SHORT COMMUNICATION

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THE DETERMINATION OF ETHANOL IN BLOOD AND URINE BY MASS FRAGMENTOGRAPHY

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Introduction

The determination of ethanol in body fluids is important for toxicological purposes and from a medicolegal point of view, in establishing the degree of intoxication. Although gas-liquid chromatography (GLC) has been described as a routine method for estimation of ethanol in biological fluids [1–3], the method suffers from the inherent disadvantage of lack of specificity. GLC methods are based upon relative retention times, and it is a well established fact that there are numerous volatile components in biological fluids which could possibly have the same retention time as ethanol and thus cause interference, leading to erroneous results. We now describe a specific, sensitive, rapid and reliable method for the quantitative determination of ethanol in blood and urine, by mass fragmentography [4].

Materials and Method

Instrumentation and operating conditions

A Varian model 1200 gas chromatograph was coupled by means of an all-glass membrane separator to a Finnigan 1015 quadrupole mass spectrometer, interfaced to the ACME computer system of the Stanford University Medical School [5]. GLC separations were carried out using a 6 foot coiled glass column (I.D. 1/8 inch) packed with Porapak Q (80–100 mesh), helium flow rate (50 ml/min). Column temperature (isothermal, 170°).

Reagents


(2) Ethanol standard solution. 23 mg of anhydrous ethanol was diluted with water to 25 ml.
Method

Ethanol (40 g) was mixed with orange juice (150 ml) and consumed by a subject during a period of 20 min. 200 \( \mu l \) samples of blood were withdrawn periodically from the antecubital vein, mixed with 90 \( \mu l \) of the \([^2]H_2\) ethanol standard solution (internal standard) and an aliquot (1–2 \( \mu l \)) injected into the gas chromatograph. Data acquisition was commenced after 80 s. 200 \( \mu l \) urine samples were also obtained from the same subject periodically and analyzed by the above method.

Calibration curves for the quantitation of ethanol were constructed as follows. To each of 7 vials containing 10, 25, 50, 100, 150, 250 and 500 \( \mu l \) of the ethanol standard solution, was added 90 \( \mu l \) of \([^2]H_2\) ethanol standard solution. The solutions were mixed and each sample processed in the GC–MS–computer system [5–7]. Quantitation was achieved by plotting the ratio of the peak areas of the selected fragment ions of undeuterated and deuterated ethanol against the amount of ethanol added (Fig. 1).

Results and Discussion

In the technique of mass fragmentography [4], the mass spectrometer monitors only characteristic preselected ions in the mass spectrum of the compound and its deuterated analog (the internal standard). In the case of ethanol, the ions selected were \( m/e \) 31 (CH\(_2\)=OH) and 45 (CH\(_3\)CH=OH) and for \([^2]H_2\) ethanol, \( m/e \) 33 (C\(_2^2\)H\(_2\) = \( \hat{\text{O}}\)H) and 49 (C\(_2^2\)H\(_4\) = \( \hat{\text{O}}\)H), respectively. In conjunc-
tion with its characteristic retention time, the use of two fragment ions for the quantitation of ethanol virtually eliminates any ambiguity resulting from the presence of interfering ions. In our method, up to 25 integer mass values anywhere in the mass range of 10–750 amu may be monitored sequentially. Details of the instrumentation and its application to mass fragmentography have been published elsewhere [5–7]. Fig. 2 shows a typical mass fragmentogram of ethanol and \( [^{2}H_{5}] \) ethanol. Fig. 3 represents ethanol decay profiles in blood and urine respectively after oral ingestion of ethanol.

Although the GC–MS—computer system described is fairly expensive to use on a routine basis for the analysis of ethanol, the method described is
specific and capable of quantitating as little as 5 ng of ethanol. The % standard deviation over 5 values was 3.7% for m/e 31:33 and 1.5% for m/e 45:49.

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References