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Note

Rapid determination of methionine in physiological fluids

J. MZIK
C.S.I.R.O., Division of Animal Production, P.O. Box 239, Blacktown, N.S.W. (Australia)
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Methods involving the use of lithium citrate buffers[1,2] permit the determination of methionine in 3 h. However, if the concentration of methionine alone is to be determined in many samples, these procedures become tedious. This paper describes two rapid procedures for the determination of methionine; the samples are loaded on to a partially equilibrated column, which accelerates the elution of unwanted acidic amino acids and permits methionine to be completely resolved within 1 h.

MATERIALS AND METHODS

The test samples consisted of sheep plasma, deproteinized with sulphosalicylic acid[3]. Norleucine (0.5 μmole/ml) was added as an internal standard. The analyses were performed on a TSM amino acid analyser equipped with type C-3 (crosslinked) cation-exchange resin (Technicon, Tarrytown, N.Y., U.S.A.). Details of the buffer composition are given in Table I.

TABLE I
COMPOSITION AND pH OF THE LITHIUM CITRATE BUFFERS

Component	*System A*		*System B (buffer 1)*
	Buffer 1	*Buffer 2*	
Lithium concentration (*M*)	0.29	0.28	0.27
Citrate concentration (*M*)	0.48	0.47	0.45
Methyl Cellosolve (%, v/v)	0	1.75	1.00
Thiodiglycol (%, v/v)	0.1	0.1	0.1
pH (±0.01)	3.30	3.90	3.10

The plasma samples were loaded into cartridges containing 0.1 ml of type C-3 resin, from which they were subsequently eluted with the equilibrium buffer (buffer 1) into columns that had previously been washed for 6 min with 0.3 *N* lithium hydroxide solution and then for 14 min with equilibrium buffer at a pump pressure ranging from 250 to 350 lb./in.^{2}.

In these studies, two systems were used. With system A, two buffers were

needed for the operation of one column (41 × 0.5 cm), a buffer change being made after 8 min. A column temperature of 42° and a flow-rate of 25.8 ml/h were maintained throughout the analyses. System B involved the use of one buffer and a single column (27 × 0.5 cm) kept at 50°; the flow-rate was 28 ml/h.

Quantitation of methionine levels by reference to norleucine was achieved by the peak height method[4,5].

RESULTS AND DISCUSSION

Under the conditions described, methionine was eluted in 43 min with system A (Fig. 1a) and 35 min with system B (Fig. 1b). The total analysis cycles were 85 and 80 min, respectively. The analysis time was shortened by the introduction of a second column programmed for sequential operation, and two samples were analysed every 110 min. The recovery of methionine added to sheep plasma (triplicate samples in the range 7.5–30 nmole) was 98.5 (±1.65%) with system A and 98.1 (±1.75%) with system B. A reduction of the volume of equilibrium buffer shortened the elution time of methionine and produced a better distribution of peaks than could be obtained with the conventional 5½-h cycle. The unique feature of this method was the partial regeneration of the column at the end of each analysis. When a new sample was applied to the column, the lower part of the resin bed still contained lithium hydroxide, so that those amino acids which emerged first from the sample cartridge were even more rapidly eluted on reaching the alkaline zone. By the time the slower moving methionine, isoleucine, leucine and norleucine reached this region, the lithium hydroxide had been displaced by equilibrium buffer. The positions of the moving boundaries of lithium hydroxide were detected by the accompanying darkening of the resin, and this served as a basis for the formulation of the sample loading programme.

System B (a modification of A) permits an adequate separation of methionine, isoleucine, leucine and norleucine with a smaller resin column, which is normally

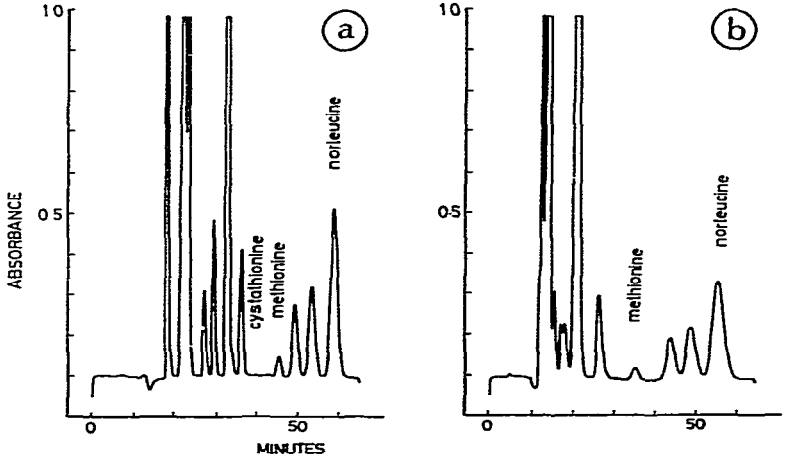

Fig. 1. Analysis of 0.2 ml of deproteinized ovine plasma containing 3.2 nmole of methionine and 50 nmole of norleucine using (a) system A and (b) system B.

used for the separation of basic amino acids. An optimal resolution efficiency is thus secured with minimal changes to the existing equipment.

The operating conditions described here can serve only as a guide, and slight changes may be necessary owing to variations in the length of the tubing connections.

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