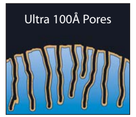
Choosing a Stationary Phase

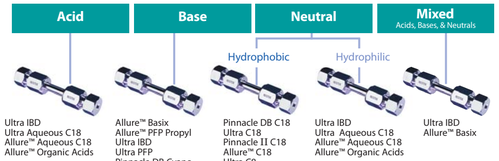
- Most critical decision in column selection.
- Based on analyte solubility, chemical differences among analytes.
- Analyte solubility dictates mode of separation:
  Reversed phase - analytes soluble in water and/or polar organic solvent (e.g., acetonitrile, methanol).
  Mobile phase more polar than stationary phase.
  Normal phase - analytes soluble in nonpolar solvent (e.g., hexane).
  Mobile phase less polar than stationary phase.

Pore Size

- Small molecules (<2000MW) generally analyzed on material with pore size ≤200Å (typical size: 60Å–180Å).
- Large molecules (>2000MW) generally analyzed on material with pore size 200–300Å.
- Surface area inversely related to pore size (the smaller the pores the larger the surface area).
- Stationary phase loading directly related to surface area (the larger the surface area the higher the stationary phase loading).



Analyte Functional Group



Choosing a Mode

Sample Characteristics

Sample soluble in:	Ionic Strength	Analytes primarily differ by:	Recommended Mode:
water and/or polar organics	nonionic	hydrophobicity/hydrophilicity	RP
	weakly ionic	hydrophobicity/hydrophilicity	RP with ion suppression
	nonionic/weakly ionic	size (>2000MW)	SEC (GFC)
	strongly ionic	hydrophobicity/hydrophilicity	RP-IP, IEX
nonpolar organics	nonionic	hydrophobicity/hydrophilicity	NP
	weakly ionic	hydrophobicity/hydrophilicity	NP with ion suppression
	nonionic/weakly ionic	size (>2000MW)	SEC (GPC)

RP - reversed phase
SEC - size exclusion chromatography
GFC - gel filtration chromatography
RP-IP - reversed phase-ion pair ion exchange
NP - normal phase
GPC - gel permeation chromatography
IEX - ion exchange

Analytical Column Dimensions

ID

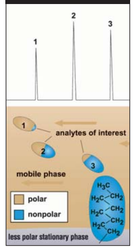
- Theoretically, resolution and pressure are independent of ID, if the mobile phase flow rate is adjusted to maintain the same linear velocity.
- Smaller column ID can increase sensitivity if sample is limited.
- Smaller column ID can reduce / eliminate need for flow splitting in LC/MS.
- Column ID <3.2mm requires reducing extra column volume (tubing, injector, detector flow cell).

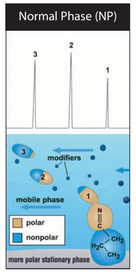
Length

- If all else is equal: shorter columns provide faster analyses and less backpressure; longer columns provide greater resolution
- Efficiency (N) is directly related to column length, but is a square root factor in resolution: a 4-fold increase in length is needed to double resolution

$[R = {}^{1}/_{4}\,(\alpha\text{-}1 / \alpha)\,(\sqrt{N})\,(k' / k'+1)]$

α: selectivity k': capacity





Particle Size

- Analytical separations generally are on 5µm or smaller particles.
- Semi-preparative and preparative analyses generally are on 7µm or larger particles.
- Smaller particles provide greater efficiency (N) than larger particles (a 3µm particle provides ~50% greater efficiency than a 5µm particle).
- Smaller particles create higher backpressure.

Steps in Scouting a Method

1 Choose stationary phase based on chemistry of analytes.
2 Use a 150 x 4.6mm, 5µm column; determine appropriate mobile phase strength needed (2<k'<5) by using a gradient test run.
3 If resolution is close to requirements, optimize column dimensions and conditions. If large gain in resolution is needed change mobile phase composition or stationary phase.

Optimum Flow Rate

ID (mm)	5µm Particles Flow Rate (mL/min.)	3µm Particles 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

Restek's Exclusive Trident™ Integral System

- Convenient and economical leak-free guard cartridge system, extremely easy to install.
- Versatile configuration protects against all levels of contamination.
- Integral design eliminates troublesome tubing connections.

The system's foundation consists of the analytical column configured with our exclusive Trident™ end fitting and XF fitting. This configuration contains the standard internal frit as well as a replaceable cap frit, which easily can be changed without disturbing the packed bed. Changing the external frit can reverse the effects of accumulated particles, such as high backpressure or peak distortion. To obtain this basic configuration, simply order any Restek HPLC column, and add the suffix -700 to the catalog number for the column. (Nominal additional charge.)

For maximum protection against contaminants and particulate matter, the system can be configured with an integral guard cartridge holder (XG-XF), a guard cartridge, and a replaceable external frit. To obtain this configuration, simply order any Restek HPLC column, add the suffix -700 to the catalog number for the column, and order the appropriate XG-XF male fitting (cat.# 25026 or 25062, below) and Trident™ guard cartridges (refer to our catalog or website).

Description	qty.	cat.#
XG-XF Fitting for 10mm Guard Cartridge	ea.	25026
XG-XF Fitting for 20mm Guard Cartridge	ea.	25062
Replacement XF Filter Fitting	ea.	25024
Replacement Cap Frits: 4mm, 2.0µm	5-pk.	25022
Replacement Cap Frits: 4mm, 0.5µm	5-pk.	25023
Replacement cap frits: 2mm, 2.0µm	5-pk.	25057

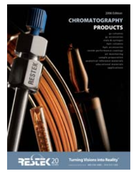
Column with Trident™ Integral Inlet Fitting (to order add "-700" to catalog number of column) and guard cartridge, XG-XF fitting, cap frit, and XF end fitting.

Remove the XF end fitting and install the guard cartridge in the end of the column.

Add the XG-XF fitting (order cat.#25026 for 10mm guard cartidges, cat.#25062 for 20mm guard cartridges).

Re-install the XF end fitting with cap frit.

The cap frit can be easily replaced if it becomes contaminated/plugged.



Please see Restek's Annual Chromatography Products Guide *(lit. cat.# 580021) for more HPLC columns and accessories.*

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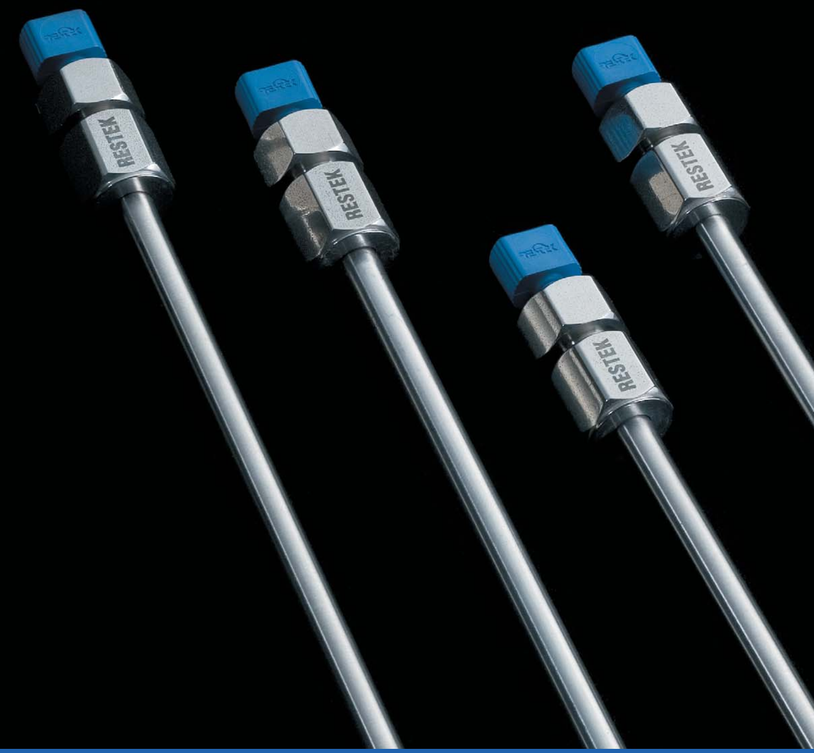
INNOVATIVE PRODUCTS

HPLC Column Selection Guide



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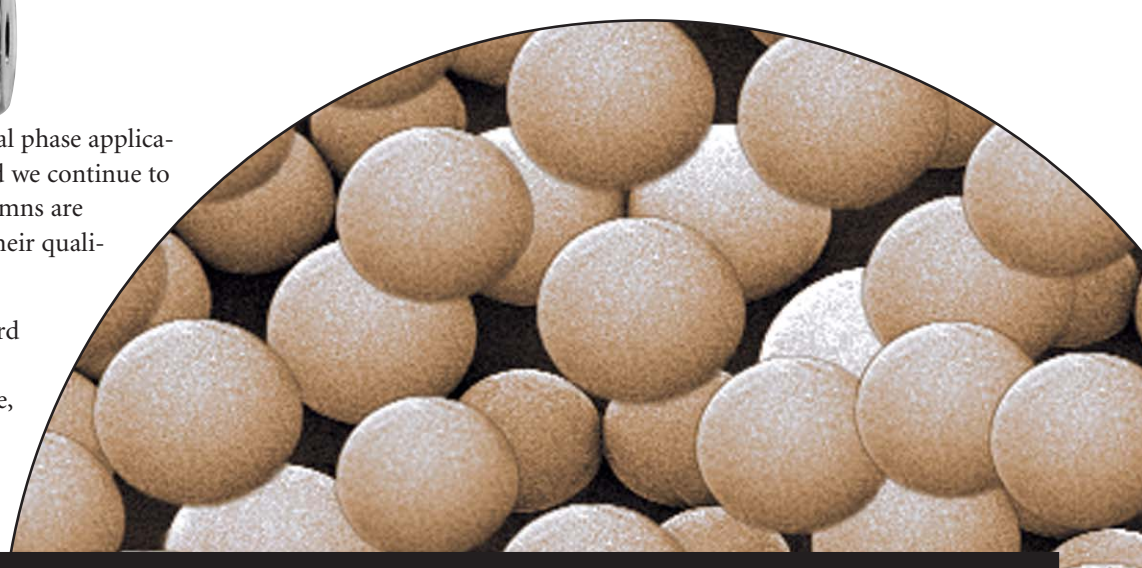
INNOVATIVE PRODUCTS

HPLC Column Selection Guide

Uniform, spherical Restek silica allows optimal packing, for better reproducibility and lower back pressure, extending column life.



Restek Corporation manufactures a variety of columns for reversed phase or normal phase applications. The Allure™ and Ultra column product lines contain 20 different phases, and we continue to develop additional unique HPLC products. Pinnacle II, Pinnacle DB, and Viva columns are based on silica we manufacture in our own laboratories, so you can be assured of their quality, from raw material synthesis through packing and testing.

We pack bonded phases into a wide range of column dimensions, including standard analytical columns in lengths of 30 to 250mm, internal diameters of 1.0 to 4.6mm, and particle sizes of 3 or 5µm. Preparative and semi-preparative columns are available, as well. Hundreds of column choices can make column selection overwhelming. The chart below, and the information on the back of the chart, are general guidelines to help simplify the column selection process.

Restek HPLC Column	End Cap?	Pore Size (Å)	Carbon load (%)	Applications	Chromatographic Properties	Similar Phases	USP Code
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 Corp. Monomeric C18 bonding.	Thermo-Hypersil® BDS C18	**L1**
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 Corp. Monomeric C8 bonding. Similar to Pinnacle DB C18, but the shorter alkyl chain provides less hydrophobic retention.	Thermo-Hypersil® BDS C8	**L7**
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 Corp. Cyano bonding.	Thermo-Hypersil® BDS Cyano	**L10**
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 Corp. Phenyl bonding.	Thermo-Hypersil® BDS Phenyl	**L11**
Pinnacle DB Silica	NA	140	NA	Normal phase mode of separation.	Highly base-deactivated spherical silica manufactured by Restek Corp.	Thermo-Hypersil® BDS	**L3**
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 Corp.	Thermo-Hypersil® ODS; Spherisorb ODS (Waters)	**L1**
Pinnacle II PAH	Y	110	P	Maximum resolution of polynuclear aromatic hydrocarbons.	Proprietary stationary phase; resolves 16 PAHs in US EPA Method 610. Silica manufactured by Restek Corp.	Unique	—
Pinnacl™ 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 Corp.	Thermo-Hypersil® C8	**L7**
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 Corp.	Thermo-Hypersil® Cyano; Spherisorb Cyano (Waters)	**L10**
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 Corp.	Thermo-Hypersil® Phenyl; Spherisorb Phenyl (Waters)	**L11**
Pinnacle II Amino	N	110	2	Excellent general purpose amino phase. Excellent choice for carbohydrate analysis.	Silica manufactured by Restek Corp.	Thermo-Hypersil® Amino; Spherisorb Amino (Waters)	**L8**
Pinnacle II Biphenyl	Y	110	P	Multiple aromatic ring structures; excellent for explosives.	Silica manufactured by Restek Corp. Unique biphenyl phase.	Unique	—
Pinnacle II Silica	NA	110	NA	Ideal for polar analytes.	Superior value phase for normal phase separation of polar analytes. Lower retention than Ultra C18. Silica manufactured by Restek Corp.	Thermo-Hypersil® Silica	**L3**
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 (Phenomenex); Betamax Neutral (Thermo-Keystone)	**L1**
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**
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 (Keystone); Maxsil CN (Phenomenex)	**L10**
Allure™ PFP Propyl	Y	60	17	Ideal for MS, ELSD, or NPD detection of beta-blockers halogenated compounds, nucleosides, nucleotides, purines, pyrimidines, tricyclic antidepressants	A pentafluorophenyl phase with a propyl spacer. Highly retentive for basic analytes.	Discovery HS F5 (Supelco)	**L43**
Allure™ Organic Acids	N	60	NA	Excellent resolution of challenging organic acids.	Single 30cm column performs equally to two C18 columns in series. (AOAC Method 986.13)	Unique	—
Allure™ Biphenyl	Y	60	23	Multiple ring structure; excellent for aromatic and unsaturated compounds. Increased retention, relative to traditional phenyl phases.	High purity, highly retentive phase for aromatic and unsaturated compounds.	Unique	—
Allure™ Silica	NA	60	NA	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 (Phenomenex)	**L3**
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 (Supelco); Symmetry C18 (Waters); Luna C18 (Phenomenex); Zorbax C18 (Agilent); Kromasil C18; Lichrosphere RP-18; Inertsil ODS-2; Develosil C18	**L1**
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.	ODS-AQ (YMC); Aquasil C18 (Thermo-Keystone); Aqua C18 (Phenomenex)	**L1**
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.	Symmetry Shield (Waters); Discovery ABZ & ABZ+ (Supelco); Prism (Keystone)	—
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 (Phenomenex); Symmetry C8 (Waters); Kromasil C8	**L7**
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) (Supelco); Delta-Pak C4 (Waters)	**L26**
Ultra C1	NA	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 (Waters)	**L13**
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 (Alltech); Develosil Cyano; Luna CN (Phenomenex)	**L10**
Ultra Phenyl	Y	100	10	Ideal for fatty acids, polynuclear 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 (Alltech); Supelcosil Phenyl (Supelco)	**L11**
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 (Alltech); Develosil NH2 (Phenomenex)	**L8**
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 (Thermo-Keystone); Fluosep-RP Phenyl (ES Industries)	**L43**
Ultra Silica	NA	100	NA	Ideal for normal phase applications.	High purity, high surface area. Normal phase separations.	Zorbax Sil (Agilent)	**L3**
Ultra Carbamate	P	100	P	Rapid analysis of carbamates.	Proprietary stationary phase can process up to twice as many samples per hour, compared to a conventional C18 phase.	Unique	—
Ultra Quat	P	100	P	Proprietary phase for the analysis of paraquat and diquat.	High purity silica.	Unique	—
Viva Wide Pore C18	Y	300	9	Proteins and other higher molecular weight compounds.	Highly base deactivated wide pore packing that exhibits excellent peak shape for a wide range of compounds. Excellent general purpose column for large molecules. Silica manufactured by Restek Corp.	Jupiter 300 C18 (Phenomenex); Zorbax 300SB-C8 (Agilent); Symmetry 300 C18 (Waters)	**L1**
Viva Wide Pore C8	Y	300	5	Proteins and other higher molecular weight compounds. Less retentive than C18 phase.	Highly base-deactivated wide pore packing for analyzing large molecules and biomolecules. Silica manufactured by Restek Corp.	Zorbax 300 SB-C8 (Agilent)	**L7**
Viva Wide Pore C4	Y	300	3.5	Proteins and other higher molecular weight compounds. Less retentive than C18 and C8 phases.	Highly base-deactivated wide pore packing for analyzing large molecules and biomolecules. Silica manufactured by Restek Corp.	Symmetry 300 C4 (Waters); Jupiter 300 C4 (Phenomenex)	**L26**
Viva Wide Pore PFP Propyl	Y	300		Proteins and other higher molecular weight compounds. Highly retentive for basic and halogenated compounds.	Highly base-deactivated wide pore packing for analyzing large molecules and biomolecules. Silica manufactured by Restek Corp.	Unique	**L13**
Viva Wide Pore Biphenyl	Y	300	6.7	Proteins and other higher molecular weight compounds. Highly selective and retentive for aromatic and halogenated compounds.	Highly base-deactivated wide pore packing for analyzing large molecules and biomolecules. Silica manufactured by Restek Corp.	Unique	—
Viva Wide Pore Silica	—	300	NA	Normal phase applications for highly retained high molecular weight compounds.	Silica manufactured by Restek Corp.	YMC-Pack Silica 300 (Waters)	**L3**
pHidelity™ C18	P	200	P	Hydrophobic C18 phase suitable for analyzing a wide range of compounds; enhanced stability under extreme pH conditions.	Excellent stability under extreme pH conditions. True C18 selectivity in a silica-based stationary phase.	Unique	—

P= proprietary, NA= not applicable
pH range: Pinnacle II and Pinnacle DB columns, except amino and cyano phases, Viva C18 columns — 2.5–10; Allure, Ultra, and Pinnacle II and Pinnacle DB amino and cyano phases — 2.5–7.5. Temperature limit, all columns: 80°C.
Typical column lifetime is 1000–2000 column volumes when operating at pH and/or temperature extremes.

Call Restek Technical Service Now for the Right Column for Your Application: 800-356-1688 or 814-353-1300, ext. 4.