LAH 0448 11/90 DP/LS/RS/AA/DV

## Sensitivity Limits for Amino Acid Detection Using the Pico•Tag (Phenylisothiocyanate) Procedure

The Pico•Tag® method, based on the formation of PITC derivatives, is one of the most popular approaches to the qualitative and quantitative analysis of complex mixtures of amino acids. Using a system as configured by Waters, detection limits of less than 1 pmol can be readily attained. Columns can be used for upwards of 500 analyses. While this procedure provides slightly less sensitivity than reported for some fluorescent derivatives (e.g. OPA, FMOC, Dansyl Chloride), the performance, reliability, ease of operation and the support from Waters has led to hundreds of satisfied users.

Two papers by Furst et. al. appeared recently in which the authors compared various methods of amino acid analysis by reverse phase HPLC. In one paper<sup>1</sup>, the summary table indicates a sensitivity of 50 pmole using PITC chemistry, while in the other paper<sup>2</sup> the summary table indicates 5 pmole sensitivity. Also, they report that the separation is satisfactory for only 150 analyses. Prospective customers who have read the Furst papers may feel that the PITC/Pico•Tag procedure cannot provide the same level of sensitivity that fluorescence techniques can provide. These reports are clearly misleading and in discussions with customers the following points should be made.

There are several possible reasons why Furst did not attain the sensitivity of 1 pmol, including:

- 1) They did not use the Waters™ system, e.g. the Model 440 detector with the patented Taper-Cell® for smoother baselines and the Pico•Tag workstation.
- 2) Trace impurities in their PITC reagent or buffer may have lead to an elevated baseline, which reduces sensitivity. They used a Spherisorb ODS, 3µ column with phosphate buffers, rather than the Waters application specific column and acetate buffers to separate the derivatives.

In addition to their erroneously high sensitivity estimates, these authors make other misleading statements:

1) "Since UV detection has to be employed, the sensitivity of the PITC-method is not as high as with fluorescence detection".

The fact is that although fluorescence detection can frequently provide better sensitivity than UV detection, it is not <u>always</u> the case. All of the factors must be considered when comparing two different assays. In this case, important factors to consider include the completeness of the conversion of the compounds of interest to their derivatives, the intensity of the detector light source, and the absorbance and fluorescence of the derivatives.

2) "The reproducibility of the method ranged between 2.6% and 5.5% (C.V.) for all amino acids except histidine (6.3%) and cystine (10.0%). PTC-cystine also revealed an inferior linearity and therefore the determination of free cystine is not practical with this method."

A relative standard deviation of 3.85% for cystine and 1.85% for histidine has been reported<sup>3</sup> using the Pico•Tag method. Without having all of the experimental details, it is not possible to speculate why Furst et.al. observed such anomalies.

Furst et. al. appear to favor the OPA and FMOC methodologies for the determination of amino acids, even though those methods cannot measure cystine.

In an upcoming Lab Highlight we will compare and contrast the four commonly used techniques for amino acid quantitation in some detail.

## References

The following trademark is the property of Millipore Corporation, Bedford, MA 01730 USA:

The following registered trademarks are the property of Millipore Corporation, Bedford, MA 01730 USA: Pico Tag. Taper-Cell

<sup>1.</sup> P. Furst, L. Pollack, T.A. Graser, H. Godel and P. Stehle, J. Liq. Chrom. 12 (14) (1989) 2733.

<sup>2.</sup> P. Furst, L. Pollack, T.A. Graser, H. Godel and P. Stehle, J. Chrom., 499 (1990) 557.

<sup>3.</sup> B.A. Bidlingmeyer, S.A. Cohen and T.L. Tarvin, J. Chrom., 336 (1985) 93.