

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.

Onyx 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:** See method

### Post-Column Conditions

**Post-Column System:** Onyx PCX or 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

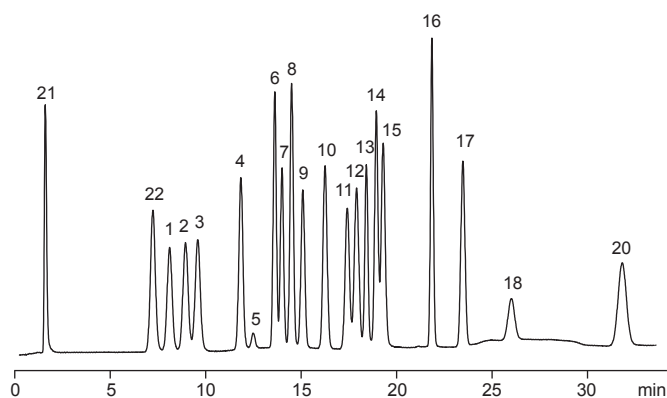


Fig 1. Chromatogram of oxidized feeds hydrolysate standard

- |                 |                  |                       |
|-----------------|------------------|-----------------------|
| 1 Aspartic Acid | 9 Valine         | 17 Lysine             |
| 2 Threonine     | 10 Methionine    | 18 Tryptophan         |
| 3 Serine        | 11 Isoleucine    | 19 Ammonia            |
| 4 Glutamic Acid | 12 Leucine       | 20 Arginine           |
| 5 Proline       | 13 Norleucine    | 21 Cysteic Acid       |
| 6 Glycine       | 14 Tyrosine      | 22 Methionine Sulfone |
| 7 Alanine       | 15 Phenylalanine |                       |
| 8 Cystine       | 16 Histidine     |                       |

Method for Oxidized Feeds Hydrolysate Samples				
Time	Na270, %	Na425, %	Na640, %	RG011, %
0	100	0	0	0
4.0	100	0	0	0
15.0	0	100	0	0
16.0	0	0	100	0
31.0	0	0	100	0
31.1	0	0	0	100
33.0	0	0	0	100
33.1	100	0	0	0
40.0	100	0	0	0

Column Oven Program	
Time	Temp, °C
0	55
12	55
17	70
32	70
33	55

**Method for Protein Hydrolysate Samples**

Time	Na315, %	Na425, %	Na640, %	RG011, %
0	100	0	0	0
4.0	100	0	0	0
15.0	0	100	0	0
16.0	0	0	100	0
31.0	0	0	100	0
31.1	0	0	0	100
33.0	0	0	0	100
33.1	100	0	0	0
40	100	0	0	0

**Column Oven Program**

Time	Temp, °C
0	46
4	46
9	70
32	70
33	46

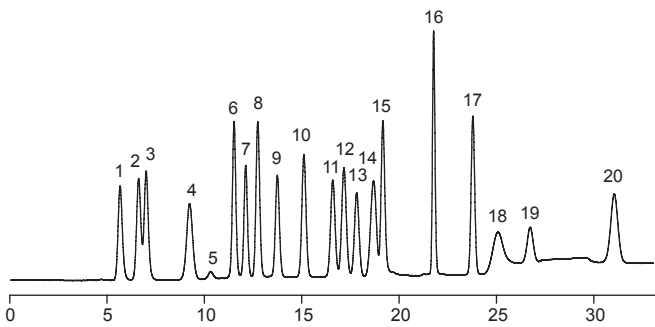


Fig 2. Chromatogram of protein hydrolysate standard

- |                 |                  |                       |
|-----------------|------------------|-----------------------|
| 1 Aspartic Acid | 9 Valine         | 17 Lysine             |
| 2 Threonine     | 10 Methionine    | 18 Tryptophan         |
| 3 Serine        | 11 Isoleucine    | 19 Ammonia            |
| 4 Glutamic Acid | 12 Leucine       | 20 Arginine           |
| 5 Proline       | 13 Norleucine    | 21 Cysteic Acid       |
| 6 Glycine       | 14 Tyrosine      | 22 Methionine Sulfone |
| 7 Alanine       | 15 Phenylalanine |                       |
| 8 Cystine       | 16 Histidine     |                       |