# **INNOVATIVE PRODUCTS**

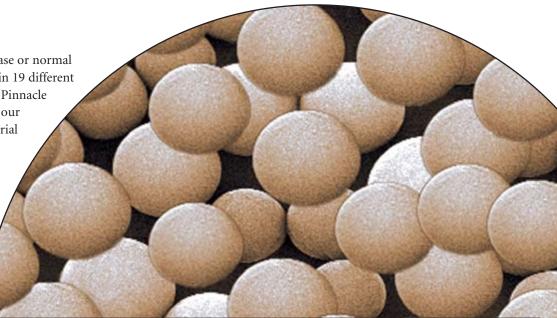
# **HPLC Column Selection Guide**



The uniform spherical shape of Restek silica allows optimal packing, for better reproducibility and lower back pressure, resulting in extended column life.

Restek Corporation manufactures a variety of columns for reversed phase or normal phase applications. The Allure™ and Ultra column product lines contain 19 different phases, and we continue to develop additional unique HPLC products. Pinnacle  $\mathbf{II^{\scriptscriptstyle TM}}$  and Pinnacle  $^{\scriptscriptstyle TM}$  DB 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  $5\mu m.$ Preparative and semi-preparative columns are available, as well. These 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.



End	Pore Size	Carbon				USP
Cap?	(Å)	Load (%)	Applications  Hydrophobic C18 phase suitable for a wide range of	Chromatographic Properties  Highly base-deactivated spherical silica manufactured	Similar Phases	Code
Y	140	11	compounds, neutral through weakly basic.	by Restek Corp. Monomeric C18 bonding.	Hypersil* BDS C18	L1
Υ	140	6	hydrophobic retention. Less retention can be useful for shortening analysis time, if resolution is adequate.	Monomeric C8 bonding. Similar to Pinnacle DB C18, but the shorter alkyl chain provides less hydrophobic retention.	Hypersil® BDS C8	L7
Υ	140	4	Suitable for a wide range of compounds, from acidic through weakly basic. Also useful for confirmation of analyses on C18 or C8. Can be used in normal phase or reversed phase mode of separation.	Cyano bonding on highly base-deactivated spherical silica manufactured by Restek Corp.	Hypersil* BDS Cyano	L10
NA	140	NA	Normal phase separations.	Highly base-deactivated spherical silica manufactured by Restek Corp.	Hypersil' BDS	L3
Υ	110		Superior general purpose C18 for neutral and acidic analytes.	Intermediate carbon load and surface area, suitable for a wide range of neutral to acidic compounds. Silica manufactured by Restek Corp.	Hypersil® ODS; Spherisorb® ODS	L1
Υ	110		Maximum resolution of polynuclear aromatic hydrocarbons.	Proprietary stationary phase; resolves 16 PAHs in US EPA Method 610. Silica manufactured by Restek Corp.	Unique	
Υ	110	/	Superior general purpose C8 for neutral and acidic analytes.	Provides shorter retention times for hydrophobic compounds than C18. Silica manufactured by Restek Corp.	Hypersil* C8	L7
Υ	110	4	Superior general purpose cyano for weakly basic analytes. Can be used in normal phase or reversed phase mode of separation.	More rugged than bare silica for normal phase analyses. Silica manufactured by Restek Corp.	Hypersil <sup>®</sup> Cyano; Spherisorb <sup>®</sup> Cyano	L10
Υ	110		Superior general purpose phenyl for neutral to weakly basic analytes.	Offers unique selectivity versus traditional alkyl chain phases, especially for aromatic compounds. Silica manufactured by Restek Corp.	Hypersil <sup>®</sup> Phenyl; Spherisorb <sup>®</sup> Phenyl	L11
N	110		Excellent general purpose amino phase. Excellent choice for carbohydrate analysis.	Silica manufactured by Restek Corp.	Hypersil* Amino; Spherisorb* Amino	L8
NA	110		Superior value phase for normal phase separation of polar analytes.	Lower retention than Ultra C18. Silica manufactured by Restek Corp.	Hypersil* Silica	L3
Υ	60	27	Ideal for MS and light-scattering detection of neutral to slightly polar non-ionic solutes. Excellent for basic compounds, 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. Extensive deactivation.	Ultracarb <sup>*</sup> C18; Betamax <sup>*</sup> Neutral	L1
N	60	Р	Ideal for analyses that require >90% water in the mobile phase. Excellent for highly water soluble or poorly organic soluble compounds, water-soluble vitamins, 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.		L1
Υ	60		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 <sup>®</sup> Base; Maxsil <sup>®</sup> CN	L10
Υ	60	17	Excellent for beta-blockers, halogenated compounds, nucleosides, nucleotides, pyridines, pyrimidines, or tricyclic antidepressants. Ideal for MS, ELSD, or NPD detection.	A pentafluorophenyl phase with a propyl spacer. Highly retentive for basic analytes.	Discovery HS F5	
N	60	Р	Excellent resolution of challenging organic acids.	Single 30cm column performs equally to two C18 columns in series. (AOAC Method 986.13)	Unique	
NA	60		Highly retentive phase for polar analytes by normal phase separation.	High purity, very high surface area.	Maxsil* Si	L3
Υ	100	20	Ideal for anilines, barbiturates, carbonyls, fat-soluble vitamins, fatty acids, glycerides, phthalates, PTH amino acids, steroids, other acids.	Very retentive, high-purity packing material exhibits excellent peak shape for a wide range of compounds. Excellent first choice general purpose reversed phase column.	Discovery C18; Symmetry C18; Luna* C18; Zorbax* C18; Kromasil* C18; Lichrospher* RP-18; Inertsil* ODS-2; Develosil* C18	L1
N	100	15	Ideal for analyses that require >90% water in the mobile phase. Excellent for highly water soluble or poorly organic soluble compounds, water-soluble vitamins, organic acids.	Highly retentive and selective for reversed phase separations of polar analytes. Extensively base deactivated. Compatible with highly aqueous (up to 100%) mobile phases.	ODS-AQ Aquasil C18 Zorbax* SB-AQ	L1
N	100	12	A polar group assists in deactivating surface silanols and contributes to unique selectivities for acids, bases, zwitterions, and polar compounds.	Intrinsically base-deactivated (IBD) phase, with a polar group embedded in the alkyl bonded phase. Provides unique selectivity while reducing or eliminating the need for mobile phase additives.	Symmetry Shield; Discovery ABZ & ABZ+; Prism <sup>*</sup> ; HyPurity Advance	
Υ	100	12	Excellent peak shape for a wide range of compounds. Selectivity and peak shape similar to Ultra C18, but less hydrophobic retention.	Very retentive, high-purity, base-deactivated reversed phase packing.	Luna <sup>*</sup> C8; Symmetry C8; Kromasil <sup>*</sup> C8	L7
Υ	100	9	Ideal for peptides, small proteins, hormones.	Exceptionally stable C4 packing, with high bonding coverage and silanol base-deactivation. Shorter retention than C18 or C8.	Supelcosil Butyl (C4); Delta-Pak® C4	L26
NA	100	5	Alternative selectivity to Ultra C18 or C8, 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
NA Y	100	5	polar analytes. Shortest chain alkyl phase available for	mobile phase conditions. Least retentive reversed phase hydrocarbon	Spherisorb* C1  Platinum* CN; Develosil* Cyano; Luna* CN	L13
		8	polar analytes. Shortest chain alkyl phase available for reversed phase separations.  Excellent for basic pharmaceuticals, steroids (normal or	mobile phase conditions. Least retentive reversed phase hydrocarbon packing.  High-purity cyano phase with reduced silanol activity. Often a better choice than C18 for basic pharmaceuticals. Cyano is the most stable	Platinum* CN; Develosil" Cyano;	
Y	100	8 10	polar analytes. Shortest chain alkyl phase available for reversed phase separations.  Excellent for basic pharmaceuticals, steroids (normal or reversed phase conditions), or other basic compounds.  Ideal for fatty acids, polynuclear aromatic hydrocarbons,	mobile phase conditions. Least retentive reversed phase hydrocarbon packing.  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 separations.  High-purity, highly retentive, base-deactivated phase with alternate	Platinum* CN; Develosil* Cyano; Luna* CN	L10
Y	100	5 8 10 2	polar analytes. Shortest chain alkyl phase available for reversed phase separations.  Excellent for basic pharmaceuticals, steroids (normal or reversed phase conditions), or other basic compounds.  Ideal for fatty acids, polynuclear aromatic hydrocarbons, purines and pyrimidines, polar aromatics.  Superior general purpose amino phase.	mobile phase conditions. Least retentive reversed phase hydrocarbon packing.  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 separations.  High-purity, highly retentive, base-deactivated phase with alternate selectivity to hydrocarbon phases, especially for aromatic analytes.  Recommended for normal phase analyses of mono- and disaccharides and other similar compounds. Can also serve as a weak anion	Platinum* CN; Develosil* Cyano; Luna* CN  Platinum* Phenyl; Supelcosil Phenyl  Platinum* Amino;	L10
Y	100	5 8 10 2	polar analytes. Shortest chain alkyl phase available for reversed phase separations.  Excellent for basic pharmaceuticals, steroids (normal or reversed phase conditions), or other basic compounds.  Ideal for fatty acids, polynuclear aromatic hydrocarbons, purines and pyrimidines, polar aromatics.  Superior general purpose amino phase. Ideal for carbohydrates.	mobile phase conditions. Least retentive reversed phase hydrocarbon packing.  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 separations.  High-purity, highly retentive, base-deactivated phase with alternate selectivity to hydrocarbon phases, especially for aromatic analytes.  Recommended for normal phase analyses of mono- and disaccharides and other similar compounds. Can also serve as a weak anion exchanger, with aqueous buffers.  A pentafluorophenyl phase. Unique selectivity by interaction with functional groups of organohalogens or other basic analytes.  High purity, high surface area.	Platinum* CN; Develosil* Cyano; Luna* CN  Platinum* Phenyl; Supelcosil Phenyl  Platinum* Amino; Develosil* NH <sub>2</sub> Fluophase* PFP;	L10 L11 L8
Y Y N	100 100 100	5 8 10 2 7 NA	polar analytes. Shortest chain alkyl phase available for reversed phase separations.  Excellent for basic pharmaceuticals, steroids (normal or reversed phase conditions), or other basic compounds.  Ideal for fatty acids, polynuclear aromatic hydrocarbons, purines and pyrimidines, polar aromatics.  Superior general purpose amino phase. Ideal for carbohydrates.  Ideal for taxol and precursors, halogenated compounds, amines, esters, or ketones.	mobile phase conditions. Least retentive reversed phase hydrocarbon packing.  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 separations.  High-purity, highly retentive, base-deactivated phase with alternate selectivity to hydrocarbon phases, especially for aromatic analytes.  Recommended for normal phase analyses of mono- and disaccharides and other similar compounds. Can also serve as a weak anion exchanger, with aqueous buffers.  A pentafluorophenyl phase. Unique selectivity by interaction with functional groups of organohalogens or other basic analytes.	Platinum* CN; Develosil* Cyano; Luna* CN  Platinum* Phenyl; Supelcosil Phenyl  Platinum* Amino; Develosil* NH <sub>2</sub> Fluophase* PFP; Fluosep-RP* Phenyl	L10 L11 L8 L43
Y Y N Y NA	100 100 100 100	5 8 10 2 7 NA P	polar analytes. Shortest chain alkyl phase available for reversed phase separations.  Excellent for basic pharmaceuticals, steroids (normal or reversed phase conditions), or other basic compounds.  Ideal for fatty acids, polynuclear aromatic hydrocarbons, purines and pyrimidines, polar aromatics.  Superior general purpose amino phase. Ideal for carbohydrates.  Ideal for taxol and precursors, halogenated compounds, amines, esters, or ketones.  Ideal for normal phase applications.	mobile phase conditions. Least retentive reversed phase hydrocarbon packing.  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 separations.  High-purity, highly retentive, base-deactivated phase with alternate selectivity to hydrocarbon phases, especially for aromatic analytes.  Recommended for normal phase analyses of mono- and disaccharides and other similar compounds. Can also serve as a weak anion exchanger, with aqueous buffers.  A pentafluorophenyl phase. Unique selectivity by interaction with functional groups of organohalogens or other basic analytes.  High purity, high surface area.	Platinum* CN; Develosil* Cyano; Luna* CN  Platinum* Phenyl; Supelcosil Phenyl  Platinum* Amino; Develosil* NH <sub>2</sub> Fluophase* PFP; Fluosep-RP* Phenyl  Unique	L10 L11 L8 L43
Y Y N Y NA P	100 100 100 100 100	5 8 10 2 7 NA P	polar analytes. Shortest chain alkyl phase available for reversed phase separations.  Excellent for basic pharmaceuticals, steroids (normal or reversed phase conditions), or other basic compounds.  Ideal for fatty acids, polynuclear aromatic hydrocarbons, purines and pyrimidines, polar aromatics.  Superior general purpose amino phase. Ideal for carbohydrates.  Ideal for taxol and precursors, halogenated compounds, amines, esters, or ketones.  Ideal for normal phase applications.  Rapid analysis of carbamates.	mobile phase conditions. Least retentive reversed phase hydrocarbon packing.  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 separations.  High-purity, highly retentive, base-deactivated phase with alternate selectivity to hydrocarbon phases, especially for aromatic analytes.  Recommended for normal phase analyses of mono- and disaccharides and other similar compounds. Can also serve as a weak anion exchanger, with aqueous buffers.  A pentafluorophenyl phase. Unique selectivity by interaction with functional groups of organohalogens or other basic analytes.  High purity, high surface area.  Proprietary stationary phase can process up to twice as many samples per hour, compared to a conventional C18 phase.	Platinum* CN; Develosil* Cyano; Luna* CN  Platinum* Phenyl; Supelcosil Phenyl  Platinum* Amino; Develosil* NH <sub>2</sub> Fluophase* PFP; Fluosep-RP* Phenyl  Unique  Unique	L10 L11 L8 L43
	Y Y Y NA Y Y N NA Y N N N N N N N N N N	Y 140 Y 140 Y 140 NA 140 Y 110 Y 110 Y 110 Y 110 N 110 N 110 N 60 N 60 Y 60 N	Y 140 11 Y 140 6 Y 140 6 Y 140 4 NA 140 NA Y 110 13 Y 110 P Y 110 7 Y 110 4 Y 110 6 N 110 2 NA 110 NA Y 60 27 N 60 P Y 60 12 Y 60 17 N 60 P NA 60 NA Y 100 15 N 100 15	Y         140         11         Hydrophobic C18 phase suitable for a wide range of compounds, neutral through weakly basic.           Y         140         6         Applications similar to Pinnacle* D8 C18, but with less hydrophobic retention. Less retention can be useful for shortening analysis time, if resolution is adequate.           Y         140         4         Suitable for a wide range of compounds, from acidic through weakly basic. Also useful for confirmation of analyses on C18 or C8. Can be used in normal phase or reversed phase mode of separation.           NA         140         NA         Normal phase separations.           Y         110         P         Maximum resolution of polynuclear aromatic hydrocarbons.           Y         110         P         Maximum resolution of polynuclear aromatic hydrocarbons.           Y         110         A sanitytes.         Superior general purpose C18 for neutral and acidic analytes.           Y         110         4         analytes.           Y         110         4         analytes.           Y         110         4         analytes.           N         110         2         Excellent general purpose phenyl for neutral to weakly basic analytes.           N         110         2         Excellent general purpose phenyl for neutral to weakly basic analytes.           N         10         2 <td>  Y   140</td> <td>y 140 17 depted and a value canable for a value range of compound, recent of evolve) residents of the propound control of the value of the propound propound control of the propound propound propound control of the propound propound</td>	Y   140	y 140 17 depted and a value canable for a value range of compound, recent of evolve) residents of the propound control of the value of the propound propound control of the propound propound propound control of the propound

P= proprietary NA= not applicable

All columns: recommended pH range is 2.5–7.5; temp. limit  $80^{\circ}\text{C}$ . Column lifetimes can be shortened by operating at pH and/or temperature extremes. Call Restek Now for the Right Column for Your Tough Analysis 1-800-356-1688





### **Choosing a Stationary Phase**

- · Most critical decision in column selection.
- Analyte solubility dictates mode of separation:
- Based on analyte solubility and chemical differences among analytes.
- 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  $\leq 200$ Å (typical size:

Ultra 100Å Pores

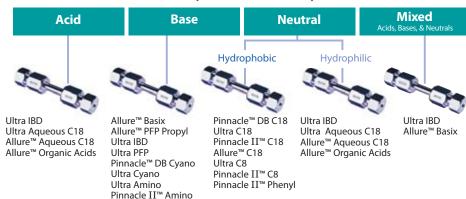
Allure™ 60Å Pores

Reversed Phase (RP)

Normal Phase (NP)

- · Large molecules (>2000mw) generally analyzed on material with pore size  $\geq 300$ Å.
- 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

# **Column Dimensions**

- · 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: therefore, a 4-fold increase in length is needed to double res-

 $[R = \frac{1}{4} (\alpha - 1 / \alpha) (\sqrt{N}) (k' / k' + 1)]$ 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**

		5µm Particles	$3\mu$ m Particles
	ID (mm)	Flow Rate (mL/min.)	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

# **Trident™ Direct Guard Column System**

Unlike "one size fits all" guard systems, the Trident™ Direct system gives you the power to select the right level of protection for your analysis. The system offers three levels of protection and guard cartridges in four dimensions, with a variety of bonded phases to match your analytical column. The economical, leak-free cartridge design provides an unprecedented combination of convenience, economy, and reliability. The foundation of the Trident™ Direct system is a reusable direct connect holder that easily attaches to any HPLC column using CPI- or Waters®-style end fittings.\* The system is available in the following configurations to match different protection level needs: in-line filter, in-line filter with holder for 10mm guard cartridge, and in-line filter with holder for 20mm guard cartridge. The guard cartridges are available in 2.1 and 4.0mm ID and are interchangeable within the appropriate length holder. Use 4.0mm ID guard with 4.6 or 4.0mm ID analytical column, 2.1mm ID guard with 2.1 - 3.2mm ID analytical column.



Description	qty.	cat.#
High-pressure filter	ea.	25082
10mm guard cartridge holder without filter	ea.	25083
10mm guard cartridge holder with filter	ea.	25084
20mm guard cartridge holder without filter	ea.	25085
20mm guard cartridge holder with filter	ea.	25086
Connection tip for Waters®-style end fittings	ea.	25088
PEEK® tip standard fittings	ea.	25087
Replacement cap frits: 4mm, 2.0 $\mu$ m	5-pk.	25022
Replacement cap frits: 4mm, $0.5\mu$ m	5-pk.	25023
Replacement cap frits: 2mm, 2.0 $\mu$ m	5-pk.	25057

\*The standard PEEK® tip in Trident™ Direct systems is compatible with Parker®, Upchurch®, Valco®, and other CPI-style fittings. To use Trident™ Direct systems with Waters®-style end fittings, the tip must be replaced with cat.# 25088.



Easy to Use, Low Dead Volume—The Ultimate Combination of Convenience and Column



Please see Restek's Chromatography Products Catalog for more HPLC columns and accessories.

Restek Trademarks: Allure, Pinnacle, Pinnacle II, Viva, Trident, Turning Visions into Reality, Restek logo.

Other Trademarks: Aquasil, Betamax, BioBasic, Fluophase, Hypersil, Prism, HyPurity Advance (Thermo Electron Corp.); Delta-Pak, Spherisorb, Symmetry, Waters (Waters Corp.); Develosil, Jupiter, Luna, Maxsil, Ultracarb (Phenomenex, Inc.); Discovery, Supelcosil (Sigma-Aldrich Co.); Fluosep-RP (ES Industries); Inertsil (GL Sciences, Inc.); Kromasil (Eka Chemicals); LiChrospher (Merck KgaA); Parker (Parker Instrumentation Division); PEEK (Victrex plc); Platinum (Alltech Associates, Inc.); Upchurch (Upchurch Scientific); Valco (Valco Instruments Co., Inc.); Zorbax (Agilent Technologies, Inc.).

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HPLC Column Selection Guide









**Turning Visions into Reality**<sup>™</sup>