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The use of an external standard in the quantitative evaluation of amino acid chromatograms using the Technicon system

The quantitative determination of the amino acids in physiological fluids may be invalidated by the presence of ninhydrin-positive substances which are eluted at or near the position of the chosen internal standard. An external standard may be used to estimate the presumptive area of an internal standard making the following assumptions.

The area beneath the tracing of the peak of an internal standard is determined, first, by the molar extinction coefficient in the ninhydrin reaction; second, in any one chromatogram, by the proportions of the volumes of sample and ninhydrin reagent mixed by the proportioning pump, the condition of the ninhydrin reagent and the flow through the colorimeter relative to waste at the pre-colorimeter debubbling system. In all quantitative work it is necessary to assume that these characteristics of the analytical system remain virtually constant throughout the chromatogram. Thirdly, by the ratio of the flow through the column to the sample flow through the analytical system. There is, in addition, a small mixing effect at the point where the sample is withdrawn.

The area of 100 nmoles of norleucine added as an internal standard therefore approaches a maximum value when the flow rate through the column is exactly equal to the flow rate through the proportioning pump; a condition which, for technical reasons is never used, the flow rate through the column being adjusted to be greater than the sample flow rate through the proportioning pump.

The presumptive area of an internal standard is therefore equal to the area of an equal amount fed into the analytical system as an external standard multiplied by the ratio of the sample flow rate through the proportioning pump to the flow rate through the column.

The average flow rate through the column is found by measuring the volume of eluting buffer and dividing by the time run. The area of 100 nmoles of norleucine and the calibration of the proportioning pump is achieved by running 5 ml of 20 m*M* norleucine from a calibrated fine-pointed centrifuge tube, measuring the area under the square wave produced and the time taken.

The area obtained in this way is equivalent to the area under a normal chromatographic peak integrated by the trapezoidal rule[1] so that it is necessary to have calibrating chromatograms with internal standards which have been integrated in this way.

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1 H. W. HOLY, *Techniques in Amino Acid Analysis*, Technicon Instrument Co. Chertsey, p. 147.

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