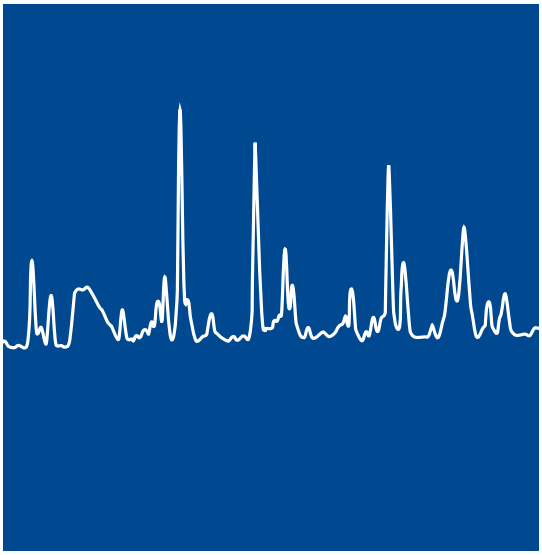

Discovery Sciences

VYDAC® MS Columns

Pioneer in Bio-separations

- **Unique Selectivity and Exceptional Resolution**
  Proprietary silica & bonding technology
- **Maximum Sensitivity and Recovery Increased Peak Capacity**
  Ideal peak capacity for biomolecules <10K and oligonucleotides >30mer
- **30-years of Proven Performance**
  History of quality and reproducibility

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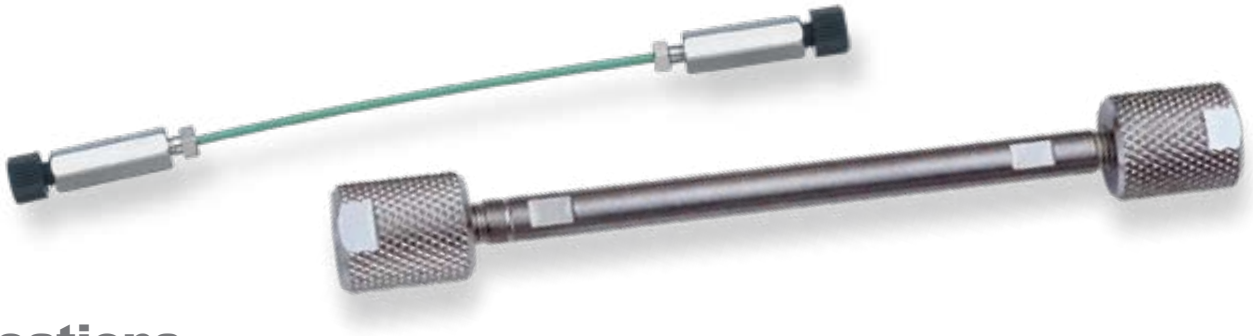
Great Separations Require Selectivity

Use the separating power of Vydac® MS columns to see what you are missing!

The Vydac® MS family of columns offers a new standard of excellence in reversed-phase technology for large biomolecule separations. Since the beginning of the biotechnology revolution, Vydac® reversed-phase columns have been the gold standard for separating intact proteins and the peptides resulting from protein digestion – peptide maps.

The secret to any reversed-phase HPLC separation is the separation factor, a measure of relative retention and peak shape. The New Vydac® MS reversed-phase columns are now setting a new and even higher standard for protein and peptide separations. Based on specially treated large pore silica and enhanced with a new proprietary bonding process, Vydac® MS reversed-phase columns offer better separation of protein therapeutic drugs from degradation products or process-related proteins than other reversed-phase columns. Better separation of the very large numbers of peptides from protease digests required for proteomic analysis result in more peptide, and hence more protein, identifications.

Vydac® MS columns are the latest development in the ongoing effort to provide the best reversed-phase HPLC columns for the separations of proteins and peptides. If you are monitoring protein therapeutic drugs for degradation products such as deamidated asparagine or oxidized methionine or if you are separating difficult, hydrophobic proteins, MS columns offer superior selectivity and peak shape. If you are separating the peptides in a peptide map, Vydac® MS columns offer unique selectivity. If you are separating the very large numbers of peptides necessary to identify proteins as possible drug targets, Vydac® MS columns separate more peptides and lead to the identification of more proteins than other reversed-phase columns. Whether you work with protein therapeutic drugs, proteomics or basic research, Vydac® MS columns are the answer to better protein and peptide separations.



Applications

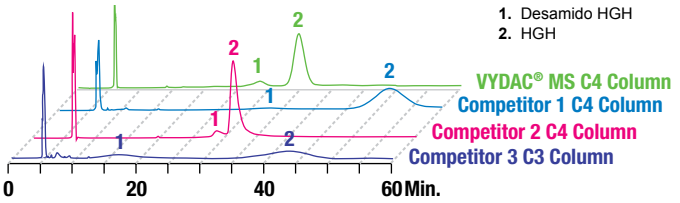
Protein Deamidation

The deamidation of human growth hormone has been monitored for many years by reversed-phase HPLC using Vydac® columns (Ref: Riggin, Dorulla, and Miner, Analytical Biochemistry 167, 199-209 (1987)). As illustrated in **Figure 1**, Vydac® MS columns are superior to other columns for monitoring the deamidation of growth hormone because of superior selectivity and peak shape.

Deamidated proteins

One of the most commonly monitored degradation processes of protein therapeutic drugs is deamidation. When proteins are modified by deamidation, an asparagine residue is modified to an aspartic acid residue or its isoform, isoaspartic acid (Ref: Manning, Patel and Borchardt, Pharmaceutical Research 6, 903-917 (1989)). The change in a single polar side chain is particularly by reverse phase, which interacts with the non-polar regions of the large compound. Deamidated proteins are often less biologically active than the native form which reduces drug potency, and are therefore critical to detect and require the selectivity of Vydac® MS columns.

Human Growth Hormone (hGH) Assay on 300Å C4 Columns



Columns:	VYDAC® MS C4 column **214MS54**, Competitor 1 C4, Competitor 2 C4, and Competitor 3 C3 (all 300Å, 4.6 x 250 mm packed with 5µm except 4µm for Competitor 1)
Flow Rate:	0.5 mL/min.
Eluent:	Isocratic, 71% 50 mM Tris, pH 7.5, 29% n-propanol
Absorbance:	220 nm
Column Temp:	45°C
Injection Vol:	20 µL of a 1 mg/mL preparation

Figure 1: The Vydac® column provides the overall best performance characteristics (good recovery, resolution, and peak shape) for the common important assay of HGH and desamido HGH. A USP peak symmetry < 1.8 is required for HGH.

Hydrophobic proteins

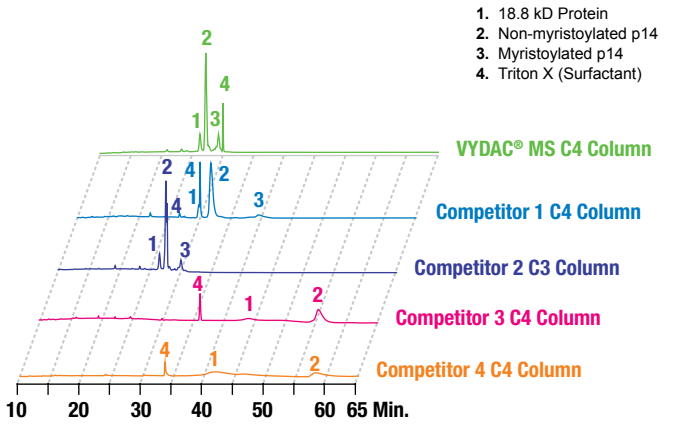
Many proteins are bound to the cell membrane and are very hydrophobic. Although hydrophobic proteins are particularly difficult to separate, Vydac® MS columns provide excellent selectivity and peak shape for these molecules (**Figure 2**). In this case, a hydrophobic transmembrane protein was separated from other cellular components and a synthetic myristoylated derivative.

Hydrophobic proteins

Many "membrane-associated" proteins function as cell surface receptors, providing a message transfer center for extra-cellular molecules to signal internal cellular processes and call them to action. Because cell surface receptors play a critical role in so many cellular processes they are potential targets for drug molecules. To understand the role membrane-associated proteins play in disease states and in a quest to identify drug targets, hydrophobic, "membrane-associated" proteins must be isolated and identified. Hydrophobic post-translational modifications such as this provide anchors to bind "membrane-associated" proteins to the cell membrane. Similar modifications may be added to a protein synthetically to enhance protein performance.

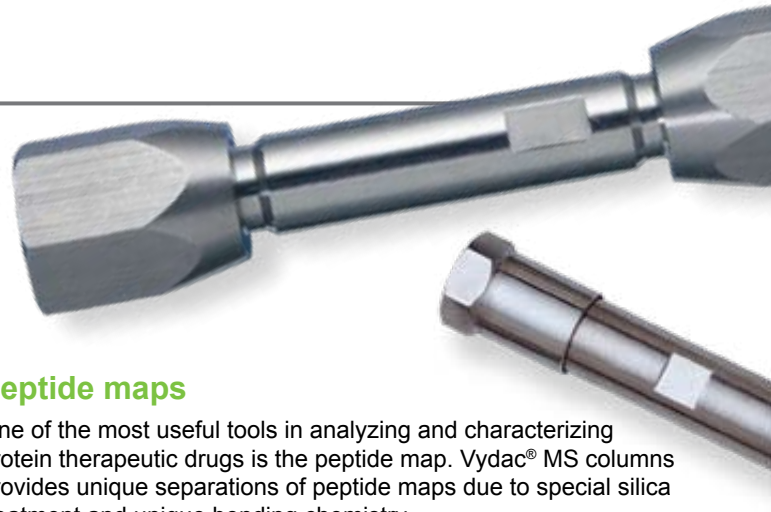
Transmembrane Protein p14 on 300Å C4 Columns

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



Columns: VYDAC® MS C4 column **214MS54**, Competitor 1 C4, Competitor 2 C3, Competitor 3 C4, and Competitor 4 C4 (all 300Å, 4.6 x 250 mm packed with 5µm except 4µm for Competitor 1)
Flow Rate: 1.0 mL/min.
Eluent: **A** = 0.1% v/v trifluoroacetic acid in H2O
B = 0.085% v/v trifluoroacetic acid in Acetonitrile
Gradient Vydac®, Comp. 1, & Comp. 2: (**min.,% B**): (0,20), (20,60), (25,80), (45,80)
Gradient Comp. 3 & Comp. 4: (**min.,% B**): (0,20), (20,60), (25,80), (45,80), (50,90), (80,90)
Absorbance: 215 nm
Column Temp: Ambient, 25°C
Injection Vol: 50 µL

Figure 2: The Vydac® column provides better separation and recovery for a highly hydrophobic membrane protein (RRV p14) and its fatty acid modified (myristolyated) form, a component of a potentially new vaccine delivery system.



Peptide maps

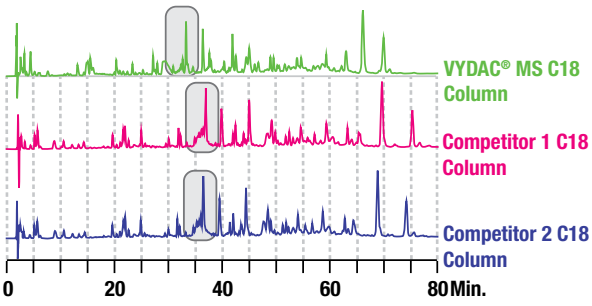
One of the most useful tools in analyzing and characterizing protein therapeutic drugs is the peptide map. Vydac® MS columns provides unique separations of peptide maps due to special silica treatment and unique bonding chemistry.

The separation of the peptides of fetuin, a glycoprotein, exhibits a different pattern on Vydac® MS columns compared to other C18 columns (**Figure 3**), revealing some peaks otherwise not seen. The improved selectivity for peptides on the Vydac® MS columns results in better primary structure definition and easier identification of degradation products and other protein characteristics.

Peptide maps

A therapeutic protein drug is digested with a protease, typically trypsin, producing a number of peptides which are then separated by reversed-phase HPLC. Peptide maps provide a great deal of information about a protein. They confirm primary structure, help identify product related impurities such as deamidation or oxidation and are useful in confirming disulfide bond linkages and determining glycosylation.

Tryptic Digest of Fetuin on 300Å C18 Columns



Columns: VYDAC® MS C18 column **238MS5415**, Competitor 1 C18, and Competitor 2 C18 (all 300Å, 5µm, 4.6 x 150 mm)
Flow Rate: 1.0 mL/min.
Eluent: **A** = 0.1% v/v trifluoroacetic acid in H2O
B = 0.085% v/v trifluoroacetic acid in Acetonitrile
Gradient: (**min., % B**): (0, 4), (5, 4), (80, 40), (90, 90), (100, 90)
Absorbance: 215 nm
Column Temp: Ambient, 22°C
Sample: 30 µL of a 6 µg/µL digest (180 µg total peptide load)

Figure 3: A sample of bovine fetuin, a 36 kD glycoprotein, was digested with trypsin. Some of the sample components interfere with the separation of peptides on the Competitor 1 and Competitor 2 columns, appearing as a chromatographic "hump" with peaks riding on top. The unique selectivity of Vydac® MS columns solves separation problems.

Ordering Information

	50mm	100mm	150mm	250mm
3µm C18				
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 C18*				
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
1.0mm	218MS5105	218MS5110	218MS5115	218MS51
2.1mm	218MS5205	218MS5210	218MS5215	218MS52
4.6mm	218MS5405	218MS5410	218MS5415	218MS54
10mm				218MS510
5µm C18 Monomeric*				
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
1.0mm	238MS5105	238MS5110	238MS5115	238MS51
2.1mm	238MS5205	238MS5210	238MS5215	238MS52
4.6mm	238MS5405	238MS5410	238MS5415	238MS54
5µm C8				
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
1.0mm	208MS5105	208MS5110	208MS5115	208MS51
2.1mm	208MS5205	208MS5210	208MS5215	208MS52
4.6mm	208MS5405	208MS5410	208MS5415	208MS54
10mm				208MS510
5µm C4				
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
1.0mm	214MS5105	214MS5110	214MS5115	214MS51
2.1mm	214MS5205	214MS5210	214MS5215	214MS52
4.6mm	214MS5405	214MS5410	214MS5415	214MS54
10mm				214MS510
5µm Diphenyl				
75µm	219MS5.07505	219MS5.07510	219MS5.07515	219MS5.07525
150µm	219MS5.1505	219MS5.1510	219MS5.1515	219MS5.1525
300µm	219MS5.305	219MS5.310	219MS5.315	219MS5.325
500µm	219MS5.505	219MS5.510	219MS5.515	219MS5.525
1.0mm	219MS5105	219MS5110	219MS5115	219MS51
2.1mm	219MS5205	219MS5210	219MS5215	219MS52
4.6mm	219MS5405	219MS5410	219MS5415	219MS54
10mm				219MS510

*Guards also available for 5µm C18.

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