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Analysis of Phosphorylated Amino Acids Using the Hitachi L-8900 Amino Acid Analyzer

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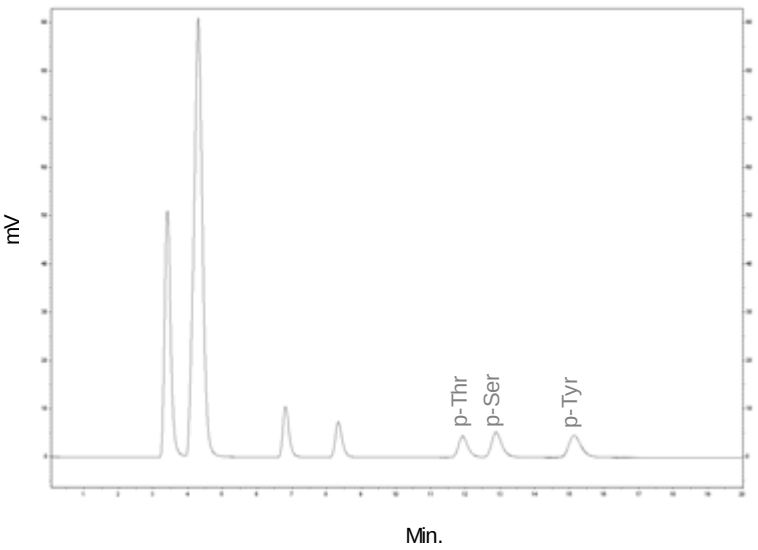
Phosphorylated amino acids are post-translationally modified amino acids used to regulate cellular processes. Analysis of these amino acids can be accomplished by protein hydrolysis followed by amino acid analysis. Due to the low pKa values of phosphorylated amino acids, anion-exchange methods are often preferable to the cation-exchange methods used for standard amino acids.

Here we describe a method for analysis of phosphorylated serine, threonine, and tyrosine. After anion-exchange separation, the analytes are derivatized with ninhydrin and detected by absorbance at 570 nm. The Hitachi L-8900 Amino Acid Analyzer, which utilizes a post-column derivatization system, is capable of analysis of phosphorylated serine, threonine, and tyrosine within 20 minutes.

Experimental Conditions

Component	Conditions
Mobile Phase	24 mM phosphate buffer, pH 4.0 in 8% CH_3OH, Flow Rate = 0.75 mL/min. Post-Column: 50:50 Ninhydrin:Buffer (Wako) Flow Rate: 0.4 mL/min.
Column	SUPELCOSIL LC-SAX1, 4.6 x 250 mm, 5 µm
Temperature	Column Oven: 25° C Reaction Column: 135° C
Detection Wavelength	570 nm
Injection Volume	20 µL
Standard	Amino Acid Standard (Sigma AA-S-18), o-phospho-L-serine, o-phospho-L-threonine, and o-phospho-L-tyrosine, 100 µM each in 0.02 N HCl

Results- Chromatograph of Amino Acid Standard



Results – Linearity (10 - 200 µM)

Amino Acid	R^2
p-Threonine	0.9999
p-Serine	0.9994
p-Tyrosine	0.9999

Results – Reproducibility (100 µM, N = 5)

Amino Acid	Retention Time (min.)	Peak Area (µV/sec.)
p-Threonine	0.45% = RSD	3.6% = RSD
p-Serine	0.47% = RSD	3.4% = RSD
p-Tyrosine	0.49% = RSD	6.2% = RSD

Discussion

Hitachi's Amino Acid Analyzer is effective at simultaneous analysis of multiple phosphorylated amino acids in under 20 minutes.

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