APPLICATION NOTE

Reverse-phase Peptide Standards

Application 2057 LC

Dependence of peptide separations on side chains of amino acids

The retention times, and the resulting selectivity, of peptide standards seems to be affected by the kind of side chains attached to amino acid residues in any given peptide sequence. Amino acids can have different kinds of side chains, e.g. aliphatic, aromatic, basic, acidic, and sulphur-containing side-chains. We selected sequences varying in amino acid residues with aliphatic side chains. Hence, a reverse-phase peptide standard mixture routinely employed for monitoring column performance, efficiency, selectivity, and resolution of columns used for the analysis of proteins and peptides is selected for this study. S1-S5 are 5 C-terminal amide decapeptides, 4 of which are N-alpha-acetylated with sequence variations as shown in S2-S5. S1 contains a free N-alpha-amino group. Very subtle differences in side chains of aliphatic amino acids like glycine, alanine, and valine change the hydrophobicity of the different peptides resulting in altered selectivity differences. Excellent selectivity, sharp peaks, and high resolution between very similar peptides is achieved when using Polaris C18-A..

Column : Polaris C18-A (250 x 4.6 mm, 5 µm)

Conventional Part nr.: A2000250X046
ChromSep Part nr.: A2000250C046

Eluent : Solvent A: H2O + 0.1% TFA,
Solvent B: CH3CN + 0.1% TFA
Gradient : 5% - 45% B in 20 min (2% B/min)
Flow : 1.0 ml/min,
Temperature : ambient
Detection : 210 nm,

Peak Identification

1. S1: NH$_2$-Arg-Gly-**Ala**^{3}**-Gly**^{4}-Gly-Leu-Gly-Leu-Gly-Lys-Amide
2. S2: Ac-Arg-Gly-**Gly**^{3}**-Gly**^{4}-Gly-Leu-Gly-Leu-Gly-Lys-Amide
3. S3: Ac-Arg-Gly-**Ala**^{3}**-Gly**^{4}-Gly-Leu-Gly-Leu-Gly-Lys-Amide
4. S4: Ac-Arg-Gly-**Val**^{3}**-Gly**^{4}-Gly-Leu-Gly-Leu-Gly-Lys-Amide
5. S5: Ac-Arg-Gly-**Val**^{3}**-Val**^{4}-Gly-Leu-Gly-Leu-Gly-Lys-Amide