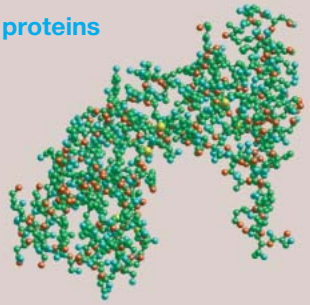
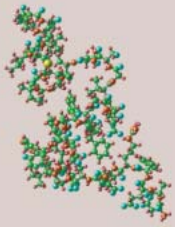
High-Performance Chromatography Columns

proteins



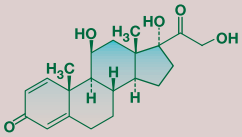
peptides

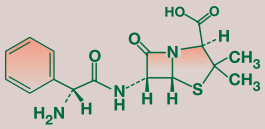


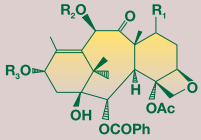
nucleic acids

■ **bioseparations**

USP methods

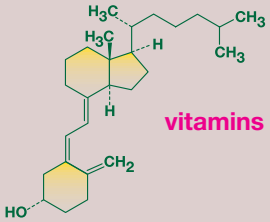






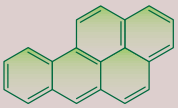
research

■ **pharmaceuticals**

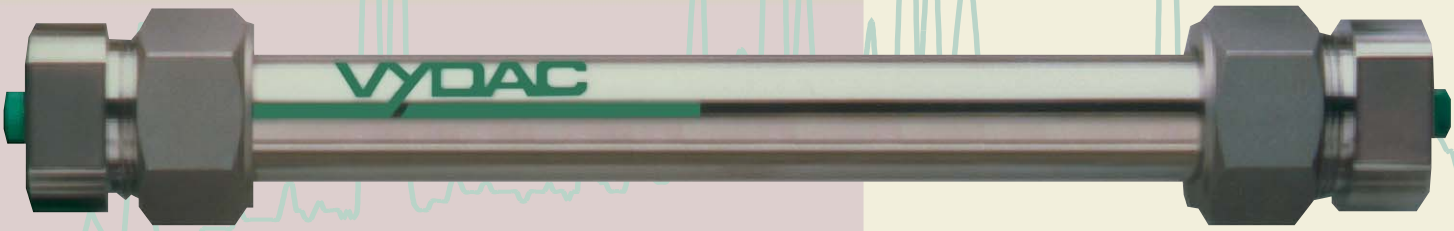


vitamins

EPA methods



■ **food and environment**



◆ **analytical columns**
◆ **preparative columns & bulk media**

1998/1999

VYDAC®

ISO 9001 Certified

Table of Contents

VYDAC®

We've Been Busy!

New!

Here's What's New

New Products, New Applications...

In 1998 Vydac celebrates 27 years of providing the highest quality HPLC columns and separation products to the scientific community. We're proud of that history, but we're not standing still. 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 new and improved applications for existing Vydac products, details of which can be found throughout this catalog.

and More!

That's not all. We've also become **ISO 9001 certified** to assure we have the procedures and systems necessary to assure quality and consistency in products and services, and we've initiated a **World Wide Web Site** where customers can quickly access answers to frequently asked questions, get technical tips, and find the latest information about Vydac products and applications.

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.

Don't miss these additions!

Pages 10-11

259VHP Polymer Reversed-Phase

A highly chemically resistant, heat-stable reversed-phase provides the solution for applications that require harsh conditions for chromatography, or for column cleaning, regeneration, or sanitization.

Pages 16-17

238TP Monomeric C_{18} Reversed-Phase

A new monomeric-bonded C_{18} reversed-phase provides alternative selectivity that can result in improved resolution of some samples and reveal additional peaks in complex samples, for example partial hydrolysates of proteins and carbohydrates.

Page 22

3-Micron Reversed-Phase Materials

Faster analysis of polypeptides is now possible on these small-particle versions of Vydac's proven 300Å TP-silica based reversed-phase chemistries.

Pages 24-25

Columns for LC/MS

A new section highlights Vydac's microbore reversed-phase columns for hyphenated techniques.

Pages 38-41

218MR C_{18} Reversed-Phase for Multi-Ring Pharmaceuticals

Specifically designed with special quality control to assure USP-compliant analytical separations of complex multi-ring pharmaceuticals.

Pages 54-61

Columns and Bulk Adsorbents for Preparative and Process LC

Identical reversed-phase chemistries to those you use for laboratory separations are now available in 10-15 micron and 15-20 micron sizes, packed in columns, or in multi-kilo drum quantities for scaling up preparative and process liquid chromatography.

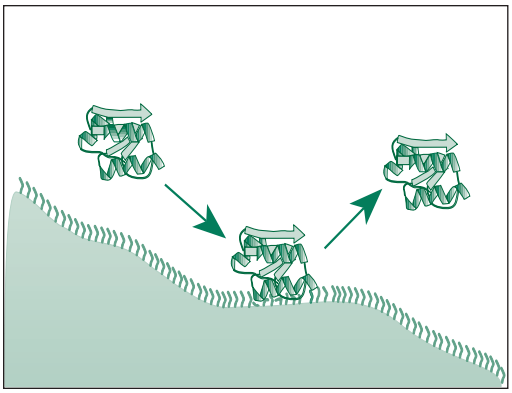
Analysis and Purification of Polypeptides by Reversed-Phase HPLC

Reversed-phase HPLC is a valuable tool for the analysis and purification of proteins and peptides. It is effective in separating **peptide fragments** from enzymatic digests, in the separation and purification of **natural and synthetic peptides,** and in purifying **proteins as large as 100 kd** for characterization.

Why is reversed phase so widely used?

The answer is resolution! RP-HPLC is able to resolve very similar polypeptides, some of which differ by a single amino acid residue. The separation of insulin variants (below) is an example of the high resolution capabilities of reversed-phase HPLC. Some insulin variants differ by as little as a single amino acid. For instance, rabbit insulin has a threonine where human insulin has a serine - a difference of one methylene group in a single residue - and RP-HPLC is able to separate these two variants! In another instance, the purification of insulin-like growth factor (IGF), RP-HPLC separated native IGF from its oxidized methionine derivative - a difference of a single oxygen atom in a molecule of nearly 5800 MW!

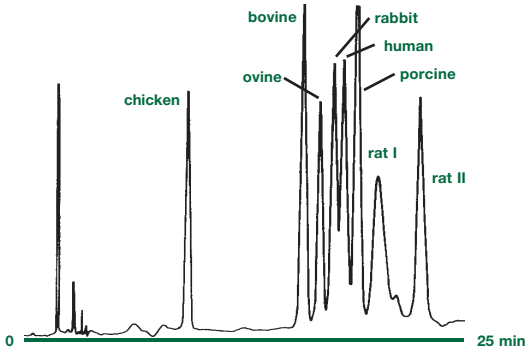
The power and popularity of reversed-phase HPLC is in its high resolving power!



Polypeptides are large molecules and cannot "partition" into the stationary phase as do small molecules. Polypeptides adsorb onto the hydrophobic "reversed-phase" surface from the mobile phase and remain adsorbed until the organic component of the mobile phase reaches a critical concentration. At that time the polypeptide desorbs and is carried by the mobile phase to the column exit. There is little further interaction with the reversed-phase surface. Polypeptides elute quickly once the critical organic concentration is reached, which accounts for the sharp peaks and high resolution achieved with this technique.
X. Geng and F.E. Regnier, J. Chrom 296, 15-30 (1984)

Insulin variants

The separation of insulin variants, some of which differ by a single amino acid, demonstrates the ability of reversed-phase HPLC to separate very similar polypeptides.



Conditions

Column: Vydac 214TP54 (C_4, 5µ, 4.6mmID x 250mmL).
Eluent: 27-30 % ACN with 0.1% TFA over 25 minutes.
From J. Rivier and R. McClintock, J. Chrom. 268, 112-119 (1983)

Separation conditions

Polypeptides are eluted from reversed-phase columns using aqueous mobile phases containing an ion pairing agent and an organic modifier. The most commonly used **ion pairing agents** include:

- ❏ trifluoroacetic acid (TFA)
- ❏ heptafluorobutyric acid (HFBA)
- ❏ phosphoric acid
- ❏ triethylamine phosphate (TEAP)

Ion pairing agents are added to both gradient solvents at concentrations of 0.05 to 0.2 %.

Trifluoroacetic acid is by far the most commonly used ion pairing agent because of its excellent separation capabilities, low UV absorbance, and high volatility for easy removal in peptide isolation. TFA is also very effective in solubilizing hydrophobic polypeptides.

HFBA is effective in the chromatography of basic proteins, and TEAP has shown unique selectivity for a variety of peptides.

The most commonly used organic modifiers are:

- ❏ acetonitrile (ACN)
- ❏ isopropanol (IPA)
- ❏ ethanol (EtOH)

Acetonitrile offers low viscosity, excellent UV absorption characteristics, and high volatility for easy removal. Isopropanol is used either alone or in combination with acetonitrile (1:2 to 2:1) to elute large or hydrophobic proteins. Ethanol is most often used in large-scale process applications.

Gradients in the organic modifier concentration are normally used to obtain sharp peaks and optimum selectivity. Initial organic concentrations range from 1-2% for hydrophilic peptides to 20-40% for large or hydrophobic proteins. While most peptides will elute in 70% organic modifier or less, large or hydrophobic proteins may require as high as 85% organic to elute.

The gradient slope (percent change in organic modifier per unit volume or time) is normally around 1-2% per minute, however very shallow gradients - as low as .05 - .2 % per minute are used to separate complex mixtures or very similar peptides.

Polypeptides are usually detected by UV absorption at wavelengths from 210 to 220 nm, where the peptide bond absorbs. Higher wavelengths such as 280 nm are sometimes used to monitor proteins with aromatic residues such as tryptophan.

References

1. **The Importance of Silica Type for Reversed-Phase Protein Separations,** J.D. Pearson, N.T. Lin and F.E. Regnier. *Anal. Biochem, 124, 217-230 (1982)*
2. **Retention Model for Proteins in Reversed-phase Liquid Chromatography**, X. Geng and F.E. Regnier, *J. Chrom. 296, 15-30 (1984)*
3. **Reversed-phase High-Performance Liquid Chromatography of Insulins from Different Species,** J. Rivier and R. McClintock, *J. Chrom. 268, 112-119 (1983)*
4. **Reversed-Phase Chromatography of Interleukin-2 Muteins**, M. Kunitani, P. Hirtzer, D. Johnson, R. Halenbeck, A. Boosman and K. Koths, *J. Chrom. 359, 391-402 (1986)*

Keys to Well-Characterized Biotechnology Products

Recent changes in FDA rules simplify the regulatory treatment of "well-characterized biotechnology products"*. HPLC often plays a crucial role in characterizing biotechnology products as to purity, potency and identity.

Keys to defining a "well-characterized biotechnology product" by HPLC are:

✓ ***Selectivity:*** To monitor biotechnology products for purity and identity, important impurities must be separated from the major product; digest fragments with minor changes must be resolved from normal fragments. Resolving minor impurities such as deamidation products and oxidized methionine variants place the ultimate demands on HPLC column selectivity.

✓ ***Stability:*** Column selectivity must be constant over hundreds of injections to ensure robust and reliable assays. Only columns which are physically and chemically stable and which maintain selectivity over time are practical for monitoring purity, potency or identity of "well-characterized biotechnology products".

✓ ***Reproducibility:*** Column selectivity and sample resolution should remain the same when used columns are replaced with columns from a new batch. HPLC columns used to monitor purity, potency or identity of "well-characterized biotechnology products" must be reproducible from batch to batch.

** The term "specified product" is favored for future use to eliminate the subjective aspect of "well characterized."*

Selectivity

Selectivity is the primary measure of column performance; a column must separate key impurities to be useful for an assay or purification.

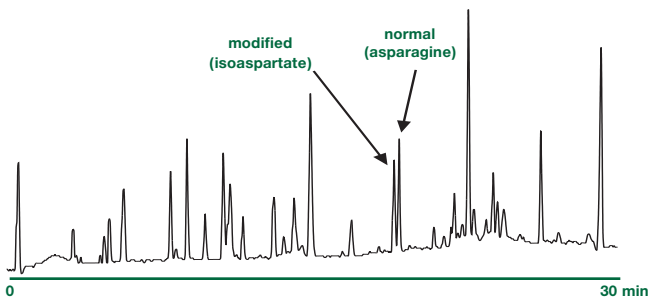
Selectivity and resolution of peptides and proteins are very sensitive to column characteristics such as the silica matrix and bonding chemistry. Vydac reversed-phase HPLC columns for bioseparations are made with the 300 angstrom pore diameter silica that pioneered the separation of polypeptides over a decade ago and continues to be the standard against which all polypeptide separations are compared. Vydac's unique polymeric bonding chemistry offers unrivaled selectivity for separating polypeptides.

Vydac reversed-phase columns have been used to separate all types of polypeptides including nearly identical insulins (Ref. 3, pg. 3), interleukin muteins (Ref. 4, pg. 3) and deamidated peptides (see figure below).

Vydac selectivity is illustrated in the figure below by the separation of two very similar digest peptide fragments where asparagine is converted to isoaspartate by deamidation.

Separation of tryptic digest fragments from normal and deamidated bovine somatotropin

Conditions:
Column: Vydac 218TP54 (C_{18}, 5µ, 4.6mmID x 250mmL).
Eluent: 0 - 15 % ACN over 20 min., 15 - 21% ACN over 12 min., 21 - 48% ACN over 27 min., 48 - 75% ACN over 4 min; in 0.1% aqueous TFA at 2.0 mL/min.
From: Schlittler, et.al., Ninth ISPPP, Abstract 621 (1989)



For details on how HPLC column selectivity can be optimized to best characterize your biotechnology product, request Vydac's Technical Report:

Column Selectivity: The Primary Key to Achieving High-Performance HPLC Separations of Proteins and Peptides

Stability

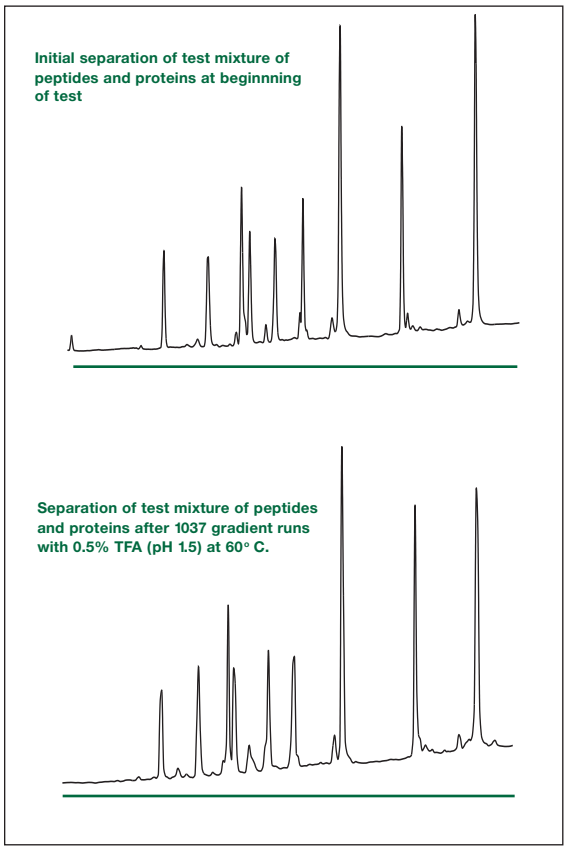
Column stability is the second measure of column performance. Stable columns result in constant column selectivity and sample resolution over hundreds of sample injections, thus ensuring robust and reliable assays for monitoring purity, potency or identity of biotechnology products.

HPLC column stability in polypeptide separations requires that columns retain their selectivity even under the somewhat harsh conditions of peptide analysis – pH 2 or less with trifluoroacetic acid. Vydac reversed-phase HPLC columns have long been known for unusually long column lifetimes under these demanding conditions, often exceeding a thousand sample injections per column. The extended lifetime of Vydac columns is attributed to the exceptionally stable silica matrix and to unique, polymeric bonded phases.

To show how column stability results in reliable assays for proteins and peptides, Vydac tested a 214TP54 (C_4, 5 micron, 4.6mmID x 250mmL) column under extreme elution conditions of pH 1.5 with 0.5% trifluoroacetic acid at a temperature of 60 degrees C – well outside the ranges of normal polypeptide chromatography. Over 1000 gradient runs produced no change in column selectivity; resolution between all of the peptides and proteins tested remained the same – a remarkable indication of long-term column stability (see figures at right)!

Column stability test procedure:

To provide an extreme test of column stability, over 1000 repeat gradients were run on a Vydac 214TP54 (C_4, 5 micron, 4.6mmID x 250mmL) column under extreme conditions of pH and temperature. Gradients were run from 0 - 100% B over 80 minutes at 1.0 mL/min at a ***temperature of 60° C. The starting eluent (A) contained 0.5% TFA – pH 1.5 – in water. The final eluent (B) contained 0.45% TFA in acetonitrile.*** *The unusually high concentration of TFA – 0.5% – and unusually high temperature – 60° C – ensure an extreme test of column stability. A test mixture consisting of six peptides (oxytocin, bradykinin, angiotensin II, eledoisin-related peptide, neurotensin and angiotensin I) and three proteins (ribonuclease, insulin and lysozyme) were run every 50 - 100 gradients under the test conditions.* ***Results:*** *The chromatographic separation of the peptides and proteins is nearly identical after 1037 gradient runs to that obtained during the initial separation.* ***The column did not degrade, and selectivity and resolution remained unchanged over 1037 gradient runs under the extreme conditions of pH and temperature used.***



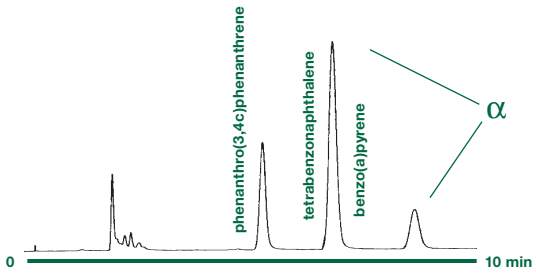
Reproducibility

Batch-to-batch column reproducibility is the third measure of column performance. Reproducible HPLC columns are essential in the development of robust assays of biotechnology products.

Column selectivity and sample resolution should remain the same when used columns are replaced with columns from a new batch. Columns which retain selectivity and separation characteristics from batch to batch reduce hassle and ensure reliable assays over the lifetime of the biotechnology product.

Batch-to-batch column reproducibility depends on absolute control over the manufacture of the silica matrix and the bonding chemistry. Vydac manufactures HPLC silicas in its own facility and maintains absolute control over the production process through in-process quality control and Statistical Process Control measurements. These ensure highly reproducible, high quality silica particles.

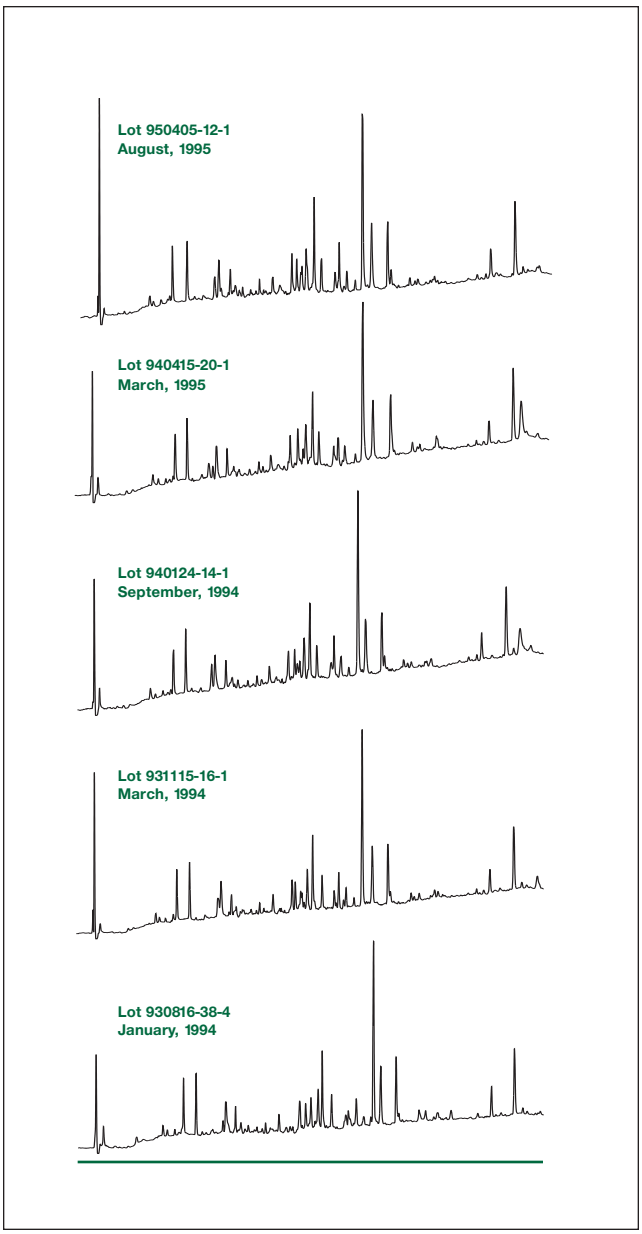
Vydac uses very sensitive tests to monitor and control carbon loading on reversed-phase materials; tests which surpass conventional methods of measuring carbon loading such as carbon microcombustion. One such test used to monitor the carbon load of polymerically bonded C_{18} is based on a test developed by the U.S. NIST (National Institute for Standards and Technology), involving the isocratic separation of three polyaromatic hydrocarbons and the calculation of a resolution factor - α - between two of these (see figure). Vydac is able to precisely control the carbon load of polymerically bonded C_{18} through this highly sensitive test.



Column: 218TP5415 (C_{18}, 5µ, 4.6mmID x 150mmL)
Eluent: 85:15 acetonitrile:water
Sample: three polyaromatic hydrocarbons (NIST SRM 869)

Vydac Application Note #9703, "Developing a Robust Reversed-Phase Method for Analysis of Polypeptides", discusses the importance of reversed-phase HPLC in research, production and quality control of polypeptide therapeutics as well as its pivotal role in developing robust assays for well-characterized biotechnology-derived therapeutics. The publication emphasizes the need for column stability and reproducibility and describes methods for optimizing analyses by selection of ion-pairing agents, buffers, and eluant pH.

Available free on request from Vydac.



Highly sensitive tests enable precise control over reversed-phase carbon load and result in unparalleled batch-to-batch column reproducibility for Vydac columns used in the analysis and purification of proteins and peptides. Batch-to-batch column reproducibility is evidenced by the separation of peptide fragments from the tryptic digest of beta-lactoglobulin A. This digest was chromatographed on Vydac 218TP54 columns (C_{18}, 5 micron, 4.6mmID x 250mmL) from several lots of materials produced over more than a year (see figure). The digest maps performed on columns produced from January 1994 to August of 1995 are nearly identical - evidence of Vydac's exceptional batch-to-batch column reproducibility.

Column reproducibility test procedure:

The separation of peptide fragments from an enzymatic digest is a very effective test of batch-to-batch reproducibility of reverse phase columns. A tryptic digest of beta-lactoglobulin A was chromatographed on Vydac 218TP54 (C_{18}, 5µ, 4.6mmID x 250mmL) columns from the following five lots: lot 930816-38-4 produced in January 1994; lot 931115-16-1 produced in March 1994; lot 940124-14-1 produced in September 1994; lot 940415-20-1 produced in March 1995; and lot 950404-12-1 produced in August 1995. Conditions were: 0 - 30% acetonitrile with 0.1% TFA over 60 minutes at 1.0 mL/min and 30° C.

Conclusion: Vydac 218TP54 columns show remarkable lot-to-lot reproducibility.

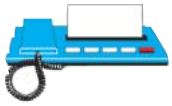
To place an order, obtain further information, or for technical assistance regarding Vydac products

In USA:

Telephone

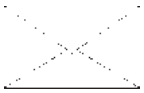
760-244-6107
- or -
TOLL-FREE
1-800-247-0924

FAX



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

Mail



VYDAC®
The Separations Group
17434 Mojave Street
Hesperia, California 92345

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.

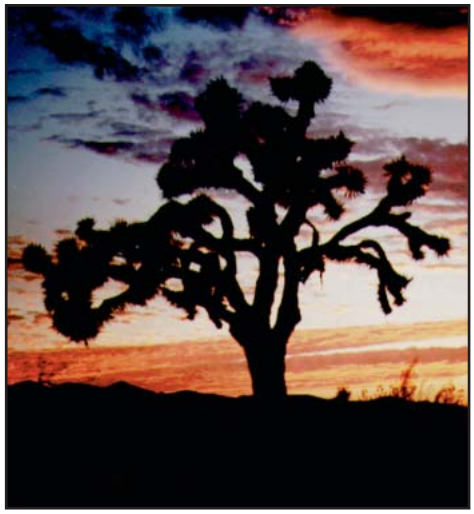
To locate the distributor for your area, visit Vydac on the World Wide Web at http://www.vydac.com

- or -

Contact Vydac directly.

Phone: USA 760-244-6107
FAX: USA 760-244-1984

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, which we obtained in 1997.

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.

VYDAC®

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Hesperia, CA 92345 USA

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Fax: (760) 244-1984
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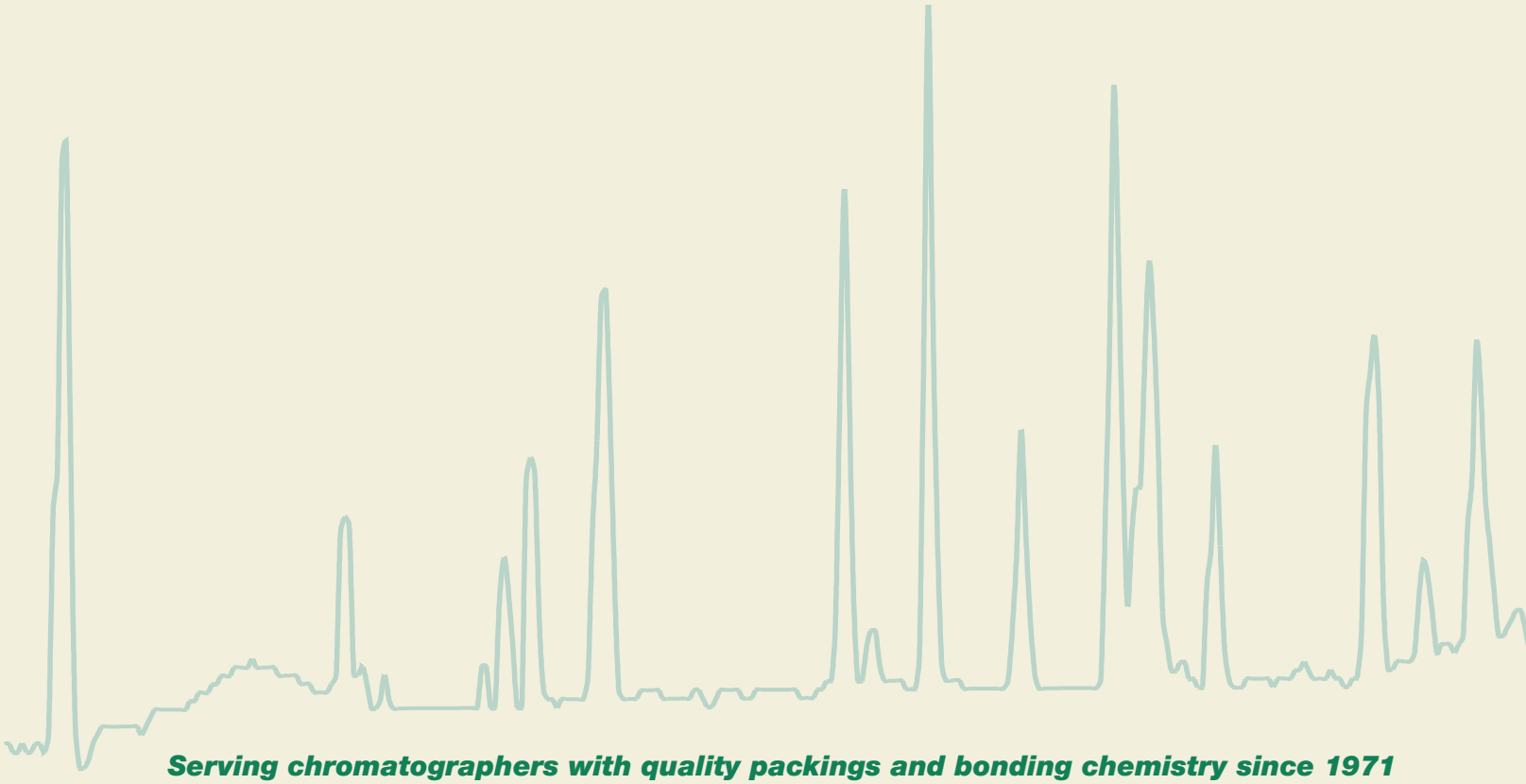
In USA, phone (800) 247-0924
or fax (888) 244-6610, toll-free.

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