Analysis in the ion exchange tradition

from Greg Ogden

Amino acid analysis by ion exchange chromatography is just as important an analytical tool in 1986 as it was in the late 1950s, when Moore and Stein first described an automated AAA instrument.

In contrast to more recently developed techniques for amino acid determination, which rely on reverse phase HPLC separation of derivatized amino acids, the traditional method employs ion exchange chromatography followed by post-column derivatization with ninhydrin or, where higher sensitivity is required, ortho-phthalaldehyde, for detection. Whilst the basic technique has changed relatively little from that presented in Moore and Stein's original publication[1], significant developments in methodology and instrumentation have left their mark on today's generation of dedicated amino acid analysers.

With ion exchange chromatography, separation of the native amino acids requires minimal sample preparation and in some cases amounts to merely filtering prior to injection. The post-column ninhydrin reaction takes place in a low volume, high temperature reaction coil, where the optimized reaction conditions give full colour development in about one minute. Upon reaction with ninhydrin, most components produce a complex which absorbs strongly at 570 nm; however, the imino acids, proline and hydroxyproline, form a complex with different absorbance characteristics. Therefore a dual channel detector is required, with the second channel set to 440 nm.

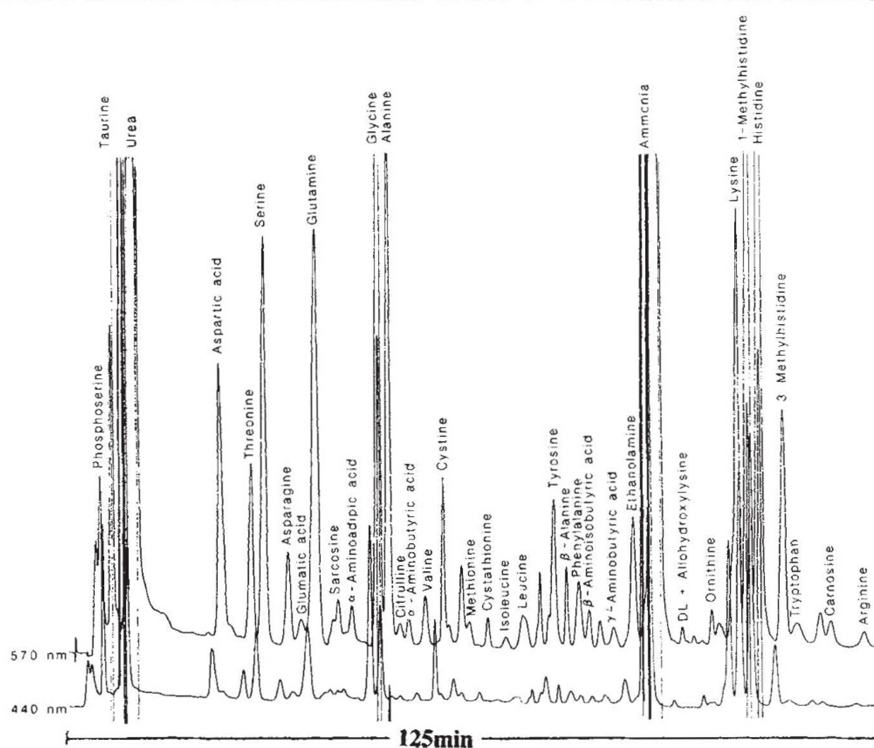

Fig. 1 Chromatogram showing the separation of human urine (40 μl) on an LKB Alpha Plus amino acid analyser using a lithium citrate buffer system.

From hydrolysate beginnings

Traditional protein and peptide characterization begins with sample hydrolysis in 6 M HCl followed by evaporation or lyophilization and injection in a *p*H 2.2 buffer[2,3]. Separations of protein hydrolysates, which typically contain 18 components or less, can then be achieved using a system of three sodium citrate buffers[4]. Careful optimization of chromatography parameters will effect the separation of unusual components such as contaminants in impure samples, artefacts introduced by the protein chemist (for example *S*-carboxymethylcysteine, homoserine and homoserine lactone), or as naturally occurring compounds (for example post-translationally modified amino acids).

The other classical application for amino acid determination is the analysis of free amino acids in physiological fluids such as urine, plasma, serum and cerebrospinal fluid.

Physiological samples are inherently more complex than protein hydrolysates. In contrast to the 18 components found in most protein hydrolysates, physiological fluids can contain between 25 and 50 amino acids and other ninhydrin-positive compounds. Owing to this complexity, a system of five lithium citrate buffers has been developed to effect the separation of physiological fluids and related sample[6].

Sample preparation for most physiological fluids is limited to deproteinization, usually with 5-sulphosalicylic acid[7], followed by filtration, although some samples may also require subsequent *p*H adjustment for optimal separation. Sample preparation procedures have also been published for the analysis of plant and animal tissues[8,9]. Figure 1 shows an example of a physiological fluid separation obtained using the lithium citrate buffer system.

Future analysis

There is no reason to suppose that current trends towards faster, more sensitive analysis will not continue. Reverse-phase HPLC methods have gained in popularity because they appear to offer the increase in speed and sensitivity which some workers seem to require. However, the traditional, dedicated amino acid analyser can also benefit from advances in HPLC technology, and the late 1980s will without doubt see a new generation of Moore and Stein machines employing miniaturized components, higher operating pressure and smaller and more efficient ion-exchange resins and microbore columns. These advances, coupled with developments in buffer and reagent systems, will mean that the proven reliability and reproducibility of the established technique will continue to survive as the method of choice for a great many workers. □

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