

Column

Watch

This month's "Column Watch" is the first of a two-part series that examines new column introductions at Pittcon 2004. In part I, Ron Majors discusses high performance liquid chromatography (HPLC) columns and packings for reversed-, normal-, and bonded-phase; ion-exchange; ion; and size-exclusion chromatography. He also looks at specialty HPLC columns.

New Chromatography Columns and Accessories at the 2004 Pittcon Conference, Part I

Pittcon 2004 — the 55th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy — returns to the gigantic McCormick Place in Chicago, Illinois on 7–12 March 2004. This year's event will host more than 900 instrument manufacturers and laboratory suppliers in more than 2700 booths. In addition to attending the exposition, the conferees can listen to 1200 oral presentations, view more than 900 posters, check out 50 seminar rooms, or attend one of 240 short courses.

Pittcon was last held in Chicago in 1996 and that year still stands as having the largest attendance in the history of the conference. Pittcon 2004 could well surpass the attendance figures for the meetings held in the South the last several years. Undoubtedly, Pittcon remains the most important yearly international analytical exhibition where companies introduce their latest instruments, instrument accessories, columns, sample preparation products, and other consumable products. Because many past attendees have purchased one or more new products within three months after attending the show, most exhibitors attempt to maximize their booth traffic to meet as many customers as possible.

The purpose of this report is to provide information about many of the new separation consumables and accessory products that will be displayed at Pittcon 2004. In some cases, products that were introduced during 2003 but after Pittcon 2003 (1,2) could be included for reasons of completeness. The information is based upon manufacturers' responses to a questionnaire mailed in December 2003. Because of space limitations and the fact that some of the manufacturers did not respond to the questionnaire, this report cannot be considered an exhaustive listing of all the new products that will be introduced in Chicago. However, over the years, these Pittcon introduction summaries have provided a good source of information that

would be difficult for one individual to gather during the four days of the exhibition. In addition, the products introduced have shown definite correlations to current research, development, and application activity in the separation sciences.

As in previous years, columns and other products recommended by their manufacturers primarily for biomolecule separations or sample preparation are denoted in the tables with the designation *BIO*. Some of these products can be used for general high performance liquid chromatography (HPLC) separations as well, but their main emphasis is for biological samples. I will cite specific information about bioapplications wherever it is appropriate. Because microbore HPLC columns (1.0–2.1 mm i.d.) are now numerous, they will be covered in the individual modes whereas capillary (, 1.0 mm i.d.) and nano columns (, 0.15 mm i.d.) have been placed in the specialty HPLC columns category because they require special instrumentation to get their full performance. This year I have extended the columns and sample preparation accessories to include various kits that are designated to solve specific applications problems.

In this month's coverage, I will describe new introductions in the areas of high performance liquid, reversed-phase, normal- and bonded-phase-, ion-exchange and ion, size-exclusion, large- and preparative-scale, and specialty chromatography columns. Next month, I will look at gas chromatography (GC) columns, sample preparation products, and hardware, accessories, and kits for chromatography and sample preparation.

Trends and Highlights

General: Again this year, the largest number of products will be introduced for the separation, sample preparation, and liquid chromatography–mass spectrometry (LC–MS) measurement of biomolecules — still the most active area in chromato-

Companies Listed in This Column

Agilent Technologies, Inc., Wilmington, Delaware
 Allsep Technologies (SIELC), Prospect Heights, Illinois
 Alltech Associates, Inc., Deerfield, Illinois
 Argonaut Technologies, Inc., Foster City, California
 BIA Separations (Astec), Whippany, New Jersey
 BioChrom Labs, Inc., Terre Haute, Indiana
 Bischoff Chromatography, Leonberg, Germany
 Chiral Technologies, Inc., Exton, Pennsylvania
 Cluzeau-Info-Labo, Sainte Foy La Grande, France
 Daiso Co. Ltd., Osaka, Japan
 Dionex Corp., Sunnyvale, California
 ES Industries, West Berlin, New Jersey
 GL Sciences, Tokyo, Japan
 Grace Vydac, Hesperia, California
 Isco, Lincoln, Nebraska
 Macherey-Nagel GmbH & Co. KG, Düren, Germany
 Merck KGaA, Darmstadt, Germany
 MicroSolv Technology Corp., Long Branch, New Jersey
 Nanostream, Inc., Pasadena, California
 Nacalai Tesque, Kyoto, Japan
 New Objective, Inc., Woburn, Massachusetts
 Optimize Technologies, Inc., Oregon City, Oregon
 OraChrom, Woburn, Massachusetts
 Phenomenex, Torrance, California
 Polymer Laboratories, Amherst, Massachusetts
 Restek Corp., Bellefonte, Pennsylvania
 Scivex/Upchurch Scientific, Oak Harbor, Washington
 SGE Inc., Austin, Texas
 Shiseido Co., Ltd., Tokyo, Japan
 Showa Denko, Kawasaki, Japan
 Thermo Electron Corp., Runcorn, United Kingdom
 Tosoh Bioscience LLC, Montgomeryville, Pennsylvania
 Varian, Inc., Palo Alto, California
 Waters, Milford, Massachusetts
 ZirChrom Separations Inc., Anoka, Minnesota

Table I: New series of HPLC columns at Pittcon 2004

Product Name	Supplier	Analytical	Prep	Guard	Bulk
Alltima HP	Alltech	Yes	Yes	Yes	No
CapCell Pak UG, MG, ACR, AQ, DD	Shiseido	Yes	Yes	Yes	Yes
Epic High Density reversed phase	ES Industries	Yes	Yes	Yes	Yes
EVA Evanescence capillary	ES Industries	Yes	No	No	No
Isolute Flash Si II	Argonaut Technologies	Yes	Yes	No	No
Javelin Express	Thermo Electron	Yes	No	No	No
Microbore HPLC column format	Alltech	Yes	No	Yes	No
Pinnacle DB	Restek	No	No	No	Yes
PLRP-S, PL-SAX, etc. capillary columns	Polymer Laboratories	Yes	Yes	Yes	No
Ultra-Fast high temperature precolumns	Zirchrom Separations	Yes	No	No	Yes
*PS-DVB 5 poly(styrene-divinylbenzene).					

graphic research and applications. Products for high-throughput chromatography and sample preparation abound. New breeds of low-bleed phases for both GC and LC-MS were noted. Monolith technology has begun to spread into preparative LC and sample preparation. Application-specific solutions with everything needed to perform sample prep and analysis also are in vogue.

HPLC columns: Reversed-phase HPLC maintains its dominance and a significant number of reversed-phase columns are being debuted, with an increasing number

of C18 phases still being introduced. Particles and column internal diameters are becoming smaller and column lengths are becoming shorter. More monolithic silica columns will be introduced but a greater number of polymeric monoliths for analytical and preparative LC will appear. As far as I was able to determine from the questionnaire responses, no irregularly shaped silica gel packed columns in analytical sizes will be introduced at Pittcon this year, so unless stated otherwise, assume that all the packings are spherical in nature. The number of specialty columns will be near the all-time

Modes	Phases	Comments
Reversed and normal phase	C8, C18, C18 high load, embedded, C18 EPS, cyano, silica	Family based on high purity, Type B silica, 3 and 5 mm; pore size: 180 Å; stainless steel and PEEK construction; pH range 1–10; low bleed, recommended for LC–MS applications; EPS 5 extended polar selectivity.
Reversed and normal phase, cation exchange	C1, C8, C18, phenyl, amino, strong cation exchange	Polymer-coated silica particles, phases have combined benefits of silica and polymer; low density of phase allows rapid mass transfer; various coverage of C18 bonded phases provides different selectivity and retention; particle sizes: 3–20 mm; pore sizes: 80–300 Å.
Reversed phase	C4 SD, C8, C18, C18MS, C18SD, polar (C18-ether)	SD = superdensity; phases with high-density monomeric bonding (in excess of 4 mmol/m ²); based on ultra high purity Type B silica; particle sizes: 3, 5, 10 mm and larger; pore size: 120 Å; surface area: 230 or 350 m ² /g; pH range 1–12; cartridge design hardware in a wide variety of dimensions.
Reversed and normal phase	C4, C8, C18, polar-embedded (ether), fluoro cyano, phenyl	Capillary and nano-LC columns packed with 3–5 mm bonded high-purity silica particles; pore sizes: 120, 200, 300, 1000, and 2000 Å; useful for LC–MS and proteomics studies; <i>BIO</i> .
Normal phase	Silica	Flash chromatography column in following sizes: 2 g/6 mL; 2 g/15 mL; 5 g/25 mL; 10 g/25 mL; 10 g/70 mL; 25 g/150 mL; 50 g/150 mL; 70 g/150 mL; compatible with flash chromatography systems; 40- and 100-g columns also available; FlashMaster and FlashVac manifold systems accommodate all of these columns; 40–70 mm particles; 60-Å pore size; surface area: 500 m ² /g; recommended for purification of target compounds from organic reaction mixtures.
Reversed phase	C18, cyano, fluophase, polar retention	Several C18 phases with various surface properties are available; endcapped and polar end capped; phase coverage: 4–20% depending upon phase; pore sizes: 100, 190, 300 Å; particle size: 5 mm; dimensions: 50 mm \times 2.1-mm; recommended for high-throughput screening for drug discovery and metabolites.
Reversed and, normal phase, cation and anion exchange, hydrophilic interaction, aqueous SEC	Phases from all key column families	Narrow-bore columns for solvent reduction and LC–MS use; available for all proprietary families: Alltima, Alltima HP, Platinum, Prevail, and ProSphere; inner diameters of 1.0, 2.1, and 3.0 mm and lengths as short as 10 mm; 1.0 mm in ProSphere only; guard columns and custom columns available for all configurations.
Reversed and normal phase	C8, C18, cyano	Bulk packing for those who want to pack their own and scale up from analytical columns; DB is a deactivated packing material for analysis of basic compounds; particle size: 5 mm; pore size: 140 Å each lot is thoroughly tested to specific chemical and physical properties.
Reversed phase, ion exchange	Reversed phase, strong and weak anion, strong and weak cation	Standard polymeric phases now available in capillary dimensions (50 and 150 \times 0.30 mm); stainless steel construction; rigid and coated PS–DVB*; 3- and 5-mm particles with 100-, 300-, 1000-, and 4000-Å pore sizes; recommended for LC–MS of peptides and proteins; high stability with no particle bleed; <i>BIO</i> .
Reversed and normal phase, cation exchange, anion exchange	C18-, polybutadiene-, carbon-, polystyrene-coated zirconia	Recommended for high-temperature HPLC; particle sizes: 3, 5, 7, 10, and 25 mm; pore size: 300 Å; stainless steel construction; columns are compatible with all column ovens; column dimensions: 20 mm \times 4 mm for fast LC.

high, with protein–peptide analysis columns representing about half of the introductions. Others such as chiral, sugar–oligosaccharide, and affinity columns are among the most popular. Pittcon 2004 seems to have a much larger number of preparative LC columns and bulk particles than earlier Pittcons.

Gas chromatography: GC columns this year are highlighted with a continued introduction of lower-bleed phases that provide enhanced sensitivity, lower baseline drift during temperature programming and improved MS capability. A large number

of application-specific columns will be introduced in the petrochemical, environmental, drug testing, and food–flavor markets. Also to be introduced is a tiny packed capillary column–oven combination that weighs only a few ounces.

Sample preparation: For the first time, the number of 96-well plates for solid phase extraction (SPE) and filtration will exceed the number of introductions in cartridge format. Protein precipitation plates for drugs in biological fluids continue to be developed. The pipette tip format for submicroliter isolation and cleanup of pro-

teins and peptides is receiving more attention. Application-specific products include a column for removal of high-abundance proteins from human plasma, a dioxin cleanup system, SPE cartridges and 96-well plates for drugs-of-abuse cleanup, and an extraction column for oil and grease in environmental samples.

Accessories: Questionnaire responses included 25 new accessories for LC, GC, capillary electrophoresis (CE), capillary electrochromatography (CEC), and sample preparation. System components such as valves, pumps, and tubing for nano- and

Table II: Reversed-phase chromatography columns at Pittcon 2004

Product Name	Supplier	Type	Base Material	Functional Group	Particle Diameter (µm)	Pore Size (Å)
Acclaim PA Polar Advantage	Dionex	Polar-embedded	Silica	C16 and sulfonamide	3, 5	120
Cosmosil 5C18-PAQ	Nacalai Tesque	Polymeric-coated	Silica	C18	5	120
Denali	Grace Vydac	Regular	Silica	C18	5–20	120
Hypercarb 3-µm	Thermo Electron	Regular	Graphitized carbon	None	3	250
Nucleodur 100-5 CN-RP	Macherey-Nagel	Regular	Silica	Cyano	5	110
Nucleodur C18 Pyramid	Macherey-Nagel	Polar-encapped	Silica	C18	5	110
Primesep D	Allsep-SIELC	Regular	Silica	Unspecified	5, 10, 20	Unspecified
ProntoSIL 120 C8 ace-EPS	Bischoff	Polar-embedded	Silica	C18, amide	3, 5, 10	120
ProntoSIL Hypersorb ODS	Bischoff	Regular	Silica	C18	3, 5, 10	120
ProntoSIL Spheribond ODS 1 and 2	Bischoff	Regular	Silica	C18	3, 5, 8, 10	80
Pursuit C8	Varian	Regular	Silica	C8	3, 5, 10, 20	200
Pursuit Diphenyl	Varian	Regular	Silica	Diphenyl	3, 5, 10, 20	200
Swift RP-all	Isco	Regular	PS-DVB monolith	Phenyl	Not applicable	>500
Synergi Fusion-RP	Phenomenex	Polar-embedded	Silica	C18 + polar ligand	2, 4, 10	80
Turbo ODS-MS	CIL Cluzeau Info Labo	Regular	Silica	C18	2	120
Zirchrom-MS	ZirChrom Separations	Polymer-coated	Zirconia	Polybutadiene	3, 5, 7, 10, 25	300
Zorbax Eclipse XDB-CN	Agilent Technologies	Regular	Silica	Cyano	3.5, 5	80

capillary LC will be introduced to supplement the columns already in the marketplace. Guard columns for LC and GC are popular. A quick GC liner replacement device will debut that is designed to save time and prevent burnt fingers. CE is alive and well and several products for controlling the electroosmotic flow will be shown. A clever carbonate removal system will

help to increase the sensitivity in ion chromatography.

New Series of HPLC Columns and Packings

Each year at Pittcon, manufacturers introduce entire new families of HPLC columns. For the sake of brevity, I have elected to cover those series here instead of

listing each column under individual headings in the tables. Table I covers those new series of HPLC columns to be displayed at Pittcon 2004. In some cases, the series are major extensions of existing products in the companies' offerings but cover an entire mode or group of phases, while in other cases the series are new to the market.

Comments

Recommended for separation of acidic, basic, and neutral compounds; sulfonamide coupled through ether linkage; recommended pH range of 2–8 but may be used at pH 1 for short periods of time; compatible with 100% aqueous mobile phases; 2.1- and 4.6-mm i.d. standard but can be supplied in capillary to nano formats.

Type B silica is only partially coated with C18 chains; contains a polymeric linking for acid resistance; stable retention time in 100% aqueous mobile phase; 11% carbon content with endcapping; specific surface area of silica: 300 m²/g.

Recommended for separation of acidic and basic small molecules; carbon coverage of 19–20% (3 mmol/m²); nano- to preparative-scale dimensions for linear scaleup, 75- μ m particles and 100-mm i.d. columns also available; guard columns and bulk packing available.

Porous graphitic carbon that is recommended for very polar compounds and high-pH separations; expands range of product offering; capillary to analytical dimensions.

Endcapped phase recommended for reversed-phase applications; cyanopropyl functionality with intermediate polarity; 7% carbon coverage; available in capillary to preparative dimensions; guard cartridges available; separate version of column for normal phase is shipped in heptane.

Recommended for very polar compounds (drug metabolites, organic acids); may be used in 100% aqueous mobile phase; phase coverage: 14% carbon; low bleed for LC–MS. Recommended for the reversed-phase separation of hydrophobic and acidic compounds in plasma and other biofluids; packing excludes plasma proteins but allows interaction of small molecules with stationary phase; microbore and conventional analytical dimensions.

Recommended for separation of polar compounds with new polar selectivity; base deactivated; 12% carbon coverage; Type B silica; 14–300 mm lengths, 2.0–50 mm internal diameters available; stainless steel and PEEK construction.

Recommended as alternative to Hypersil ODS (Thermo Separations); 9.5% carbon coverage, Type A silica; available from 14–300 mm lengths, 2.0–50 mm internal diameters stainless steel and PEEK construction.

Recommended as alternative to Spherisorb ODS 1 and ODS 2 (Waters); 7.0% carbon coverage (ODS 1) and 12% (ODS 2), Type A silica; 14–300 mm lengths, 2.0–50 mm internal diameters; stainless steel and PEEK construction.

Recommended for improving selectivity for polar analytes; higher phase density and lower hydrophobicity than C18; phase coverage: 4.4 mmol/m²; nano- to preparative-scale LC sizes; guard columns and bulk packings available.

Recommended for analytes with double bonds or aromatic groups (π - π selectivity); nano- to preparative-scale LC sizes available; guard columns and bulk packings available.

Monolith column recommended for protein and peptide separations; pH range 0–14; column dimensions: 50 mm \times 2.1 mm for fast LC; column tubing construction: stainless steel; *BIO*.

Balanced hydrophobic and polar selectivity; enhanced retention of polar compounds; low bleed for MS; pH range 1.5–10; 12.2% carbon coverage; can be used in 100% aqueous mobile phase; capillary to preparative dimensions with cartridge design; guard columns and bulk packing

Short columns (13–50 mm lengths) recommended for high-speed LC and LC–MS; capillary to analytical internal diameters; based on high purity Type B silica.

High retention of basic compounds using mixed-mode mechanisms, especially for LC–MS applications; 1–10 pH range and temperatures as high as 80 °C; 3.1% carbon coverage, 30 m²/g surface area; available in 1-, 2-, and 3-mm nonporous particles; capillary to preparative dimensions.

XDB 5 extra densely bonded; recommended for both reversed- and normal-phase applications; less retention of nonpolar compounds in reversed-phase chromatography; based on Type B silica; complements Zorbax StableBond-CN; 50–250 mm \times 2.1–4.6 mm dimensions.

This year most of the new families to be introduced are based upon low-dispersion columns: narrow-bore (microbore), capillary or short, conventional internal diameter columns.

This observation is not just for these new series in Table I but for the individual modes as well. The advantage of all of these columns is their ability to interface to

MS systems because of their ability to operate at lower flow rates than typical analytical columns. With microbore columns and especially with capillary columns, specialized capillary LC instruments or modifications of existing HPLC instruments often are needed to optimally use these small columns (3). Other advantages of these smaller internal diameter

columns include increased sensitivity in sample-mass-limited situations and solvent savings because of the low flow-rate requirements. The short conventional columns are useful for high-throughput HPLC and also offer solvent savings.

Another trend noted is a move toward smaller particle sizes, especially particles with 3- μ m diameters, for polymeric and polymer-coated packings. When the particles become smaller, efficiency rises dramatically but so does the pressure drop. Polymeric packings traditionally have been considered to have lower efficiency than equivalent size silica-based particles, and this movement toward smaller particles helps to offset some of the efficiency differences found when the polymeric packings are compared with 5- μ m silica bonded phases. Polymeric packings normally provide an increased pH range.

One interesting series is the Epic High Density bonded reversed-phase columns from ES Industries. For silica gel porous packings, roughly 8-mmoles/m² of surface silanols are available for bonding organosilane. Monomeric phases are prepared by the reaction of monochloro- or monoethoxy-silanes with these silanols. Due to steric reasons, a typical dimethyloctadecylsilane (C18) phase could react with one quarter to one third of these available silanols, leaving a number of unreacted silanols that can interact with basic compounds. Sometimes, some of the silanols can be reduced by reaction with a small silylating reagent such as trimethylchlorosilane, a technique known as endcapping. ES Industries has developed bonding techniques that permit the reaction of more than half of the available silanols (4 mmoles/m²) with alkyl chain silane reagents as long as C₁₈. Such high bonded-phase coverage protects the underlying surface from attack by basic solution and raises the upper pH limit. In addition, higher coverage implies greater retention and different selectivity compared with reversed phases that have lower percentages of carbon loading. Peak shape should be improved by shielding of the remaining silanols, many of which are buried deep within pores.

Reversed-Phase Chromatography

As in years past, many new reversed-phase chromatography columns will be introduced at Pittcon 2004 (see Table II). In fact, if I include the reversed-phase chromatography columns to be introduced under the series (Table I) and specialty

Product Name	Supplier	Type	Base Material	Functional Group	Particle Diameter (mm)
Inertsil AX	GL Sciences	Anion	Silica	Diethylamine	5
Inertsil CX	GL Sciences	Cation	Silica	Benzenesulfonic acid	5
IonPac AS19	Dionex	Anion	Ethylvinylbenzene-55% crosslinked with DVB*	See comments	7
IonPac CS18	Dionex	Cation	Ethylvinylbenzene-55% crosslinked with DVB	Carboxylic acid	6
PL-SAX, -WAX, -SCX, -WCX	Polymer Laboratories	Cation, anion	PS-DVB	Various	5
Shodex MS-IEC Me Series	Showa Denko	Cation, anion	Polyhydroxy-methacrylate	See comments	5-8
Universal Cation	Alltech Associates	Cation	Silica	Unspecified	3

*DVB 5 divinylbenzene

(Table IV) categories, this year's total listings of new products or lines of products indicates the continuing strong popularity of the technique. Of the packings to be introduced, 65% are silica-based packings, 28% are polymeric- or polymer-coated materials, and 7% are based upon other materials such as zirconia or monoliths, or the material was not specified. What is quite interesting this year is that a greater number of larger particles will be introduced. Last year, in absolute numbers, 80% of the packings were smaller than 5 mm but this year only 57% of the packings to be introduced have diameters smaller than 5 mm. A total of 21% of reversed-phase chromatography packings to be introduced have diameters larger than 10 mm, indicating that the preparative market could be having a resurgence (see the preparative section). These larger particles find use in wide-bore columns, usually 21.2 mm i.d. and greater, for which the use of microparticulates could become cost prohibitive.

In terms of phase chemistry, the C18 phase is the leading chemistry (approximately 40% of total column introductions) with polymer and polymer-coated phases a distant second (14% of total, mostly poly[styrene-divinylbenzene] [PS-DVB]). Others include polar-embedded (9%), cyano (9%), could also include use in normal phase), C8 (7%), and phenyl and

diphenyl (7%). The others were 5% or less.

Due to the large number of reversed-phase chromatography materials, this year I classified the phases according to their suggested chemistry or application emphasis of the manufacturer based upon the information they provided. For example, if a column is listed as a "regular" type, the manufacturer did not specify any unusual characteristics or applications. Another popular phase type, the polar-embedded phases, is recommended for use in low organic, highly aqueous environments—even with mobile phases containing 100% water. This type of phase incorporates a polar functional group (this year amide, sulfonamide, and proprietary unspecified polar groups) somewhere in the bonding process into the alkyl bonded phase chain itself or endcaps the silica surface with a hydrophilic reagent (polar endcapping). The phase is primarily hydrophobic but has hydrophilic groups near the surface or in the alkyl chain that can be wetted with polar eluents.

To illustrate an example of one of the newer lines of reversed-phase chromatography columns, Figure 1 depicts the separation of opiates using a 5-mm d_p DENALI C18 column (Grace Vydac). The separation was performed at pH 7 using isocratic conditions and shows a good separation of these structurally related compounds.

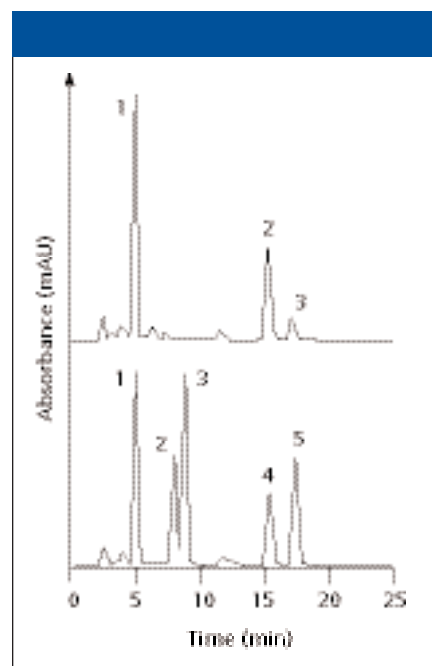


Figure 1: Isocratic separation of opiates on a 250 mm x 4.6 mm, 5- μ m d_p , 120-Å DENALI C18 column (Grace Vydac). Mobile phase: 50:50 methanol-water containing 20 mM dibasic potassium phosphate (pH 7); flow rate: 1.0 mL/min; temperature: ambient (22 °C); detection: UV absorbance at 254 nm; sample volume: 10 mL containing 10 mg of each analyte. Upper trace peaks: 1 5 morphine, 2 5 ethylmorphine, 3 5 ethylmorphine (impurity). Lower trace peaks: 1 5 hydromorphone, 2 5 oxycodone, 3 5 codeine, 4 5 ethylmorphine, 5 5 nalorphine.

Pore Size (Å)	Comments
100	Recommended for protein separations; 18% carbon coverage; available in nano- to preparative-scale dimensions; guard columns available; <i>BIO</i> .
100	Recommended for the separation of proteins; 12% carbon coverage; available in nano- to preparative-scale dimensions; guard columns available; <i>BIO</i> .
Unspecified	Functional group: alkanol quaternary amine functionalized latex with 65-nm diameter; recommended for separation of inorganic anions, especially bromate and others in drinking water and wastewater by EPA Methods 300.0 and 300.1 Parts A/B; uses hydroxide eluents (generated using the Eluent Generator with water) resulting in low backgrounds and detection limits; dimensions: 250 mm \times 2 or 4 mm; guard column available; PEEK hardware.
Unspecified	Recommended for separation of ammonium, alkanolamines, and methylamines from Group I and II inorganic cations from wide variety of matrices; low capacity, can be used with suppressed- and with nonsuppressed-conductivity detection; hydrophobic surface provides better resolution of alkylamines and alkanolamines; can be operated at high temperature and with LC-MS due to low bleed; dimensions: 250 mm \times 2 or 4 mm; guard column available; PEEK hardware.
1000, 4000	Recommended for separations of peptides, proteins, and oligonucleotides; rigid particles with wide gaps; new particle and column size (50 mm \times 4.6 mm) for high speed, high-performance separations; weak-anion and -cation phases are completely new; stainless steel construction; <i>BIO</i> .
Unspecified	Four types of ion exchangers: strong (quaternary ammonium) and weak (diethylaminoethyl) anion, strong (sulfopropyl) and weak (carboxymethyl) cation; recommended for pre-separation of small amounts of protein before detailed analysis; dimensions: 50 mm \times 0.3 mm, stainless steel hardware; <i>BIO</i> .
Unspecified	Stationary phase consists of proprietary copolymer and is recommended for Group I and II cation HR IC (single run) and transition metal analysis by ion chromatography; small particle provides rapid separations and high resolution; stainless steel or PEEK hardware; guard columns available.

Normal Bonded-Phase and Adsorption Chromatography Columns

Normal-phase chromatography still has its place in the separations laboratory, especially for water-sensitive samples, analytes that are insoluble in water, certain geometric isomers that are difficult to separate by reversed-phase chromatography, and for class separations. Organic solvents used in normal phase separations are more MS-friendly than some of the typical buffers used in HPLC. In reversed-phase chromatography, HPLC operating conditions sometimes must be modified dramatically to allow compatibility with MS detection. Daiso is a producer of silica gel whose primary chromatography business is selling bulk materials to the OEM market. The company has added a super wide-pore spherical silica gel to its portfolio. The packings have pore diameters of 1000 Å and 2000 Å that make them suitable for the separation of very large molecules. After suitable derivatization, proteins, oligonucleic acids, and other biomolecules can be separated. Containers with as much as 10 kg of silica packings are available with particle sizes ranging from 3 to 60 mm for analytical and preparative columns.

Cyano- and amino-derivatized packings can be used with reversed-phase solvents as lower-retention phases or with organic solvents in the normal-phase mode. Compa-

nies introducing cyano and amino phases this year include Alltech, Thermo Electron, Agilent Technologies, and Macherey-Nagel. Silica columns for the adsorption mode will be introduced by Alltech, Argonaut Technologies, Shiseido, Agilent Technologies, Waters, and Merck. Details of these normal phase products are featured in Tables I and II and in the preparative section.

Ion-Exchange and Ion Chromatography Columns

In looking at all the column inputs received, 17 new ion-exchange and ion chromatography columns — cation-exchange (9) and anion columns (8) — will be introduced at Pittcon 2004. Table III shows 13 of those entries (the rest are located in other sections). Continuing with the trend of the past few years, polymer-based columns still are in the majority compared with silica-based columns (13:4 ratio). Polymeric columns have a larger pH operating range and can be used and cleaned with harsh reagents such as sodium hydroxide, which is favored by biochemists to remove residual proteins. Silica-based ion exchangers usually are introduced by companies that have core competency in silica gel chemistry. In general, silica-based packings usually display higher efficiency than polymeric-based packings. Both PEEK and stainless steel column hardware

will be used. For ion chromatography applications, PEEK hardware seems to be preferred because any potential cations that might be leached from the stainless steel are absent. The applications for the ion-exchange offerings this year are split between the separation of small molecules and ions and the separation of biomolecules. Columns designed for separating biomolecules usually have larger average pore diameters, and I have assigned several of these columns to the specialty columns section.

Ion chromatography is used to separate trace inorganic and low molecular weight organic anions and cations, and usually it is performed isocratically with either suppressed or nonsuppressed conductivity detection. In principle, ion chromatography is exactly the same as ion-exchange chromatography. In the nonsuppressed mode, the ionic strength must be kept low to prevent detector saturation; thus, low-capacity ion exchangers frequently are employed. Several of this year's products were recommended for ion chromatography of small ions. An example of one of the newest ion chromatography columns is from Alltech Associates. Figure 2 shows the separation of mono- and divalent cations in less than 8 min on the company's 3-mm d_p Universal Cation HR column using a simple mobile phase and conductivity detection.

Table IV: HPLC specialty columns at Pittcon 2004

Product Name	Supplier	Type	Applications	Base Material	Functional Group	d_p (mm)
Allure Organic Acids	Restek	Reversed phase	Organic acids	Silica	Proprietary	5
BioSuite Peptide Analysis	Waters	Reversed phase	Peptide mapping, LC-MS	Silica	C18	3.5
Brio uPLC cartridge	Nanostream	Reversed phase	High throughput analyses	Derivatized polyolefins	C18	8–10
Capcell Pak C18 MG	Shiseido Fine Chemicals	Reversed phase	Melatonin analysis	Silica	C18	5
CarboPac PA200	Dionex	Anion exchange	Oligosaccharides	EVB-DVB*	Difunctional quaternary amine	5.5
Ceramospher RU-2	Shiseido Fine Chemicals	Chiral	Chiral	Sodium magnesium silicate	Ruthenium complex	5, 20
Chiral CD-Ph	Shiseido Fine Chemicals	Chiral	Chiral	Silica	β -Cyclodextrin	5, 20
Chiralpak IA	Chiral Technologies	Chiral	Chiral	Silica	Derivatized	5
Chromolith Cap Rod	Merck/EM Science	Reversed phase	Proteins, peptides	Silica monolith	C18	N/A
Cogent Type C4-300	MicroSolv Technology	Multimode	Proteins	Silica	C4	4
Conductive Sample Trap	Upchurch Scientific	Trapping column	LC-MS	Silica	C18 and strong cation exchange	5
Everest	Grace Vydac	Reversed phase	Proteins, peptides	Silica	C18	5–30
Inertsil WP300 C4	GL Sciences	Polar embedded RPC	Proteins	Silica	C4	5
Monolith Nano columns	Dionex	Reversed phase monolith	Proteins, peptides	PS-DVB	PS-DVB	N/A
OPTI-PAK Capillary Trap	Optimize Technologies	Trapping column	Biological sample analysis	Silica, DVB	Various	5
P-CAP, R,R, and S,S	BIA Separations (Astec)	Chiral	Broad-range chiral separation	Silica	Diamine	5, 10

*EVB-DVB 5 ethylvinylbenzene-divinylbenzene

Size-Exclusion Chromatography

Following a dearth of new products in this category at last year's Pittcon, this year will see the introduction of four new size-exclusion chromatography (SEC) packing materials or new configurations of older materials. Three of the four are for aqueous SEC. Joining the rest of the modes for new products for protein and other biomolecular separations and purification are new biocompatible materials from Alltech Associates and BioChrom Labs. The Alltech line, called ProSphere SEC columns, is based upon packing materials with a rigid hydrophilic backbone. With

average pore diameters of 125, 250, and 450 Å, their products cover a wide molecular weight range (roughly 5×10^3 – 7×10^6 for globular proteins). Particle sizes of 4, 5, and 8 mm provide a range of column efficiencies. Analytical (300 mm \times 4.6 mm) and semipreparative (300 mm \times 7.8 mm) columns along with protecting guard columns are offered.

BioChrom's BioGPC 1000 and 2000 packings are based upon a PS-DVB base particle that is made biocompatible with a hydrophilic coating containing hydroxy groups. The 10-mm particles are packed into 316 stainless steel columns with a 7.8-

mm inner diameter and 150- and 250-mm lengths. The columns are recommended for the high-speed separation of low to medium molecular weight biopolymers (BioGPC 1000) and high molecular weight biopolymers (BioGPC 2000). Guard columns and bulk amounts of the packing are available.

The other aqueous-compatible packing, called PL Aquagel-OH, (Polymer Laboratories) is a smaller-particle-size version of the company's 7-mm packing. The 5-mm macroporous column with a 1000-Å pore size and a hydrophilic surface is recommended for the separation of low molecu-

Pore Size (Å)	Comments
60	Recommended for organic acid analysis, especially in fruit juice according to the AOAC Method 986.13 using phosphate buffer, pH 2.5, and UV detection at 226 nm; column dimensions: 150, 250, or 300 mm \times 4.6 mm; guard columns available; compatible with 100% aqueous mobile phases.
100, 300	PA-A and PA-B columns provide different separation selectivities; PA-A column (100-Å pore size) is useful for polar peptides and for LC-MS using formic acid eluents; PA-B column (300-Å pore size) is recommended for complex digests containing hydrophobic, hydrophilic, and basic peptides, especially with TFA-containing eluents; columns specifically tested in peptide mapping applications; lengths as long as 250 mm; <i>BIO</i> .
100	Cartridge incorporates 24 columns in a highly parallel LC system called Nanostream Veloce; recommended for high throughput analytical separations for drug development and discovery; packing material is embedded inside of 30 mm \times 0.5 mm and 80 mm \times 0.5 mm channels; cartridge dimensions: 15 cm \times 23 cm \times 1 cm.
Unspecified	Column designed for Nanospace SI-2 semimicro LC system; internal diameters: 1.0, 1.5, and 2.0 mm; precolumn derivatization method for melatonin.
Microporous	EVB 5 ethylvinylbenzene; DVB 5 divinylbenzene; crosslinked with 45% DVB; recommended for separation of charged and neutral oligosaccharides derived from glycoproteins; pH stability 0–14; can be used for high pH separations of carbohydrates as anions using pulsed amperometric detection; ion-exchange capacity: 110 mequiv/g; column: 3 mm \times 250 mm; guard column available; <i>BIO</i> .
40	Recommended for the separation of acidic, basic, and neutral compounds; bonded tris(1,10-phenanthroline) ruthenium II complex, surface area: 300 m ² /g; high loadability and good temperature stability; microbore to preparative dimensions; guard columns available; can be used in aqueous or aqueous-organic mobile phases
Unspecified	Recommended for chiral separation of acidic, basic, and neutral compounds; high loadability and wide temperature capability; microbore to preparative dimensions; guard columns available.
Wide pore	Column is recommended for chiral resolution with a wide solvent tolerance, for both aqueous and nonaqueous mobile phases; dimensions: 250 mm \times 4.6 mm; preparative columns will be available in late 2004. Dimensions: 150 mm \times 100 mm; designed for proteomics and LC-MS applications; fully endcapped; provided with instructions and certificate of analysis.
300	Type C silica with silicon-carbon surface bonds (no surface silanols); can be used in reversed-phase, normal-phase, and HILIC (aqueous normal phase) modes; no protein binding or denaturing; 3% carbon loading; capillary to prep dimensions; guard columns and bulk packing available; <i>BIO</i> .
300 and 85, respectively	Capillary cartridge design for in-line, on-column voltage application for electro-spray LC-MS; requires voltage to be applied to tip; can serve as upstream ground to prevent electrospray voltage from damaging other sensitive LC equipment; stainless steel holder dimensions: 2.5 cm \times 100 mm; two separate phases (modes) available with different pore sizes; fittings connect with 360-mm o.d. capillary tubing.
300	Wide-pore Type A silica specifically designed for separation of proteins and peptides; novel silica treatment and bonding to provide high coverage of 4–4.5 mmol/m ² (5.75–6.75% carbon); nano- to preparative-scale column dimensions: 75-mm to 100-mm internal diameters available; guard columns and bulk packing available; <i>BIO</i> .
300	Recommended for proteins, high recovery for basic and hydrophobic proteins; formic acid is the recommended modifier for LC-MS; can be used at low pH; available in nano- to preparative-scale dimensions; guard columns; <i>BIO</i> .
Unspecified	Column available in 5 cm \times 100 mm fused-silica column placed in a monolith protective housing; pH range 2–7.5; normalized to 250,000 plates/m; <i>BIO</i> .
Unspecified	Recommended for sample preparation and cleanup (preconcentration, desalting, and detergent removal); new design has all excess swept volume eliminated, resulting in a packed bed that can be introduced directly into the flowpath, which eliminates dilution and dispersion effects; can be used in multidimensional column-switching system; volumes range from 0.25 to 5 mL; <i>BIO</i> .
200	Recommended for use in normal-phase solvent systems; brush polycyclic amine polymer phase in R,R and S,S configurations for possible elution order reversal; sample capacity: 1–5 mg/g packing; available in microbore to preparative dimensions and bulk packing; can use volatile organic solvents for preparative-scale chromatography and LC-MS; stainless steel column construction.

lar weight water-soluble polymers. This smaller particle provides an improvement in efficiency and, therefore, resolution. The stainless steel column dimensions are 300 mm \times 7.5 mm.

Polymer Laboratories also has the only organic SEC columns introduced at Pittcon 2004. The PlusPore range is based upon high-pore volume PS-DVB beads with 3–5- μ m particle size and a 10⁵-Å average pore size, making it suitable for a wide range of synthetic organic polymers, resins, and prepolymers. With the multi-pore technology employed, these columns exhibit higher resolution than conventional

beads. The company offers two dimensions in these stainless steel columns: 250 mm \times 4.6 mm and 300 mm \times 7.8 mm.

Specialty HPLC Columns

Specialty columns are HPLC columns that have been developed for specific separations that are difficult to achieve on standard columns. However, sometimes manufacturers will use a standard column but test it specifically for a certain compound class and provide a recommended set of chromatographic conditions. In some cases, the specialty column comes as part of a “total solution” kit with reagents, stan-

dards and method. Most specialty columns will come with a test chromatogram from an analysis performed at the factory before shipment, and some are guaranteed. Table IV shows 32 specialty columns that will be presented at Pittcon 2004. Silica gel-based specialty columns outnumber polymer-based columns by almost two to one.

The largest single category of specialty columns is that designated for the separation of biomolecules, mainly proteins and peptides. Approximately one half of the specialty columns in Table IV are in this category. It should be noted that a few other columns for protein separations are

Table IV: HPLC specialty columns at Pittcon 2004, continued

Product Name	Supplier	Type	Applications	Base Material	Functional Group
PicoFrit columns 50-mm	New Objective	Reversed phase, cation exchange	LC-MS	Silica	C18 and other
Primesep A, B2 and C	Allsep Technologies	Mixed mode	Polar compounds	Silica	See comments
Primesep-100 and -200	Allsep Technologies	Reversed phase	Polar compounds	Silica	Unspecified acidic phase
ProntoSIL Chiral AX	Bischoff	Anion exchange	Chiral	Silica	Quinine, quinidine
ProteCol Trap	SGE	Trapping column	Preconcentration of dilute samples	Silica	C18
ProteoPep II PicoFrit	New Objective	Reversed phase	Peptide mapping and purification	Silica	C18
Rhinophase-AB	Zirchrom Separations	Ion exchange- affinity	Monoclonal antibody	Zirconia	EDTPA (see comments)
Shodex Sugar KS-807	Showa Denko	Size exclusion	High molecular weight sugars	PS-DVB	Sulfonic
Styros rA	Orachrom	Antibody affinity	IgG in serum	PS-DVB/methacrylate	Recombinant Protein A
Sucrebond I	Shiseido Fine Chemicals	Reversed phase	Monosaccharide, sugar alcohols	PS-DVB	PS-DVB
Swift RP-C4	Isco	Reversed phase monolith	Proteins	Polymethacrylate	C4
Swift RP-SP	Isco	Reversed phase monolith	Proteins, peptides	PS-DVB	Phenyl
TSKgel BioAssist Q and S	Tosoh Bioscience	Ion exchange	Large biomolecules	Polymethacrylate	Anion, cation (see comments)
Turbo BS-C23	Cil Cluzeau Info Labo	Mixed mode	Acidic compounds	Silica	See comments
Ultra Carbamate	Restek	Reversed phase	Carbamate pesticides	Silica	Proprietary

d_p (mm)	Pore Size (Å)	Comments
Unspecified	Unspecified	Fused-silica nanocolumns with 50- μ m internal diameter, 360- μ m outer diameter and 10- and 15- μ m tips; integrated frit reduced clogging; spray directly from column to eliminate band broadening and possible postcolumn loss; available with various packing materials or customer-supplied chemistries; <i>BIO</i> .
5, 10, 20, 50	Unspecified	Primesep A has unspecified strongest acidic phase, reversed-phase-cation-exchange mixed mode mechanisms; Primesep B2 has an unspecified weakly basic phase (pH range 0.5–7), used with ammonium acetate buffers, mixed mechanisms of reversed phase and anion exchange; Primesep C has combined reversed-phase properties with weakly acidic functional group; modes of reversed phase, normal phase, ion exchange, ion exclusion, and HILIC are possible depending on mobile phase; can retain polar compounds without ion-pair reagents; microbore to preparative dimensions available.
5, 10, 20	Unspecified	A reversed-phase column with embedded strong acidic (100) or medium acidic (200) functional groups; can also be used for normal phase, HILIC, cation exchange and anion exclusion; can be used with 100% aqueous; retains polar compounds, especially strongly basic, without use of ion-pair reagents; microbore to preparative dimensions; 200 column possesses complex formation properties for some metal ions.
5, 10, 20	Unspecified	Recommended for chiral separation of acidic analytes, especially hydroxy acids; QN 5 quinine derivative and QD 5 quinidine derivative; can be used in polar organic (normal- and reversed-phase mode) and aqueous eluents; stable at pH 2–8; microbore to preparative
3	120 or 300	Recommended for drug discovery and proteomics applications for capillary LC; fused silica-lined PEEK (PEEKsil) used for both column and connecting tubing with integrated design to minimize dead volume and for robustness; PEEKsil material provides flexibility and smooth inert flow path; 10 mm \times 150 or 300 mm; equipped with 10-mm length of PEEKsil inlet and outlet tubings; <i>BIO</i> .
5	300	Fused-silica packed columns with dimensions of 50- and 75- μ m i.d. \times 360- μ m o.d. with 10- and 15- μ m tips for direct connection to mass spectrometer inlet; recommended for hydrophobic and hydrophilic peptides; <i>BIO</i> .
3, 4, 7, 10, 25	300	EDTPA 5 ethylenediamine- <i>N,N,N',N'</i> -tetra(methylenephosphonic)acid; recommended for purification of monoclonal antibodies by pseudoaffinity separation mechanism; biocompatible phase with pH range 1–14; temperatures as high as 50 °C; maximum binding capacity for hIgG is 55–65 mg IgG/mL of phase; available in packed columns, bulk or in research and production kits; <i>BIO</i> .
30	Unspecified	SEC column for molecular weight distribution analysis of hyaluronic acid and other water-soluble, high molecular weight polymers; estimated exclusion limit 2×10^8 g/mol; analysis more rapid than traditional viscosity method; column dimensions: 300 mm \times 8.0 mm.
Unspecified	Gigaporous	Recommended for fast isolation of most human, rabbit, cow, guinea pig, dog, and pig IgG directly from undiluted serum; no leaching of; immobilized ligand from matrix; total covalent chemistry starting with simulated monolithic matrix to the coatings and functionalization; fully pervious, non-obstructed pores with uniform distribution; high capacity and strong binding of IgG; can be cleaned with harsh reagents such as sodium hydroxide; dimensions: 33–150 mm \times 2–4.6 mm; PEEK and stainless steel construction; <i>BIO</i> .
7	Unspecified	Column is used in conjunction with pulsed amperometric detector for high sensitivity analysis of carbohydrates; wide pH range; column dimensions: 35, 150, and 250 mm \times 2.0 mm.
N/A	> 500	Monolith capillary column; pH range 0–12; column dimensions: 150 mm \times 0.32 mm; column construction: fused-silica tubing with PEEK sleeve; <i>BIO</i> .
N/A	> 500	Monolith capillary column; pH stable 0–14; column dimensions: 150 mm \times 0.20 mm; column construction: fused-silica tubing with PEEK sleeve; <i>BIO</i> .
7, 10, 13	4000 (AEX) 1200 (CEX)	Macroporous resin with large pores allow for access to the binding sites for large molecules resulting in improved capacity and resolution; anion exchanger has a combination of secondary and tertiary amine groups and cation exchanger has sulfopropyl ligands; 0.1 mequiv/L of ion-exchange capacity; PEEK hardware; analytical and preparative dimensions available; <i>BIO</i> .
2	80	Column recommended for separation of acidic molecules that are more retained than basic compounds; the hybrid bonded phase contains an embedded amino group between a C ₆ and a C ₁₈ alkyl chain; both endcapped and nonendcapped versions available; 25% carbon phase coverage; analytical column lengths from 13 to 50 mm; stainless steel and packed fused-silica construction; guard and preparative columns available.
3	100	Column is specifically tested for the 10 carbamate pesticides listed in EPA Method 531.1 using 4-bromo-3,5-dimethylphenyl- <i>N</i> -methyl carbamate internal standard and water-methanol-acetonitrile gradient and UV detection at 220 nm; separation in under 10 min; column dimensions: 4.6 \times 50 mm.

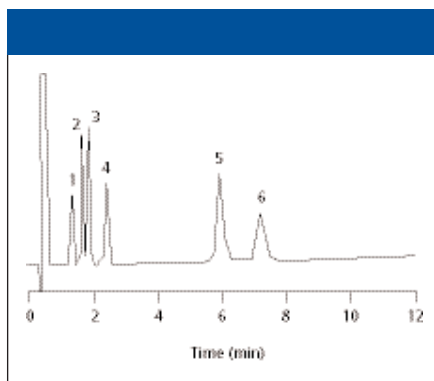


Figure 2: Separation of monovalent and divalent cations using a 53 mm \times 7.0 mm, 3- μ m d_p Universal Cation HR column (Alltech). Mobile phase: 3 mM methanesulfonic acid; flow rate: 2.5 mL/min; detection: conductivity. Peaks: 1.5 lithium, 2.5 sodium, 3.5 ammonium, 4.5 potassium, 5.5 magnesium, 6.5 calcium.

covered in the ion-exchange and reversed-phase chromatography sections. Several of these biochromatography columns were designed for direct and easy interfacing to an MS detector. Capillary, nano, and trapping columns specifically designed for LC-MS use are included in the specialty columns category this year and will be introduced by several companies. Capillary and nano columns, with internal diameters as small as 0.05 mm, have major advantages for the small samples encountered in

protein digests because they can provide good sensitivity in sample-limited cases and can be interfaced directly to the MS source. The use of two-dimensional capillary chromatography coupled with MS is becoming an important replacement technique for two-dimensional gel electrophoresis. By first fractionating a peptide digest on a cation-exchange capillary with subsequent desalting (via a reversed-phase chromatography trap column) and transfer of fractions to a reversed-phase chromatography nanocolumn and then to an MS or MS-MS system might be the preferred technique once the methodology proves rugged and reproducible. It should be noted that for optimum chromatographic performance, capillary- and especially nano-LC columns require specialized HPLC instruments or major modifications to existing analytical instruments.

Affinity chromatography is among the most selective techniques in separation science. The Rhinophase-AB stationary phase (Zirchrom) is used primarily for the large-scale purification of monoclonal antibodies. The biocompatible stationary phase, termed a pseudo-affinity phase because the stationary phase is not an antigen, is useful for small- and large-scale purification of biomolecules. Compared with soft gels, the rigid material can be run at very high mobile phase linear velocities that result in increased sample throughput. Figure 3

depicts the fast preparative purification of IgG₁ in a cell culture supernatant using a 10-g bed of Rhinophase-AB. The total turnaround time was only 15 min, at least two orders of magnitude faster than could be achieved on a soft gel column. A simple vacuum manifold was used to pull the various solutions through the packed bed.

Other significant application columns were those for chiral compounds and sugars-oligosaccharides. Of particular interest is the use of high pH to separate and detect sugars in their anion form. For this application, polymeric packings that can withstand high pH are used with pulsed amperometric detection to separate trace amounts of carbohydrates derived from glycoproteins. Both Dionex and Shiseido will be introducing products in this application area.

Acknowledgement

I would like to thank the manufacturers and distributors who kindly furnished the requested information in advance of Pittcon 2004, thus allowing a timely report of new product introductions. For those manufacturers who would like to be considered for inclusion into Pittcon 2005 coverage, please send the name of the primary company contact, mailing address, fax number, and e-mail address to "Column Watch" Pittcon 2005, LCGC, 485 Route 1 South, Building F, Iselin, NJ 08830.

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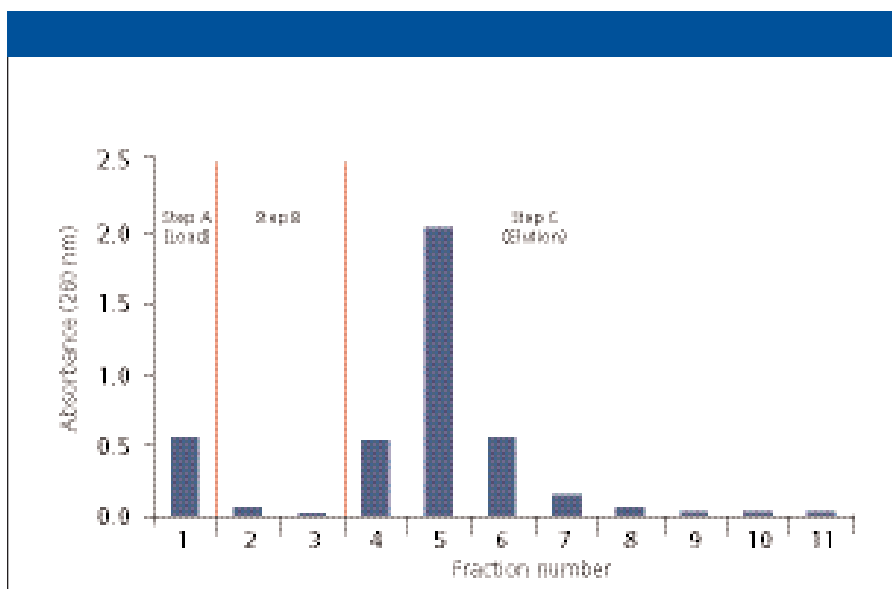


Figure 3: Ultrafast preparative purification of IgG₁ using Rhinophase-AB stationary phase (Zirchrom). Step A: 20 mM MES buffer, 4 mM EDTPA, 50 mM sodium chloride (pH 4.0). Step B: 20 mM MES buffer, 4 mM EDTPA, 50 mM sodium chloride (pH 4.0). Step C: 20 mM MES buffer, 4 mM EDTPA, 2.0 M sodium chloride (pH 4.0). Flow rate: 60 mL/min; injection volume: 31.6 mL serum-free cell culture supernatant diluted fourfold with loading buffer (3.98 mg of monoclonal antibody); stationary phase in tube: 10 g. Total analysis time: 15 min.

Ronald E. Majors

"Sample Prep Perspectives" Editor
Ronald E. Majors is business development manager, Consumables and Accessories Business Unit, Agilent Technologies, Wilmington, Delaware, and is a member of LCGC's editorial advisory board.



Direct correspondence about this column to "Sample Prep Perspectives," LCGC, Woodbridge Corporate Plaza, 485 Route 1 South, Building F, First Floor, Iselin, NJ 08830, e-mail lcgcedit@lccmag.com.