Reversed-Phase Columns for LC/MS

Using trifluoroacetic acid (TFA) for ion pairing is common practice in reversed-phase separation of peptides and proteins. TFA in the mobile phase has the most important effect of improving peak shapes. It overcomes peak broadening and asymmetry (tailing) that are believed to result from mixed-mode interactions of the variety of polar, ionic and hydrophobic sites on peptide molecules with hydrophobic bonded phases and residual polar groups on silica surfaces.

TFA is believed to exert its effects by pairing with positive charged and polar groups on peptides and proteins to mask these sites from polar interactions and bring them to the hydrophobic reversed-phase surface. TFA may similarly mask unbonded polar regions of the adsorbent. It can be demonstrated that TFA is retained on reversed-phase adsorbents and interacts with both the column and polypeptides, as reported in *Vydac Advances* for Spring, 1997 (Ref. 1).

TFA is often preferred over other ionic modifiers because its volatility permits easy removal from preparative fractions. The UV absorbance spectrum of TFA peaks below 200 nm and thus creates minimal interference with detection of peptides at low wavelengths (Ref. 2).

Altering TFA concentration changes reversed-phase selectivity for peptides in subtle ways (also reported in Ref. 1). These changes can be exploited to optimize separations or increase the information obtained from complex chromatograms – peptide fingerprints for example.

TFA is most often added to mobile phases at a concentration of 0.1%. This concentration produces good peak shapes with most reversed-phase columns, whereas TFA concentrations much below that level produce noticeable peak broadening and tailing.

LC/MS

In the past ten years, reversed-phase chromatography coupled to electrospray mass spectrometry has become a valuable tool for molecular weight determination and detailed structure analysis of peptides and proteins. Unfortunately, TFA-containing mobile phases have a suppressive effect on ion generation, reducing the sensitivity and analytical reliability of LC/MS techniques (Ref. 3). This suppressive effect can be partially overcome by post-column additive techniques, but these significantly complicate the chromatographic system. Alternatively, a 10-fold reduction in TFA concentration will practically eliminate suppression, but this generally produces significant reduction in chromatogram quality.

New LC/MS-Grade Columns

Recognizing the need, Grace Vydac developed new columns that produce peptide and protein separations with excellent peak sharpness and symmetry using only a fraction of the TFA concentration previously required. These columns were based on Grace Vydac's high-purity synthetic 300 Å pore-size silica with polymeric C18 and C4 bonded phases. A proprietary silica treatment reduced the dependence on TFA.

Figure 1 shows a separation of four peptides on the VYDAC® 218MS54 LC/MS-grade C18 column. Note that peak shapes and symmetry are maintained over a 10-fold reduction in TFA concentration. Reductions in retention times occur as a result of less activity of TFA in bringing polar groups on sample molecules to the bonded C18 phase at lower TFA concentrations. The effect is more pronounced for angiotensin II by virtue of its positive-charged arginine side chain, which results in different selectivity and, in fact, a reversal of elution order with oxytocin at 0.01% TFA.

Separation of Peptides

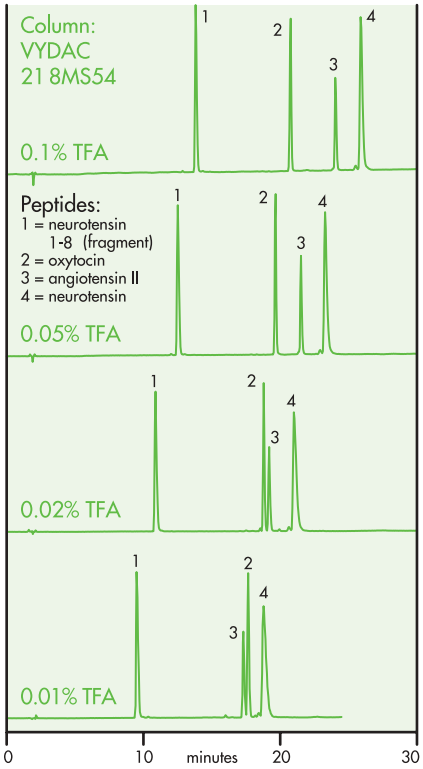

Figure 1. *Separation of peptides on LC/MS-grade C18 column with various TFA concentrations. Column: VYDAC 218MS54 C18 300 Å 5 µm 4.6 mm ID x 250 mm L. Detection: UV absorption, 220 nm. Mobile Phase: A = 5% acetonitrile in water with TFA as indicated (v/v). B = 95% acetonitrile in water containing same TFA concentration as in A. Flow: 1.5 mL/min. Gradient: Linear from 0 to 20% B over 20 minutes, then to 100% B in 5 minutes.*

Figure 2 shows a separation of two peptides and two proteins on the VYDAC 214MS54 LC/MS-grade C4 column. Peak shapes for the peptides are maintained down to 0.01% TFA for the peptides, and down to 0.02% TFA for bovine and human insulin, with some noticeable broadening at 0.01%. The basis for greater dependency of the protein peaks on TFA is not known. Furthermore, a change in selectivity with reversal of elution order is seen for oxytocin and angiotensin II, similar to that seen on the C18 column.

Separation of Peptides and Proteins

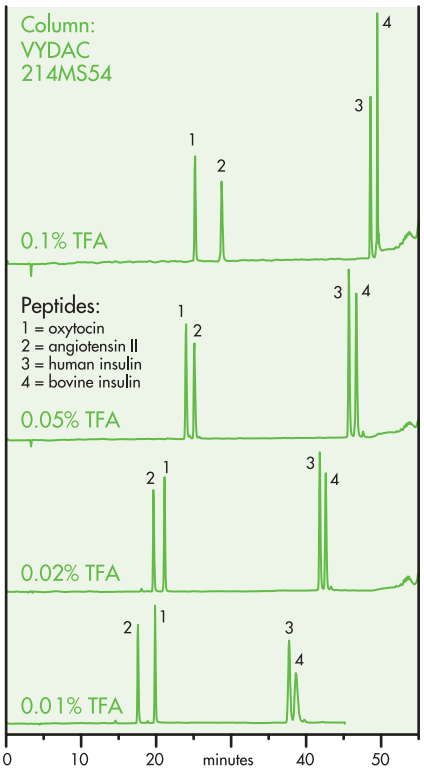

***Figure 2**. Separation of two peptides and two proteins on LC/MS-grade C4 column with various TFA concentrations. Column: VYDAC 214MS54 C4 300 Å 5 µm 4.6 mm ID x 250 mm L. Detection: UV absorption, 220 nm. Mobile Phase: A = 5% acetonitrile in water with TFA as indicated (v/v). B = 95% acetonitrile in water containing same TFA concentration as in A. Flow: 1.0 mL/min. Gradient: Linear from 0 to 10% B over 15 minutes, then to 25% B in 30 minutes, and finally to 100% B in 5 minutes.*

Additional LC/MS-Grade Adsorbents

Recently, Grace Vydac has introduced three additional LC/MS-grade bonded phases – a monomeric C18, a C8, and a diphenyl – providing a full range of reversed-phase selectivities for protein and peptide HPLC.

Nano, Capillary, and Microbore Columns Available

VYDAC 218MS and 214MS LC/MS-grade columns are available in small diameter analytical, microbore, and capillary columns for operation at lower flow rates when 100% feed to a mass spectrometer is desired.

References

1. "What is 0.1% TFA?" Vydac Application Note #9804.
2. Vydac Handbook of Analysis and Purification of Peptides and Proteins by Reversed-Phase HPLC, 2nd Edition, page 7.
3. A. Apffel, S. Fischer, G. Goldberg, P.C. Goodley, and F.E. Kuhlmann, "Enhanced sensitivity for peptide mapping with electrospray liquid chromatography - mass spectrometry in the presence of signal suppression due to trifluoroacetic acid-containing mobile phases." J. Chrom. A, 712 (1995) 177-190.

Ordering Information

Adsorbent / Column Size	C4 (polymeric)	C8 (polymeric)	C18 (polymeric)	C18 (monomeric)	diphenyl
75 µm ID x 50 mm L	214MS5.07505	208MS5.07505	218MS5.07505	238MS5.07505	219MS5.07505
75 µm ID x 100 mm L	214MS5.07510	208MS5.07510	218MS5.07510	238MS5.07510	219MS5.07510
75 µm ID x 150 mm L	214MS5.07515	208MS5.07515	218MS5.07515	238MS5.07515	219MS5.07515
75 µm ID x 250 mm L	214MS5.07525	208MS5.07525	218MS5.07525	238MS5.07525	219MS5.07525
150 µm ID x 50 mm L	214MS5.1505	208MS5.1505	218MS5.1505	238MS5.1505	219MS5.1505
150 µm ID x 100 mm L	214MS5.1510	208MS5.1510	218MS5.1510	238MS5.1510	219MS5.1510
150 µm ID x 150 mm L	214MS5.1515	208MS5.1515	218MS5.1515	238MS5.1515	219MS5.1515
150 µm ID x 250 mm L	214MS5.1525	208MS5.1525	218MS5.1525	238MS5.1525	219MS5.1525
300 µm ID x 50 mm L	214MS5.305	208MS5.305	218MS5.305	238MS5.305	219MS5.305
300 µm ID x 100 mm L	214MS5.310	208MS5.310	218MS5.310	238MS5.310	219MS5.310
300 µm ID x 150 mm L	214MS5.315	208MS5.315	218MS5.315	238MS5.315	219MS5.315
300 µm ID x 250 mm L	214MS5.325	208MS5.325	218MS5.325	238MS5.325	219MS5.325
500 µm ID x 50 mm L	214MS5.505	208MS5.505	218MS5.505	238MS5.505	219MS5.505
500 µm ID x 100 mm L	214MS5.510	208MS5.510	218MS5.510	238MS5.510	219MS5.510
500 µm ID x 150 mm L	214MS5.515	208MS5.515	218MS5.515	238MS5.515	219MS5.515
500 µm ID x 250 mm L	214MS5.525	208MS5.525	218MS5.525	238MS5.525	219MS5.525
1.0 mm ID x 250 mm L	214MS51	208MS51	218MS51	238MS51	219MS51
2.1 mm ID x 250 mm L	214MS52	208MS52	218MS52	238MS52	219MS52
4.6 mm ID x 250 mm L	214MS54	208MS54	218MS54	238MS54	219MS54

Nano & Capillary Diameters Standard and Microbore Diameters

To place an order, call your local Grace Vydac distributor.

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