



Column

Watch

This month's "Column Watch" is the conclusion of a two-part series in which Ron Majors examines the trends in column introductions at Pittcon 2003. In part II, he discusses large- and preparative-scale chromatography columns, gas and supercritical fluid chromatography columns, thin-layer chromatography and capillary electrophoresis products, sample preparation products, and hardware and accessories for chromatography and sample preparation.

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Column Watch Editor

New Chromatography Columns and Accessories at the 2003 Pittsburgh Conference, Part II

Pittcon 2003 — the 54th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy — ended its stay in Orlando, Florida, last month. Approximately 24,000 attendees visited the conference and participated in its presentations and exhibition. The purpose of this two-part report is to provide information about many of the new consumable and accessory separation products that were displayed at the show (1). In some cases, products that were introduced during 2002 but after Pittcon 2002 have been included for reasons of completeness. The information is based upon manufacturers' responses to a questionnaire mailed in December 2002. Because of space limitations and the fact that some manufacturers did not respond to the questionnaire, this report cannot be considered an exhaustive listing of all new products introduced in Orlando this year.

As in previous years, columns and other products recommended by their manufacturers primarily for biomolecule separations are denoted in the tables with the designation *BIO*. Some of these products can be used for general-purpose high performance liquid chromatography (HPLC) separations as well, but their main applications are in bioseparations. I cite specific information about bioapplications where appropriate.

In last month's column, I described new introductions for high performance liquid, reversed-phase, normal- and bonded-phase, ion-exchange and ion, size-exclusion, and specialty chromatography columns (1). This month, I will discuss large- and preparative-scale chromatography columns, gas chromatography (GC) columns, thin-layer chromatography (TLC) and capillary electrophoresis (CE) products, sample preparation products, and small hardware and accessories for chromatography and sample preparation.

Large- and Preparative-Scale Chromatography Columns

The definition of preparative chromatography always has been fuzzy and in the eyes of the beholder, because the mass of the injected or collected sample often is dependent upon the amount available or the intended need. For some, a few micrograms of material is sufficient for further characterization or use, but for others, 10–100 g represents preparative amounts.

Flash chromatography is a throwback to the old days of preparative chromatography when chromatographers spent hours filling and unpacking glass columns to separate crude synthetic mixtures or natural products. At that time, gravity feed systems were the norm; they had huge reservoirs placed overhead, and the column effluent was collected manually or by a crude time-based fraction collector. Nowadays, convenient prepacked flash columns and cartridges are available with predetermined packing masses and various dimensions. In fact, several companies provide fully automated or nearly automated flash chromatography instruments to make the whole process easy and convenient. To fill the need for flash columns, companies have responded by producing prepacked flash columns to fit these instruments.

The latest entry is the Chromabond Flash cartridges from Macherey-Nagel. These columns are packed with 10–50 μm d_p irregular or spherical packings with more than 30 phase chemistries. These phases match the company's TLC plates, so method development and purity checks can be performed during the flash chromatography experiments. Solid-phase extraction (SPE) cartridges with the same materials also can be purchased.

Fullerenes continue to be an area of active research, and researchers often require preparative quantities for further

study. Nacalai Tesque responded to this need by developing the 250 mm × 50 mm packed Cosmosil 10PBB columns that are quite specific for fullerenes. The 10- μm d_p columns with 120-Å pores have a pentabromobenzyl-bonded moiety and can be used for many separations with pure toluene, a relatively inexpensive solvent. Fullerenes also can be solubilized with carbon disulfide and 1,2,4-trichlorobenzene. The columns can separate the higher fullerenes, as well as metallofullerenes and their derivatives.

Another area of great interest is the preparation of larger quantities of pure enantiomeric compounds. Responding to this trend, Chiral Technologies and its parent company Daicel Chemical Industries have extended their range to include higher capacity, semipreparative versions of their popular Chiralpak phases by supplying them in 250 mm × 30 mm column dimensions. These chiral polysaccharide-based polymer particles (5- μm d_p , 1000-Å pores) are derivatized with various chiral functionalities such as cellulose tris(3,5-

dimethylphenyl carbamate), cellulose tris(4-methylbenzoate), amylose tris(3,5-dimethylphenyl carbamate), and [(*S*)- α -methylbenzyl carbamate]. These columns are recommended especially for high-throughput supercritical fluid chromatography (SFC) separation of various chiral compounds such as pharmaceuticals. Separations of acidic compounds can be achieved without the use of acidic additives that can interfere with product recovery.

Preparative chromatography has found widespread use in the purification of pro-

teins and polypeptides from biological origins. This year BIA Separations displayed biopurification columns based on the company's polymeric monolith technology. The company introduced a CIM 800-mL tube and a CIM 8-L tube monolith cartridge for very large-scale (industrial) production. The monolith, a poly(glycidylmethacrylate-*co*-ethyleneglycoldimethacrylate) copolymer, was fabricated into two sizes of a tube configuration: 150 mm × 65 mm with a 105-mm o.d. and an 800-mL bed volume and 33 cm × 24 cm with a 30-cm

o.d. and an 8-L bed volume. The monoliths are functionalized with diethylamino for anion-exchange purifications and epoxy functional groups that can be used to immobilize antigens or other reactive compounds. With comparable analytical columns available, biochromatographers now have the ability to optimize their separations on a smaller monolith column and then scale them to industrial-scale production and purification. A demonstrated binding capacity of 24.0 ± 4.0 mg/mL wet support translates to nearly 200 g of

purified product per run for the 8-L bed cartridge. This bed also can withstand flow rates as great as 2 L/min to provide high-throughput possibilities.

Gas Chromatography Columns

Seven companies contributed new GC column information for Pittcon 2003 and presented 28 columns and phases (Table I). Several of the column introductions were low-bleed stationary phases that allowed better detection limits and less baseline drift during temperature-programmed sep-

Table I: Gas chromatography columns at Pittcon 2003

Product Name	Supplier	Type	Stationary Phase	Film Thickness (μm)	Inner Diameter (mm)	Length (m)	Comments
Chiraldex B-DP and G-DP columns	Astec	WCOT	See comments	0.125	0.25	10, 20, 30, 40, 50	Phase: B-DP = 2,3-di- <i>o</i> -propionyl-6- <i>o</i> -tertbutylsilyl- β -cyclodextrin; G-DP = 2,3-di- <i>o</i> -propionyl-6- <i>o</i> -tertbutylsilyl- γ -cyclodextrin; recommended for analyzing aromatic and aliphatic chiral amines, chiral acid esters, and chiral lactones; columns have broad, general chiral selectivity and good speed and efficiency for polar chiral analyte analysis; 200/220 °C temperature limits.
FactorFour low-bleed columns	Varian Middelburg	WCOT	VF-1ms, VF-5ms, VF-Xms, VF-23ms	0.1–1.0	Various	Various	Four-times-lower published bleed specifications than earlier phases; suggested for trace applications in GC and GC-MS analyses; low background; available phases include VF-1ms = 100% polydimethylsiloxane, VF-5ms = 5% phenyldimethylpolysiloxane, VF-Xms = high percentage arylene-modified polysiloxane, and VF-23ms = 50% cyanopropylmethylpolysiloxane; can withstand temperatures as high as 375 °C.
FactorFour VF-DA columns	Varian Middelburg	WCOT	Nonpolar	0.25	0.20	12	Recommended for the separation of drugs of abuse with MS detection; high-stability phase with very high resistance for repeated splitless methanol injections; low background for MS measurements.
HayeSep D Valcoplot columns	VICI Gig Harbor Group	PLOT	HayeSep D- and Q-divinylbenzene	10–20	0.32, 0.53	Not specified	Recommended for the separation of light gases; provides similar retention indices as packed columns; –50 to 250 °C temperature range.
HayeSep R Valcoplot columns	VICI Gig Harbor Group	PLOT	HayeSep R divinylbenzene– <i>N</i> -2-pyrrolidinone	10–20	0.32, 0.53	Not specified	Recommended for the separation of halogen- and sulfur-containing compounds, water, alcohols, free fatty acids, esters, ketones, and aldehydes; –50 to 250 °C temperature range; provides similar retention indices as packed columns.
Heliflex AT-1ms columns	Alltech Associates	WCOT	100% polydimethylsiloxane	0.25	Various	Various	Columns recommended for MS analyses; low-bleed characteristics; 360 °C upper temperature limit; same selectivity as other 100% dimethylpolysiloxane phases.
InertCap GC capillary columns	GL Sciences	WCOT	See comments	0.1, 0.25, 0.5, 1.5	0.25, 0.32, 0.53	Various	Polydimethylsiloxane phases include 1 version = 100%, 5 version = 5% phenyl, 1- and 5-MS versions of latter two, 17 version = 50% phenyl, 35 version = 35% phenyl, 1701 version = 14% cyanopropylphenyl, and 1301 version = 6% cyanopropylphenyl; other phases include WAX = polyethyleneglycol and FFAP = free fatty acid phase; new family of columns recommended for analyzing a wide variety of polar and nonpolar volatile compounds.
Rt-2560 FAME columns	Restek	WCOT	Biscyanopropyl	0.2	0.25	100	Column is recommended for the separation of <i>cis</i> and <i>trans</i> isomers of fatty acid methyl esters; can separate saturated and unsaturated fatty acid methyl esters; 250 °C upper temperature limit; 312.5:1 phase ratio; also food industry fatty acid methyl ester mix (reference materials) of 37 components designed with AOAC Method 996.06 in mind.
Select Mineral Oil columns	Varian Middelburg	WCOT	Nonpolar, high cross-linking	Proprietary	0.32	15	Recommended for C ₅ –C ₄₄ mineral oil analysis; stable at temperatures as high as 400 °C.
Select Permanent Gases/CO ₂ columns	Varian Middelburg	PLOT	Bonded-phase polymer and MolSieve 5A	Not specified	0.32, 0.53	10, 25, 50	Columns recommended for the separation of permanent gases and carbon dioxide at percent and parts-per-million levels; two systems available, including an HR version for high resolution of argon and oxygen and a standard column for fast analysis of permanent gases and carbon dioxide; stable at temperatures as high as 300 °C; two lengths per column, one set 10 m × 0.32 mm and 50 m × 0.53 mm and the other set 25 m × 0.32 mm and 50 m × 0.53 mm; lengths and inner diameters are optimized to run together so separation of permanent gases on MolSieve is obtained before first peak is eluted from porous polymer column.
ShinCarbon ST columns	Restek	Micropacked	Carbon	Not applicable	1	2	Column is recommended for analyzing permanent gases — oxygen, nitrogen, methane, carbon monoxide, and carbon dioxide — and C ₁ –C ₇ hydrocarbons.
SolGel-WAX columns	SGE	Sol gel	Polyethylene glycol	1.0	0.53	30	A high-temperature polar phase recommended for analyzing volatile compounds, alcohols, esters, and solvents; can withstand temperatures as high as 300 °C.
Zebtron ZB column additions	Phenomenex	WCOT	See comments	0.5–3.0 phase dependent	0.25, 0.53	Various	Four new capillary column products; 624 VOC column is designed for volatile organic compound analysis as per U.S. Environmental Protection Agency methods, phase is 6% cyano–94% methylpolysiloxane, available in 75- and 105-m lengths, 3- μm d_f film; ZB-1 PONA-50 and PONA-100 columns are designed for the analysis of petroleum process products such as refinery gas and unleaded gasoline, phase is 100% dimethylpolysiloxane, 0.25- and 0.50- μm d_f and 50- and 100-m lengths, respectively, can withstand temperatures as high as 350 °C; ZB-1 SIM DIST column is designed for American Society for Testing and Materials Test Method D-2887, 10-m, 0.53-mm, and 2.65- μm d_f , can withstand temperatures as high as 360 °C; all columns are solvent rinseable.

Companies Listed in This Column

Advanced Separation Technologies (Astec), Whippany, New Jersey
 Agilent Technologies, Inc., Wilmington, Delaware
 Alltech Associates, Inc., Deerfield, Illinois
 BIA Separations, Ljubljana, Slovenia
 Chiral Technologies, Inc., Exton, Pennsylvania
 Daicel Chemical Industries, Ltd., Tokyo, Japan
 Dionex Corp., Sunnyvale, California
 GL Science, Tokyo, Japan
 Hamilton Co., Reno, Nevada
 Harvard Apparatus, Inc., Holliston, Massachusetts
 Horizon Technology, Inc., Atkinson, New Hampshire
 Iris Technologies LLC, Lawrence, Kansas
 Jordi FLP, Bellingham, Massachusetts
 Kimble/Kontes, Vineland, New Jersey
 Macherey-Nagel GmbH & Co. KG, Düren, Germany
 Merck KGaA, Darmstadt, Germany
 MicroSolv Technology Corp., Long Branch, New Jersey
 Nacalai Tesque, Kyoto, Japan
 OraChrom, Woburn, Massachusetts
 Orochem Technologies Inc., Westmont, Illinois
 Phenomenex, Torrance, California
 Polymer Laboratories, Amherst, Massachusetts
 Quadrex Corp., Woodbridge, Connecticut
 Restek Corp., Bellefonte, Pennsylvania
 SEDERE, Alfortville, France
 SeQuant AB, Umeå, Sweden
 SGE Inc., Austin, Texas
 Spark Holland BV, Emmen, The Netherlands
 Supelco, Bellefonte, Pennsylvania
 Thermo Electron Corp., Runcorn, United Kingdom
 United Chemical Technologies, Bristol, Pennsylvania
 Valco Instruments Co., Inc., Houston, Texas
 Varian, Inc., Palo Alto, California
 Varian Middelburg, Middelburg, The Netherlands
 VICI Gig Harbor Group, Gig Harbor, Washington
 Waters Corp., Milford, Massachusetts
 ZirChrom Separations Inc., Anoka, Minnesota

arations. Two companies, GL Science and Varian, introduced entire new lines of capillary columns, the latter with greatly improved bleed specifications compared with those of previous phases. Capillary columns with inner diameters ranging from 0.20 mm (small bore) to 0.53 mm (large or megabore) were the most popular sizes. This year, most introductions were wall-coated open tubular (WCOT) columns, but three porous-layer open tubular (PLOT) columns and one micro-packed column debuted.

Many of the general-purpose GC capillary columns displayed at Pittcon 2003 were cross-linked alternatives to popular coated phases. Cross-linked, chemically bonded, or immobilized phases are more stable, have lower bleed, and usually can operate at higher temperatures than can coated phases. Another advantage of bonded phases is their ability to be rinsed by solvents. If columns become contaminated by high-boiling-point compounds, inorganic compounds, or other nonvolatile impurities that can cause drifting baselines, bleed, and other undesirable occurrences, users can disconnect the columns, wash them (outlet to inlet direction) with various solvents, and place them back into the gas chromatograph for additional use.

Many applications-specific columns were displayed at Pittcon 2003. Columns for analyzing drugs of abuse, mineral oil, chiral compounds, free fatty acids and fatty acid methyl esters, permanent gases, petroleum-related assays (for example, hydrocarbons, simulated distillation, paraffins, olefins, naphthenes, and aromatic compounds), and environmental compounds (for example, volatile organic compounds) were among those columns submitted. These columns usually are tested with the compounds to be assayed, and the manufacturers ship test chromatograms with the columns. Figure 1 shows a chromatogram of drugs of abuse analyzed by the Varian FactorFour VF-DA column. Note the good resolution of the amphetamines, which often are present at very low concentrations in biological fluids. Mass spectrometry (MS) is the favored detection technique for drugs-of-abuse analysis because of its high sensitivity and selectivity.

The newest type of capillary column introduced at Pittcon 2003 was a thicker-film SGE SolGel-WAX column, which joins the sol gel-based columns from last year's Pittcon. Although sol gels are used to

Text continues on page 344.

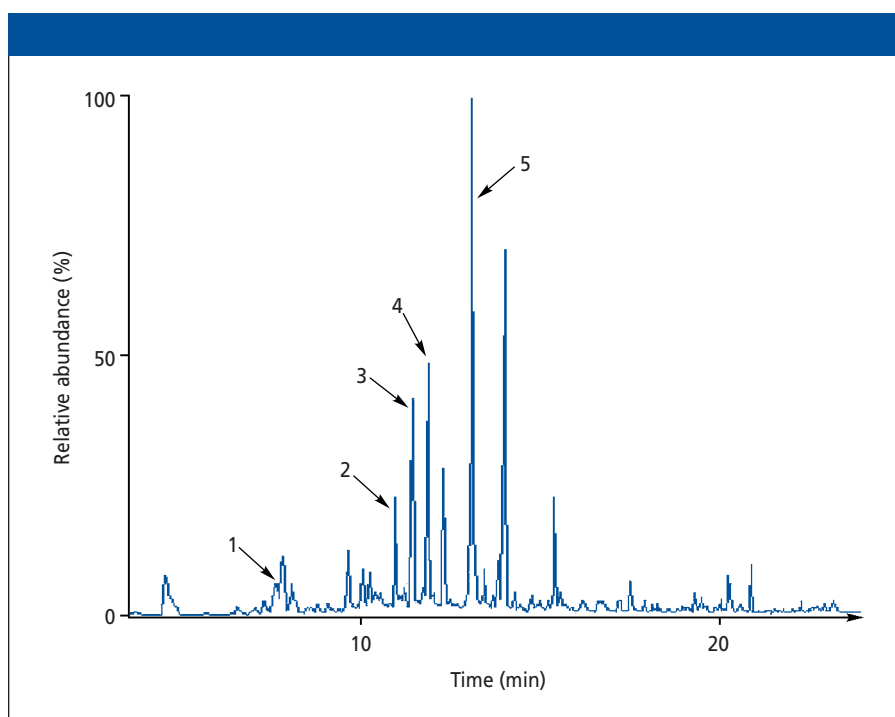


Figure 1: Separation of drugs of abuse on a FactorFour VF-DA GC capillary column with MS detection. Column: 12 m × 0.20 mm FactorFour VF-DA; temperature program: 70 °C for 2 min, 70–200 °C at 20 °C/min, 200–260 °C at 5 °C/min, 320 °C for 2 min. Peaks: 1 = amphetamine, 2 = 3,4-methylenedioxy-amphetamine (MDA), 3 = 3,4-methylenedioxy-methamphetamine (MDMA), 4 = 3,4-methylenedioxy-ethylamphetamine, 5 = cotinine. (Courtesy of Varian.)

Table II: Sample preparation products at Pittcon 2003

Product	Supplier	Product Type	Modes	Base Material	Functional Group	Dimensions	Comments
AccuBOND II ENV PS-DVB cartridges	Agilent Technologies	SPE cartridges	Reversed phase	PS-DVB*	PS-DVB	1.0 g/6 mL	Polymeric packing recommended for the extraction of analytes from environmental samples (especially trace phenols in water) and pharmaceutical analytes; prewashed packing, frits, and cartridge bodies for cleaner extracts; 75-150 µm particle size; 600-m ² /g surface area for high capacity (caffeine 320 mg/g; benzylalkonium chloride 370 mg/g); 520-Å pore diameter.
Chromabond SPE cartridge	Macherey-Nagel	SPE cartridge	Reversed and normal phase, cation and anion exchange, hydrophilic interaction	Silica	Various	1-50 g in 1-150 mL	Flash chromatography particles sold in polypropylene cartridges with more than 30 phases available; same packings used in TLC plates for method development and purity checks.
Clean-up Scavengers products	United Chemical Technologies	Scavenger product	Covalent bonding	Silica	See comments	1-, 3-, 6-, 10-, 25-, and 75-mL cartridges	A family of scavengers for various reactant molecules used in combinatorial synthesis; phases include THX = thiopropyl for alkylating agents, ICN = isocyanate (for amines, alkoxides, and other nucleophiles), TAX = triacetic acid for chelation of metal ions, and PAX = polyimine (for acids, sulfonyl chlorides, isocyanate, and other electrophiles); reactions by covalent bonding; 40-60 µm particle sizes; 60-Å pore size; cartridges and 96-well plates available.
Disposable Buchner funnels	Supelco	SPE and filtration	Adsorption, reversed and normal phase	SPE packed-bed polypropylene	See comments	55-, 70-, 90-, and 110-mm bed	Prepacked disposable Buchner funnels have a two-piece polypropylene body with 55-110 mm diameters; the lower half is a funnel, the upper half is tightly packed with adsorbent between two polyethylene frits with a heat-sealed retaining ring to further compress the bed; silica, magnesium sulfate, Celite, Florisil, polyamide, alumina, and charcoal sorbents available; similar to SPE but on a larger scale (masses of packing: 12.5 g/55 mm, 25 g/70 mm, 50 g/90 mm, 100 g/110 mm).
DryDisk membrane	Horizon	Separation membrane	Drying agent	PTFE† membrane	None	65-mm membrane	A hydrophobic membrane using Gore-Tex process filtration media designed to remove residual water from methylene chloride extractions; replaces sodium sulfate drying agent; high sample throughput; reduces laboratory waste and preparation time; works on emulsions.
Focus SPE 96-well plate	Varian	96-well SPE plate	Reversed phase	Functionalized PS-DVB	Proprietary	10 mg/1-mL well	Especially recommended for the extraction of drugs from biological matrices but can be used for other applications; can retain polar and nonpolar analytes; Power Rinse feature allows 10-20% organic wash without loss of analyte, which provides reduced ion suppression and cleaner extracts for LC-MS; simpler extraction protocols and enhanced polar recovery; 44-µm average particle size; 70-Å pore size; pH 0-14 range; 140 µg/10 µL bed analyte capacity.
InertSep cartridge	GL Sciences	SPE cartridge	Reversed phase	Methacrylate-PS-DVB	Methacrylate-PS-DVB	230 mg/6 mL	A water-wettable phase that requires no preconditioning; recommended for analyzing both hydrophilic and hydrophobic compounds; polymer is a methacrylate-PS-DVB copolymer; spherical particle; 40- and 70-µm particle size; 85-Å pores; 630 m ² /g; 1-, 3-, and 6-mL cartridge volume.
Oasis µElution SPE plate	Waters	96-well plate	Mixed mode	DVB-NVP‡	HLB, MCX, MAX	96-well plate format	Recommended for cleanup of small molecules in small volumes of biological matrices; microelution tips allow elution volumes as small as 25-µL for an increased concentration without the need for evaporation (direct injection); HLB = hydrophilic-lipophilic balanced reversed-phase sorbent; MCX = mixed-mode cation-exchange (sulfonic)-reversed phase; MAX = mixed mode anion exchange (quaternary amine)-reversed phase; 30-µm particle size; 80-Å pore size.
Orpheus silica sorbents	Orochem Technologies	SPE family of sorbents	Reversed and normal phase, ion exchange, mixed mode	Silica	Various	Various	A family of silica sorbents available in cartridges, disks, columns, and 96-well plates; 60- and 300-Å pore sizes; specific phases for analyzing drugs of abuse in biological fluids and large mass cartridges for environmental samples.
Polyamide media	Jordi	SPE bulk packing	Hydrophilic interaction	Polyamide	Polyamide	Not applicable	Highly hydrophilic packing with 10,000-Å pores; can withstand pH 0-14 values and high temperature.
Prevail C18 SPE cartridge	Alltech Associates	SPE cartridge	Reversed phase	Silica	Modified C18	1.5-, 4-, and 8-mL cartridges	Water-wettable phase for use with 100% aqueous samples; requires no preconditioning; 35-75 µm particle sizes; 500-m ² /g surface area; 60-Å pores; 11% carbon load; sizes: 0.1 g/1.5 mL, 0.5 g/4 and 8 mL, 1g/8 mL in Extra Clean syringe barrel and 0.3, 0.5, and 0.9 g in Maxi-Clean format.
Protain cartridge	ZirChrom Separations	In-line protein removal cartridge	Mixed mode	Zirconia	Polybutadiene	20 mm × 2.1 and 4.6 mm	Recommended for the removal of proteins from biological fluids; proteins will be strongly retained by multimodal interactions (reversed phase and cation exchange); 5-, 10-, and 25-µm particle sizes; 300-Å pore size; compatible with any type of HPLC column; disposable cartridge requires a holder and can be changed in less than 1 min; <i>BIO</i> .
Protein filter plates	Orochem Technologies	Protein removal	Precipitation	Polypropylene	None	96-well plate size	Protein crash and protein precipitate flowthrough filter 96-well plates are used to remove protein material from biological fluids in a high-throughput environment; sample is mixed with acetonitrile, which precipitates the protein; the supernatant fluid is filtered through a graded polypropylene filter and collected in a collection plate; made for automation; 1- and 2-mL formats.
PRP-1 SPE	Hamilton	SPE cartridges and 96-well plates	Reversed phase	PS-DVB	PS-DVB	100, 150, 1000, 10,000 mg	Polymeric SPE phase with 30-50 µm particle size distribution; 100-Å pores; polymeric material has higher sample capacity than silica-based phases; spherical particles display good flow characteristics; recommended for the purification and isolation of synthetic compounds or natural products.
Strata-X-C	Phenomenex	SPE cartridges and 96-well plates	Cation exchange	Functionalized PS-DVB	Strong cation exchange	Cartridges: 1-, 3-, and 6-mL cartridges; 10- and 30-mg/well in 96-well plates	Recommended for the extraction of basic drugs from biological fluids; ion-exchange mechanism allows for aggressive cleanup of samples; larger capacity than silica-based packings; 33-µm average particle size; 80-Å pores; a typical application is the extraction of amphetamines from urine.
StratoSpheres plugs	Polymer Laboratories	Scavenger product	Covalent bonding	Polymer	Various	48-well and 96-well sizes	Recommended for solution and solid-phase synthesis or for peptide synthesis; various functionalized high-capacity resins are uniformly loaded into porous composite polymeric plugs; the plugs can be used in solution with agitation or in 48- or 96-well plates; little swelling with various solvents; easy to handle; 13 chemistries available.
Styre Screen DBX cartridge	United Chemical Technologies	Polymeric SPE cartridge	Mixed mode	PS-DVB	C8 and sulfonic	1-, 3-, 6-, 10-, 25-, and 75-mL cartridges	Because of its mixed mode, this product can extract acidic, basic, and neutral compounds with a single cartridge; recommended for drugs-of-abuse applications; complete protocols for the SAMHSA-5 drugs-of-abuse classes provided for 30 mg sorbent mass in 1-, 3-, and 6-mL cartridge volumes; high-capacity polymer; 30-µm average particle size; pH 1-14 stability.
StyrosZyme Papain column	OraChrom	In-line digestion column	Enzymatic hydrolysis	PS-DVB-methacrylate	Immobilized papain	50-150 mm × 0.20-2.1 mm	Gigaporous porous matrix packed into column that can be used for on-line enzymatic fragmentation of IgG into two Fab and Fc fragments; hard-gel simulated monolithic material that has been coated and functionalized; immobilization of enzyme negates enzyme contamination typical of solution techniques; stainless steel or PEEK§ construction; <i>BIO</i> .
StyrosZyme Pepsin column	OraChrom	In-line digestion column	Enzymatic hydrolysis	PS-DVB-methacrylate	Immobilized pepsin	50-150 mm × 0.20-2.1 mm	Gigaporous porous matrix packed into column used for on-line enzymatic fragmentation of IgG into F(ab') ₂ and Fc fragments; hard-gel simulated monolithic material that has been coated and functionalized; immobilization of enzyme negates enzyme contamination typical of solution techniques; stainless or PEEK construction; <i>BIO</i> .
SuPerScreen Disc SPE disks	Orochem Technologies	SPE disks	Reversed and normal phase, strong anion and cation exchange, mixed mode	Silica	C3, C8, C18, phenyl, silica, ammonium, diol, cyano, strong anion and cation exchange	6- and 8-mm disks	Glass fiber disks with long and short fibers for better porosity; 14-mg nominal loading capacity for 8-mm disk; 8-mg nominal capacity for 6-mm disk; C8-strong cation exchange and strong cation exchange-strong anion exchange mixed modes; accessories include frits, caps, and depth filter.
ZIC-HILIC SPE cartridge	SeQuant	SPE cartridge	Hydrophilic interaction	Silica	Zwitterionic (see comments)	25 mg to 2 g	Quaternary amine and sulfonic group functionalities; zwitterionic covalently bonded stationary phase takes advantage of weak electrostatic interactions with charged analytes; recommended for the isolation of cationic and hydrophilic compounds.

*PS-DVB = poly(styrene-divinylbenzene).

†PTFE = polytetrafluoroethylene.

‡DVB-NVP = divinylbenzene-*N*-vinylpyrrolidone.

§PEEK = polyetheretherketone.

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prepare high-efficiency HPLC columns, the technology only recently has been used to prepare coated GC capillary columns. One advantage of sol gels is that they effectively shield the active sites, so the only interaction occurs between the sample and the phase.

Other New Chromatographic Products

TLC is an old technique, yet it is still in widespread use for separations. The technique can separate complex mixtures quickly, follow the course of chemical reactions, measure the purity of preparative fractions, provide rapid scouting for HPLC solvent systems, and, under the right circumstances, provide quantitative data.

This year, Macherey-Nagel introduced its nano-Adamant precoated, high performance TLC plate. The hard-layer, silica-gel plate is abrasion resistant because of its improved binding system. It also provides a narrower spot because of a narrower particle-size distribution of the base silica, and it has a brilliant UV 254-nm indicator for increased sensitivity. The standard layer thickness is 0.25 mm and the following sizes are available: 2.5 cm × 7 cm; 5 cm × 10 cm; 5 cm × 20 cm; 10 cm × 10 cm; and 20 cm × 20 cm.

CE is in widespread use and new products still come to market. Nacalai Tesque introduced two new CE capillaries. One called Cosmosmile (+) is coated with a cationic polymer for separations based upon electroosmotic-flow reversal. The second Cosmosmile (-) is a dextran sulfate-coated capillary that provides pH-independent electroosmotic flow. The capillaries' dimensions are 40 and 120 cm × 50 μm.

Sample Preparation Products

As Table II indicates, Pittcon 2003 saw another productive year with 21 new sample preparation products or product families displayed. Most of these products fit into the SPE category, with formats ranging from the traditional cartridges (a majority) and disks to 96-well plate configurations. Polymeric SPE phases this year outnumbered the silica-based product introductions. Several scavenger products were introduced. Scavenger products are similar to SPE devices, except that the bonded groups on the packing are chemically reactive and therefore can be used to remove excess reactants, especially in combinatorial chemistry preparations.

In the past several years, the 96-well SPE and filter plate designs have proliferated. The predominant driving force has been the high-throughput requirements placed upon sample preparation, chromatography, and MS laboratories by the pharmaceutical

industry for drug screening and combinatorial chemistry needs. As workloads increase to hundreds of samples per day, the need for rapid turnaround of analytical results dictates automation, and the manufacturers of robotics and liquid-handling systems have geared up to meet the demand.

This year four new products or families of products debuted. The 96-well filter plate, sometimes called a *crash plate*, is used for the automated filtration of large numbers of samples. Most often used in the measurement of drugs and drug metabolites in serum or blood plasma, each well of the filter plate has a membrane filter. Analysts first add serum (and sometimes a small amount of trifluoroacetic acid) to each well in the plate and then add three times the volume of acetonitrile into each well and mix. The protein in the serum precipitates — crashes — and the supernatant is filtered into a collection plate for additional processing. Because no selective interactions occur in this process, as in SPE, and all nonprecipitated compounds remain in the supernatant, the extract might not be as clean compared with that obtained from SPE, but much less method development is required. If liquid chromatography (LC)–MS or LC–MS–MS is used, the extract cleanliness can be sufficient after protein precipitation and filtration.

Figure 2 depicts an interesting comparison for the cleanup of amitriptyline in plasma. In this setup, the chemists used optimized generic methods for each approach — protein precipitation, SPE using Waters Oasis HLB cleanup (nonspecific), and SPE using Waters Oasis MCX cleanup (specific). The MCX product is a mixed-mode cation-exchange (SO₃) reversed phase, and its selective ion-exchange functionality gives the cleanest extract providing nine times the sensitivity compared with the protein precipitation method. Evaporation of the organic eluent in the final elution step would have increased the sensitivity even more.

The Supelco prepacked disposable Buchner funnel, depicted in Figure 3, is an interesting combination of SPE and a filter funnel. Based upon the mass of its sorbent material, the product actually is closer to a packed flash chromatography column than to an SPE disk, but the product should be useful for cleanup and filtration of many types of sample matrices with its variety of included packings.

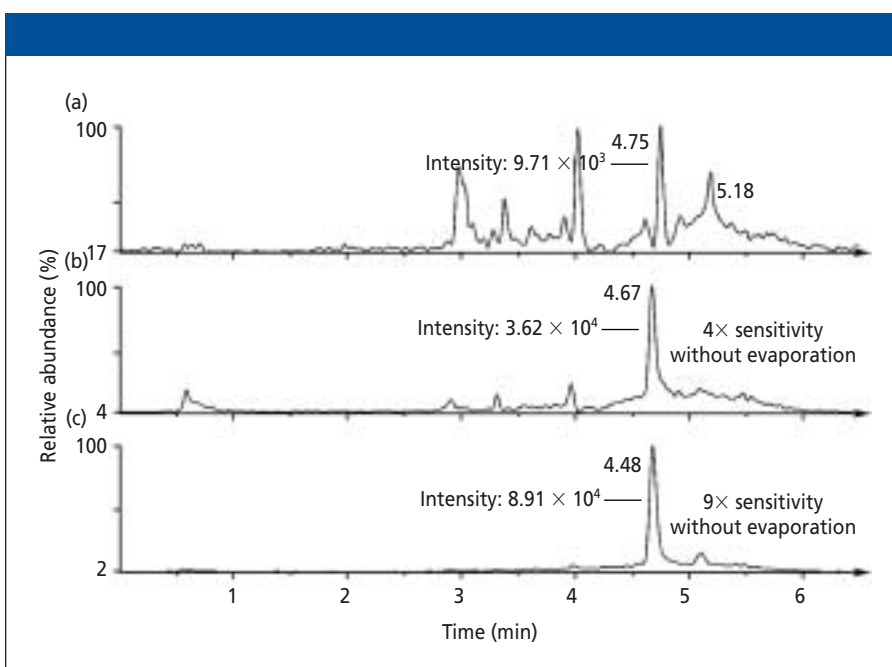


Figure 2: Comparison of (a) protein precipitation and (b) Oasis HLB SPE and (c) Oasis MCX SPE cleanup of amitriptyline in plasma. Sample: amitriptyline spiked into plasma at 0.1-ng/mL concentration. Column: 30 mm × 2.1 mm, 3.5-μm *d_p* Xterra MS C18; mobile-phase A: water–0.5% ammonia; mobile-phase B: acetonitrile–0.5% ammonia; gradient: 5 to 95% B in 1 min; flow rate: 0.2 mL/min; temperature: ambient; detection: MS. (Courtesy of Waters Corp.)

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Table III: Chromatography and sample preparation accessories at Pittcon 2003

Product Name	Supplier	Application Area	Product Type	Suggested Applications	Comments
Capillary LC-MS optimization kit	Thermo Electron	LC-MS	Kit to interface LC and MS	General LC-MS	All-in-one consumables kit that contains column (all standard Thermo phases), sleeves, fittings, tubing, and ferrules; 100 mm × 0.10, 0.18, and 0.32 mm column dimensions; matched components maximize the sensitivity of LC-MS analyses; conveniently packaged; easy to install with detailed instructions.
CD-100A gas leak detector	Quadrex	GC	Gas leak detector	Locating combustible and liquid vapors	Handheld unit capable of locating many combustible, noncombustible, and toxic gas leaks in a system; red LED visual and audible indicators; battery powered; small footprint; 18-in. extendable arm to detect leaks in hard-to-reach areas; 50-ppm methane sensor sensitivity.
CR-TC continuously regenerated trap columns	Dionex	LC	Trap column for ion chromatography	Continuous electrolytic reconditioning of trap columns	This on-line packed trap column bed contains an anion or cation exchange resin with electrodes situated to supply hydronium or hydroxide ions required for regeneration; designed to be used with automatic eluent generator modules for electrolytic generation of eluents; 50- μ m packing material contains alkyl quaternary amine for anion trap and sulfonic acid for cation trap; traps remove anionic or cationic contaminants from source water; helps to maintain constant, low-noise background.
Deans switching kit	Agilent Technologies	GC	Column switching accessory	Analyzing complex organic mixtures	Kit contains hardware components (samples contact Restek Sulfinert only), software, and installation instructions; requires an Agilent model 6890 gas chromatograph with electronic pressure control, two detectors, a pneumatics-control module or an auxiliary electronic pressure control module; two GC columns; system allows simplified, fast, and precise two-dimensional GC analysis in which the peaks of interest from the first column are cut and directed to the second column with different stationary phases; balanced gas flows managed by electronic pressure control; typical applications include determination of oxygenates and aromatics in gasoline, solvent purity determinations, and determination of thiophene in benzene.
EasiVial polymer standards kit	Polymer Laboratories	GPC*	Calibration standards	Convenient calibration of GPC columns	Standards kit includes three 4-mL vials each containing four accurately preweighed polymer standards; packaged in 30 vials (10 of each type); avoids weighing process; the molecular weights of the standards are chosen to obtain baseline resolution on most columns; users can obtain 12-point calibration with only three injections.
Flex-Column chromatography columns	Kimble/Kontes	LC	Glass columns	Flash column chromatography	Polypropylene reservoirs and column outlets permanently connected to borosilicate glass barrel; have 20- μ m porosity polyethylene frits at outlet; Luer-lock inlets and outlets; 30 different sizes; 4–170 cm × 0.7–2.5 cm column dimensions; accessories include adapters, valves, and fittings.
Micro DispoDialyzer	Harvard Apparatus	Sample preparation	Disposable microdialyzer	Buffer exchange, detergent removal and concentration	A disposable dialyzer for small-sample volumes from 5 μ L to 100 μ L; various molecular weight cutoff membranes available from 100 Da to 50,000 Da; also useful for purification of proteins and removal of excess radiolabeled or polymerase chain reaction primers; after sample is placed in sample chamber, the unit can be placed in beaker containing a dialysis buffer (it floats); after dialysis, the DispoDialyzer is inverted in microcentrifuge tubes and centrifuges briefly to recover sample; <i>BIO</i> .
Miniature air sampling canisters	Restek	GC sample preparation	Gas sampling devices	Sampling volatile organic compounds	Canisters are available in 400-cc and 1-L sizes; quick-connect fittings; compatible with sampling instruments; 1-L canister can sample air according to U.S. Environmental Protection Agency Methods TO-14 and TO-15; recommended for controlled sampling of indoor air, personal air sampling, and emergency response sampling; these canisters are smaller sampling devices with reduced purchase and shipping costs.
Mobile Phase Handling systems	Kimble/Kontes	LC	HPLC solvent reservoirs and accessories	Handling LC mobile phases	Systems consist of conical bottom reservoirs (250 mL–20 L), integrated filtration (47- or 90-mm filters), sparging, and solvent delivery; airtight and chemically resistant; plastic safety coating also blocks UV radiation.
MS/FID splitter	SGE	GC-MS	Flow splitter	Splits flow between mass spectrometer and gas chromatograph flame ionization detector	Glass-lined tee piece ensures inertness; supplied with bracket, fused-silica tee, nuts and ferrules; 1:1 split ratio; can be used with other detector combinations such as mass spectrometer and electron-capture detector; allows monitoring of GC effluents by flame ionization detection and MS simultaneously.
Nanovolume valves	Valco Instruments	HPLC	Small-volume valve	Nanoliter injections	20,000-psi electronically actuated valves with $\frac{1}{32}$ -in. fittings and 0.10-mm ports; available versions include an external-loop version (injection volumes as low as 40 nL) and an internal volume version (fixed volume of 4 or 10 nL); includes stainless steel nuts and ferrules.
PEEK tubing	MicroSolv	LC	HPLC connecting tubing	Most HPLC applications	Different-colored tubing sold in 5- and 10-ft. rolls; $\frac{1}{16}$ in. o.d. × 0.005-, 0.007-, 0.010-, 0.020- and 0.030-in. i.d.; can withstand pressures as high as 5000 psi; biocompatible; PEEK tubing cutter available.
RapiGest SF reagent	Waters	LC sample preparation	Protein solubilization reagent	Aiding enzymatic protein digestion	Reagent is an anionic surfactant that helps solubilize and unfold the substrate protein to make it more amenable to cleavage without denaturation or inhibiting proteolytic enzymes such as trypsin; increases peptide recovery; facilitates digestion of proteolytically resistant proteins; increases solubility of hydrophobic proteins and peptides; <i>BIO</i> .
Sample enrichment/Sample cleanup valves	Agilent Technologies	LC sample preparation	Automated switching valve system	On-line sample cleanup and concentration	Valving configurations available for enrichment, matrix stripping, column selection, solvent selection, and column regeneration; recommended for LC and LC-MS applications; increases sample throughput; higher separation performance possible using multidimensional LC; system software control; capillary kits available for easy setup.
Sample handling system	Polymer Laboratories	GPC sample preparation	Robotic platform	Sample dilution and injection	System integrates both sample preparation and autosampling functions; can be interfaced with any GPC unit; ambient to high temperature capability; sample-filled vials loaded in blocks in x-y array with as many as 96 vial positions; robotic arm dispenses defined volume of solvent, initiates heating and stirring, and initiates injection by heated injection valve (optional filtration); can withstand temperatures as high as 220 °C.
SilTite ferrule and nut	SGE	GC	GC capillary column ferrule	Column sealing	Stainless steel metal ferrule and nut expand and contract at same rate during GC oven temperature cycles to eliminate leaks.
Symbiosis SPE and autosampling system	Spark Holland	LC sample preparation	SPE and autosampler system	LC-MS	System comprises an autosampler, a stacker for sample trays, a column switching system, and a solid-phase extractor; performs high-speed injection mode with reduced cycle time (28-s wash time); integrated cooling for stacker and autosampler; generic sampling protocols; SPE-LC-MS automation.
UNEXAS direct-injection system	Iris Technologies	LC sample preparation	Universal extraction and sample preparation system	SPE interface to a liquid chromatograph	The system is designed for the direct injection of biological samples for the analysis of pharmaceutical analytes; proteins are directed to waste and analytes to the analytical column; unit for on-line extraction comprises pumps, switching valves, controlling software, and a LiChrospher ADS restricted-access media column (Merck); an autosampler and an HPLC detector are required to complete fully automated analytical system.
VFS Variable Flow Splitter	SEDERE	LC-MS or NMR†	Variable-flow splitter	Flow splitting to LC detectors	Variable-flow splitter reduces the volume from an HPLC flow stream to the volume needed for a mass spectrometer or other detector; a rapidly switching rotor is used to periodically divert the desired aliquot to the detector and optimize the performance of the detector; 10:1 to 100,000:1 user-selected split ratio; split ratio is independent of mobile-phase viscosity, tubing length, pressure, or temperature.
Zebtron GC column cage and box	Phenomenex	GC	GC capillary column cage	GC column support	Newly designed cage and box for Zebtron GC capillary columns; easier to use; all-metal sturdy construction.
Zephyr 500HR digital gas flowmeter	Phenomenex	GC	Gas flowmeter	Real-time flow measurement of gas streams	Microprocessor controlled; seven gas NIST-traceable calibrations; 2% reading accuracy and 0.01–500 cc/min measuring range; autoranging; can measure air, nitrogen, oxygen, hydrogen, helium, argon-methane, and carbon dioxide; interchangeable fittings; performs a wide variety of flow measurements, including capillary and packed GC column flow, split ratio determinations, and detector gas flow; flow to traps and sorbent tubes; bypass valve isolates solid-state flow sensor from gas stream during autozeroing sequence.

*GPC = gel-permeation chromatography.

†NMR = nuclear magnetic resonance spectroscopy.

Text continued from page 344.

Another newer approach is the on-line sample preparation, digestion, removal, and enrichment of protein samples in conjunction with proteomics studies and biological matrices. Several products in Table II fit into these categories. Particularly noteworthy are the OraChrom in-line digestion columns with different immobilized enzymes that will selectively hydrolyze IgG with different fragmentation products.

Chromatography and Sample Preparation Accessories

Table III lists new hardware products and accessories for HPLC, GC, and sample preparation. All the products are practical devices designed to make chromatographers' jobs easier. I will cover each area individually.

HPLC and ion chromatography products: An eclectic mix of new LC-oriented products made their appearance at Pittcon 2003. Products for use in LC-MS, which is one of the more rapidly growing areas in chromatography, included optimized kits that contain all the hardware and columns necessary to perform capillary LC-MS and a variable flow splitter that diverts aliquots of flow to an MS detector to reduce overall solvent volume. A continuously regenerated trap column, a unique accessory for ion chromatography, makes ion chromatography eluent generation much easier in that only water is needed to feed the system.

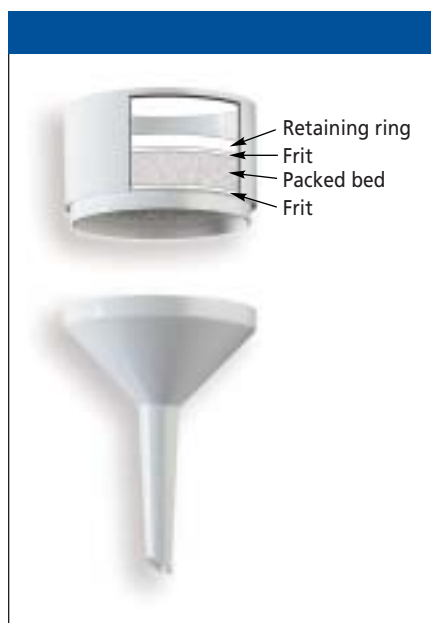


Figure 3: Schematic of a prepacked disposable Buchner funnel. (Courtesy of Supelco.)

GC-SFC products: Leak detectors, gas flowmeters, and other small gas-measurement accessories help to keep GC systems trouble free and ensure data reliability. A newly designed column cage, a new stainless steel ferrule combination, and a convenient flow splitter are among the hardware items of note.

Sample preparation products: Several sample preparation and autosampling and convenient column valving systems integrate sample preparation and handling with the mainframe HPLC instrument to save time, improve analytical precision, and minimize manual sample-handling errors. A simple disposable microdialysis system enables a convenient method for cleaning up biological samples. For GC air sampling, smaller volume inert canisters are more convenient to handle and easier to ship than older models.

Acknowledgment

I would like to thank the manufacturers and distributors that kindly furnished the requested information in advance of Pittcon 2003, thus allowing a timely report of new product introductions. For those manufacturers who would like to be considered for inclusion in Pittcon 2004 coverage, please send the name of the primary company contact, mailing address, fax number, and e-mail address to "Column Watch" Pittcon 2004, LCGC, 859 Willamette Street, Eugene, OR 97401.

Reference

- (1) R.E. Majors, *LCGC* 21(3), 240-259 (2003).

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Erratum

The x axis of Figure 2 was incorrectly labeled in last month's "Column Watch" (*LCGC* 21[3], 246 [2003]). The x axis should have been labeled Time (s). *LCGC* regrets the error and apologizes for any confusion it may have caused.