Waters
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TEN MINUTE RESOLUTION OF ALL PTH AMINO ACIDS ON NOVA-PAK™ C_{18} COLUMN

A common and complex chromatographic problem routinely encountered in the biochemistry laboratory is the separation of PTH amino acids which are the end product of the sequential Edman degradation of peptides and proteins. New developments in protein and peptide isolation, especially HPLC methods, and improved technology in peptide/protein sequence analysis have increased the requirements for speed and sensitivity in a PTH separation. Consequently, improved chromatography of the PTH derivatives is very desirable in order to provide low picomole detection limits and to maintain pace with one or more sequencers without tieing up multiple instrument systems.

The resolution of all the PTH amino acids on many bonded C_{18} reversed phase columns is complicated by the especially difficult separations of Met and Val and the group consisting of Phe, Lys and Ile. Previously, most published procedures have used small percentages of THF in acetonitrile in order to get any selectivity for the Met-Val separation, and long analysis times and elevated temperature (50-60°C) improved the separation of Lys/Phe/Ile. Another approach to improve selectivity has been the use of cyanopropyl columns. However, some workers have noticed batch-to-batch reproducibility problems with this stationary phase, and typically the basic PTH derivatives afford poorer peak shapes. A better approach is to use a NOVA-PAK™ C_{18} column (Figure 1) and substitute isopropanol for THF to obtain a favorable selectivity change in the relative retention of PTH Met and Val. In addition a less toxic, much more stable solvent is utilized.

Using a step gradient with two M6000A pumps and an M680 gradient controller a NOVA-PAK™ C_{18} column can separate 25 PTH amino acids in under 10 minutes. In addition low picomole detection (Figure 2) is possible. The very rapid analysis is very significant since it makes it possible for one system to analyze all the fractions from two sequencers.

It is also quite easy to translate the conditions for the two-pump system to a system consisting of just an M590 programmable pump equipped with a low pressure switching valve. The chromatography shown in Figure 1 requires just two solvents. Solvent 1 is the 80% aqueous buffer in Figure 2, and Solvent 2 is 50% solvent A, 25% water and 25% isopropanol. Note also that the methyl esters of Asp, Glu and carboxymethyl cysteine are well resolved in the system, as well as their corresponding free acids. The esters are the derivative of importance for a sequencer equipped with an autoconverter, while the acids are produced by most manual conversion methods.

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PTH Amino Acids on NOVA-PAK™ C_{18} Column

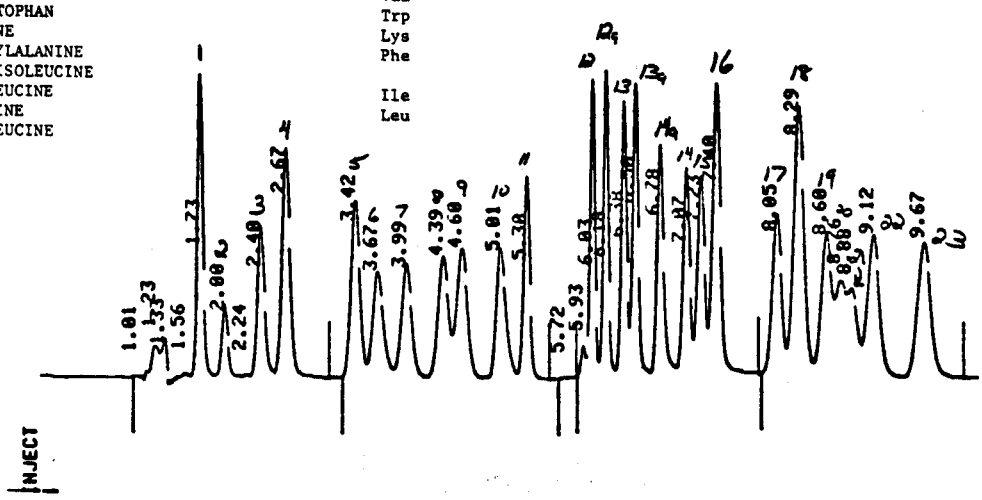
TABLE 1

IDENTIFICATION OF PTH DERIVATIVE PEAKS IN THE FIGURES

1.	ASPARTIC ACID	Asp
2.	CYSTEIC ACID	
3.	CARBOXYMETHYL CYSTEINE	
4.	GLUTAMIC ACID	Glu
5.	ASPARAGINE	Asn
6.	HISTIDINE	His
7.	SERINE	Ser
8.	THREONINE	Thr
9.	GLUTAMINE	Glu
10.	ARGININE	Arg
11.	GLYCINE	Gly
12.	ALANINE	Ala
12a.	ASPARTIC ACID METHYL ESTER	
13.	TYROSINE	Tyr
13a.	GLUTAMIC ACID METHYL ESTER	
14.	PROLINE	Pro
14a.	CARBOXYMETHYL CYSTEINE METHYL ESTER	
15.	METHIONINE	Met
16.	VALINE	Val
17.	TRYPTOPHAN	Trp
18.	LYSINE	Lys
19.	PHENYLALANINE	Phe
20.	ALLOISOLEUCINE	
21.	ISOLEUCINE	Ile
22.	LEUCINE	Leu
23.	NORLEUCINE	

FIGURE 1

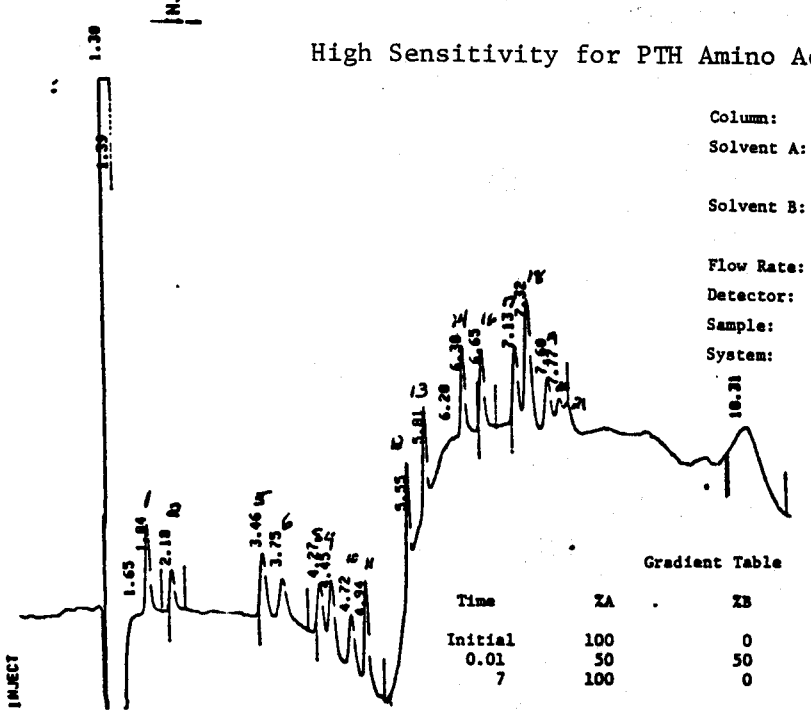
Column: NOVA-PAK™ C_{18}
Solvent 1: 80% 25 mM Sodium Acetate pH 5.15
20% Acetonitrile
Solvent 2: 25% H_2O
25% Isopropanol
50% Solvent 1
Flow Rate: 1 ml/min
Detector: Waters M440, 254 nm, at 0.5 AUFS
Sample: 1-2 nmol each derivative
System: M590 programmable pump with a low pressure switching valve



High Sensitivity for PTH Amino Acids - 10 pmol

FIGURE 2

Column: NOVA-PAK™ C_{18}
Solvent A: 80% 25 mM Sodium Acetate pH 5.15
20% Acetonitrile
Solvent B: 50% H_2O
50% Isopropanol
Flow Rate: 1 ml/min
Detector: Waters M440, 254 nm, at 0.01 AUFS
Sample: 10 pmol each PTH derivative
System: Two M6000A pumps plus an M720 System Controller



Gradient Table

Time	%A	%B	Flow	Curve
Initial	100	0	1	*
0.01	50	50	1	11
7	100	0	1	11