Rapid fully automated determination of gamma-aminobutyric acid by ion exchange chromatography

With the increasing importance that is now being attributed to gamma-aminobutyric acid (GABA) as an inhibitory neurotransmitter in a number of invertebrate and vertebrate species [1] many laboratories are becoming interested in performing a large number of GABA determinations. GABA can be measured by various chromatographic and electrophoretic techniques which separate also other amino acids, and also by specific enzymatic methods (see ref. [2] for review). By micro and sub-micro modifications of the original enzymatic assay GABA concentrations of the order of 10^{-11} and 10^{-14} molar respectively can be detected [3,4]. However, all the methods described in the literature are manual, more or less time-consuming, and permit to handle only a limited number of samples per day.

The system described in the present report permits to perform about 60 fully automated GABA determinations per day by ion exchange chromatography, and may result most useful unless micro or sub-micro amounts of GABA have to be measured. The sensitivity of the method could be increased by introducing some modifications, as in the case of conventional amino acid analysis [5].

Methods

The analyses were performed on a Beckman mod. 121 automatic amino acid analyzer. This instrument is provided with a rotary table, holding up to 72 samples which can be refrigerated if necessary. 0.5 ml aliquots are automatically aspirated from the sample coils on the rotary table and injected into the columns. The system employed utilized two

TABLE 1

Program statements and conditions of analysis for GABA determination on a Beckman model 121 automatic amino acid analyzer

Program statements		Program time (min)	
		step time	elapsed time
Equilibration column 1 and 2	column 2 to coil	8	8
Load* sample column 1, equil. column 2	»	2.5	10.5
Inject sample column 1, equil. column 2	»	10	20.5
Regeneration column 1, equil. column 2	column 1 to coil	3	23.5
Equilibration column 1, equil. column 2	»	8	31.5
Load* sample column 2, equil. column 1	»	2.5	34
Inject sample column 2, equil. column 1	»	10	44
Regeneration column 2, equil. column 1	column 2 to coil	3	47

Conditions of analysis

Resin type : Aminex A 7
Column size : 15 × 0.9 cm
Height of resin : 6 cm
Column flow rate : 50 ml/h
Temperature : 38°C
Sodium citrate buffer : pH 4.6, Na conc. 0.38 M.

* Includes sample aspiration and measurement of the sample volume.

(*) La Redazione non si ritiene responsabile delle opinioni espresse dagli Autori nelle Brevi Note.

short columns, one of which was regenerated while the other was in analysis. The program statements and the conditions of analysis are reported in Table 1. Peak areas were calculated by the Beckman mod. 125 automatic digital integrator.

Results

The chromatogram reported in Fig. 1 shows that, in the analytical conditions employed, GABA is completely separated from other amino acids of a standard amino acid mixture.

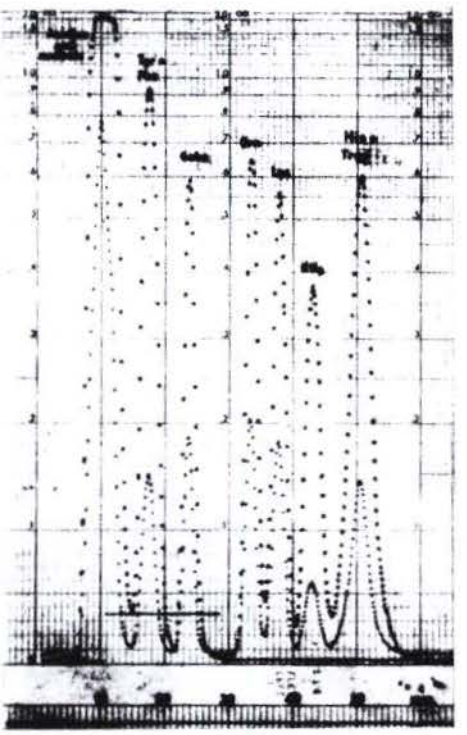

Fig. 1. — Separation of GABA from other amino acids of a standard solution, in the analytical conditions reported in Table 1.

Fig. 2 shows a series of chromatograms performed in the conditions reported in Table I as they appear on the recorder chart. It will be noted that only a small part of the chromatogram, including the peak of GABA, is recorded.

Fig. 3 shows that when multiple analyses are performed on a standard solution of GABA at room temperature, the response remains constant for about 8 hours, then decays at a rate of approximately 2.7% hour, probably due to degradation of GABA. This phenomenon was not present when the rotary table containing the sample coils was refrigerated at 8°C. In these conditions, taking as 100 the average of the first 10 of 72 sequential analyses of the same standard solution of GABA (with a S. D. of $\pm$ 1.6), the average of the last 10 analyses was 100 $\pm$ 2.3. Table 2 shows the per cent deviation from the mean observed on 100 sequential determinations of a 0.25 mM GABA solution performed under

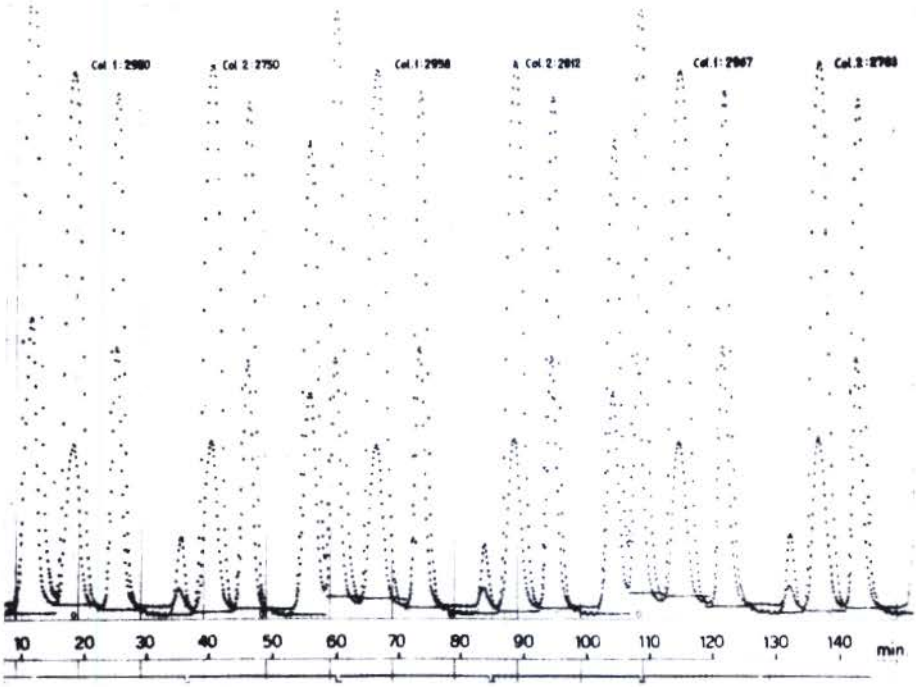

Fig. 2. — Recording of sequential automated GABA analyses, on two chromatographic columns, using the program statements and analytical conditions reported in Table 1. Super-cript numbers are those calculated by the digital integrator.

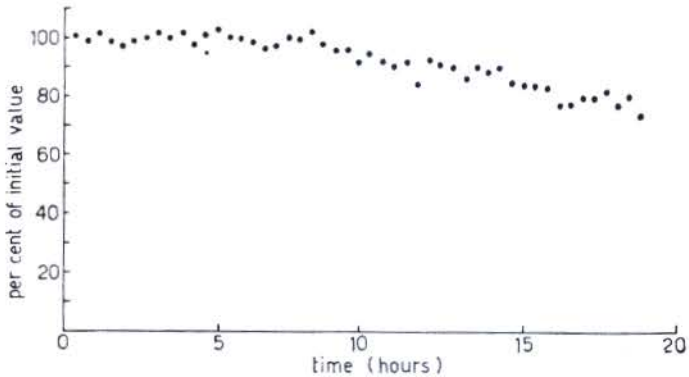

Fig. 3. — Time dependent decay in the response when multiple analyses of the same 0.25 mM GABA solution are performed without refrigerating the sample coils on the rotary table. The average of the values obtained on 10 determinations at time zero (first determinations of 10 series) was taken as 100.

TABLE 2

Per cent deviation from the mean value of 100 sequential automated GABA determinations

Deviation	Number of determinations
Between 0 and 1 per cent	53
» 1 and 2 »	20
» 2 and 3 »	19
» 3 and 4 »	4
» 4 and 5 »	4
S. D. on 100 determinations: 1.78 %	Total 100

A 0.25 mM standard solution of GABA was used to load the sample coils. The rotary table was kept refrigerated. Peak areas were calculated by an automatic digital integrator.

conditions of refrigeration. The deviation was within 1% in more than half of the analyses, and between 3% and 5% in only 9 determinations. No apparent correlation was observed between magnitude of the deviation and time of analysis.

Discussion

The system described is based on the short elution time of GABA in the analytical conditions employed and on automatic sampling and column regeneration. In these conditions the time spent by the operator is reduced to a minimum. The system could also be operated manually if an instrument with automatic sampling is not available. In this case approximately 16 determinations could be performed in 8 working hours, and the time spent by the operator would be of about 5 min twice an hour.

It may be useful to stress the importance of refrigerating the rotary table containing the sample coils to avoid deterioration of the preparation, when operating in automated conditions. If GABA is determined on perchloric acid tissue extracts, brought to the correct pH by KOH, care has to be taken to make the pH adjustments and subsequent centrifugation at about 4°C, in order to avoid the precipitation of part of the potassium perchlorate in the refrigerated sample coils. We often noted that perchlorate precipitation taking place when samples are cooled or frozen is accompanied by a significant decrease in the concentration of GABA in the decanted fluid.

It has also to be reminded that the columns should be repacked after the analysis of not more than 144 samples (2 full trays), even when perfectly clear extracts are used.

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