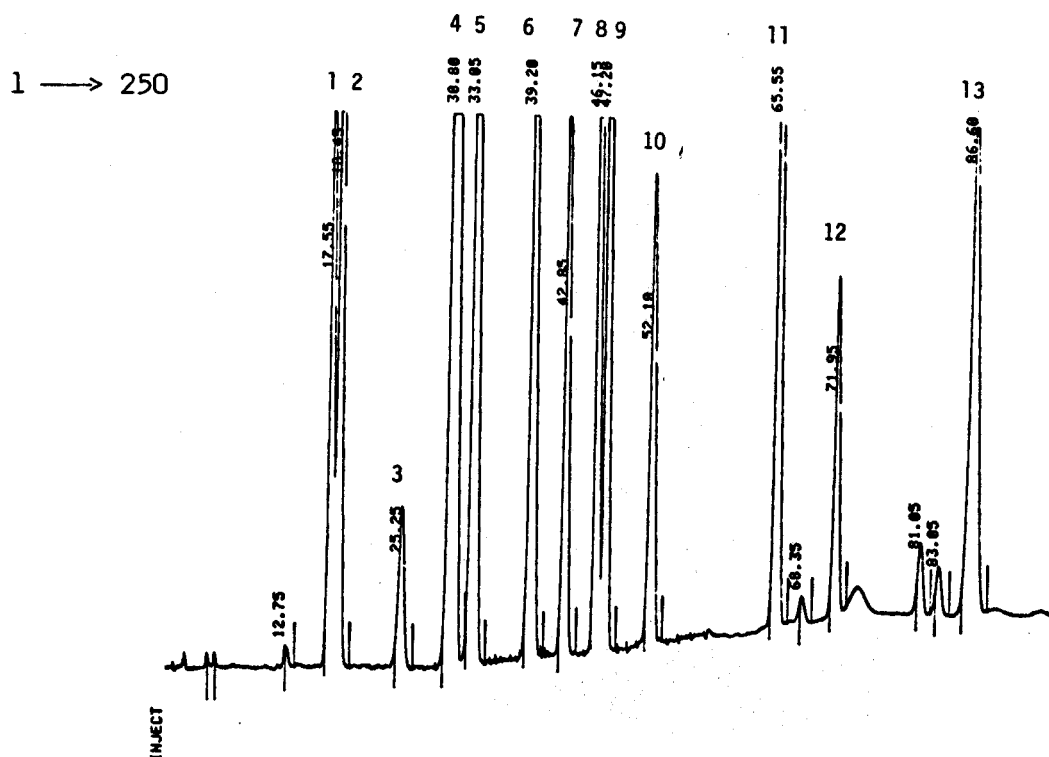


ANALYSIS OF INTRAVENOUS AMINO ACID SOLUTION USING NINHYDRIN DETECTION

Producers of intravenous amino acid solutions, like other pharmaceutical manufacturers, are required by law to verify the potency of their products prior to release for distribution. Thus, analysis of intravenous amino acid solutions requires a high level of precision and accuracy, and frequently a wide dynamic range of detection as well. The Waters HPLC Amino Acid Analysis System, with the ninhydrin detection option, meets both of these requirements.

The chromatogram below shows a typical IV formulation. The sample was diluted 1→250, and injected. Analysis conditions were as follows:

Sample: 10% Amino Acid Infusion,
1 to 250 (v/v)
Column: Waters AAA, 25cm x 4.6mm
Column Temp: 62°C
Eluents: A: 0.2N Na⁺, pH 3.15 (Pickering P/N Na315)
B: 1.0N Na⁺, pH 7.40 (Pickering P/N Na740)
Gradient: 0-80% B, 45 min., Curve 8
80-100% B, 60 min., Curve 8
Run Time: 105 minutes
Injection Volume: 50µl
Detection: Ninhydrin @ 546nm (M440 @ 0.5AUFS)



Amounts of each amino acid injected ranged from 3.5 to 37 nmoles.

Table 1 lists the CV's for each amino acid from five replicate injections. Note that outstanding precision is attained with single channel monitoring at 546nm, including proline.

TABLE 1

COEFFICIENTS OF VARIATION FOR FIVE REPLICATE INJECTIONS

1. Threonine	0.4%
2. Serine	0.3%
3. Proline	1.3%
4. Glycine	0.9%
5. Alanine	0.6%
6. Valine	0.3%
7. Methionine	0.6%
8. Isoleucine	0.3%
9. Leucine	0.1%
10. Phenylalanine	0.8%
11. Lysine	0.5%
12. Histidine	0.6%
13. Arginine	0.5%