Number 23 June 6, 1977

Citation Classics

Piez K A & Morris L. A modified procedure for the automatic analysis of amino acid. *Analytical Biochemistry* **1**:187-201, 1960.

The Sparkman, Moore, and Stein procedure for amino acid analysis[1] was modified by using a continuous salt and pH gradient for elution and separation on a single column of all the amino acids in protein hydrolysates, including hydroxyproline and hydroxylysine. [The *SCI*® indicates that this paper was cited 690 times in the period 1961-1975.]

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January 18, 1977

"As the title of the article by me and Louise Morris indicates, it reports a modified method based on the earlier Moore and Stein procedure for the ion exchange analysis of amino acids which had been automated by Spackman, Moore and Stein. Our reason for modifying what was already a highly developed method and one of the most important procedures in modern biochemistry was twofold. First, it seemed to be an advantage to be able to do a complete analysis on a single column in one run rather than two runs on separate columns as required by the Spackman procedure. Second, we wanted to analyze for hydroxyproline, hydroxylysine and other amino acids not resolved by the two-column method. This was done by using a continuous gradient, which has greater flexibility, rather than a step gradient.

"We were helped greatly in construction by Stanford Moore who kindly provided us with the plans for his instrument. Our instrument was built in the NIH shops and was used continuously for more than ten years. As is well known the Spackman procedure was quickly adopted by instrument manufacturers. Our modification came into commercial use more slowly because it was technically more difficult. With improved instrumentation and computer operation, the single-column method is now usually the method of choice. The continuous gradient is no longer necessary since instruments now allow multiple small steps which serve the same purpose. I don't believe they serve as well, but automatic instrumentation to produce the precise gradients required has not developed.

"Perhaps the most important aspect of the publication to me is that the method was my first step into the collagen and connective tissue biochemistry field. That has resulted in publications which I would like to think are more important contributions than a method, even though individually they may be cited less frequently. Still, all advances depend on methods."

1. **Spackman D H, Stein W H & Moore S.** Automatic recording apparatus for use in the chromatography of amino acids. *Analytical Chemistry* **30**(7):1190-1206, 1958.