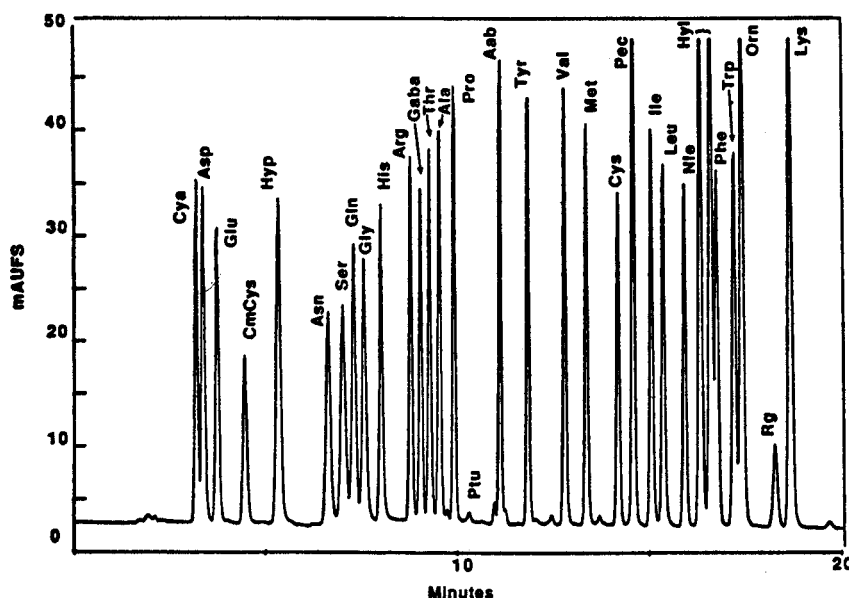


Very High Resolution Pico-Tag® Analysis

Waters Pico-Tag Chemistry Package for hydrolyzates (P/N 07360) uses the high efficiency Pico-Tag column (3.9 x 150 mm, P/N 88131) and guarantees a minimum resolution 85%, (with many peaks baseline resolved) yet there are customers who desire even more resolution. "That separation is very good, but I don't need a twelve minute separation. Are there analysis conditions that provide baseline resolution?" The answer to this should be an unqualified "yes"!

The key to very high resolution is the 3.9 x 300 mm Pico-Tag Free Amino Analysis Column (p/n 10950). These columns are extremely efficient ($N_{avg} > 22,000$) and provide the horsepower for superb separations. Using the gradient shown below, rapid analysis is still possible with a sample injected every forty minutes. In Figure 1, not only are all the hydrolyzate amino acids separated in 20 minutes, but a number of other important amino acids and derivatives are resolved in the same run. These include cysteine derivatives cysteic acid (Cya), carboxymethylcysteine (CmCys) and pyridylethylcysteine (PEC), hydroxyproline (Hyp) and hydroxylysine (Hyl), and the amides asparagine (Asn) and glutamine (Gln). Except for the extended run time, all other specifications for the Pico-Tag separation are met or exceeded -- and this is still guaranteed! Note that only the gradient conditions and column temperature have been adjusted for the new column. It is also important to note that the separation still employs the same high quality hydrolysis eluents (Pico-Tag A and B) available from Waters.

Figure 1



AMINO ACID STANDARD MIX

Column

Pico-Tag®

Free Amino Acid

Eluents

Pico-Tag A and B

Temperature

46 °C

**Gradient Table - Hydrolyzate Amino Acids
3.9 X 300 mm Pico-Tag Column**

<u>Time</u>	<u>Flow</u>	<u>%A</u>	<u>%B</u>	<u>Curve No.</u>
Init.	1.000	100	0	*
1.0	1.000	100	0	6
21.0	1.000	56	44	5
21.5	1.000	0	100	6
22.0	1.500	0	100	6
26.0	1.500	0	100	6
26.5	1.500	100	0	6
39.0	1.500	100	0	6
39.5	1.000	100	0	6

To summarize the benefits of the two columns:

Pico-Tag Hydrolysis Column (3.9 x 150 mm)

- Fast, 12 minute analysis, 21 minutes between injections
- Detection limits ≤ 1 pmol
- Lower cost
- Lower backpressure

Pico-Tag Free Amino Acid Analysis Column (3.9 x 300 mm) for Hydrolysis Analysis

- Baseline resolution
- Detection limits 1-2 pmol
- Extra resolution provides peak capacity for non-standard amino acids

* * *

The following trademarks are the property of Millipore Corporation, Bedford, MA 01730 USA: Nova-Pak, Pico-Tag, Waters.