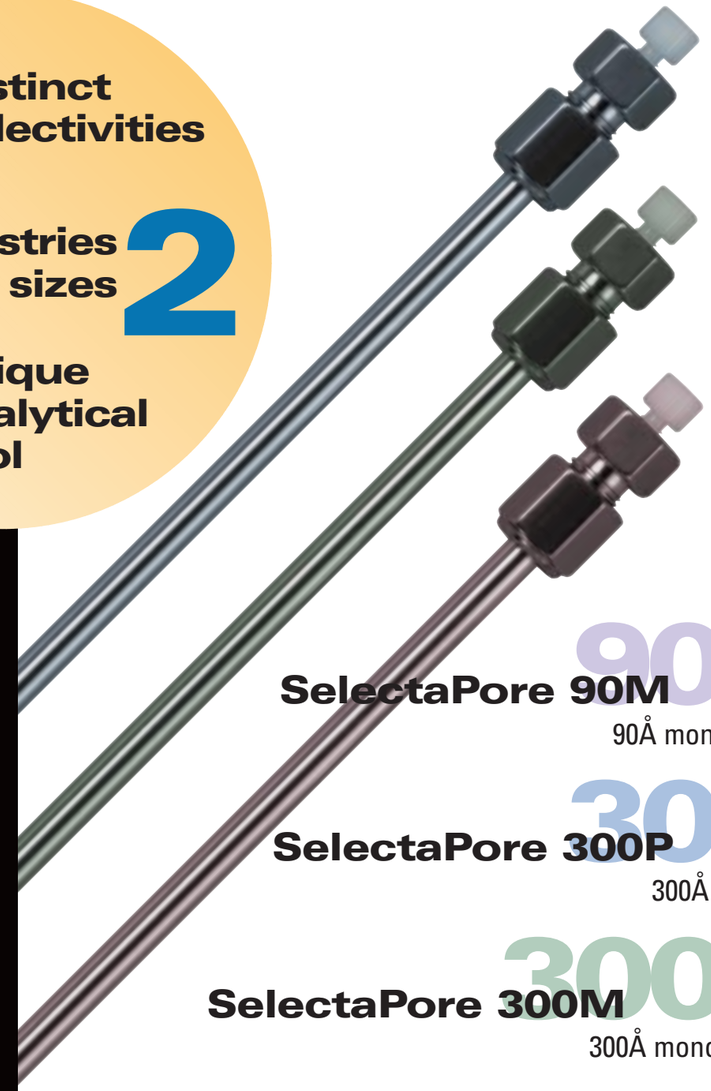
new SelectaPore™

SEPARATION POWER FOR ADVANCED PHARMACEUTICAL DEVELOPMENT

3 distinct selectivities

2 C18 chemistries & pore sizes

1 unique analytical tool



SelectaPore 90M
90Å monomeric C18

SelectaPore 300P
300Å polymeric C18

SelectaPore 300M
300Å monomeric C18

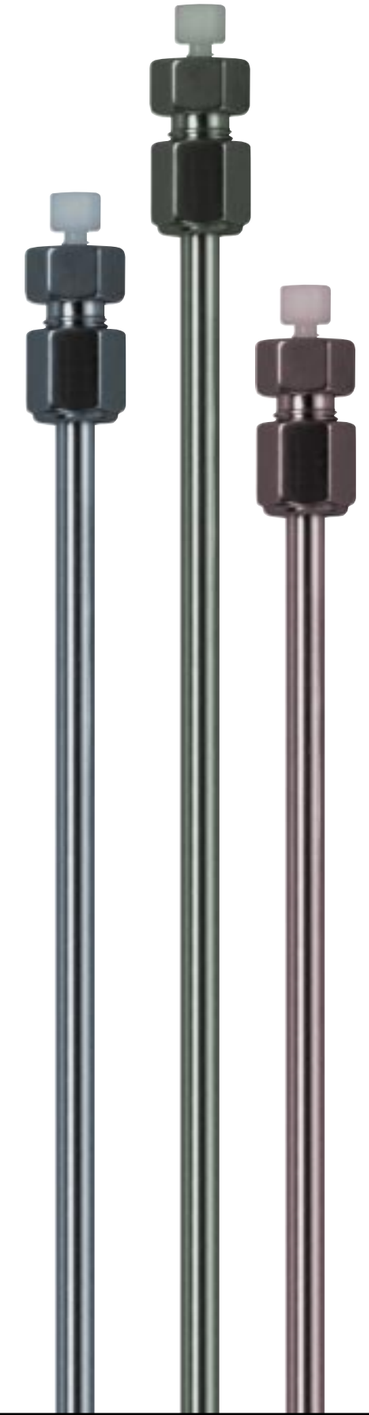
VYDAC®

Generic C18

Reversed-phase HPLC on a C18 column is the method most frequently used for purity analysis of small-molecule pharmaceuticals. C18 columns used for small-molecule separations are ordinarily based on 90Å to 120Å pore-size silica gel. Selection of a C18 column to use for a specific application is often based on previous experience, manufacturers' recommendations, and performance attributes such as efficiency, durability, and reproducibility.

USP specifies functional selectivity tests to determine suitability for standard analyses. But little science exists regarding how to determine if a column has the necessary selectivity for separations essential to successful new drug development, even though

"selectivity is the most critical single attribute affecting the ability to detect unknown and unforseen contaminants in a novel product or process."



SelectaPore C18

- two pore sizes
- two C18 chemistries
- three distinct selectivities

Although reversed-phase columns based on 300Å pore-size silica have long been used for larger analytes such as peptides, proteins, and oligonucleotides, it has not been widely realized that they also perform well in small-molecule analyses. In fact, compared to small-pore adsorbents their lower surface areas per gram reduce retentivity and improve selectivity for very hydrophobic compounds. As a side benefit, analytes elute at lower solvent concentrations, reducing solvent costs and waste. Vydac's unique offering of C18 reversed-phase columns based on both 90Å and 300Å pore-size silicas provides a valuable option for retention and selectivity.

In addition,Vydac uses two different C18 bonding techniques. Bonding with monofunctional silane produces a "monomeric" C18 phase on both 90Å and 300Å silicas. Using polyfunctional silane results in a more complex "polymeric" bonded phase on 300Å silica. (Polymeric bonding is not practical for 90-120Å silicas because it results in near total occlusion of the pores.) Monomeric and polymeric C18 provide another variation with subtle differences in retention and selectivity.

SelectaPore columns are offered in three varieties:

SelectaPore 90M 90Å pore, monomeric C18

SelectaPore 300M 300Å pore, monomeric C18

SelectaPore 300P 300Å pore, polymeric C18

They are produced by C18 bonding followed by end-capping on special high-purity 90Å and 300Å silica gels modified by a proprietary process that reduces residual polarity and produces symmetrical peaks for basic analytes. All SelectaPore columns are stable and produce highly reproducible separations.

Trial use of the three different SelectaPore reversed-phase columns is recommended when developing methods or screening products for impurities. This provides the best chance for optimizing separations. It also reduces chances that critical impurities that can affect drug performance and approval will be accidentally missed due to peak overlap. An economical kit is available to simplify ordering the necessary SelectaPore columns.

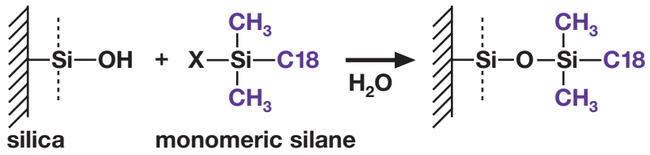
Two Surface Areas

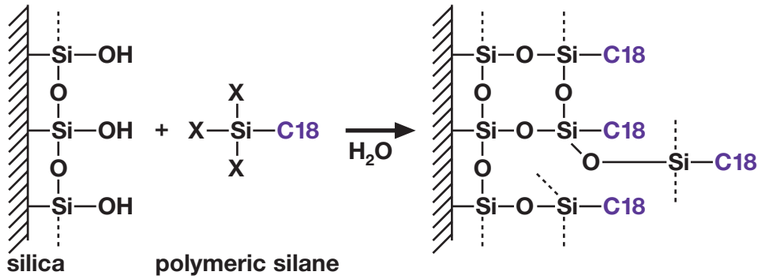
Vydac high-purity spheroidal 90Å and 300Å silica gels are the base matrices for SelectaPore columns. Total surface area is a function of pore size and is derived from mercury porosimetry data. The following table shows a comparison of total surface area for Vydac 90Å and 300Å silica gels.

Pore size	Total surface area
90Å	250 m^2/g
300Å	70 m^2/g

Two Bonding Chemistries

Monomeric silanes produce a single layer (monomeric) C18 coating on the silica gel surface. Polymeric silanes produce a more complex multilayer (polymeric) C18 coating.





Separation Power for Drug Development

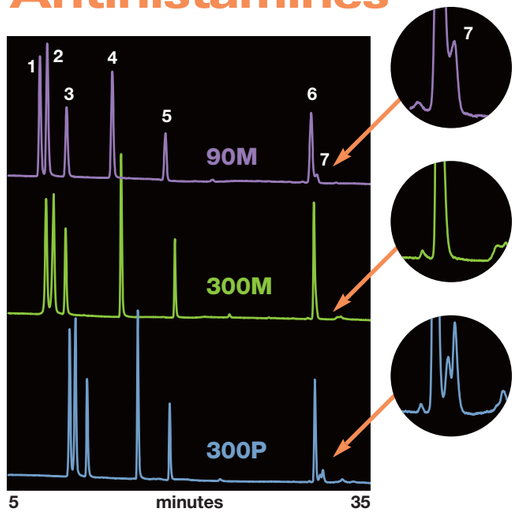
- find best resolution
- reveal sample details
- detect hidden impurities

Although SelectaPore C18 columns provide options for routine analyses, their power for drug development lies not in resolving major identifiable peaks, but in selectivity differences for minor contaminants.

Real-world purity assurance is based on the ability to find small peaks. An unexpected peak at the 1% level can be the most important peak in a chromatogram. Yet it will be impossible to find if it coelutes with product. Failure to detect such an impurity early in development can spell disaster down the road.

SelectaPore columns, with their unique combination of monomeric and polymeric bonding chemistries, provide alternative selectivities that increase the probability of discovering contaminants at an early stage. Using all three can reveal sample details and detect impurities that may remain hidden when only one type of C18 is used. Examples of these points appear in chromatograms on the following three pages.

Antihistamines



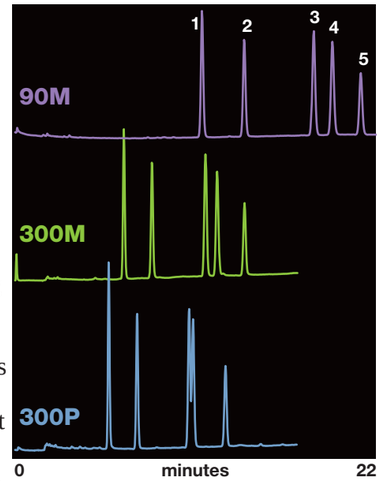
This separation of antihistamines demonstrates the power of diverse C18 selectivities. Had the separation been run on either SelectaPore 90M or SelectaPore 300M alone, at least one impurity in the final peak would have been missed. SelectaPore 300P clearly resolves two impurities.

Conditions of chromatography: All columns 4.6mmID x 250mmL. Detection: 260 nm. Flow rate: 1.0 mL/min. SelectaPore 90M: Gradient linear 12% to 42% acetonitrile over 35 minutes in 0.1% TFA (v/v). SelectaPore 300M and 300P: Gradient linear 5% to 32% acetonitrile over 35 minutes in 0.1% TFA (v/v).

Peaks: 1. pheniramine, 2. doxylamine, 3. methapyraline, 4. chlorpheniramine, 5. orphenadrine, 6. diphenylpyraline, 7. promethazine.

Barbiturates

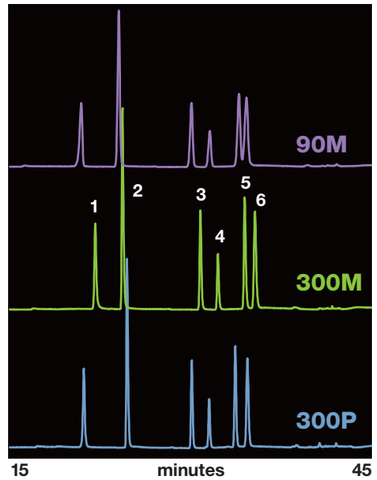
These five barbiturates are resolved on both SelectaPore 90M and 300M. SelectaPore 300M provides faster elution, using less solvent. SelectaPore 300P provides alternative selectivity but does not resolve peaks 3 and 4.



Conditions of chromatography were identical for all three 4.6mmID x 250mmL columns. Detection: 210 nm. Flow: 1.5 mL/min. Mobile phase A: 20% acetonitrile in 50 mM KH_2PO_4, pH 2.51. Mobile phase B: 90% acetronitrile. Gradient: linear 0% to 20% B over 20 minutes. Then to 100% B in 5 minutes.

Peaks: 1. butalbital, 2. phenobarbital, 3. mephobarbital, 4. secobarbital, 5. amobarbital.

Antidepressants

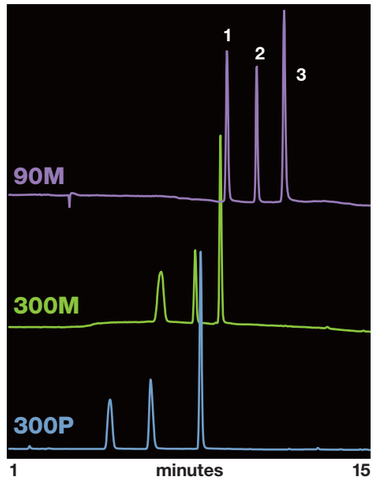


The antidepressants in this mixture separate on all three columns. Temazepam and diazepam are baseline resolved on SelectaPore 300M and 300P, and selectivities are similar although selectivities for other compounds, including contaminants, may be significantly different.

Conditions of chromatography: All columns 4.6mmID x 250mmL. Detection: 260 nm. Flow rate: 1.0 mL/min. Mobile phase A: 20 mM KH_2PO_4, pH 2.0, 5% ACN. Mobile phase B: 80% ACN. SelectaPore 90M: Gradient linear, 9% to 56% B over 35 minutes, then to 100% B in 5 minutes. SelectaPore 300M and 300P: Gradient linear, 0% to 46% B over 35 minutes, then to 100% B in 5 minutes.

Peaks: 1. bromazepam, 2. medazepam, 3. oxazepam, 4. lorazepam 5. temazepam, 6. diazepam.

Tryptophan Derivatives



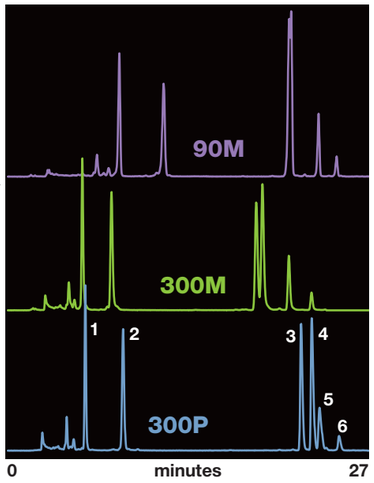
This separation behaves as expected for small hydrophilic compounds. The best retention and peak sharpness are obtained on SelectaPore 90M.

Conditions of chromatography were identical on the three 4.6mmID x 250mmL columns. Detection: 254 nm. Flow rate: 1.5 mL/min. Mobile phase A: 50 mM KH_2PO_4, pH 4.59. Mobile phase B: 90% ACN. Gradient: Hold 1% B for 3 minutes. Then linear to 15% B over 7 minutes. Then linear to 35% B over 5 minutes.

Peaks: 1. 5-hydroxytryptophan, 2. 5-hydroxytryptamine, 3. tryptophan.

Fat-Soluble Vitamins

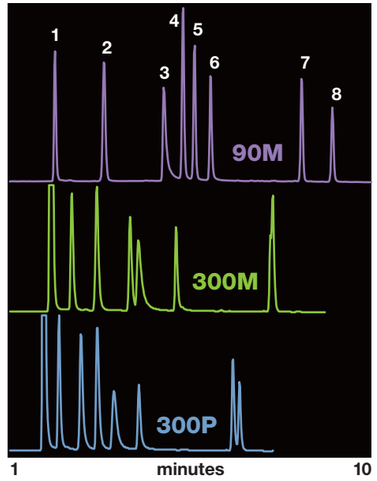
Fat-soluble vitamins are best resolved by SelectaPore 300P. Interestingly, vitamins D_2 and D_3 are more highly retained on SelectaPore 300P than on SelectaPore 90M. In this case, the chemistry of the polymeric C18 overcomes the effect of lower surface area.



Conditions of chromatography were identical for all three 4.6mmID x 250mmL columns. Detection: 295 nm. Flow: 1.0 mL/min. Mobile phase A: 75% MeOH, 25% H_2O. Mobile phase B: 70% ACN, 30% MeOH. Gradient: Hold 50% B for 5 minutes. Then linear to 100% B over 10 minutes. Hold 100% B for 15 minutes.

Peaks: 1. vitamin A (all-*trans*-retinol), 2. vitamin A acetate, 3. vitamin D_2 (calciferol), 4. vitamin D_3 (cholecalciferol), 5. vitamin E (α-tocopherol), 6. vitamin E acetate.

Water-Soluble Vitamins

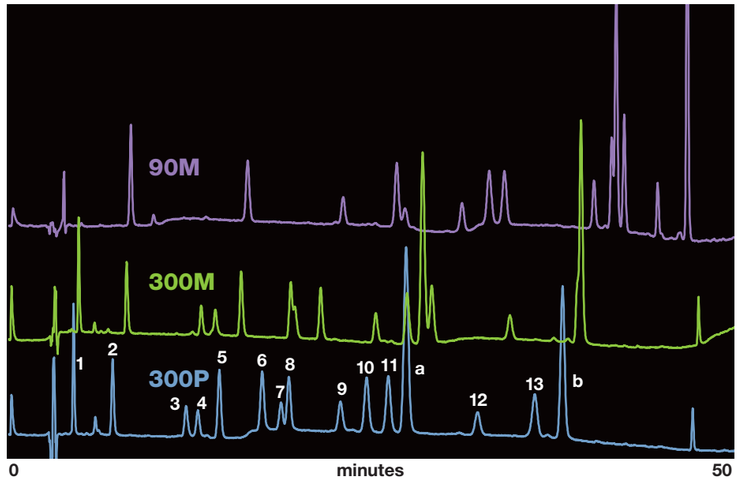


The water-soluble vitamins are best resolved on SelectaPore 90M. Although peaks elute faster on Selectapore 300M and 300P, there are significant differences in selectivity which, in this case, produce less resolution.

Conditions of chromatography were identical for all three 4.6mmID x 250mmL columns. Detection: 254 nm. Flow: 1.5 mL/min. Mobile phase A: 2.5% acetonitrile in 0.1 M KOAc, pH 5.4. Mobile phase B: 50% acetronitrile. Gradient: linear 5% to 100% B over 15 minutes.

Peaks: 1. ascorbic acid (C), 2. niacin, 3. pyridoxine (B6), 4. thiamine (B1), 5. nicotinamide (B3), 6. folic acid (M), 7. cyanocobalamin (B12), 8. riboflavin (B2).

Taxanes

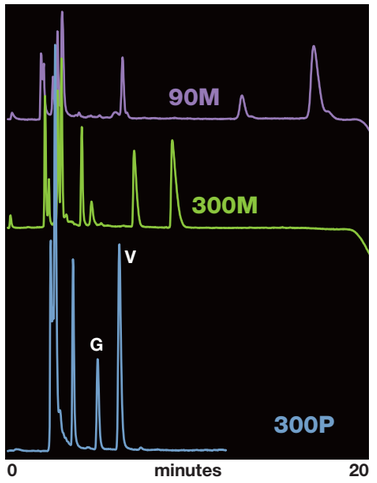


Peaks
1. 10-deacetylbaccatin III
2. baccatin III
3. 10-deacetyl-7-xylosyltaxol B
4. taxinine M
5. 10-deacetyl-7-xylosyltaxol
6. 10-deacetyl-7-xylosyltaxol C
7. 10-deacetyltaxol
8. 7-xylosyltaxol
9. cephalomannine
10. 10-deacetyl-7-epitaxol
11. paclitaxel
12. taxol C
13. 7-epitaxol
a. 3'-fluorophenyl-10-deacetyltaxol
b. 3'-fluorophenyltaxol

This mixture of 13 taxanes and two fluorinated taxol derivatives shows best resolution on SelectaPore 300P. SelectaPore 300M provides significantly different selectivity, which may be useful in some analyses. Retention of many sample components on SelectaPore 90M is too strong for the small-pore C18 column to be useful in displaying the entire range of components in this sample.

Conditions of chromatography were identical for all three 4.6mmID x 250mmL columns. Detection: 254 nm. Flow: 1.0 mL/min. Mobile phase A: 50 mM NaOAc, pH 6.8. Mobile phase B: acetronitrile. Gradient: Hold at 34% B for 2 minutes. Then linear to 44% B over 30 minutes. Then linear to 60% B over 10 minutes. Then linear to 100% B in 10 minutes.

Penicillins G and V



Penicillins G and V are somewhat unstable in aqueous mobile phases. These relatively hydrophobic molecules are strongly retained on SelectaPore 90M. Separation on either SelectaPore 300M or 300P provides a faster analysis, less chance for decomposition on the column, and greater sensitivity. However, peak shape is far better on SelectaPore 300P.

Conditions of chromatography were identical for all three 4.6mmID x 250mmL columns. Detection: 254 nm. Flow: 1.0 mL/min. Mobile phase: 60:40 water:ACN with 1% HOAc. Isocratic.

VYDAC®

SelectaPore™

Ordering information

Cat. No.	Description	
SelectaPore 90M	90 Å, 5 µm, monomeric C18	
201SP54	Column	4.6mmID x 250mmL
201SP5415	Column	4.6mmID x 150mmL
201SP52	Column	2.1mmID x 250mmL
201SP5215	Column	2.1mmID x 150mmL
SelectaPore 300P	300 Å, 5 µm, polymeric C18	
218WP54	Column	4.6mmID x 250mmL
218WP5415	Column	4.6mmID x 150mmL
218WP52	Column	2.1mmID x 250mmL
218WP5215	Column	2.1mmID x 150mmL
SelectaPore 300M	300 Å, 5 µm, monomeric C18	
238WP54	Column	4.6mmID x 250mmL
238WP5415	Column	4.6mmID x 150mmL
238WP52	Column	2.1mmID x 250mmL
238WP5215	Column	2.1mmID x 150mmL
SelectaPore Kit		
200SPK54	Includes one of each 4.6mmID x 250mmL column: 201SP54, 218WP54, & 238WP54	

Other analytical and preparative column and particle sizes available on request.

To place an order

contact your local Vydac distributor (listing on website)

or phone: **1-800-247-0924** or (760) 244-6107

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