NEW!

Low-TFA Reversed-Phase Columns

for Applications Including Electrospray MS Analysis

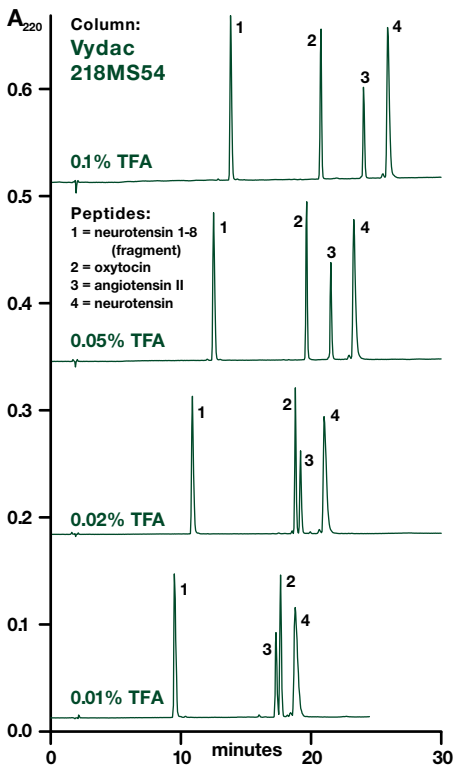

Figure 1. Separation of peptides on Low-TFA C18 column with various TFA concentrations. Column: Vydac 218MS54 C18 300Å 5µm 4.6mmID x 250mmL. **Detection:** UV absorption, 220 nm. **Mobile Phase:** A = 5% acetonitrile in water with TFA as indicated (v/v). B = 95% acetonitrile in water containing same 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.

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 peptide molecules having a variety of polar, ionic and hydrophobic sites with hydrophobic bonded phases and residual polar 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, for example, peptide fingerprints.

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

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can be partially overcome by postcolumn 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 a significant reduction in chromatogram quality.

New Low-TFA Columns

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

Figure 1 shows a separation of four peptides on the 218MS54 Low-TFA 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.

Figure 2 shows a separation of two peptides and two proteins on the 214MS54 Low-TFA 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%. A change in selectivity with reversal of elution order is seen for oxytocin and angiotensin II, similar to that seen on the C18 column. The basis for greater dependency of the protein peaks on TFA is not known, but one could speculate that, in addition to intermolecular effects, TFA may have effects on molecular conformation that make it more difficult for smaller peptide molecules to access multiple sites on the adsorbent surface.

Small-Diameter and Microbore Columns Available

Vydac 218MS and 214MS Low-TFA columns are available in smaller diameter analytical and microbore columns for operation at lower flow rates for 100% feed to mass spectrographic detection.

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***, 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.

To place an order, call 800-247-0924 or contact your local Vydac distributor.

Low-TFA Reversed-Phase Columns

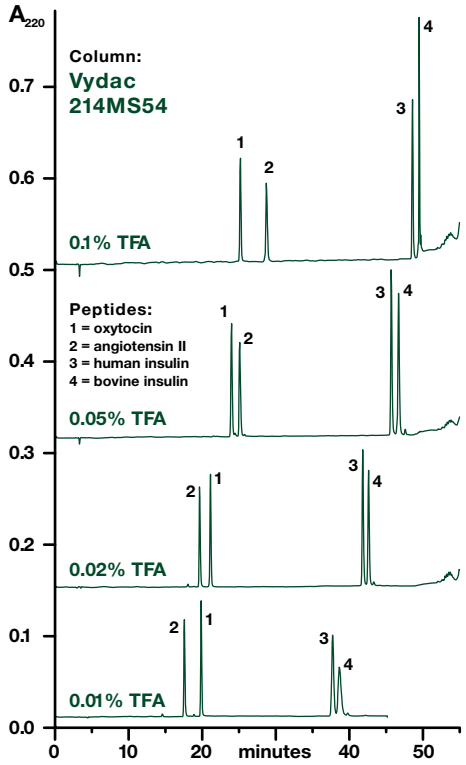

Figure 2. Separation of two peptides and two proteins on Low-TFA C4 column with various TFA concentrations.
Column: Vydac 214MS54 C4 300Å 5µm 4.6mmID x 250mmL. **Detection:** UV absorption, 220 nm. **Mobile Phase:** A = 5% acetonitrile in water with TFA as indicated (v/v). B = 95% acetonitrile in water 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.

Ordering Information

Cat.No.	Description
	C4:
214MS54	Column, Low-TFA C4 Reversed Phase, 300Å, 5µm, 4.6mm ID x 250mm L
214MS52	Column, Low-TFA C4 Reversed Phase, 300Å, 5µm, 2.1mm ID x 250mm L
214MS51	Column, Low-TFA C4 Reversed Phase, 300Å, 5µm, 1.0mm ID x 250mm L
	C18:
218MS54	Column, Low-TFA C18 Reversed Phase, 300Å, 5µm, 4.6mm ID x 250mm L
218MS52	Column, Low-TFA C18 Reversed Phase, 300Å, 5µm, 2.1mm ID x 250mm L
218MS51	Column, Low-TFA C18 Reversed Phase, 300Å, 5µm, 1.0mm ID x 250mm L

Other column sizes are available for analytical and preparative applications.

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