The Influence of C18 Ligand Type on the Peptide Selectivity of Silica-Based Reversed-Phase Columns

Introduction

Fifteen years ago, the introduction of synthetic, high purity silica with wide pores made the chromatographic separation of peptides possible and practical. The separation selectivity of peptides on silica-based reversed-phase columns is not only determined by the nature of silica, but also by the type of silanes and bonding chemistry used. This provides us an important tool to design columns with different selectivity to suit different separation needs. In this report, we demonstrate that different silanes applied on the same silica have different selectivity for peptide separations. The comparison results show that these reversed-phase columns can be used complementally for peptides which are difficult to separate.

Effect of Ligand Type

Vydac TP is a high-purity 300Å pore-size silica produced from purified organic silicates.

Historically, protein/peptide reversed-phase adsorbents produced from TP silica have had "polymeric" bonded phases – synthesized from polyfunctional silanes which produce crosslinking of the hydrophobic phase on the silica surface. This produces columns with long lifetimes and no measurable stationary phase leaching. Vydac 218TP is a C18 reversed-phase adsorbent produced in this way.

Chemistry of Monomeric and Polymeric Bonding

$X = CH_3O,\ CH_3CH_2O,\ Cl$
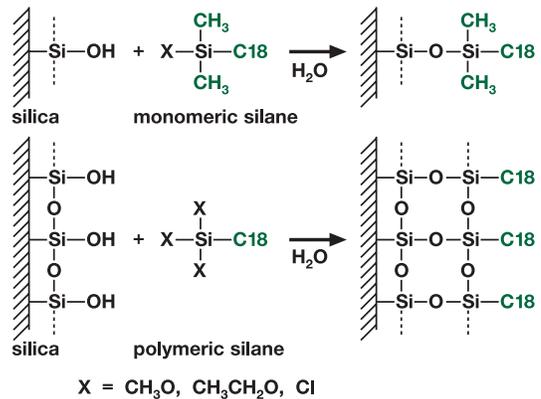
More recently, we have bonded a "monomeric" C18 ligand on the same silica which is later exhaustively endcapped. The resulting adsorbent is designated Vydac 238TP. Figure 1 shows a comparison of peptide separations on the polymeric and monomeric bonded phases. Note the

■ greater resolution in the 18-26 minute range on the monomeric phase (238TP54)

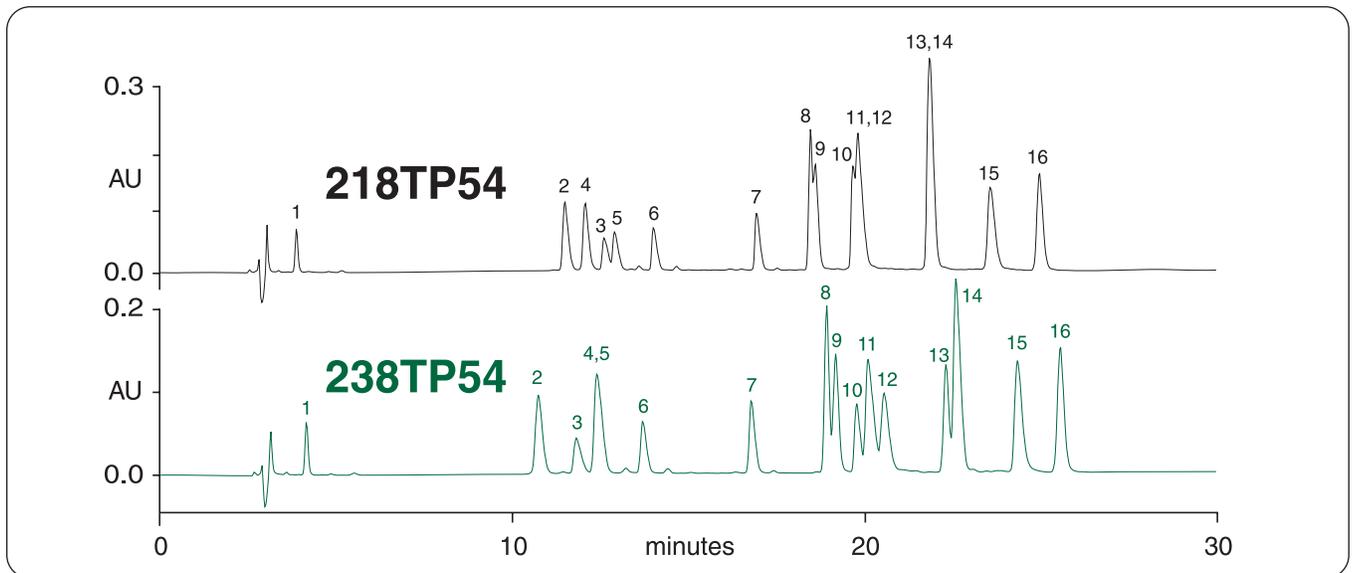■ better selectivity for this mixture in the 10-15 minute range on the polymeric phase (218TP54)

Figure 1. Synthetic peptides on two 300Å C18 silica reversed-phase columns. Both columns were 4.6 mm ID x 250 mm L. Chromatographic conditions were identical. Conditions: 1.0 mL/min, absorbance at 220 nm, gradient from 10% to 40% ACN with 0.1% TFA (w/v) over 30 minutes. Sample: Mixture of standard peptides, listed with peak identification: 1>GY, 2>VYV, 3> neurotensin fragment 1-8 (pELYENKPR), 4>acRGGGGLGLGK-NH2, 5>RGAGGLGLGK-NH2, 6>acRGAGGLGLGK-NH2, 7>acRGVGGLGLGK-NH2, 8>oxytocin (CYIQNCPLG-NH2), 9>met enkephalin (YGGFM), 10>bradykinin (RPPGFSPFR), 11>acRGVVGLGLGK-NH2, 12>neurotensin fragment 8-13 (RRPYIL), 13>angiotensin II (DRVYIHPF), 14>leu enkephalin (YGGFL), 15>neurotensin (pELYENKPRRPYIL), and 16>angiotensin I (DRVYIHPFHL).
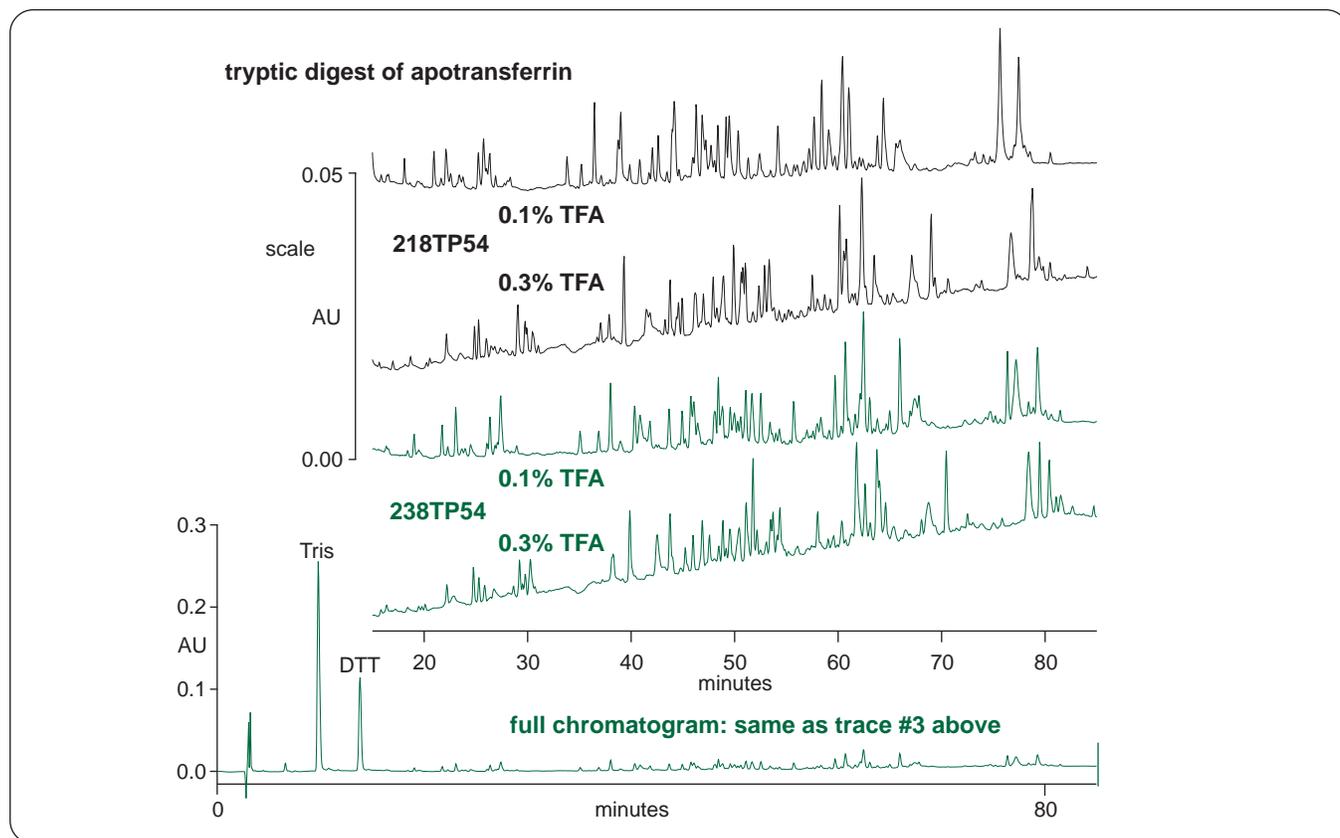
Figure 2. Effects of reversed-phase column type and TFA concentration. The bottom trace is a complete chromatogram. The upper traces show the region from 15 to 85 minutes, in which virtually all tryptic peptides emerge, for four individual runs with the vertical scale expanded to facilitate comparison. Samples identical for all runs. Conditions: 1.0 mL/min, absorbance at 215 nm, TFA concentration (w/v) as indicated, gradient from 0% to 50% ACN over 100 minutes. Both columns were 4.6mm ID x 250mm L. Sample: Tryptic digest of apotransferrin.

Effect of Modifier

Figure 2 shows that changing the concentration of TFA modifier in the mobile phase is another way to change selectivity. For reproducible runs it is important that TFA concentration be clearly specified (v/v or w/v) and carefully controlled.

It can be seen from the chromatograms in Figures 1 and 2 that under identical operating conditions 218TP and 238TP exhibit comparable retention of peptides, but subtle differences in selectivity. This can help in separating peptides that are not resolved or incompletely resolved if only one reversed-phase column type is tried.

ORDERING INFORMATION:

Cat. No.	Description
238TP54	Column, Octadecyl (C18), Monomeric, 5µm, 300Å, 4.6mm ID x 250mm L
218TP54	Column, Octadecyl (C18), Polymeric, 5µm, 300Å, 4.6mm ID x 250mm L

Larger columns are also available.

To place an order, call The Nest Group 800.347.6378

Conclusions

1) "Polymeric" silica-based reversed-phase adsorbents are produced using polychlorosilanes and have a cross-linked hydrophobic layer on the silica surface. Vydac 218TP is an example.

2) "Monomeric" silica-based reversed-phase adsorbents are produced using monochlorosilanes and have a simpler hydrophobic layer on the silica surface. Vydac 238TP is an example.

3) Polymeric and monomeric C18 columns have different selectivities for peptide mixtures.

4) Exploiting these selectivity differences provides an easy way to improve peptide analyses and facilitate peptide purifications.

5) Changing the TFA concentration also has dramatic effects on selectivity. For reproducibility, TFA concentrations must be carefully specified (v/v or w/v) and controlled.

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