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## Note

# Determination of biogenic amines in intestinal contents by ion-exchange chromatography

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For a study of amino acid and amine metabolism in the alimentary tract of pigs, it was necessary to develop a convenient method for the quantitative analysis of several amines which are the primary decarboxylation products of amino acids. Since the ion-exchanger Zeo-Karb 226 × 4½% DVB used previously by several workers[1–3] is no longer commercially available, a new method of analysis has been developed on a short column of Technicon Chromobeads A resin.

## EXPERIMENTAL

A Technicon amino acid analyzer was used with a 24 × 0.60 cm column packed with Chromobeads A (Technicon, Brussels, Belgium). Four buffers were used and their compositions are presented in Table I.

TABLE I
COMPOSITION OF EACH BUFFER

| *Reagent* | *Buffer* | | | |
|---|---|---|---|---|
| | *A* | *B* | *C* | *D* |
| pH | 4.10 | 7.50 | 8.20 | 11.50 |
| Sodium citrate dihydrate (g/l) | 10.51 | 10.51 | 58.20 | 38.75 |
| Sodium chloride (g/l) | 25.05 | 25.05 | 80.00 | 50.00 |
| 2 *N* Sodium hydroxide (ml/l) | 17.85 | 17.85 | 40.00 | 22.50 |
| Brij solution (ml/l) | 10.00 | 10.00 | 10.00 | 10.00 |
| Propanol-2 (ml/l) | — | — | — | 100.00 |

### *Sample preparation*

*Amine standard.* A 1 m*M* solution of basic amino acids and amines containing α-amino-β-guanidinopropionic acid as internal standard was prepared in distilled water and adjusted to pH 4.10 with HCl (p.a.).

*Intestinal material.* After centrifugation (10 min, 3000 *g*), the clear supernatant from an aliquot of intestinal material was concentrated to dryness in a rotating evaporator and solubilized in distilled water. After adding an appropriate amount of

internal standard and adjusting the pH to 4.10 with HCl (p.a.), an aliquot was directly applied to the column. Further purification of the samples was not needed.

*Method*

A flow-rate of the buffers of 50 ml/h and a column temperature of 65° were maintained throughout the separation of the amines. Buffer A (pH 4.10) was changed to B (pH 7.50) after running for 10 min; buffer C (pH 8.20) was used after the elution of lysine (57 min) and buffer D (pH 11.50) was used after the elution of putrescine (140 min). After the introduction of an intestinal sample, the extraneous ninhydrin-positive components such as neutral and dicarboxylic amino acids proceeded through the column much more rapidly than the amines and were diverted to the waste during the first 45 min of the analysis. Measurements were made at 570 nm with a 15-mm flow cell. Table II shows the mean elution times of some basic amino acids and amines.

TABLE II

ELUTION TIMES OF SOME BASIC AMINO ACIDS AND AMINES

| *No.* | *Compound* | *Mean elution time (min) (n = 10)* | *Coefficient of variation* |
|---|---|---|---|
| 1 | Lysine | 57 | 6.7 |
| 2 | Ammonia | 67 | 4.0 |
| 3 | Internal standard | 77 | 5.0 |
| 4 | Arginine | 86 | 5.1 |
| 5 | Histamine | 122 | 4.2 |
| 6 | Putrescine | 140 | 3.3 |
| 7 | Cadaverine | 176 | 2.8 |
| 8 | Tyramine | 196 | 4.6 |
| 9 | Phenylethylamine | 208 | 4.2 |
| 10 | Agmatine | 219 | 4.5 |
| 11 | Tryptamine | 281 | 5.5 |

TABLE III

MOLAR RESPONSES (± S.E.) RELATIVE TO α-AMINO-β-GUANIDINOPROPIONIC ACID

Molar response is the ratio of the peak area produced by unit molar quantity of a ninhydrin-positive compound to the peak area produced by unit molar quantity of α-amino-β-guanidinopropionic acid.

| *Compound* | *Mean ± S.E. (n = 10)* |
|---|---|
| Lysine | 1.51 ± 0.05 |
| Ammonia | 0.65 ± 0.06 |
| Arginine | 1.18 ± 0.05 |
| Histamine | 0.61 ± 0.01 |
| Putrescine | 1.18 ± 0.03 |
| Cadaverine | 0.78 ± 0.02 |
| Tyramine | 0.58 ± 0.02 |
| Phenylethylamine | 0.68 ± 0.02 |
| Agmatine | 0.84 ± 0.02 |
| Tryptamine | 0.23 ± 0.01 |

## RESULTS

In Table III are presented the molar responses relative to $\alpha$-amino-$\beta$-guanidino-propionic acid of some basic amino acids and amines. Fig. 1 shows a typical standard chromatogram of a prepared mixture of basic amino acids and amines. The amount of each amino acid or amine present is 0.25 $\mu$mole. Fig. 2 shows a chromatogram of amines found in the terminal ileum of a pig. It was not possible to eliminate completely the small shift in baseline due to the introduction of buffer C, which often occurred after the elution of arginine. However, this did not affect the resolution and the quantitative determination of the amines. The reproducibility of this technique is similar to that obtained in amino acid analysis.

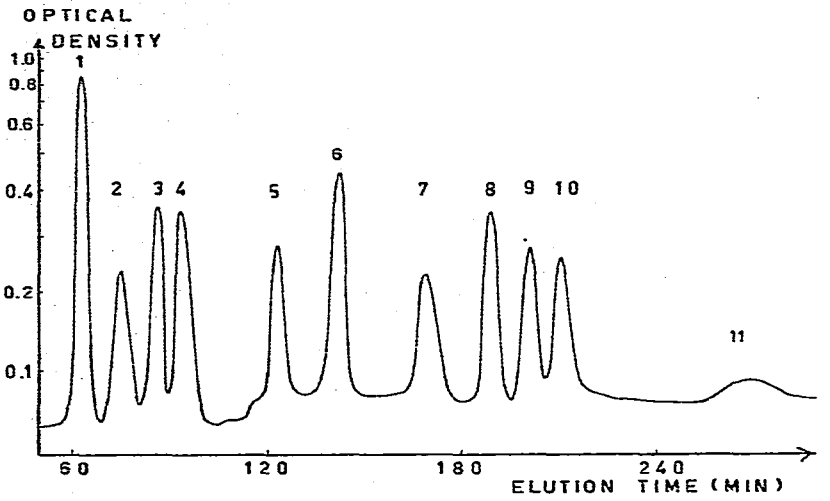


Fig. 1. Test mixture of basic amino acids and amines. The peaks are as in Table II.

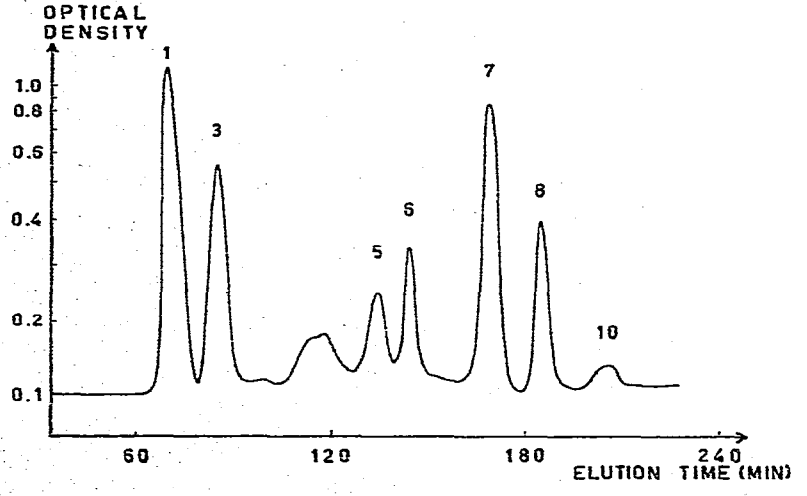


Fig. 2. Amines found in the terminal ileum of a pig, with 0.25 $\mu$mole of internal standard added. The peaks are as in Table II.

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