmer
For the Better

DANI

INTRODUCING DISCOVER-GC[®]

THE SOLID PHASE FTIR
FOR COMPLEX MIXTURES

IR DETECTION SYSTEM
A BEAM OF CONFIDENCE



FLAVOURS & FRAGRANCES

AGRICULTURE

PHARMACEUTICAL

FOOD & BEVERAGE

FORENSIC DRUGS

ENVIRONMENT

CHEMICALS

MATERIAL SCIENCE

FEATURING :

- Complete integration of analytical operations from sample input to GC operation, data collection, spectral library search and report generation.
- Identification of unknown in complex samples
- Unequivocal identification of structural isomers
- Confirmation of GC-MS IDentification via orthogonal technique
- Natural product characterization & competitive analysis.

CONTACT :

USA & Canada: Conor.Sullivan@dani-instruments.com

International : Jeff.Kearney@dani-instruments.com

DANI Instruments, Inc., Marlborough, MA 01752

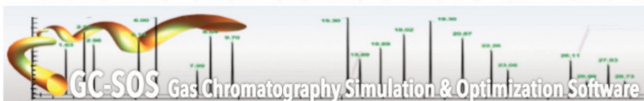
Ph. +1 508-281-6232 www.dani-instruments.com

LIMITED TIME OFFER:

- ORDER BY JULY 4, 2017
TO RECEIVE A 15% DISCOUNT
- ORDER BY SEPT 4, 2017 TO
RECEIVE A 10% DISCOUNT
- ORDER NOW AND TAKE UP
TO 12 WEEKS FOR DELIVERY

DANI

Gas Chromatography Method Development Magic Dust!



GC-SOS simulation & optimization software is the ultimate solution for developing highly efficient GC methods.

Save serious time and money by reducing development time from hours to minutes, & developing more efficient methods than ever before (guaranteed).

New version more powerful and easy to use:

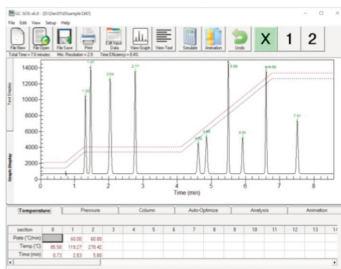
- Temperature, Column Size, Pressure & Flow simulation
- Flexible input: use 1-3 training runs -w- up to 5 temperature segments (use your existing method as a training run in many cases)
- Auto-Optimization: proprietary numerical algorithm provides a highly optimized method in seconds
- Animation Viewer: dynamic view of separations (*great teaching tool*)

**Download a
free demo
today at:**

www.GC-SOS.com

The 4S Company

Scientific Software, Services & Support
Web: www.GC-SOS.com
Email: support@GC-SOS.com



LCGC[®]
north america

MANUSCRIPTS: For manuscript preparation guidelines, see chromatographyonline.com/lcg-author-guidelines, or call The Editor, (732) 596-0276. LCGC welcomes unsolicited articles, manuscripts, photographs, illustrations, and other materials but cannot be held responsible for their safekeeping or return. Every precaution is taken to ensure accuracy, but LCGC cannot accept responsibility for the accuracy of information supplied herein or for any opinion expressed.

SUBSCRIPTIONS: For subscription and circulation information: LCGC, P.O. Box 6168, Duluth, MN 55806-6168, or call (888) 527-7008 (7:00 a.m.–6:00 p.m. central time). International customers should call +1-218-740-6477. Delivery of LCGC outside the United States is 14 days after printing. For single and back issues, call (800) 598-6008 or (218) 740-6480. (LCGC Europe and LCGC Asia Pacific are available free of charge to users and specifiers of chromatographic equipment in Western Europe and Asia and Australia, respectively.)

CHANGE OF ADDRESS: Send change of address to LCGC, P.O. Box 6168, Duluth, MN 55806-6168; alternately, send change via e-mail to magazines@superfill.com or go to the following URLs:

- Print: <http://ubmsubs.ubm.com/?pubid=LCGC>
- Digital: <http://ubmsubs.ubm.com/?pubid=LCGC&V=DIGI>

Allow four to six weeks for change. **PUBLICATIONS MAIL AGREEMENT No.** 40612608. Return all undeliverable Canadian addresses to: IMEX Global Solutions, P.O. Box 25542, London, ON, N6C 6B2, CANADA. Canadian GST number: R-124213133RT001.

C.A.S.T. DATA AND LIST INFORMATION: Contact Ronda Hughes, tel. (218) 464-4430, e-mail Ronda.Hughes-Stovern@ubm.com.

REPRINTS: Reprints of all articles in this issue and past issues of this publication are available (500 minimum). Call 877-652-5295 ext. 121 or e-mail bkolb@wrights-media.com. Outside US, UK, direct dial: 281-419-5725. Ext. 121

MARKETING DEVELOPMENT/CLASSIFIED: Contact Tod McCloskey, tel. (440) 891-2739, fax (440) 826-2865.

RECRUITMENT: Contact Tod McCloskey, tel. (440) 891-2739, fax (440) 826-2865.

INTERNATIONAL LICENSING: Contact Maureen Cannon, tel. (440) 891-2742, fax (440) 891-2650, or e-mail Maureen.Cannon@ubm.com.

**Alliance for
Audited Media**

Connectiv

© 2017 UBM. All rights reserved. No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical including by photocopy, recording, or information storage and retrieval without permission in writing from the publisher. Authorization to photocopy items for internal/educational or personal use, or the internal/educational or personal use of specific clients is granted by UBM for libraries and other users registered with the Copyright Clearance Center, 222 Rosewood Dr. Danvers, MA 01923, 978-750-8400 fax 978-646-8700 or visit <http://www.copyright.com> online. For uses beyond those listed above, please direct your written request to Permission Dept. fax 440-756-5255 or email: Maureen.Cannon@ubm.com.

UBM Americas provides certain customer contact data (such as customer's name, addresses, phone numbers, and e-mail addresses) to third parties who wish to promote relevant products, services, and other opportunities that may be of interest to you. If you do not want UBM Americas to make your contact information available to third parties for marketing purposes, simply call toll-free 866-529-2922 between the hours of 7:30 a.m. and 5 p.m. CST and a customer service representative will assist you in removing your name from UBM Americas lists. Outside the U.S., please phone 218-740-6477.

LCGC North America does not verify any claims or other information appearing in any of the advertisements contained in the publication, and cannot take responsibility for any losses or other damages incurred by readers in reliance of such content.

To subscribe, call toll-free 888-527-7008. Outside the U.S. call 218-740-6477.

UBM Americas (www.ubmlifesciences.com) is a leading worldwide media company providing integrated marketing solutions for the Fashion, Life Sciences and Powersports industries. UBM Americas serves business professionals and consumers in these industries with its portfolio of 91 events, 67 publications and directories, 150 electronic publications and Web sites, as well as educational and direct marketing products and services. Market leading brands and a commitment to delivering innovative, quality products and services enables UBM Americas to "Connect Our Customers With Theirs." UBM Americas has approximately 1000 employees and currently operates from multiple offices in North America and Europe.

Sciencix

SERVING ROYALTY. EXCEEDING EXPECTATIONS. EVERY MOMENT.

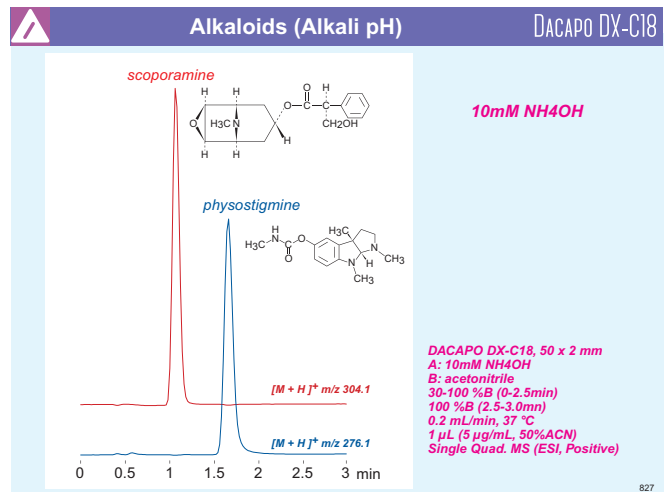
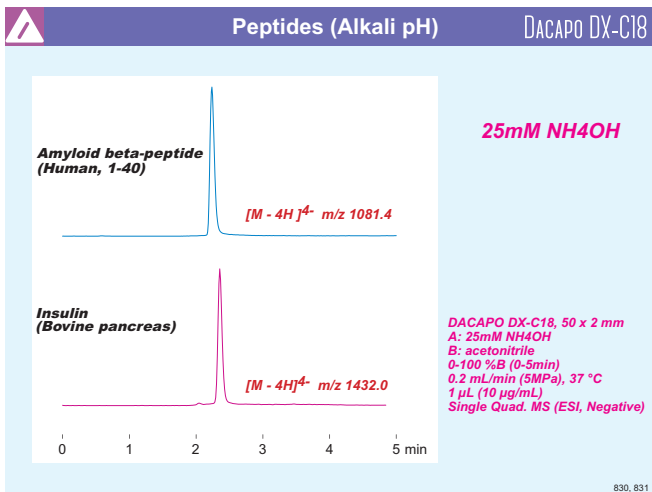
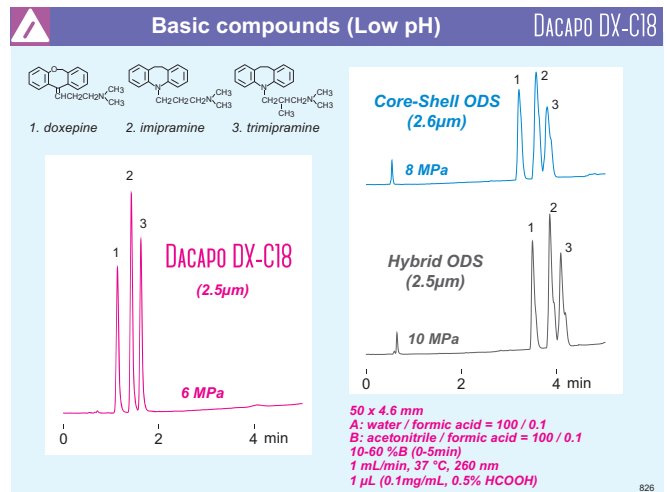
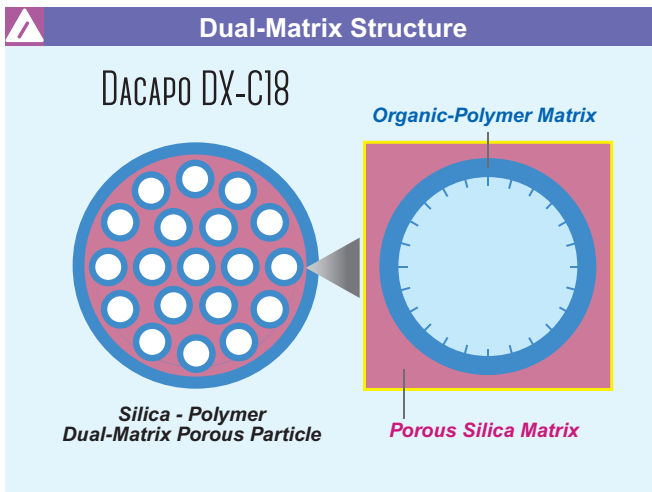


- Provider of top brand HPLC instrumentation products
- Equivalent to corresponding OEM products
- Serving customers for over 30 years
- Reduce product repair expenses by 15% to 30%
- Lifetime Warranty on manufacturing defects

www.sciencix.com 800.682.6480 sales@sciencix.com



We are pleased to announce our newest column, **Dacapo DX-C18**. This column features the latest in particle technology from Imtakt, called Dual Matrix. This technology takes polymeric surface treatment to a whole new level. Starting with a silica-based particle, we grafted a surface polymeric layer to protect the silica from high pH attack and to improve peak shape for basic compounds. Then we went crazy and added several more layers on top of that, for even more protection. If you are looking for a high pH C18 column, Dacapo DX-C18 with Dual Matrix technology will certainly be a column you will be interested in.



Particle Size 2.5µ | Pore Size: 130Å | pH Range: 1-12 | Pressure: 500 bar



PINNACLE PCX
(Reliably Sensitive)

Glyphosate Analysis in Food
The Experts for 30 Years

www.pickeringlabs.com



Sonntek, Inc.
www.sonntek.com PH: 201-236-9300 Email: sonntek@aol.com

WE CAN HELP!!

Let **SONNTEK**
BREAK you out of your CELL
in *Service Charge Hell!*

Reliable and Honest
Service, Lamps and Systems
for your BIO applications and more!

A K T A TRAZ



LCGC[®]
north america

485F US Highway One South, Suite 210
Iselin, NJ 08830

(732) 596-0276

Fax: (732) 647-1235

Michael J. Tessalone

Vice President/Group Publisher
Michael.Tessalone@ubm.com

Edward Fantuzzi

Publisher
Edward.Fantuzzi@ubm.com

Stephanie Shaffer

Sales Manager
Stephanie.Shaffer@ubm.com

Michael Kushner

Senior Director, Digital Media
Michael.Kushner@ubm.com

Laura Bush

Editorial Director
Laura.Bush@ubm.com

Megan L'Heureux

Managing Editor
Meg.L'Heureux@ubm.com

Stephen A. Brown

Group Technical Editor
Stephen.Brown@ubm.com

Cindy Delonas

Associate Editor
Cindy.Delonas@ubm.com

Kristen Moore

Webcast Operations Manager
Kristen.Moore@ubm.com

Vania Oliveira

Project Manager
Vania.Oliveira@ubm.com

Sabina Advani

Digital Production Manager
Sabina.Advani@ubm.com

Kaylynn Chiarello-Ebner

Managing Editor, Special Projects
Kaylynn.Chiarello.Ebner@ubm.com

Dan Ward

Art Director
dward@hcl.com

Anne Lavigne

Marketing Manager
Anne.Lavigne@ubm.com

Ronda Hughes

C.A.S.T. Data and List Information
Ronda.Hughes-Stovern@ubm.com

Wright's Media

Reprints
bkolb@wrightsmedia.com

Maureen Cannon

Permissions
Maureen.Cannon@ubm.com

Jesse Singer

Production Manager
jsinger@hcl.com

Wendy Bong

Audience Development Manager
Wendy.Bong@ubm.com

Matt Blake

Audience Development Assistant Manager
Matt.Blake@ubm.com



Time for a new LC-MS/MS? Make a smart investment with Shimadzu.

Maximize return on investment with Shimadzu triple quad systems.

Shimadzu offers the ultimate combination of speed, sensitivity, and uptime to maximize your productivity while minimizing costs.

Consolidate your old instruments and save time and money with Shimadzu.

Learn more about Shimadzu's LC-MS/MS.
Call (800) 477-1227 or visit us online at
www.ssi.shimadzu.com/LCMSMS

Order consumables and accessories on-line at <http://store.shimadzu.com>
Shimadzu Scientific Instruments Inc., 7102 Riverwood Dr., Columbia, MD 21046, USA

- Ultra-fast LC-MS/MS allows comprehensive panels significantly faster than competitors
- Superior sensitivity reduces the amount of internal standards used
- Consolidating old instruments onto a faster platform reduces service contract costs

LCGC®

north america

solutions for separation scientists



Cover photography by Joe Zugic, Joe Zugic Photography

Cover materials courtesy of Advanced Materials Technology, Agilent Technologies, MicroSolv Technology, Nacalai USA, PerkinElmer, Restek Corporation, Regis Technologies, Phenomenex, Showa Denko America, W.R. Grace & Co., and ZirChrom Separations

WEB SEMINARS

Automated SIFT-MS: High-Throughput Volatile Compound Analysis for the Laboratory

Mark Perkins, PhD, Anatune, and Vaughan Langford, PhD, Syft Technologies

Demystifying Software Validation: What Is It Really and When Do I Need to Do It?

Loren Smith, Agilent Technologies

www.chromatographyonline.com/LCGCwebseminars

NEW ON LCGCtv

Translating Analytical QbD to the Real World

Reed Harris, Genentech

Like LCGC on Facebook:
www.facebook.com/lcgmagazine



Follow LCGC on Twitter:
https://twitter.com/LC_GC







Join the LCGC Group on LinkedIn
<http://linkd.in/LCGCgroup>



CONTENTS

COLUMNS

- 226**  **COLUMN WATCH**
New Chromatography Columns and Accessories for 2017
David S. Bell
Our annual review of new liquid chromatography (LC) columns and accessories, including an analysis of the overall trends.
- 240**  **LC TROUBLESHOOTING**
Count the Cost, Part II: Increasing Resolution by Increasing Retention
John Dolan
Learn how to find the “sweet spot” in terms of retention for a liquid chromatography separation and how to estimate how much a change in mobile-phase percent organic will affect retention.
- 246**  **PERSPECTIVES IN MODERN HPLC**
New HPLC Systems and Related Products: A Brief Review
Michael W. Dong
A review of the new high performance liquid chromatography (HPLC) instruments, modules, and software that were introduced at Pittcon 2017 and during the year prior, including descriptions of their innovative features and significant benefits.
- 278**  **THE ESSENTIALS**
Hydrophobic Interaction Chromatography for Biopharmaceutical Analysis
HIC employs nondenaturing conditions, does not require the use of organic solvents or high temperatures, and is carried out at physiological pH, thus allowing protein structure to be preserved.
- PEER-REVIEWED ARTICLE**
- 258** **Maximization of Selectivity in Reversed-Phase Liquid Chromatographic Method Development Strategies**
Melvin R. Euerby, Mark Fever, Jennifer Hulse, Matthew James, Patrik Petersson, and Colin Pipe
The approach described here will help chromatographers produce highly efficient method development strategies for reversed-phase LC separations in a relatively short time frame.

DEPARTMENTS

Peaks of Interest 224 Products & Resources . . . 273 Ad Index 277

LCGC North America (ISSN 1527-5949 print) (ISSN 1939-1889 digital) is published monthly by UBM Life Sciences, 131 West First Street, Duluth, MN 55802-2065, and is distributed free of charge to users and specifiers of chromatographic equipment in the United States and Canada. Single copies (prepaid only, including postage and handling): \$15.50 in the United States, \$17.50 in all other countries; back issues: \$23 in the United States, \$27 in all other countries. LCGC is available on a paid subscription basis to nonqualified readers in the United States and its possessions at the rate of: 1 year (13 issues), \$74.95; 2 years (26 issues), \$134.50; in Canada and Mexico: 1 year (13 issues), \$95; 2 years (26 issues), \$150; in all other countries: 1 year (13 issues), \$140; 2 years (26 issues), \$250. Periodicals postage paid at Duluth, MN 55806 and at additional mailing offices. POSTMASTER: Please send address changes to LCGC, P.O. Box 6168, Duluth, MN 55806-6168. PUBLICATIONS MAIL AGREEMENT NO. 40612608, Return Undeliverable Canadian Addresses to: IMEX Global Solutions, P. O. Box 25542, London, ON N6C 6B2, CANADA Canadian GST number: R124213133R1001. Printed in the USA.

~~Eliminate gas cylinders~~ today!

Discover a better way with VICI DBS gas generators.

ELIMINATE HIGH PRESSURE GAS CYLINDERS TO:

- ✗ Improve lab safety
- ✗ Reduce lab expenses due to costly gas refills and cylinder rental fees
- ✗ Prevent instrument downtime due to running out of cylinder gas

DISCOVER HOW VICI GAS GENERATORS:

- ✓+ Are the smart safe alternative
- ✓+ Can pay for themselves immediately by saving money on gases
- ✓+ Provide an endless supply of research grade gas
- ✓+ Include a 2 year warranty



Hydrogen



Nitrogen



Zero Air

VICI DBSTM
www.vicidbs.com

713-263-6970 | infousa@vicidbs.com

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 MilliporeSigma, Bellefonte, Pennsylvania
Dennis D. Blevins Agilent Technologies, Wilmington, Delaware
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 Leyden, Zoeterwoude, The Netherlands
André de Villiers Stellenbosch University, Stellenbosch, South Africa
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
Francesco Gasparrini Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive, Università "La Sapienza," Rome, Italy
Joseph L. Glajch Momenta Pharmaceuticals, Cambridge, Massachusetts
Davy Guillaume University of Geneva, University of Lausanne, Geneva, Switzerland
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 Tasmania, Hobart, Tasmania, Australia
John V. Hinshaw Serveron Corporation, Beaverton, Oregon
Kiyokatsu Jinno School of Materials Science, Toyohashi University of Technology, Toyohashi, Japan
Ira S. Krull 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. McGuffin Department of Chemistry, Michigan State University, East Lansing, Michigan
Mary Ellen McNally E.I. du Pont de Nemours & Co., Wilmington, Delaware
Imre 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 Waters Corporation, Milford, 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

Hybrid Particle Performance – PEEK-Lined Columns

Particle size

1.9 μm
3 μm
5 μm



YMC now offers all of YMC-Triart's advantages in columns that combine the strength of stainless steel with the inert behavior of PEEK-lined column tubes and PEEK-frits.

YMC-Triart is based on a hybrid-silica stationary phase support, well known for its chemical stability, as well as its physical and thermal durability.

Benefits

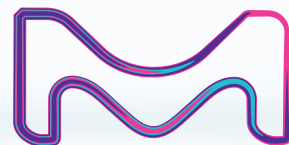
- YMC-Triart's reproducibility
- Excellent peak shape
- Choice of 6 different phases = 6 different selectivities
- Ideal for HPLC and UHPLC – including LC/MS of metal coordination compounds and other difficult-to-chromatograph species.

YMC
AMERICA, INC.

Phone: +1.610.266.8650
 Email: info@ymc-america.com
 Web: www.ymc-america.com
 Store: store.ymc-america.com

- Six distinct selectivities.
- Robust chemical, physical, and thermal durability.
- Columns for: UHPLC – HPLC.

Milli-Q®
Lab Water



A touch of intelligence

Milli-Q® IQ 7000 purification system

- New ultrapure water system with intuitive touch screen
- Innovative Q-POD® dispenser designed to maximize efficiency
- Environmentally friendly mercury-free UV oxidation lamps



emdmillipore.com/milliq-iqsystem

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

MilliporeSigma, Milli-Q, and the vibrant M are trademarks and Q-POD is a registered trademark of Merck KGaA, Darmstadt, Germany. Copyright © 2017 EMD Millipore Corporation. All Rights Reserved. 2017-02003

**MILLIPORE
SIGMA**

PEAKS of Interest



Shuang Yang Receives CACA 2017 Young Investigator Award

Shuang (Jake) Yang was presented with the Chinese American Chromatography Association (CACA) 2017 Young Investigator Award on Tuesday, March 7, at a networking dinner event in Chicago, Illinois. The event was part of the 2017 Pittcon conference which took place March 6–9. The CACA Young Investigator Award recognizes outstanding contributions to the development of separation science and its applications, especially in the field of chromatography.



Shuang (Jake) Yang receiving the Chinese American Chromatography Association's 2017 Young Investigator Award from Dr. Perry Wang of the US FDA, the award committee chair.

Yang received his BS degree from the Department of Applied Chemistry at Harbin Institute of Technology in China in 1994. He earned his MS degree from the Department of Materials Science and Engineering at Shanghai Jiaotong University in China, in 1997. With the grant of a full scholarship from the National University of Singapore, in 2003 Yang received a second MS degree from the

Department of Materials at that university. In 2008, working under the supervision of Prof. Don DeVoe, Yang received his PhD from the University of Maryland at College Park (UMCP). His postdoctoral training took place under DeVoe at UMCP (2008–2010) and Prof. Hui Zhang at Johns Hopkins University in Baltimore, Maryland (2010–2013). Following his postdoctoral research, Yang joined the faculty at Johns Hopkins as a research associate in the Department of Pathology.

Yang is currently a staff fellow in the Laboratory of Bacterial Polysaccharides, in the Center for Biological Evaluation and Research at the U.S. Food and Drug Administration in Silver Spring, Maryland.

Yang's major contributions to separation science include the development of a microfluidic platform for the analysis of peptides or proteins using capillary electrophoresis (CE) and liquid chromatography (LC); the integration of isoelectric focusing (IEF) with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for high-throughput analysis of intact proteins; the development of a chemoenzymatic platform for the quantitative analysis of glycans, glycosite, occupancy, intact glycopeptide; the development of an innovative 2D approach for online separation of glycans by mass spectrometry (MS); and the design and synthesis of isobaric tags for glycan quantification by LC–MS.

Yang has published 41 peer-reviewed papers in journals and has given more than 20 oral presentations at international conferences. He has been a reviewer for many journals, including *Analytical Chemistry*, *Lab on a Chip*, and *Electrophoresis*. Additionally, Yang has four U.S. patents, has contributed to three chapter books, and has obtained several awards in separation and analytical chemistry. ■

LC|GCtv NEW VIDEOS FROM LCGC



REED HARRIS ASKS: HOW CERTAIN ARE YOU THAT YOU HAVE DONE YOUR ASSESSMENT OF CRITICAL QUALITY ATTRIBUTES WELL?

Reed Harris from Genentech discusses how the uncertainty scale helps to determine the most important factors when assessing critical quality attributes.

Other recent LCGC TV interviews include:

- Paola Dugo from the University of Messina talks about her work using the same stationary phase in both dimensions of a comprehensive 2D LC separation, and how using different gradients in the two dimensions can add to the separation power.
- Susan Olesik from The Ohio State University talks about her work developing enhanced fluidity liquid chromatography and its advantages over conventional LC.

Visit www.chromatographyonline.com/lcgc to see these videos and more.

SPECIAL ISSUE HIGHLIGHTS



CURRENT TRENDS IN MASS SPECTROMETRY

Founded in 2003, *Current Trends in Mass Spectrometry*, a quarterly supplement to *LCGC*, examines developments in mass spectrometry and MS-hyphenated methods and their application to analytical problems in many fields. Included in this recent issue are articles that discuss the following topics:

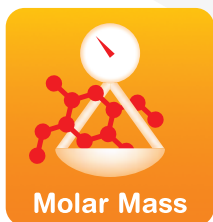
- A new peptide mapping method developed specifically for mAb characterization that employs optimal enzyme pH for robustness, but with short digestion times and time-course elements to minimize and monitor deamidation–isomerization, respectively
- A method for advanced antibody–drug conjugate structural characterization using sheathless capillary electrophoresis–tandem mass spectrometry using complementary approaches
- A review of the use of mass spectrometry in the clinical laboratory. The use of MS can lead to accurate, selective, and precise quantitative methods by detecting new classes of compounds with greater efficiency and sensitivity than is possible with older, established technologies.

Find this issue online at www.chromatographyonline.com/special-issues-03-01-2017-0

Absolute Molecular Weights and Sizes with your UHPLC...



and our μ DAWN™ MALS Detector



Molar Mass

Absolute molar masses: 200 g/mol to 1×10^7 g/mol



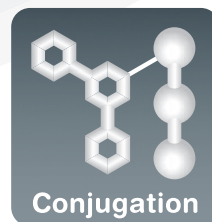
Size

RMS radii (r_g): 10 to 50 nm



Conformation

Shapes and branching ratios



Conjugation

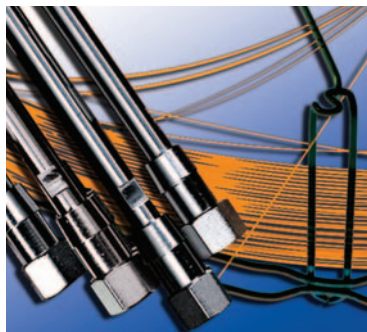
Protein conjugates, copolymers and more

The Solution is Light™

www.wyatt.com | info@wyatt.com | 805-681-9009



WYATT
TECHNOLOGY



COLUMN WATCH

New Chromatography Columns and Accessories for 2017

Our annual review of new liquid chromatography (LC) columns and accessories introduced at Pittcon and throughout the previous year.

In accordance with recent tradition, this article is intended to cover chromatography columns and accessories commercially released after Pittcon 2016 through this year's conference held in Chicago March 5–9, 2017. *LCGC* once again sent out a survey in early 2017 asking vendors to supply them with any products launched after Pittcon 2016. Instrumentation is covered in this issue of *LCGC* (1), and gas chromatography and sample preparation will be covered in the May issue. Because information for this installment of “Column Watch” is obtained sporadically over the course of many months, it is very possible that some information has been missed. Readers are encouraged to check with specific vendor sites for additional products as well as more detailed information regarding what is covered here.

The vendors that responded to the survey with high performance liquid chromatography (HPLC) and ultra-high-pressure liquid chromatography (UHPLC) columns and accessories are listed in Table I. The list contains very well-known vendors such as Waters and Agilent Technologies, but also several lesser-known vendors. The product range is also highly varied from traditional reversed-phase columns using fully porous materials to specialty columns. As in years past, with such a varied list, further discussion requires some breakdown of the different offerings. Similar to 2016, one differentiation can be made based on offerings that target small or large molecules. Within the small-molecule realm three subcategories could be established: reversed-phase, hydrophilic

interaction liquid chromatography (HILIC), and chiral offerings. Each of these will be discussed separately. For large-molecule products, subareas involving reversed-phase mode and size-exclusion chromatography are identified and discussed. Columns commercialized using ion-exchange chromatography for both small and large molecules will be discussed separately. Furthermore, there were products launched for supercritical fluid chromatography (SFC) that deserve some discussion. An interesting and potentially game-changing microchip chromatography device based on pillar array technology is also featured. Lastly, several accessories relating to HPLC are noted.

Small-Molecule Separations Reversed-Phase Chromatography

The product offerings assigned to the small-molecule, reversed-phase category are listed in Table II. A total of 22 new surface chemistries are noted from the list. Continuing recent trends, many of the new columns are intended to round out selectivity or particle size offerings within existing lines. Of the 22 columns listed, nine are manufactured using superficially porous particles (SPP), indicating that the particle architecture continues to garner attention. Enhancements of fully porous particles (FPP) are also noteworthy. For example, Phenomenex's Luna Omega phase reportedly includes a thermal modification that provides improved performance, inertness, and robustness as compared to conventional FPP products. Another theme consistent with 2016 is the continuing effort to increase column stability

David S. Bell is the editor of *Column Watch*.



Spot-On Automation

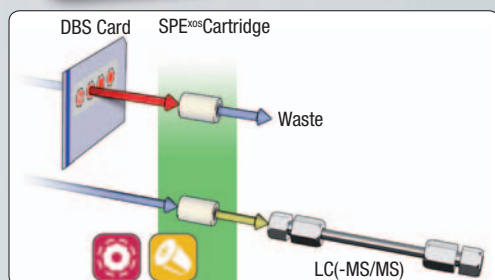
GERSTEL DBS Autosampler (DBSA) with SPE^{XOS} module (right) for direct coupling to LC-MS/MS



The GERSTEL Dried Blood Spot Autosampler (DBSA) performs efficient and highly accurate DBS desorption combined with SPE clean-up, addition of internal standard, and LC/MS analysis - or runs as an independent WorkStation (DBSA Collect) for vials or 96 well plates.

Available for Forensic Toxicology, Doping Analysis, Pharmaceutical Research, and Metabolomics, The DBS A performs:

- Reproducible desorption of up to 240 DBS cards
- Addition of internal standard (optional)
- SPE clean-up with replaceable cartridges (optional)
- 100 % analyte transfer to the LC column for low LODs
- Optical image recognition of blood spot and card ID
- WorkStation DBSA Collect for vials or 96 well plates
- Directly coupled to LC/MS under MAESTRO control



Schematic diagram of DBS flow-through desorption (FTDTM) and SPE clean-up.

***The instruments are NOT for use in diagnostic procedures.**

What can we do for you*?

for wider pH range utility. Agilent Technologies (HPH-C18), ChromaNik Technologies (SunShell C18), and Imtakt Corporation (DZ-C18) all launched phases with enhanced pH stability. Separation Methods Technologies commercialized an interesting set of short-chain reversed-phase chemistries (C1, C2, and C4). The company contends that their proprietary bonding strategy provides “proper deactivation” of silanols as well as resistance to acid hydrolysis that plagues many short-chain surface chemistries. Another interesting trend from this year’s offerings is the presence of several “polar C18” chemistries. Phenomenex launched a polar C18 stationary phase in both the Kinetex and Luna Omega lines and Waters added its T3 chemistry to the Cortec brand. In each case the surface modification is noted to impart a balanced retention for hydrophilic and hydrophobic molecules. Phenomenex also came out with a chemistry denoted as PS C18 which is reported to include a positively charged modification to the surface that aids in the retention of acidic analytes. More complete, full line offerings were launched by Agilent (InfinityLab Poroshell) and Restek (Force Performance).

Hydrophilic Interaction Chromatography

HILIC, which is often employed for the retention and separation of very polar compounds, continues to show growth. Table III lists product offerings in the HILIC

Table I: 2017 LCGC new product survey responding vendors

Company	Product
Advanced Chromatography Technologies Limited	ACE Amino columns
Advanced Materials Technology, Inc.	HALO 1000Å C4, 2.7-µm columns
Agilent Technologies	InfinityLab Poroshell 120 1.9-µm columns
	Six different chemistries: InfinityLab Poroshell 120 EC-C18 1.9-µm columns
	InfinityLab Poroshell 120 EC-C8 1.9-µm columns
	InfinityLab Poroshell 120 Phenyl-Hexyl 1.9-µm columns
	InfinityLab Poroshell 120 PFP 1.9-µm columns
	InfinityLab Poroshell 120 HPH-C18 1.9-µm columns
InfinityLab Poroshell 120 HILIC 1.9-µm columns	
Analytical Sales & Services, Inc.	MonoSLEEVE single-column heater controller
Chiral Technologies	CHIRALPAK IA-U columns
	CHIRALPAK IC-U columns
	CHIRALPAK IG columns
Chromanik Inc. Japan	SunShell C18, 2-µm columns
ES Industries	ChromegaChiral CCU columns
	ChromegaChiral CCX columns
	Epic SCX columns
	MacroSep Bio Gold C18, C8, C4, PFP, naphthyl, biphenyl, diphenyl, and HPR columns
Fortis Technologies	SpeedCore BIO C18 Peptide columns
	SpeedCore BIO Protein C18, C8, and C4 columns
Hilicon	iHILIC-Fusion(P) column
Imtakt Corporation	Dacapo DX-C18 column
Jordi Labs LLC	Jordi Resolve GPC columns
Macherey-Nagel	Nucleodur C18 Gravity-SB columns
	Nucleodur π ² columns
Microsol Technology Corporation	Cogent Amide HPLC column
Mott Corporation	PerfectPeak HPLC-UHPLC mixers
Optimize Technologies	EXP Trap columns
PharmaFluidics	µPAC chip separation device
Phenomenex	Clarity Oligo-SAX columns
	Clarity Oligo-XT C18 columns
	Kinetex Polar C18 columns
	Luna Omega C18 columns
	Luna Omega Polar C18 columns
	Luna Omega PS C18 columns
	Lux AMP columns
Lux i-Cellulose-5 columns	
Yarra 1.8u SEC-X300 columns	

HPLC Columns for Organic Acid Analysis

Over 25 years of experience providing high quality polymeric HPLC columns for the analysis of samples containing carbohydrates and organic acids.



Benson
polymeric™



bensonpolymeric.com
775.356.5755



SELECTRA® U/HPLC Columns

Reproducible, Robust & Selective

Bringing Our Superior
Phases to you
in U/HPLC Format



DA – Unique Polyaromatic Phase

C18 – Traditional Reverse Phase

Aqueous C18 – Traditional Reverse Phase with Enhanced Selectivity

C8 – Less Retentive than Standard C18 Column

PFPP – Operates in Reverse Phase, Normal Phase, or HILIC Mode

EtG – Maximum retention for polar EtG/EtS alcohol metabolites

Available in 1.8, 3, and 5 μ m particle sizes

800.385.3153 www.unitedchem.com

Table I (continued): 2017 LCGC new product survey responding vendors

Company	Product	Company	Product
PolyLC Inc.	PolyHeptyl A columns	Shodex	HK-404L columns
	PolyHexyl A columns		LW-803 columns
	PolyPentyl A columns		VC-50 2D columns
	VN-50 2D columns		
Prolab Instruments GmbH	ZircoFit UHPLC fitting system for fused-silica capillaries	Thermo Fisher Scientific	IonPac AS23 columns
Restek	Force Performance Biphenyl columns		IonPac CS20 columns
	Force Performance C18 columns	Vici	VICI Jour JR-BPR3 back-pressure regulator
	Force Performance Fluorophenyl columns	Waters	CORTECS Shield RP18 columns
SMT C1B-TE - Specialty Teicoplanin columns	CORTECS T3 columns		
SMT MEB C1, C2 and C4 columns	Torus SFC columns		
Separation Methods Technologies	SMT PAH1 columns	Welch Materials Inc.	Ghost-Buster column
		YMC Co. Ltd.	YMC-Pack Diol SEC columns

category. The seven new entries in this category match the number of new columns reported in 2016. It is interesting to note that three of the seven entries are built on polymeric phases. HILICON introduced a polymeric version in its iHILIC-Fusion line. Shodex commercialized

two polymerically based HILIC phases. The VN-50 2D diol phase is designed for oligosaccharide analysis, and the VC-50 2D carboxyl chemistry is reportedly applicable to monoamine type neurotransmitters and oral anti-diabetic drugs. The company also notes that the PEEK

Table II: Reversed-phase columns for small-molecule separations

Company	Product Name	Stationary Phases	Particle Sizes (μm)	Particle Type
Agilent Technologies	InfinityLab Poroshell 120	EC-C18, EC-C8, phenyl-hexyl, PFP, HPH-C18	1.9	SPP
ChromaNik Technologies Inc	SunShell	C18	2	SPP
Imtakt Corporation	Dacapo	DX-C18	2.5	FPP
Macherey-Nagel GmbH & Co.	Nucleodur	C18 Gravity-SB	1.8, 3, and 5	FPP
Macherey-Nagel GmbH & Co.	Nucleodur	π 2	5	FPP
Phenomenex	Kinetex	Polar C18	2.6	SPP
	Luna Omega	PS C18, polar C18	1.6, 3, and 5	FPP
	Luna Omega	C18	1.6	FPP
Restek Corporation	Force Performance LC Columns	C18, biphenyl, and fluorophenyl	1.8, 3, and 5	FPP
Separation Methods Technologies	SMT PAH1	Proprietary	1.7, 3, 5, and 10	FPP
Separation Methods Technologies	SMT-MEB	C1, C2, and C4	1.7, 3, 5, and 10	FPP
Waters	CORTECS	Shield RP18, T3	1.6, 2.7	SPP

housing reduces undesired interactions with the column hardware. MicroSolv Technology continues to fill out its line of silica hydride based phases with a new amide chemistry. Fully porous— and superficially porous—based HILIC phases continue to be promoted. Advanced Chromatography Technologies added an amino chemistry to its FPP ACE offering, and both Agilent Technologies (bare silica) and ChromaNik Technologies (amide) added to their respective SPP lines.

Chiral Chromatography

Eight new chiral phases were introduced after Pittcon 2016. This number is a significant increase over the same time period from Pittcon 2015 through the “New Chromatography Columns and Accessories for 2016” column installment where only three

entries were noted (2). Cellulose- and amylose-based chiral phases dominate the list provided in Table IV. Chiral Technologies extended its line of meta-immobilized 5- μm phases through the introduction of Chiralpak IG. The company also introduced amylosic and cellulosic phases on 2- μm FPP particles designed for UHPLC. ES Industries continued to fill out its ChromegaChiral line with two new chemical modifications of amylose that provide what the company refers to as unique enantiomeric separation behavior. Phenomenex introduced an immobilized cellulosic phase, Lux i-Cellulose-5, which is designed to be more robust for strong organic solvents than coated polysaccharide phases. The company also launched the Lux-AMP phase. The proprietary chemistry is specifically designed for the enan-

tiomeric separation of amphetamine and methamphetamine. Separation Methods Technologies reported the development of a teicoplanin-based chiral stationary phase. The company reports that the column is useful for performing a wide variety of chiral separations with amino acids, carboxylic acids and neutral compounds. Like 2016, it is clear from these new product introductions that amylose and cellulose phases based on FPP particles continue to dominate the field. New this year is the introduction of sub-2- μm chiral columns (Chiral Technologies); however, SPP technologies have still not entered this realm.

Large-Molecule Separations

The separation of large molecules such as peptides, proteins, and oligonucleotides often requires larger pore

Dimensions (mm)	Comments
50 × 2.1 to 150 × 3.0	Robust superficially porous 1.9- μm particles and optimized column loading give manageable pressure, which allow columns to be run at optimum linear velocities or the use of longer columns without exceeding column or instrument pressure rating. This feature leads to faster run times or improved resolution. Benefits are decreased costs from higher throughput or improved data quality and decreased costs from reduced re-work. This particle and loading technology also provides long column lifetimes under UHPLC conditions. Benefits are decreased costs because of fewer column purchases, reduced workflow disruption, and less re-work. For the HPH-C18 phase, an organic compound is integrated into the porous layer during particle manufacture to give stability at extremes of pH.
50 × 2.1 to 150 × 2.1	Stable over a pH range from 1 to 10. More than 300,000 plates per meter. Symmetrical peak shape for basic, acidic and metal chelating compounds.
Not specified	Hybrid silica and polymeric particle dual-matrix technology provides high pH stability
30 × 2 to 250 × 4.6	Hydrophobic C18 phase with distinct polar selectivity for improved resolution of early eluted compounds.
50 × 2 to 250 × 4.6	Hydrophobic phase with alternative selectivity compared to classical C18 modifications. The biphenylpropyl ligand provides π - π and hydrophobic interactions.
Common analytical lengths for 2.1, 3.0, and 4.6 mm i.d.	Balanced retention of polar and nonpolar compounds under reversed-phase or 100% aqueous conditions.
1 mm to 2.1 mm i.d. (1.6 μm), 2.1 mm to 4.6 mm i.d. (3 and 5 μm) common analytical lengths. Preparative dimensions also available	Polar C18: Balanced retention of nonpolar compounds alongside enhanced retention of polar compounds under reversed phase or 100% aqueous conditions. PS C18: Positively charged ligands on surface aid in the retention of acidic compounds through ionic interactions, while the C18 ligand promotes general reversed-phase retention.
1 mm to 2.1 mm i.d. common analytical lengths	Thermally modified fully porous particles provide greater performance, inertness, robustness, and performance compared to conventional fully porous products.
30 × 2.1 to 100 × 3.0 (1.8 μm), 30 × 2.1 to 150 × 4.6 (3 μm), and 50 × 2.1 to 250 × 4.6 (5 μm)	Fully scalable from 1.8 μm to 3 and 5 μm for easy method transfer. Long-lasting and reproducible even under the stress of elevated UHPLC pressures and rapid cycling.
50 × 2.1 to 300 × 50	For the separation of structurally similar isomers of polyaromatic hydrocarbons (PAHs)
50 × 2.1 to 300 × 50	Columns with short carbon chains for rapid analysis of basic, neutral, and mildly acidic drugs and small molecules and biomolecules. These columns provide excellent baseline resolution for small basic molecules, including therapeutic drugs. The bonding scheme ensures proper deactivation substrate silanols and resistance to acid hydrolysis.
30 × 2.1 to 150 × 3.0 (1.6 μm) and 30 × 2.1 to 150 × 4.6 (2.7 μm)	Shield RP18: The embedded polar group helps to “shield” residual surface silanols, reducing peak tailing for basic analytes and providing alternative selectivity for polyphenolic compounds such as flavanoids. T3: 100% aqueous compatible reversed-phase column designed to give balanced retention to both polar and nonpolar compounds

sizes than are typically used in small molecule applications. Full characterization of these complex molecules often requires multiple modes of separation, including reversed-phase chromatography, size-exclusion chromatography (SEC), ion-exchange chromatography, and hydrophobic interaction chromatography (HIC).

Reversed-Phase Chromatography

In last year's report, columns intended for reversed-phase analysis of large molecules was dominated by chemical modification of SPP particles. That trend continues this year. However, a new line of columns from ES

Industries (MacroSep BIO-Gold) demonstrates that new lines based on fully porous particles are still being developed. New columns intended for reversed-phase chromatography of biomolecules are presented in Table V. Also included in the table is a new set of offerings from PolyLC, Inc. for HIC.

Advanced Materials Technologies introduced a 1000-Å C4 column intended for very large molecules such as monoclonal antibodies (mAbs). The larger pore size as compared to many "large-molecule" columns (typically 160–400 Å) provides less restriction into the pores and thus

can provide higher resolution of minor variants of mAbs. The column is based on SPP technology. Fortis Technologies reports the expansion of its SPP portfolio with SpeedCore BIO Peptide (160 Å) and SpeedCore BIO Protein (300 Å) columns. Also based on wider pore SPP technology, these offerings are intended for improved separations of peptides and proteins, respectively. Oligonucleotides also fall into this category of large molecules and offer their own set of chromatographic challenges. Phenomenex recently launched Clarity Oligo-XT, an organo-silica SPP phase intended for the reversed-phase characterization

Table III: HILIC columns for small-molecule separations

Company	Product Name	Stationary Phases	Particle Sizes (µm)	Particle Type	
Advanced Chromatography Technologies	ACE Amino	Amino	1.7, 3, and 5	FPP	
Agilent Technologies	InfinityLab Poroshell 120 HILIC	Unbonded	1.9	SPP	
HILICON AB	iHILIC-Fusion(P)	Amide, phosphate, quaternary ammonium	5	Polymeric	
MicroSolv Technology Corporation	Cogent Amide	Amide	4	Silica hydride	
Shodex	VN-50 2D	Diol	5	Polymeric	
	VC-50 2D	Carboxyl	5	Polymeric	

Table IV: Chiral columns for small-molecule separations

Company	Product Name	Stationary Phases	Particle Sizes (µm)	Particle Type	
Chiral Technologies, Inc. (Daicel Corporation)	Chiralpak IG	Amylose tris(3-chloro-5-methylphenylcarbamate)	5	FPP	
	Chiralpak IA-U and Chiralpak IC-U	Amylose tris(3-chloro-5-methylphenylcarbamate) and Cellulose tris(3-chloro-5-methylphenylcarbamate)	<2	FPP	
ES Industries	ChromegaChiral CCU	Methylbenzylcarbamate and 3-chloro-4-methylphenylcarbamate-amylose	3, 5, 10, and 20	FPP	
	ChromegaChiral CCX	Methylbenzylcarbamate and 3,5-dimethylphenylcarbamate-amylose	3, 5, 10, and 21	FPP	
Phenomenex	Lux AMP	Proprietary	3	FPP	
	Lux i-Cellulose-5	Cellulose tris(3,5-dichlorophenylcarbamate)	3, 5	FPP	
Separation Methods Technologies, Inc.	SMT CIB-TE	Teicoplanin	5	FPP	

and purification of synthetic oligonucleotides. The column is reported to be stable at high pH, which is often used in oligonucleotide separations.

HIC likely deserves its own category within the large-molecule realm. However, for the sake of space it is lumped in here with the reversed-phase offerings. HIC is often a valuable tool used to separate polar variants of proteins. HIC, however, traditionally uses high concentrations of nonvolatile buffers, rendering it incompatible with mass spectrometry (MS) detection. A presentation by Andrew Alpert of PolyLC, Inc., at HPLC 2016 described efforts to

render HIC MS friendly (3). Using more-hydrophobic analogs of a poly(propyl aspartamide) stationary phase, Alpert demonstrated that proteins could be denatured in MS-friendly(er) mobile phases that may lead to a possible marriage of the two techniques (4). Since that time, PolyLC, Inc., has launched three versions of the polyaspartamide phases discussed at HPLC 2016 (pentyl, hexyl, and heptyl) that permit MS-compatible HIC for proteomics and quality control of biotherapeutics.

Size-Exclusion Chromatography–Gel Permeation Chromatography

SEC continues to witness resurgence largely as a result of the focus on large-molecule therapeutics. SEC resolves analytes based on molecular size and is often used as a complementary technique to reversed-phase analysis of protein. Gel permeation chromatography (GPC) uses the same process of separation by molecular size. Separation by molecular size in aqueous mobile phases is commonly referred to as SEC, whereas separation in organic solvents such as tetrahydrofuran is normally referred to as GPC. Columns used in SEC and GPC are often characterized by strict control of pore size and by inert surface chemistry. Control

Dimensions (mm)	Comments
Not disclosed	Highly reproducible and ultrainert amino phase for reversed-phase, normal-phase, weak anion-exchange, and HILIC modes. Proprietary bonding technology provides robustness with excellent column lifetimes.
50 × 2.1 to 150 × 3.0	Robust superficially porous 1.9- μ m particles and optimized column loading.
20 × 2.1 to 250 × 4.6	Unique selectivity from enhanced hydrophilic partitioning, weak electrostatic interaction, and hydrogen bonding capacity; HILIC applications at high pH.
100 × 2.1 plus additional dimensions	Stability of column with greater column lifetime and unique selectivity.
150 × 2.0	Diol-functionalized PVA-based HILIC column designed for oligosaccharide analyses.
150 × 2.0	Packed in PEEK, this column is designed for monoamine type neurotransmitters and oral anti-diabetic drugs. The polymer column housing decreases interactions between compounds and column hardware.

Dimensions (mm)	Comments
100 × 4.6 to 250 × 10	Meta immobilized columns offer mechanistic advantages in chiral recognition, higher success rates using fewer columns. The columns are designed for robustness and extended column life.
50 × 3.0 and 100 × 3.0	These columns are designed to be used with UHPLC systems to achieve ultrafast and high-resolution separation of enantiomers.
Capillary to preparative	Packed in PEEK, this column is especially designed for monoamine type neurotransmitters and oral anti-diabetic drugs. The polymer column housing decreases interactions between compounds and column hardware.
Capillary to preparative	
Common analytical lengths for 3.0- and 4.6-mm i.d. columns	Designed and tested for chiral separation of amphetamine and methamphetamine enantiomers.
Common analytical lengths for 2.1-, 3.0-, and 4.6-mm i.d. columns; preparative dimensions available	Immobilized portion of this new chiral stationary phase greatly increases column robustness by tolerating strong organic solvents such as DMSO, DCM, ethyl acetate, MTBE, and tetrahydrofuran to be injected onto the column.
50 × 2.1 to 300 × 22.1	SMT CIB-TE column is made through bonding of the antibiotic, teicoplanin with 1,6-diisocyanatohexane, using SAM Total Coverage technology, both patented and proprietary. This covalently bonded teicoplanin column is extremely useful for performing a wide variety of chiral separations with amino acids, carboxylic acids, and neutral compounds.

Table V: Reversed-phase separations for large-molecule separations

Company	Product Name	Stationary Phases	Particle Sizes (μm)	Particle Type	
Advanced Materials Technology	HALO 1000Å C4	C4	2.7	SPP	
ES Industries	MacroSep BIO-Gold HPLC Columns	C18, C8, C4, PFP, naphthyl, biphenyl, diphenyl, HPR	1.9, 3, 5, and 10	FPP	
Fortis Technologies	SpeedCore BIO Peptide	C18	2.6	SPP	
	SpeedCore BIO Protein	C18, C8, C4	3.5	SPP	
Phenomenex	Clarity Oligo-XT	C18	1.7, 2.6, and 5	SPP	
PolyLC, Inc.	PolyPentyl A, PolyHexyl A and PolyHeptyl A	Polypentylaspartamide, polyhexylaspartamide, and polyheptylaspartamide	2, 3, and 5	FPP	

Table VI: SEC columns for large-molecule separations

Company	Product Name	Stationary Phases	Particle Sizes (μm)	Particle Type	
Jordi Labs LLC	Jordi Resolve GPC columns	Divinylbenzene	5 and 13	Monodisperse polymeric	
Phenomenex	Yarra 1.8u SEC-X300	Proprietary	1.8	FPP	
Shodex	HK-404L	Styrene-divinylbenzene	3.5	Polymeric	
Shodex	LW-803	Not disclosed	3	FPP	
YMC Co. Ltd.	YMC-Pack Diol SEC	Dihydroxypropyl	2	FPP	

Table VII: Strong cation-exchange columns for large-molecule separations

Company	Product Name	Stationary Phases	Particle Sizes (μm)	Particle Type	
ES Industries	Epic SCX	Aromatic sulfonic acid	1.8, 3, 5 and 10	FPP	
Phenomenex	Clarity Oligo-SAX	Quaternary amine	5	Nonporous	
Thermo Fisher Scientific	IonPac AS23-4 μm	Not disclosed	4	Not disclosed	
	IonPac CS20	Not disclosed	Not disclosed	Not disclosed	

of the pore size dictates how molecules of various sizes can diffuse into the pores while the inertness of the surface limits secondary interactions that may interfere with molecular size separation and determination.

Table VI lists the SEC and GPC column offerings launched since

Pittcon 2016. The number of new offerings is similar to those reported last year. Jordi Labs released a divinylbenzene GPC phase in both 3- and 13- μm particle sizes that promises high efficiency, high capacity, and lower back pressure. Shodex continued to expand its offering with

the release of two new chemistries on polymeric supports. The HK-404L phase is intended for rapid GPC analysis, and the LW-803 product is designed for antibody and antibody aggregate separations. Smaller-particle designs continue to infiltrate the SEC-GPC market in an attempt

Dimensions (mm)	Comments
20 × 2.1 to 250 × 4.6	Higher resolution of minor variants of mAbs and narrower peak widths by 12–40% compared to current products.
50 × 2.1 to 300 × 4.6	MacroSep BIO-Gold packings are based on ultrahigh-purity spherical silica, state-of-the-art high density bonding, and full endcapping intended for the separation of high-molecular-weight compounds such as proteins and peptides.
Not disclosed	An addition to the SpeedCore line of columns intended for peptide analysis.
Not disclosed	Additional phases to the SpeedCore line of columns intended for protein analysis.
Common lengths with 2.1 mm i.d. (1.7 μm), common lengths for 2.1 and 4.6 mm i.d. (2.6 and 5 μm); semipreparative and preparative dimensions also available	Designed to provide rugged high performance and high pH stability for the LC–MS characterization of synthetic DNA and RNA samples as well as purification of these target oligos.
Capillary to process scale	These materials are unique in permitting HIC of proteins in native structure with volatile (MS-compatible) mobile phases for top-down proteomics and quality control.

Dimensions (mm)	Comments
300 × 7.8	High efficiency, high separation capacity, and lower back pressure with greater bed stability.
Common analytical lengths for 4.6 mm	For separation and characterization of mAb aggregates, antibody–drug conjugates (ADCs) and biosimilars. Biomolecule separation range 10–700 kDa complements existing Yarra 1.8 μm SEC-X150.
300 × 8	Monodisperse styrene–divinylbenzene particles create an environment for rapid analyses on both traditional GPC systems as well as micro-GPC and UHPLC (APC) systems. The HK-404L is a mixed-bed linear type column for separations ranging from 100–1,000,000 Da. No need to purchase expensive APC XT columns.
300 × 8	LW-800 series is specially designed for antibody and antibody aggregates separations. The ultralow shed makes this series ideal for multiangle light scattering systems.
150 × 4.6 and 300 × 4.6	The 2-μm particle exhibits greater resolution than standard 3- and 5-μm materials, which allows smaller diameter and shorter length columns to be used. This increases throughput while decreasing mobile-phase consumption.

Dimensions (mm)	Comments
Not disclosed	Epic SCX uses a high density bonding technology that provides for superior cation loading and robustness.
Common analytical lengths for 4.6 mm i.d.	Innovative high capacity strong anion-exchange column delivers high-resolution analysis of synthetic oligonucleotides.
Not disclosed	Advanced capabilities to detect oxyhalides and common inorganic anions in drinking water, groundwater, wastewater, and other diverse sample matrices.
Not disclosed	Designed to offer choice performance for the analysis of inorganic cations and amines. The columns use gradient ion chromatography to offer high selectivity for detecting a variety of alkyl and alkanol amines.

to enhance resolution and speed. Phenomenex extended its line of sub-2-μm SEC columns with the release of the Yarra 1.8 μm SEC-X300 column. The column's 300-Å pore size is intended for the characterization of mAb aggregates, antibody–drug conjugates (ADCs) and biosimilars with

a separation range of 10–700 kDa. Similarly, YMC Co. Ltd. launched a 2-μm diol phase that reportedly exhibits greater resolution than standard 3- and 5-μm materials.

Ion-Exchange Chromatography
Ion-exchange chromatography is a

separation technique that exploits strong interactions between opposite charges of a surface and an analyte. Various surface supports may be modified to carry permanent (strong cation and anion exchange) or variable (weak cation and anion exchange) charge that can be used

Table VIII: Accessories for liquid chromatography

Company	Product Name	Comments
Analytical Sales and Service, Inc.	MonoSLEEVE	MonoSLEEVE is the next generation of the original HotSleeve controller. With its new advanced "plug-and-play" technology, the controller can accommodate single HotSleeve Plus or AgileSleeve Plus column heaters of different sizes without having to recalibrate to each individual sleeve.
Mott Corporation	PerfectPeak HPLC–UHPLC mixers	HPLC–UHPLC mixers are designed to provide better sensitivity by greatly reducing baseline noise and maximizing lower limits of detection. The mixers are pressure rated up to 20,000 psig and reportedly maintain high efficiency over a wide range of flows.
Optimize Technologies, Inc.	EXP Trap columns	The EXP Trap column system enables chemists to quickly remove detergents or salts that can affect the ionization process in MS work. This trapping technique can concentrate the sample directly on-line thus allowing for increased recovery of precious sample material compared to off-line techniques. On-line trapping readily lends itself to automation for high-throughput analysis in UHPLC–MS applications. The company's Free-Turn architecture allows users to change cartridges by hand without breaking fluid connections on the holder inlet–outlet.
Prolab Instruments GmbH	Zircofit	Zircofit is a novel capillary fittings system that allows users to assemble their custom-length or custom-volume connections within minutes. Using a simple technique, a special tool presses the composite sleeves onto the bare ends of fused-silica capillaries with 10–75 μm i.d. They then fit into 1/16-in. HPLC ports, achieving a pressure rating of 20,000 psi (patent pending).
VICI AG International	VICI Jour JR-BPR3 back-pressure regulator	As an extension in the line of the VICI Jour back-pressure regulators, the new JR-BPR3 offers an extended application range. Pressures from 90 to 300 bar (1300–4300 psi) are easily manually adjustable. The stainless steel made body and the inert membrane enables the usage of any HPLC mobile phase and of supercritical carbon dioxide. The back-pressure regulator can also be mounted into any column oven that maintains temperatures up to 80°C. Additionally an installation using a 17-mm panel hole is possible.
Welch Materials, Inc.	Ghost-Buster column	This column is designed to be installed between the gradient mixer and the sample injector for removal of impurities in the mobile phase that contribute to ghost peaks. Without any contact with the sample and an easy installation, the column provides superior performance in removing ghost peaks from the baseline, leading to significantly enhanced resolution and minimal impact to established chromatography methods. Because of these unique features, the Ghost-Buster column can be easily adopted in a broad spectrum of application methods.

to interact with and separate analytes with the opposite charge. As with SEC, ion-exchange chromatography is often used as a complementary tool for the analysis of large molecules. As shown in Table VII, however, the product launches this year are dominated by columns intended for small-molecule analysis. ES Industries released the Epic SCX column, which the company claims provides superior cation loading and stability that results from a high density bonding technology. Thermo Fisher Scientific also released two new chemistries in its IonPac line. According to the company, the AS23 phase provides advanced capabilities to detect oxyhalides among other inorganic anions in various water matrices of interest and the CS20 product provides enhanced performance for the analysis of inorganic cations and amines. The lone "large-molecule" ion-exchange product release came from Phenomenex. The Clarity

Oligo-SAX phase is interestingly built on a nonporous, 5- μm particle and is intended for the high-resolution analysis of synthetic oligonucleotides.

Supercritical Fluid Chromatography

SFC continues to receive interest because of improvements in instrument design, the desire to perform greener separations, and the high resolution and speed the technique often provides. The lone line of new SFC phases reported since Pittcon 2016 comes from Waters Corporation. Several surface modifications, including 2-picolamine, diethylamine, diol, and 1-aminoanthracene built on Water's bridged ethyl hybrid (BEH) particle technology were released under the brand name Torus. The company claims that the particle is designed especially for SFC and is enhanced by a two-stage bonding process that provides stable retention and unique selectivity.

Pillar-Array Separation Device

An interesting and potentially game-changing separation device was featured at Pittcon 2017. PharmaFluidics launched the first microchip chromatography device that is manufactured using lithographic techniques. The device features a perfectly ordered backbone that is formed by etching interstitial volume out of a silicon wafer. The company reports that the device produces highly reliable and robust reversed-phase, nano-LC separations with unprecedented peak capacity. It is certainly possible that this development may provide a revolutionary advance for liquid chromatography.

Liquid Chromatography Accessories

Although most of the attention in this series of column installments goes to the development of new columns, accessories play a vital role in enabling liquid separations. Table VIII lists several LC-related

The FFF - MALS Platform

Next Level Nano, Bio and Polymer Analysis



POSTNOVA

**NEW
With SEC Option!**



NovaFFF Software

MALS	DLS	UV	RI	ICP-MS

Contact us for more information: www.postnova.com

accessories that have been recently developed. Analytical Sales and Service, Inc., reported the release of the MonoSLEEVE column heater device, which features advanced “plug-and-play” technology. Mott Corporation launched HPLC–UHPLC static mixers intended to supply better sensitivity by greatly reducing baseline noise and improving lower limits of detection. According to the company, the mixers are easy to install, are pressure rated up to 20,000 psi, and can maintain high efficiency over a wide range of flow rates. Optimize Technologies released a series of trap columns under the brand name EXP. On-line trapping facilitates automation for high-throughput analysis and can be used for multidimensional chromatography processes. High-pressure capillary connections often frustrate the practitioner. ProLab Instruments has developed a novel capillary fittings system called Zircofit that is designed to allow users to assemble

connections within minutes. The company reports the achievement of 20,000-psi pressure ratings with this design. VICI AG International launched a new back-pressure regulator, JR-BPR3, which reportedly extends the application range of the company’s existing line. The company claims the new regulator is manually adjustable between 90 bar and 300 bar and can be mounted into any column oven that maintains temperatures up to 80 °C. Lastly, ghost peaks in gradient LC can not only be a nuisance, but may also interfere with detection and quantitation of target analytes. The source of ghost peaks is often impurities in solvents used in mobile phases. Welch Materials has developed what it calls the Ghost-Buster column to help alleviate the issue. The column, packed with proprietary material, is installed between the gradient mixer and the sample injector and is intended to remove contaminants from the mobile phase and

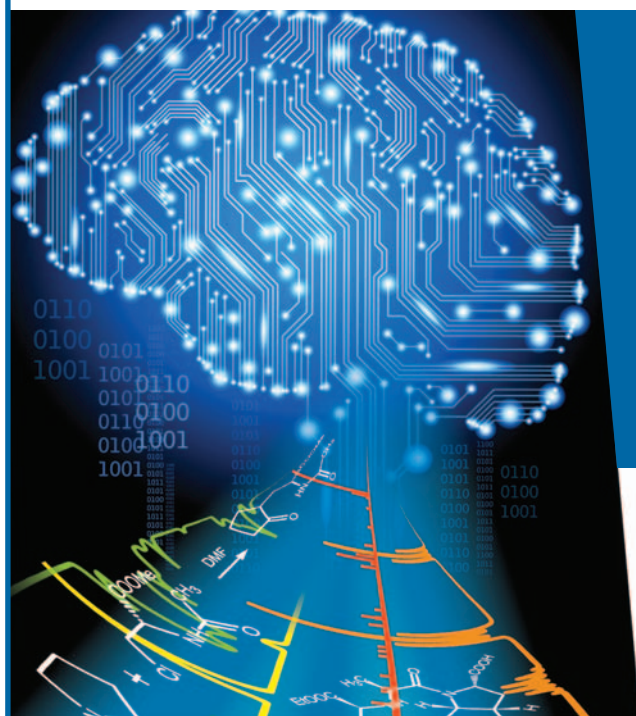
thus reduce or eliminate undesired responses in gradient analyses.

Conclusions

Overall, the products released over the past year represent evolutionary developments. The greatest number of new columns falls into the category of line extensions or “gap-filling” new product lines for individual companies based on existing accepted technologies. New known chemical modifications of both SPPs and FPPs of various particle sizes continue to be developed, and these modern particles continue to find utility in niche separation modes such as HILIC and chiral. Several reported treatments of FPP surfaces may lead to more revolutionary products, and several new chemistries, especially hydrophobic–hydrophilic balanced phases, may offer unique and powerful selectivities.

As opposed to last year, there seems to be less emphasis on large-molecule separations in terms of the number of phases that have been launched.

From Data to Intelligence



Unify chromatographic, spectral, and chemical data for greater efficiency and productivity.

Capture, search, and access interpreted data to gain insights.

Share knowledge and collaborate more effectively.

ACD/Spectrus Platform

Let Your Data Live On



ACD/Labs

www.acdlabs.com/LetYourDataLiveOn

That said, several developments in large-molecule separations could play a key role in the future of HPLC. An emphasis on even larger-pore-size particles may, for example, enable improved analysis of mAbs and other biotherapeutics. Techniques complementary to reversed-phase chromatography such as SEC-GPC and ion-exchange chromatography continue to grow at a steady pace. It is noteworthy, however, that no affinity chromatography columns were reported to be released over the past year. Is it possible that the emphasis on large-molecule separations is slowing?

The commercialization of pillar-array nano-LC devices may be the most revolutionary development over the past year. It will be interesting to see if the device leads us to more widespread acceptance of chip-based separations.

Acknowledgment

Product reviews would not be possible without the contributions and cooperation of the manufacturers

that have responded to the *LCGC* survey. Their effort is greatly appreciated. Although *LCGC* has made every attempt to include every submission in the series of review articles, it is possible that some have been missed. If there have been omissions or if you want to be sure to be included in the 2018 review series, please contact Laura Bush, Editorial Director, *LCGC* North America, at laura.bush@ubm.com.

References

- (1) M.W. Dong, *LCGC North Am.* **35**(4), 246–257 (2017).
- (2) A. Alpert, *A Series of New Materials for Direct HIC-MS Analysis of Proteins in Top-Down Proteomics*, presented at the 44th International Symposium of High performance Liquid Phase Separations and Related Techniques (HPLC 2016), San Francisco, California, 2016.
- (3) D.S. Bell, *LCGC North Am.* **34**(9), 700–709 (2016).
- (4) D.S. Bell, *LCGC North Am.* **34**(4), 242–252 (2016).

David S. Bell is senior manager of separations technologies and workflow development at MilliporeSigma (formerly Sigma-Aldrich/Supelco). With a B.S. degree from SUNY Plattsburgh and a PhD in



Analytical Chemistry from The Pennsylvania State University, Dave spent the first decade of his career within the pharmaceutical industry performing analytical method development using various forms of chromatography and electrophoresis. During the past 15 years, working directly in the chromatography industry, Dave has focused his efforts on the design, development, and application of stationary phases for use in HPLC and hyphenated techniques. In his current role at MilliporeSigma, Dr. Bell's main focus has been to research, publish, and present on the topic of molecular interactions that contribute to retention and selectivity in an array of chromatographic processes. Direct correspondence to: dave.bell@sial.com

For more information on this topic, please visit www.chromatographyonline.com/majors

PUT YOUR
LCMS
ON A
LOW SALT
DIET

Learn More at
Cogent-HPLC.com

Higher Yield in Prep

Less Salt Required for Polars

MANUFACTURED BY:

MICROSOLV
TECHNOLOGY CORPORATION

COGENT
HPLC Columns™



LC TROUBLESHOOTING

Count the Cost, Part II: Increasing Resolution by Increasing Retention

We'll learn how to find the "sweet spot" in terms of retention for a liquid chromatography separation as well as how much retention change can be expected for a selected change in mobile-phase percent organic or column temperature.

This is the second installment in a series about how to use chromatography fundamentals to estimate the impact of various parameter changes on liquid chromatography (LC) separations. In the first discussion (1) we looked at the influence of the column plate number (N) and saw that for most situations, starting method development with a 10,000-plate column makes the most sense. This is a good compromise between separation power, pressure, and run time. The effect of further changes in column length (L) or particle diameter (d_p) can be easily estimated without doing the actual experiments.

This month, we'll continue the discussion with an emphasis on the influence of the retention factor (k) on the separation, expressed as resolution (R_s). We'll consider the general case, specific cases, and how to estimate the influence of a change in mobile-phase percent organic (%B) or temperature on retention. As with the prior installment of this series, we'll limit ourselves to isocratic separations and reversed-phase conditions to simplify the discussion, but much of this installment will apply to gradients and other separation modes.

The Influence of Retention on Resolution

Once again, we'll use the fundamental resolution equation to guide the discussion:

$$R_s = \frac{1}{4} N^{0.5} (\alpha - 1) (k / [1 + k]) \quad [1]$$

(i) (ii) (iii)

where α is the selectivity between two peaks with k -values of k_1 and k_2 :

$$\alpha = k_2 / k_1 \quad [2]$$

Although we could use retention time (t_R), it is usually simpler to use the retention

factor in discussions like the present one. Recall that the retention factor is calculated from t_R and from the column dead time (t_0 or t_M) as:

$$k = (t_R - t_0) / t_0 \quad [3]$$

From equation 1, we can see that resolution is a function of $k/(1+k)$ (term *iii*). Thus, R_s will increase with k , but not linearly. For example, if $k = 1$, $k/(1+k) = 0.5$, whereas if $k = 100$, $k/(1+k) \approx 1.0$. I find that this relationship is easier to understand in a graphic representation, such as in Figure 1, where retention, expressed as k , is plotted against resolution, expressed as $k/(1+k)$. Initially resolution increases rapidly with increases in k , then flattens out as k exceeds ~ 5 .

Target Ranges for k


The relationship between k and R_s shown in Figure 1 can help us to decide in advance what is the desired range of k -values we would like to aim for in a separation. Let's look at three different aspects of this: the improvement in R_s we get for some increase in k , the cost of an increase in k to increase R_s , and the stability of the separation as it relates to k .

The Advantages of Increased k -Values

The plot of Figure 1 shows us that for small values of k , R_s increases rapidly with increased k . For example, a change of k from 0.5 to 1.0 (black dots in Figure 1) increases the relative resolution by 50% from 0.35 to 0.53. A further doubling of k from 1.0 to 2.0 gives only a 33% increase in resolution. A fivefold increase of k from 2 to 10 increases resolution by another third, but a change of k from 10 to 20 only changes resolution by 5%. This tells us that if we want to use k as a lever to improve R_s , it will be most effective at small values of k .

John Dolan and **Dwight Stoll** are the coeditors of *LC Troubleshooting*, and Dolan is the author of this installment.

The heart of every autosampler



Your sample depends on it

More than 50 years of precision liquid handling experience. Proven accuracy. Continuous research and development. Choose an autosampler syringe from Hamilton Company and be confident in your results.

Hamilton offers a line of syringes designed to work with a wide range of the most popular autosamplers from Agilent, CTC PAL, Spark Holland and more. Each syringe is expertly handcrafted to maximize sample integrity, process efficiency and new long-life syringe technology.

For more information, visit

www.ham-info.com/0804-1

or call toll free **1-888-525-2123**.

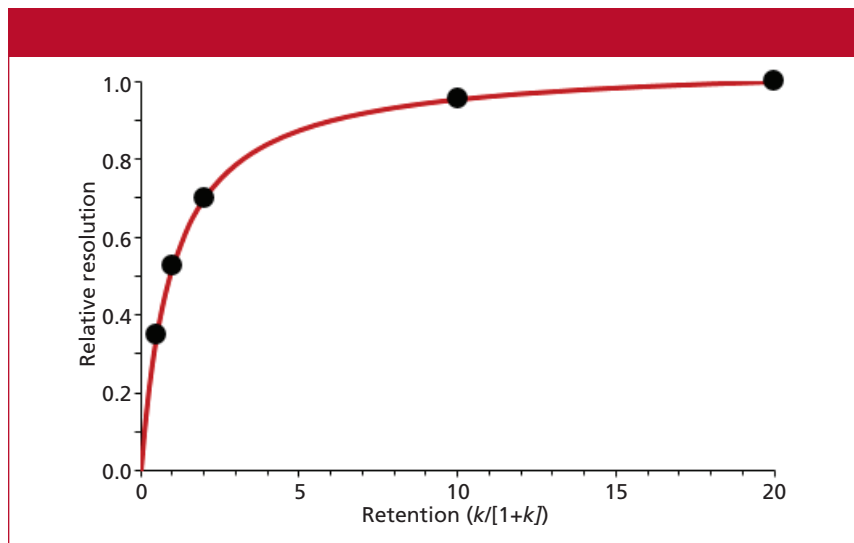


Figure 1: Plot of retention factor versus resolution (expressed as $k/[1+k]$) based on equation 1. Black dots represent results for $k = 0.5, 1, 2, 10,$ and 20 .

The Cost of Increased k -Values

Another way to look at the data of Figure 1 is to consider how much it costs for an increase in k and compare this to the gain in resolution we might get for that cost. Because most LC methods are run in an automated mode, the cost of analysis time

usually is not significant as long as a batch of samples can be run in a reasonable time, such as a 14-h (840-min) overnight run. As an example, let's consider a method run on a 150 mm \times 4.6 mm column at 2.0 mL/min and a sample batch size (comprising both samples and calibration standards) of 100 injections. Under these conditions, $t_0 \approx$

0.75 min; we can rearrange equation 3 and solve for retention time:

$$t_R = t_0(1 + k) \quad [4]$$

If we use the same examples as above with $k = 0.5, 1, 2, 10,$ and 20 (black dots on Figure 1), we can use equation 4 to calculate retention times of 1.3, 1.5, 2.3, 8.3, and 16 min, respectively. (I've rounded my numbers here, so your results may vary a bit if you repeat these calculations.) These translate to batch run times 100 times larger, of 113, 150, 225, 825, and 1575 min. You can see that methods where the last peak is eluted with $k > 10$ would exceed the allotted 14-h window.

Another potential cost of adjusting k has to do with detection limits for a method. Longer retention times mean broader peaks (peak width is directly proportional to retention time under isocratic conditions). If peak area is assumed constant, peak height will be inversely proportional to peak width (or retention time). This relationship means that doubling retention, as when k is increased from 10 to 20, will halve the peak height. For trace analysis,

DAISOGEL Introducing the NEW 5 μ m Semi-PREP Column

Simplified Scale-up from mg to grams

The world's largest HPLC silica gel manufacturer proudly introduces the new 5 μ m Semi-PREP column.

- Higher resolution than previous 10 μ m series.
- Full lineup of DAISOGEL's cutting edge modification technology.
- Optimized and reproducible column packing, individually tested.
- Available ID 10, 20 & 30 mm and lengths of 50, 100 & 150 mm.

Visit our web site today
www.daisogelUSA.com



DAISO Fine Chem USA, Inc.

+1-310.540.5312

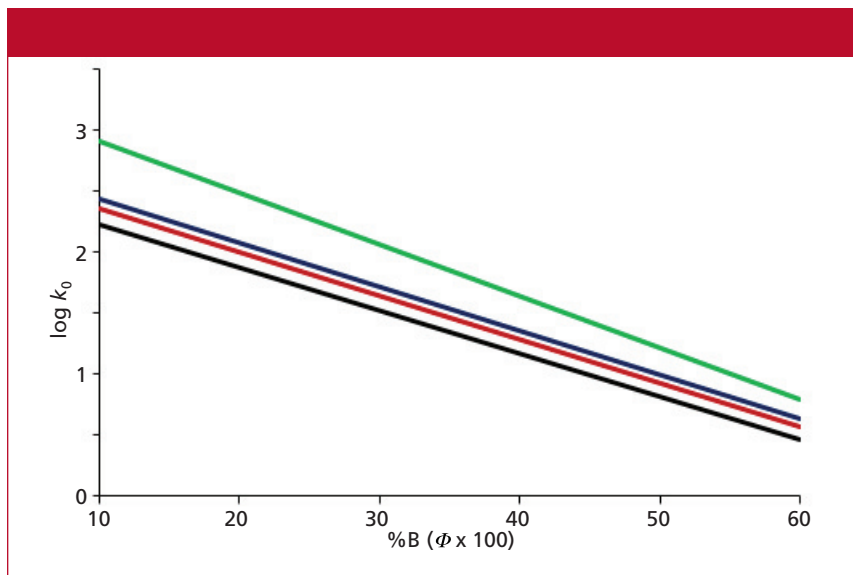


Figure 2: Plot of $\log k$ versus %B according to equation 5. Data for a “regular” sample from reference 5 are triazine herbicides separated on a C18 column in mixtures of methanol–water as the mobile phase.

peak height, not peak area, is the limiting factor for detection limits. Clearly smaller k -values are desired for taller peaks.

A final cost-related factor is that of solvent costs. The purchase price, preparation costs, and disposal costs all add up. Shorter

run times (smaller k -values) will reduce solvent costs.

Stability of Separation

The general curve shape for Figure 1 is one we often see in chemistry, such as

when tracking reaction completion. It is common to see a rapid rise in the dependent variable with a small change in the independent variable, then with continuing increases in the independent variable, the change in the dependent one levels off. The same happens here for changes in resolution as k is increased. For values of $k > 5$, there is only a small increase in resolution for each successive increase in k , whereas for $k < 2$, the changes are quite dramatic. Values of k can change because of normal laboratory variation. For example, as discussed below (Rule of Three), a 1% change in %B (the organic content of the mobile phase) can be expected to change k by approximately 10–15% for a 500-Da sample. A 1% error in mobile-phase preparation certainly is within the normal expectations of laboratory variation. Because the curve of Figure 1 is quite steep at low k -values compared to higher ones, small k -value samples will be more susceptible to significant changes in resolution caused by normal fluctuations in mobile-phase composition. For this reason, it is best to avoid conditions that give $k < 2$ for maximum method robustness.

A BOLD approach to CHROMATOGRAPHY

At Optimize, we love innovation and problem solving. We take a small-batch approach to everything we do. We make the best products for ease of use and extreme performance.

Proudly designed and manufactured in Oregon since 1985.



optimize technologies
INNOVATIVE HPLC, UHPLC & LC/MS PRODUCTS

www.optimize-tech.com

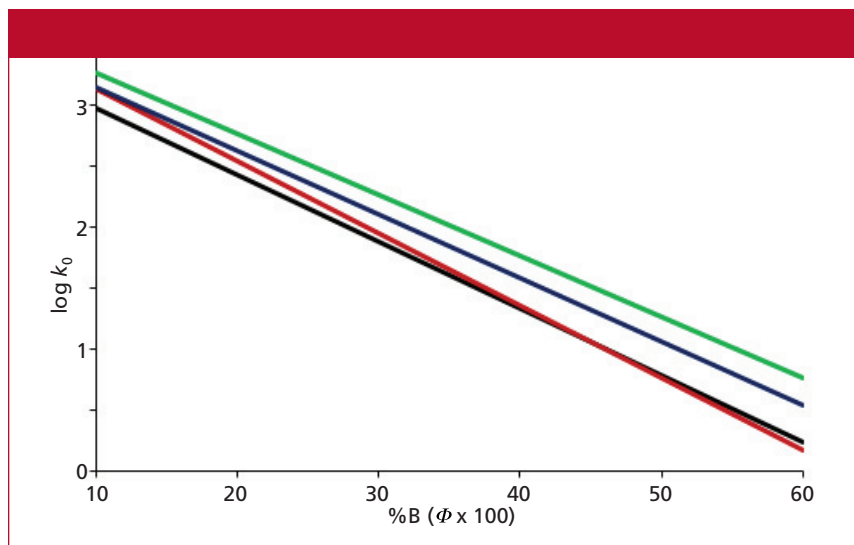


Figure 3: Plot of $\log k$ versus %B according to equation 5. Data for an “irregular” sample from references 6 and 7 are substituted aromatics separated on a C18 column with mixtures of acetonitrile–water as the mobile phase.

Selection of k -Range Is a Compromise

After considering the above discussion, it is clear that selection of an ideal value of k has tradeoffs—robustness, resolution, run time, detection limits, solvent expense, and so forth. Another complicating factor is that the typical sample contains components that vary somewhat in retention time, so we would like to fit the sample into a range so that the k -value of the first peak is not too small and the k -value of the last one is not too large. Based on the above discussion, a range of $2 \leq k \leq 10$ is ideal, being a good compromise between robustness and run time. However, some or many samples, depending on the application, have too broad a polarity range to fit within this k -range. In such cases, extending the range to $1 \leq k \leq 20$ is reasonable. When the ratio of the k -values of the first and last peaks exceeds 20–40, a gradient method usually will be favored over isocratic.

k Is Not the Whole Story

The data in Figure 1 imply that an increase of k will always increase resolution, but this conclusion is a rather broad generality that tends to fail in the case of specific samples. To understand this relationship better, we can classify samples as “regular” and “irregular” (see discussion of reference 2 for more details). It has been shown (3,4) that the relationship between k and the mobile-phase percent organic (%B, expressed as Φ , where $\Phi = \%B \times 0.01$):

$$\log k = \log k_0 - S \Phi \quad [5]$$

Where k_0 is the k -value of a solute at 0% B and S is the slope of the plot, and is a characteristic of the solute. S can be estimated as follows:

$$S \approx 0.25 MW^{0.5} \quad [6]$$

where MW is the molecular weight in daltons. For some types of samples, plots of equation 5 appear as in Figure 2. Here it can be seen that the $\log k$ versus S plots are parallel or tend to fan out from each other as %B is reduced. In a case like this, the chromatogram would spread out, with increasing resolution at smaller values of %B, as might be expected from the behavior observed in Figure 1. This kind of sample has been referred to as a “regular” sample. Regular samples tend to be those closely related in structure, such as homologs or isomers. The sample of Figure 2 is a group of structurally related triazine herbicides.

Many, if not most, samples include components that are not as closely related as those just discussed. Such samples vary in core structure as well as in the type and number of functional groups. It is common for plots of equation 5 for these samples to appear more like that of Figure 3. In Figure 3, compounds represented by the black, blue, and green lines behave much like those of Figure 2 in that they are roughly parallel. However, the compound represented by the red line moves relative to the others. At ~45% B, the red and black lines cross, so the two compounds would

be coeluted. This is also the crossover point for the red and black compounds; at %B > 40%, the red compound comes out first and at %B < 40% B, it comes out after the black one. In contrast to the expected behavior of Figure 2, the compounds represented by the black and blue lines converge as %B is reduced, so their resolution gets worse, not better, with increased k . Samples in which the slopes of the $\log k$ versus %B plots differ significantly are referred to as “irregular” samples.

The information we gather from Figures 1 and 3 can be valuable guides for LC method development. Figure 1 tells us that we’ll get the “best” chromatographic behavior with $2 \leq k \leq 10$ (or $1 \leq k \leq 20$ if the sample won’t fit within this restricted retention range). This is a good general guideline. However, samples that behave as the one in Figure 3 are common enough that we can’t assume that larger k -values (longer retention) will always improve the separation. Sometimes α (equation 2) will remain relatively constant as %B is changed and sometimes it will vary significantly. This tells us that fine-tuning k can be a powerful tool to fine-tune the resolution.

Predicting Retention

Another useful tool that we can use to save experimental runs is to estimate the change in retention (expressed as k) for a selected change in %B. We can do this estimation quite accurately based on two experimental runs that allow us to make plots as in Figures 2 and 3. For example, chromatograms run at 30% B and 50% B would allow us to measure retention for each compound, convert it to k and plot the results as in Figure 2 or 3. Alternatively, we could use equations 5 and 6, along with a single experimental run and knowledge (or guess) of the analyte molecular weights to construct similar plots with a little less accuracy (depending on how many assumptions we make). Equation 6 tells us that a 400-Da compound will have an S -value of approximately 5, which is a convenient number to use as an average or typical S -value for small molecule samples (for example, <1000 Da). From equation 5, we can construct an estimate of the change in k (Δk) for a given change in %B ($\Delta\%B$, where $\Delta\%B = 10\%$ is equivalent to $\Delta\Phi = 0.1$):

$$\Delta k = 10^{(\Delta\Phi S)} \quad [7]$$

Thus for $S = 5$ and $\Delta\%B = 10\%$, we get $\Delta k = 10^{(0.1 \times 5)} \approx 3$. This is what is often referred to as “The Rule of Three,” where a 10% change in the organic solvent concentration of the mobile phase is expected to change k by approximately threefold. This is a very handy tool for estimating how much retention will change when the mobile phase is modified. I used a similar estimate for the earlier discussion of the effect of a 1% change in %B due to normal variation in the laboratory.

Although I won't go into detail here (see discussion in Section 2.3.2.2 of reference 2 for more information), we can get plots similar to Figure 2 or 3 if we plot $\log k$ versus $1/T$, where T is temperature in kelvins. From such data, we can generate a rule of thumb that an increase in temperature of 1 °C will decrease k by 1–2%. This rule of thumb can be useful to help estimate the effect of a change in column temperature on retention and also alerts us to the importance of column temperature control if we want stable retention times.

Conclusions

We have examined the influence of the retention factor, k , on resolution. After we considered the risks (costs) and benefits of various values of k , using Figure 1 as a guide, we concluded that we are likely to get the “best” chromatography and a good balance of costs (time, solvent costs, resolution, and so forth) if we can adjust the chromatographic conditions to achieve $2 \leq k \leq 10$, or if the sample won't fit into this retention range, $1 \leq k \leq 20$. When $k < 1$, retention tends to be less stable, resolution is very sensitive to small changes in k , and there is more chance of interferences from the unretained materials at t_0 . When $k > 20$, run times become excessive and peak heights suffer. When samples won't fit in a retention range of $1 \leq k \leq 20$, a gradient is likely a better choice.

We also saw that while it is a general pattern that resolution increases with an increase in k (as in Figure 1 or 2), there are many cases in which the sample components vary enough that changes in relative retention and even peak reversals can be seen with either increases or decreases in %B. This means that even if the sample is eluted in the $1 \leq k \leq 20$ range, fine-tuning %B can be a useful tool to move peaks around relative to each other.

Finally, we saw that we can further save

the cost of experimental runs by estimating the change in retention or k for a specific change in %B or temperature. If two experimental runs are made, such as at 30% B and 50% B for Figures 2 and 3, quite accurate estimates can be made for the change in retention that can be expected with a specific change in %B.

Often if we start method development with a column that generates $N \approx 10,000$ plates (1), find conditions that generate $1 \leq k \leq 20$ for our sample components, and then fine-tune %B for the best retention, we will obtain a satisfactory separation. If this is the case, method development can be a fairly simple process. If we have optimized these two factors (terms *i* and *iii* of equation 1) and are not successful, it is time to look for help in term *ii* of equation 1, as will be discussed in the next installment of this series.

References

- (1) J.W. Dolan, *LCGC North Am.* **35**(3), 170–173 (2017).
- (2) L.R. Snyder, J.J. Kirkland, and J.W. Dolan, *Introduction to Modern Liquid Chromatography*, 3rd edition (Wiley, 2010), section 2.5.2.1.
- (3) K. Valkó, L.R. Snyder, and J.L. Glajch, *J. Chromatogr. A* **656**, 501–520 (1993).
- (4) L.R. Snyder, J.W. Dolan, and J.R. Gant, *J. Chromatogr. A* **165**, 3–30 (1979).
- (5) T. Braumann, G. Weber, and L.H. Grimme, *J. Chromatogr. A* **261**, 329–343 (1983).
- (6) N.S. Wilson, M.D. Nelson, J.W. Dolan, L.R. Snyder, R.G. Wolcott, and P.W. Carr, *J. Chromatogr. A* **961**, 171–193 (2002).
- (7) N.S. Wilson, M.D. Nelson, J.W. Dolan, L.R. Snyder, and P.W. Carr, *J. Chromatogr. A* **961**, 195–215 (2002).

John W. Dolan

“LC Troubleshooting” Editor John Dolan has been writing “LC Troubleshooting” for LCGC for more than 30 years. One of the industry's most respected professionals, John is currently a principal instructor for LC Resources in McMinnville, Oregon. He is also a member of LCGC's editorial advisory board. Direct correspondence about this column via e-mail to LCGCedit@ubm.com



For more information on this topic, please visit www.chromatographyonline.com

Trust
Jordi Labs
for your
Resolve GPC
columns and
Analytical
Service needs.

Jordi  Labs
MATERIAL SOLUTIONS. UNCOMPROMISING INTEGRITY.

- Resolve GPC Columns
- Regulatory Testing
- GPC Services



This installment highlights new high performance liquid chromatography (HPLC) systems, modules, and software that were introduced at Pittcon 2017 and during the year prior, and describes their innovative features and significant benefits.

PERSPECTIVES IN MODERN HPLC

New HPLC Systems and Related Products: A Brief Review

This annual review of new high performance liquid chromatography systems and related products covers products introduced during the last 12 months. The majority of the products were launched at Pittcon 2017.

The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) is one of the world's largest conference on laboratory science. The 69th Pittcon was held at McCormick Place in Chicago, the largest conference center in the United States, from March 5 to 9, 2017. The last time Pittcon was held in Chicago was in 2014 (and 2011 prior), and it will be there again in 2020. Note that Chicago, Orlando, Philadelphia, New Orleans, and Atlanta are the cities selected for hosting Pittcon, with Chicago in the rotation every 3 years and the others about every 6 years. This frequency makes sense since Chicago is the third largest city in the U.S. and is also an industrial, agricultural, financial, transportation, and communication center. We had great weather this year at the conference with many sunny days and spring-like temperature in the 50s.

Pittcon is impressive with ~2000 presentations, 100+ short courses, and a huge exposition with over 800 exhibitors, including over 120 new manufacturers displaying for the first time. Although the number of attendees has been dropping in recent years, registration hit 13,300 after three days. With open admission on Thursday, a total of above 14,100 was expected, which is

very good news for the conference and an indication of a healthier economy and positive outlook for instrumentation. This year's conference featured a plenary lecture by Dr. Karl Deisseroth, a professor of bioengineering at Stanford University, on insights on optogenetic proteins using electron microscopy and mass spectrometry (MS). Once again, Pittcon 2017 provided a "Mecca" for analytical chemists and a premier venue for instrument manufacturers.

Megatrends in HPLC Equipment

The perfect marriage of ultrahigh-pressure liquid chromatography (UHPLC) and MS instruments continues to be the megatrend in chromatography in recent years (1–5), even though this is another off year for new UHPLC system introductions. With many second-generation UHPLCs debuted by all major manufacturers in prior years (2,3), this year we are seeing mostly line extensions, application-specific systems, modules (particularly new MS or MS add-ons), and software products.

Table I lists the new product introductions (arranged alphabetically by vendor name), followed by more-detailed descriptions or commentaries.

New HPLC and UHPLC Systems and Line Extensions

New HPLC, UHPLC, supercritical fluid chromatography (SFC), ion chromatography (IC), modules, chromatography data systems (CDS), and related software are summarized in Table I and described with more details below.

Michael W. Dong

is the editor of *Perspectives in Modern HPLC*.

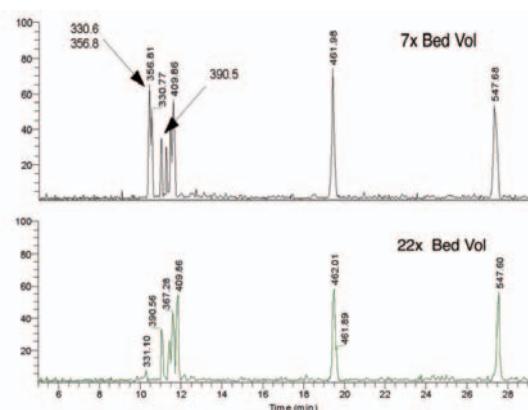
New! Semi-Reusable SPE Formats



BioPureSPN™ Color Coded ~ Reusable Spin Columns

VOLUME EFFECTS ON SPE

Desorption Increases From Excessive
Sample & Wash Volumes



Column: TARGA® C18, 300µm x 30mm Trap
Sample Solvent: 0.1% FA water (volumes as shown). Trap
Column in 0.1% FA water
Gradient: 98% water, 0.1% FA, 2% ACN to ACN, 0.1% FA

A Chemistry For Every Compound

Affinity - Empty - ERLIC - HILIC - IEX - RPC - SEC

Product Formats	Sample Capacity	Packed Volume	Void Volume	Elution Volume
UltraMicroSpin™ Column	3-30 µg	30 µL	12 µL	2-25 µL
MicroSpin™ Column	5-60 µg	60 µL	25 µL	5-50 µL
MACROspin™ Column	30-300 µg	190 µL	90 µL	25-180 µL
96-Well MicroSpin™ Plate	3-30 µg	60 µL	25 µL	5-50 µL
96-Well MACROspin™ Plate	30-300 µg	190 µL	90 µL	25-180 µL
Empty -Reactors™ /w caps			200 µL	
Page*Eraser™ Filters		0.1µL	5-200 µL	
HisSep™ Histidine Removal	200-500 µg			

New Products

BioPureSPN™ PROTO™ C18	3-300 µg	20-200 µL	10-100 µL	5-200 µL
BioPureSPN™ PROTO™ C4	3-300 µg	20-200 µL	10-100 µL	5-200 µL
BioPureSPE™ TARGA® C18	3-300 µg	20-200 µL	10-100 µL	5-200 µL
iSPE® Fusion™ HILIC	0.4 - 1 g	50µL-1mL	50-500 µL	50µL-1mL

The Nest Group, Inc.

Not Just Columns ... Answers!

45 Valley Road, Southborough, MA 01772 USA
E-mail: sales@nestgrp.biz

For more Info: www.nestgrp.com

BioPureSPN™ is a trademark of The Nest Group, Inc.
HisSep™ is a trademark of ID Biologics
iSPE® is a registered trademark of HILICON AB
PROTO™ trademark of Higgins Analytical, Inc.
TARGA® is a registered trademark of Higgins Analytical, Inc.
UltraMicroSpin™, MicroSpin™, MACROspin™ are trademarks of Harvard Apparatus, Inc.

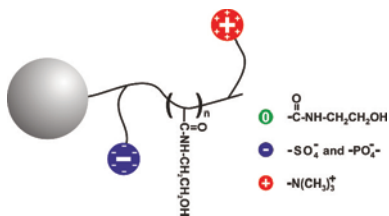


HILICON®

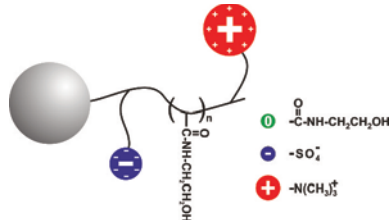
Nucleotides and Polar Metabolites

Use High pH on Polymeric Fusion(P) for Enhanced Phospho- Detection or 1.8µm Fusion for Resolution

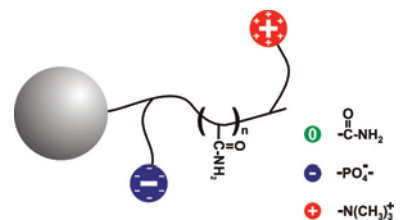
iHILIC Fusion



iHILIC Fusion(+)

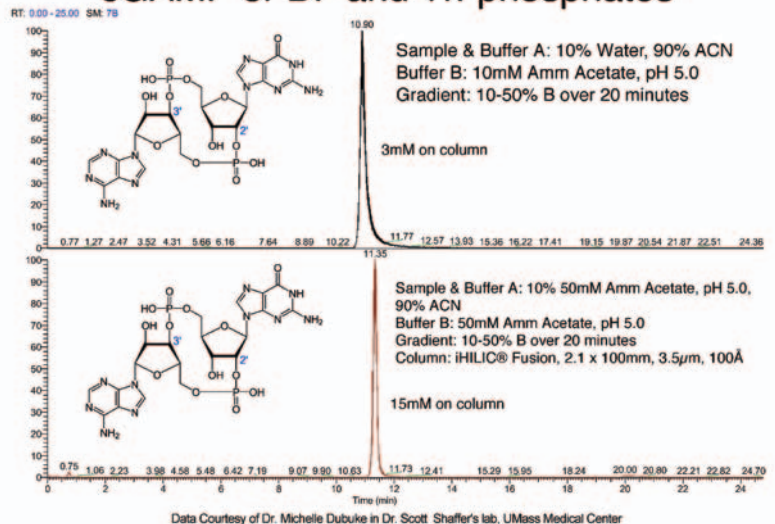


iHILIC Fusion(P)



- * Complementary selectivity for polar differences
- * Reliable analyses with excellent reproducibility and robustness of chemistry
- * Ultra-low column bleed for stable baselines
- * Polymeric Fusion(P) using 10-50mM ammonium bicarbonate

Effect of Buffer Strength When Separating cGAMP or Di- and Tri-phosphates



Columns available with 1.8µm, 3.5µm, or 5µm particles and SPE formats

www.nestgrp.com/iHILIC.shtml

iHILIC® is a registered trademark of HILICON AB

Table I: New HPLC product introductions at Pittcon 2017 or the prior year		
Vendor	Product	Description and Comments
ACD/Labs	AutoChrom 2016 version	Software includes overlaid logD curves, enhanced column data bases, and better connectivity to Chromeleon 7, Empower, and other MS data systems.
ACD/Labs	LUMINATA	LUMINATA is a new multitechnique software product that offers comprehensive data aggregation for impurity characterization.
ACD/Labs	MetaSense	MetaSense is an LC–MS software solution to facilitate identification of predicted and unexpected metabolites.
Agilent	1260 and 1220 Infinity II systems	Updated platforms for Agilent’s Infinity II family of HPLC and UHPLC systems.
Agilent	1260 Infinity II SFC	Offers an improved autosampler and an updated control module with an optional binary pump.
Agilent	1290 Infinity II preparative fraction collector	Enables collection of up to 432 fractions or volumes up to 5.9 L triggered by time-, peak- or mass-based events.
Agilent	OpenLab CDS 2.1	This version of OpenLAB CDS provides a networked or standalone environment that combines ease of use and regulatory compliance.
Analytical Sales and Services	Sentinel	A fluid-level indicator for monitoring up to three bottles of waste or mobile phases.
Biotech AB	DEGASi Compact and Prep+ degassers	DEGASi Compact with small internal membrane volume and DEGASi Prep+ supports preparative HPLC and flash chromatography.
Bonna-Agela	OCTOPUS purification system	A flexible modular purification system for both flash and preparative HPLC with automatic fraction collection and many detection modes.
Bruker	Elute LC series	UHPLC systems provide pressures from 700 to 1300 bar for LC–MS analysis.
Cecil Instruments	Electrochemical Detection Adept HPLC	A modular HPLC system with electrochemical detection that supports DC-amperometric, scanning and pulsed modes.
ChromPerfect	ChromPerfect SL CDS	ChromPerfect for small laboratories that controls up to four chromatographs and is compatible to tablets control and data analysis.
Cromite	A portable HPLC–UV system	A portable, battery operated HPLC–UV system with novel approaches to column and sample probe design.
DataApex	Clarity v.7.2 CDS	An updated software version operating under Windows 10 with enhanced instrument connectivity, and MS extensions.
ECOM	ECF 2096 fraction collector	An automated collector that collects up to 48 tubes of 8-mL volumes.
FF Runge GmbH	Mikron detector series	Very small photometric, fluorimetric, and conductivity HPLC detectors based on LED technology.
JASCO	HPLC–MS and SFC–MS	JASCO offers both LC-4000 Series HPLC–MS and SFC–MS systems with an add-on of Advion’s Expression SQ MS system.
JASCO	Method scouting software	JASCO offers method scouting software for automated solvent and column screening (up to 10 solvents and 10 columns).
JM Science	Sonic reservoir system	A monitoring system for HPLC reservoirs and waste containers using sonic detection and LED display.
Knauer	Azura Analytical and Semi-prep HPLC	A family of analytical and semipreparative HPLC systems (200, 400, 700, and 1000 bar) in stainless and biocompatible formats.
Metrohm	Eco IC	The Eco IC is a new ion chromatograph designed for routine analysis of anions, cations, and polar compounds in environmental testing and education.
PerkinElmer	QSight MS/MS system	The QSight triple-quadrupole system is a self-cleaning analyzer for testing food contaminants in compliance with regulatory standards.
Prolab Instruments	ZirconiumHT UHPLC system	The Zirconium High-Throughput UHPLC system is a micro-UHPLC system with novel trapping configuration for 2D applications.
Sciex	X500B QTOF MS	A compact QTOF system for standardized biotherapeutics characterization.

Table I: New product introductions (continued)

Vendor	New HPLC and related product	Description and Comments
Shimadzu	Cannabis Analyzer for Potency	Shimadzu's Cannabis Analyzer for Potency is an HPLC–UV system designed specifically for quantitative determination of cannabinoid content.
Shimadzu	CLAM-2000 automation module for LC/MS/MS	CLAM-2000 clinical laboratory automation module automatically performs the sample preparation necessary for analyzing blood and other biological samples by LC–MS/MS.
Shimadzu	LCMS-8045	This system performs demanding routine quantitative analyses in food safety and environmental testing.
S-Matrix	Fusion QbD v. 9.8.0	A version of Fusion QbD software for HPLC method development using design of experiments with interfaces to most HPLC systems.
Thermo Scientific	Vanquish Flex binary pump	A biocompatible binary pump for the Vanquish Flex UHPLC system rated at 1000 bar (15,000 psi).
Thermo Scientific	Q Exactive HF LC–MS/MS system	A compact orbital trap hybrid LC–MS/MS system capable of a maximum resolution of 240,000 FWHM.
Thermo Scientific	Chromeleon 7.2 SR5 CDS	This version of Chromeleon is a multitechnique, multivendor scalable platform for GC, LC, IC, and MS applications with many enhancements.
Thermo Scientific	AppsLab methods library	The latest AppsLab library is a searchable online repository for access to chromatography and MS applications including analytical methods, chromatograms, and related compound information.
Waters/Sotax	Acquity H-Class UPLC for dissolution testing	Waters offers an automated injector for dissolution based on Sotax's Xtend Dissolution Line.
Waters	Empower Cloud	Waters announced cloud-based Empower 3 software available through Amazon Web Services.
Wyatt	ViscoStar III	An online viscometer detector offers sensitive measurements of intrinsic viscosity for macromolecular size and polymer branching.

Agilent 1260 and 1220 Infinity II systems—Two years ago Agilent introduced the 1290 Infinity II LC systems. This year, Agilent completed the InfinityLab LC Series with the 1260 and 1220 Infinity II systems, all to deliver full method compatibility. Innovative 1290 Infinity II LC technology is leveraged with the 1260 Infinity II Multisampler with dual-needle sampling and Vialsampler. A larger column oven, which fits 30-cm columns and can accommodate four columns with a single switching valve, and a range of detectors are common to both systems. The 1260 Infinity II pumps have a 600-bar pressure limit and are available in either stainless steel or bioinert format. The 1220 Infinity II system remains an integrated 600-bar system available with an isocratic or a dual-channel gradient pump that can be paired with a manual injector or an autosampler, a variable-wavelength UV detector or a diode-array detector, and external special detectors including a mass-

selective detector. All systems can be operated with Agilent OpenLab CDS Software as well as third-party software with the universal Agilent Instrument Control Framework.

Bruker Elute LC series—Bruker introduces the Elute LC series systems for LC–MS analysis: SP (700 bar), UHPLC (1300 bar for ultrafast analysis), OLE (for online extraction) and HT (high-throughput with the new PAL3 autosampler).

Cromite portable HPLC—Cromite introduced a handheld, portable HPLC system that consists of an isocratic pump with a 275-bar or 4000-psi pressure limit and a max flow rate of 4 mL/min, and a UV–vis detector with an light-emitting diode (LED) source that provides up to four customizable wavelengths. The system is designed with novel approaches to mobile-phase storage and sample introduction and preparation, and it includes a single-ended leak-proof column. The system weighs 2.5 lb (1 kg) and has overall dimensions of

6.8 × 3.2 × 3.4 in. (173 × 81 × 86 mm). The device can be controlled from a PC, smartphone, or tablet via Bluetooth and can be powered off a 12-VDC power supply or external battery. The company is targeting applications in education, environmental and production QC testing with this offering. Figure 1 shows an example chromatogram obtained using the Cromite portable HPLC system.

Knauer Azura Analytical and Semi-Prep HPLC—Knauer offers the Azura as a system solution for analytical to preparative HPLC. The analytical systems are available with binary or quaternary pumps (stainless steel at 700 and 1000 bar as well as a biocompatible, ceramic version at 400 bar). The semipreparative 200-bar systems are capable of flow rates as high as 50 mL/min and are available in both stainless and biocompatible formats. These systems can be configured with appropriate autosamplers, column ovens, and UV–vis, refractive-index, and diode-array detectors. The systems

can be controlled by a controller, a mobile tablet, or a chromatography data system (CDS) such as OpenLAB EZChrom, ClarityChrom, and Chromeleon.

Prolab Instruments ZirconiumHT—The Zirconium High-Throughput UHPLC system is a micro-UHPLC system with a novel trapping column configuration for loading and analyzing samples at the same time using only one binary pump. Suggested applications are 2D micro and nano-LC analyses.

Shimadzu Cannabis analyzer—The Shimadzu Cannabis analyzer is a HPLC–UV system designed specifically for quantitative determination of cannabinoid content for potency with three method packages optimized for high-throughput, high sensitivity, and high resolution. Figure 2 shows an example chromatogram for potency testing of 11 cannabinoid standards on the system.

Sotax Xtend On-Line Dissolution with Waters Acquity H-Class UPLC—For those who have been

waiting for a modernized replacement of the Alliance-based 2695D automated dissolution analyzer, the wait is over. Waters, in conjunction with Sotax, now offers an automated UHPLC system based on the Acquity H-Class UPLC system and Sotax's dissolution testing apparatus with Sotax's sample manager (SAM) to allow sample withdrawals, fractions collection, and autoinjections from USP dissolution systems.

Bonna-Agela OCTOPUS purification system—Bonna-Agela Technologies (acquired by Danaher last year) introduced a flexible and easy-to-use modular purification system for both flash and preparative HPLC with a family of four pumps (1–200 mL/min and pressure limits of 14–200 bar [200–3000 psi]) that can be equipped with automatic fraction collection, an autosampler, a column oven–switcher, and UV–vis, photodiode-array, evaporative light scattering, refractive-index, or MS detection.

Agilent 1260 Infinity II SFC—The Agilent 1260 Infinity II SFC system

now offers an improved Multisampler autosampler, which uses an innovative feed injection principle that allows 0.1–90 μL of injection volume range with the higher sample capacity of 432 2-mL vials. The 1260 Infinity II SFC binary pump can provide pressures of 600 bar at up to 5 mL/min. The Infinity II SFC control module now offers full flow into evaporative light scattering or MS detection systems. A novel low dispersion nozzle with variable back-pressure settings enables outstanding peak shapes in this case.

Metrohm Eco IC—The Eco IC ion chromatograph is designed for routine analysis of anions, cations, and polar compounds in environmental testing and education applications. The system is complete with a suppressor, a conductivity detector, software, and an optional autosampler with up to 36 samples.

HPLC Modules

Cecil Instruments Electrochemical Detection Adept HPLC system—Cecil



Leverage the outstanding inertness, low bleed, and high reproducibility of Rxi® 3-in-1 technology to gain:

- **Accurate Data**
- **The Right Results Fast**
- **Maximized Instrument Uptime**

Put Rxi® columns to work in your lab.

Visit www.restek.com/rxi and order yours today.



Rxi® columns come with an unbeatable guarantee.

That is Restek® Pure Satisfaction.

RESTEK

Pure Chromatography

www.restek.com/rxi

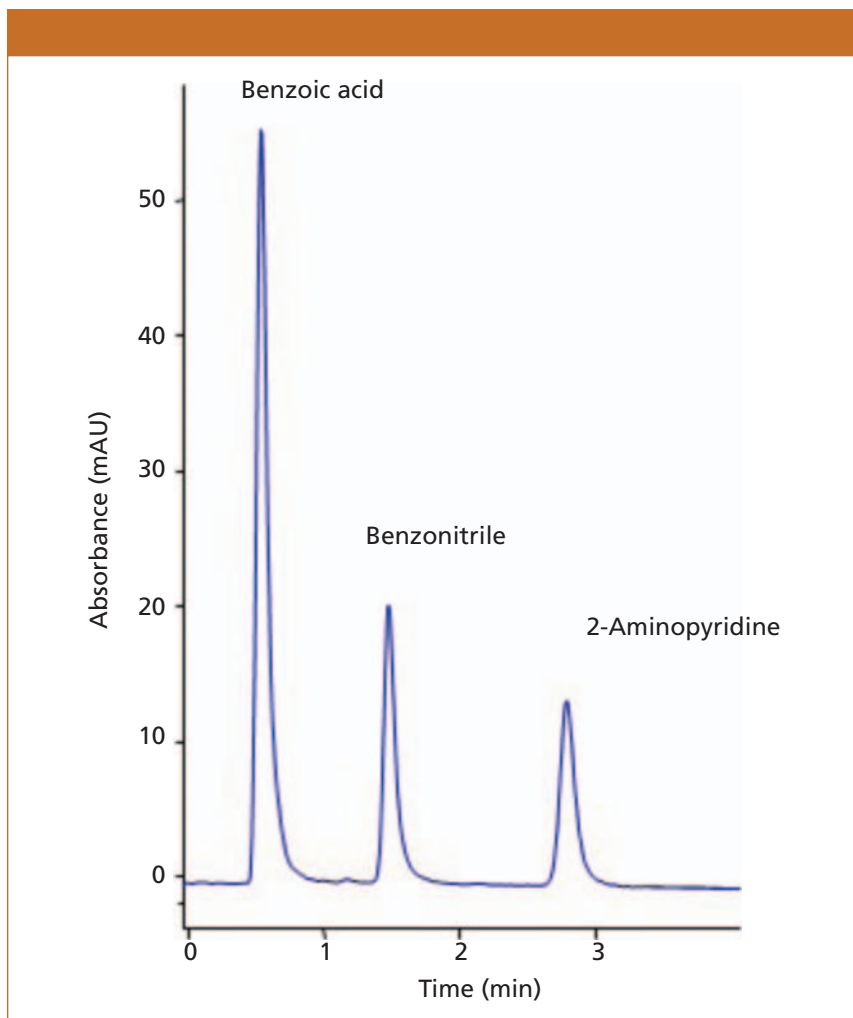


Figure 1: An example chromatogram obtained using Cromite's portable HPLC system. Column: 100 mm × 3 mm, 5- μ m d_p Cromite C18; mobile phase: 70:30:0.5 (v/v/v) water-acetonitrile-acetic acid; detection: UV absorbance at 275 nm; flow rate: 1 mL/min.

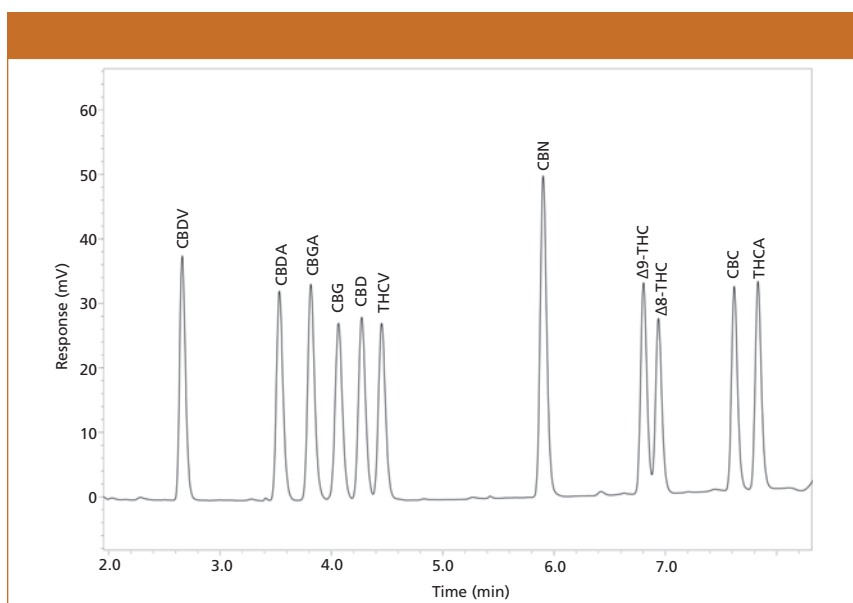


Figure 2: An example chromatogram of a 10.0-mg/L standard mixture of 11 cannabinoids separated on the Shimadzu Cannabis analyzer using the instrument's high-sensitivity method. Gradient elution conditions with acid-modified water and acetonitrile were used with a C18 column.

Instruments offers a modular HPLC system with electrochemical detection that supports DC-amperometric, scanning, and pulsed modes.

FF Runge Mikron detector series—These very compact photometric, fluorimetric, and conductivity HPLC detectors are based on LED technology.

Thermo Scientific Vanquish Flex binary pump—The Vanquish Flex binary system now features a binary high-pressure gradient pump with 2 × 3 solvent channels (1000 bar or 15,000 psi) and low gradient delay volume capable of delivering flow rates of up to 8 mL/min. The entire system provides a biocompatible flow path.

Wyatt ViscoStar III—The Wyatt ViscoStar III viscometer detector offers measurements of intrinsic viscosity for sensitive macromolecular size and conformation for polymer branching analysis. The detector uses Mark-Houwink-Sakurada parameters and hydrodynamic radii intrinsic viscosity calculations using Wyatt's ASTRA software package in size-exclusion chromatography.

Mass Spectrometry

JASCO HPLC-MS and SFC-MS—JASCO offers the Advion Expression compact single-quadrupole MS system for its HPLC-MS (LC-4000 Series) and SFC-MS (SFC-400 Series SFC) systems with mass-triggered fraction collection and integrated system control using the ChromNAV CDS.

PerkinElmer QSiight MS/MS system—The QSiight triple-quadrupole system is a highly sensitive dual source self-cleaning LC-MS/MS analyzer that enables laboratories to test complex samples for food contaminants (such as pesticides) for regulatory compliance. QSiight provides a complete pesticide solution from sample preparation, extraction to analysis, and reporting.

Sciex X500B QTOF—Sciex launches the compact X500B QTOF system designed to bring simplicity, performance, and robustness for biotherapeutic analyses. The new SCIEX OS software interface and BioPharma-View 2.0 processing software provide an integrated LC-MS solution for experts or novices performing standard biotherapeutic characterization.

A Q&A

MarvelXACT: A Worry-Free Fitting System for Liquid Chromatography



Eric Beemer
Senior Development Engineer
and Inventor of MarvelXACT
IDEX Health & Science

Chromatographers need and want repeatable, reproducible results. A key aspect of ensuring such reproducibility is avoiding guess-work when tightening fittings. To learn more about a recent innovation in liquid chromatography connections called MarvelXACT, LCGC recently spoke with Eric Beemer, senior development engineer at IDEX Health & Science.

LCGC: Why did IDEX develop MarvelXACT and what kind of field feedback led to it?

Beemer: At IDEX, we've done a lot of Voice of the Customer (VOC) analysis in the field and have had close communication with customers to understand the pain points of chromatography users and to develop a set of criteria from which to work. We also have significant chromatography experience from our internal lab work and incorporated that knowledge into the MarvelXACT specifications.

We wanted to develop a “worry-free” fitting. By “worry-free,” I mean no carryover, no peak broadening, or any kind of tailing—just repeatable chromatography. I think that's what all chromatographers want. The leaks that we tend to see with fittings currently available on the market have a big impact on troubleshooting time and chromatography results.

We also wanted to create a fitting that is highly re-usable. Ultra high-performance liquid chromatography (UHPLC) is typically run at very high pressures, and there's very few tubing material and fitting technology out there that lends itself to such pressure. If fittings are highly re-usable, labs don't have to discard items every time they change columns or connections, thereby increasing the value of our new technology.

Ease of use is also important. Although we have some existing finger-type connections that don't require tools, concerns about whether they are properly assembled still exist. With MarvelXACT, the connection is not a concern anymore because it enables an exact, proper connection every time.

LCGC: What are the key features of MarvelXACT?

Beemer: One key feature is its torque-limiting mechanism. Torque is important because if you don't have enough torque, leaks can develop—and with leaks come bad chromatography.

By applying enough torque, you maximize the fitting's sealing potential. If you have too much torque, you can damage the port or the tubing itself. Once the tubing is damaged, an increase in back pressure usually occurs, which can be detrimental for achieving the flow rates that might be required by the analysis.

SPONSORED BY



With MarvelXACT, by having a torque-limiting “click” mechanism, the proper amount of torque is provided to maximize the seal while preventing too much torque, which can limit the tubing’s re-usability and the functionality. The click also provides good feedback; you know it has been properly assembled. It’s like your automobile’s gas cap. When you hear the click, you know that you’ve assembled it properly and that it will seal every time.

Another key feature is face-sealing. Face-sealing is basically how the end of the tubing seals at the bottom of the port. No cones, ferrules, or other components are needed. A load is applied to the back of the seal to create the seal on the front end. MarvelXACT connections are available in either stainless steel or all PEEK flow path options, the latter providing a flow path that is smooth and very inert, which is important for “sticky” applications like protein separations.

LCGC: IDEX launched a new connection about a year ago called MarvelX. What are the similarities and differences between MarvelXACT and MarvelX?

Beemer: MarvelXACT is really just the advanced version of MarvelX. It still utilizes the same tubing and face-sealing

element, but MarvelXACT builds upon MarvelX by adding the torque-limiting mechanism to make it easier to use. Nonetheless, much of the mechanism for creating a seal and the resulting chromatographic results are the same as those previously obtained on the proven MarvelX technology. MarvelXACT has been extensively tested to verify a superior product incorporating these new elements.

LCGC: Is MarvelXACT a patented technology?

Beemer: Yes; both the torque mechanism and the fitting have been granted patents. The tubing itself and the face sealing element is patent pending.

LCGC: What is IDEX’s vision on MarvelX technology? What should users expect for the near future?

Beemer: IDEX Health & Science is committed to the growth of the MarvelX product family as evidenced by the expansion of the face-sealing MarvelX technology used in MarvelXACT. We’re looking at ways we can use different tubing internal diameters and are experimenting with different materials such as a new fused silica flow path to try and open up other applications of the technology.

IDEX Health & Science is the global authority in fluidics and optics, bringing life to advanced optofluidic technologies with products, people, and engineering expertise. The company is respected worldwide for solving complex problems and delivering complete path innovation for analytical, diagnostic, and biotechnology applications for the life sciences market. As a genuine and trustworthy partner, IDEX Health & Science solves its customers’ most demanding challenges with the industry’s most extensive portfolio of state-of-the-art components and capabilities that are unrivaled in breadth, quality, performance, and design. IDEX’s vision of the complete path goes far beyond just meeting its customers’ needs—it anticipates them, with intelligent solutions for life.

Product offerings include: connections, valves, pumps, degassers, column hardware, manifolds, flow cells, microfluidics consumables, sensors, integrated fluidic systems, optical filters, lenses, shutters, laser & light engines, and integrated optical systems. For more information, visit: www.idex-hs.com



Figure 3: A screen shot of the Agilent OpenLab 2.1 CDS showing four tiled panels on data processing, chromatograms, peak analysis, and processing methods.

Sciex QTRAP 6500+ LC-MS/MS—This combined quadrupole-ion trap system includes the highly sensitive triple-quadrupole 6500+ MS/MS system for high-throughput LC-MS/MS analysis for both small- and large-molecule quantitation. The MS system is equipped with the multicomponent IonDrive technology for enhancing sensitivity and is capable of six orders of magnitude of linearity, up to 20,000-u/s scan rates and 5-ms polarity switching.

Shimadzu LCMS-8045—Shimadzu's LC-MS/MS-8045 system is a mid-range triple-quadrupole MS system that performs routine quantitative analyses in food safety and environmental testing. The instrument features a scan speed of 30,000 u/s without the loss of mass accuracy and a polarity switching time of 5 ms. It is operated using Shimadzu's LabSolutions software. A dual electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI) source is available.

Shimadzu CLAM-2000 clinical laboratory automation module for LC-MS—The CLAM-2000 system

automatically performs all of the sample preparation procedures necessary for analyzing blood (from blood collection tubes) and other biological samples by LC-MS/MS for therapeutic drug monitoring and testing of abused drugs.

Thermo Scientific Q Exactive HF—This compact quadrupole-orbital trap hybrid LC-MS/MS system designed for rapid quantitation of proteins, peptides, lipids, metabolites, and contaminants is capable of scan rates up to 18 Hz and a maximum resolution of 240,000 FWHM.

HPLC Accessories

Agilent 1290 Infinity II preparative fraction collector—This automated open-bed fraction collector allows efficient and flexible automated collection of up to 432 fractions or volumes up to 5.9 L triggered by time-, peak-, or mass-based mode.

Analytical Sales and Services Sentinel—The Sentinel is a fluid-level indicator for monitoring up to three bottles of waste or mobile-phase containers and is equipped with status display and alarm functions.

Biotech AB DEGASi Compact and Prep+ degassers—Two new degassers were introduced: The DEGASi Compact degasser with a small internal volume and the DEGASi Prep+ degasser that supports preparative HPLC, flash chromatography, and high-throughput applications.

ECOM ECF 2096 fraction collector—The ECF 2096 automated fraction collector collects up to 48 tubes of 8-mL volumes.

JM Science Sonic reservoir system—A monitoring system for up to three HPLC reservoirs and waste containers using sonic detection and an LED display.

Chromatography Data System and Other HPLC-Related Software

Agilent OpenLAB CDS 2.1—This newest version of OpenLAB CDS provides a networked or standalone environment that combines ease of use with rigorous regulatory compliance. This latest platform is an LC-, GC-, LC-MS-, and GC-MS-capable CDS for the QC laboratory with strong data integrity and audit trail review capabilities. Version 2.1 contains many new features including an extended support for non-Agilent instruments and improvements MS quantitation, peak integration and reporting. Figure 3 shows a screen shot of the Agilent OpenLAB CDS with four tiled panels for data processing, chromatograms, peak analysis, and processing methods.

ChromPerfect SL—ChromPerfect introduces a CDS for small or mobile laboratories that controls up to four chromatographs with web-based and Windows 10 operating systems. It is compatible with tablets with data processing, reporting, and analysis functions.

DataApex Clarity v.7.2 CDS—The Clarity v.7.2 advanced CDS operates under Windows 10 and can be connected to four instruments and up to 32 data channels. Its optional control modules provide integrated control of selected instruments. Extensions are available for diode-array detectors, gel permeation chromatography (GPC) analysis, MS (single quadrupole and time of flight), and system suitability testing.

**COUNTER CURRENT & CENTRIFUGAL
PARTITION CHROMATOGRAPHY, PLUS HPLC,
IL-LC AND SFC FOR ADVANCED SAMPLE
PREPARATION MG TO TONNES PER ANNUM:**



QUATTRO CCC & CPC + PARTITRON CPC

Why consider Liquid-Liquid Chromatography for your preparation?

- Save ~90% process chromatography/ extraction, solvent/column costs
- Ideal HPLC-MS pre-prep; no column induced degradation or adsorption
- Uniquely allows infinitely polar to infinitely non-polar prep in one run
- Easily scale-up from milligram to your process scale, with no target loss
- Orthogonal selectivity options compared to HPLC / Flash / SFC
- No solubility problems; solvents available include Ionic Liquids (IL)
- IL / SFC / SFC-MS / MS / MS-MS / HPLC / HPLC-MS compatible
- High crude sample mass loading (typically 5 to 40g per 1000ml capacity)
- Inject up to 10+ % coil volume without excessive band spreading
- High pressure (1000psi+) + high "G" (80-150)(250)(350+) options
- Only manufacturers of both hydro-dynamic & hydrostatic CCC
- NEW DEDICATED IONIC LIQUID CHROMATOGRAPHY / EXTRACTION CCC/CPC
- NEW DUAL-FLOW CCC OPTIONS, SINGLE COIL 4 INLET / OUTLET OPTIONS



Partitron CPC with 4 to 6 litre demo rotor available for short-term hire, at discounted prices, for non-ATEX or ATEX research or process trial applications, prior to purchase consideration.



Take crude tar like sample. CCC or CPC can potentially obtain pure targets in one run, without fear of column contamination or compound loss.



Quattro CCC & CPC for use standard solvent extractions/chromatography, and for use with ionic liquids, liquid ion exchange or chelating agents. Option remote electronics/touchscreen computer for use extreme-hazards glove-box applications.

GUIDE PRICING TO ASSIST YOUR BUDGETING.

Scale-up mg/g/10's grams, Laboratory Scale to Multiple Tonnes Per Annum, Process Scale.

If your Liquid-Liquid Chromatography, CCC or CPC budget below UK POUNDS:

20,000 Consider Laboratory-Scale Quattro CCC or CPC IntroPrep™

50,000 Consider Laboratory-Scale Quattro CCC or CPC QuikPrep™ or LabPrep™

100,000 Consider PilotPrep™, Non-Atex 2 to 4.5 Litre, CCC, or CPC (Uniquely we use Titanium CPC rotors)

140,000 Consider multiple PilotPreps™, Non-Atex 2 to 4.5 Litre, CCC or CPC

Or consider Atex rated ProcessPrep™ 6+ Litre CPC (Uniquely we use Titanium CPC rotors)

500,000 Consider 25 Litre Partitron CPC™ or multiple Quattro PilotPrep™/ProcessPrep™

Website: www.quattroprep.com • Email: aecs@gmx.com

Contact AECS-QuikPrep Ltd. Managing Director personally on Int. UK Code: 783 876 1524

NEW EXCLUSIVE COUNTRY DISTRIBUTORS REQUIRED.

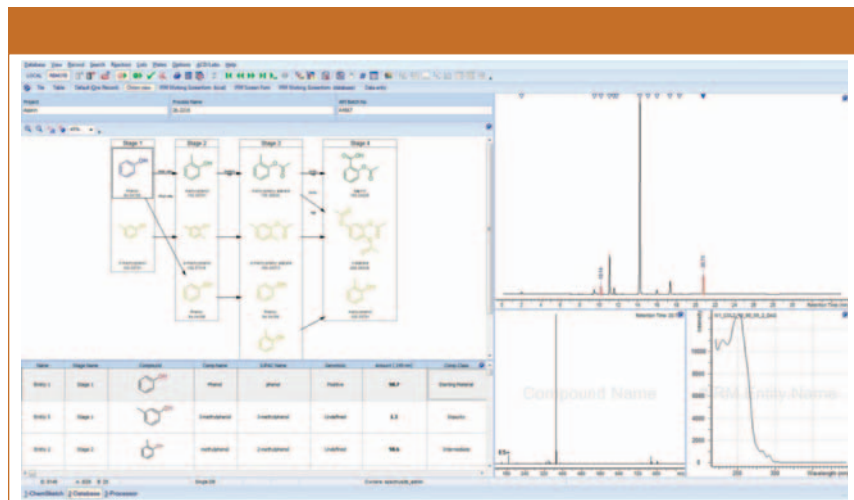


Figure 4: A screen shot of ACD/Labs LUMINATA software displaying multiple panels of an overall synthetic scheme of a drug substance, a table of potential impurities, and the data associated with each impurity (HPLC chromatogram, UV, and mass spectra).

Waters Empower Cloud—Waters announced a release of cloud-based Empower 3 (feature release 4) available through Amazon Web Services in late 2017. This cloud-based CDS platform is particularly suited for pharmaceutical companies to reduce costs for implementation and maintenance and can support globally all subsidiaries and contract organizations.

ACD/Labs AutoChrom 2016 version—This automated HPLC method development software uses predictive simulation and can interface with both UV and MS detection. This version includes overlaid logD curves, better peak tracking, and an enhanced column data base with Tanaka parameters and superficially porous particle columns. It also has better connectivity to Chromeleon 7, Empower, and other MS data systems.

ACD/Labs LUMINATA—LUMINATA multitechnique software offers comprehensive data aggregation for impurity characterization and knowledge management applied to pharmaceutical development applications. Figure 4 shows a screen shot from this software displaying multiple panels including the overall synthetic scheme of the drug substance, a table of potential impurities, and the data associated with each impurity (HPLC chromatogram, UV, and mass spectra).

ACD/Labs MetaSense—MetaSense software uses LC–MS data as part of its workflow in metabolite iden-

tification. It facilitates faster, easier, and more accurate detection and identification of predicted and unexpected metabolites. Built on the ACD/Spectrus platform, MetaSense offers functionalities including the facilitation of decision-support and effective knowledge sharing throughout organizations as well as the ability to support LC–MS analytical data from most instrument vendors.

JASCO method scouting software—JASCO offers a method scouting software for automated solvent and column screening with up to 10 solvents and 10 columns. It also supports multiple detectors and can be configured for use with up to 30 columns and parallel screening.

S-Matrix Fusion QbD method development software v. 9.8.0—The Fusion QbD software for automated LC method development using principles of quality by design (QbD) and design of experiments (DOE) has been updated to include an enhanced user interface with an integrated chromatogram viewer, new project management capabilities with extended data security, additional support for GC and capillary electrophoresis (CE) method development, and increased instrument support for most HPLC and UHPLC systems, including hybrid systems.

Thermo Scientific Chromeleon 7.2 SR5 CDS—The Chromeleon CDS is a multitechnique, multivendor scalable platform for GC, LC, IC, and

MS applications and includes a unique workflow automation called eWorkflows. The new platform is optimized for large-scale installation for thousands of users and instruments with data integrity features for regulatory compliance. This latest platform is designed particularly for MS analysis including biotherapeutics and is equipped with capabilities for automated target mass analysis for both LC–MS and GC–MS.

Thermo Scientific AppsLab methods library—The latest AppsLab library is a searchable online repository for easy access to chromatography and MS application databases including analytical methods, chromatograms and related compound information (1886 applications and 380 ready-to-use eWorkflows for Chromeleon CDS).

Concluding and Personal Remarks

Most analytical chemists come to Pittcon for professional development and networking. My schedule this year included teaching several short courses on HPLC, UHPLC, and method development, and chairing an invited symposium on UHPLC method development in pharmaceutical analysis. My networking events included a Chrom Mixer sponsored by Chrom Forum Delaware Valley in memory of Jack Kirkland (more than 500 attendees), a networking dinner event with the Chinese American Chromatography Association (CACA) attended by more than 120 conferees (featuring a lecture by Dr. Perry Wang of the US FDA on the perfect marriage of HPLC and MS), and the annual Pittcon presidential reception, where I personally thanked many Pittcon staff and program chairs.

I spent considerable time on the exposition floor to verify information on new HPLC products for this column installment. I returned home Thursday night, slept late and woke up to find 4 inches of snow on the ground in Connecticut. We were lucky as the weather can be unpredictable in early March in Chicago, as well as the Northeast. Rumor says that next year will be the last year for Pittcon in Orlando because the vendors com-

plained about conference attendees visiting Disney World instead of the exposition. I love Orlando because of the excellent restaurants and the warmer weather—and I plan to visit both the exposition and the theme parks.

This installment provides a brief and nonexhaustive overview of new HPLC product introductions (systems, modules, MS, CDS, software, and related products) at Pittcon 2017 and during the prior year. Readers should contact manufacturers for additional technical details. Note that new HPLC columns are covered separately in *LCGC*'s "Column Watch" annual review by David Bell. The opinions expressed in this installment are the author's own and bear no reflections on those of *LCGC* magazine, the Pittsburgh Conference, or any other organizations.

Acknowledgments

I wish to give thanks to the marketing and editorial staff of *LCGC* and all

HPLC manufacturers for their timely responses to my numerous requests for information. I am particularly grateful to Davy Guillaume and Szabolcs Fekete of U. Geneva, Harika Vemula of U. Missouri at Kansas City, David Van Meter from EAG Laboratories, Hernan Fuertes from Ardelyx, and Matt Mullaney from Pentec Health for their invaluable editorial and technical input.

References

- (1) C.H. Arnaud, *Chem. & Eng. News* **94**(24), 29–35 (2016).
- (2) M.W. Dong, *LCGC North Amer.* **34**(4), 262–273 (2016).
- (3) M.W. Dong, *LCGC North Amer.* **33**(4), 254–261 (2015).
- (4) D. Guillaume and M.W. Dong, *Amer. Pharm. Rev.* **16**(4), 36–43 (2013).
- (5) D. Guillaume and M.W. Dong (Eds.), *UHPLC: Where We Are Ten Years After Its Commercial Introduction, Trends in Anal. Chem.* **63**, 1–188 (2014).



Michael W. Dong is a principal of *MWD Consulting*, which provides training and consulting services in HPLC and UHPLC, pharmaceutical analysis, and drug quality. He was formerly

a Senior Scientist at Genentech, Research Fellow at Purdue Pharma, and Senior Staff Scientist at Applied Biosystems/Perkin-Elmer. He holds a PhD in Analytical Chemistry from City University of New York. He has more than 100 publications and a best-selling book in chromatography. He is an editorial advisory board member of *LCGC* North America. Direct correspondence to: LCGCedit@ubm.com

For more information on this topic, please visit www.chromatographyonline.com/column-perspectives-modern-hplc

Shodex™
CAPTURE THE ESSENCE



OHPak LB Series Flattest Baseline for Light Scattering

Other GFC polymer column baseline
Shodex OHPak LB-806M baseline

shodexhplc.com

Maximization of Selectivity in Reversed-Phase Liquid Chromatographic Method Development Strategies

The selectivity of different combinations of organic modifiers, pH, and types of reversed-phase liquid chromatography (LC) materials has been characterized using Tanaka column characterization, linear solvent energy relationships (LSER), and selectivity correlations. The three characterization techniques highlighted the potential complementary selectivity of these phases and conditions as well as the type and dominancy of some of the retention mechanisms involved. Subsequently, selectivity differences were proven to be valid in the practical separation of acids, bases, and neutral analytes. This paper aims to help chromatographers to produce highly efficient method development strategies for reversed-phase LC separations in a relatively short time frame.

A range of columns are available on the market for small molecule reversed-phase liquid chromatography (LC) method development strategies. These types of columns are purported to possess complementary selectivities in order to maximize the probability of separating all components in a mixture. Reversed-phase LC materials complementary to the ubiquitous and standard C18 include pentafluorophenyl (PFP), phenyl, cyano (CN), extended polar selectivity, and polar embedded group (PEG) phases. In addition to a hydrophobic retention mechanism, these types of phases can offer additional (augmented) retention mechanisms such as π - π , dipole-dipole, dipole-induced dipole, hydrogen bonding, ion-ion, ion-dipole, ion-induced dipole, and steric interactions (1) when compared to conventional C18 phases. Retention of analytes on reversed-phase LC materials is dependent on the dominancy or proportion of each stationary-mobile phase-analyte interaction that occurs.

There is a growing trend within many industries, and particularly within the pharmaceutical sector, to utilize reversed-

phase LC column and solvent switching strategies to rapidly assess which combination of stationary and mobile phase is the most suitable to progress into retention modeling and optimization studies (2).

This article presents an unbiased and in-depth evaluation of a representative range of complementary stationary phases from a single manufacturer that are recommended for reversed-phase LC method development strategies. The five totally porous stationary phases investigated are based on the same silica, utilizing the same monofunctional bonding and endcapping technologies that generate phases of approximately the same surface coverage and hydrophobicity. It is well known that reversed-phase LC materials that possess the same bonded ligand on differing base silica can yield products with differing chromatographic properties (3). Hence, there was a need to limit the study to a single manufacturer's range of materials so that the underlying silica chemistry would not confuse any interpretations and that any differences in chromatographic selectivity observed would be attributed only to the differ-

**Melvin R. Euerby, Mark Fever,
Jennifer Hulse, Matthew James,
Patrik Petersson, and
Colin Pipe**

ences in the type of ligands. It is expected that similar deductions regarding selectivity should be valid in respect of another manufacturer's method development phases. In addition, the use of high-pH-stable phases (both totally porous and superficially porous) has been evaluated in terms of chromatographic selectivity as a function of pH, organic modifier, and stationary-phase chemistry. Empirical-based approaches (that is, principal component analysis [PCA], column characterization, and selectivity correlations) and a model-based approach (linear solvent energy relationships [LSER]) have been employed to provide a scientific rationale for the use of complementary stationary- and mobile-phase combinations.

Experimental

Experimental work was performed on Agilent 1260 and 1290 LC systems. All columns were of 50 mm × 2.1 mm format and a particle size of 2.5 and 3 μm, respectively, for the superficially porous and totally porous materials unless otherwise stated. Table I details the columns investigated. As mentioned previously, columns from the same manufacturer were chosen so that differences in chromatographic selectivity observed would be attributed solely to the differences in the type of ligands and not different silica chemistries from different manufacturers.

Their integrity was assessed periodically throughout the runs by injecting a standard of toluene. pH measurements were recorded in the aqueous fraction of the mobile phase. PCA was performed using Simca-P+ version 11.5 software (Umetrics). To give all variables the same importance, the variables were "auto scaled"; that is, the average was subtracted from each variable and each variable was divided by its standard deviation. Physical properties, logD, and pK_a determinations of analytes were performed using ACD/Percepta v 2015 (ACD/Labs). Multivariable regression analysis, Fisher F test, and selectivity correlations were performed using Microsoft Excel.

Results and Discussion

Column chromatographic properties and hence the selectivity of stationary phases are commonly assessed by comparing the retention differences between analytes of well-defined physical or chemical properties—see reference 4 for a review on column characterization databases and procedures. Popular protocols include the PQRI (5,6), NIST 870 methodology (6,7), University of Leuven (8), the supercritical fluid chromatography method (9), and the Tanaka (10) plus its extended variant (3,11–14) approaches. All of these approaches are equally valid in comparing column selectivity. The extended Tanaka approach determines chromatographic parameters that are associated with hydrophobicity (k_{PB} , α_{CH_2}), steric or shape selectivity ($\alpha_{T/O}$), hydrogen bonding capacity ($\alpha_{C/D}$), ion-exchange capacity at pH 2.7 and 7.6 ($\alpha_{B/P}$), aromatic selectivity ($\alpha_{TNB/T}$), phenolic selectivity ($\alpha_{P/BA}$), and dipole–dipole interactions ($\alpha_{1,2-/1,4-DNB}$).

An alternative approach is that of LSER, which involves a series of analytes of well-defined structural properties chromatographed on each column using either methanol or acetonitrile (15,16). Multiple regression analysis of the analyte's retention against their Abraham's solvatochromic solute parameters (17) allows a number of stationary phase properties to be determined.

Brighter separations

State-of-the-art HPLC Columns for small molecules bringing you reliable, brilliant results



- **Monolithic - Chromolith®**
Low backpressure – Long lifetime – Suitable for dirty samples – Available in micro format
- **Fused core particles - Ascentis® Express** – Efficiency – Peak symmetry – Wide range of phases
- **Fully porous particles - Purospher® STAR** – 100% aqueous phase compatibility – pH stability 1.5 – 10.5 – Column/batch reproducibility – High loadability

sigma-aldrich.com/hplc-columns

MILLIPORE SIGMA

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

Copyright © 2017 EMD Millipore Corporation. All Rights Reserved. MilliporeSigma and the vibrant M are trademarks and Chromolith and Purospher are registered trademarks of Merck KGaA, Darmstadt, Germany. Ascentis is a registered trademark of Sigma-Aldrich Co. LLC. or its affiliates.

Table I: Extended Tanaka parameters for columns from a single manufacturer

Generic Column Name	Column Name	k_{PB}	α_{CH2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{B/P}$ pH 7.6	$\alpha_{B/P}$ pH 2.7	$\alpha_{TNB/T}$	$\alpha_{P/BA}$	$\alpha_{1,2-DNB/1,4-DNB}$
TPP C18	ACE C18	5.44	1.48	1.56	0.38	0.32	0.11	0.16	0.98	0.98
TPP Phenyl	ACE C18-AR	4.50	1.42	1.74	0.75	0.35	0.12	0.62	1.00	0.95
TPP PFP	ACE C18-PFP	4.33	1.38	3.09	0.53	0.30	0.10	0.26	1.20	1.74
TPP Cyano	ACE CN-ES	2.19	1.30	4.57	0.17	0.22	0.05	0.37	1.75	1.85
TPP PEG	ACE C18-Amide	4.09	1.41	2.07	0.26	0.21	0.08	0.24	1.62	1.14
High pH TPP C18	ACE SuperC18	7.93	1.49	1.46	0.44	0.18	0.11	0.16	0.99	1.18
High pH SPP C18	ACE UltraCore SuperC18	6.33	1.49	1.46	0.39	0.32	0.09	0.14	0.99	1.19
High pH SPP Phenyl	ACE UltraCore SuperPhenylHexyl	2.63	1.36	1.11	0.68	0.32	0.09	0.62	1.00	1.15

TPP = Totally porous particle (ACE range)
SPP = Superficially porous particle (ACE UltraCore range)



THE BEST SURFACE FOR ANY HPLC FLOW PATH

Get drastically better reliability, sensitivity, and uptime from any LC system by coating the entire flow path with **Dursan**[®]. Our patented surface technology completely eliminates unwanted surface interactions and ensures sample integrity in the toughest conditions.

- **Better reliability** - bio-inert for superior resolution and accuracy
- **Longer column lifetime** - corrosion resistant in any pH environment
- **More uptime** - proteins and other bio-molecules won't stick to the surface



Game-Changing Coatings™

LEARN MORE:
SilcoTek.com/LC

An assessment of the chromatographic selectivity between two sets of stationary- and mobile-phase conditions can be conveniently derived from the correlation of the retention times of a structurally diverse set of analytes chromatographed using both LC conditions (18).

This article will describe the characterization of a range of complementary reversed-phase LC phases (based on the same base silica) using the extended Tanaka, chromatographic selectivity, and LSER approaches. It will also assess the practical usability of these reversed-phase materials in method development strategies.

Tanaka Column Characterization Approach: PCA (19,20) is a general tool for interpretation of large data tables. In PCA the number of variables (the column characterization parameters) is reduced by a projection of the objects (the stationary phases) onto a smaller number of new variables termed principal components (PC). The PCs are orientated so that the first PC describes as much as possible of the original variation between the objects. The second PC is orientated in an orthogonal manner to the first PC and is directed to describe as much as possible of the remaining variation and so on. The projection of objects onto a PC is called scores—by plotting the scores for two PCs it is possible to graphically find similarities and differences between objects. The distance between objects in a score plot shows if they are similar or different. How much of each of the original variables that are included in a PC is described by so-called loadings, one for each variable. By plotting the loadings for the two PCs and superimposing it onto the score plot, it is possible to see which of

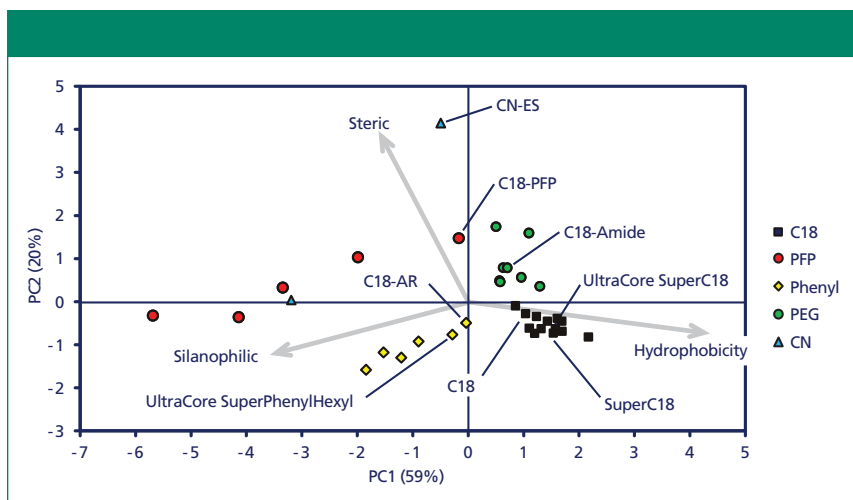


Figure 1: PCA score and loading plot of a representative range of phases using the standard Tanaka column characterization protocol. The Tanaka column characterization data have been taken from the ACD database (21).

the original variables are the most important (the longest distance from the origin) and if any variables are correlated (the same or opposite directions on a straight line through the origin). The reason why two objects are different can easily be determined with a so-called contribution plot. This type of plot shows which

variables (that is, the chromatographic parameters) cause a difference between two objects (the stationary phases).

From the PCA score plot (see Figure 1) of 13 × C18, 5 × PFP, 6 × phenyl, 8 × PEG, and 2 × cyano stationary phases, from a range of manufacturers (both superficially [SPP] and totally porous

particles [TPP] of 2.5–3.5 μm), based on their Tanaka column characterization data (column details can be supplied by the authors on request) the heterogeneous nature of the PFP, phenyl, PEG, and cyano class of phases can be clearly seen as demonstrated by the distance between them. This is attributed to the difference in silica, pore size, bonding functionality, type of endcapping, and, more importantly, differences in the silanes used. For example, it has previously been shown that the length of the alkyl spacer between the silica and the phenyl moiety can have a profound effect on the resultant chromatographic properties of the phase (11). The PCA results also highlight the diversity within different classes of phases as reported previously for PFP (12) and PEG (13). In contrast, the new generation C18 phases appear to be clustered more closely together, highlighting their more similar chromatographic properties.

The importance of using the same base silica was highlighted in Figure 2 in which identical C18 bonding was applied to a Type A and Type B silica. The acidic nature of the Type A silica compared to

Because your lab is **STRONGER** with Agilent

STRONGER... Research Results, Performance, Consistency, Confidence, Reproducibility, Resolution

Analytical scientists and clinical researchers worldwide rely on Agilent products to help fulfill their most complex laboratory demands. Whether your lab is engaged in environmental testing, pharmaceuticals, petrochemicals, academic research, medical diagnostics or food testing, Agilent's solutions will meet your full spectrum needs and improve efficiencies throughout the entire laboratory.



For more information, contact Neta Scientific today, call (800) 343-6105 or email sales@netascientific.com.



Neta Scientific
LAB SUPPLIES AND SOLUTIONS

NETASCIENTIFIC.COM



Agilent Technologies

Authorized Distributor

the Type B silica produced a markedly different selectivity and even caused irreversible adsorption of the bases remacemide and nortripyline (peaks 4 and 6) onto the Type A silica C18 phase. Therefore, if meaningful interpretations regarding ligand–mobile phase–analyte interactions are to be made, then the ligands must be bonded onto the same base silica.

The PCA score plot (Figure 3) of the six TPP (3 μm) and two SPP (2.5 μm) phases from a single manufacturer using the extended Tanaka parameters (see

Table 1) illustrates that these phases possess complementary chromatographic properties as demonstrated by the distance between them. However, the two phenyl phases (the TPP phenyl and the high-pH SPP phenyl) are chromatographically similar overall but very different from their porous and superficially porous C18 phases counterparts. The extended pH range phases, while being chromatographically similar to their conventional versions, have the added advantage that they can be employed with

high-pH mobile phases.

PCA contribution plots of each complementary phase against the standard C18 phase highlight the chromatographic differences that occur between them (data not shown). For example, the phenyl phases possessed additional aromatic selectivity (that is, π - π interactions as shown by increased $\alpha_{\text{TNB/T}}$ and $\alpha_{\text{C/P}}$ parameters [in addition to assessing hydrogen bonding selectivity, the latter term also assesses the π - π interaction of caffeine with phenyl moieties]); the PFP phase additional dipole–dipole ($\alpha_{1,2-/1,4\text{-DNC}}$), apparent steric/shape selectivity ($\alpha_{\text{T/O}}$), and aromatic selectivity; the PEG phase additional phenolic selectivity ($\alpha_{\text{P/BA}}$) and decreased silanophilic interactions ($\alpha_{\text{B/P}}$); the CN phase additional dipole–dipole, apparent steric/shape selectivity, phenolic selectivity, and decreased silanophilic interactions; and the high-pH-stable TPP C18 possessed enhanced hydrophobicity (k_{PB} , α_{CH_2}), and decreased silanophilic activity at pH 7.6 compared to the standard C18 phase.

Linear Solvent Energy Relationships (LSER): The retention of the eight phases from one manufacturer was characterized by employing LSER using Abraham's solvatochromic solute parameters (17) and studying the retention of 30 disparate solutes (22,23). The stationary phases were investigated with either methanol–water or acetonitrile–water gradients containing 20 mM ammonium formate at pH 3.

Retention time (t_{R}) in gradient reversed-phase LC can be correlated with various fundamental solute properties as described in equation 1 (22):

$$t_{\text{R}} = c + eE + sS + aA + bB + vV \quad [1]$$

where capitals indicate the solute properties while the properties of the separation conditions (system coefficients) are indicated by lower case letters in italics. The contribution from electron pair interactions is defined by eE , interactions of a dipole type by sS , hydrogen bond interactions aA and bB , and differences in cavity formation and dispersion interactions in the mobile and stationary phase by vV . Solute descriptors are E , excess molar refraction; S , dipolarity/polarizability; A , effective hydrogen bond acidity; B , effective hydrogen bond basicity; and V , McGowan's volume. The

Get Better Sensitivity by superior mixing with less volume



Perfect PEAK™

The ultimate in-line static mixer



MIXER90

Internal Volume: 90 μL

Noise Reduction: up to 98%

- › Industry-leading baseline noise reduction
- › Significantly less mixing volume than comparable mixers
- › Maintains flow efficiency from 0.1-10 mL/min
- › Easily installed in any HPLC/UHPLC unit
- › Passivated all stainless steel construction
- › Bio-inert Titanium option coming soon

Also available in 30 μL and 60 μL .
Mixers stackable up to 180 μL
for >99% noise reduction.

mott
MISSION CRITICAL PRECISION

www.mottcorp.com/mixer | info@mottcorp.com

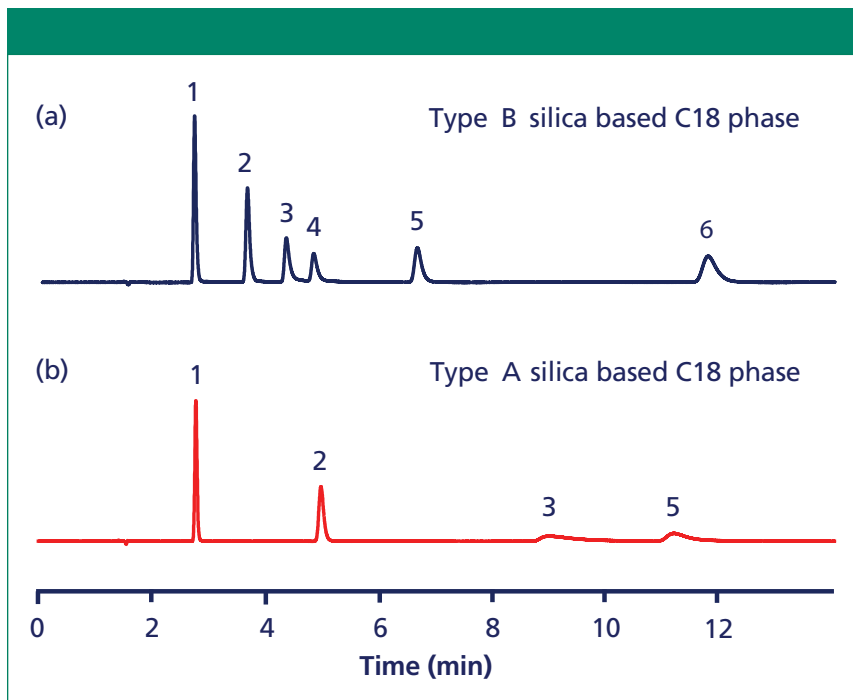


Figure 2: Isocratic chromatography of five basic analytes plus phenol as a neutral analyte using 20 mM KH_2PO_4 , pH 2.7, in 54.5:45.5 (v/v) water–methanol, 1 mL/min, 60 °C, $\lambda = 210$ nm, 150 mm \times 4.6 mm, 5- μm . (a) Type B silica–based C18, (b) Type A silica–based C18. Peaks: 1 = phenol, 2 = proprietary base X, 3 = proprietary base Y, 4 = remacemide, 5 = diphenhydramine, 6 = nortriptyline.

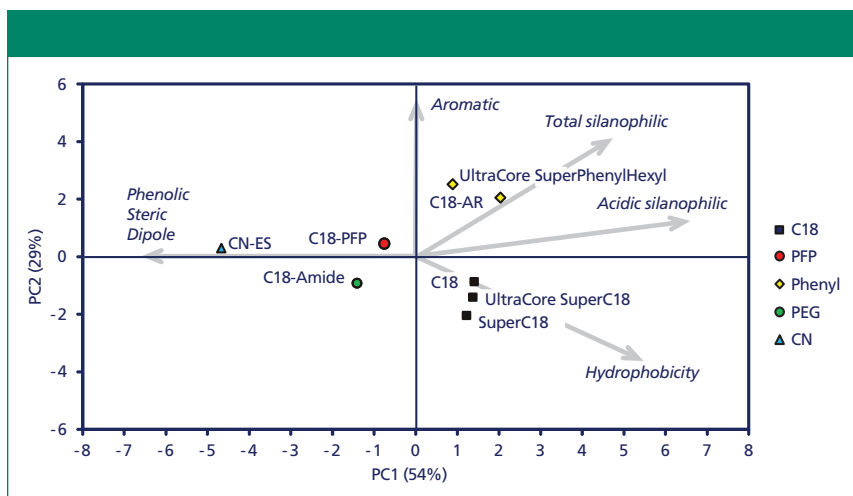


Figure 3: PCA score and loading plot of the phases using the extended Tanaka column characterization protocol.

intercept c and the regression coefficients e , s , v , a , and b are characteristic of the particular mobile and stationary phase combination and are obtained by multi-variable linear regression of the retention times against the solute descriptors.

LSER regression coefficients and their corresponding statistical descriptors using equation 1 were determined for the eight stationary phases using either methanol or acetonitrile. The goodness of fit of

the equations was acceptable ($r^2 > 0.95$) and the validity of the regression hypothesis was demonstrated for all column and mobile phase combinations by the Fisher F -test ($F > 90$) (Data can be supplied by the authors on request). By comparing the coefficients of equation 1, the relative effects of different stationary phase properties can be revealed and thus assist in the understanding of which retention mechanism is responsible for the comple-

MARKES

international

World leaders in
thermal desorption
for environmental
air monitoring

Since 1997
20
YEARS
ANNIVERSARY



Tailor your TD system to your requirements – canisters, on-line and sorbent tubes



- Maximise efficiency by running VVOC to SVOC on one platform
- Sample splitting and re-collection for easier validation and method compliance
- Reduce your costs – avoiding use of liquid cryogen

Find out more
<http://chem.markes.com/XR>



A company of the **SCHAUBURG** International Group

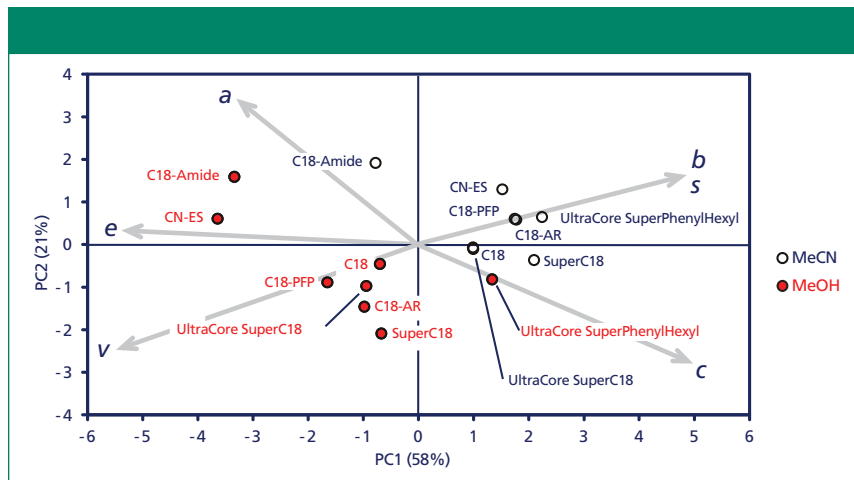


Figure 4: PCA score and loading plot of the LSER coefficients for the columns evaluated as a function of organic modifier used.

mentary selectivity observed between these reversed-phase LC materials.

Highlights from the LSER study are given below. A positive coefficient indicates that the respective molecular interaction is stronger in the stationary than in the mobile phase and hence increases retention. This is the case for *v* (measurement of cavity formation and dispersive

interactions) and *e* (n- and π -electron interactions) coefficients. The magnitude of these two coefficients indicates that cavity formation together with dispersion is far more critical than electron-involved type interactions. A negative coefficient indicates molecular forces are more favorable in the mobile phase and hence decrease retention. Hydrogen

bond donor and acceptor interactions and dipole-type forces fall into this class. The size of the coefficients highlights that solute molecular size and hydrogen bonding acceptor-basicity are the two main dominant retention controlling factors.

The *e* coefficient describes the solvated stationary phase and mobile phase ability to interact with solutes possessing n- and π -electrons. The larger *e* coefficients for the PEG, phenyl, CN, and PFP phases (compared to the C18 phase) suggest that solutes capable of donating n- and π -electrons are retained longer on these phases.

The PEG phase exhibited enhanced hydrogen bond basicity (a coefficient) than the other phases as a consequence of the amide functionality embedded in the silane ligand.

The phenyl and PFP phases displayed a greater ability to interact with solutes possessing π -electrons (*e* coefficient) via the phenyl and pentafluorophenyl functionality than the C18 phase.

When the value of these terms in either methanol or acetonitrile was analyzed



CANNABIS
SCIENCE
CONFERENCE

August 28-30, 2017

Oregon Convention Center, Portland, Oregon

CannabisScienceConference.com

JOIN US for exciting cannabis keynotes, presentations, round tables and exhibits!

The CANNABIS SCIENCE CONFERENCE pulls together cannabis industry experts, instrument manufacturers, testing labs, research scientists, medical practitioners, patients, policy makers and interested novices. Grow with us in 2017! *email Josh@jcanna.com*



PREMIER MEDIA PARTNER:



FOUNDING SPONSORS:



For a full listing of sponsors and exhibitors, please visit CannabisScienceConference.com

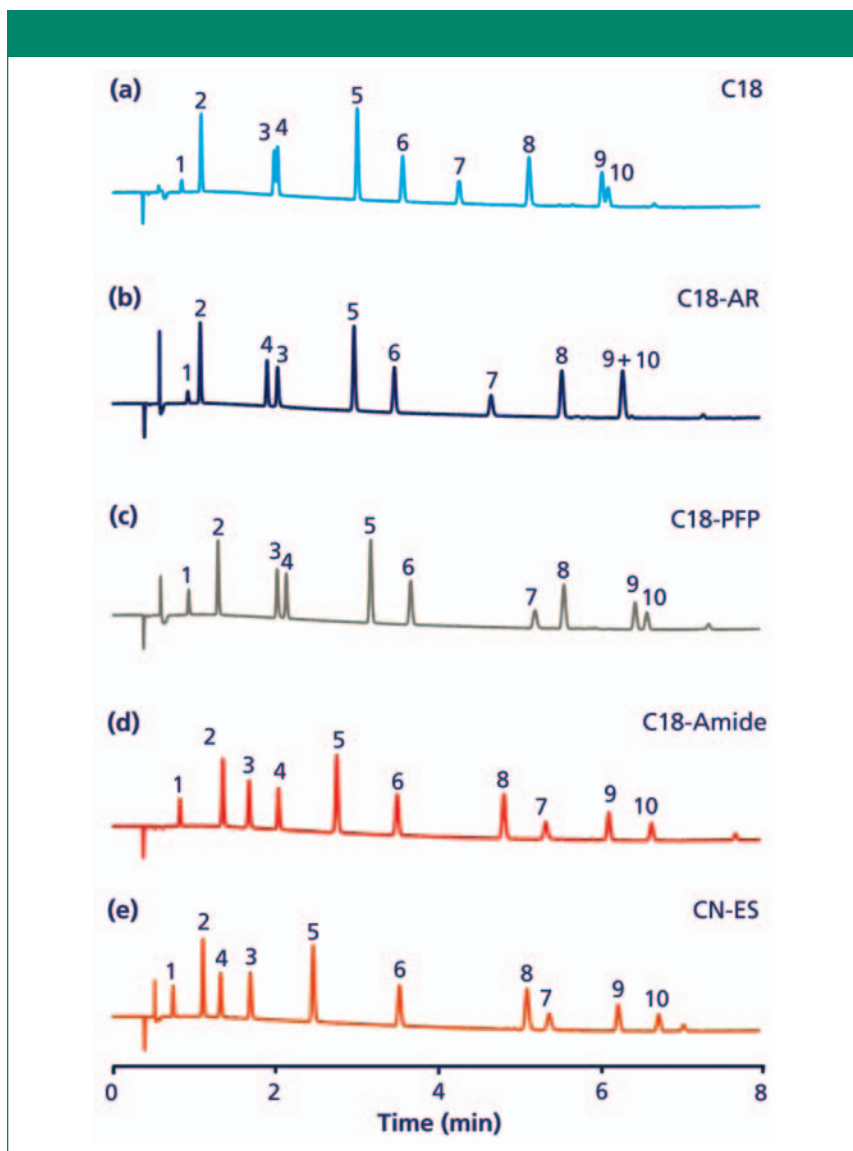


Figure 5: Gradient chromatography of paracetamol and related compounds on (a) ACE C18, (b) ACE C18-AR, (c) ACE C18-PFP, (d) ACE C18-Amide, and (e) ACE CN-ES phases (100 mm × 3 mm, 2- μ m) gradient 5–95% B in 10 min, 1.2 mL/min, 40 °C, λ = 210 nm. Mobile-phase A: 20 mM ammonium acetate in water, pH 6; mobile-phase B: 20 mM ammonium acetate, pH 6, in 9:1 (v/v) methanol–water. Peaks: 1 = 4-aminophenol, 2 = hydroquinone, 3 = 2-aminophenol, 4 = paracetamol, 5 = 2-acetamidophenol, 6 = phenol, 7 = 4-nitrophenol, 8 = 2-nitrophenol, 9 = 4-chloroacetanilide, 10 = 4-chlorophenol.

by PCA the score plot demonstrated that larger selectivity differences were observed in methanol compared to acetonitrile (Figure 4). Hence, in method development strategies, it is strongly recommended that methanol be evaluated if possible, in addition to the ubiquitously used acetonitrile.

Selectivity Correlations: A measurement of chromatographic selectivity between two sets of stationary- and mobile-phase conditions can be conveniently derived from the correlation of the retention time of a set of structurally

diverse analytes chromatographed using both sets of LC conditions.

The selectivity correlations were determined using equation 2, where r^2 = correlation coefficient between the retention times of the two phases under specified LC conditions (18). S values of 0 and 100 signify columns of equal and orthogonal retention selectivity:

$$S = 100 \sqrt{1 - r^2} \quad [2]$$

A range of 45 analytes (10 acids, 11 bases, 14 neutrals, and 10 phenols) of differing physical properties was selected

(the diversity of the set of analytes was proven by PCA score plots that highlighted the wide difference between the different classes of analytes and also within each class—data not shown) to produce the selectivity values shown in Table II.

Table II demonstrates the similarity between TPP C18 and high-pH TPP and SPP C18 phases; for example, S values of < 9 were observed between the extended pH range and the conventional C18 phases in methanol and acetonitrile; this is also true for the TPP phenyl and high-pH SPP phenyl phases. In comparison, S values between 15 and 25 are observed between the C18 phase and the four complementary phases in methanol, while the range is 11–20 for acetonitrile, suggesting reduced selectivity differences in acetonitrile compared to methanol.

The importance of organic modifier was highlighted by S values of between 17 and 30 being obtained when methanol and acetonitrile were compared with the same phases. Evaluating both stationary phase and organic modifier combinations generated S values in the range of 17 to 37, highlighting the usefulness of evaluating both stationary phase and organic modifier selectivity parameters in method development strategies.

The selectivity differences between the C18 and phenyl containing TPP and SPP phases were maintained (TPP, S = 19 and 13 in methanol and acetonitrile, respectively; SPP, S = 18 and 13 in methanol and acetonitrile, respectively), which should give the chromatographer reassurance that complementary selectivity can be maintained and exploited in either TPP or SPP range of phases.

There appeared to be only minor selectivity differences (S = 6 in either methanol or acetonitrile) between the TPP and SPP C18 phases that should be highly beneficial in method translation exercises. However, the high-pH SPP C18 possesses reduced hydrophobicity compared to its TPP variant (k_{PB} = 7.93 compared to 6.33 for the TPP and SPP, respectively). This may, in part, be attributed to their difference in surface area. The same is true for the TPP phenyl and high-pH SPP phenyl phases because low selectivity values of 8 and 4 were obtained in methanol and aceto-

GC-Orbitrap™ MS Technology for Pesticides Analysis



Amadeo R. Fernández-Alba, PhD
Professor, European Reference
Laboratory for Pesticide Residues
in Fruits and Vegetables, Analytical
Chemistry Dept.
University of Almería, Spain

Comprehensive testing for the presence of pesticide residues in food requires the application of both liquid chromatography (LC) and gas chromatography (GC) coupled to mass spectrometry. In particular, interest is growing in the use of full-scan High-Resolution Accurate Mass (HRAM) mass spectrometry (MS) such as the Thermo Scientific™ GC Orbitrap™ MS system for both the quantitation of target pesticides and non-targeted screening of unexpected pesticides, simultaneously in a single workflow. Historically, developments in GC-HRAM-MS have lagged behind those in LC-HRAM-MS, but the adoption of GC-HRAM-MS has been increasing recently. *LCGC* spoke with Prof. Amadeo R. Fernández-Alba, PhD, professor at the University of Almería in Spain, to share his thoughts on developments in GC-HRAM-MS technology and the prospects for the use of these powerful instruments for routine analysis in the future.

LCGC: Can you give us a brief overview of your experience with GC-HRAM-MS technology for pesticides analysis?

Fernández-Alba: We started working with time-of-flight high-resolution mass spectrometry (ToF-HRMS) instruments about 17 years ago. Around that time, in addition to teaching analytical chemistry, I founded the Agroalimentación (AGR) research group at the University of Almería in 1996 with the goal of facilitating the commercialization of fruits and vegetables in Europe. While the quality of ToF-HRMS data was good, retrieving and evaluating the information was time consuming and labor intensive. In addition, the mass accuracies at that time were much lower than those that can be achieved today. Since then, mass spectrometry platforms have evolved tremendously, especially with the development of the Thermo Scientific™ GC-Orbitrap™ technology enabling GC-High Resolution Accurate Mass (HRAM) mass spectrometry (MS). Important upgrades have improved systems' sensitivity, selectivity, the quantitation capabilities, and offered more comprehensive and faster software for data treatment. All these improvements allow HRMS and HRAM technologies to work with similar workflows as triple-quadrupole mass spectrometry (triple-quad MS) while offering several advantages.

LCGC: What do you consider to be the main benefits of modern GC-HRAM-MS technology?

Fernández-Alba: The first advantage is that it works in full scan every time. This capability means that we cannot only detect or identify targeted compounds, but we can also determine extra components that we did not predict we would find in our sample.

The second advantage, which is very important for routine labs, is that we can include new components within the scope of the method more easily than with triple-quad MS. In the case of triple-quad MS, we must target preselected ions to determine the voltage needed to get transitions. With GC-Orbitrap MS systems, we can work in full scan with electron ionization, which means that the inclusion of new components in the analysis is very fast and easy.

It's also important to consider that in the last year, companies have introduced new instruments that are even more sensitive than others previously available. The sensitivity that we can achieve with GC-Orbitrap MS technology is now similar to that of triple-quads.

SPONSORED BY

ThermoFisher
SCIENTIFIC

LC|GC
north america

LCGC: Please describe some specific applications of modern GC-HRAM-MS technology that you are most excited about.

Fernández-Alba: Pesticide residue analysis is one for sure important because full-scan analysis is always a benefit for identification and management of the analytical scope.

What is probably the most impressive of all new applications are those related to situations where the target list of analytes is very extensive and a non-target analysis approach is necessary. Also, laboratories can use the technology to improve the overall scope of their analytical capabilities. For instance, this might be useful for analyzing food contact materials or investigating food fraud.

One must also consider the accurate mass aspect of GC-Orbitrap MS technology. Electron ionization (EI) makes analysis with this technology possible without having to obtain MS² (MS/MS) data. This makes the analysis simpler and easier with identification criteria based on mass accuracy and ion ratio of the EI fragment ions.

LCGC: Are you aware of any limitations with using GC-HRAM-MS?

Fernández-Alba: I don't see any disadvantages at the moment. GC-HRAM-MS is a new technology for many routine labs, and it takes time to become proficient in different types of instrumentation. At the moment, the gold standard is triple-quad MS instruments. As I mentioned earlier, the sensitivity that is now possible with new GC-Orbitrap MS technology is on par with that of triple-quads, which will help speed the introduction of this new technology into laboratories.

It's only a matter of time before lab personnel will be trained in GC-HRAM-MS, and I don't anticipate difficulties with analysts adopting this new technology.

LCGC: Do you know the percentage of laboratories currently using full-scan GC-HRAM-MS and LC-HRAM-MS for pesticides analysis in Europe and the rest of the world?

Fernández-Alba: In Europe, about 20 percent of pesticide residue control laboratories are currently working with HRAM and HRMS, and these groups are primarily using it for LC.

The introduction of HRAM-MS for GC has not happened at the same rate, so the percentage of labs working with GC-HRAM-MS is much lower than LC-HRAM-MS.

Some labs may be under the outdated impression that GC-HRAM-MS is too expensive or too limited to warrant its investment. One point to consider is that typically all new technology is more expensive in the first few years after its launch because of the large R&D efforts required to advance the market in a new direction. After the initial thrust, costs are often dramatically reduced. This is also the case for HRAM-MS. Investment in this technology was once quite high in comparison to TQ. Recently, we have seen the introduction of more affordable GC-HRAM-MS instrumentation,

the Thermo Scientific™ Exactive™ GC Orbitrap GC-MS system, designed specially for routine analysis. As more laboratories become interested in using all the benefits of this new technology, the costs will hopefully be reduced even more.

In non-European countries, I don't have a clear idea of the percentage of laboratories that have adopted HRMS and HRAM-MS. I recently returned from visiting several countries in Latin America, and I would guess that the level of adoption is much lower there—around two or three percent. It is clear, however, that laboratories are very interested in taking advantage of this new full-scan technology, so I believe that the adoption of the technique in the rest of the world will soon be like that of Europe.

LCGC: Do you think GC-HRAM-MS technology will become as popular as triple-quadrupole technology for pesticides analysis?

Fernández-Alba: The analytical performance of these instruments is comparable to triple-quad technology, and GC-Orbitrap MS technology has several advantages derived from the combination of its high resolution and excellent mass accuracy. It's important to note that high resolution and high mass accuracy do not automatically go hand in hand for all technologies. This is an important consideration for laboratories because high resolution is only useful if the instrument also offers accurate mass, which is the case with GC-Orbitrap MS systems. If these two characteristics are not present in combination, one may not be able to achieve the desired results. So, yes, I believe GC-HRAM-MS will achieve similar popularity to that of triple-quad technology in routine laboratories.

LCGC: Laboratory guidelines relating to HRAM-MS are not harmonized worldwide. Do you have any thoughts on prospects for harmonization?

Fernández-Alba: In the area of pesticide residue analysis, we work with especially strict controls and guidelines for the identification of components. But, it's important to note that both HRAM-MS and triple-quad MS provide data that comply with regulatory guidelines. In the case of quantitation, the requirements are the same for both technologies. New GC-Orbitrap MS platforms completely fulfill regulatory identification criteria satisfied by triple-quad MS.

Unfortunately, laboratories do not have a clear understanding about this issue, which relates to the fact that HRAM-MS is a relatively new platform for routine laboratories. Furthermore, there is currently no clear harmonization among guidelines worldwide, which some may see as a limiting factor in the acceptance of this new platform. We are working to improve this. In general, several international guidelines are very similar, so it's only a matter of producing more information and more data to adjust and harmonize the guidelines for everyone.

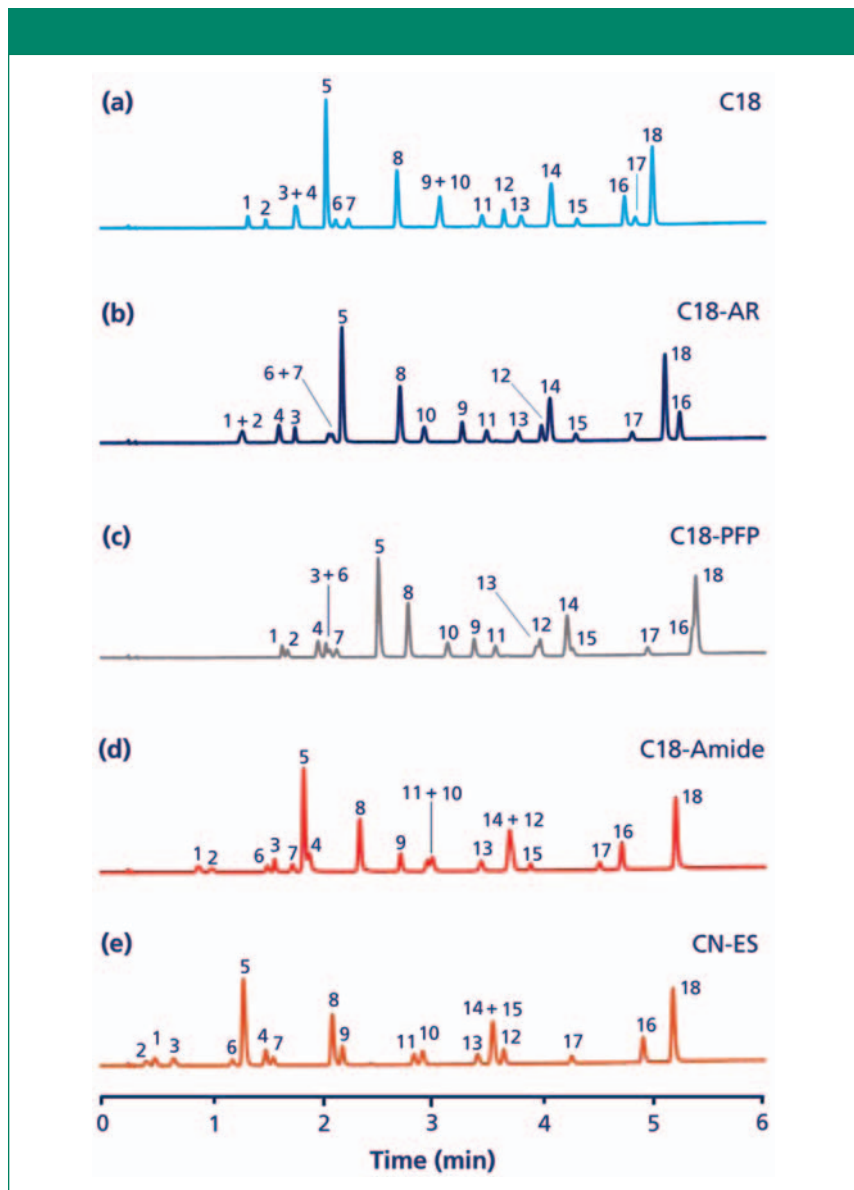


Figure 6: Gradient chromatography of 18 pharmaceutically relevant compounds on (a) ACE C18, (b) ACE C18-AR, (c) ACE C18-PFP, (d) ACE C18-Amide, and (e) ACE CN-ES phases (50×2.1 mm, $5\text{-}\mu\text{m}$) gradient 3 to 100% B in 5 min, 0.6 mL/min, 60°C , $\lambda = 214$ nm. Mobile-phase A: 20 mM KH_2PO_4 , pH 2.7 in water; mobile-phase B: 20 mM KH_2PO_4 , pH 2.7 in 35:65 (v/v) water–methanol. Peaks: 1 = sotalol, 2 = salbutamol, 3 = *N*-acetylprocainamide, 4 = 3-hydroxybenzoic acid, 5 = pindolol, 6 = methylphenylsulfonide, 7 = benzyl alcohol, 8 = quinoxaline, 9 = quinine, 10 = *p*-cresol, 11 = 1,2-dimethoxybenzene, 12 = furoseamide, 13 = anisole, 14 = methylbenzoate, 15 = remacemide, 16 = nimesulide, 17 = ethylbenzoate, 18 = diflunisal.

nitrile respectively. Despite their similar selectivities, the SPP phenyl phase possesses significantly reduced hydrophobicity ($k_{\text{PB}} = 4.50$ compared to 2.63 for the TPP and SPP phases, respectively).

Stationary-Phase Selectivity Examples: An evaluation of the selected method development phases by either the Tanaka column characterization, LSER, or selectivity values has demonstrated that they provide the chromatographer with a range of reversed-phase columns

of complementary chromatographic selectivity to maximize the probability of achieving the desired separation. The following three examples highlight what these observed chromatographic differences equate to in practical complementary chromatographic selectivity using a range of typical gradient and isocratic liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography–ultraviolet (LC–UV) conditions (differing gradient time, temperature,

organic modifier, and low- to mid-pH conditions).

Example 1: Figure 5 highlights the selectivity differences between the five TPP stationary phases using “LC–MS friendly generic gradient conditions” (that is, methanol–ammonium acetate pH 6) in the separation of 10 paracetamol-related compounds. Three out of the five stationary phases generated separation of all 10 components. The PEG phase gave the best overall separation using these generic non-optimized conditions. It was of interest to observe that peaks 8 (2-nitrophenol) and 7 (4-nitrophenol) eluted in the order 7 before 8 in the cases of the PFP, C18, and phenyl phases while the reverse elution order was observed for the PEG and CN phases. This must, in part, be due to the 4-nitrophenol’s higher hydrogen bonding acidity, which can interact with the PEG and CN phases (that is, enhanced $\alpha_{\text{P/BA}}$ and α coefficient). The CN and phenyl phases also caused an elution reversal of peaks 3 (2-aminophenol) and 4 (paracetamol) compared to the other three phases.

Example 2: Figure 6 highlights the chromatographic selectivity differences between the five TPP phases using the UV popular conditions of a methanol–potassium phosphate pH 2.7 generic gradient in the separation of 18 pharmaceutically relevant compounds (including acids, bases, and neutral components).

While it is of academic interest to hypothesize and understand which retention mechanism is responsible for the enhanced retention, from a practical viewpoint only, these chromatograms quickly inform the chromatographer of the number of peaks that are in the sample and that no one phase can separate all the components under these conditions. However, in this example, the C18 and phenyl phases are very good starting points for further retention modeling and optimization studies. It can be clearly seen in the C18 chromatogram that peaks 1 and 2 are separated while 3 and 4 are coeluted; in comparison peaks 1 and 2 are coeluted and 4 and 3 are separated on the phenyl phase.

Example 3: Figure 7 highlights the chromatographic selectivity differences between the five TPP phases using an isocratic methanol–water separation

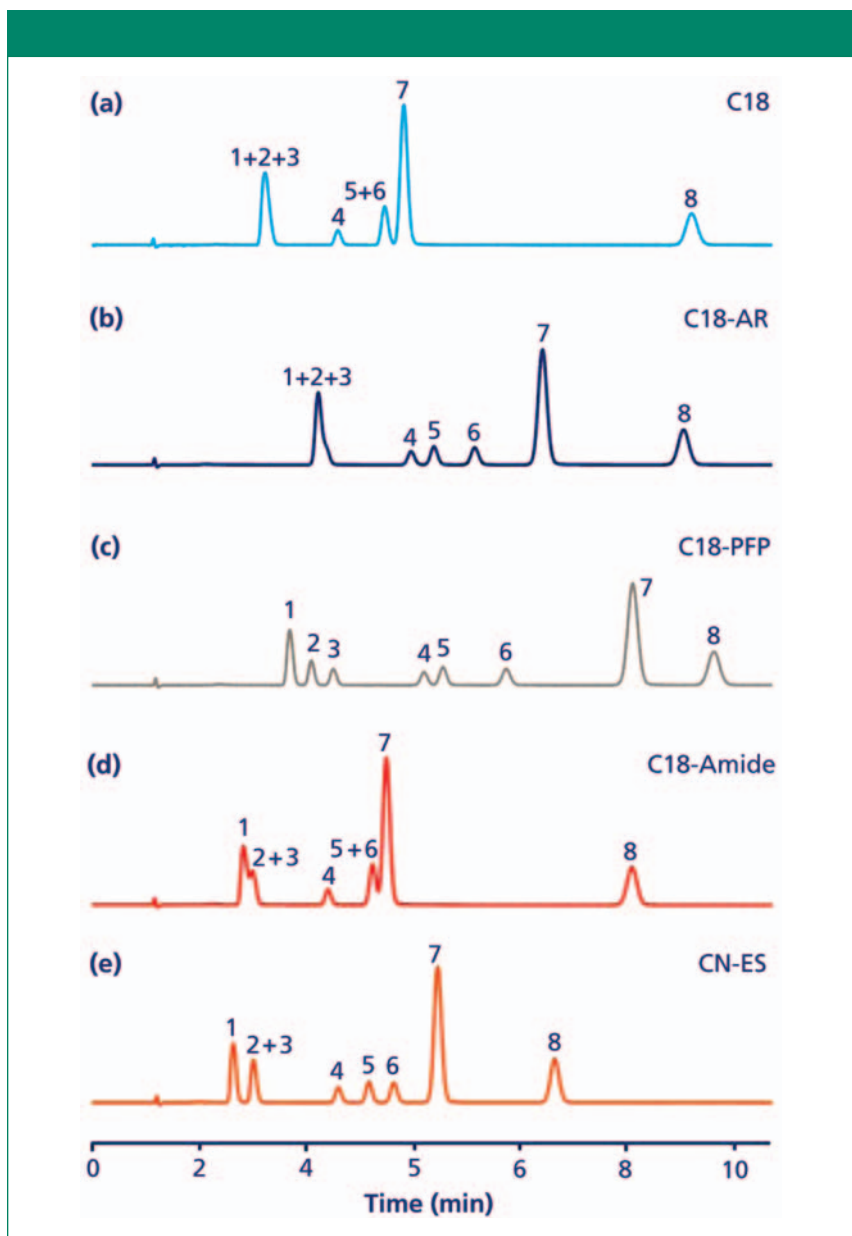


Figure 7: Isocratic chromatography of mono-, di-, and trimethoxybenzene regioisomers using 1:1 (v/v) methanol–water, 0.21 mL/min, 40 °C, $\lambda = 210$ nm, 50 mm \times 2.1 mm, 3- μ m column. (a) ACE C18, (b) ACE C18-AR, (c) ACE C18-PFP, (d) ACE C18-Amide, and (e) ACE CN-ES. Peaks: 1 = 1,2,3-trimethoxybenzene, 2 = 1,2-dimethoxybenzene, 3 = 1,2,4-trimethoxybenzene, 4 = 1,4-dimethoxybenzene, 5 = methoxybenzene, 6 = 1,3-dimethoxybenzene, 7 = 1,3,5-trimethoxybenzene, 8 = toluene.

of a range of methoxybenzene regioisomers under the specified conditions. It is clear that the hydrophobic retention mechanism of the C18 phase alone is insufficient to differentiate and separate these components. Only the PFP phase successfully separated all the components. The C18, phenyl, and PEG phases did not provide complete separation of the 1,2,3- from the 1,2,4-trimethoxybenzene regio-isomer, while the PFP and CN phases afforded excellent separation. It is believed that

the high steric or shape selectivity term ($\alpha_{T/O}$) associated with the later phases is responsible for the separation of these regio-isomers. In comparison, the 1,2-, 1,4-, and 1,3-dimethoxybenzene regioisomers were easily separated on all the phases. Examples 1, 2, and 3 show that significant selectivity differences can be practically observed with S values between 10 and 25.

Mobile-Phase pH Selectivity: Over the last 10 years the chromatographic market place has seen a steady rise in

the number of phases that can withstand high-pH conditions. This is an extremely attractive situation for the chromatographer because the ionization of acidic or basic analytes can now be switched on or off and hence retention controlled. The use of pH can dramatically change chromatographic selectivity as can be seen in the case of the high-pH TPP C18 phase selectivity values: in either methanol or acetonitrile, selectivity values > 80 have been obtained for a set of 45 structurally diverse analytes (containing acidic, basic, neutral, and phenolic components) when low- and high-pH conditions are compared (see Table III). These are extremely high selectivity values compared against the typical values of 10–37 achieved when changes are made to the stationary phase or the organic modifier is changed from methanol to acetonitrile.

The usefulness of this approach can be seen in Figure 8 where the chromatographic elution profile of a mixture of neutral, basic, and phenolic components can be changed at low and high pH (24). The unionized bases (that is, nizatidine, *N*-acetylprocainamide, salbutamol, and reserpine) eluted later at pH 10.7 compared to their ionized forms at pH 3, while the ionized phenolic analytes (methyl parabens and *p*-cresol) were eluted earlier at pH 10.7 compared to pH 3. There was little movement of the neutral species (quinoxaline, piperine, toluene, and felodipine). These movements result in a totally different elution profile as shown in Figure 8. The elution order is not in line with the log D values of the analytes at either pH conditions (see Figure 8 caption), suggesting that there are other retention mechanisms in operation as well as hydrophobicity even on a high-purity C18 phase.

On closer inspection of the retention time correlations for the TPP or SPP version of the extended-pH-range C18 material (see Figure 9 for the SPP version) at low and high pH, it can be seen that the neutral components exhibit a near perfect correlation (S value = 1). The retention time correlations for these phases exhibited excellent complementary selectivity at low and high pH for basic, acidic, and phenolic analytes but not for neutrals.

Table II: Selectivity values at pH 3 for the TPP and SPP stationary- and mobile-phase combinations

		Methanol							
		TPP C18	TPP Phenyl	TPP PFP	TPP Cyano	TPP PEG	High-pH TPP C18	High-pH SPP C18	High-pH SPP Phenyl
Methanol	TPP C18	0	19	15	25	18	7	5	19
	TPP Phenyl		0	15	27	28	18	18	8
	TPP PFP			0	20	19	15	14	16
	TPP Cyano				0	19	23	25	25
	TPP PEG					0	20	20	28
	High pH TPP C18						0	6	17
	High pH SPP C18							0	18
	High pH SPP Phenyl								0
		Acetonitrile							
		TPP C18	TPP Phenyl	TPP PFP	TPP Cyano	TPP PEG	High-pH TPP C18	High-pH SPP C18	High-pH SPP Phenyl
Acetonitrile	TPP C18	0	13	11	15	20	9	6	14
	TPP Phenyl		0	8	17	26	11	12	4
	TPP PFP			0	13	23	9	10	9
	TPP Cyano				0	17	16	16	16
	TPP PEG					0	24	23	26
	High pH TPP C18						0	6	12
	High pH SPP C18							0	13
	High pH SPP Phenyl								0
		Methanol							
		TPP C18	TPP Phenyl	TPP PFP	TPP Cyano	TPP PEG	High-pH TPP C18	High-pH SPP C18	High-pH SPP Phenyl
Acetonitrile	TPP C18	26	26	28	28	31	21	23	24
	TPP Phenyl	33	26	32	31	37	28	30	24
	TPP PFP	31	28	30	27	34	26	29	26
	TPP Cyano	28	28	27	18	29	24	27	25
	TPP PEG	25	28	24	18	17	22	25	27
	High pH TPP C18	32	31	33	31	36	26	29	28
	High pH SPP C18	27	27	29	29	33	23	25	25
	High pH SPP Phenyl	33	26	31	30	37	28	31	21

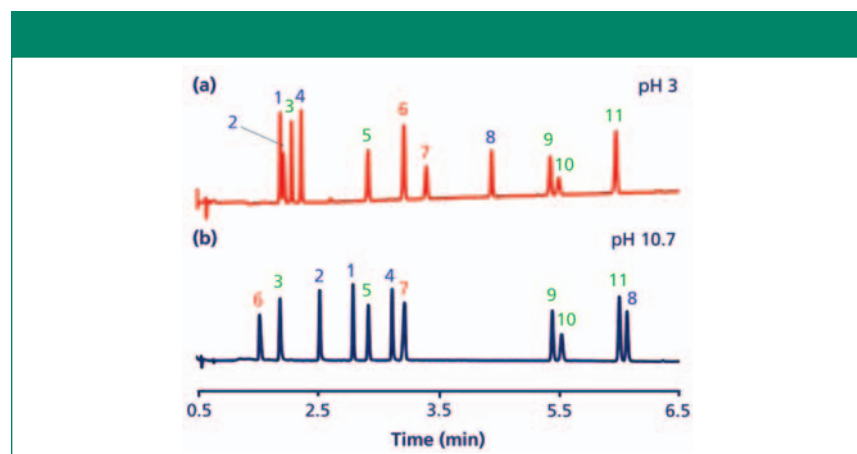


Figure 8: Gradient chromatography of a mixture of 11 bases, phenols, and neutral components on an ACE SuperC18 phase (50 mm × 2.1 mm, 3- μ m column) gradient 3 to 100%B in 7 min, 0.42 mL/min, 40 °C, λ = 254 nm. (a) Mobile-phase A: 10 mM ammonium formate in water, pH 3; mobile-phase B: 10 mM ammonium formate, pH 3, in 90:10 (v/v) acetonitrile–water; (b) mobile-phase A: 18 mM ammonia in water pH 10.7; mobile-phase B: 18 mM ammonia, pH 10.7, in 90:10 (v/v) acetonitrile–water. Peaks (logD at pH 3 and 10.7): 1 = nizatidine (-3.01/0.15), 2 = salbutamol (-2.49/0.15), 3 = amiloride (0.95/1.03), 4 = N-acetylprocainamide (-1.8/1.28), 5 = quinoxaline (1.45/1.45), 6 = methyl paraben (2.14/0.19), 7 = *p*-cresol (2.09/1.48), 8 = reserpine (1.09/4.16), 9 = piperine (2.55/2.55), 10 = toluene (2.61/2.61), 11 = felodipine (4.68/4.86). Peak assignments in red, blue, and green = phenolic, basic and neutral constituents, respectively (24).

As a consequence, many leading stationary phase manufacturers have recently introduced additional stationary phase chemistries that are also stable at high-pH conditions to rectify this deficiency. For example, the high-pH SPP phenyl phase was designed to complement the high-pH SPP C18 phase. Table III highlights that both phases possess excellent selectivity differences at low and high pH (S values > 80) for the same organic modifier. The organic modifier selectivity with both phases at either low or high pH exhibited $S > 23$. The stationary phase selectivity values between the two phases at either low or high pH were observed to be > 11 for acetonitrile and methanol, respectively. The practical significance of these S values is borne out in Figure 10, which illustrates the separation of seven neutral components at low pH with acetonitrile on the high-pH SPP phenyl phase compared to the

Table III: Selectivity values for the TPP and SPP phases at pH 3 and 10.7 in methanol or acetonitrile

	High-pH SPP C18 Acetonitrile pH 3	High-pH SPP C18 Methanol pH 3	High-pH SPP C18 Acetonitrile pH 10.7	High-pH SPP C18 Methanol pH 10.7	High-pH SPP Phenyl Acetonitrile pH 3	High-pH SPP Phenyl Methanol pH 3	High-pH SPP Phenyl Acetonitrile pH 10.7	High-pH SPP Phenyl Methanol pH 10.7	High-pH TPP C18 Acetonitrile pH 3	High-pH TPP C18 Methanol pH 3	High-pH TPP C18 Acetonitrile pH 10.7	High-pH TPP C18 Methanol pH 10.7
High-pH SPP C18 Acetonitrile pH 3	0	25	82	82	13	25	86	85	6	23	82	84
High-pH SPP C18 Methanol pH 3		0	84	80	31	18	87	84	29	6	84	83
High-pH SPP C18 Acetonitrile pH 10.7			0	26	83	84	11	23	84	85	6	24
High-pH SPP C18 Methanol pH 10.7				0	83	84	23	11	83	82	28	9
High-pH SPP Phenyl Acetonitrile pH 3					0	23	85	85	12	28	83	85
High-pH SPP Phenyl Methanol pH 3						0	86	83	28	17	84	84
High-pH SPP Phenyl Acetonitrile pH 10.7							0	22	87	88	12	27
High-pH SPP Phenyl Methanol pH 10.7								0	86	85	24	13
High-pH TPP C18 Acetonitrile pH 3									0	6	83	86
High-pH TPP C18 Methanol pH 3										0	85	85
High-pH TPP C18 Acetonitrile pH 10.7											0	24
High-pH TPP C18 Methanol pH 10.7												0

high-pH SPP C18 under alkaline conditions in methanol. Peaks 2–6 exhibited a completely different elution order on the two systems.

Conclusions

The column characterization procedures investigated have demonstrated measurable differences between reversed-phase LC columns specifically designed for method development purposes. It has been shown that these differences do result in complementary chromatographic selectivity that the method developer can practically exploit in development strategies to fully resolve

separations. These differing reversed-phase materials, which possess additional retention mechanisms to that of hydrophobicity, when used in combination with differing organic modifiers at differing pH values, have been shown to be of great benefit in the practical resolution of moderately complex separations.

The development of robust column and solvent switching technologies with software generated methods and sequences that most of the major instrument manufacturers offer, in combination with complementary columns and mobile phases or pH, now permits the

chromatographer to evaluate a wider and more comprehensive method development space in a relatively short timeframe. In addition, the ability to identify stationary- and mobile-phase combinations with complementary chromatographic selectivity will be of major importance in the rapidly developing field of two-dimensional reversed-phase LC.

Acknowledgments

The authors would like to thank Dr. A.P. McKeown of Advanced Chromatography Technologies Ltd for supplying the ACE range of columns.

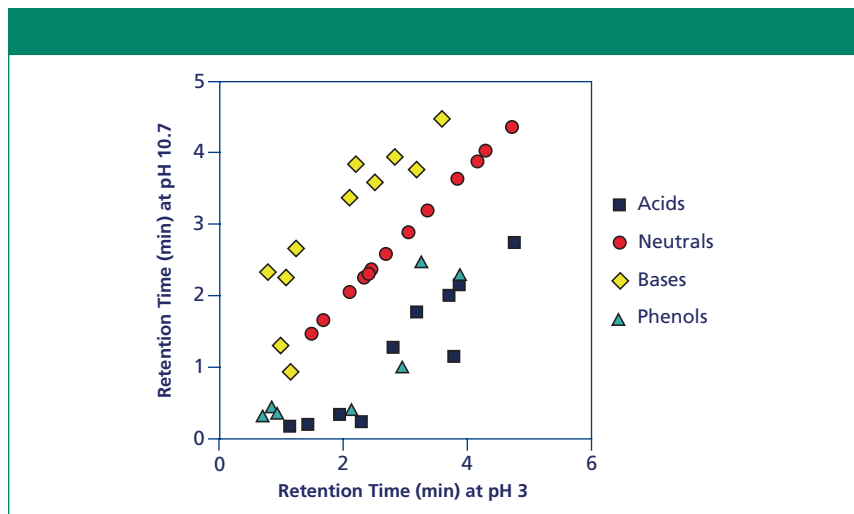


Figure 9: Correlation of 45 analytes (10 acids, 11 bases, 10 phenols, and 14 neutrals) on the ACE UltraCore SuperC18 phase using gradient chromatography (50 mm × 2.1, 3- μ m column) 3 to 100%B in 6 min, 0.6 mL/min, 60 °C. Mobile-phase A: 20 mM ammonium formate, pH 3, in water or 18 mM ammonia, pH 10.7, in water; mobile-phase B: 20 mM ammonium formate, pH 3, in 1:9 (v/v) water–methanol or 18 mM ammonia, pH 10.7, in 1:9 (v/v) water–methanol. Overall S value = 80, for the neutral components S = 1.

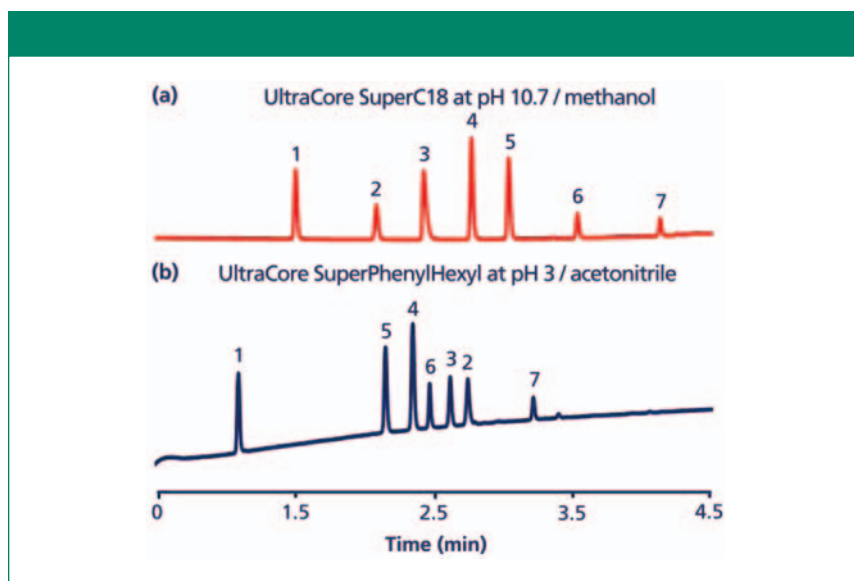


Figure 10: Gradient chromatography of a mixture of seven neutral analytes using a 3 to 100%B in 6-min gradient, 0.6 mL/min, 60 °C, λ = 214 nm. (a) ACE UltraCore SuperC18 phase (50 mm × 2.1 mm, 2.5- μ m column) using mobile-phase A 18 mM ammonia in water, pH 10.7, and mobile-phase B 18 mM ammonia, pH 10.7, in 90:10 (v/v) methanol–water; (b) ACE UltraCore SuperPhenylHexyl phase (50 mm × 2.1 mm 2.5- μ m column) mobile phase A 20 mM ammonium formate in water, pH 3, and mobile-phase B 20 mM ammonium formate, pH 3, in 90:10 (v/v) acetonitrile–water. Peaks: 1 = caffeine, 2 = 1,3,5-trinitrobenzene, 3 = 1,2-dinitrobenzene, 4 = dimethylphthalate, 5 = 1,2,3,4-tetrahydro-1-naphthol, 6 = corticosterone, 7 = piperine.

References

- (1) J.L.E. Reubsæet and K Jinno, *Trends in Analytical Chemistry* **17**, 157 (1998).
- (2) *HPLC Made to Measure. A Practical Handbook for Optimization*, S. Kromidas, Ed. (Wiley-VCH, 2006).
- (3) M.R. Euerby and P. Petersson, *J. Chromatogr. A* **994**, 13 (2003).
- (4) T. Taylor, *LCGC North America* **30**, 442 (2012).
- (5) L.R. Snyder, J.W. Dolan, and P.W. Carr, *J. Chromatogr. A* **1060**, 77 (2004).
- (6) HPLC Column Classification, Pharmacopeial Forum **31**, 637 (2005).
- (7) <https://www-s.nist.gov/srmors/certificates/870.pdf>
- (8) T. Iványi, Y. Vander Heydan, D. Vsičky, P. Baten, J. De Beer, I. Lázár, D.L. Massaet, E. Roets, and J. Hoogmartens, *J. Chromatogr.*

A **954**, 99 (2002).

- (9) E. Lesellier, *J. Chromatogr. A* **1266**, 34 (2012).
- (10) K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Arki, and N. Tanaka, *J. Chromatogr. Sci.* **27**(2989), 721.
- (11) M.R. Euerby, P. Petersson, W. Campbell, and W. Roe, *J. Chromatogr. A* **1154**, 138 (2007).
- (12) M.R. Euerby, A.P. McKeown, and P. Petersson, *J. Sep. Sci.* **26**, 295 (2006).
- (13) M.R. Euerby and P. Petersson, *J. Chromatogr. A* **1088**, 1 (2005).
- (14) C. Markopoulou, T. Tweedlie, D. Watson, G. Skellern, H. Reda, P. Petersson, H. Bradstock, and M.R. Euerby, *Chromatographia* **70**, 705 (2009).
- (15) L.A. Lopez and S.C. Rutan, *J. Chromatogr. A* **965**, 301 (2002).
- (16) M. Reta, P.W. Carr, P.C. Sadek, and S.C. Rutan, *Anal. Chem.* **71**, 3484 (1999).
- (17) M.H.J. Abraham, *J. Pharm. Sci.* **83**, 1085 (1994).
- (18) U.D. Neue, J. E. O’Gara, and A. Mendez, *J. Chromatogr. A* **1127**, 161 (2006).
- (19) L. Eriksson, E. Johansson, N. Kettaneh-Wold, and S. Wold, *Multi- and Megavariate Data Analysis: Principles and Applications* (Umetrics AB, Umeå, Sweden, 2001).
- (20) K. Esbensen, S. Schönkopf, and T. Midtgaard, *Multivariate Analysis in Practice* (Camo AS, Trondheim, Norway, 1994).
- (21) ACD Column selector <http://www.acdlabs.com/resources/freeware/colsel/>
- (22) K. Valkó, M. Plass, C. Bevan, D. Reynolds, and M.H. Abraham, *J. Chromatogr. A* **797**, 41 (1998).
- (23) Á. Sándi and L. Szepeszy, *J. Chromatogr. A* **818**, 1 (1998).
- (24) ACE SuperC18 Brochure, Application No. 1510, <http://www.ace-hplc.com/products/product.aspx?id=2814>, accessed 24 July 2015.

Melvin Euerby is the head of R&D at Hichrom Ltd and professor at the University of Strathclyde and the Open University.

Mark Fever is a research scientist at Hichrom Ltd.

Colin Pipe is a research scientist at Hichrom Ltd.

Jennifer Hulse is an application scientist at Hichrom Ltd.

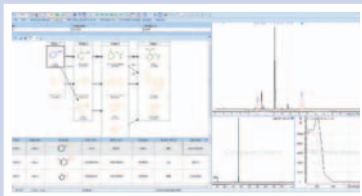
Matthew James is an application Scientist at Hichrom Ltd.

Patrik Petersson is a principal scientist at Novo Nordisk A/S and Associate Professor at Uppsala University. ■

PRODUCTS & RESOURCES

Informatics system

ACD/Labs' LUMINATA informatics system is designed to enable organizations to establish effective impurity control strategies based on assembled analytical and chemical information, in a single environment. According to the company, the system provides data aggregation and visualization for substance impurity characterization.



ACD/Labs,

Toronto, Ontario, Canada.
www.acdlabs.com

Viscosity detector

The ViscoStar III viscosity detector from Wyatt Technology is designed for use in conjunction with gel permeation chromatography systems and multiangle light scattering detectors. According to the company, the detector can determine the molar mass, size, and conformation of all types of macromolecules, including polymers and proteins.

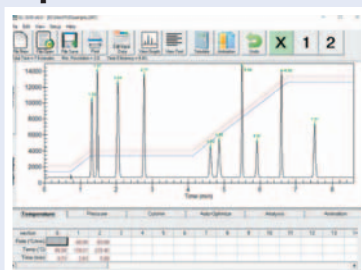
Wyatt Technology,

Santa Barbara, CA.
www.wyatt.com



GC simulation and optimization software

The 4S Company's GC-SOS software is designed for use by GC method developers and instructors. According to the company, in addition to temperature, column size, pressure, and flow simulation, the updated version features flexible input, auto-optimization, and animation view capabilities.



The 4S Company,

Cincinnati, OH.
www.GC-SOS.com

HPLC columns

DAISOGEL Semi-PREP and PREP columns are designed as a scale-up tool for purification scientists. According to the company, the silica gel columns are available in 5- μm and 10- μm particle sizes with various pore sizes and diverse modification phases. Available pore diameters of 100–300 Å are applicable to analytes ranging from small molecules to large peptides.

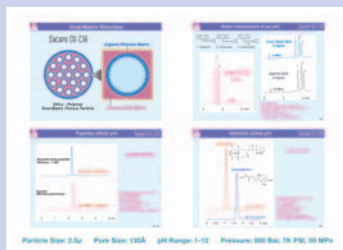
DAISO Fine Chem USA, Inc.,

Torrance, CA.
www.daisogelUSA.com



HPLC column

The Decapo DX-C18 HPLC column from Imtakt is designed with Dual Matrix particle technology. According to the company, the column consists of silica-based particles with a grafted surface polymeric layer to protect the silica from high pH attack and to improve peak shape for basic compounds.



Imtakt USA,

Portland, OR.
www.ImtaktUSA.com

GPC columns

Jordi Gel DVB Resolve 13- μm GPC columns from Jordi Labs are designed with a novel packing material for the high-temperature molecular weight analysis of polyethylene and other polyolefins. According to the company, the column packing is based on 100% divinylbenzene and uses a new synthetic process that results in a monodisperse polymer column packing.

Jordi Labs, Mansfield, MA. jordilabs.com

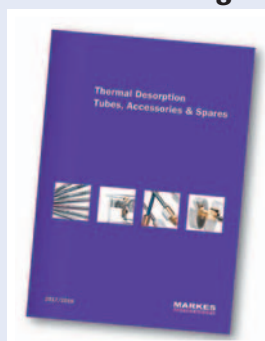


Thermal desorption consumables catalog

A 2017–2018 catalog from Markes International describes thermal desorption tubes, accessories, and spares and contains products for VOC and SVOC monitoring and analysis. According to the company, the catalog is available as an interactive online version (chem.markes.com/TDcat), or as a hard copy from enquiries@markes.com.

Markes International Ltd.,

Llantrisant, UK.
www.markes.com



HPLC column information guide

MicroSolv's *Cogent HPLC Column Information Guide* is a 48-page technical brochure that focuses on how to use aqueous normal-phase HPLC mode for polar and some nonpolar compounds as well as using silica hydride columns for reversed-phase HPLC and for certain circumstances in normal-phase HPLC. The brochure also includes ordering information for stainless steel columns, PEEK columns, guard columns, prep columns and other accessories.

MicroSolv Technology Corp.,

Eatontown, NJ.
mrtc-usa.com/index.aspx



Water purification system

The Milli-Q IQ 7000 water purification system from MilliporeSigma is designed to provide ultrapure water at flow rates as high as 2 L/min. According to the company, the system has ech2O mercury-free UV lamps for the photooxidation of organic contaminants, and purification cartridges that are 33% smaller than previous versions.

MilliporeSigma,
Billerica, MA.
www.emdmillipore.com



Static mixers

PerfectPeak HPLC and UHPLC static mixers are designed to provide baseline noise reduction and lower detection limits. According to the company, the mixers are made from passivated stainless steel and can be retrofitted into any HPLC or UHPLC system. The mixers are available in 90-, 60-, and 30- μ L versions.

Mott Corporation,
Farmington, CT.
www.mottcorp.com/mixer



Syringe filters

Agilent Captiva syringe filters, available from Neta Scientific, are designed to provide sample integrity, accuracy, optimal instrument performance, extended column lifetime, and less system downtime. A range of pore sizes, formats, and membranes is available.

Neta Scientific, Inc.,
a distributor for Agilent Technologies
Hainsport, NJ.
www.netascientific.com



Hydrogen generators

PG-H2 Plus hydrogen generators from VICI DBS Instruments are available in six models that reportedly produce over 600 cc/min of clean, 99.9996% pure hydrogen. According to the company, features include a PEM electrochemical cell protection system with microprocessor controls, a water hydration circulation purification system, USB and RS capability with software options for remote monitoring and control, and a liquid crystal display with touch-screen visuals for system status checks.

VICI Valco Instruments Co., Inc., Houston, TX. www.vici.com



Online GC software

Restek's EZGC online method development software is designed for the development of new gas chromatography methods in minutes directly from a desk. According to the company, the software is available in English, Portuguese, Spanish, French, Italian, Dutch, German, Japanese, and Chinese languages.

Restek Corporation,
Bellefonte, PA.
www.restek.com



HPLC rotor seals

Sciencix's HPLC rotor seals are designed to keep HPLC systems performing at an optimal level. According to the company, the seals are equivalent to the corresponding OEM parts.

Sciencix,
Burnsville, MN.
www.sciencix.com



Cannabis potency analyzer

Shimadzu's Cannabis Analyzer for Potency HPLC system is designed specifically for quantitative determination of cannabinoid content. According to the company, the analyzer integrates instrument hardware, analytical workflows, and supplies, including an analytical column, guard columns, mobile phase, and a CRM standard mixture.

Shimadzu Scientific Instruments,
Columbia, MD.
www.ssi.shimadzu.com



Size-exclusion chromatography column

Shodex's PROTEIN LW-803 silica-based size-exclusion column is designed for the analysis of enzymes and proteins—specifically antibodies. According to the company, the column can analyze proteins of several hundred thousand daltons.

Shodex/Showa Denko America,
New York, NY.
www.shodexhplc.com



Bioinert coating

Dursan bioinert coating from SilcoTek Corporation is designed to improve the speed, resolution, and reliability of liquid chromatography instruments by eliminating the negative effects of a metal flow path. According to the company, the coating can be applied to pumps, columns, frits, and other sample transfer components.

SilcoTek Corporation,
Bellefonte, PA.
www.silcotek.com/lc



Protein A column

The TSKgel Protein A-5PW analytical column from Tosoh Bioscience LLC is designed for the rapid separation and robust quantification of a variety of antibodies. According to the company, monoclonal antibodies can be captured and accurately quantitated in less than 2 min.

Tosoh Bioscience LLC,
King of Prussia, PA.
www.tosohbioscience.com



QuEChERS push-through cartridges

UCT's Quick QuEChERS push-through cartridges are designed to eliminate the need for shaking and centrifugation of extracts during sample cleanup. According to the company, the approach is customizable and fully automatable.

UCT LLC,
Bristol, PA.
<https://sampleprep.unit-edchem.com/products/quechers/quick-quechers-2459>



Dual-injection autosampler

The Master COMBO dual-injection autosampler from DANI Instruments is designed to increase sample throughput by using two syringes in one autosampler to double sample throughput. According to the company, common high-throughput applications include pesticides, FAME, and detailed hydrocarbon analysis.

DANI Instruments, Inc.,
Marlborough, MA.
www.Dani-Instruments.com



Ludger

Seeking regulatory approval for your glycosylated biopharmaceutical?

Ludger's expertise can help you achieve success. We work with over half of the world's top pharma companies*

*GEN, 2016

Glycoprofiling Services

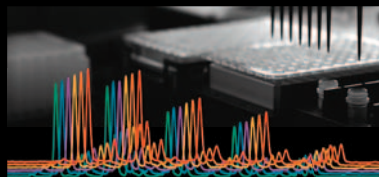
Detailed analysis of both innovator drugs and biosimilars by UHPLC, MS, and LC-MS

Technology

Reliable kits and reagents for glycan sample preparation including standards, clean-up and labeling kits

High-Throughput Glycomics

Collaborative R&D programmes to study glycosylation in disease and for biomarker discovery



Dr. Claire Morgan
Head of Business Dev.
claire.morgan@ludger.com



Dr. Louise Royle
Head of Glycoprofiling
louise.royle@ludger.com

Ludger www.ludger.com

Autosampler syringes

Autosampler syringes from Hamilton Company designed to be used with Spark Holland HPLC autosamplers reportedly provide chemical inertness, reduced friction, and an inert flow path. According to the company, an exact fit between the PTFE plunger tip and the inner diameter of the glass barrel creates a leak-free seal required for accurate autosampler operation.



Hamilton Company,
Reno, NV.
www.hamiltoncompany.com

Pressurized liquid extraction system

FMS' PLE system is an automated pressurized liquid extraction system that is designed for the rapid extraction of a variety of compounds from environmental, biological, and food samples. According to the company, the system is a green, direct replacement for Soxhlet extractions and can reduce solvent consumption and extraction time by 70%.



FMS, Inc.,
Watertown, MA.
www.fms-inc.com

Sample pretreatment system

Shimadzu's Clinical Laboratory Automation Module (CLAM 2000) is designed as a fully integrated sample pretreatment module for LC-MS. According to the company, the system automatically performs all of the processes necessary for analyzing blood and other biological samples, ranging from scanning in information from blood collection tubes to sample pretreatment and LC-MS analysis.



Shimadzu Scientific Instruments,
Columbia, MD; www.ssi.shimadzu.com

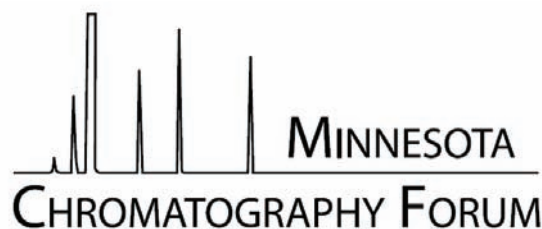
Preparative system

The Quattro countercurrent and centrifugal partition chromatograph and extractor from AECS-QuikPrep is designed to work with and complement standard flash and HPLC laboratory and process instrumentation. According to the company, standard biphasic solvents, ionic liquids, liquid chiral selectors, and ion exchangers may all be used.



AECS-QuikPrep Ltd.,
London, UK.
www.quattroprep.com

38th Annual MN Chromatography Forum Spring Symposium



May 9 - 11, 2017

Earle Brown Heritage Center Minneapolis, MN

May 9-10 Short Courses

Advanced HPLC & UHPLC Method Development
Comprehensive GC & GC-MS Troubleshooting
Analytical Sampling and Sample Prep for Chromatography

May 10 (1pm) Complimentary Open House

Equipment Exhibition
Vendor Seminars
Refreshments

May 11 Keynote Presentation:

Dr. Paul Mahaffy, NASA

"Exploration of Martian Habitability with the Curiosity Rover"
Technical Presentations
Equipment Exhibition

For further information and
online registration information visit:

www.minnchrom.com

AD INDEX

ADVERTISER	PAGE	ADVERTISER	PAGE
ACD/Labs	238	Neta Scientific	261
AECS-QuikPrep	255	Optimize Technologies	243
Benson Polymeric, Inc.	228	PerkinElmer Corporation	CV2
Cannabis Conference	264	Pickering Laboratories	218
DAISO Fine Chem, Inc.	242	Postnova Analytics GmbH	237
DANI Instruments	215	Restek Corporation	250
Gerstel GmbH & Co. KG	227	Sciencix	216
Hamilton Company	241	Shimadzu Scientific Instruments	CV4, 219
HPLC	277	Showa Denko America, Inc.	257
IDEX Health & Science	252-253	SilcoTek Corporation	260
Imtakt USA	217	Sonntek, Inc.	218
ISCC & GCxGC	CV3	The 4S Company	216
Jordi Labs	245	The Nest Group	246A-B/INSERT
Markes International Ltd	263	Thermo Fisher Scientific	266-267
MicroSolv Technology Corporation	239	Tosoh Bioscience	CVTIP
MilliporeSigma	223, 259	UCT, LLC	229
Minnesota Chromatography Forum	276	VICI Harbor Group	221
Mott Corporation	262	Wyatt Technology Corporation	225
MS Noise	249	YMC America, Inc.	222



HPLC 2017 PRAGUE

18-22 June 2017 | CZECH REPUBLIC

FUN FUNDAMENTALS	HYP HYPHENATIONS	APP APPLICATIONS	YOU YOUNG & TUTORIALS
----------------------------	----------------------------	----------------------------	---------------------------------

Key Dates

March 6, 2017
Abstract deadline for Best Poster Award
March 20, 2017
Deadline for early registration payment
April 17, 2017
Abstract deadline for poster presentations

Registration Fees

	Early	Regular
Academic	650 €	790 €
Industry	900 €	1 080 €
Student	240 €	340 €
Student + 2 Short Courses	360 €	460 €



THE ESSENTIALS

Excerpts from LCGC's professional development platform, CHROMacademy.com

Hydrophobic Interaction Chromatography for Biopharmaceutical Analysis

Hydrophobic interaction chromatography (HIC) is a milder form of reversed-phase liquid chromatography (LC). Separation of analytes is based on hydrophobic interactions with the stationary phase; therefore, the elution order in HIC enables proteins to be ranked on the basis of their relative hydrophobicity. HIC employs nondenaturing conditions, does not require the use of organic solvents or high temperatures, and separations are carried out at physiological pH, which allows the preservation of protein structure. Historically HIC has been used for the determination of the relative hydrophobicity of proteins and was applied on a preparative scale for protein purification; it is now applied at all stages of the purification process, including high-yield capture, polishing monoclonal antibodies (mAbs), characterization of mAbs and antibody–drug conjugates (ADCs), removal of truncated species from full-length forms, separation of active and inactive forms, and clearing viruses.

HIC analysis is carried out using a reversed salt gradient, starting with a high salt concentration and moving to a low salt concentration to facilitate protein elution. When starting at a high salt concentration, proteins are retained on the stationary phase because the hydrophobic interaction between the solute and stationary phase is increased by the salt in the buffer, which reduces the solvation of the proteins; as solvation decreases hydrophobic regions become exposed and are adsorbed to the stationary phase. The more hydrophobic the molecule the less salt is needed to promote binding.

The mobile phase is typically an aqueous solution of a nondenaturing salt such as ammonium sulfate (1–2 M) or sodium chloride (3 M) and a buffer to control pH (usually phosphate, $6 \leq \text{pH} \leq 7$). High concentrations of salt, especially ammonium sulfate, may precipitate proteins; therefore, solubility should be checked under the initial gradient (binding) conditions. The strength of the interaction between the protein and stationary phase decreases with increasing pH as a result of increased charge on the protein caused by the ionization of acidic groups. This effect can vary depending on the protein; thus, pH can impact the level of protein binding and selectivity. Changes in pH do not have a significant effect over moderate ranges.

Stationary phases generally consist of silica bonded with ligands of relatively limited hydrophobicity (for example, butyl, phenyl, ether, amide, or propyl). Straight-chain alkyl ligands exhibit hydrophobic character, whereas aryl ligands provide mixed-mode behavior where both aromatic and hydrophobic interactions are possible. The choice of ligand type will be determined empirically. Normally stationary phases are nonporous; however, there are also some porous polymethacrylate-based particles available. HIC columns can be operated at pressures in the 100–400 bar range, and are typically packed with 5-, 3-, or 2.5- μm particles.

HIC can be applied to the analysis of mAb heterogeneity resulting from post-translational modifications (Table 1). HIC has found particular applicability to the analysis of ADCs. ADCs contain a lipophilic cytotoxic drug attached to a monoclonal antibody; the mAb affords the selectivity while the drug provides the efficiency of treatment. Separation occurs since most cytotoxins are hydrophobic. The hydrophobicity of the ADC depends

Table 1: Common PTMs, with expected retention times relative to the principal intact protein peak

Modification	HIC Peak
Aspartate isomerization	Pre-peak
Asparagine deamidation	Post-peak + pre-peak
Oxidation	Pre-peak
PyroGlu from Glu ($-\text{H}_2\text{O}$)	Post-peak
PyroGlu from Gln ($-\text{NH}_3$)	Post-peak
Succinimide	Post-peak
Sugar	Pre-peak
C-terminal lysine	Pre-peak
Aggregation	Post-peak
Fragmentation	Variable

on the number of conjugated drugs; therefore, ADCs with different drug-to-antibody ratios (DAR) can be separated in HIC. In terms of elution order, mAbs that contain no drug will be eluted first and those with the highest number of drugs attached will be eluted last. The relative distribution of the ADC species can be determined on the basis of peak area percentages. The weighted DAR can then be determined based in the percentage peak area information and the drug load numbers. Because of the high salt concentrations used, HIC is generally incompatible with mass spectrometry (MS) detection (although successful studies have implemented HIC–MS analysis of intact proteins using ammonium tartrate as an MS-compatible salt in place of the more traditional ammonium sulfate). Multidimensional chromatography using a reversed-phase LC desalting step before MS analysis allows online characterization of ADCs as the ADCs dissociate into their respective subunits under the denaturing reversed-phase LC conditions.

More Online:

Get the full tutorial at

www.CHROMacademy.com/Essentials
(free until May 20).

41st International Symposium on Capillary Chromatography & 14th GCxGC Symposium

FORT WORTH, TEXAS USA

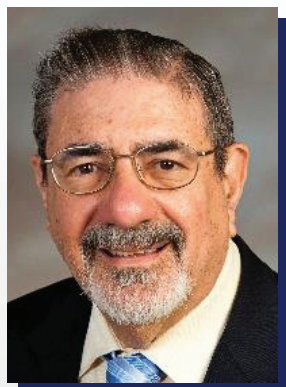
MAY 14-19, 2017

www.isccgxcgc.com

We invite you to join the 41st International Symposium on Capillary Chromatography (ISCC) held in conjunction with the 14th GCxGC Symposium. The 2017 meetings will be hosted at the Hilton Fort Worth from May 14 - 19, 2017 and will include a top-level speaker lineup, excellent short courses, and highly attended poster sessions.

Poster abstracts are still being accepted!

The 2017 meetings will be highlighted with plenary lectures by:



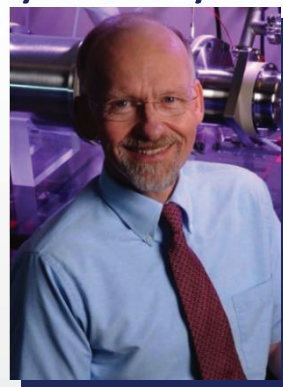
Dr. Richard N. Zare

Marguerite Blake Wilbur Professor in
Natural Science at Stanford University



Dr. Pat Sandra

Emeritus Professor, Organic
Chemistry, Ghent University
Research Institute for
Chromatography, Founder & President



Dr. Richard D. Smith

Director of Proteome Research at
Pacific Northwest National
Laboratories

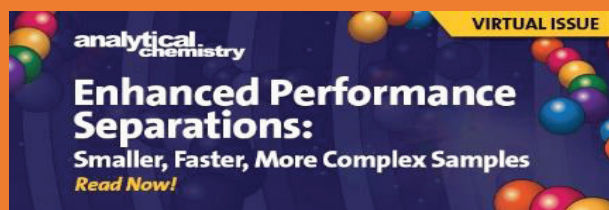
Topics of interest will include but not be limited to the following:

- Pharmaceutical, Forensic & Process Analysis
- Contaminants in Food and the Environment
- Biomarker Discovery and Protein Analysis
- Petroleum, Energy & Resource Development
- Ionic Liquids in Chemical Analysis
- Novel Stationary Phase Chemistry
- Software and Data Analysis
- Atmospheric and Air Analysis
- Lab-On-A-Chip and Microfluidics
- Novel Detection Techniques
- Multidimensional Separations
- Miniaturized & Portable Systems

Four short courses to choose from as part of ISCC & GCxGC 2017!

- GCxGC Short Course
- Sample Prep Techniques for Capillary Chromatography
- Ionic Liquids in GC & MS
- Basic HPLC

The virtual issue in *Analytical Chemistry* resulting from presentations at the 2015 Fort Worth meetings can be accessed via our website. We are pleased to announce that papers presented at the 2017 meetings will be eligible for another virtual issue of *Analytical Chemistry*.



www.isccgxcgc.com

LC Systems

i-Series



Imagine an HPLC Designed to Deliver Reliable Results with Minimum Effort



intuitive — Easy-to-Understand Instrument Control

- A unity between colorful touchscreen GUI and hardware
- Easy-to-see status indicators for Ready, Run, and Error



innovative — Advanced Interactive Design

- Load samples and start analysis immediately via the touch screen
- Monitor run status and results remotely with your smart phone



intelligent — Smart Automation

- Easy methods migration
- Automatic power economy mode

Learn more about Shimadzu's i-Series.
Call (800) 477-1227 or visit us online at
www.ssi.shimadzu.com/iseries

Order consumables and accessories on-line at <http://store.shimadzu.com>
Shimadzu Scientific Instruments Inc., 7102 Riverwood Dr., Columbia, MD 21046, USA