

# Vydac® 201TP and 202TP C18

## Specialty Reversed Phases for PAH and Vitamins

- 201TP: The standard for PAH analysis in all types of environmental samples. Vydac® 201TP columns separate the EPA 16 priority pollutants in less than 20 minutes.
- 202TP3405: Rapid analysis to separate the 16 priority pollutant PAHs in under 10 minutes
- 202TP54 and 202TP5415: For the analysis of derivatized PAHs

VYDAC



7110

Vydac® 201TP and 202TP columns were developed specifically for the separation and quantification of PAHs required by environmental regulations, current and future. Beyond the 16 EPA priority pollutant PAHs, Vydac® PAH columns are used to separate many other PAHs, such as methylated naphthalenes.

### Vydac® 201TP and 202TP C18 Specifications

Phase	Base Material	Particle Shape	Particle Size	Pore Size	Surface Area	Carbon Load	Phase Type	Endcapped?	USP L-code
201TP C18	Silica	Spheroidal	5, 7, 10, 10–15, 15–20µm	300Å	70–90m²/g	8%	Polymeric	No	L1
202TP C18	Silica	Spheroidal	3, 5, 10µm	300Å	60–90m²/g	9%	Polymeric	No	L1

### Vydac® 201TP

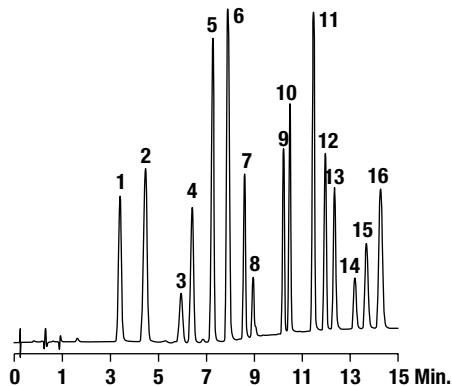
Vydac® 201TP HPLC columns have long been the standard for the analysis of PAHs in water, air, soil, automotive exhaust, and food. They were used to establish standard reference materials, measure air quality, measure PAHs in sediments, quantify PAHs in food, and study high molecular weight PAHs. They have also been used in the study of shape selectivity of reversed-phase materials.

#### Priority Pollutants PAHs

#### In Accordance with

#### EPA Methods 505, 550.1, 610, and 8310

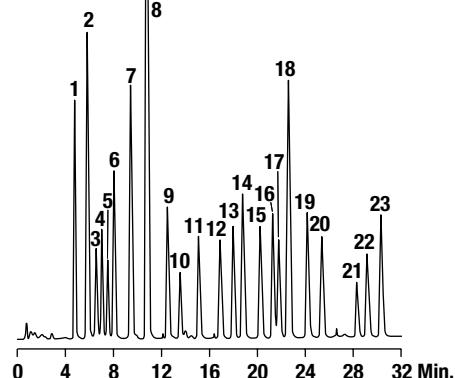
- |                   |                            |
|-------------------|----------------------------|
| 1. Naphthalene    | 9. Benz[a]anthracene       |
| 2. Acenaphthylene | 10. Chrysene               |
| 3. Acenaphthene   | 11. Benzo[b]fluoranthene   |
| 4. Fluorene       | 12. Benzo[k]fluoranthene   |
| 5. Phenanthrene   | 13. Benzo[a]pyrene         |
| 6. Anthracene     | 14. Dibenz[ah]anthracene   |
| 7. Fluoranthene   | 15. Benzo[ghi]perylene     |
| 8. Pyrene         | 16. Indeno[1,2,3-cd]pyrene |



**Column:** Vydac® C18, 5µm, 4.6 x 150mm  
(Part No. 201TP5415)  
**Flow Rate:** 1.5mL/min  
**Mobile Phase:** A: Water B: ACN  
**Gradient:** Hold 50% B for 3min, then 50 to 100% B in 7min  
**Detector:** UV at 254nm

#### PAHs Beyond the EPA Priority Pollutants

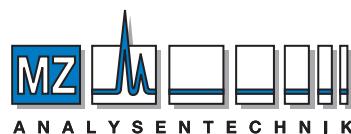
- |                          |                                    |
|--------------------------|------------------------------------|
| 1. Naphthalene           | 13. Benz[a]anthracene              |
| 2. Acenaphthylene        | 14. Chrysene                       |
| 3. 1-Methylnaphthalene   | 15. Benzo[b]naphtho[2,1-d]thiopen  |
| 4. 2-Methylnaphthalene   | 16. 7,12-Dimethylbenz[a]anthracene |
| 5. Acenaphthene          | 17. Benzo[e]pyrene                 |
| 6. Fluorene              | 18. Benzo[b]fluoranthene           |
| 7. Phenanthrene          | 19. Benzo[k]fluoranthene           |
| 8. Anthracene            | 20. Benzo[a]pyrene                 |
| 9. Fluoranthene          | 21. Dibenz[ah]anthracene           |
| 10. Pyrene               | 22. Benzo[ghi]perylene             |
| 11. Benzo[c]phenanthrene | 23. Indeno[1,2,3-cd]pyrene         |
| 12. Cyclopenta[cd]pyrene |                                    |



**Column:** Vydac® C18, 5µm, 4.6 x 150mm  
(Part No. 201TP5415)  
**Flow Rate:** 1.0mL/min  
**Mobile Phase:** A: Water B: ACN  
**Gradient:** 50 to 100% B over 30min  
**Column Temp:** 30°C  
**Detector:** UV at 254nm

### more applications

To view our complete searchable chromatogram database visit  
[www.discoverysciences.com/chromdb/](http://www.discoverysciences.com/chromdb/)



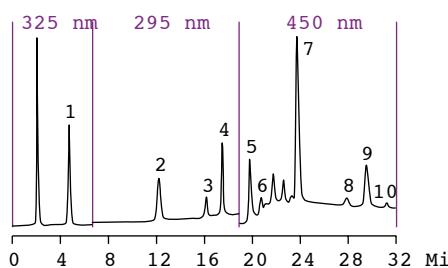
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**Vydac® 201TP (continued)****Vitamins**

Vydac® 201TP columns also have application in the analysis of carotenoids, retinoids, and vitamins.

**Retinol,  $\alpha$ -Tocopherol, and  $\beta$ -Carotene in Serum**

1. All-trans-retinol (vitamin A)
2. Tocol
3.  $\gamma$ -Tocopherol
4.  $\alpha$ -Tocopherol (vitamin E)
5. Lutein
6. Zeaxanthin
7. Cryptoxanthin
8.  $\alpha$ -Carotene
9. All-trans- $\beta$ -carotene
10. Cis- $\beta$ -carotene



**Column:** Vydac® 201TP C18, 5 $\mu$ m, 4.6 x 250mm  
(Part No. 201TP54)

**Flow Rate:** 1.5mL/min

**Mobile Phase:** A: 15:75:10 Buffer/Methanol/n-Butanol  
B: 2:88:10 Buffer/Methanol/n-Butanol  
Buffer = Aqueous 0.02 M NH<sub>4</sub>OAc, pH 3.5

**Gradient:** 100% A for 3min, then 0 to 100% B over 15min,  
then hold 100% B

**Detector:** UV, programmed wavelengths

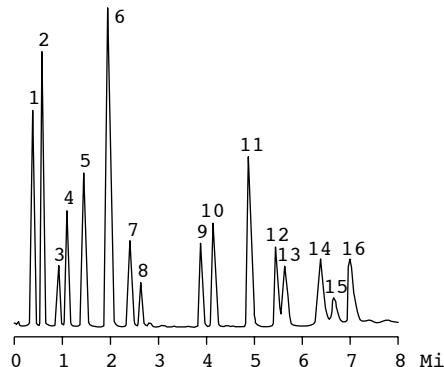
Chromatogram reproduced with author's permission. From W.A. MacCrehan and E. Schonberger, *Clin. Chem.*, 33(9), 1585-1592 (1987).

**Vydac® 202TP**

Built on top of the success of 201TP, 202TP columns are ideal for derivatized PAH samples or high throughput PAH analyses.

**Rapid Analysis of Polyaromatic Hydrocarbons**

1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[a]anthracene
10. Chrysene
11. Benz[b]fluoranthene
12. Benz[k]fluoranthene
13. Benz[a]pyrene
14. Benz[ghi]perylene
15. Dibenz[ah]anthracene
16. Indeno[1,2,3-cd]pyrene



**Column:** Vydac® 202TP C18, 5 $\mu$ m, 4.6 x 50mm  
(Part No. 202TP3405)

**Flow Rate:** 3.0mL/min

**Mobile Phase:** A: Water B: ACN

**Gradient:** 40 to 95% B in 8min

**Column Temp:** 30°C

**Detector:** UV at 254nm

**201TP C18 Analytical Columns**

Particle Size	Columns					Recommended Guards	
	i.d.	50mm	100mm	150mm	250mm	Guard Kit <sup>1</sup>	Guard Cartridge <sup>2</sup>
5 $\mu$ m	1.0mm	<b>201TP5105</b>	—	<b>201TP5115</b>	<b>201TP51</b>	<b>201GK51T</b>	<b>201GD51T</b>
	2.1mm	<b>201TP5205</b>	<b>201TP5210</b>	<b>201TP5215</b>	<b>201TP52</b>	<b>201GK52T</b>	<b>201GD52T</b>
	3.2mm	—	—	<b>201TP5315</b>	<b>201TP53</b>	<b>201GK54T</b>	<b>201GD54T</b>
	4.6mm	<b>201TP5405</b>	<b>201TP5410</b>	<b>201TP5415</b>	<b>201TP54</b>	<b>201GK54T</b>	<b>201GD54T</b>
10 $\mu$ m	4.6mm	—	—	<b>201TP10415</b>	<b>201TP104</b>	<b>201GK104T</b>	<b>201GD104T</b>

<sup>1</sup>A guard kit includes a holder and one guard cartridge. <sup>2</sup>Guard cartridge units include two guard cartridges.

**202TP High-Carbon-Load C18 Analytical Columns**

Particle Size	Columns					Recommended Guards	
	i.d.	50mm	100mm	150mm	250mm	Guard Kit <sup>1</sup>	Guard Cartridge <sup>2</sup>
3 $\mu$ m	3.2mm	—	—	<b>202TP3315</b>	—	—	—
5 $\mu$ m	4.6mm	—	—	<b>202TP5415</b>	<b>202TP54</b>	—	<b>202GD54T</b>

<sup>1</sup>A guard kit includes a holder and one guard cartridge. <sup>2</sup>Guard cartridge units include two guard cartridges.

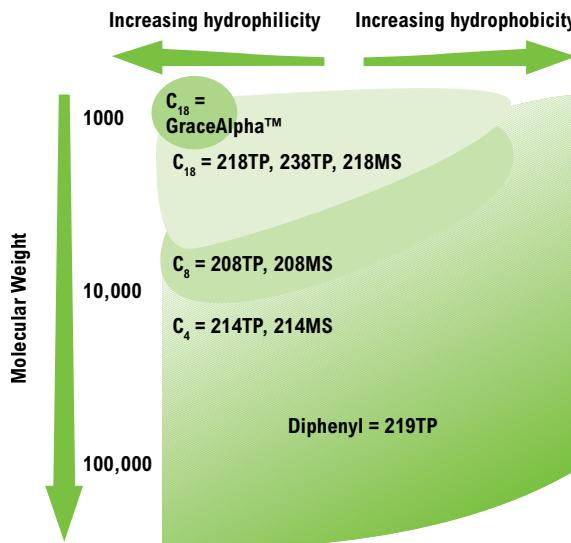
## Vydac® Large Molecule Columns

Vydac® has always been a trusted name in bioseparations, now, with technology acquired by Grace over the past few years, we have expanded this expertise further. New Grace® large molecule columns range from nano, capillary to micro, analytical to preparative columns.

Separate biomolecules from small peptides to large intact proteins with the Vyadac® family of products which includes reversed-phase, normal-phase, ion-exchange, and affinity phases. Our extensive applications library offers solid method development guidance, and our technical experts provide insight to even the most unusual separation challenges. Whether your primary analysis consideration is speed, MS compatibility, resolution, or recovery, we have a solution.

### Column Selection for Polypeptides

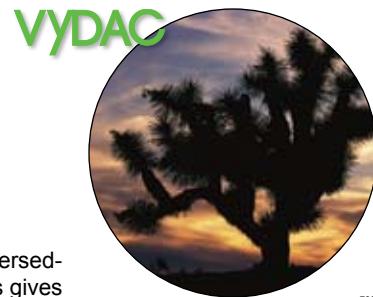
The reversed-phase column for a polypeptide separation should be selected based on the hydrophobicity of the polypeptide being chromatographed and molecular weight as a secondary consideration.



Phase	Simple Enzymatic Digests (<12 proteins)	Complex Enzymatic Digests (>12 proteins)	Biomolecules 0.5K MW	Biomolecules 5-10K MW	Biomolecules >10K MW	Un-denatured, Intact Proteins	Antibodies	Oligonucleotides	Oligosaccharides	Comments
218MS (C18) See page 85–87	●									Polymeric bonding highest hydrophobic interaction and unique geometric selectivity
238MS (C18) See page 85–87	●									Monomeric bonding offers increased peptide interaction and generally yields higher peak counts. Different selectivity compared to 218MS.
208MS (C8 See page 85–87			●			●				Lower hydrophobicity is better for larger molecules
214MS (C4) See page 85–87				●	●	●	●	●		Ideal for hydrophobic proteins, or when minimal organic is desired
219MS (Diphenyl) See page 85–87		●	●	●	●					Lowest capacity, highly selective for proteins with aromatic sidechains
Everest® C18 See page 88–89	●						●			Maximum surface coverage for highest resolution of complex samples
ProZap™ See page 93–94			●	●	●	●	●	●		Sub 2µm, in Expedite™ format optimized for fast analysis
218TP (C18) See page 90–92	●	●								First generation media with extensive applications library
214TP (C4) See page 90–92				●	●	●				First generation media with extensive applications library

### related products

Looking for large molecule prep columns?  
See pages 156–161.



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# Vydac® MS Introduction

## New Generation Columns with Unsurpassed Resolution, Sensitivity, and Recovery

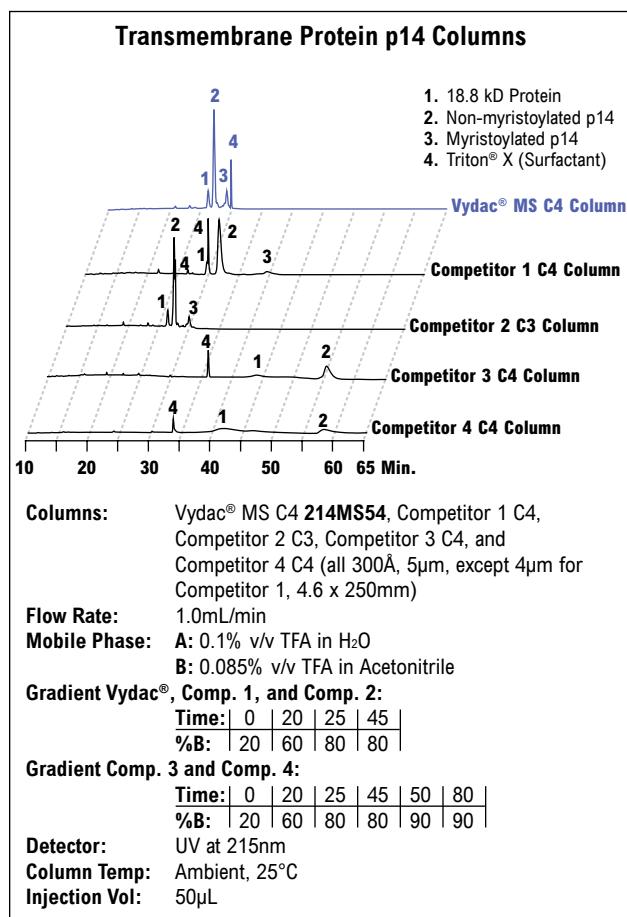
- Unique selectivity reveals peaks otherwise masked by other C18 columns
- Excellent peak shape with little or no TFA
- High protein recoveries make scale-up easy

Vydac® MS columns are the latest development in the ongoing effort to provide the best reversed-phase HPLC columns for biomolecule. A surface treatment and proprietary bonding process gives Vydar® MS columns a unique selective not found anywhere else. A variety of reversed phases makes this product line suitable for small peptides to large intact, undenatured proteins.

MS Specifications										
Phase	Base Material	Particle Shape	Particle Size	Pore Size	Surface Area	Carbon Load	Phase Type	Endcapped?	USP L-code	
208MS C8	Silica	Spheroidal	5µm	300Å	70m²/g	5%	Polymeric	Yes	L7	
214MS C4	Silica	Spheroidal	5µm	300Å	70–110m²/g	3%	Polymeric	Yes	L26	
218MS C18	Silica	Spheroidal	3, 5, 10, 10–15µm	300Å	60–110m²/g	8%	Polymeric	Yes	L1	
238MS C18	Silica	Spheroidal	5µm	300Å	70m²/g	4%	Monomeric	Yes	L1	
219MS Di-Phe	Silica	Spheroidal	5µm	300Å	70m²/g	4%	Polymeric	Yes	—	

## Unique Selectivity

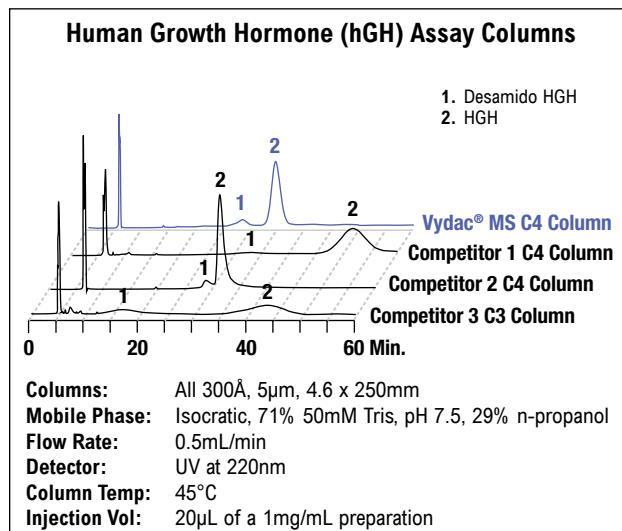
A sample of bovine fetuin, a 36kD glycoprotein, was digested with trypsin. Some of the sample components interfere with the peptide separation on the Competitor 1 and Competitor 2 columns and appear as a chromatographic "hump" with peaks riding on top. The unique selectivity of Vydar® MS columns solves these separation problems



Reptilian reovirus RRV p14 sample courtesy of Drs. Roberto J. de Antuono and Roy Duncan, Dalhousie University, Halifax, Nova Scotia.

## Unsurpassed Resolution and Peak Symmetry

Vydac® MS C4 columns provide the overall best performance for hGH and desamido hGH analysis. Competitive columns show significant, undesirable interaction of hGH with the stationary phase.



## more info

For additional protein and peptide applications, see application section pages 429–441.

## more applications

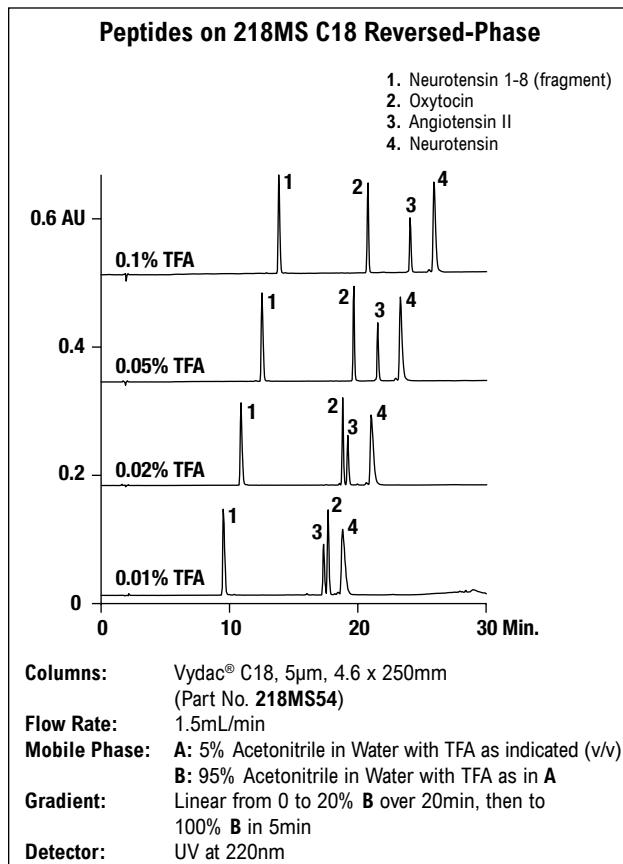
To view our complete searchable chromatogram database visit [www.discoverysciences.com/chromdb/](http://www.discoverysciences.com/chromdb/)



## Vydac® MS Columns

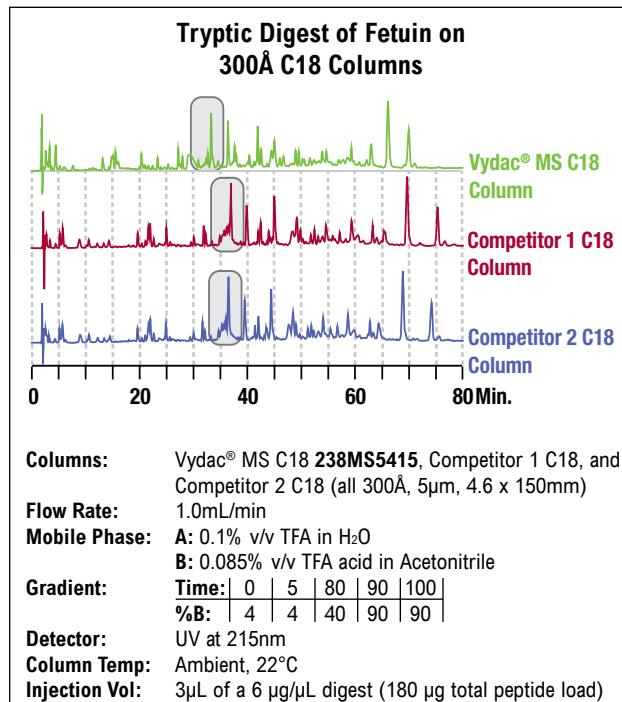
### Excellent Peak Shape with Little or No TFA

It is common practice for protein and peptide separations to include an acidic modifier such as TFA in the mobile phase. TFA masks basic entities, reducing mixed-mode retention, and improving peak symmetry. TFA also changes retention and selectivity for different analytes, and its concentration can be adjusted to optimize a separation. Unfortunately TFA is UV absorbent and contributes background at low UV wavelengths. Also it is especially problematic with electrospray MS where it interferes with ion generation, called "quenching", and reduces MS sensitivity.



### Hydrophobic Proteins

Transmembrane proteins are hydrophobic proteins which bind to cell membranes and are particularly difficult to separate. Vydac® MS columns provide excellent selectivity and peak shape for these molecules. In this case, a hydrophobic transmembrane protein was separated from a synthetic myristoylated derivative and other cellular components.

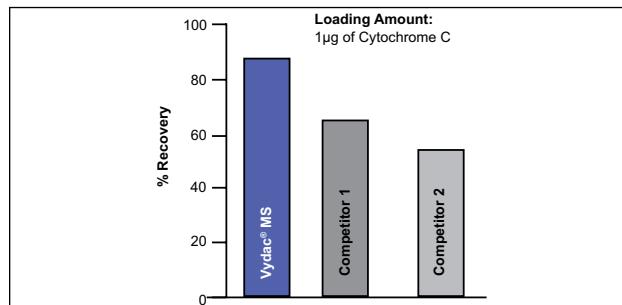


The Vydac® column provides better separation and recovery for a highly hydrophobic membrane protein (RRV p14) and its fatty acid modified (myristoylated) form, a component of a potentially new vaccine delivery system.

### High Protein Recovery for Higher Sensitive, Ideal for Preparative Chromatography

Surface chemistry reduces adsorption of proteins for higher recoveries and also increases mass loading.

### % Recovery at Low Protein Load: Vydac® MS C4 vs. 2 Competitors



Vydac® MS C4 column provides more than 20% more loading of Cytochrome C.



### more applications

To view our complete searchable chromatogram database visit [www.discoverysciences.com/chromdb/](http://www.discoverysciences.com/chromdb/)



# Vydac® MS Columns

## Vydac® MS Analytical Columns

Particle Size	i.d.	Columns					Recommended Guards	
		50mm	100mm	150mm	250mm	Guard Kit <sup>1</sup>	Guard Cartridge <sup>2</sup>	
<b>214 MS C4</b>								
5μm	1.0mm	214MS5105	214MS5110	214MS5115	214MS51	214GK51MS	214GD51MS	
	2.1mm	214MS5205	214MS5210	214MS5215	214MS52	214GK52MS	214GD52MS	
	3.2mm	—	—	—	214MS53	214GK54MS	214GD54MS	
	4.6mm	214MS5405	214MS5410	214MS5415	214MS54	214GK54MS	214GD54MS	
<b>208 MS C8</b>								
5μm	1.0mm	208MS5105	208MS5110	208MS5115	208MS51	208GK51MS	208GD51MS	
	2.1mm	208MS5205	208MS5210	208MS5215	208MS52	208GK52MS	208GD52MS	
	3.2mm	—	—	—	208MS53	208GK54MS	208GD54MS	
	4.6 mm	208MS5405	208MS5410	208MS5415	208MS54	208GK54MS	208GD54MS	
<b>218 MS Polymeric C18</b>								
5μm	1.0mm	218MS5105	218MS5110	218MS5115	218MS51	218GK51MS	218GD51MS	
	2.1mm	218MS5205	218MS5210	218MS5215	218MS52	218GK52MS	218GD52MS	
	3.2mm	—	—	—	218MS53	218GK54MS	218GD54MS	
	4.6mm	218MS5405	218MS5410	218MS5415	218MS54	218GK54MS	218GD54MS	
<b>238 MS Monomeric C18</b>								
5μm	1.0mm	238MS5105	238MS5110	238MS5115	238MS51	238GK51MS	—	
	2.1mm	238MS5205	238MS5210	238MS5215	238MS52	238GK52MS	238GD52MS	
	3.2mm	—	—	—	238MS53	238GK54MS	238GD54MS	
	4.6mm	238MS5405	238MS5410	238MS5415	238MS54	238GK54MS	238GD54MS	
<b>219 MS Diphenyl</b>								
5μm	2.1mm	—	219MS5210	—	219MS52	219GK52MS	—	
	3.2mm	—	—	—	219MS53	219GK54MS	219GD54MS	
	4.6mm	219MS5405	219MS5410	219MS5415	219MS54	219GK54MS	219GD54MS	

<sup>1</sup>A guard kit includes a holder and one guard cartridge; <sup>2</sup>Guard cartridge units include two guard cartridges.

## Vydac® MS Nano and Capillary Columns

	i.d.	50mm	100mm	150mm	250mm
<b>214 MS C4</b>					
5μm	75μm	214MS5.07505	214MS5.07510	214MS5.07515	214MS5.07525
	150μm	214MS5.1505	214MS5.1510	214MS5.1515	214MS5.1525
	300μm	214MS5.305	214MS5.310	214MS5.315	214MS5.325
	500μm	214MS5.505	214MS5.510	214MS5.515	214MS5.525
<b>208 MS C8</b>					
5μm	75μm	208MS5.07505	208MS5.07510	208MS5.07515	208MS5.07525
	150μm	208MS5.1505	208MS5.1510	208MS5.1515	208MS5.1525
	300μm	208MS5.305	208MS5.310	208MS5.315	208MS5.325
	500μm	208MS5.505	208MS5.510	208MS5.515	208MS5.525
<b>218 MS Polymeric C18</b>					
3μm	75μm	218MS3.07505	218MS3.07510	218MS3.07515	218MS3.07525
	150μm	218MS3.1505	218MS3.1510	218MS3.1515	218MS3.1525
	300μm	218MS3.305	218MS3.310	218MS3.315	218MS3.325
	500μm	218MS3.505	218MS3.510	218MS3.515	218MS3.525
5μm	75μm	218MS5.07505	218MS5.07510	218MS5.07515	218MS5.07525
	150μm	218MS5.1505	218MS5.1510	218MS5.1515	218MS5.1525
	300μm	218MS5.305	218MS5.310	218MS5.315	218MS5.325
	500μm	218MS5.505	218MS5.510	218MS5.515	218MS5.525
<b>238 MS Monomeric C18</b>					
5μm	75μm	238MS5.07505	238MS5.07510	238MS5.07515	238MS5.07525
	150μm	238MS5.1505	238MS5.1510	238MS5.1515	238MS5.1525
	300μm	238MS5.305	238MS5.310	238MS5.315	238MS5.325
	500μm	238MS5.505	238MS5.510	238MS5.515	238MS5.525

## Vydac® MS Guard Cartridges

Packing	i.d. x Length	Qty.	Part No.
C18 Polymeric, 5μm*			
Capillary Guard**	1.0 x 10mm	2	214GD51MS
C4, 5μm* Capillary Guard**	0.150 x 10mm	ea	214MS5C0115
	1.0 x 10mm	2	214GD51MS

\*All-Guard™ holder required. Other particle sizes available.  
\*\*1.5μm and 5μm particles and other dimensions are available.

## Vydac® MS Guard Cartridges (continued)

Packing	Qty.	Part No.
Capillary Guard Cartridge Holder		
Guard Holder for 0.100mm and 0.150mm Guards	ea	GR-3710E
Guard Holder for 0.300mm and 0.500mm Guards	ea	GR-3710A
Guard Holder for 1mm Guards	ea	GCH1

# Vydac® Everest® Columns Introduction

## High Resolution for Complex Peptide Samples

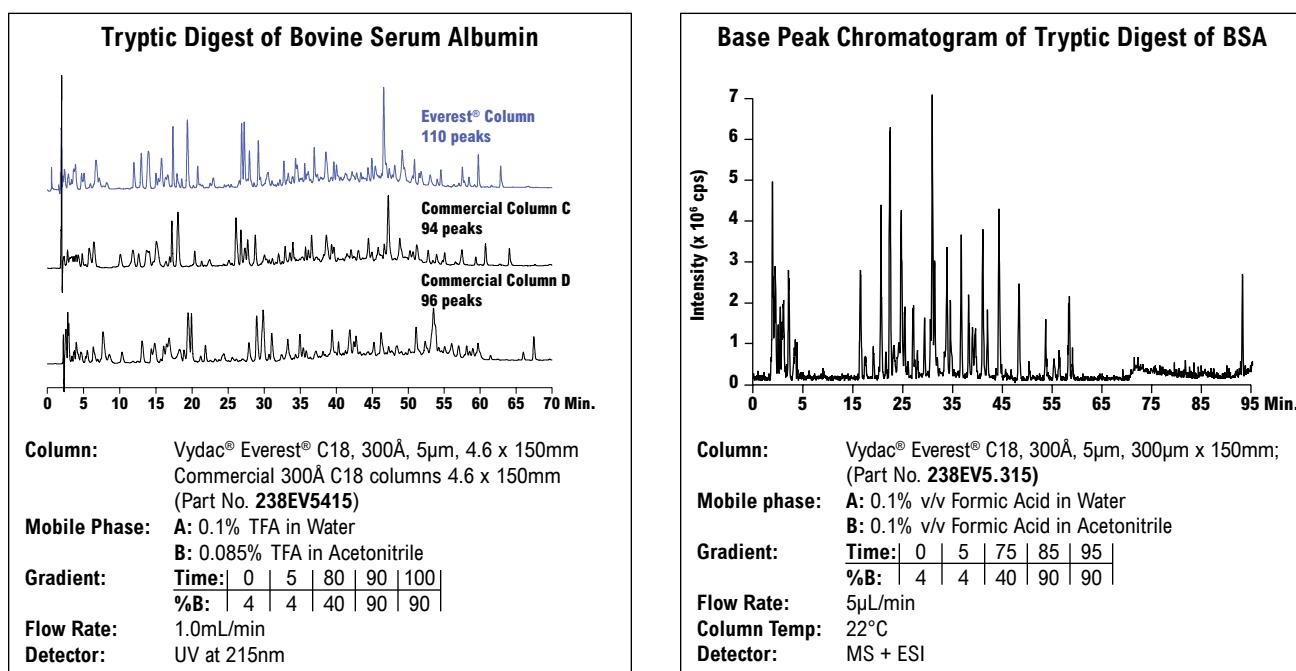
- High resolution of complex peptide digests
- Unique selectivity for both hydrophilic and hydrophobic peptides
- Exceptional microbore sensitivity with little or no TFA in the mobile phase

Everest® columns provide exceptionally high resolution for complex peptide digest separations. Unique selectivity and sensitivity are the result of new bonding technology that improves C18 surface coverage and deactivates residual silanols. Previously, the best 300Å C18 chemistries have had carbon coverage in the 2.8 to 3.6µmol/m<sup>2</sup> range. Everest® C18 coverage is in excess of 4µmol/m<sup>2</sup> and approximates the theoretical limit based on surface area. This increased shielding of the base silica increases column life and reduces the amount of TFA required to shield the silica.



7189

Everest® Specifications									
Phase	Base Material	Particle Shape	Particle Size	Pore Size	Surface Area	Carbon Load	Phase Type	Endcapped?	USP L-code
238EV C18	Silica	Spheroidal	5, 10, 10–15, 15–20µm	300Å	70–110m <sup>2</sup> /g	6%	Monomeric	Yes	L1



Everest® columns outperform competitor columns at higher peptide loads, by providing higher-resolution separations (average of 17% higher peak counts than competitor columns tested).

The Everest® column performs exceptionally well with no TFA in the mobile phase, ensuring excellent microbore sensitivity.

### Everest® 300Å C18

Particle Size	i.d.	Columns					Recommended Guards	
		50mm	100mm	150mm	250mm	Guard Kit <sup>1</sup>	Guard Cartridge <sup>2</sup>	
<b>Everest® Analytical Columns 238EV C18</b>								
5µm	1.0mm <sup>3</sup>	<b>238EV5105</b>	—	<b>238EV5115</b>	<b>238EV51</b>	—	—	
	2.1mm <sup>3</sup>	<b>238EV5205</b>	<b>238EV5210</b>	<b>238EV5215</b>	<b>238EV52</b>	—	<b>238GD52EV</b>	
	4.6mm <sup>3</sup>	<b>238EV5405</b>	—	<b>238EV5415</b>	<b>238EV54</b>	<b>238GK54EV</b>	<b>238GD54EV</b>	
<b>Everest® Capillary Columns 238ZU C18</b>								
5µm	75µm	<b>238EV5.07505</b>	<b>238EV5.07510</b>	<b>238EV5.07515</b>	—	—	—	
	150µm	—	<b>238EV5.1510</b>	<b>238EV5.1515</b>	<b>238EV5.1525</b>	—	—	
	300µm	<b>238EV5.305</b>	<b>238EV5.310</b>	<b>238EV5.315</b>	<b>238EV5.325</b>	—	—	
	500µm	<b>238EV5.505</b>	—	<b>238EV5.515</b>	—	—	—	

<sup>1</sup>A guard kit includes a holder and one guard cartridge. <sup>2</sup>Guard cartridge units include two guard cartridges. <sup>3</sup>Titanium frits are standard in column diameters 4.6mm and smaller.

### Everest® Guard Cartridges

Packing	i.d. x Length	Qty.	Part No.
C18, 5µm* Capillary Guard**	0.300 x 10mm	ea	<b>238EV5C0130</b>
	1.0 x 10mm	2	<b>238GD51EV</b>

\*All-Guard™ holder required. Other particle sizes available.

\*\*1.5µm and 5µm particles and other dimensions are available.

### Everest® Guard Cartridges (continued)

Packing	Qty.	Part No.
Capillary Guard Cartridge Holder		
Guard Holder for 0.100mm and 0.150mm Guards	ea	<b>GR-3710E</b>
Guard Holder for 0.300mm and 0.500mm Guards	ea	<b>GR-3710A</b>
Guard Holder for 1mm Guards	ea	<b>GCH1</b>



# Vydac® Everest® Columns

## Tryptic Digest of Cytochrome C

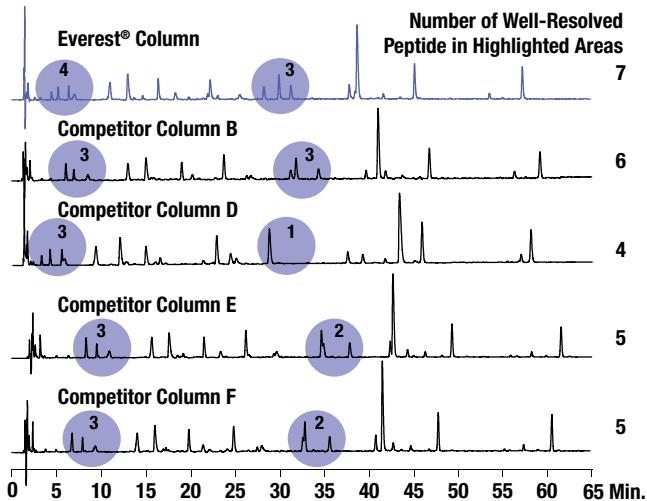
**Columns:** Everest® 238EV5415 and 4 competitor 300Å reversed-phase columns, all C18, 5µm, 4.6 x 150mm

**Flow Rate:** 1.0 mL/min

**Mobile Phase:** A: 0.1% v/v TFA in water  
B: 0.085% v/v TFA in ACN

**Gradient:** Time: 0 | 75 | 85 | 95  
%B: 4 | 40 | 90 | 90  
hold 90% B for 10 min

**Detector:** UV at 215nm



For a tryptic digest of Cytochrome C, an Everest® column offers unique selectivity which allows the best separation of a group of hydrophilic and hydrophobic peptides. To assess resolution, chromatograms of tryptic digests of several proteins on an Everest® column and several other commercial 300Å C18 reversed-phase columns were compared. Peak counts were based on detection by automated chromatography software with parameters set identically for all columns. In the analyses of a tryptic digest of cytochrome c, specific regions of the chromatogram were examined for the number of peaks resolved. The Everest® column demonstrated unique selectivity for both hydrophilic and hydrophobic peptides.

## Tryptic Digest of Fetuin (a Glycoprotein)

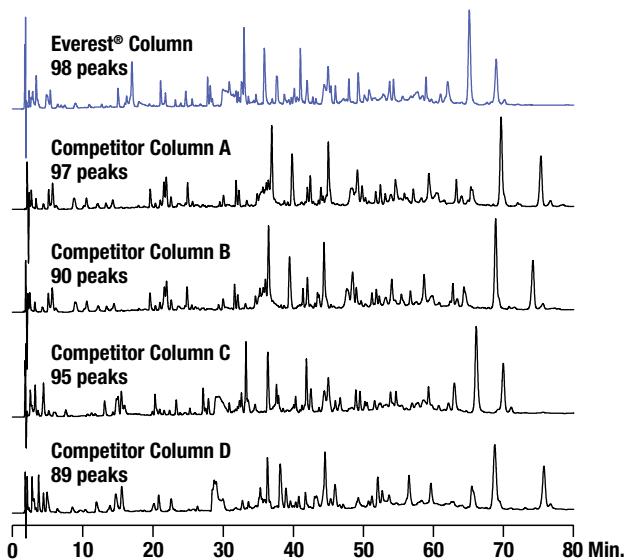
**Columns:** Everest® 238EV5415 and 4 competitor 300Å reversed-phase columns, all C18, 5µm, 4.6 x 150mm

**Flow Rate:** 1.0 mL/min

**Mobile Phase:** A: 0.1% v/v TFA in water  
B: 0.085% v/v TFA in ACN

**Gradient:** Time: 0 | 5 | 80 | 90 | 100  
%B: 4 | 4 | 40 | 90 | 90

**Detector:** UV at 215nm



A tryptic digest of fetuin injected at high load on an Everest® column provided higher peak counts compared to four commercial columns. Peak numbers shown are the average of three separations on each column.

## more applications

To view our complete searchable chromatogram database visit [www.discoverysciences.com/chromdb/](http://www.discoverysciences.com/chromdb/)



## Vydac® TP Columns

### Industry Standard for Polypeptide Separations

- Referred in over 9000 patents, Vydac® 300Å TP is the industry-standard, for peptide, protein, and large molecule separations
- Polymeric bonded phases have exceptionally long column lifetime and negligible phase leaching
- Extensive applications library based on over two decade's experience

VYDAC



7110

Vydac® TP reversed-phase material consists of aliphatic groups bonded to the surface of 300Å pore diameter silica. The large pores of the 300Å TP silica give polypeptide molecules complete access to the interior of the silica pores. The unique process by which we manufacture Vydac® TP silica results in high-purity, synthetic silica with carefully controlled characteristics. Vydac® TP silica is the standard that has defined large pore HPLC silica for polypeptide separations for nearly two decades.

Vydac® TP Columns										
Phase	Base Material	Particle Shape	Particle Size	Pore Size	Surface Area	Carbon Load	Phase Type	Endcapped?	USP L-code	
101TP Sil	Silica	Spheroidal	5, 10, 10–15, 15–20µm	300Å	70–110m²/g	—	unbonded	—	L3	
201TP C18	Silica	Spheroidal	5, 7, 10, 10–15, 15–20µm	300Å	70–90m²/g	8%	Polymeric	No	L1	
202TP C18	Silica	Spheroidal	3, 5, 10µm	300Å	60–90m²/g	9%	Polymeric	No	L1	
208TP C8	Silica	Spheroidal	3, 5, 7, 10, 10–15, 15–20µm	300Å	60–110m²/g	5%	Polymeric	Yes	L7	
214TP C4	Silica	Spheroidal	3, 5, 7, 10, 10–15, 15–20µm	300Å	60–110m²/g	3%	Polymeric	Yes	L26	
218TP C18	Silica	Spheroidal	3, 5, 7, 10, 10–15, 15–20µm	300Å	60–110m²/g	8%	Polymeric	Yes	L1	
219TP Di-Phe	Silica	Spheroidal	3, 5, 7, 10, 10–15, 15–20µm	300Å	60–110m²/g	4%	Polymeric	Yes	—	
238TP C18	Silica	Spheroidal	3, 5, 7, 10, 10–15, 15–20µm	300Å	60–110m²/g	4%	Monomeric	Yes	L1	

### Vydac® 218TP C18 Columns

Vydac® 218TP is a polymerically bonded endcapped n-octadecyl reversed-phase based on 300Å TP silica.

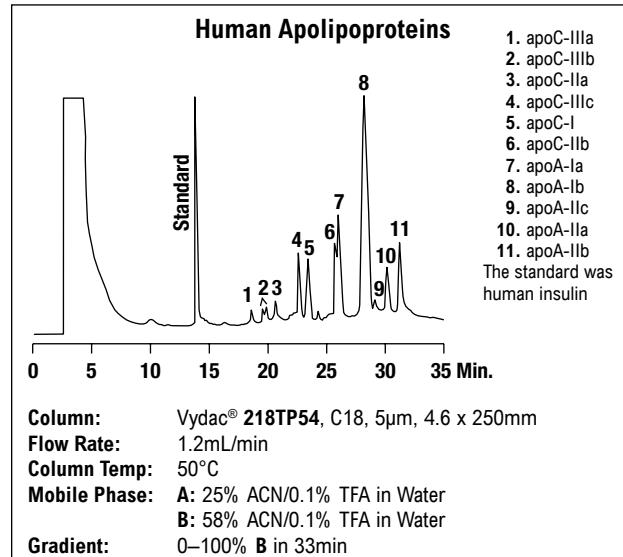
#### Applications

Vydac® 218TP reversed-phase columns are recommended for the separation of:

- Small polypeptides less than 4000–5000 MW
- Enzymatic digest fragments
- Natural and synthetic peptides
- Multi-ring compounds

Specific examples include:

- Tryptic digests
- S. aureus V8 digests
- Synthetic peptides
- Natural peptides
- Peptide studies



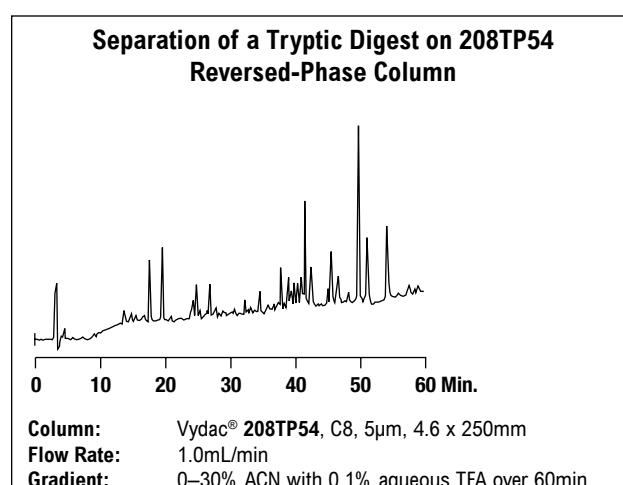
### Vydac® 208TP C8 Reversed-Phase

Vydac® 208TP is a polymerically bonded endcapped n-octyl reversed-phase based on 300Å TP silica.

#### Applications

Vydac® 208TP reversed-phase columns are recommended for the separation of:

- Polypeptides up to 10,000–20,000 MW
- Enzymatic digest fragments
- Natural and synthetic peptides



## Vydac® 214TP C4

Vydac® 214TP is a polymerically bonded endcapped n-butyl reversed-phase based on 300Å TP silica. 214ATP is a less extensively endcapped C4 that has been found more suitable for resolution of degradation products in analysis of biosynthetic human growth hormone.

### Applications

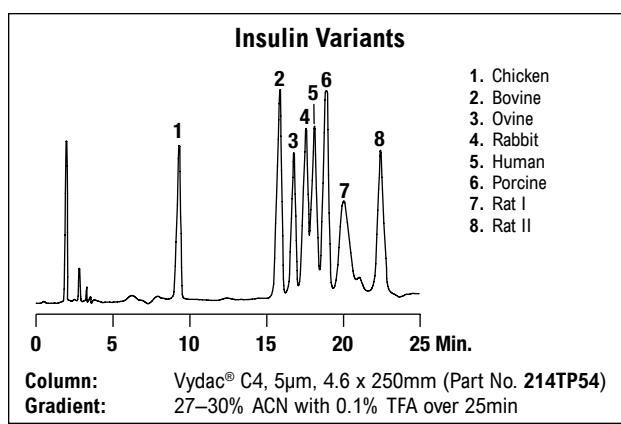
- Glycoproteins
- Hemoglobin variants
- Histones
- Human growth hormone
- Insulin variants
- Membrane proteins

## Vydac® 219TP Diphenyl Reversed-Phase

Vydac® 219TP is a polymerically bonded endcapped diphenyl reversed-phase based on 300Å TP silica. It combines moderate retentivity with unique selectivity.

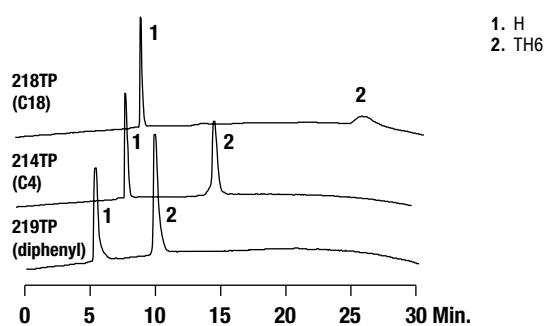
### Applications

- Polypeptides with aromatic side chains
- Large, hydrophobic proteins
- Membrane-spanning peptides
- Lipid peptides
- Fusion proteins from inclusion bodies



From J. Rivier and R. McClintock, *J. Chrom.* 268, 112-119 (1983).

## Comparison of Polypeptide Separations on C18, C4, and Diphenyl Reversed-Phase Columns



Columns: Vydar® 218TP5415 (C18), 214TP5415 (C4) and 219TP5415 (diphenyl), all 5µm, 4.6 x 150mm  
Gradient: 10–90% ACN with 0.1% TFA over 30min  
Sample: 18-residue helical peptide (1) and six-helix template assembled synthetic protein (2)

## Vydac® 238TP C18 Reversed-Phase

Vydac® 238TP is a monomerically bonded endcapped n-octadecyl reversed-phase based on 300Å TP silica.

### Applications

Monomerically bonded C18 provides an alternative to 218TP polymeric C18 with subtle differences in selectivity. The combination of these adsorbents can reveal analytes that may be hidden on a single C18 column.

### more info

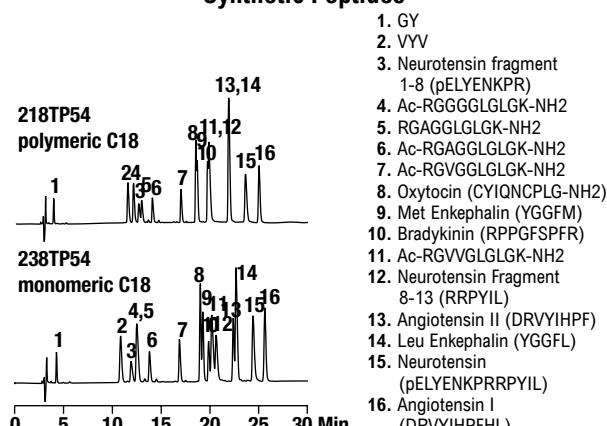
For more protein and peptide applications, see the application section pages 429–441.

### more applications

To view our complete searchable chromatogram database visit [www.discoverysciences.com/chromdb/](http://www.discoverysciences.com/chromdb/)



## Synthetic Peptides



Columns: Vydar® 218TP54 and 238TP54, both C18, 5µm, 4.6 x 250mm  
Flow Rate: 1.0mL/min  
Gradient: 10–40% ACN with 0.1% TFA (w/v) over 30min  
Detector: UV at 220nm

## Vydac® TP Columns

### Vydac® TP Analytical Columns

Particle Size	i.d.	Columns					Recommended Guards	
		50mm	100mm	150mm	250mm	Guard Kit <sup>1</sup>	Guard Cartridge <sup>2</sup>	
<b>218TP C18</b>	3 $\mu$ m	4.6mm	<b>218TP3405</b>	<b>218TP3410</b>	—	—	<b>218GK34</b>	<b>218GD34</b>
	5 $\mu$ m	1.0mm	<b>218TP5105</b>	<b>218TP5110</b>	<b>218TP5115</b>	<b>218TP51</b>	—	—
		2.1mm	<b>218TP5205</b>	<b>218TP5210</b>	<b>218TP5215</b>	<b>218TP52</b>	<b>218GK52</b>	<b>218GD52</b>
		3.2mm	<b>218TP5305</b>	<b>218TP5310</b>	<b>218TP5315</b>	<b>218TP53</b>	<b>218GK54</b>	<b>218GD54</b>
		4.6mm	<b>218TP5405</b>	<b>218TP5410</b>	<b>218TP5415</b>	<b>218TP54</b>	<b>218GK54</b>	<b>218GD54</b>
<b>208TP C8</b>	3 $\mu$ m	4.6mm	<b>208TP3405</b>	<b>208TP3410</b>	—	—	<b>208GK34</b>	<b>208GD34</b>
	5 $\mu$ m	1.0mm	<b>208TP5105</b>	<b>208TP5110</b>	<b>208TP5115</b>	<b>208TP51</b>	<b>208GK51</b>	<b>208GD51</b>
		2.1mm	<b>208TP5205</b>	<b>208TP5210</b>	<b>208TP5215</b>	<b>208TP52</b>	<b>208GK52</b>	<b>208GD52</b>
		3.2mm	<b>208TP5305</b>	<b>208TP5310</b>	<b>208TP5315</b>	<b>208TP53</b>	<b>208GK54</b>	<b>208GD54</b>
		4.6mm	<b>208TP5405</b>	<b>208TP5410</b>	<b>208TP5415</b>	<b>208TP54</b>	<b>208GK54</b>	<b>208GD54</b>
<b>214TP C4</b>	3 $\mu$ m	4.6mm	<b>214TP3405</b>	<b>214TP3410</b>	—	—	<b>214GK34</b>	<b>214GD34</b>
	5 $\mu$ m	1.0mm	<b>214TP5105</b>	<b>214TP5110</b>	<b>214TP5115</b>	<b>214TP51</b>	<b>214GK51</b>	—
		2.1mm	<b>214TP5205</b>	<b>214TP5210</b>	<b>214TP5215</b>	<b>214TP52</b>	<b>214GK52</b>	<b>214GD52</b>
		3.2mm	<b>214TP5305</b>	<b>214TP5310</b>	<b>214TP5315</b>	<b>214TP53</b>	<b>214GK54</b>	<b>214GD54</b>
		4.6mm	<b>214TP5405</b>	<b>214TP5410</b>	<b>214TP5415</b>	<b>214TP54</b>	<b>214GK54</b>	<b>214GD54</b>
<b>214ATP C4 Columns</b>	5 $\mu$ m	2.1mm	—	—	—	<b>214ATP52</b>	—	—
		4.6mm	—	—	—	<b>214ATP54</b>	—	—
<b>219TP Diphenyl</b>	5 $\mu$ m	1.0mm	<b>219TP5105</b>	<b>219TP5110</b>	<b>219TP5115</b>	<b>219TP51</b>	—	—
		2.1mm	<b>219TP5205</b>	<b>219TP5210</b>	<b>219TP5215</b>	<b>219TP52</b>	<b>219GK52</b>	<b>219GD52</b>
		3.2mm	<b>219TP5305</b>	<b>219TP5310</b>	<b>219TP5315</b>	<b>219TP53</b>	<b>219GK54</b>	<b>219GD54</b>
		4.6mm	<b>219TP5405</b>	<b>219TP5410</b>	<b>219TP5415</b>	<b>219TP54</b>	<b>219GK54</b>	<b>219GD54</b>
	—					—		
<b>238TP C18</b>	3 $\mu$ m	4.6mm	<b>238TP3405</b>	<b>238TP3410</b>	—	—	<b>238GK34</b>	<b>238GD34</b>
	5 $\mu$ m	1.0mm	<b>238TP5105</b>	<b>238TP5110</b>	<b>238TP5115</b>	<b>238TP51</b>	<b>238GK51</b>	<b>238GD51</b>
		2.1mm	<b>238TP5205</b>	<b>238TP5210</b>	<b>238TP5215</b>	<b>238TP52</b>	<b>238GK52</b>	<b>238GD52</b>
		3.2mm	<b>238TP5305</b>	<b>238TP5310</b>	<b>238TP5315</b>	<b>238TP53</b>	<b>238GK54</b>	<b>238GD54</b>
		4.6mm	<b>238TP5405</b>	<b>238TP5410</b>	<b>238TP5415</b>	<b>238TP54</b>	<b>238GK54</b>	<b>238GD54</b>

NOTE: Additional column diameters and lengths are available on request. Please contact Grace Davison Discovery Sciences to discuss your requirements.  
<sup>1</sup>A guard kit includes a holder and one guard cartridge; <sup>2</sup>Guard cartridge units include two guard cartridges.

### related products

For prep Vydac® TP Columns, see our prep section  
pages 158–160.



### related products

Looking for HPLC column prefilters? See page 111.



### related products

Need high-pressure polymeric fittings?  
See pages 112–114  
for our full selection of  
high-pressure fittings.

6673

### technical assistance

Contact Tech Support: Phone: 1.800.255.8324 (North America)  
Email: [contact.alltech@grace.com](mailto:contact.alltech@grace.com)  
Online: [www.discoverysciences.com](http://www.discoverysciences.com)

### related products

Need HPLC tubing?  
See pages 384–391.



5535

# Vydac® ProZap™ C18

## Ultra Fast Protein and Peptide Separations

- 10 times faster bioseparations than traditional column formats
- Ultra-high efficiency 1.5μm packing
- Fast protein and peptide separations with conventional HPLC systems

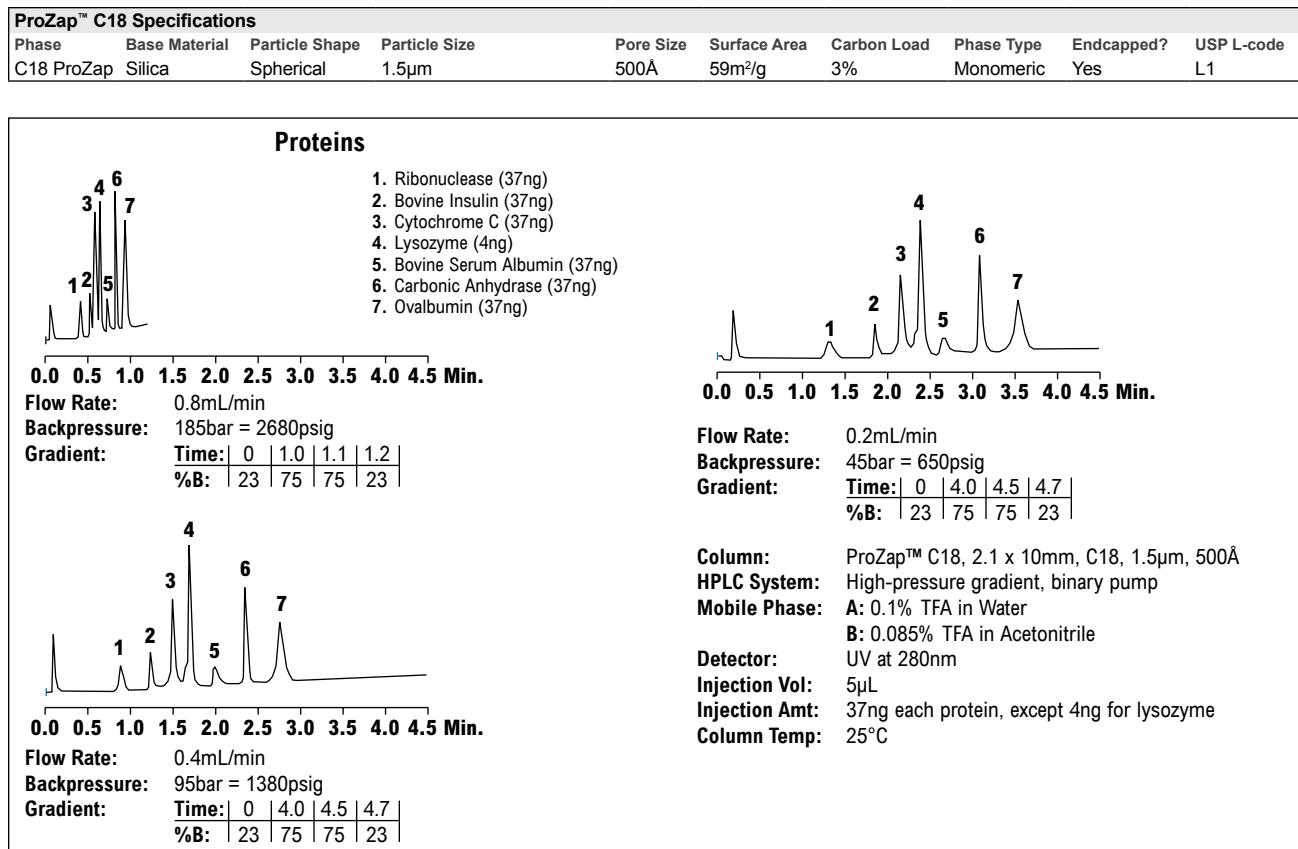
ProZap™ 1.5μm, 500Å packings are ideal for fast bioseparations in life science applications. The combination of ProZap™ packings and short, 10mm Expedite™ column hardware delivers not only faster sample throughput, but also low back pressures suitable for conventional LC systems.



7279

## Optimization of Gradient Parameters for Fast Protein Analysis

Short ProZap™ columns are the perfect tool for fast reversed-phase protein separations. Under gradient conditions, longer columns only increase run time and do not increase resolution to improve the separation. Proteins adsorb at the head of the column and then desorb once the critical mobile phase concentration is reached. Since the proteins do not interact with the full length of the packed bed, short columns are sufficient for full resolution. Therefore, proteins of broad molecular weight ranges can be separated in less than one minute by combining short columns, higher flow rates, and fast, modified gradients. For best results high-pressure mixing should be used with fast gradients.



Seven proteins were tested on a 10mm ProZap™ column. By increasing flow rate and reducing gradient time, total run time is reduced from 4.5 minutes to 1.2 minutes.

### more info

For more information about the Expedite™ hardware format, see page 31.



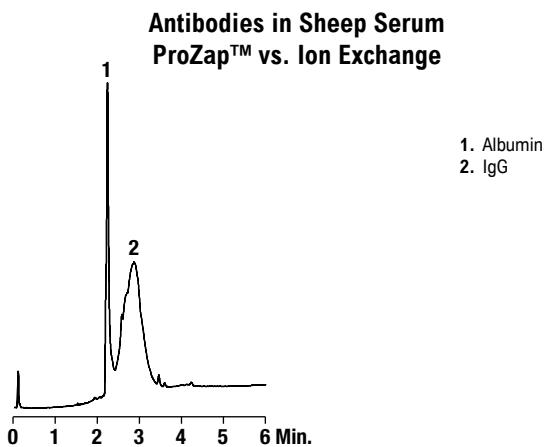
### more applications

To view our complete searchable chromatogram database visit [www.discoverysciences.com/chromdb/](http://www.discoverysciences.com/chromdb/)

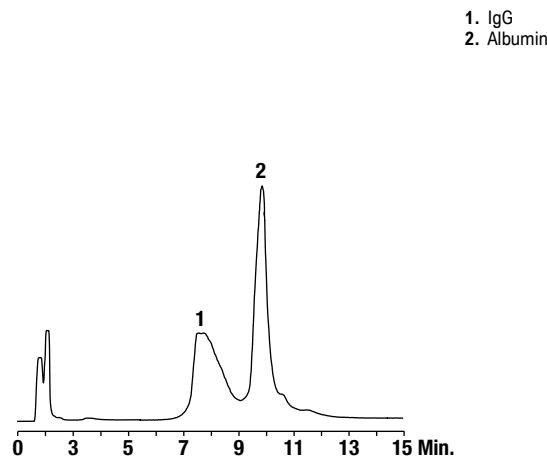


## analytical hplc

## Vydac® ProZap™ Columns

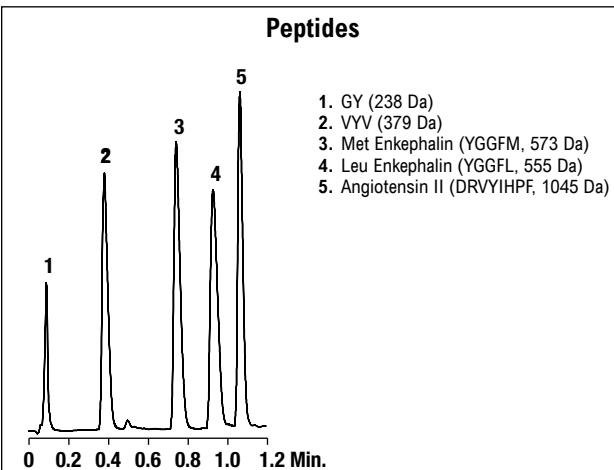


**Column:** ProZap™ C18, 2.1 x 10mm, C18, 1.5 $\mu$ m, 500Å  
**HPLC System:** High-pressure gradient, binary pump  
**Mobile Phase:** A: 0.1% TFA in Water  
 B: 0.085% TFA in 90:10 n-propanol:water  
**Gradient:** Time: 0.0 | 6.0 | 6.5 | 7.0 |  
 %B: 5 | 75 | 75 | 5 |  
**Flow Rate:** 0.5mL/min  
**Detector:** UV at 280nm  
**Injection Vol:** 5 $\mu$ L  
**Column Temp:** 75°C  
**Backpressure:** 170bar = 2465psig

**Separation of Antibodies in Sheep Serum**

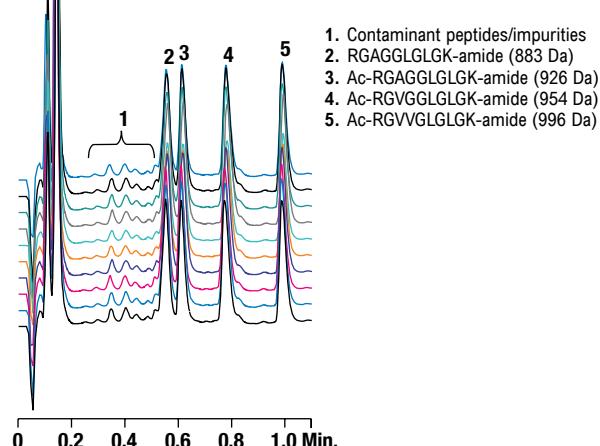
**Column:** DEAE-type anion exchange, 7.5 x 50mm  
**Mobile Phase:** 10mM HEPES/TEA, pH 8.0  
**Gradient:** 0 to 0.5 M NaCl in 20min

Vydac® ProZAP™ columns separate intact IgG antibodies (150 kDa) rapidly. Other traditional HPLC methods, such as ion exchange take longer and typically involve the use of non-volatile buffers.



**Column:** ProZap™ C18, 2.1 x 10mm, C18, 1.5 $\mu$ m, 500Å  
**HPLC System:** High-pressure gradient, binary pump  
**Mobile Phase:** A: 0.1% TFA in Water  
 B: 0.085% TFA in 80:20 Acetonitrile:Water  
**Gradient:** Time: 0.0 | 0.1 | 0.7 | 1.1 | 1.2 |  
 %B: 4 | 15 | 20 | 50 | 4 |  
**Flow Rate:** 0.8mL/min  
**Detector:** UV at 215nm  
**Injection Vol:** 5 $\mu$ L  
**Injection Amt:** 600ng each, except 200ng for GY  
**Column Temp:** 25°C  
**Backpressure:** 180bar = 2610psig

Peptides under one minute.

**Synthetic Peptides—10 Injections**

**Column:** ProZap™ C18, 2.1 x 10mm, C18, 1.5 $\mu$ m, 500Å  
**HPLC System:** High-pressure gradient, binary pump  
**Mobile Phase:** A: 0.1% TFA in Water  
 B: 0.085% TFA in 80:20 Acetonitrile:Water  
**Gradient:** Time: 0.0 | 0.1 | 0.7 | 1.1 | 1.2 |  
 %B: 4 | 15 | 20 | 50 | 4 |  
**Flow Rate:** 0.8mL/min  
**Detector:** UV at 215nm  
**Injection Vol:** 5 $\mu$ L  
**Injection Amt:** 500ng each  
**Column Temp:** 25°C  
**Backpressure:** 180bar = 2610psig

Reproducibly separate synthetic peptides can be in one minute.

**ProZap™ (1.5 $\mu$ m)**

Dimension	2.1 x 10mm	2.1 x 20mm	4.6 x 10mm	4.6 x 20mm	7 x 33mm
Part No.	35585	35587	35586	35588	35589