Waters
Lab Highlights
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WATERS ANNOUNCES PICO·TAG™ ANALYSIS

A PRECOLUMN DERIVATIZATION METHODOLOGY FOR AMINO ACID ANALYSIS

In recent years the use of reverse-phase HPLC has become increasingly widespread for the separation and quantitation of derivatized amino acids. This rising popularity is especially evident with those researchers involved with low level amino acid analysis of rare or valuable samples as well as laboratories that are overburdened with samples that conventional ion-exchange systems are too slow and too insensitive to handle.

The most prevalent reagents used for precolumn techniques have been ortho-phthalaldehyde (OPA) and dansyl or dabsyl chloride. However, OPA does not react with proline and hydroxyproline, and derivative stability is a problem, while the latter two reagents cause very large reagent peaks and can yield multiple derivatives with several amino acids, most notably histidine and tyrosine.

FIGURE 1

Column: PICO·TAG™ Reverse Phase
Eluents: A = PICO·TAG™ A
B = PICO·TAG™ B
Flow: 1 ml/min.
Gradient: 0-46% B in 10 minutes, Curve 5
Sample: 250 pmol of each amino acid derivatized with PITC
Detection: UV 254 nm @ 0.1 AUFS

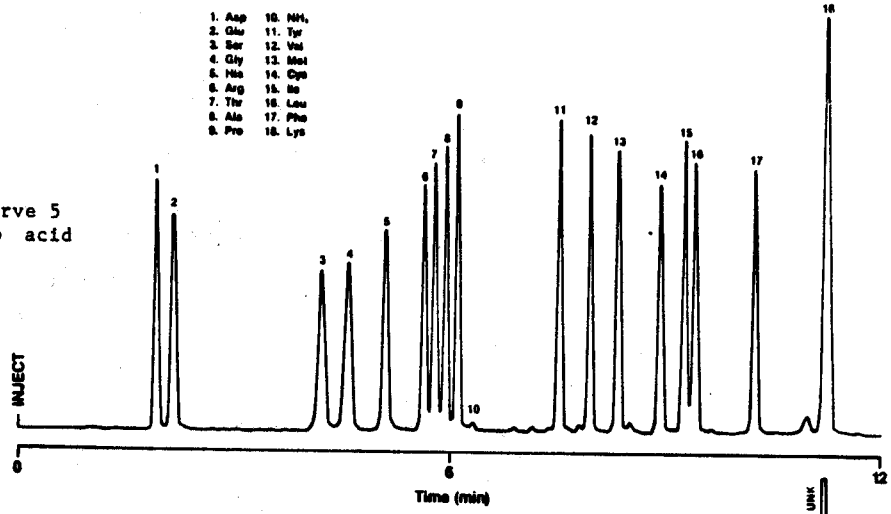
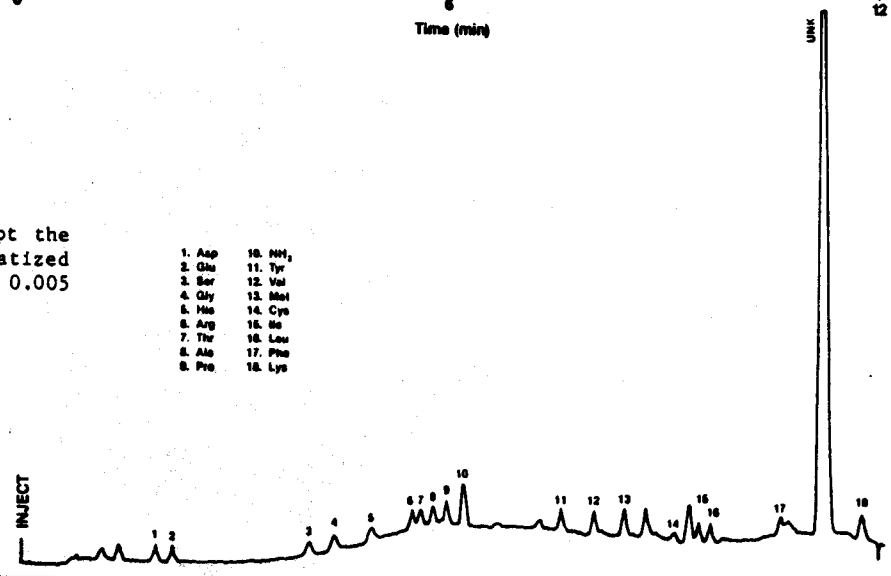


FIGURE 2

Conditions same as in Figure 1, except the sample was 1 pmol each of the derivatized mixture, and the detector was set at 0.005 AUFS.



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Now Waters introduces the PICO·TAG™ Method, a complete, packaged methodology using phenyl isothiocyanate (PITC), the well-known Edman sequencing reagent. This remarkable technique solves the most pressing problems of the other precolumn methods, yet pushes the speed and sensitivity to levels that will satisfy even the most demanding scientist (1,2).

Figure 1 shows the separation of 250 pmol of amino acid standard. Note the excellent resolution achieved throughout even though the analysis is complete in under twelve minutes. Of course, proline (Peak 9) responds equally as well as the primary amino acids since the same chromophore is produced. Very high sensitivity is illustrated in the chromatogram in Figure 2 (only 1 picomole of standard!).

Other outstanding features of the PICO·TAG™ Method include quantitative reaction, excellent reproducibility and linearity in the range 10-500 picomoles, very good derivative stability even at room temperature, and complete reagent volatility for low interference after vacuum drying. Typical PICO·TAG™ results are shown in Table I with a comparison to an ion-exchange analysis of myoglobin.

TABLE 1

COMPARISON OF PICO-TAG™ WITH ION-EXCHANGE ANALYSIS:

SPERM WHALE MYOGLOBIN

	RATIO FOUND		
	PICO-TAG™	ION-EXCHANGE	THEORETICAL
Asp	8.0	8.7	8
Glu	18.5	21.3	19
Ser	5.7	5.8	6
Gly	11.3	11.3	11
His	11.2	11.7	12
Arg	4.5	4.1	4
Thr	4.2	5.1	5
Ala	17.1	17.3	17
Pro	4.0	-	4
Tyr	3.0	3.4	3
Val	6.8	6.3	8
Met	2.0	2.1	2
Ile	8.3	6.9	9
Leu	17.8	17.2	18
Phe	5.9	6.2	6
Lys	18.6	18.5	19

All this is available in a complete package that includes a novel sample preparation module, the PICO·TAG™ Work Station. This combination vacuum/oven system makes the hydrolysis and derivatization of twelve samples simultaneously a simple procedure. In addition, cumbersome flame-sealing of individual samples is eliminated. The PICO·TAG™ package also includes all the reagents needed for derivatization and high quality eluents for low level, high sensitivity analysis.

1. Bidlingmeyer, B. A., Cohen, S. A. and Tarvin, T. L., J. Chromatogr. accepted for publication.
2. Koop, D. R., Morgan, E. T., Tarr, G. E. and Coon, M. J., J. Biol. Chem. 257, (1982), 8472.