

30-MINUTE AMINO ACID ANALYSIS OF HYDROLYZED SAMPLES

Pickering Laboratories specializes in the manufacturing of cation-exchange columns and eluants for amino acid analysis. No other technique has been shown to match the reproducibility and selectivity of ion-exchange analysis with post-column Ninhydrin detection. Nor is there a chromatography technique that provides as much information; the 570/440 nm signal ratio for each amino acid is a constant and so offers information about peak purity.

Pinnacle PCX post-column derivatization system provides a unique opportunity to combine eluant gradient capabilities of modern HPLC instruments with column temperature gradients. As might be expected, this capability also reduces the analysis time.

We introduce an accelerated Amino Acid analysis method for hydrolyzed samples that utilizes temperature and eluant gradients.

METHOD

Analytical Conditions

Column: High-efficiency Sodium cation-exchange column, 4.6 x 110 mm,

Catalog number 1154110T

Flow Rate: 0.6 mL/min

Mobile Phase: Na315, Na740, RG011

Post-Column Conditions Post-Column System: Pinnacle PCX

Reactor Volume: 0.5 mL

Temperature: 130 °C

Reagent: Trione Ninhydrin reagent

Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids 440 nm for secondary amino acids

Runtime: 30 min

Equilibration Time: 9 min

COLUMN OVEN PROGRAM			
TEMP °C			
46			
46			
55			
70			
70			
46			

HPLC PROGRAM			
TIME	Na315 %	Na740 %	RG011 %
0	100	0	0
7.3	100	0	0
15	53	47	0
15.1	0	100	0
28	0	100	0
28.1	0	0	100
30	0	0	100



Chromatogram of Protein Hydrolysate Standard

1 Aspartic Acid 2 Threonine

Serine

Proline

Glycine

Alanine

Glutamic Acid

3

4

5

6

7

- 8 Cystine 9
 - Valine
- 10 11
- 12 Leucine
- 13 Tyrosine
- 14
- Methionine Isoleucine
 - 18 Arginine 19

16

17

15 Lysine

Histidine

Ammonia

Tryptophan

Phenylalanine