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ordering **note**

For assistance in selecting an HPLC column, please contact Restek Technical Service at 814-353-1300 or 800-356-1688 (ext. 4) or support@restek.com.

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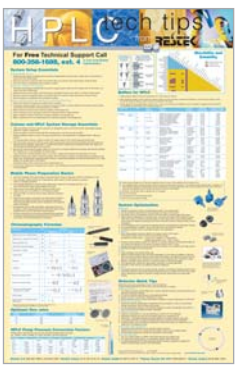

HPLC Tech Tips Wall Chart
Almost everything you need to remember about HPLC, condensed into 3 feet by 2 feet: mobile phase basics, buffers (types, pKa values, pH ranges, formula masses, more), miscibility and solubility chart (invaluable!), system setup and optimization, detector tips, pressure conversion factors, most-used chromatographic equations, and column storage essentials.

Call Restek at 800-356-1688 or 814-353-1300, ext. 5, or contact your Restek representative, to request your free copy!

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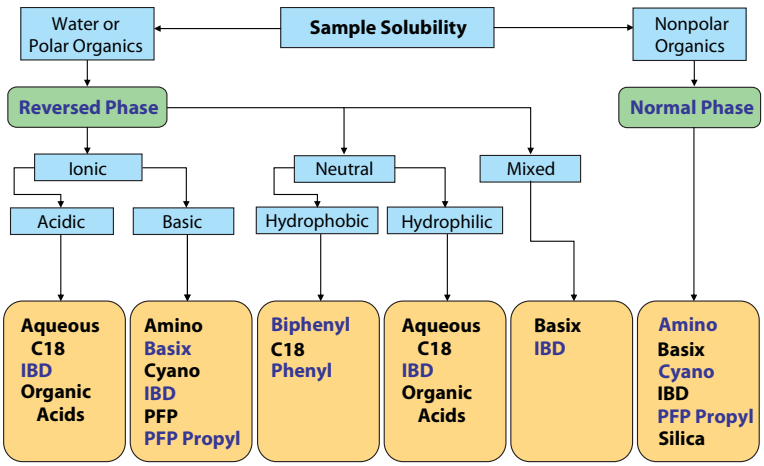
Selecting an HPLC Column

Choosing the best column for your application requires consideration of stationary phase chemistry, retention capacity, particle size, and column dimensions. Identifying the best stationary phase for your separation is the most critical step of column selection, and your decision should be based on sample solubility and the chemical differences among the compounds of interest. Figure 1 is a handy tool for stationary phase selection.

Figure 1 Stationary phase selection.



Reversed phase columns (e.g. alkyl, phenyl, cyano) work well for water-soluble hydrophobic compounds. Some stationary phases incorporate both polar and nonpolar functionality and can be used in either reversed phase or normal phase modes (e.g. Ultra IBD, Allure® Basix, and Allure® PFP Propyl). While straight chain alkyl stationary phases (e.g. C18) are historically the most commonly used, many newer phases provide better separations. Alkyl phases are best suited for analyzing neutral compounds with a high ratio of carbon:heteroatoms where the major distinction among analytes is their hydrophobicity. However, for analyzing compounds that are highly polar, aromatic, or halogenated, nonalkyl stationary phases often provide significantly better selectivity (Figure 2).

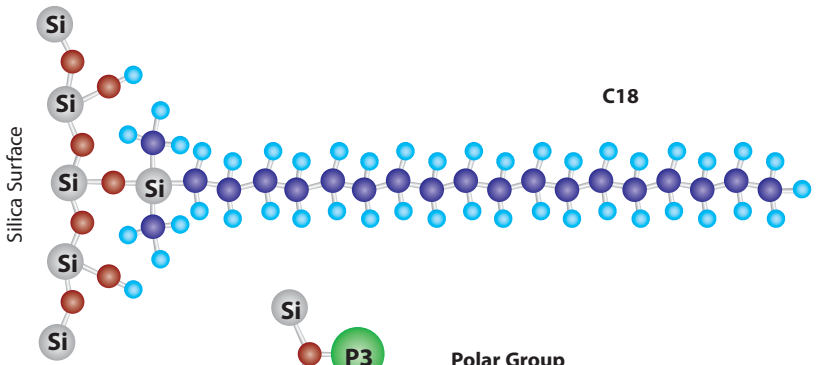
Retention capacity is another important consideration and is influenced by surface area and carbon load (% carbon in the packing material). Allure® columns were designed for maximum retention using a high density of ligands bonded to a large surface area silica. Ultra, Kromasil®, Pinnacle™ II and Pinnacle™ DB columns have the same high ligand density, but are more moderately retentive due to their lower surface areas. Surface area is inversely proportional to pore size; thus, larger pore sizes result in less retention. However, wide pore (e.g. 300Å) packings, such as Viva, are ideal when analyzing larger molecules, as a larger pore size is necessary to allow the analytes to 'fit' into the pores.

Particle size and column dimensions also influence column choice. In selecting a particle size, the primary consideration is efficiency (plates/meter) versus column pressure. A 3µm column will have approximately 50% more efficiency than a 5µm column, if all other conditions are constant for both columns. As particle size is further decreased (e.g. <2µm), theoretically, efficiencies will increase proportionally, based on the Van Deemter equation (and the usable flow rate range is much wider). Please note that column backpressure also increases as particle size decreases. Column dimensions include internal diameter and length, where the most commonly used internal diameter (ID) for HPLC columns is 4.6mm. In theory, resolution and pressure should be independent of column ID as long as flow rate is adjusted to maintain the same mobile phase linear velocity (flow rate is proportional to column cross-sectional area). Table I shows the approximate optimum flow rates for four column IDs.

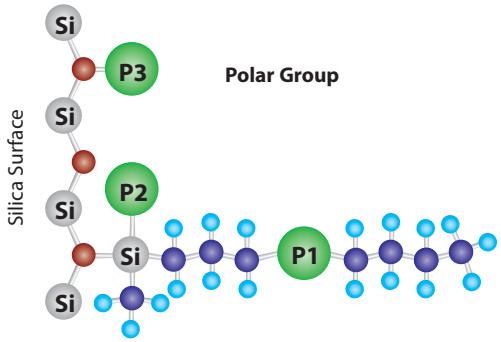
Table I Approximate optimum flow rates for various analytical column IDs.

	5µm Particles	3µm Particles
ID (mm)	Optimum Flow Rate (mL/min.)	Optimum Flow Rate (mL/min.)
4.6	1.00	1.5
3.2	0.50	0.73
2.1	0.20	0.31
1.0	0.05	0.07

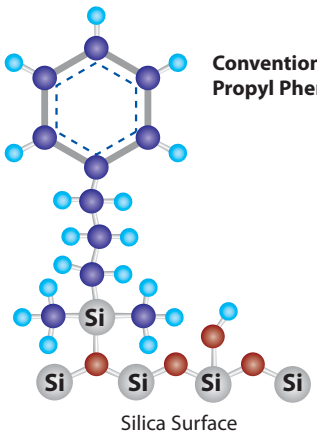
Figure 2 Stationary phase comparison.

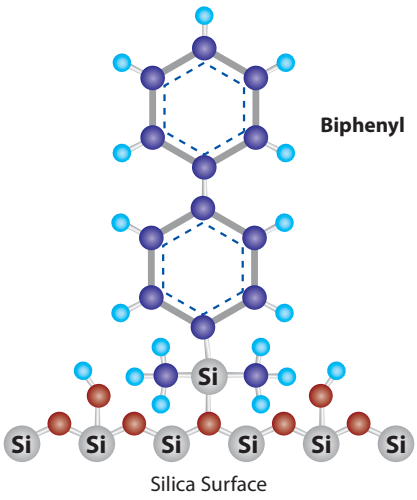


Alkyl phases (e.g. C18)
Alkyl-based stationary phases, such as C18, are best suited for analyzing hydrophobic molecules with a high carbon:heteroatom ratio.

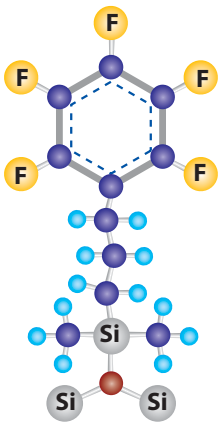


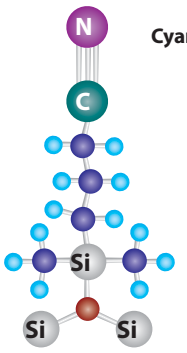
Alkyl phase with polar functional group
An alkyl-based stationary phase with either an embedded polar group (P1), a polar side chain (P2), or a polar end-cap (P3), has significantly greater interaction with polar compounds than a traditional alkyl phase.





Phenyl & Biphenyl phases
Phenyl stationary phases interact with compounds containing aromatic groups or unsaturated bonds through π-π interactions. The biphenyl stationary phase has even greater interaction due to the higher concentration of aromatic rings.





Cyano & Fluorinated phases
Fluorinated phases, such as the pentafluorophenyl propyl (PFP propyl), and cyano-based phases interact strongly with basic, nitrogen-containing and halogenated analytes.

Restek HPLC Column	End Cap?	Pore Size (Å)	Carbon load (%)	Applications	Chromatographic Properties	Similar Phases	USP Code	Page #
Pinnacle™ DB C18	Y	140	11	Hydrophobic C18 phase suitable for analyses of a wide range of compounds, from acidic through slightly basic.	Highly base-deactivated spherical silica manufactured by Restek. Monomeric C18 bonding.	Hypersil® BDS C18, Zorbax® Eclipse XDB-C18, Spherisorb® ODS	L1	310
Pinnacle™ DB Aqueous C18	—	140	6	Ideal for applications that require highly aqueous mobile phases, such as organic acids and water-soluble vitamins.	Highly selective phase for polar analytes. Compatible with highly aqueous (up to 100%) mobile phases. Silica manufactured by Restek.	Aquasil C18, AQUA® C18, Hypersil® Gold AQ, YMC® ODS-Aq	L1	313
Pinnacle™ DB C8	Y	140	6	Applications similar to Pinnacle™ DB C18, but with less hydrophobic retention. Less retention can be useful for shortening analysis time, if resolution is adequate.	Highly base-deactivated spherical silica manufactured by Restek. Monomeric C8 bonding. Similar to Pinnacle™ DB C18, but the shorter alkyl chain provides less hydrophobic retention.	Hypersil® BDS C8, Spherisorb® C8	L7	310
Pinnacle™ DB PFP Propyl	Y	140	6	Exhibits excellent peak shapes for a wide range of compounds, including nucleosides, nucleotides, and halogenated compounds.	Highly base-deactivated spherical silica manufactured by Restek. Unique pentafluorophenyl phase with a propyl spacer.	Discovery® HS F5	L43	312
Pinnacle™ DB Biphenyl	Y	140	8	Excellent choice for the analysis of steroids, tetracyclines, drug metabolites, and other compounds that contain some degree of unsaturation.	Highly base-deactivated spherical silica manufactured by Restek. Unique reversed phase material that displays both increased retention and selectivity for aromatic and/or unsaturated compounds when compared to conventional alkyl and phenyl phases.	Unique	L11	312
Pinnacle™ DB Cyano	Y	140	4	Suitable for a wide range of compounds, from acidic through slightly basic. Also useful for confirmation of analyses on a C18 or C8 column. Can be used in normal phase or reversed phase mode of separation.	Highly base-deactivated spherical silica manufactured by Restek. Cyano bonding.	Hypersil® BDS Cyano, Spherisorb® Cyano, Zorbax® Eclipse XDB-CN	L10	311
Pinnacle™ DB Phenyl	Y	140	5.3	Suitable for polar aromatic compounds, fatty acids, purines and pyrimidines.	Highly base-deactivated spherical silica manufactured by Restek. Phenyl bonding.	Hypersil® BDS Phenyl, Spherisorb® Phenyl Zorbax® Eclipse XDB-Phenyl	L11	311
Pinnacle™ DB Silica	—	140	—	Normal phase mode of separation.	Highly base-deactivated spherical silica manufactured by Restek.	—	L3	313
Pinnacle™ II C18	Y	110	13	Superior general purpose C18 for non-basic analytes.	Intermediate carbon load and surface area, suitable for a wide range of neutral to acidic compounds. Silica manufactured by Restek.	Hypersil® ODS	L1	314
Pinnacle™ II PAH	Y	110	—	Maximum resolution of polycyclic aromatic hydrocarbons.	Proprietary stationary phase; resolves 16 PAHs in US EPA Method 610. Silica manufactured by Restek.	Unique	—	314
Pinnacle™ II C8	Y	110	7	Superior general purpose C8 for non-basic analytes.	Provides shorter retention times for hydrophobic compounds than C18. Silica manufactured by Restek.	Hypersil® C8	L7	315
Pinnacle™ II Cyano	Y	110	4	Superior general purpose cyano for weakly-basic analytes. Used in either normal or reversed phase analyses.	More rugged than bare silica for normal phase analyses. Silica manufactured by Restek.	Hypersil® CPS	L10	315
Pinnacle™ II Phenyl	Y	110	6	Superior general purpose phenyl for neutral analytes.	Offers unique selectivity versus traditional alkyl chain phases, especially for aromatic compounds. Silica manufactured by Restek.	Hypersil® Phenyl	L11	316
Pinnacle™ II Amino	N	110	2	Excellent general purpose amino phase. Excellent choice for carbohydrate analysis.	Silica manufactured by Restek.	Hypersil® APS 2 Amino, Spherisorb® Amino	L8	316
Pinnacle™ II Biphenyl	Y	110	—	Multiple aromatic ring structures; excellent for explosives.	Silica manufactured by Restek. Unique biphenyl phase.	Unique	L11	317
Pinnacle™ II Silica	—	110	—	Ideal for polar analytes.	Superior value phase for normal phase separation of polar analytes. Lower retention than Ultra C18. Silica manufactured by Restek.	Hypersil® Silica	L3	317
Allure® C18	Y	60	27	Ideal for MS and light-scattering detection of neutral to slightly polar solutes. Separates basic compounds, showing good deactivation; excellent for explosives or steroids.	Most retentive phase for hydrophobic and slightly polar analytes. Mobile phase containing higher percentage of organic modifier contributes to higher sensitivity in ESI-based LC/MS.	Ultracarb® C18, BetaMax® Neutral, Discovery® C18	L1	318
Allure® Aqueous C18	N	60	—	Ideal for analyses that require >90% water in the mobile phase. Excellent for highly water soluble or poorly organic soluble compounds. Excellent for water-soluble vitamins and organic acids. More retention than Ultra Aqueous columns.	Highly retentive and selective for reversed phase separations of polar analytes. Highly base deactivated. Compatible with highly aqueous (up to 100%) mobile phases.	Unique	L1	319
Allure® AK	Y	60	—	Ideal for the analysis of aldehydes and ketones as DNPH derivatives.	Highly retentive, highly selective phase, developed specifically for the analysis of aldehydes and ketones as DNPH derivatives.	Unique	—	321
Allure® Basix	Y	60	12	Ideal for LC/MS of basic solutes. Excellent for basic pharmaceuticals or other amine-containing compounds.	Highly retentive phase for analytes containing amino functionality.	BetaMax® Base, Maxsil™ CN	L10	318
Allure® PFP Propyl	Y	60	17	Ideal for MS, ELSD, or NPD detection of nucleosides, nucleotides, purines, pyrimidines, or halogenated compounds.	A pentafluorophenyl phase with a propyl spacer. Highly retentive for basic analytes. Excellent for beta-blockers, halogenated compounds, nucleosides, nucleotides, pyridines, pyrimidines, tricyclic antidepressants.	Discovery® HS F5	L43	319
Allure® Organic Acids	N	60	—	Excellent resolution of challenging organic acids.	Single 30cm column performs equally to two C18 columns in series. (AOAC Method 986.13)	Unique	—	320
Allure® Biphenyl	Y	60	23	Multiple ring structure; excellent for aromatic and unsaturated compounds. Increased retention over traditional phenyl phases.	High purity, highly retentive phase for aromatic and unsaturated compounds.	Unique	L11	320
Allure® Silica	—	60	—	Highly retentive phase for normal phase separation.	High purity, highly retentive phase for normal phase separation of polar analytes. Very high surface area.	Maxsil™ Si	L3	321
Ultra C18	Y	100	20	Ideal for anilines, barbiturates, carbonyls, fat-soluble vitamins, fatty acids, glycerides, phthalates, PTH amino acids, steroids, other acids.	A very retentive, high-purity phase that exhibits excellent peak shape for a wide range of compounds. Recommended as a general purpose reversed phase column.	Discovery® C18, Symmetry® C18, Hypersil® Gold C18, Luna® C18, Zorbax® C18, Kromasil® C18, LiChrospher RP®-18, Inertsil® ODS-2, Develosil® C18	L1	322
Ultra Aqueous C18	N	100	15	Ideal for analyses that require >90% water in the mobile phase. Excellent for highly water soluble or poorly organic soluble compounds. Excellent for water-soluble vitamins and organic acids.	Highly retentive and selective for reversed phase separations of polar analytes. Highly base deactivated. Compatible with highly aqueous (up to 100%) mobile phases.	AQUA® C18, Aquasil C18, Hypersil® Gold AQ, YMC® ODS-Aq	L1	323
Ultra IBD	N	100	12	A polar group assists in deactivating surface silanols and contributes to unique separation selectivities for acids, bases, zwitterions, and other polar compounds.	One of a group of intrinsically base-deactivated (IBD) phases, with a polar group within, or intrinsic to, the alkyl bonded phase. Provides unique selectivity and high level of base deactivation while reducing or eliminating the need for mobile phase additives.	SymmetryShield, Discovery® ABZ & ABZ+, Prism™	—	323
Ultra C8	Y	100	12	Selectivity and peak shape similar to Ultra C18, but less hydrophobic retention.	Very retentive, high-purity, base-deactivated reversed phase packing that exhibits excellent peak shape for a wide range of compounds.	Luna® C8, Symmetry® C8, Hypersil® Gold C8	L7	322
Ultra C4	Y	100	9	Ideal for peptides and small proteins.	Exceptionally stable C4 packing, with high bonding coverage and silanol base-deactivation. Exhibits shorter retention than C18 or C8 phases.	Supelcosil™ Butyl (C4), Delta-Pak™ C4	L26	324
Ultra C1	—	100	5	Alternative selectivity to Ultra C18 or C8 columns, especially for polar analytes. Shortest chain alkyl phase available for reversed phase separations.	Exceptionally stable C1 packing resists hydrolysis, even under acidic mobile phase conditions. Least retentive reversed phase hydrocarbon packing.	Spherisorb® C1	L13	324
Ultra Cyano	Y	100	8	Excellent for basic pharmaceuticals, steroids (normal or reversed phase conditions), or other basic compounds.	High-purity cyano phase with reduced silanol activity. Often a better choice than C18 for basic pharmaceuticals. Cyano is the most stable bonded phase for normal phase mode.	Platinum™ CN, Develosil® Cyano, Luna® CN, Hypersil® Gold CN	L10	325
Ultra Phenyl	Y	100	10	Ideal for fatty acids, polycyclic aromatic hydrocarbons, purines and pyrimidines, and polar aromatics.	High-purity, highly retentive, base-deactivated phase with alternate selectivity to hydrocarbon phases, especially for aromatic analytes.	Platinum™ Phenyl, Supelcosil™ Phenyl, Betasil® Phenyl	L11	325
Ultra Amino	N	100	2	Superior general purpose amino phase. Ideal for carbohydrates.	Recommended for normal phase analyses of mono- and disaccharides and other similar compounds. Can also serve as a weak anion exchanger, with aqueous buffers.	Platinum™ Amino, Develosil® NH2	L8	326

pH ranges and temperature limits: see product listings on pages listed here.
Column lifetime will be shorter when operating at pH and/or temperature extremes.

Continued on next page. . .

Physical Characteristics of HPLC Columns; USP Descriptions

HPLC COLUMNS

Physical Characteristics of HPLC Columns; USP Descriptions

Restek HPLC Column	End Cap?	Pore Size (Å)	Carbon load (%)	Applications	Chromatographic Properties	Similar Phases	USP Code	Page #
Ultra PFP	Y	100	7	Ideal for taxol and precursors, or halogenated compounds, amines, esters, or ketones.	A pentafluorophenyl phase. Unique selectivity by interaction with functional groups of organohalogens or other basic analytes.	Fluophase® PFP, Fluosep®-RP Phenyl, Curosil® PFP	L43	326
Ultra Silica	—	100	—	Ideal for normal phase applications.	High purity, high surface area.	—	L3	327
Ultra Carbamate	—	100	—	Rapid analysis of carbamates.	Proprietary stationary phase can process up to twice as many samples per hour, compared to a conventional C18 phase.	Unique	—	327
Ultra Quat	—	100	—	Proprietary phase for the analysis of paraquat and diquat and other quaternary amines.	High purity silica.	Unique	—	328
Kromasil® C18	Y	100	20	A good all-purpose C18 phase for a wide range of water-soluble compounds.	High purity phase with excellent peak shape for a wide range of compounds. Good general pupose reversed phase column.	Discovery® C18, Symmetry® C18, Hypersil® Gold C18, Luna® C18, Zorbax® C18, LiChrospher RP®-18, Inertsil® ODS-2, Develosil® C18	L1	332
Kromasil® C8	Y	100	12	Selectivity similar to Kromasil® C18, but less hydrophobic retention.	High purity, reversed phase packing for a wide range of compounds.	Luna® C8, Symmetry® C8, Hypersil® Gold C8	L7	333
Kromasil® C4	Y	100	8	Selectivity similar to Kromasil® C18, but less hydrophobic retention.	High purity, reversed phase packing for a wide range of compounds. Less retentive than C18 and C8.	Supelcosil™ Butyl (C4), Delta-Pak™ C4	L26	332
Kromasil® C1	Y	100	—	Alternate selectivity to alkyl phases, especially for polar analytes.	High purity, reversed phase packing for a wide range of compounds. Less retentive than C18, C8, and C4.	Spherisorb® C1	L13	333
Kromasil® Phenyl	Y	100	14	Ideal for aromatic compounds, PAHs, and purines/pyrimidines.	High purity, base deactivated reversed phase packing. Alternate selectivity to alkyl phases.	Platinum™ Phenyl, Supelcosil™ Phenyl, Betasil® Phenyl	L11	333
Kromasil® Amino	Y	100	1.7% nitrogen	Excellent choice for carbohydrate analysis.	High purity, base deactivated reversed phase packing. Alternate selectivity to alkyl phases.	Platinum™ Amino, Develosil® NH2	L8	333
Kromasil® Silica	—	100	—	Good choice for normal phase applications.	High purity, base deactivated packing.	—	L3	333
Viva Wide Pore C18	Y	300	9	Proteins and other higher molecular weight compounds.	Silica manufactured by Restek.	BioBasic® 18, Symmetry® 300 C18, Jupiter® 300 C18, Zorbax® 300 OSB C18, Synchropak® C18, 208 TP C18	L1	329
Viva Wide Pore C8	Y	300	5	Proteins and other higher molecular weight compounds. Less retentive than C18 phase.	Silica manufactured by Restek.	BioBasic® 8, Zorbax® 300 OSB C8, Synchropak® C8, 208 TP C8	L7	329
Viva Wide Pore C4	Y	300	3.5	Proteins and other higher molecular weight compounds. Less retentive than C18 and C8 phases.	Silica manufactured by Restek.	BioBasic® 4, Symmetry® 300 C4, Jupiter® 300 C4, Synchropak® C4, 208 TP C4	L26	330
Viva Wide Pore Biphenyl	Y	300	6.7	Exhibits excellent peak shape for a wide range of compounds; ideal for large molecule and biomolecule assays.	Silica manufactured by Restek.	Unique	L11	330
Viva Wide Pore PFP Propyl	Y	300	5	Exhibits excellent peak shape for a wide range of compounds, including nucleosides, nucleotides, and halogenated compounds.	Silica manufactured by Restek.	Unique	L43	330
Viva Wide Pore Silica	—	300	—	Normal phase applications for highly retained high molecular weight compounds.	Silica manufactured by Restek.	—	L3	331
pHidelity® C18	—	140	—	Hydrophobic C18 phase suitable for analyzing a wide range of compounds; enhanced stability under basic and acidic conditions.	Excellent stability under extreme pH conditions. True C18 selectivity in a silica-based stationary phase.	Unique	—	308

pH ranges and temperature limits: see product listings on pages listed here.
Column lifetime will be shorter when operating at pH and/or temperature extremes.

US Pharmacopoeia Cross Reference

L1	Octadecyl silane chemically bonded to porous silica or ceramic microparticles, 1.7 to 10µm in diameter, or a monolithic rod. *Pinnacle™ DB C18 (p. 310), Pinnacle™ DB Aqueous C18 (p. 313), Pinnacle™ II C18 (p. 314), Allure® C18 (p. 318), Allure® Aqueous C18 (p. 319), Ultra C18 (p. 322), Ultra Aqueous C18 (p. 323), Viva C18 (p. 329), Kromasil® C18 (p. 332)*
L3	Porous silica particles, 5 to 10µm in diameter. *Pinnacle™ DB Silica (p. 313), Pinnacle™ II Silica (p. 317), Allure® Silica (p. 321), Ultra Silica (p. 327), Viva Silica (p. 331), Kromasil® Silica (p. 333)*
L7	Octylsilane chemically bonded to totally porous silica particles, 1.7 to 10µm in diameter. *Pinnacle™ DB C8 (p. 310), Pinnacle™ II C8 (p. 315), Ultra C8 (p. 322), Viva C8 (p. 329), Kromasil® C8 (p. 333)*
L8	An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 3 to 10µm in diameter. *Pinnacle™ II Amino (p. 316), Ultra Amino (p. 326), Kromasil® Amino (p. 333)*
L10	Nitrile groups chemically bonded to porous silica particles, 3 to 10µm in diameter. *Pinnacle™ DB Cyano (p. 311), Pinnacle™ II Cyano (p. 315), Allure® Basix (p. 318), Ultra Cyano (p. 325)*
L11	Phenyl groups chemically bonded to porous silica particles, 1.7 to 10µm in diameter. *Pinnacle™ DB Phenyl (p. 311), Pinnacle™ DB Biphenyl (p. 312), Pinnacle™ II Phenyl (p. 316), Pinnacle™ II Biphenyl (p. 317), Allure® Biphenyl (p. 320), Ultra Phenyl (p. 325), Viva Biphenyl (p. 330), Kromasil® Phenyl (p. 333)*
L13	Trimethylsilane chemically bonded to porous silica particles, 3 to 10µm in diameter. *Ultra C1 (p. 324), Kromasil® C1 (p. 333)*
L26	Butyl silane chemically bonded to totally porous silica particles, 3 to 10µm in diameter. *Ultra C4 (p.324), Viva C4 (p.330), Kromasil® C4 (p.332)*
L43	Pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 5 to 10µm in diameter. *Pinnacle™ DB PFP Propyl (p. 312), Allure® PFP Propyl (p. 319), Ultra PFP (p. 326), Viva PFP Propyl (p. 330)*

tech tip

Managing High Backpressure

High backpressure is one of the most common problems encountered in HPLC analyses. Normal column backpressure is observed after a new column has been installed and equilibrated with mobile phase. Unfortunately, this pressure often will increase as the column is used because particles collect on the column inlet frit. These particles can be sample impurities, mobile phase contaminants, or materials from the injector or autosampler rotor seal.

In addition to increasing backpressure, particles on the frit can cause split peaks, peak tailing, and, eventually, over-pressure shut-down. In some circumstances, these problems can be corrected by back-flushing the column. However, in many cases the result is an unusable column.

To minimize backpressure problems, all samples and mobile phase solvents must be filtered before use, and rotor seals should be changed on a routine basis. Along with these preventive measures, it is advisable to use precolumn filters such as the Trident guard column protection system. Particles build up on the inexpensive, replaceable frit in the filter, instead of on the permanent frit at the column inlet.

free literature

HPLC Column Selection Guide

A useful chart to keep with your workbooks, or post on a wall. Quickly scan important characteristics of Restek HPLC columns. Includes brief, practical guidelines for choosing stationary phase, particle size, pore diameter, and column dimensions. Also includes USP designations for each phase and lists similar phases from other suppliers.

Call Restek at 800-356-1688 or 814-353-1300, ext. 5, or contact your Restek representative, to request your free copy!

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