



# **MS RP-HPLC Columns Provide Unique Selectivity and High Recovery for Protein and Peptide Separations**

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# Abstract

Based on specially treated large pore silica and enhanced with a proprietary bonding process, Vydac® MS reversed-phase (RP) HPLC columns offer superior performance for proteins and peptides. The MS C4 column provides the best overall performance characteristics (sensitivity, resolution, and peak symmetry) for the common important assay of human growth hormone (HGH) and desamido HGH. Although hydrophobic membrane proteins are generally difficult to separate, the MS C4 column provides superior resolution and recovery for a reptilian reovirus p14 protein and myristoylated form, a component of a potentially new vaccine delivery system. RP-HPLC of the tryptic digest of fetuin exhibits unique selectivity for peptide mapping on a MS C18 column compared to other C18 columns, revealing some peaks otherwise not seen. The improved separation for peptides on the MS columns results in better primary structure definition and easier identification of degradation products and other protein characteristics.

## Human Growth Hormone (HGH) on C4 Columns

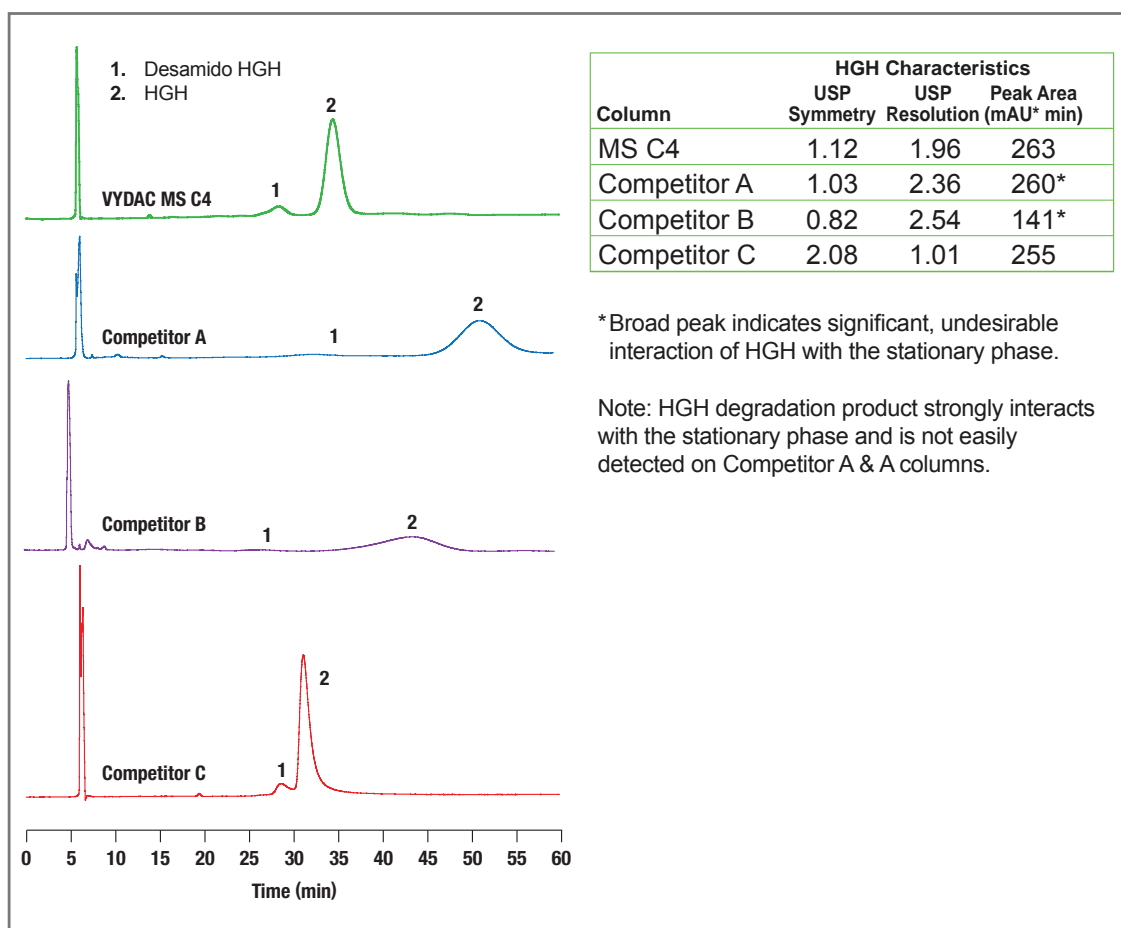


Figure 1

HPLC Conditions	
Column:	All 300Å, 4.6 x 150mm, packed with 5µm
Flow Rate:	0.5 mL/min
Eluent:	Isocratic, 71% 50 mM Tris, pH 7.5, 29% n-propanol
Absorbance:	220 nm
Column Temperature:	45°C
Injection Volume:	20 µL of a 1 mg/mL preparation

The deamidation of HGH has been monitored for many years by RP-HPLC (Riggen et al, 1987). The MS C4 column provides the overall best performance characteristics (sensitivity, resolution, and peak shape) for the common important assay of HGH and desamido HGH.

## Transmembrane Protein p14 on C4 Columns

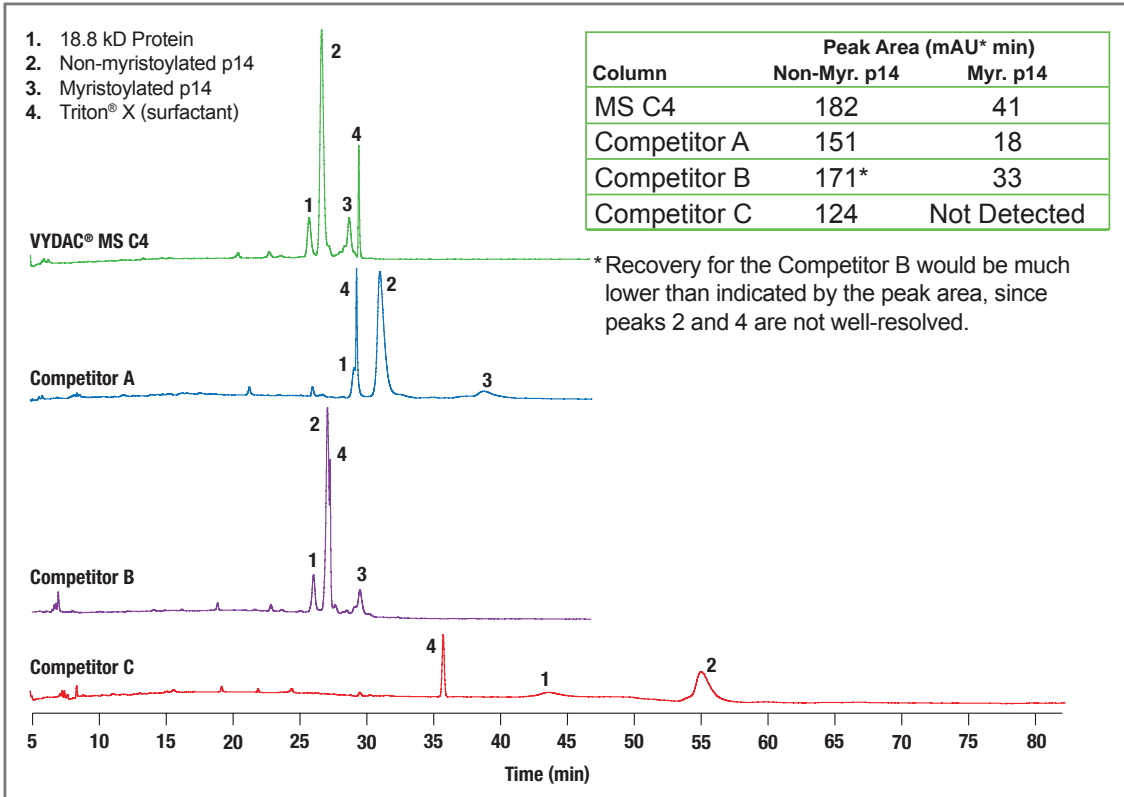


Figure 2

HPLC Conditions	
<b>Column:</b>	All 300Å, 4.6 x 250mm, packed with 5 µm except 4 µm for Competitor A
<b>Flow Rate:</b>	1.0 mL/min
<b>Eluent:</b>	A = 0.1% v/v TFA in H <sub>2</sub> O B = 0.085% v/v TFA in Acetonitrile
<b>Gradient:</b>	MS C4, Competitors A & B (min, % B): (0, 20), (20, 60), (25, 80), (45, 80) Competitor C (min, % B): (0, 20), (20, 60), (25, 80), (50, 90), (80, 90)
<b>Column Temperature:</b>	Ambient, 25°C
<b>Injection Volume:</b>	50 µL

The MS C4 column provides better resolution 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 (Nguyen, de Antueno, and Duncan, manuscript in prep.).

## Recovery & Resolution of Different p14 Samples: MS vs. Leading C4

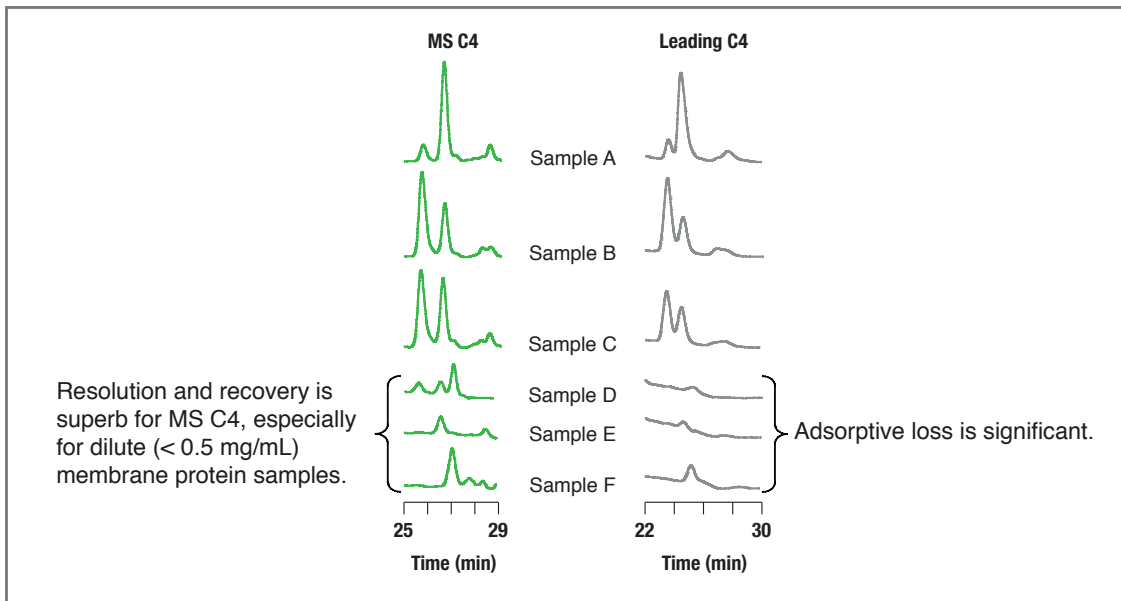


Figure 3

## Tryptic Digest of Bovine Fetuin on C18 Columns

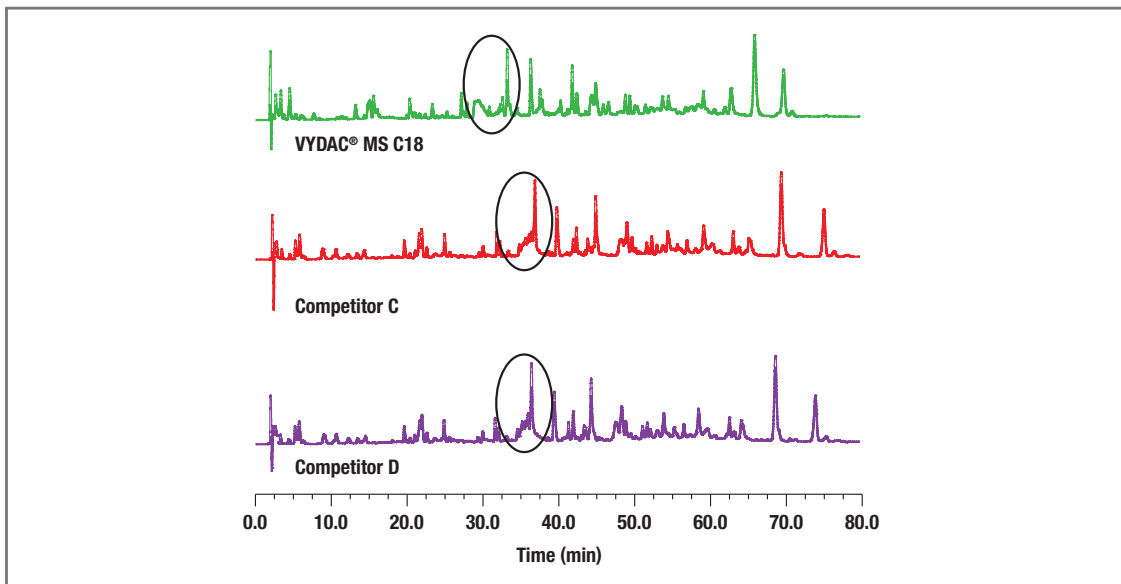


Figure 4

### HPLC Conditions

<b>Column:</b>	All 300Å, 5 µm, 4.6 x 150 mm
<b>Flow Rate:</b>	1.0 mL/min
<b>Eluent:</b>	A = 0.1% v/v TFA in H <sub>2</sub> O
	B = 0.085% v/v TFA in Acetonitrile
<b>Gradient, (min, %B):</b>	(0, 4), (5, 4), (80, 40), (90, 90), (100, 90)
<b>Absorbance:</b>	215 nm
<b>Column Temperature:</b>	Ambient, 22°C
<b>Sample:</b>	30 µL of a 6 µg/µL digest (180 µg total peptide load)

The 36-kD glycoprotein was digested with trypsin. Some of the sample components interfere with the separation of peptides on the Competitor C & W columns, appearing as a chromatographic “hump” with peaks riding on top. However, owing to its unique selectivity, the MS C18 column does not exhibit this problem, with the peptide peaks being well separated from this broad signal.

## Recovery of Proteins: MS vs. Leading Columns

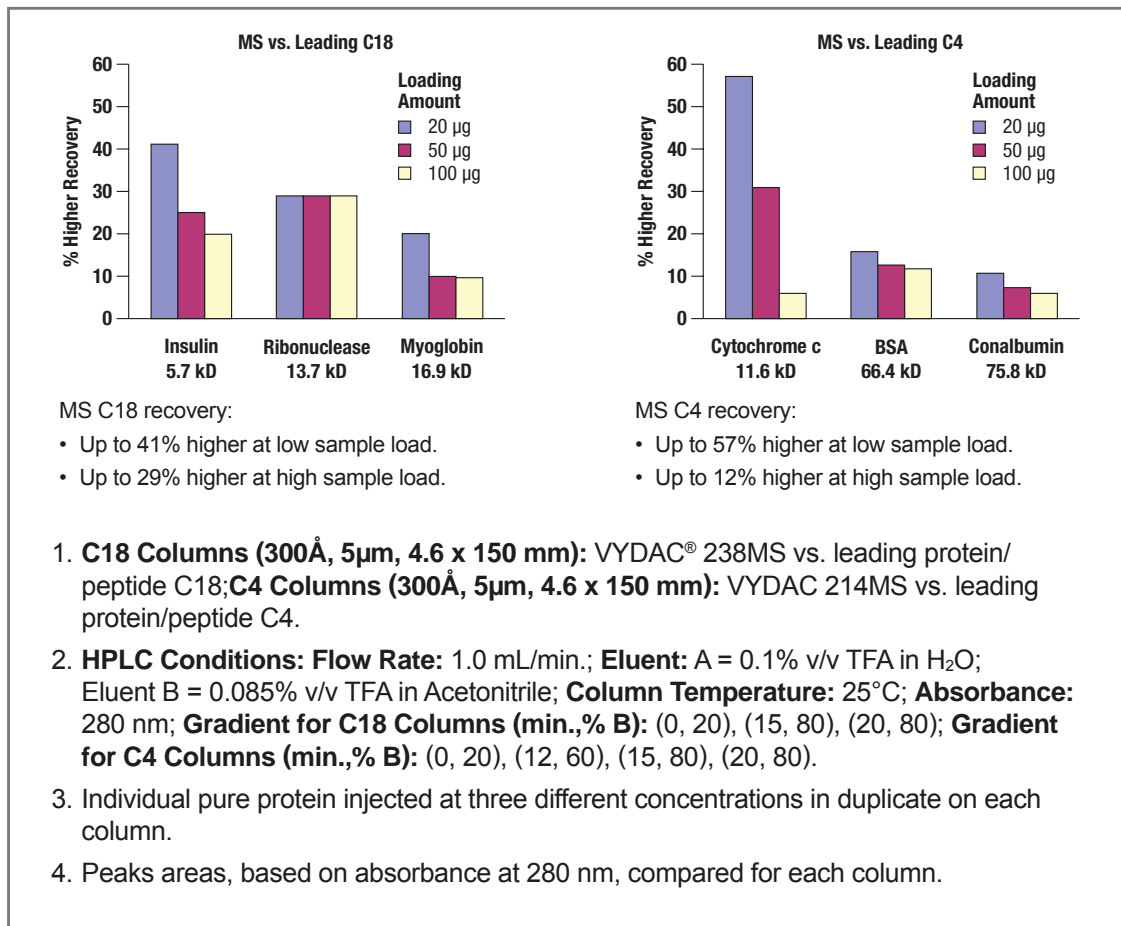


Figure 5

## Recovery of Protein at Low Sample Loads: MS vs. C4 Columns

Protein	% Recovery		% Lower vs. MS	
	1 µg	5 µg	1 µg	5 µg
<b>Cytochrome c</b>				
MS C4	88	69	—	—
Competitor A	59	61	-33	-12
Competitor B	65	67	-25	-2
Competitor C	54	57	-38	-17
<b>BSA</b>				
MS C4	67	98	—	—
Competitor A	39	84	-41	-15
Competitor B	53	92	-21	-6
Competitor C	56	88	-16	-11

Conalbumin	% Recovery		% Lower vs. MS	
	1 µg	5 µg	1 µg	5 µg
MS C4	86	97	—	—
Competitor A	61	93	-28	-3
Competitor B	72	96	-16	-1
Competitor C	80	94	-7	-2

1. **C4 Columns:** All 300Å, 5 µm, 4.6 x 150 mm.
2. **HPLC Conditions: Flow Rate:** 1.0 mL/min.; **Eluent:** A = 0.1% v/v TFA in H<sub>2</sub>O; Eluent B = 0.085% v/v TFA in Acetonitrile; **Column Temperature:** 25°C; **Absorbance:** 280 nm; **Gradient (min.,% B):** (0, 20), (12, 60), (15, 80), (20, 80).
3. Individual pure protein injected on each column. Peaks area (at 280 nm) measured.
4. Individual pure protein injected with no column on HPLC system. 0.005" ID tubing used in place. Peaks area (at 280 nm) measured.
5. Peak areas (#3/#4) normalized to estimate relative recovery.

Better recovery is observed for MS C4, especially at low sample load of 1 µg.

## Conclusion

Vydac® MS columns provide:

- The best overall performance characteristics (sensitivity, resolution, and peak symmetry) for the important assay of human growth hormone.
- Unique selectivity for peptide mapping.
- Superb separation and recovery for transmembrane protein p14.
- Higher recovery for a broad range of proteins.

## References

Nguyen, R.T., de Antueno, R., and Duncan, R., in prep. (2007) Development of Novel and Rapid HPLC and LC-MS Methods for the Analysis of p14 Fusion-Associated Small Transmembrane (FAST) Protein. *J. Chrom.*

Riggin, R.M., Dorulla, G.K., and Miner, D.J. (1987) A reversed-phase high-performance liquid chromatographic method for characterization of biosynthetic human growth hormone. *Anal. Biochem.* 167, 199-209.

## Acknowledgement

We thank Drs. Roberto J. de Antueno and Roy Duncan, Dalhousie University, Halifax, Nova Scotia, for the reptilian reovirus RRV p14 samples.

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