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L-2,4-Diaminobutyric acid as a standard in the estimation of lysine in maize samples

Part of a maize-breeding program required the estimation of lysine in the maize samples by automated amino acid analysis, following acid hydrolysis. Such programs require the screening of a large number of samples, making it necessary as far as possible to expedite both the preparation of samples and the subsequent chromatography stages.

As in this case it is the absolute amount of a single amino acid which is required, rather than the molar ratios of the amino acids, time-consuming, quantitative techniques would normally be necessary in the steps following acid hydrolysis. A more expeditious procedure, coupled with a gain in accuracy, can be achieved, however, by the use of an internal standard. Contributions to the overall error, estimated by the use of an internal standard, are due to: (1) mechanical losses after acid hydrolysis; (2) manipulative errors in applying sample to the column; and (3) possible changes in colour development. As allowance is made for these errors, absolute manipulative precision only becomes necessary during standardisation runs and in the addition of the internal standard to the samples.

As an internal standard for the short column chromatographic system, WALSH AND BROWN[1] have proposed the use of 2-aminoguanidopropionic acid, an analogue of arginine, which is eluted between ammonia and arginine. These workers suggested adding the internal standard to the samples just prior to acid hydrolysis. In the routine determination of lysine in this laboratory, however, we preferred to add the standard immediately after the acid hydrolysis and used L-2,4-diaminobutyric acid

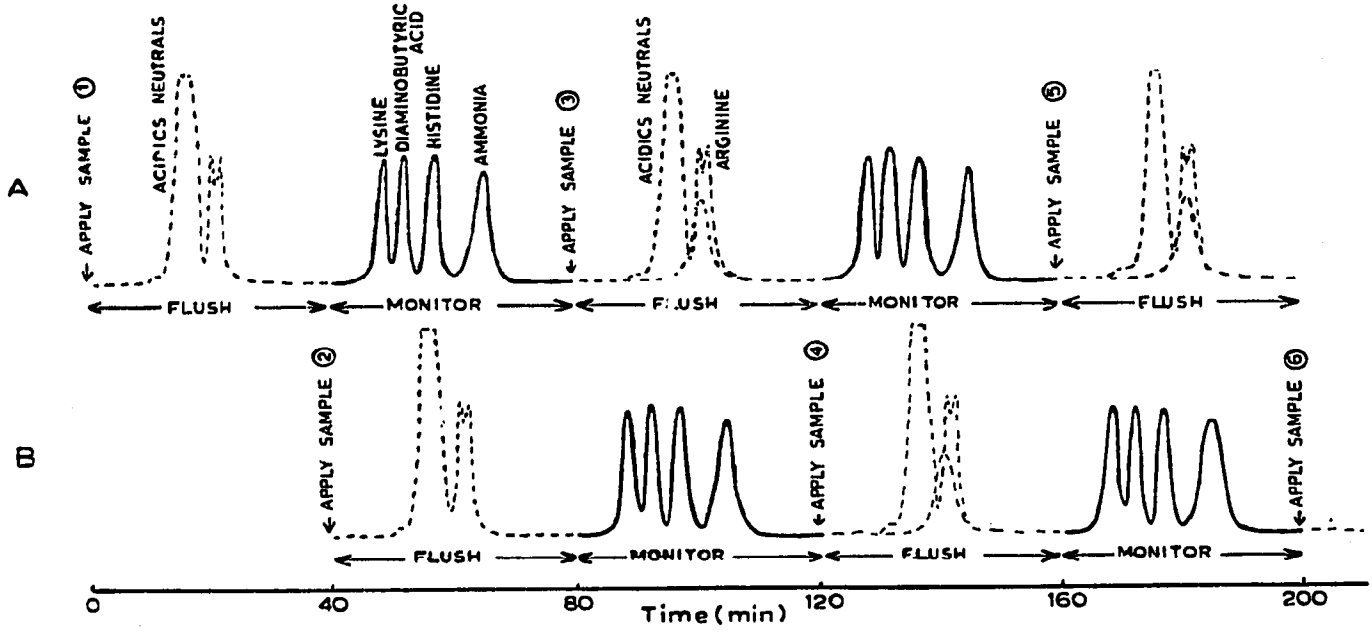

Fig. 1. Time schedule for the sequential operation of two identical columns (A and B) for the analysis of lysine. Analyser, Beckman Model 120 B with accelerated analysis accessory; operating conditions, standard conditions for the analysis of basic amino acids; columns, 0.9 × 10 cm; resin, Beckman type PA-35.

(DAB) as a standard; this is a lysine analogue which is eluted between lysine and histidine (Fig. 1). The DAB (Sigma chemical Co., Mo. U.S.A.) was oven-dried before use and was made up into two solutions, *viz.* (1) a stock solution (2 mmoles/l), consisting of DAB (38 mg) dissolved in buffer of pH 2.2 and made up to 100 ml with the same buffer, and (2) a standard calibration solution, consisting of 10 ml of stock solution diluted to 100 ml with buffer of pH 2.2.

For standardisation runs the standard calibration solution was mixed (1:1) with the standard amino acid calibration mixture (Beckman Instruments Inc.), 1 ml of the mixture being applied to the column. For sample analysis a volume of stock solution was thoroughly mixed with each sample after acid hydrolysis, the volume being selected so that the resultant mixture contained approximately equal amounts of lysine and DAB. Samples were subsequently evaporated and analysed in the usual manner.

Although the elution position of DAB necessitates the use of a slightly longer short column in order to avoid overlap with lysine and histidine it is a particularly suitable standard in that it permits the total elution time to be divided into three equal intervals, both lysine and DAB being eluted in the second interval. This fact can be exploited by arranging two short columns and two pumps to be operated in succession according to the time schedule outlined in Fig. 1. We have placed a selector valve on the inlet side of pump No 1 on the Beckman 120 B amino acid analyser so that this can be switched to pump the buffers either for long column analyses or the buffer of pH 5.28 for use on the second short column described here.

Using the two-column system outlined above it is theoretically possible to analyse one sample every 40 min, although in our experience ten samples per 8-h day is a more practicable figure*.

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* *Note.* During preparation of this manuscript another short column standard, ε-Aminocaproic acid, which could be used in a similar manner, has been described[2].