2000/2001

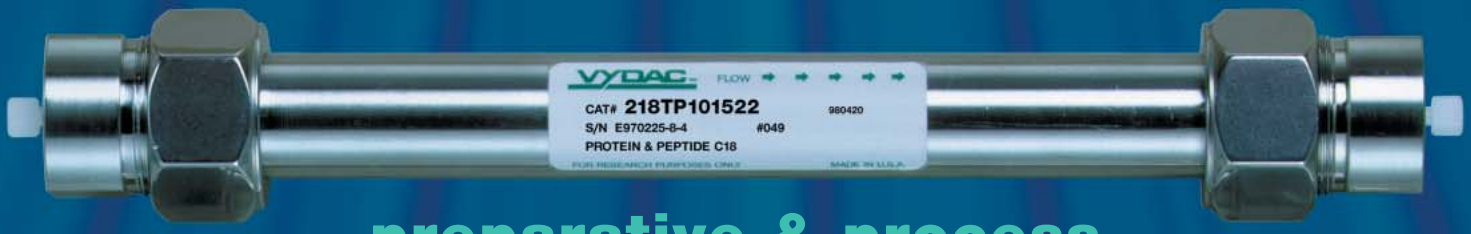
HPLC

COLUMNS AND BULK MEDIA

VYDAC®

pharmaceuticals nucleic acids proteins LC/MS peptides food & beverage environmental

analytical & microbore



preparative & process

ISO 9001 Certified

Table of Contents

VYDAC®

New Products for 2000/2001

With the year 2000 Vydac celebrates 29 years of providing the highest quality HPLC columns and separation products to the scientific community. In the two years since publication of our last general catalog, we've introduced the new products and improvements highlighted on this page. In addition, we've worked with customers to develop a variety of applications for existing Vydac products, details of which can be found throughout this catalog.

As you browse our catalog, we hope you'll find information and products that can be useful to your work.

Do you have ideas for other ways Vydac can be of service, for example other new products or applications? Please call or Email us! We'd like to hear from you, and we're eager to help.

Upgraded Website

Vydac's website has been significantly upgraded since the publication of our last catalog. It is designed to serve as a convenient source for technical and product information and provides a convenient way to keep up with new literature and developments from Vydac.

Pages 2-9

Vydac SelectaPore™ Columns for Pharmaceutical Analysis

New SelectaPore columns provide a choice of three different C_{18} reversed-phase selectivities for optimizing small-molecule pharmaceutical analyses – especially important during development for screening and characterizing new drug products.

Pages 18-20 and 68

8 µm and 15 µm Polymer Reversed Phase: Chemical & Heat Resistant Adsorbents for Preparative HPLC

Vydac's 259VHP polymer reversed-phase materials provide separation power for difficult to purify peptides and proteins by allowing chromatography under aggressive conditions – alkaline pH, high temperature, and a variety of mobile-phase modifiers. 259VHP columns are also particularly suited for biopharmaceutical purifications because they can be cleaned and sanitized by aggressive reagents. Two new particle sizes are offered for preparative scaleup.

Pages 34-37

New LC/MS Reversed-Phase Columns with Lower Ion-Pair Requirements

Ion-pair reagents such as TFA at the concentrations typically used can suppress ion generation and thus complicate MS detection. New Vydac LC/MS reversed-phase columns take advantage of proprietary silica modifications to reduce requirements for ion-pair reagents and eliminate suppression.

Page 70

New Convenient Pre-column Filter

These low-cost, low-dead-volume disposable filters attach quickly at the column inlet, making it easier to assure that columns are protected from particulates.

Pages 70-71

Microbore Guard Columns

Convenient cartridge-type guards are now available in 1.0 mm i.d. for protection of microbore reversed-phase columns.

New!

Pharmaceutical Analysis: Vydac SelectaPore™ C_{18} Reversed-Phase Columns

Background

Reversed-phase HPLC on a C_{18} column is the method most frequently used for purity analysis of small-molecule pharmaceuticals. C_{18} columns used for small-molecule separations are ordinarily based on 60 Å to 120 Å pore-size silica gel. Selection of a C_{18} column to use for a specific application is often based on previous experience, manufacturers' recommendations, and performance attributes such as efficiency, durability, and reproducibility. **Selectivity, however, is the key attribute determining whether a specific C_{18} column can discriminate a target analyte from contaminants and is therefore suitable for its analysis.**

The US Pharmacopeia (USP) specifies functional selectivity tests to determine suitability for **standard analyses**. These tests are based on historical knowledge and experience with specific pharmaceuticals and processes. They generally specify a minimum R value (resolution) to be achieved in separating the target analyte from a known contaminant or marker compound under defined mobile-phase conditions.

Analyses of defined pharmaceuticals, whether according to USP or a manufacturer's SOP, must be distinguished from analyses performed in the course of **new drug development**. Established procedures have as their objective determination of the presence or absence of known contaminants. Analytical procedures in the course of drug development often have as their objective the characterization of a novel process and discovery of contaminants and by-products, some of which may be unknown and unanticipated. **Selectivity and resolution are key to discovering contaminants** and developing procedures to assure their absence, control, or removal below specified levels in a new manufacturing process. But little guidance exists regarding how to determine whether a specific C_{18} column has the necessary selectivity to detect unknown and unforeseen contaminants in a new product.

- **Two pore sizes, 90 Å and 300 Å, provide surface area suited to analyte retentivity.**
- **Two C_{18} chemistries, monomeric and polymeric, create subtle differences in selectivity.**
- **Three distinct adsorbents permit C_{18} stationary phase optimization for specific analyses.**

Description

Vydac's unique offering of **three different C_{18} reversed-phase columns based on both 90 Å and 300 Å pore-size silicas** provides valuable options for retention and selectivity for both routine pharmaceutical analyses and new drug and process development.

Reversed-phase columns based on 300 Å pore-size silica have long been used for larger analytes such as peptides, proteins, and oligonucleotides. They also perform well in small-molecule analyses. Compared to small-pore adsorbents, their lower surface area per gram reduces retentivity and improves selectivity for very hydrophobic compounds. As a side benefit, virtually all small-molecule analytes elute at lower solvent concentrations than on 90 Å adsorbents with similar reversed-phase chemistry. This reduces solvent costs and solvent waste.

In addition to high-performance base silica with two different pore sizes, Vydac also uses **two different C_{18} bonding techniques**:

- Bonding with a monofunctional silane reagent produces a "monomeric" C18 phase on both 90Å and 300Å silicas.
- Using a polyfunctional silane reagent results in a more complex "polymeric" C18 bonded phase on 300Å silica.

"SelectaPore" is a trademark of The Separations Group, Inc.

The **monomeric and polymeric C_{18} phases exhibit subtle differences in retention and selectivity**. This leads to redistribution of relative peak positions in most complex separations, as seen in the taxane chromatograms on page 5. It can also lead to resolution of closely eluting peaks on one C_{18} phase type for compounds whose peaks overlap and are not resolved on the other, as seen in the separation of antihistamines on page 4.

Polymeric C_{18} bonding is not performed with the 90 Å silica because it results in near total occlusion of the pores and adsorbent characteristics with little practical utility. Thus the combination of two C_{18} bonding chemistries with two pore sizes results in **three SelectaPore column types**:

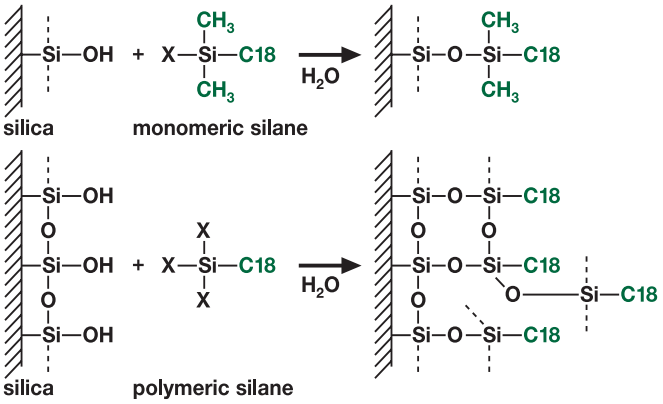
- **SelectaPore 90M** a monomeric C_{18} reversed phase with 90 Å base silica
- **SelectaPore 300M** a monomeric C_{18} reversed phase with 300 Å base silica
- **SelectaPore 300P** a polymeric C_{18} reversed phase with 300 Å base silica

Trial use of the three different SelectaPore reversed-phase columns is recommended when developing methods or screening new products for impurities. This provides the **best opportunity for detecting contaminants and optimizing separations**. It reduces the chance that any critical impurity that can affect drug performance and approval will be accidentally missed due to peak overlap.

Common Features

All three SelectaPore adsorbents begin with special **high-purity silica gels** that are modified by a proprietary process to reduce residual polarity and assure symmetrical peaks for basic analytes. All C_{18} bonding procedures are followed by **exhaustive end-capping** to assure minimal exposure of polar groups on adsorbent surfaces. SelectaPore columns are **stable** and produce **highly reproducible** separations.

Chemistry of Monomeric and Polymeric Bonding



Surface Areas

Vydac high-purity spheroidal 90 Å and 300 Å 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
90 Å	250 m^2/g
300 Å	70 m^2/g

An economical kit with a built-in discount is available to simplify ordering all three standard-dimension SelectaPore columns.

Examples of analyses on SelectaPore columns with a limited collection of samples appear on the following six pages. SelectaPore columns are suitable for routine USP analyses. The most important SelectaPore analysis could be the one you run on your new product in development.

For current information on Vydac columns for pharmaceutical analysis, obtain the **Vydac SelectaPore Column Brochure**. Available on request and also on Vydac's web site at http://www.vydac.com.

Separation Power for Drug Development

- **Find best resolution.**
- **Reveal sample details.**
- **Detect hidden impurities.**

Although SelectaPore C_{18} columns provide options for routine analysis, 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.

SelectaPore columns, with their unique combination of pore sizes, 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 C_{18} is used.

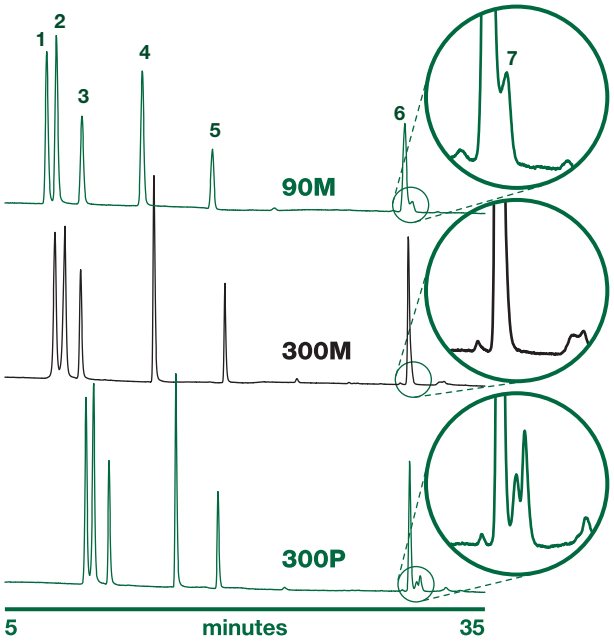
SelectaPore Ordering Information

Cat. No.	Description
	SelectaPore 90M 90 Å, monomeric
201SP54	Column, C_{18}, 90 Å, 5 µm, 4.6 mm i.d. x 250 mm
201SP5415	Column, C_{18}, 90 Å, 5 µm, 4.6 mm i.d. x 150 mm
201SP52	Column, C_{18}, 90 Å, 5 µm, 2.1 mm i.d. x 250 mm
201SP5215	Column, C_{18}, 90 Å, 5 µm, 2.1 mm i.d. x 150 mm
	SelectaPore 300P 300 Å, polymeric
218WP54	Column, C_{18}, 300 Å, 5 µm, 4.6 mm i.d. x 250 mm
218WP5415	Column, C_{18}, 300 Å, 5 µm, 4.6 mm i.d. x 150 mm
218WP52	Column, C_{18}, 300 Å, 5 µm, 2.1 mm i.d. x 250 mm
218WP5215	Column, C_{18}, 300 Å, 5 µm, 2.1 mm i.d. x 150 mm
	SelectaPore 300M 300 Å, monomeric
238WP54	Column, C_{18}, 300 Å, 5 µm, 4.6 mm i.d. x 250 mm
238WP5415	Column, C_{18}, 300 Å, 5 µm, 4.6 mm i.d. x 150 mm
238WP52	Column, C_{18}, 300 Å, 5 µm, 2.1 mm i.d. x 250 mm
238WP5215	Column, C_{18}, 300 Å, 5 µm, 2.1 mm i.d. x 150 mm
	SelectaPore Kit
200SPK54	Includes one of each 4.6 mm i.d. x 250 mm column: 201SP54, 218WP54, and 238WP54

Antihistamines

This separation of antihistamines demonstrates the power of diverse C_{18} 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.

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



Conditions

All columns 4.6 mm i.d. x 250 mm. 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). (Gradients for 300 Å columns were adjusted to lower organic solvent levels to bring peak retention times into range comparable to 90 Å column.)

Taxanes

This mixture of 13 taxanes and two fluorinated taxol derivatives shows best resolution on SelectaPore 300P. SelectaPore 300M provides significantly different selectivity, modifying relative peak positions, which may be useful in some analyses. Retention of many sample components on SelectaPore 90M is too strong for the 90 Å C_{18} column to be useful in displaying the entire range of components in this sample.

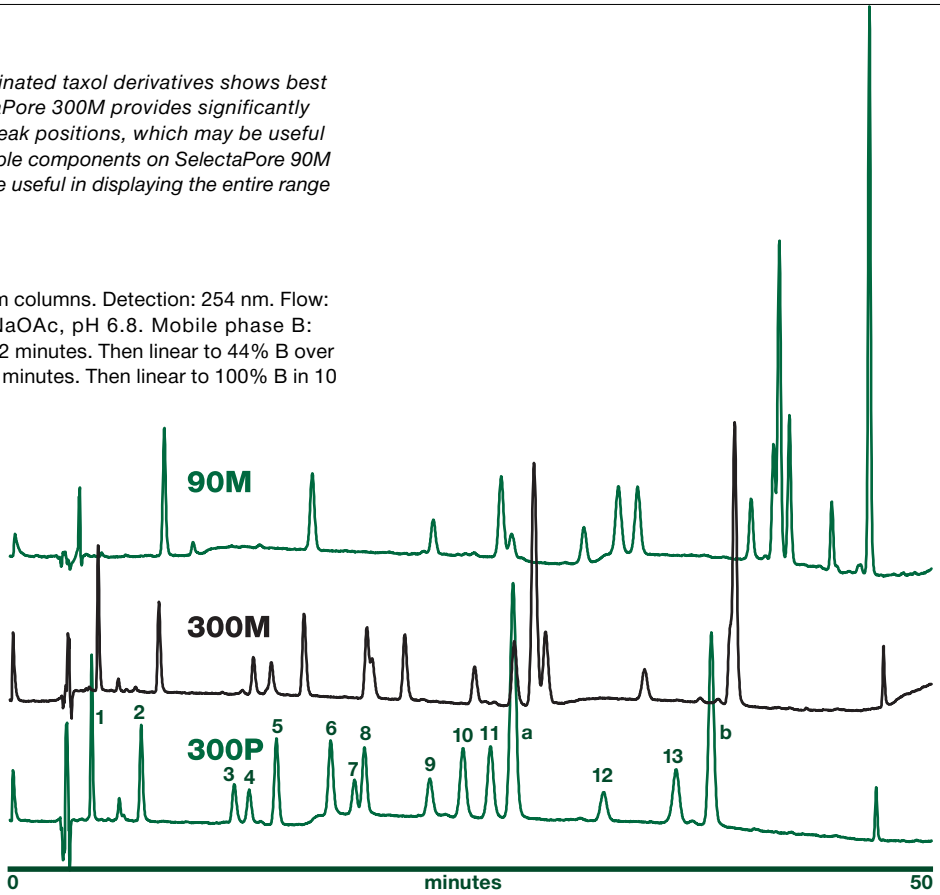
Conditions

Identical for all three 4.6 mm i.d. x 250 mm columns. Detection: 254 nm. Flow: 1.0 mL/min. Mobile phase A: 50 mM NaOAc, pH 6.8. Mobile phase B: acetonitrile. 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.

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



Barbiturates

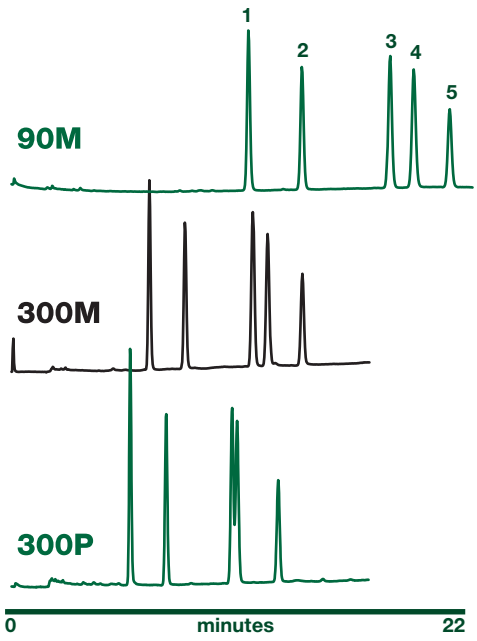
These five barbiturates are resolved on both SelectaPore 90M and 300M. SelectaPore 300M provides faster elution, using less solvent. SelectaPore 300P provides alternative selectivity but does not resolve peaks 3 and 4.

Peaks:

1. butalbital
2. phenobarbital
3. mephobarbital
4. secobarbital
5. amobarbital.

Conditions

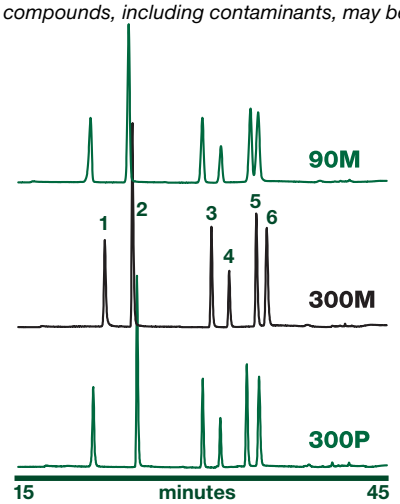
Identical for all three 4.6 mm i.d. x 250 mm columns. Detection: 210 nm. Flow: 1.5 mL/min. Mobile phase A: 20% acetonitrile in 50 mM KH_2PO_4, pH 2.51. Mobile phase B: 90% acetronitrile. Gradient: Linear, 0% to 20% B over 20 minutes. Then to 100% B in 5 minutes.



Pharmaceutical Analysis: Vydac SelectaPore™ C_{18} Reversed-Phase Columns

Anxiolytics

The anxiolytics in this mixture separate on all three columns. However, peak shapes and resolution are significantly better on the 300 Å adsorbents. Temazepam and diazepam are baseline resolved on both SelectaPore 300M and 300P, and selectivities are similar, although selectivities for other compounds, including contaminants, may be different.

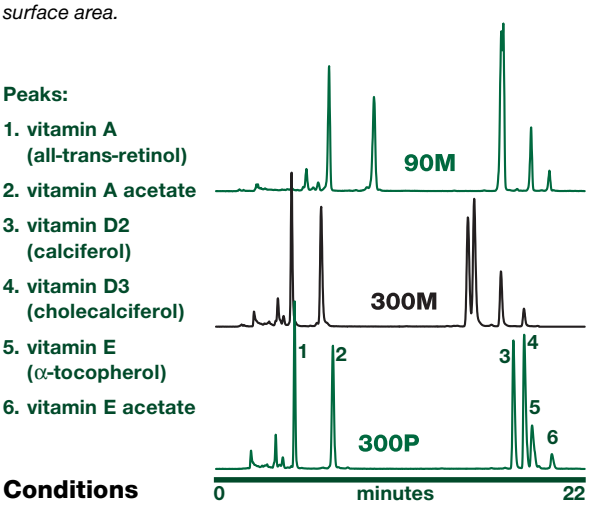


Conditions

All columns 4.6mm i.d.x 250mm. 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.

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.

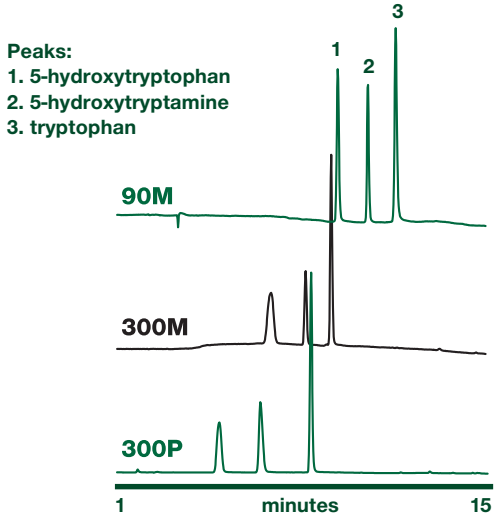


Conditions

Identical for all three 4.6 mm i.d. x 250 mm 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.

Tryptophan Derivatives

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

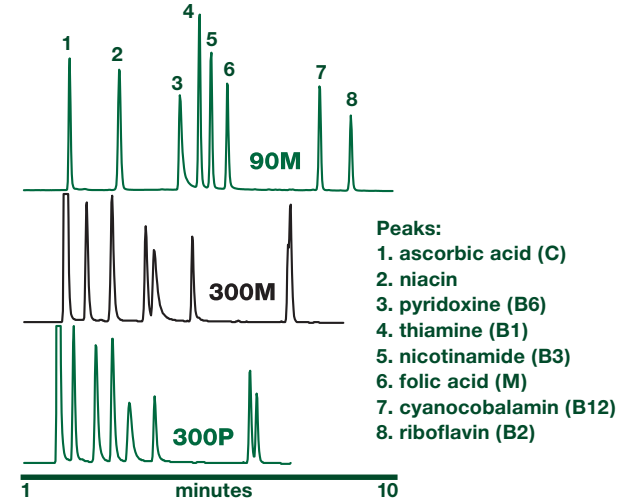


Conditions

Identical on the three 4.6 mm i.d. x 250 mm 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.

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

Identical for all three 4.6 mm i.d. x 250 mm 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.

Quality columns and chemistries for HPLC since 1971

Multi-Ring Compounds

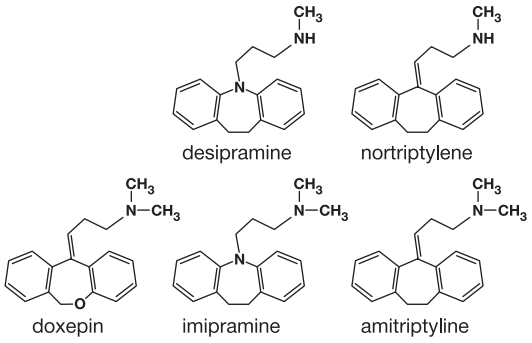
Many pharmaceuticals are complex multi-ring compounds. Reversed-phase columns based on 300 Å silica are ideally suited to analysis of these molecules because they allow complete access to the interior pore surfaces. SelectaPore 300P columns are specifically quality tested for separation of multi-ring pharmaceuticals and are guaranteed to provide performance in compliance with USP suitability requirements. Some USP analyses for which SelectaPore 300P columns can be used are illustrated here and on pages 8-9.

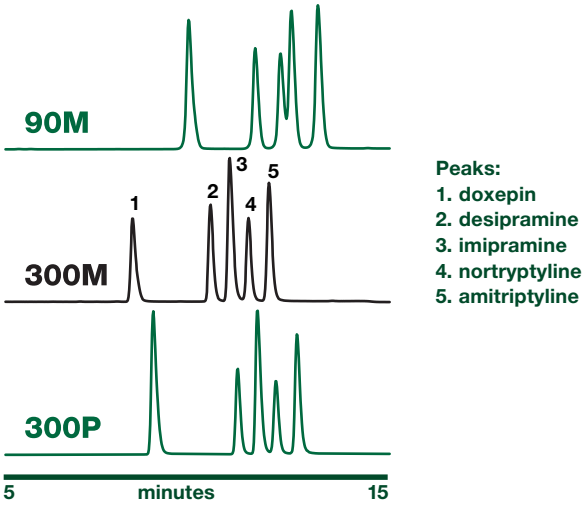
USP Methods

United States Pharmacopeia methods involving HPLC analysis generally specify column types based on particle size, phase type, and dimensions. This gives latitude to the scientist or quality control analyst in the selection of the actual column used. SelectaPore HPLC columns satisfy the Column L1 requirements for USP methods and often provide improved performance over columns traditionally used. The use of SelectaPore columns may result in sharper, more symmetrical peaks, better selectivity, faster analysis, or all of these.

Antidepressants

Vydac's SelectaPore columns separate the five tricyclic antidepressants with excellent peak symmetry. Best resolution was obtained on the 300 Å adsorbents.





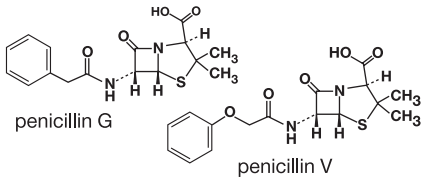
Peaks:
1. doxepin
2. desipramine
3. imipramine
4. nortryptyline
5. amitriptyline

Conditions

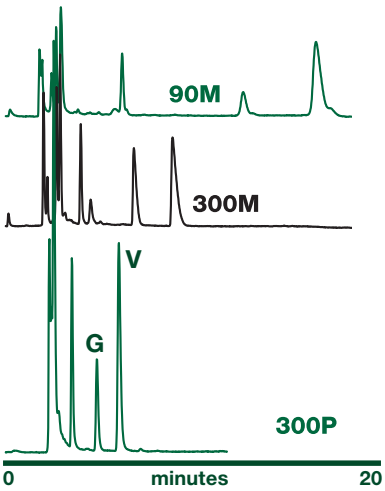
All columns 4.6 mm i.d. x 250 mm. Detection: 210 nm. Flow: 1.0 mL/min. Mobile phase: A = 25 mM KH2PO4, pH 3.0. B = 90% ACN. Gradient for SelectaPore 90M: Linear, 35% to 50% B over 15 minutes. Gradients for SelectaPore 300M and 300P: Linear, 30% to 45% B over 15 minutes.

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.



R = 4.4 on SelectaPore 300P.
USP requires R not less than 3.0.



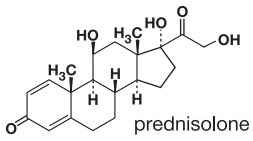
Conditions

Identical for all three 4.6 mm i.d. x 250 mm columns. Detection: 254 nm. Flow: 1.0 mL/min. Mobile phase: 60:40 water:ACN with 1% HOAc. Isocratic.

Pharmaceutical Analysis: Vydac SelectaPore™ C_{18} Reversed-Phase Columns

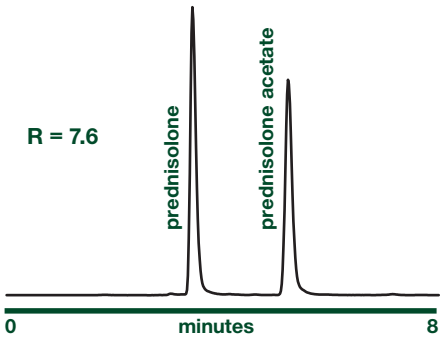
Prednisolone acetate

USP requires R not less than 2.0.



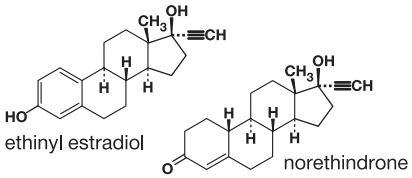
Conditions

Column: Vydac 218WP54 (C_{18}, 5 µm, 4.6 mm i.d. x 250 mm).
254 nm. Isocratic, 40% ACN in water (v/v) at 1.0 mL/min.



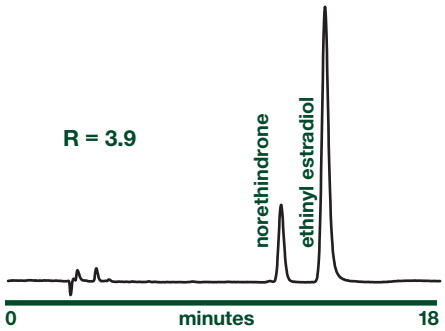
Norethindrone and ethinyl estradiol

USP requires R not less than 2.0.



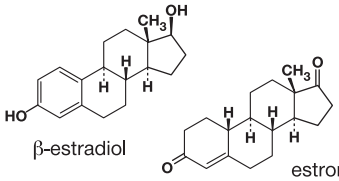
Conditions

Column: Vydac 218WP54 (C_{18}, 5 µm, 4.6 mm i.d. x 250 mm).
200 nm. Isocratic, 20 mM KH_2PO_4, pH 6.0, 35% ACN at
1.0 mL/min.



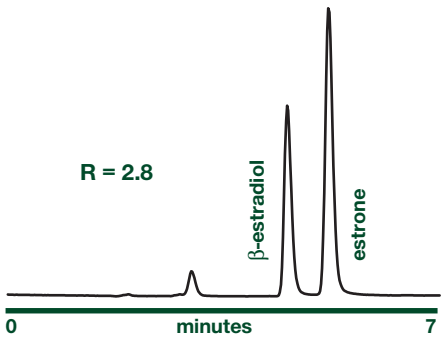
β-Estradiol

USP requires R not less than 2.0.



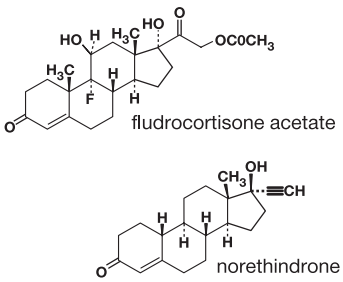
Conditions

Column: Vydac 218WP54 (C_{18}, 5 µm, 4.6 mm i.d. x 250 mm).
205 nm. Isocratic, 51:49 ACN:water at 1.0 mL/min.



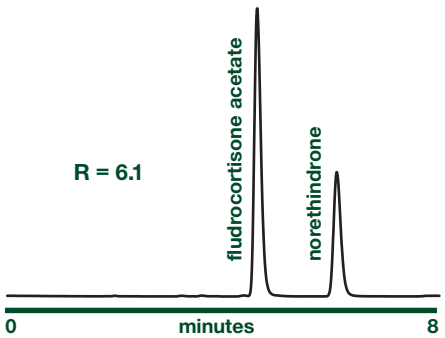
Fludrocortisone

USP requires R not less than 2.5.



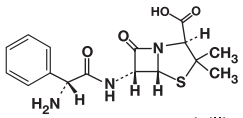
Conditions

Column: Vydac 218WP54 (C_{18}, 5 µm, 4.6 mm i.d. x 250 mm).
254 nm. Isocratic, 45:55 ACN:water at 1.0 mL/min.

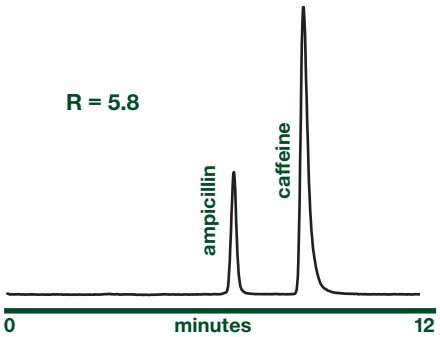


Ampicillin

USP requires R not less than 2.0.



ampicillin

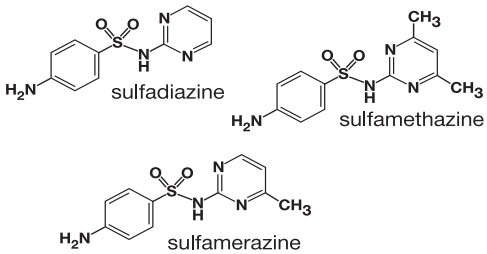


Conditions

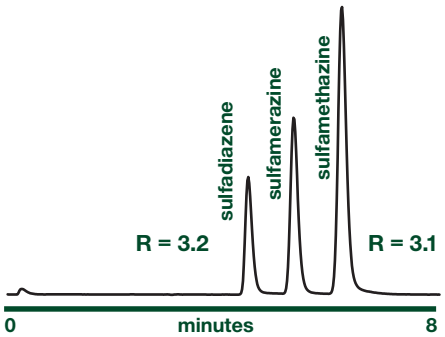
Column: Vydac 218WP54 (C_{18}, 5 µm, 4.6 mm i.d. x 250 mm). 254 nm. Isocratic 909:80:10:1 water:ACN:1M KH_2PO_4:1N acetic acid at 1.0 mL/min.

Trisulfapyrimidines

USP requires R not less than 3.0.



sulfadiazine sulfamethazine sulfamerazine



Conditions

Column: Vydac 218WP54 (C_{18}, 5 µm, 4.6 mm i.d. x 250 mm). 254 nm. Isocratic 86:13:1 water:ACN:glacial acetic acid, 1.0 mL/min.

SelectaPore Column Selection Guide

Particle Size	Column Size	SelectaPore 90M	SelectaPore 300P	SelectaPore 300M
	Bonded Phase	monomeric C18	polymeric C18	monomeric C18
	Pore Size	90 Å	300 Å	300 Å
5 µm	**Narrow Bore**			
	2.1 x 150 mm	201SP5215	218WP5215	238WP5215
	2.1 x 250 mm	201SP52	218WP52	238WP52
	Analytical			
	4.6 x 150 mm	201SP5415	218WP5415	238WP5415
	4.6 x 250 mm	201SP54	218WP54	238WP54

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

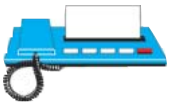
To place an order, obtain further information, or for technical assistance regarding Vydac products

In USA:

Telephone

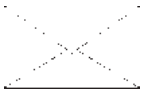
760-244-6107
- or -
TOLL-FREE
1-800-247-0924

FAX



760-244-1984
- or -
TOLL-FREE
1-888-244-6610

Mail



The Separations Group
17434 Mojave Street
Hesperia, California 92345

Web

Credit card orders can be placed on Vydac's website
http://www.vydac.com

Payment

For your convenience, Vydac columns may be purchased by purchase order, by check or by VISA, MASTERCARD or AMERICAN EXPRESS charge cards.

Shipping

Vydac columns are shipped by UPS to arrive within 2-3 working days. Federal Express or UPS Overnight shipment is available at a higher cost.

Outside USA:

Contact the Vydac distributor in your area.

To locate the distributor for your area, visit Vydac on the World Wide Web at http://www.vydac.com

- or -

Contact Vydac directly.

Phone: USA 760-244-6107
FAX: USA 760-244-1984
Email: sales@vydac.com

Vydac's Mission

Since 1971, from our location in the high desert of California, Vydac has provided the highest quality HPLC columns and separation products to the scientific community. We are proud of our past accomplishments, which include the development of the benchmark HPLC silica for separation of polypeptides. We are committed to meeting the needs of customers, not only through quality products and timely delivery, but also through personal technical support and through a fundamental commitment to "The Relentless Pursuit of Improvement" — personally, in our company, and in the products we offer. We demonstrate our commitment by continually striving to provide the most reproducible products possible, by developing new products to meet current and future separation needs, and by successfully meeting and maintaining the requirements of ISO 9001 certification.

Vydac Publications

Vydac offers a variety of informational publications describing technology and applications of HPLC for analysis and purification of pharmaceuticals, peptides, proteins, nucleic acids, environmental samples, and vitamins. A listing of Vydac publications appears on page 33 of this catalog. Printed copies of Vydac publications will be provided free on request. Publications in electronic form and current publication lists are available immediately on Vydac's web site: **http://www.vydac.com**

All locations:

Technical assistance via Email:

experts@vydac.com

Answers to frequently asked questions on Vydac's web site:

http://www.vydac.com

Quality Control

Each lot of Vydac separation material is tested for selectivity with compounds typical of the intended application. Vydac columns are individually tested for column efficiency.

Warranty

Columns are warranted to be free from manufacturing defects for 90 days. Columns that fail prematurely should be returned to Vydac. Returned columns will be repaired or replaced without charge if returned within the warranty period and the failure was due to a manufacturing defect. Please contact Vydac prior to returning a column and request a Return Authorization Number.

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In USA, phone (800) 247-0924
or fax (888) 244-6610, toll-free.

For technical information or the name of the distributor in your area, visit Vydac on the World Wide Web at http://www.vydac.com

Serving chromatographers with quality packings and bonding chemistry since 1971