HPLC peptide standard mixture

Dependence of peptide separations on sequence length and amino acid side chains

Application Note

BioPharma

Authors

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Introduction

The separation of a peptide standard mixture consisting of 5 peptides of varying length and composition was used to study this effect. The mix contained selected small peptides, e.g. a dipeptide, a tripeptide, 2 pentapeptides differing in one amino acid, and one 13-amino acid long peptide. This mixture was thus useful to study chromatographic differences arising due to a change in peptide length as well as monitor side-chain differences in peptides of the same length (e.g. methionine and leucine enkephalins). Distinct retention time differences are observed for the 5 peptides, a good base-line separation is achieved between methionine and leucine enkephalins. In contrast to other RP-HPLC columns, base-line separation between leucine enkephalin and angiotensin II is also obtained.

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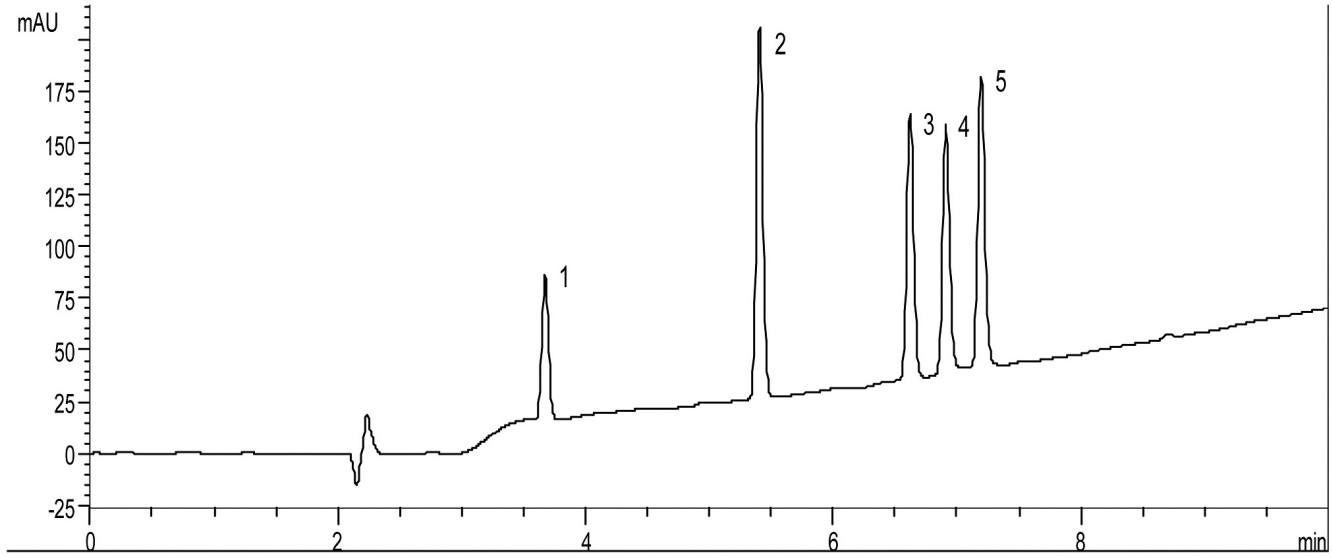
Conditions

Technique	:	Agilent Polaris C18-A, 4.6 x 150 mm, 5 µm Part no. A2000150X046
Eluent	:	Solvent A: H_2O + 0.1% TFA, Solvent B: CH_3CN + 0.1% TFA
Gradient	:	10% - 65% B in 10 min
Flow	:	1.0 mL/min
Temperature	:	ambient
Detection	:	220 nm

Peak identification

1. gly-tyr
2. vai-tyr-val
3. methionine enkephalin (tyr-gly-gly-phe-met)
4. leucine enkephalin (tyr-gly-gly-phe-leu)
5. angiotensin II (pglu-leu-tyr-glu-asn-lys-pro-arg-arg-pro-tyr-lle-leu)



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