Waters
Lab Highlights

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Pico•Tag® Free Amino Acid Analysis: How Does It Stack Up to Ion-Exchange?

New methodologies for free amino acid analysis can be subject to intense scrutiny by potential buyers, particularly with regard to chromatographic resolution and quantitative analysis. With this in mind, several customers have undertaken extensive comparative studies of their Pico•Tag systems with the ion-exchange analyzers they were replacing to assure themselves of method equivalency. These comparisons have all proved satisfactory, and we anticipate the publication of such information in the scientific literature in the future.

In the critical areas of resolution and quantitation the Pico•Tag method has no peer among the other reverse phase methods relying on precolumn derivatization procedures. But how does it compare to the benchmark ion-exchange method? Although ion-exchange has been in use for nearly 30 years, methods and instrumentation have undergone numerous significant changes in that period of time. It might appear that modifications necessarily mean improvement, but without thorough comparisons to earlier, fully evaluated systems the most recent variations should be considered to be, at best, unproven technology. For example, take a look at what a well-known expert has said concerning the newer, rapid (i.e. 2 hour) ion-exchange separations. "Even if only the common amino acids are present in the sample, there are likely to be problems of resolution owing to variations in ion-exchange resins requiring the manipulation of buffer pH and column temperature."[1] And furthermore the ". . . problem becomes more severe with more complex samples . . . when changing from sodium to lithium citrate buffers . . ."

A comparison of the standard Pico•Tag physiologic analysis (taken from a recent review article[2]) with the normal ion exchange method promoted by Beckman[3] is shown in Figure 1. For most components both systems exhibit excellent resolution; however both systems have a few peaks with something less than baseline resolution. Hence it is probably fair to state that ion-exchange resolution is no better than what the Pico•Tag system offers, and because the peak capacity (essentially the spaces for more peaks from unexpected sources) is no greater, the probability of coelutions due to extra peaks from, for example, patient ingested drugs, is equivalent.

How about quantitation? Isn't ion-exchange more thoroughly tested? Based on a thorough search of the literature, an up-to-date review on amino acid analysis in physiologic fluids contained the following cogent comment: "From the available

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information, it may be concluded that data on amino acid analysis of physiologic fluids by automated ion exchange analyzer *must, at best, be considered uncertain* due to the lack of sufficient reports on methodological data and validation in the open literature"[4] (emphasis added).

Significantly, both of these quoted articles refer to dedicated ion-exchange systems, with their deficiencies of high purchase price, high operating costs, and costly maintenance and service compared to the Pico•Tag system. In the case of HPLC-based ion-exchange analyzers, the published information for physiologic analysis is even far scarcer. Resolution can suffer terribly, particularly in the region near asparagine, glutamic acid and glutamine as well as several peak pairs in the basic region (a failing of some rapid, dedicated systems, too!). In addition, we know of no thorough study of quantitation in the scientific literature.

The Pico•Tag system may be a relative newcomer to free amino acid analysis, but with this publication and others in the literature, there is already sufficient data to make Pico•Tag analysis one of the best studied methods available.

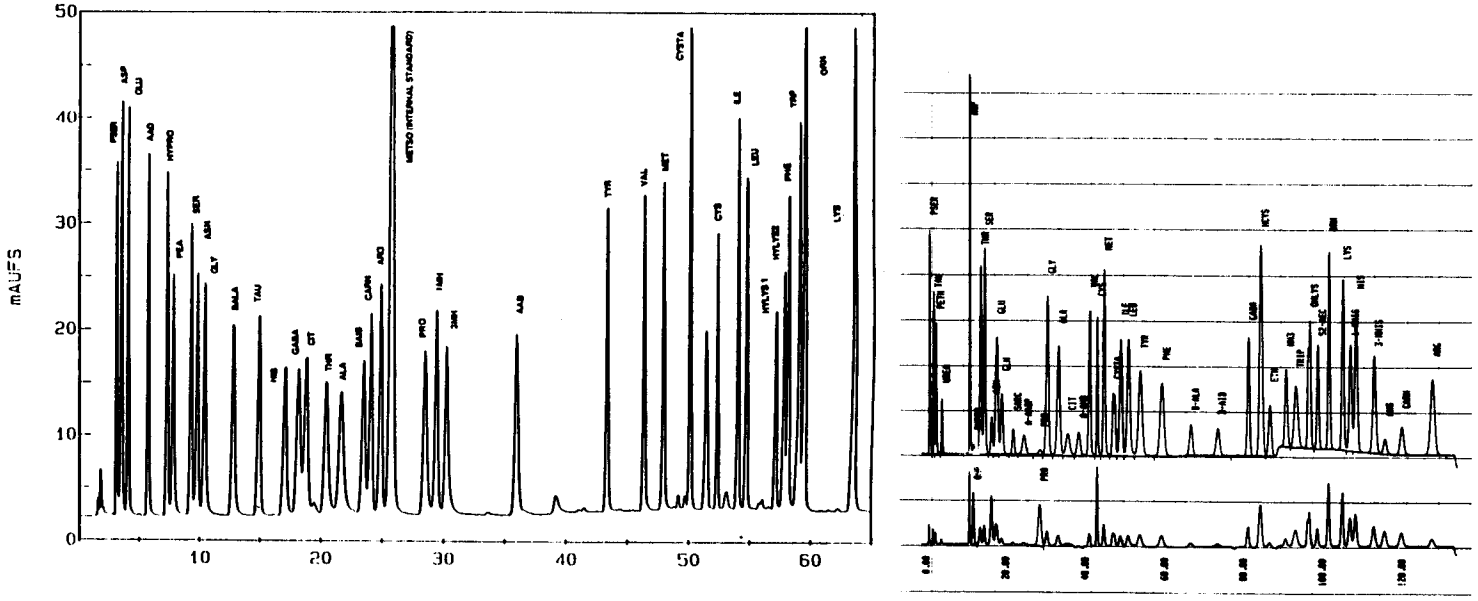

Figure 1.: **Pico•Tag Analysis**
500pmol Injected

Ion-Exchange Analysis
5nmol Injected

References

1. A.P. Williams, *J. Chromatogr.*, **373** (1986) 175.
2. S. A. Cohen and D. Strydom, *Anal. Biochem.*, **174** (1988) 1.
3. Beckman document #SB-666A, Beckman Instruments, Inc., Palo Alto, CA, (1989).
4. I.M. Moodie, G.S. Shephard and D. Labadarios, *J. High Res. Chrom.*, **12** (1989) 509.

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