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## THE QUANTITATION OF $\beta$ -AMINOISOBUTYRIC ACID IN URINE BY MASS FRAGMENTOGRAPHY

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### SUMMARY

A specific, rapid and sensitive method for the quantitation of  $\beta$ -aminoisobutyric acid in urine is described. The method is based on the analysis by quadrupole mass fragmentography of its *N*-TFA-*O*-*n*-butyl derivative using  $\alpha$ -amino octanoic acid as an internal standard. The procedure is sensitive being able to quantitate as little as 1 ng of  $\beta$ -aminoisobutyric acid and is applicable to the routine analysis of this compound in biological fluids. The analysis time, exclusive of chemical derivatization, occupies about 17 minutes.

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### INTRODUCTION

$\beta$ -Aminoisobutyric acid (BAIB) was first identified in normal urine by Crumpler and Dent<sup>1</sup>. It has been well established that the normal urinary concentration of BAIB is genetically determined<sup>2-5</sup>. Increased BAIB excretion has been reported in various conditions such as lead poisoning<sup>6</sup>, pulmonary tuberculosis<sup>7</sup>, march hemoglobinuria<sup>8</sup>, thalassemia<sup>9</sup> and Down's syndrome<sup>10</sup>. Increased levels of BAIB in urine has also been reported in people exposed to radiation<sup>11</sup>, and recovering from surgical treatment<sup>12,13</sup>. Fink *et al.*<sup>14</sup> reported the excretion of large amounts of BAIB in the urine of a patient with chronic myelogenous leukemia, during the acute phase of the disease, and demonstrated the conversion of thymine, by way of dihydrothymine and beta-ureidoisobutyric acid, to BAIB<sup>15,16</sup>.

Awapara<sup>17</sup> reported increased excretion of BAIB in the urine of leukemic patients after nitrogen mustard therapy, and proposed that the excretion of BAIB might be an index of tissue destruction after this therapy<sup>18</sup>. Lee<sup>19</sup>, on the other hand, found no increased BAIB excretion in the urine of patients with leukemia.

In a current screening program of the urine of cancer patients by gas chromatography-mass spectrometry (GC/MS) we have identified abnormal amounts of BAIB (ranging from 0.6-1.7 g/24 h) in the urine of several leukemic patients on drug therapy during relapse.

Since the current methods of quantitation of BAIB involve either paper

chromatography<sup>20</sup>, thin layer chromatography (TLC)<sup>21</sup>, or electrophoresis<sup>22,23</sup>, all of which have been criticized for their non-specificity of detection<sup>5,24</sup> we have developed a method of quantitation for BAIB in body fluids which is specific, accurate and reliable.

To demonstrate the method we chose to analyze the relatively high urinary content of BAIB from selected leukemic patients. However, the sensitivity and specificity of the method are such that BAIB levels in normal excretors can easily be determined using 0.5 ml of urine. This method should prove useful for future studies of the genetics of BAIB excretion where errors inherent in the quantitation techniques previously used may have masked relevant genetic information. Indeed some of the contradictory information in the literature<sup>10</sup> on BAIB excretion levels probably stems from the inaccuracy of the methods employed for its quantitation<sup>5,24</sup>.

#### MATERIALS AND METHODS

D,L-2-amino octanoic acid was obtained from Eastman Organic Chemicals (N.Y.). D,L- $\beta$ -aminoisobutyric acid was purchased from K and K Labs, Inc. (N.Y.). Tabsorb column packing was supplied by Regis Chemical Co., Ill.

The gas chromatograph (Varian Aerograph Model 1200)-mass spectrometer (Finnigan Quadrupole 1015) and computer system (ACME IBM 360/50 with an IBM 1800 computer system acting as an interface for data collection) used in this analytical procedure is that described previously<sup>25</sup>. The details of the use of this system for mass fragmentography using up to 25 individual ions have been published<sup>26</sup>. GLC separations were carried out on a 6'  $\times$  4 mm (int. diam.) coiled glass column, packed with Tabsorb (Regis Chemical Co.), with helium as carrier gas (60 ml/min). The column temperature was kept at 100° for 2 min, and then programmed at 4°/min to 200°. Under these conditions BAIB-*N*-trifluoroacetate-*O*-*n*-butyl ester eluted after 7.7 min and  $\alpha$ -amino octanoic acid-*N*-trifluoroacetate-*O*-*n*-butyl ester after 13.4 mins (Fig. 5).

#### PROCEDURE

All urine samples were 24 hour specimens and were frozen until assayed. To a urine sample (0.5 ml of normal urine or 0.1 ml of leukemic urine) was added 25  $\mu$ l of a solution of  $\alpha$ -amino octanoic acid (2 mg/ml) as internal standard, 1 drop of concentrated HCl, and the volume made up to 1 ml with water. The solution was extracted with ethyl acetate (3  $\times$  2 ml) and the aqueous layer evaporated to dryness. The residue was refluxed with 1 ml of 1.25 *N* HCl in anhydrous *n*-butanol for 30 min and the solvent evaporated under vacuum, 0.5 ml of 25% v/v trifluoroacetic anhydride in methylene chloride was added to the residue, and the reaction mixture was heated in a sealed vial at 60° for 15 min. The vial was cooled and the solvent removed in a stream of nitrogen. The residue was dissolved in 200  $\mu$ l of ethyl acetate and 1  $\mu$ l injected into the gas chromatograph-mass spectrometer system.

To each of 5 tubes containing 25  $\mu$ l of a solution of  $\alpha$ -aminooctanoic acid (2 mg/ml) was added 10, 20, 30, 40 and 50  $\mu$ l, respectively of an aqueous solution of BAIB (2 mg/ml). The solutions were processed by the above procedure and an aliquot (1  $\mu$ l) of each subjected to mass fragmentographic analysis. A calibration curve was

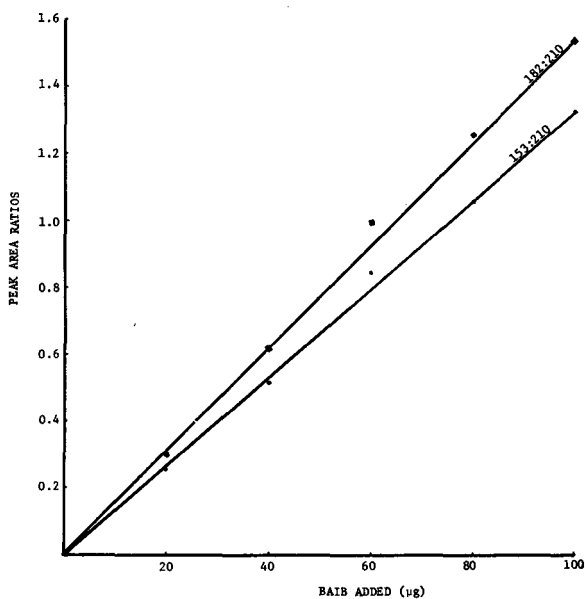


Fig. 1. Standard curve for the quantitation of BAIB.

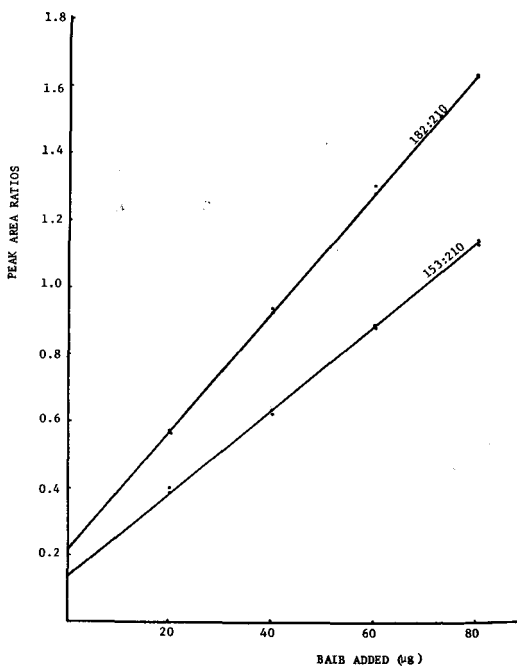


Fig. 2. Standard curve showing recovery of BAIB from normal urine.

constructed by plotting the ratio of the areas of the selected fragment ions ( $m/e$  153:210;  $m/e$  182:210) for BAIB and the internal standard ( $\alpha$ -aminooctanoic acid) against the amount of BAIB added (Fig. 1).

A similar procedure was used to quantitate BAIB in normal urine. Fig. 2 shows a calibration curve of BAIB in normal urine.

## RESULTS AND DISCUSSION

The technique of mass fragmentography uses the mass spectrometer to sense only selected characteristic ions present in the mass spectrum of that gas chromatographic effluent of interest. In the present application the ions of mass 153 and 182 in the mass spectrum (Fig. 3) of BAIB-*N*-trifluoroacetate-*O*-*n*-butyl ester (I) and the ion of mass 210 in the mass spectrum (Fig. 4) of the internal standard,  $\alpha$ -amino-octanoic acid-*N*-trifluoroacetate-*O*-*n*-butyl ester (II) were chosen. The computer system monitors only the intensity of those signals corresponding to the ions of masses 153, 182 and 210 during the analysis. At the completion of the data acquisition

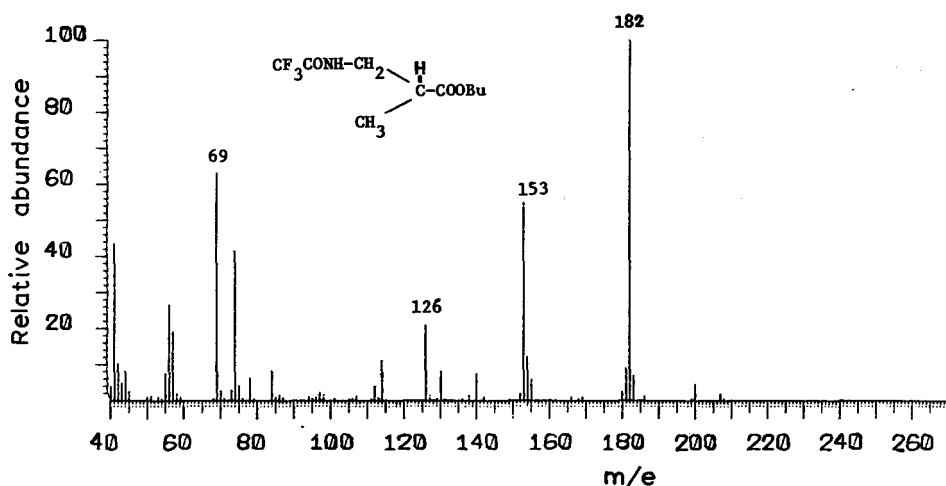


Fig. 3. Mass spectrum (70 eV) of I.

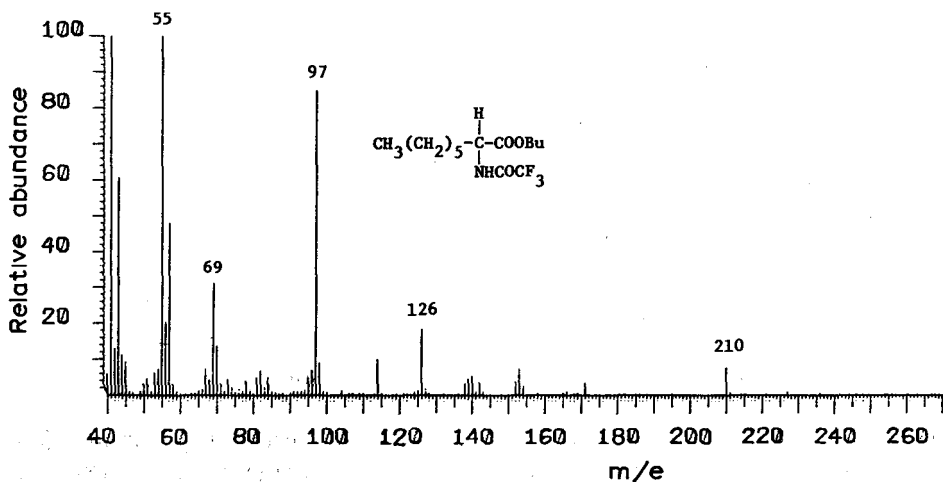


Fig. 4. Mass spectrum (70 eV) of II.

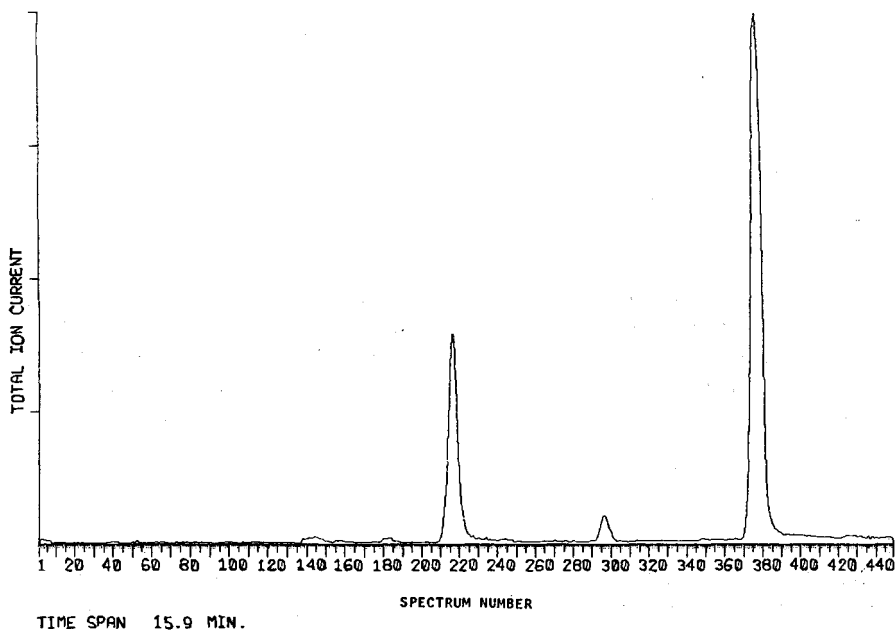


Fig. 5. Ion chromatogram of a urinary extract from a leukemic patient.

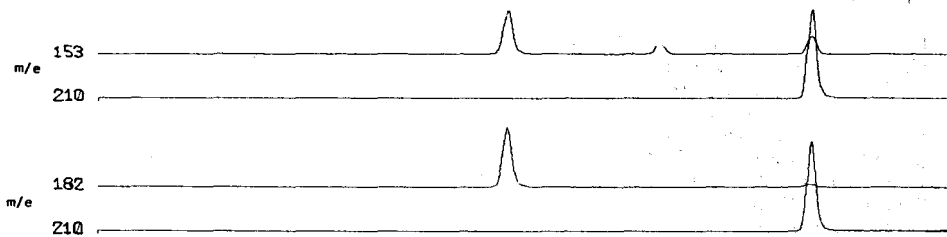


Fig. 6. Mass fragmentogram of a urinary extract from a leukemic patient.

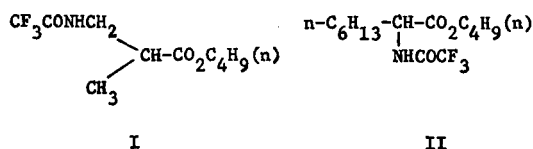
TABLE I

BAIB CONTENT (mg/100 ml) OF NORMAL AND LEUKEMIC URINES

		<i>Ions monitored</i>	
		<i>m/e 153:210</i>	<i>m/e 182:210</i>
Normal	1	2.57	2.42
	2	1.11	1.12
Leukemic	1	64.6	62.7
	2	18.1	20.2
	3	86.3	87.9

phase of the analysis (15.9 min, see Fig. 5) the computer locates the time position of the peaks associated with the ion currents of masses 153, 182 and 210 (Fig. 6). Finally the ratios of the ion current of mass 153:ion current of mass 210 and the ion current of mass 182:ion current of mass 210 are calculated and using the standard curve (Fig. 1) the result is expressed as the concentration of BAIB per 100 ml of urine

(Table I). We chose to record the intensity of two ions in the mass spectrum (Fig. 3) of I in order to produce duplicate values, per injection onto the gas chromatography column, for the quantitation of BAIB.



A typical ion chromatogram of the urine of one leukemic patient is shown in Fig. 5 and its associated mass fragmentogram is shown as Fig. 6. The results of this analytical procedure for the quantitation of BAIB in urine of normal and leukemic patients is shown in Table I. Other investigators<sup>27</sup> have reported the urinary excretion of BAIB for normal subjects to be in the range 0–2.1 mg/100 ml of urine.

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