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Bidlingmeyer B A, Cohen S **A** & **Tarvin T L.** Rapid analysis of amino acids using pre-column derivatization. *J. Chromatogr.—Biomed. Appl.* 336:93-104, 1984.
[Waters Associates, Milford, MA]

A method is described for the analysis of amino acids using derivatization with Edman's reagent, phenylisothiocyanate. Analysis time was less than 12 minutes with detection limits at the picomole level. [The *SCI*® indicates that this paper has been cited in more than 1,065 publications.]

Modern Methods for Amino Acid Analysis

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After several years of research on the fundamental behavior of proteins and peptides in reversed phase high-performance liquid chromatography (HPLC) at Northeastern University, I came to Waters Associates in 1982 with the express task of developing a new HPLC-based method for amino acid analysis. This effort arose from a collaboration among Waters, the late Emmett L. Durrum, then president of Eldex Corporation, and George Tarr at the University of Michigan. George should be considered the father of phenylisothiocyanate (PITC)-based amino acid analysis, for it was his work that formed the basis for our amino acid system, while Emmett provided the glue that wedded us at Waters to George's exciting new method.

George's extensive experience with protein chemistry had led him to apply the initial coupling step of the Edman degradation procedure[1] to amino acid analysis. This labels the amine functionality with PITC, Edman's reagent, and forms a moderately stable phenylthiocarbamyl derivative. An early publication describing (in only two paragraphs!) the analysis of free amino acids released by carboxypeptidase digestion[2] only hinted at the potential for this new technique.

At the time of our initial studies, nearly all amino acid analysis employed the classical ion-exchange method developed by S. Moore and W.H. Stein.[3,4] Commercial analyzers, essentially the first instrumental HPLC systems, have been available for over 25 years, and the methodology was well entrenched in biochemical laboratories in the early 1980s. Thus, we all realized that it would be no easy task convincing protein chemists that their comfortable but stodgy ion-exchange instruments were no longer capable of providing the highest level of performance.

At Waters, my coauthors and I began the somewhat laborious task of turning George's good science into a rugged method, useful for a wide variety of samples and convenient enough for wary researchers who often were only cursorily familiar with HPLC. It is perhaps a little difficult to remember that only 10 years ago HPLC was just beginning to make inroads in the biochemistry laboratory and was often viewed as difficult to learn and somewhat unreliable for routine use. Ease of use and reliability are both essential ingredients for amino acid analysis, and it was precisely these attributes that our work addressed. Picomole sensitivity, up to 100 times better than ion-exchange analysis, and rapid analysis times were significant advances over the traditional technique, but I believe that acceptance of the new methodology was largely dependent on our ability to provide researchers with a fully documented procedure that eliminated the need for any methods development in their own laboratories. I think it is also true that familiarity with the underlying Edman chemistry, which is described in every modern biochemistry textbook, was essential for broad-based acceptance in the biochemical world.

One curiosity in this project was the nearly simultaneous publication of similar studies by my own graduate research advisor, Bob Heinrikson, at the University of Chicago, who, unknown to us at Waters, was also applying PITC chemistry to amino acid analysis.[5] Our work led to two awards, the Millipore Prize for Innovation and an IR 100 award from *Research & Deve/opmenf* magazine. Although our original publication probably has been cited most often, subsequent studies extending the method to a wider variety of samples[6] and a recent comprehensive review[7] have been key factors in the widespread adoption of the procedure.

1. **Edman P.** Method for determination of the amino acid sequence in peptides. *Acta Chem. Scand.* 4:283-93, 1950. (Cited 815 **times.)**
2. **Koop D R, Morgan E T, Tarr** G E *&* **Coon M** J. Purification and characterization of a unique isozyme of cytochrome P-150 from liver microsomesof ethanol-treated rabbits. *J. Biol. Chem.* 257:8472-80, 1982. (Cited 375 times.)
3. **Moore S & Stein W H.** Photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol Chem.* 176:367-88. 1948. (Cited 2.490 times.)
4. **Moore S, Spackman D H,** & **Stein** W **H.** Chromalography of amino acids on sulfonated polystyrene resins: an improved system. *Anal. Chem.* 30:1185-90. 1958. (Cited 2.350 times.)
5. **Heinrikson R L & Meredith** S C. Amino acid analysis by reversed-phasc high-performance liquid chromatography: precolumn derivatizalion with phenylisothiocyanate. *Anal. Biochem.* 136:65-74, 1984. (Cited 720 times.)
6. **Cohen S A, Tarvin T L & Bidlingmeyer B** A. Amino acid analysis using pre-column derivatization with phenylisolhiocyanatc: matrix effects and tryptophan analysis. (L'ltalien J J. ed.) *Modem methods in protein chemistry.* New York: Plenum, 1988. p. 207-13.
7. **Cohen S A & Strydom D J.** Amino acid analysis utilizing phenylisothiocyanale derivatives. *Anal. Biochem.* 174:1-16, 1988.
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