Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0391 4/89
DP/LS/RS/AA/PL

Fast Physiological Pico•Tag® Methods For Selected Groups of Amino Acids

Highlight from the Sixth World Wide Technical Meeting

The Pico-Tag method for the analysis of physiological samples provides the investigator with a quantitative tool for the separation and measurement of over 35 common and unusual amino acids in under ninety minutes (including column reequilibration). While this represents a significant improvement over traditional amino acid analysis techniques, there are several applications where faster analyses of limited numbers of amino acids are desirable.

The following procedures have been abstracted from The Pico-Tag Method: A Manual of Advanced Techniques for Amino Acid Analysis (Waters P/N 23883). The manual contains the complete procedures and additional chromatograms along with a great deal of other valuable information covering many aspects of both physiological and hydrolyzate amino acid analysis.

I. Hydrophobic Amino Acids

The group of amino acids from tyrosine to lysine in the standard separation is of interest for several reasons including (1) pathologic states resulting from inborn errors of metabolism; (2) nutritional studies; (3) wound healing and trauma, and (4) hyperalimentation. The details of the separation conditions for a forty minute (injection to injection) analysis are described below.

Sample Prep: Ultrafiltration is sufficient to remove high molecular weight contaminants from plasma and urine. Most of the low molecular weight interferents found in urine samples appear in the first quarter of the chromatogram and elute prior to the hydrophobic region.

Chromatography: Figure 1 shows that the chromatography of physiological amino acid standards is compressed to approximately twenty two minutes.

Figure 1.

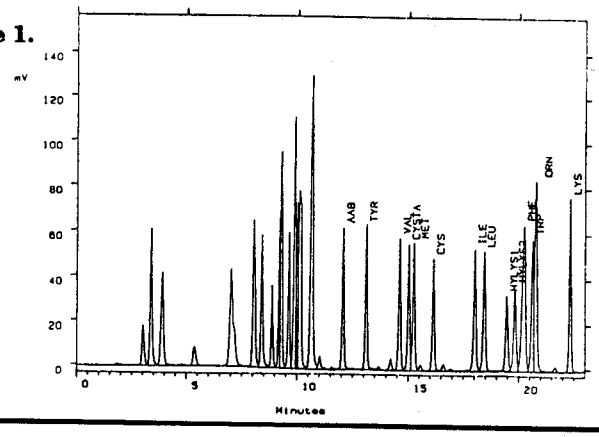

Table 1. Gradient table for hydrophobic amino acids.

Time	Flow	%A	%B	Curve
Init.	1.0	100	0	*
1.0	1.0	100	0	6
21.0	1.0	52	48	5
21.5	1.0	0	100	6
22.0	1.5	0	100	6
25.5	1.5	0	100	6
26.0	1.5	100	0	6
39.0	1.5	100	0	6
39.5	1.0	100	0	6

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Resolution of the early eluting amino acids is sacrificed in order to achieve a rapid separation of the hydrophobics. The chromatogram was generated using gradient Table 1 and using the hydrolyzate Eluent B (P/N 88108), which is a simple 60% acetonitrile solution, in place of the standard physiologic Eluent 2. Possible internal standards are ε-amino caproic acid (elutes before Tyr) and nitrophenylalanine (elutes between Reagent 2 and Lys).

II. Secondary Amino Acids

Recent research has demonstrated a direct connection between muscle and cartilidge breakdown and hydroxyproline levels in urine (and to a lesser degree in plasma). If both hydroxyproline and proline levels are required, the analysis time is 28 minutes per sample. If only hydroxyproline levels are of interest the analysis time is less than 10 minutes.

Sample Prep: Depends on the sample matrix and on whether there is a need to determine total as well as free amino acid levels. The appropriate techniques for preparation and quantitation are described in detail in The Pico-Tag Method.

Chromatography: The separation uses the standard physiologic Eluents 1 and 2. The gradient in Table 2 generates the separation shown in the chromatogram of a plasma sample (Figure 2). If only hydroxyproline is of interest the gradient in Table 3 is used for the 10 minute separation (not shown).

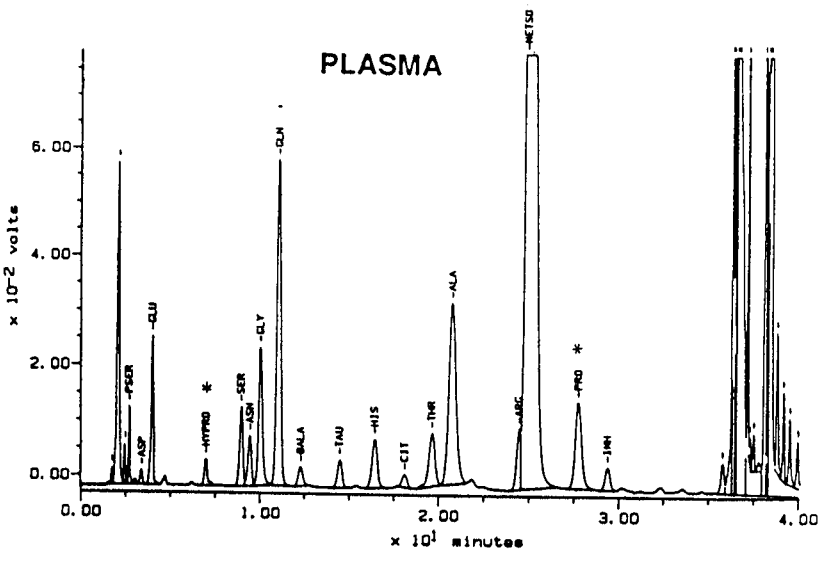

Figure 2.

Table 2. Gradient table for rapid analysis of hydroxyproline and proline.

Time	Flow	%A	%B	Curve
Initial	1.0	100	0	*
13.5	1.0	97	3	11
24.0	1.0	94	6	8
30.0	1.0	91	9	5
31.0	1.0	0	100	6
35.0	1.0	0	100	6
36.0	1.0	100	0	6
56.0	1.0	100	0	6

Table 3. Gradient table for rapid analysis of hydroxyproline.

Time	Flow	%A	%B	Curve
Init.	1.0	100	0	*
6.0	1.0	100	0	11
7.0	1.0	0	100	6
11.0	1.0	0	100	6
12.0	1.0	100	0	6
32.0	1.0	100	0	6

Note: For research use only! Not for use in *in vitro* diagnostic procedures.

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