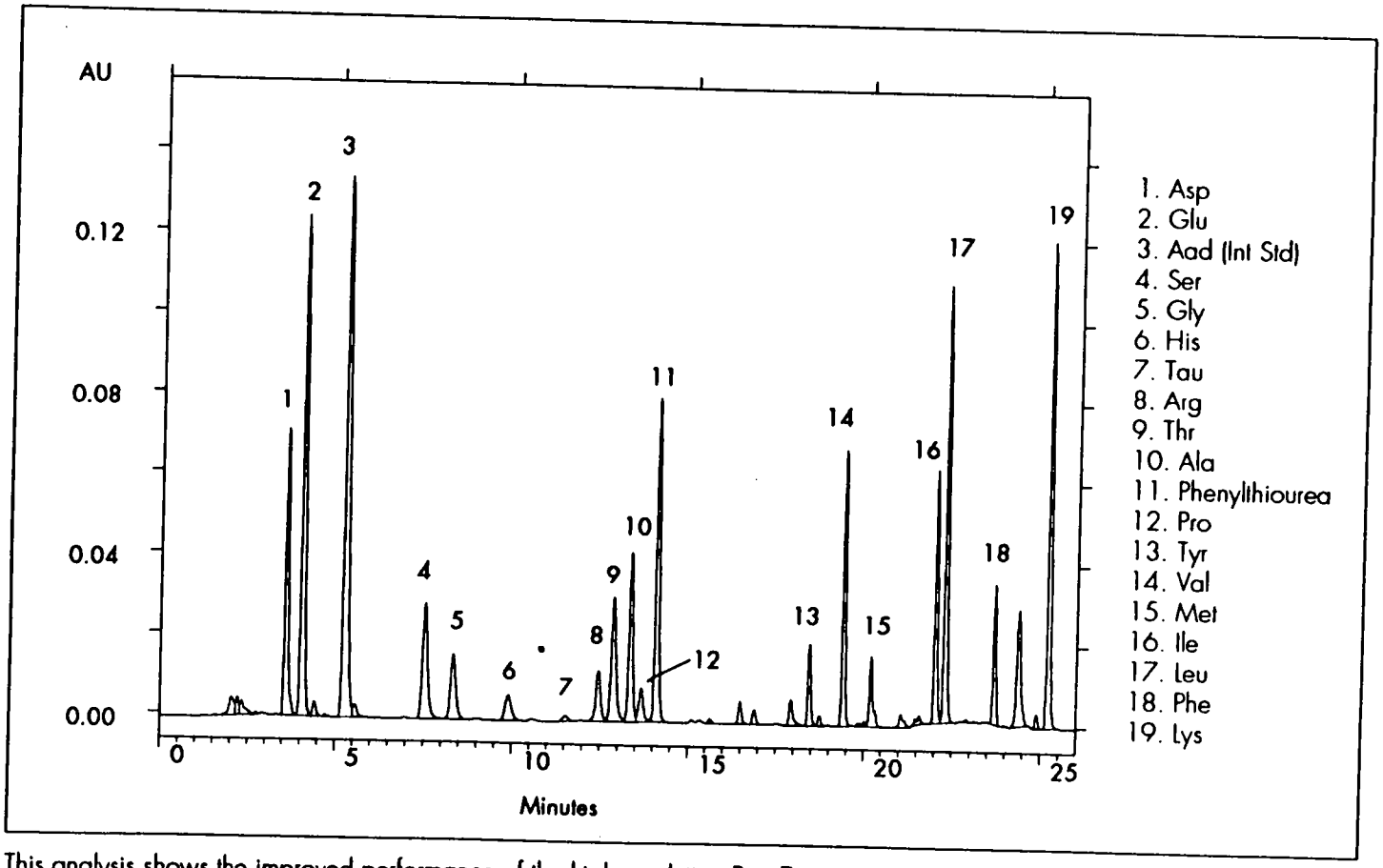
Waters Application Notebook

930530

Steve Cohen
012

Pico•Tag™ Amino Acid Analysis of Infant Formula



Conditions:

Sample: Hydrolyzed liquid infant formula

Column: Pico-Tag® Column for Free Amino Acid Analysis, (3.9 x 300 mm)

Eluent A: Pico-Tag Eluent A, adjusted to pH 6.10 with acetic acid

Eluent B: Pico-Tag Eluent B

Gradient: See details on back

Flow Rate: 1.0 ml/min (see details in notes on back)

Detection : UV @ 254 nm

Injection Volume: 20 µL

This analysis shows the improved performance of the high resolution Pico-Tag system with a food-type sample. Use of the longer 30 cm column in place of the standard 15 cm column has several key advantages including (1) the ability to easily resolve an unusual amino acid, namely Taurine (Tau) (2) improved resolution throughout, simplifying integration and quantitation (3) more separation of the initial peaks from injection artifacts

Objective:

To demonstrate the high resolving power of the Pico-Tag Free Amino Acid Analysis Column with a non-physiologic sample, liquid infant formula, and highlight the capabilities of the Pico-Tag method for the analysis of food-type samples.

Details:

Sample Preparation: Liquid infant formula concentrate was diluted 4-fold with Milli-Q water and 1 ml of the diluted sample mixed with 1 ml of concentrated HCl. The hydrolyzate sample was alternately flushed with nitrogen and evacuated three times and finally sealed under vacuum. After 22 hours at 112°C, the sample was cooled, diluted to 10ml with solution containing the internal standard, alpha-amino adipic acid (AAD). A 20 µl aliquot of this solution was then taken and derivatized using the standard Pico-Tag derivatization procedure. The derivatized amino acids were reconstituted in 100 µl of Pico-Tag diluent.

Additional Details: The column washing and re-equilibration steps can be shortened by increasing the flow rate to 1.5 ml/min during these steps (see gradient table below). Total analysis in then approximately 46 minutes. The length of the hold at 4 minutes can be adjusted to maximize resolution of the phenylthiourea (PTU, or the "ammonia peak"), where increasing the hold decreases the resolution of Ala and PTU, but increases the resolution of PTU and Pro.

Gradient:

Time (min)	Flow	%A	%B	Curve
Init.	1.0	100	0	*
4	1.0	100	0	6
21	1.0	54	46	6
27	1.0	54	46	6
27.5	1.0	0	100	6
28	1.5	0	100	6
31.5	1.5	0	100	6
32	1.5	100	0	6
45	1.5	100	0	6
45.5	1.0	100	0	6

System:

System: Standard Pico-Tag system including
- 2 x M510 pumps
- M712 WISP refrigerated autosampler
- M440 UV detector
- TCM column heater set at 35° C
- MPSA solvent conditioning system (blanket mode)
- M860 control and data system

References:

1. Liquid Chromatography Analysis of Amino Acids in Feed and Food Using a Modification of the Pico-Tag Method, a Waters manual, revised 1990.

2. Nancy E. Astephen and Steven A. Cohen, "Quantitative Determination of Amino Acid Content of Infant Formula via Derivatization with Phenylisothiocyanate; Reproducibility of a Precolumn Derivatization Method with a Complex Sample Matrix." Eleventh International Symposium on HPLC of Proteins, Peptides and Polynucleotides, October 20-23, 1991, Washington, DC USA