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Note

A rapid chromatographic determination of diaminopimelic acid with lithium citrate buffers

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The measurement of diaminopimelic acid (DAP) in acid hydrolysates of total rumen contents and its application to the estimation of bacterial growth[1] has led to the development of several methods of analysis[1–3]. However, requirements for a faster and more sensitive procedure, with the possible incorporation of an internal standard, indicated a need for an alternative technique. A rapid method for the measurement of DAP is described, whereby the need for any pretreatment of the hydrolysate sample is eliminated.

MATERIALS AND METHODS

Hydrazine sulphate was obtained from BDH (Poole, Great Britain). Other reagents were from Pierce (Rockford, Ill., U.S.A.).

The analyses were carried out on a TSM amino acid analyser equipped with a cation exchange resin type C–3 (8% cross-linked) column (41 × 0.5 cm I.D.; Technicon, Tarrytown, N.Y., U.S.A.) using a modification of a previously described procedure[4].

Two buffers were needed for the operation of the column with a buffer change occurring 12 min after sample introduction. Details of the buffer composition are shown in Table I.

TABLE I

COMPOSITION AND pH OF THE LITHIUM CITRATE BUFFERS

Buffer	*1*	*2*
Lithium conc. (*N*)	0.30	0.28
Citrate conc. (*M*)	0.50	0.47
Methyl cellosolve (%, v/v)	0	5.5
Thiodiglycol (%, v/v)	0.1	0.1
Brij-35, 30% (%, v/v)	1.0	1.0
pH (±0.01)	3.25	4.15

Recovery studies were carried out on hydrolysate samples to which 10, 20, 30 and 40 nmoles of DAP were added. Norleucine was used as an internal standard at a concentration of 0.05 nmoles per 0.1 ml of sample.

Prior to each analysis the column was washed for 12 min with 0.3 N LiOH and then for 26 min with equilibrium buffer (Buffer 1). A column temperature of 30° and a flow rate of 24 ml/h were maintained throughout the analysis. The pump pressure did not exceed 500 p.s.i.

Quantitation of DAP concentrations was achieved by the peak height method[5,6].

RESULTS AND DISCUSSION

Under the conditions described DAP was eluted in 43 min (Fig. 1) with a total analysis time of 71 min. The sharpness of the DAP peak and its low norleucine equivalent of 0.76 (S.E. for 10 samples = ±0.03) allow the measurement of 2–50 nmoles of DAP. The mean DAP recovery from triplicate samples at four concentration levels was 103.7 (±3.4%).

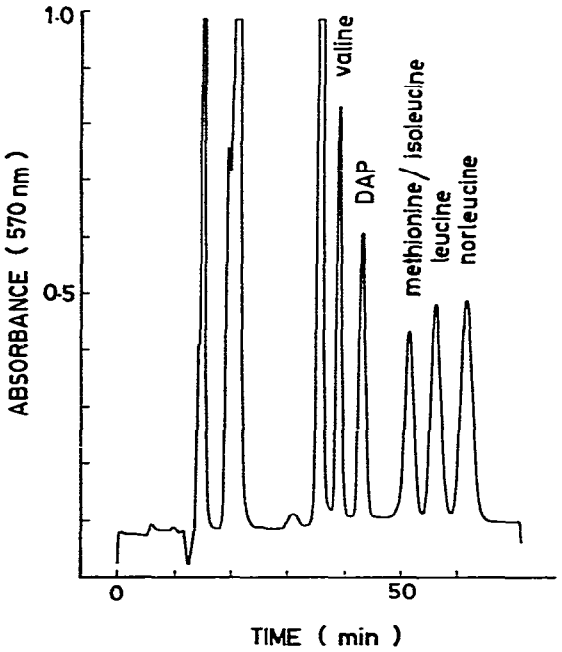

Fig. 1. Elution pattern of 0.1 ml hydrolysate sample containing 28 nmoles of DAP and 50 nmoles of norleucine.

Factors such as pH, temperature and solvent concentration in the second buffer influenced the elution time of DAP. Slight pH increases in the range 3.50–4.25 accelerated the elution of valine, methionine and norleucine in relation to DAP. An increase in the concentration of methyl cellosolve from 1.75 to 5.5% (v/v) in the second buffer extended the time interval between the elutions of valine and DAP. The elution of DAP was accelerated by reducing the volume of the first buffer and its final position was determined by an alteration in the proportion of the volumes of the two buffers. Lowering the column temperature from 45° to 20° increased the interval

between the elution times of valine and DAP but it extended the total analysis time and caused the peaks to broaden. At a temperature of 30° the broadening of the norleucine peak was minimal and complete separation of the internal standard and DAP was achieved. However, at this temperature (30°) methionine and isoleucine were eluted as a single peak. Simultaneous elution of isoleucine and methionine also occurred with increased concentration of methyl cellosolve in the second buffer.

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