INSTRUCTIONS

Peptide Retention Standard

31700

Number	Description
31700	**Peptide Retention Standard,** 1 vial, contains five C-terminal amide decapeptides denoted S1S5 having the generic formula Ac-Arg-Gly-X-X-Gly-Leu-Gly-Leu-Gly-Lys-Amide. Four of these peptides are Na-acetylated with the sequence variation as follows: Gly^3-Gly^4, Ala^3-Gly^4, Val^3-Gly^4 and Val^3-Val^4. The fifth peptide, Ala^3-Gly^4, contains a free Na-amino group. This mixture will provide 100-200 injections (approximately 0.1mg of each peptide) at 0.1 AUFS at 210nm. The Peptide Retention Standard is supplied as a dry film in a sealed vial.

Storage: Upon receipt store at -20°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Peptide Retention Standard allows accurate prediction of elution time for peptides of known amino acid composition up to 20 residues in length. This retention standard is useful for determining the relative order of peptide elution within a complex mixture, simplifying identification of specified peptides and saving time in peptide purification by increasing the prediction efficiency of peptide elution profiles. By testing the standard frequently throughout the lifetime of a column, it is possible to monitor performance characteristics such as efficiency, selectivity, and resolution during column aging. The standard can also be used to evaluate different reverse phase supports of varying n-alkyl chain lengths and ligand density, as well as columns from different manufacturers.

The use of the Peptide Retention Standard is based on a simple method that quantitatively predicts peptide retention times.[1-4] Twenty synthetic octapeptides were prepared with the following sequence: Ac-Gly-X-X-$(Leu)_3$-$(Lys)_2$-amide, where X was varied for twenty amino acids. Retention times are predicted by summing values that represent the contribution in minutes of each amino acid residue and the peptide terminal groups. These retention coefficients are derived directly from the retention times of model synthetic peptides in high performance liquid chromatographic (HPLC) separations.

Retention time is somewhat dependent on the molecular weight of the peptide. The effect of molecular weight on retention is negligible with a small peptide, but increases with the size of the molecule. The accuracy of peptide retention time prediction significantly decreases for those peptides greater than approximately 20 residues.

Figures 1 and 2 illustrate the use of the Peptide Retention Standard for evaluating reverse phase supports of varying *n-alkyl* chain lengths and density. Figure 1 shows the standard run on an Aquapore™ Reverse-Phase Butyl (C4) Column. The standard is run under identical mobile phase conditions on an Aquapore Reverse-Phase Octyl (C8) Column in Figure 2. The peptides are eluted in the same order on both columns but the retention times vary by 3 to 4 minutes for each component. Using the peptide retention standard, it is possible to predict the retention time of any peptide of known amino acid composition. This allows evaluation of various reverse phase supports for optimal resolution of peptides of interest.

It is also possible to determine the most effective mobile phase system for a peptide separation on a given column. Figures 2, 3 and 4 show the separation of the peptide components (S1-S5) on the same Aquapore Reverse-Phase Octyl (C8) Column run in three different mobile phase systems. Using the retention standard, it is possible to predict retention times, under a given set of mobile phase systems, for peptides of known amino acid composition.

The excellent resolving power and selectivity of the TFA-H_2O/TFA-Acetonitrile gradient system enhances the separation of these model peptides on a reverse phase C18 column. The amino acid coefficients, determined from HPLC of the model peptides, may be used to predict the retention time of any peptide of known composition. To ensure accuracy, a standard peptide should always be run to correct for changes in retention time due to instrument variations, column aging or varying *n-alkyl* chain length and ligand density.
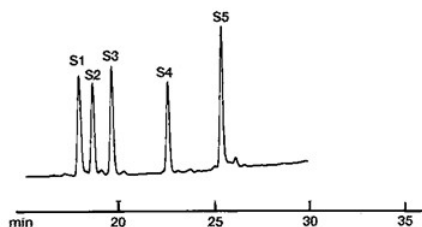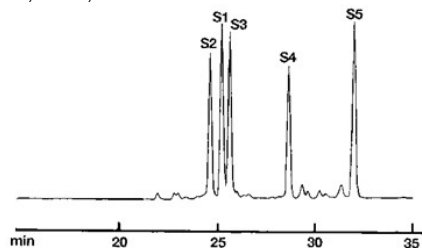
Figure 1. Peptide Retention Standard on Aquapore butyl, BU300 22cm x 4.6mm. Conditions: A) 0.1% TFA in water, B) 0.1% TFA in acetonitrile. Linear gradient, 1% B/min. 1.0mL/min, 26°C, 220nm.
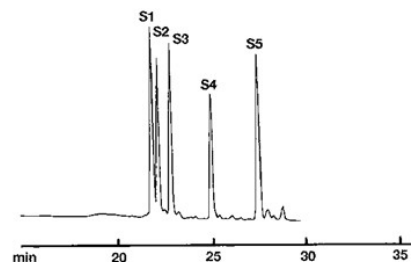
Figure 2. Peptide Retention Standard on Aquapore octyl RP-300 22cm x 4.6mm. Conditions: A) 0.1% TFA in water, B) 0.1% TFA in acetonitrile. Linear gradient, 1% B/min, 1.0mL/min, 26°C, 210nm.
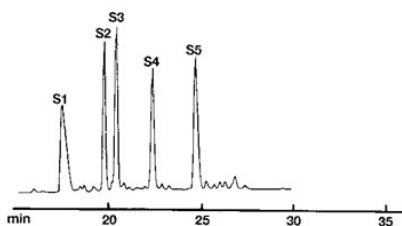
Figure 3. Peptide Retention Standard on Aquapore octyl RP-300 22cm x 4.6mm. Conditions: A) 0.1% aqueous H_3PO_4B) 0.1% H_3PO_4/acetonitrile. Linear gradient, 1% B/min, 1.0mL/min, 26°C, 210nm.

Figure 4. Peptide Retention Standard on Aquapore octyl RP-300 22cm x 4.6mm Conditions: A) 10mM $(NH_4)_2HPO_4$/0.1M $NaClO_4$, pH 7.0, B) 60% aqueous acetonitrile containing 0.1M $NaClO_4$.

Procedure

1. Dissolve contents of vial in 1mL of 0.1% TFA (trifluoroacetic acid) in water.

2. Add 5µL of 100% TFA (Product No. 53102 or 28902).

3. Inject 5-10µL onto the column. The first peak off the column is the excess TFA; this value is used for t_o. Use S4 for prediction of peptide retention. Standards S1-S5 are used to monitor column performance characteristics such as column efficiency, selectivity and resolution.

Calculation of Peptide Retention Time

The predicted retention time is based upon the following linear solvent gradient: starting composition of 100% A, followed by increasing concentration of B at 1%/min.

> A = 0.1% aqueous TFA
>
> B = 0.1% TFA in acetonitrile
>
> Flow rate: 1.0mL/min
>
> Temperature: 26°C

The predicted retention time (τ) for a peptide equals the sum of the retention coefficients (Σ Rc, see Table 1) for the amino acid residues and end groups, plus the time for elution of unretained compound (t_o) and the time correction for the peptide standard (ts) as given in the formula:

> $\tau = \Sigma R_c + t_o + t_s$

The observed retention time of peptide S4 (RtS4) within the peptide standard is used as an internal standard for the calculation of t_s where:

> $t_s = Rt_{S4} - (17.5 + t_o)$

These corrections (t_s and t_o) allow for the use of any HPLC apparatus; reverse-phase columns of any length and diameter; reverse-phase packings of any *n-alkyl* chain length and ligand density; any temperature; any flow rate.

Retention Coefficients of Amino Acid Residues

Amino Acid Residue	Retention Coefficients pH 2.0 (min)	pH 7.0 (min)	Amino Acid Residue	Retention Coefficients pH 2.0 (min)	pH 7.0 (min)
Trp	8.8	9.5	Thr	0.6	+0.3
Phe	8.1	9.0	Asp	0.2	-2.6
Leu	8.1	9.0	Gln	0.0	0.0
Ile	7.4	8.3	Ser	-0.2	-0.5
Met	5.5	6.0	Gly	-0.2	-0.2
Val	5.0	5.7	Arg	-0.6	+0.9
Tyr	4.5	4.6	Asn	-0.6	-0.8
Cys	2.6	2.6	His	-2.1	+2.2
Pro	2.0	2.2	Lys	-2.1	-0.2
Ala	2.0	2.2	α-amino	-6.9, -3.0[a]	-2.4, 0[a]
Glu	1.1	-1.3	α-COOH	-0.8	-5.2

[a]The charged α-amino group had a smaller effect in an *N*-terminal Arg residue than an *N*-terminal residue with an uncharged side chain.

General References

1. Guo, D., *et al.* (1985). Hydrophilicity parameters in peptides. 1. Prediction of peptide retention in reversed-phase HPLC (RPC). 2. Prediction of hydrophilic regions on the surface of proteins. In: Peptides-Proceedings of the Ninth American Peptide Symposium. C. M. Deber, V. J. Hruby, and K. D. Kopple, eds. Pierce Chemical Co., p. 23.

2. Guo, D., *et al.* (1986). Prediction of peptide retention times on reversed-phase high performance liquid chromatography (RPC): Determination of retention coefficients of amino acid residues using model synthetic peptides. *J Chromatogr* **359:**499-517.

3. Guo, D., *et al.* (1986). Prediction of peptide retention times on reversed-phase high performance liquid chromatography (RPC): 1. Correlation of observed and predicted peptide retention times. 2. Factors influencing retention times of peptides in RPC. *J Chromatogr* **359:**519-32.

4. Mant, C. T. and Hodges, R. S. (1986). Requirement for peptide standards in reversed-phase high performance liquid chromatography. *L C Magazine Liquid Chromatography and HPLC* **4:**250-4.

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3