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Note

A simple method for the separation of primary mono- and diamines using a standard amino acid analyzer

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During a study of amino acid metabolism in the alimentary tract of pigs and the amino acid content in dry sausages, it was found necessary to use a simple technique to separate quantitatively some mono- and diamines. Hatano *et al.*[1] reported complete resolution of 16 amines in 8 h, but without the elution of tryptamine and agmatine and using a very expensive resin. Wall[2,3] recommended the use of Zeocarb $226 \times 4^1/_2\%$ DVB for the accelerated analysis of some amines in grass silage and physiological fluids. In this paper we describe a procedure for separating completely some amines as primary decarboxylation products of amino acids.

EXPERIMENTAL

Apparatus

A Technicon amino acid analyzer was used.

Resin

Zeocarb $226 \times 4^1/_2\%$ DVB (Permutit Co. Ltd, London) was fractionated by the method of Hamilton[4] and the fraction with average bead size 24 μm was used. A 24×0.57 cm column was prepared and buffered completely before use with the starting buffer. Each filling can be used for at least 10 analyses without regeneration.

Buffers

Only two buffers are required and they were prepared from 8.74 g of potassium citrate, 60.36 g of potassium chloride, 10 ml of Brij and sufficient water to make a total volume of 1 l. Before adjusting to volume, 100 ml of *n*-propanol were added to the first buffer and 140 ml to the second. The pH of each buffer is 7.4.

Preparation of the samples

The amines were isolated from the intestinal material of pigs according to the method of Hill *et al.*[5]. The acid extract obtained was dried in a rotating evaporator, solubilized and, after adjusting the pH to 7.4, an aliquot was applied to the column. The amines from dry sausages (salami) were extracted by the same method, 30 g of dry sausage being homogenized (Virtis "45") in 100 ml of 0.05% NaOH. α-Amino-β-guanidinopropionic acid was used as an internal standard.

Elution

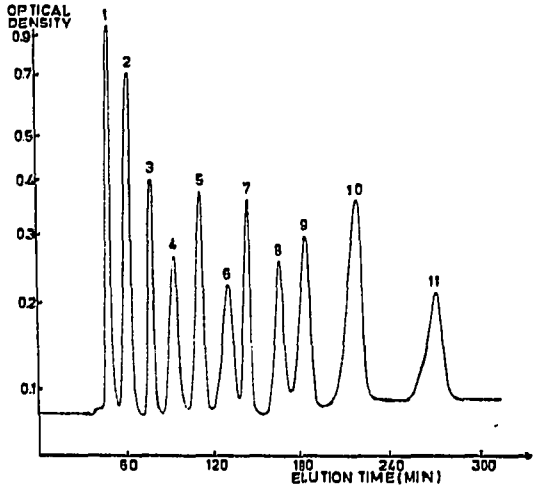
A buffer flow-rate of 42 ml/h was used and the column temperature was maintained at 43° for 103 min and automatically switched to 75° for the remainder of the analysis. The first buffer was changed for the second after 120 min, just after the elution of the internal standard. The introduction of the second buffer is necessary for the complete separation of tryptamine and cadaverine. The increase in column temperature from 43° to 75° just after the elution of tyramine results in a significant reduction in the operating back-pressure and packing of the resin column, and shortens the total elution time.

Table I lists the mean elution times of some basic amino acids and amines as observed under these conditions.

TABLE I

MEAN ELUTION TIMES OF SOME BASIC AMINO ACIDS AND AMINES FROM THE START OF THE CHROMATOGRAM

No.	*Compound*	*Time (min)*
1	Lysine	45
2	Arginine	55
3	Ammonia	72
4	Tyramine	86
5	Internal standard	103
6	Phenylethylamine	121
7	Histamine	138
8	Tryptamine	158
9	Cadaverine	175
10	Putrescine	198
11	Agmatine	254



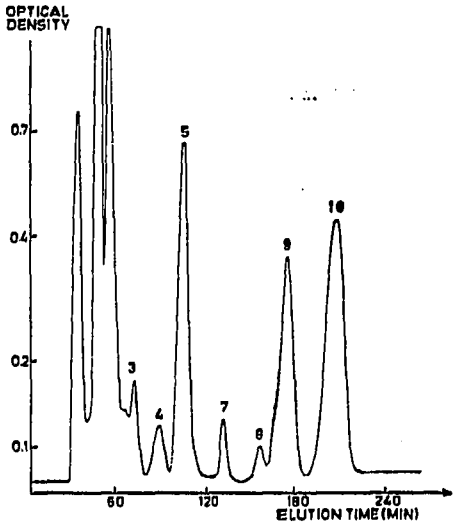


Fig. 1. Separation of a test mixture of basic amino acids and amines. Absorbance read at 570 nm. The peaks are identified as in Table I.

Fig. 2. Amines found in the caecum of a pig, with 0.15 μmole of internal standard added. The peaks are identified as in Table I.

RESULTS

Fig. 1 is a chromatogram of a standard mixture of amino acids and amines. The amount of each amino acid or amine present is 0.15 μmole and the curve represents peaks at 570 nm with a 15-mm flow cell.

Fig. 2 is a chromatogram of amines isolated from the caecum of a pig, and Fig. 3 illustrates the amines found in dry sausage extract. The reproducibility for most amines is as good as the values usually obtained in routine amino acid analysis.

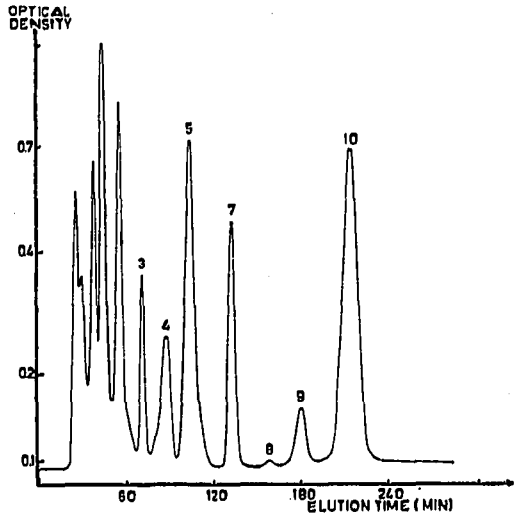


Fig. 3. Chromatographic analysis of a dry sausage extract. The peaks are identified as in Table I.

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