As indicated in *Current Contents*® (45):5-10. 8 November 1993, coauthors do not always have the same perspective on the events surrounding their *Citation Classic*® papers. For this reason, we offer twoseparatecommentaries on the Bidlingmeyer-Cohen-Tarvin paper.

This Week's Citation Classic®

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

A method is described for the high-performance liquid chromatography analysis of amino acids using gas-phase hydrolysis and derivatization with Edman's reagent, phenylisothiocyanate. Analysis time is 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.]

Improved Amino Acid Analysis

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The success of this paper was the result of a team effort where each author made timely contributions such that the sum of the pieces was greater than the individual parts. In addition, numerous other unsung heroes were involved in turning the science into a commercial product. It started in fall 1981, when Emmett Durrum, president of Eldex, visited Gary Frazier, president of Waters, and Brian Bidlingmeyer, vice president/technical director of Waters. Emmett described an idea for making a dedicated amino acid analyzer based upon a high-performance liquid chromatography (HPLC) separation using a new derivatization approach of which he had recently learned. We then had Emmett talk to a very wide audience to give the concept a broad scientific "airing." The majority of responses at the meeting were that Waters shou ld not waste timechasing this concept. While there were good reasons for that belief, Gary and Brian shared Emmett's vision. A small team was assembled to test the key aspects of the derivatization-method efficiency, reproducibility, and the ruggedness of the HPLC separation.

Emmett supplied the hydrolyzer and derivatization module. All used George Tarr, of the University of Michigan, as a consultant. Emmett realized that buried in a brief section of Tarr's then-unpublished work[1] was a potentially useful approach for free amino acid analysis.

After six months of work the hydrolysis and reaction procedures were under control, but the challenge of improving sensitivity and separation time was temporarily intensified by personnel turnovers, which brought Tom Tarvin to Waters in July 1982 and Steve Cohen in October. Fortunately, Tom's expertise in traditional amino acid analysis and Steve's biochemistry experience soon allowed us to make up for lost time. In January 1983 there was a new product meeting at Waters to review the science; and in the face of stiff opposition from many on the committee, Gary gave the go-ahead to complete the commercialization project.

We talked to researchers to whom Tarr had given his approach; however, they were not satisfied with it and returned to the traditional amino acid analyzer. We also conducted a survey of users of amino acid analyzers and met with a high degree of interest—and skepticism! Based upon this information we had two clear and consistent objectives: (1) to develop a product with a turnkey procedure for amino acid analysis, and (2) to do a thorough job on the science so that we could publish the work.

The race was on to finish the method/product. After all, if we realized the value of this approach others could also be working on it. To doublecheck our efforts, we involved groups at Harvard Medical School, Johns Hopkins University, and Carlsberg BioTech. All of these groups initially said the method was flawed. But working with them, we solved the technical issues, and our work was presented at the International Symposium on HPLC in Biological Sciences in Melbourne, Australia, on February 20-21,1984. The proceedings of the conference, and our *Citation Classic*[3] paper, were eventually published in December 1984. The commercial product was also described elsewhere.[2]

The culmination of the original vision was the "PicoTag System," which contained everything you needed to quickly, accurately, and reliably analyze amino acids. For the project team members the research was a purposefully traveled journey. Others often scoffed, but afterthe product was introduced they became supporters.

It has been gratifying to us to see the utilization of this new method contribute to new discoveries[3] and to be referred to as the first serious alternative to the ion-exchange separation of S. Moore and W.H. Stein.[4] However, solving the problems of acceptance into the marketplace was just as challenging as developing the method—but that is another story.

1. **Koop D R, Morgan E T, Tarr** G E & Coon **M J.** Purification and characterization of a unique isozyme of cytochrome P-450 from liver microsomes of elhanol-lreated rabbits. *J. Biol. Chem.* 257:8472-80. 1982. (Cited 375 times.)
2. **Cohen S A, Tarvin T L** & **Bidlingmeyer B** A. Analysis of amino acids using prccolumn derivatization with phenylisothiocyanate. *Amer. Lab.* 16:49-58, 1984.
3. **Fett J W, Strydom D J, Lobb R R, Alderman E M, Belhune J L, Riordan J F & Vallee B L.** Isolation and characterization of angiogenin, an angiogenic protein from human carcinoma cells. *Biochemistry—USA* 24:5480-6. 1985. (Cited 205 times.)
4. **Maugh** T **H II.** New tool for amino acid analysis. *Science* 225:42. 1984.

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