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Note

Accelerated column chromatography of free basic amino acids encountered in blood plasma or serum

Recently reported methods involving the use of lithium citrate buffers[1–3] enable basic amino acids found in plasma or serum to be separated in about 2 h. However, the procedure of ERTINGSHAUSEN AND ADLER[1] is not applicable to most standard analyzers, as it requires many changes of buffer and temperature. The chromatogram of a synthetic amino acid mixture obtained by MONDINO *et al.*[3] indicates that the difficulties in the separation of the ornithine–lysine–ammonia mixture were not completely overcome. In general, if a complete analysis, including acidic and neutral components[4,5], is to be performed, then the use of lithium buffers necessitates at least one additional temperature change during the run. The conversion of an ion exchanger into the lithium form results in an increase in the column pressure which may be beyond the limits of standard equipment.

Our experience with the use of lithium citrate buffers for basic amino acid chromatography directed our attention to the possibility of achieving a faster analysis time by using high-molarity sodium citrate buffers[6–8]. The separation of aromatic and heterocyclic amino acids is of an adsorbtive nature and can be speeded up by the addition of an organic solvent or by the use of high temperatures.

This paper describes a simple and rapid method for the ion-exchange separation of the main basic amino acid constituents of blood serum or plasma, based on the use of 0.6 *M* sodium citrate buffer. The elution temperature is identical with the column temperature used for acidic and neutral components. Hence, the partial co-elution of both columns can reduce the extension of the run caused by basic amino acid chromatography to about 90 min.

Equipment and methods

Three different amino acid analyzers were used. Two of the instruments, Models 4020 and 6020, were produced by the Development Works of the Czechoslovak Academy of Sciences. The Beckman Unichrom amino acid analyzer was used less frequently. The column dimensions and other operating conditions are summarized in Table I. The Ostion LG KS 0802 ion exchanger was supplied by the United Metallurgical and Chemical Works, Ústí nad Labem, Czechoslovakia, as a spherical sulphonated styrene–divinylbenzene copolymer with a particle diameter in the range 12–15 μm. This resin is nominally 8% crosslinked.

In all three modifications listed in Table I, elution with approximately 0.6 *M*

TABLE I

ANALYZER OPERATING CONDITIONS AND BUFFER COMPOSITION

Operating conditions	*Modification of basic method*		
	A	*B*	*C*
Anino acid analyzer	Model 4020	Model 6020	Beckman Unichrom
Ion exchanger	Ostion LG KS 0802	Ostion LG KS 0802	Beckman M 81
Column diameter (cm)	0.9	0.8	0.9
Column length (cm)	24	24	24
Resin bed length (cm)	17	22	20
Flow-rate (ml/h)	67	70	50
Temperature (°C)	37	50	50
Buffer pH (±0.02)[a]	4.50	4.3[c]	4.20
Sodium normality	0.60	0.5[7]	0.61
Citrate molarity	0.20	0.19	0.20
Isopropanol content (% v/v)	4	—	—
Thiodiglycol (% v/v)	0.5	0.5	0.5
Octanoic acid (%)	0.01	0.01	0.01

[a] The buffers do not contain any detergent. The buffers are titrated to the desired pH value with concentrated hydrochloric acid.

sodium citrate is used and the temperature of elution is identical with the temperature required for the completion of the analysis of acidic and neutral amino acids using long-column techniques. For this reason, the elution of basic amino acids can be started during the long-column elution and all amino acids eluted before ornithine can be led to waste as they are more effectively separated on the long column. This coincident elution further reduces the time required for the whole run so that the analyses of two physiological fluids can be started during an 8-h working shift. The method presented under A is particularly useful for instruments in which lithium citrate buffers are used for the separation of acidic and neutral amino acids and related substances[5] and which are not equipped with temperature programming.

TABLE II

ELUTION TIMES OF BASIC AMINO ACIDS FOUND IN BLOOD SERUM

Elution times are given in minutes and are not corrected for the time delay produced by the reaction coil and other analyzer components.

Substance	*Modification of basic procedure*		
	A	*B*	*C*
Ornithine	45	42	67
Lysine	49	48	79
Ammonia	59	56	89
Histidine	66	60	95
Tryptophan	80	73	118
Arginine	114	105	173

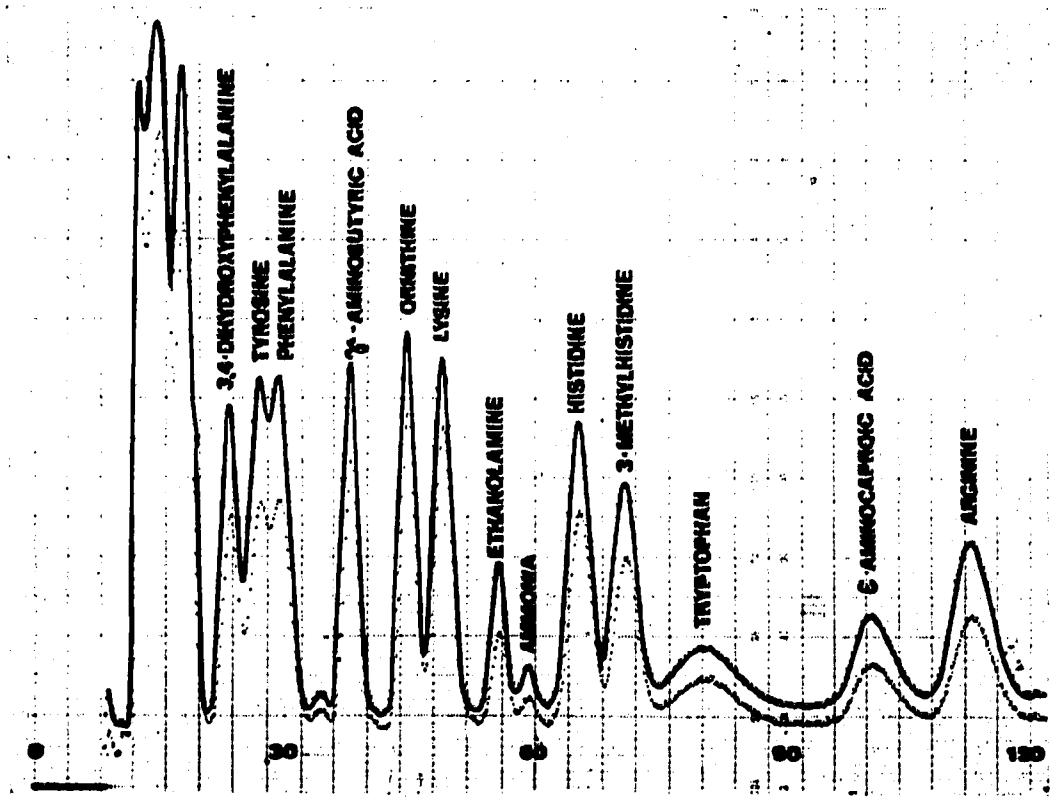

Fig. 1. Analysis of a synthetic mixture of amino acids by Method A. The mixture was applied to the column in 0.5 ml of sodium citrate buffer of pH 2.2 and contained 0.25 μmole of each amino acid, with the exception of DOPA (0.8 μmole) and E-ACA (0.3 μmole). For elution conditions, see Table I.

Results and discussion

Table II summarizes the elution times of the main basic amino acids found in blood plasma or serum. Creatinine was omitted because of its low colour constant.

In Fig. 1 is shown the chromatogram obtained with a standard mixture of amino acids by using Method A. The separation of several additional substances not included in Table II illustrates the applicability of this chromatographic method to the analysis of plant extracts[9]. The complete separations of γ-aminobutyric acid from ornithine and of ε-aminocaproic acid from arginine suggest their use as internal standards in serum analysis.

An example of rat serum analysis is shown in Fig. 2, which demonstrates the complete separation of ammonia from histidine even in the case of an increased ammonia content. The sample was deproteinized by using the method of GERRITSEN *et al.*[10].

Chromatograms obtained by Methods B and C gave substantially better resolution of tyrosine and phenylalanine.

We studied the effect of some operating conditions on the resolution of serum constituents in order to permit the application of the reported methods to resins

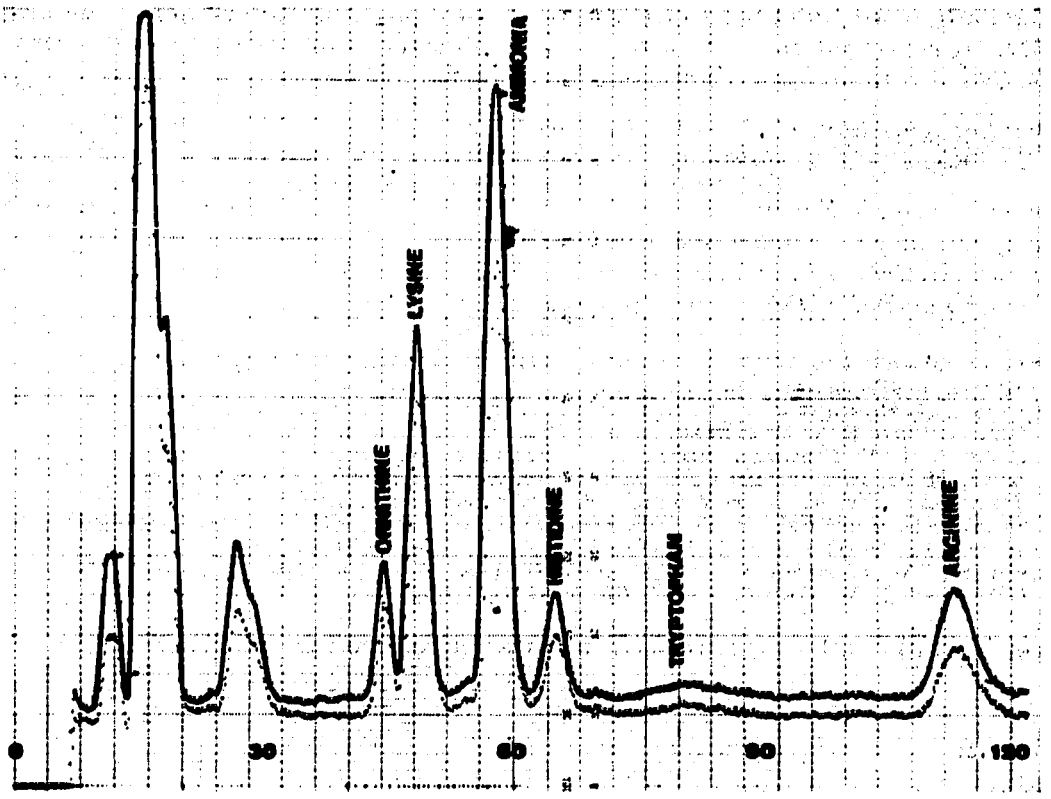

Fig. 2. Analysis of deproteinized rat blood serum. An amount of sample equivalent to 0.85 ml of serum was applied to the column. The analysis was performed by Method A.

with slightly differing elution characteristics. The observed effects are as follows.

When the pH of the elution buffer is lowered, the peak of ammonia tends to be eluted earlier. If the pH of the buffer is shifted to the basic region, the separation of ornithine and lysine, and also of ammonia and histidine, becomes inadequate.

An increase in the isopropanol content in the case of Method A leads to coincidence between the peaks of ornithine and lysine and to interference with the tryptophan results by histidine.

An increase in the column temperature used in Method A to 50° led to the coelution of histidine, ammonia and tryptophan. The separation between ornithine and lysine was decreased markedly, but the separation of tyrosine and phenylalanine was maintained. An increase in the column temperature of the solvent-free Method B to 55° led to coincidence of histidine and ammonia peaks, whereas a decrease in the column temperature to 37° had the reverse effect and the time of analysis was increased by 12 min.

The effects of the elution conditions on the resolution agree essentially with the results of LONG AND GEIGER[11], indicating that the method will be applicable to other strongly acidic cation exchangers.

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