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NEW Chromatography Columns and HPLC Systems for 2017

How Retention Influences Resolution in LC

Hydrophobic Interaction Chromatography for Biopharmaceutical Analysis

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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?

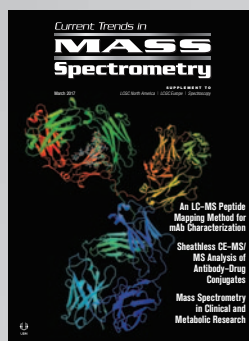
Reed Harris from Genentech discusses how the uncertainty scale helps to determine the most important factors when assessing critical quality attributes.

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

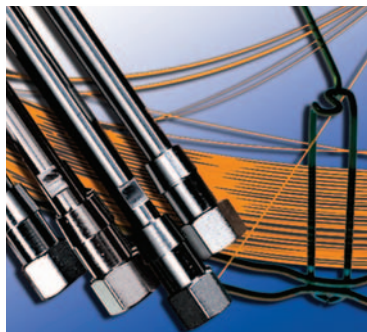


CURRENT TRENDS IN MASS SPECTROMETRY

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



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*.

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- μ m columns
Agilent Technologies	InfinityLab Poroshell 120 1.9- μ m columns
	Six different chemistries: InfinityLab Poroshell 120 EC-C18 1.9- μ m columns
	InfinityLab Poroshell 120 EC-C8 1.9- μ m columns
	InfinityLab Poroshell 120 Phenyl-Hexyl 1.9- μ m columns
	InfinityLab Poroshell 120 PFP 1.9- μ m columns
	InfinityLab Poroshell 120 HPH-C18 1.9- μ m columns
InfinityLab Poroshell 120 HILIC 1.9- μ m 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- μ m 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	μ PAC 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	

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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

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

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

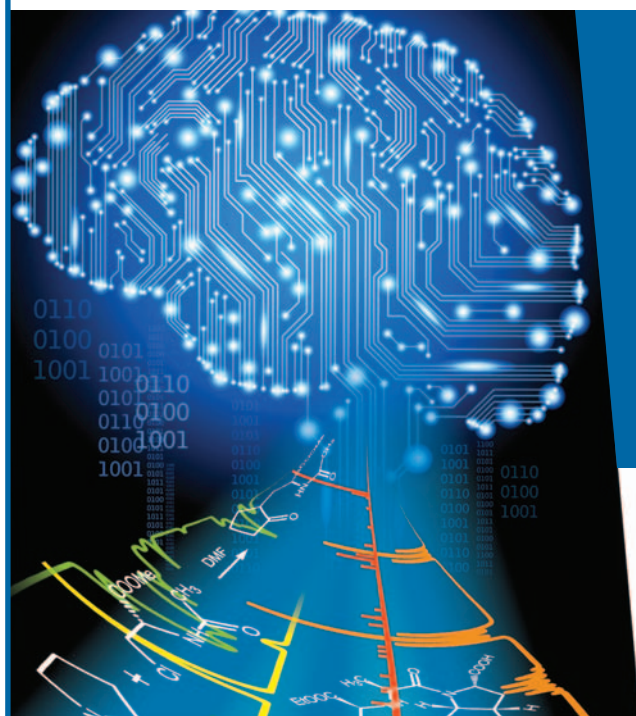
thus reduce or eliminate undesired responses in gradient analyses.

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.

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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.

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