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Accelerated analysis of amino acids in plasma: A $5^1/_4$-hour program

Rapid analysis of amino acids has the obvious advantages of increasing the number of samples that may be accomodated during any given period. The resulting decrease in the amount of buffers necessary for each individual analysis reduces the effective cost per sample. Resolution, however, should not be sacrificed in the attempt to reduce the total analysis time.

An accelerated amino acid analysis system for physiologic fluids which provides high resolution has been developed[1]. The time required for the elution of amino acids through arginine, as indicated in figures of chromatograms, was approximately 6 h, although additional time was necessary to regenerate the basic column. A 150-min program for physiologic fluids has been reported[2], but in this program threonine, serine, asparagine and glutamic acid are eluted as a single peak, as are proline and citrulline.

The most rapid separation of amino acids in physiologic fluids has been developed by MONDINO[3]. The amino acids separated on the acid–neutral column are eluted in a period of 220 min and the amino acids separated on the basic column are eluted during the same time period. The temperature of the acid–neutral column is changed during the run and it is necessary to cool the column to the original temperature (37.5°) before regenerating the column. The net time committed for each analysis is thereby somewhat in excess of the 220 min required for separation. In addition, MONDINO[3] has demonstrated that as the analysis time is reduced a secondary benefit is obtained in that the amino acids may be quantitated by measuring peak height rather than measuring the area of each peak.

Procedure

The system and methodology of ERTINGSHAUSEN AND ADLER[1] was utilized in these studies with the objectives of reducing the time of analysis and improving the elution characteristics of the individual amino acids where possible. The only modification involved was altering the pH of the buffers.

Results

The elution program developed is presented in Table I. Altering the pH of the buffers changed the elution sequence of a few amino acids from that reported[1]. Citrulline appears between glycine and alanine. Cystine (as half-cystine) is eluted immediately after alanine and is followed by α-amino-n-butyric acid. Tryptophan may be lost if the pH of the initial buffer pumped through the basic column is not adjusted carefully or if the flow from the basic column to the analytical system is not correctly timed. Ornithine is eluted after tryptophan and is followed closely by lysine, ammonia and histidine. Ammonia in samples at levels exceeding 3 μmoles/ml may partially obscure the lysine peak.

Discussion

One buffer (pH 5.25) was particularly susceptible to microbial growth which produced blockage of the acid–neutral column and excessive operating pressures.

TABLE I

ELUTION PROGRAM FOR ACCELERATED ANALYSIS OF AMINO ACIDS IN PHYSIOLOGIC FLUIDS[a]

Period No.	*Time (min)*	*Column*		*Elution sequence*[c]
		Acid–neutral[b]	*Basic*	
1	2	2.70 + 3%MC	—	—
2	42	2.70 + 3% MC	—	Cysteic acid, taurine, O-phosphoethanolamine, urea, aspartic acid, hydroxyproline, threonine, serine, asparagine
3	32	2.95	—	Glutamic acid, glutamine, sarcosine, proline, glycine, citrulline, alanine
4	32	3.40	—	Cystine, α-amino-*n*-butyric acid, valine
5	54	5.25	4.05	Methionine, isoleucine, leucine, norleucine, β-alanine, β-amino-isobutyric acid, tyrosine, phenylalanine, γ-amino-butyric acid
6	3	5.25	4.05	Tryptophan
7	57	0.3 *N* LiOH	5.27	Ornithine, lysine, ammonia, histidine
8	15	2.70 + 3% MC	5.27	Carnosine
9	48	2.70 + 3% MC	9.0	Arginine
10	10	—	4.05	—
11	10	—	4.05	—
12	10	—	4.05	—

[a] Buffers prepared according to ERTINGSHAUSEN AND ADLER[1].
[b] MC = methyl cellosolve.
[c] Analytical system receives flow from acid–neutral column during periods 1–5, flow from basic column during periods 6–12.

YOUNG AND YAMAMOTO[4] have discussed preservation of buffers, and, in contrast to their report, microbial growth was prevented by incorporating 100 mg pentachlorophenol dissolved in 10 ml methanol in each 4 l of the buffer (pH 5.25).

The instrumentation used in development of the program reported here (Technicon Amino Acid Analyzer, Model TSM-1R) was supplied with a buffer program which required 8 ½ h per sample rather than the program of ERTINGSHAUSEN AND ADLER[1], in which the analysis (but not regeneration and re-equilibration) is complete in approximately 6 h. No explanation for this difference was given. The program supplied with the instrument produced generally adequate separation although many peaks were rather short and broad at the base.

LONG AND GEIGER[5] evaluated several parameters in accelerating amino acid analysis. The accelerating effects of temperature, pH, and alcohol concentration of the buffers were most obvious for the basic amino acids. More recently, MONDINO[6] has demonstrated the role of column dimensions upon the resolving power of ion-exchange chromatography.

Other authors[1,2,3] use the time required for elution of the amino acids as the length of time required for analysis. The total elution time of the buffer program presented is 280 min. This time is in excess of that quoted by MONDINO[3] for different instrumentation.

Accelerated analysis as produced by the program reported here improved the elution characteristics of several amino acids as determined by increasing the heig and decreasing the width of the observed peak on the chromatogram. Peaks impro

most were those of cystine, α-amino-n-butyric acid, ornithine, lysine, ammonia and histidine.

Further reduction of analysis time may be possible by increasing the pH of the buffers during periods 4 and 5 for the acid–neutral column and increasing the pH and/or ionic strength of the buffers during periods 8 and 9 for the basic column.

Department of Animal Science,
North Dakota State University,
Fargo, N.D. (U.S.A.)

ROBERT L. HARROLD

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