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The Determination of Plasma Ethanol by Gas Liquid Chromatography using Trichloracetic Acid as the Protein Precipitant

Gas liquid chromatography is now a common method of estimating certain volatile compounds in body fluids [*1,2,3*]. There is, however, no general agreement regarding sample preparation prior to injection.

This paper deals with the use of trichloracetic acid as the protein precipitant in the preparation of plasma for analysis by gas liquid chromatography.

Materials

Ethanol stock: absolute alcohol. (McAlpine Pharmaceuticals Ltd., Toronto).

Ethanol working standard (100 mg/ml): 0.5 ml of absolute ethanol diluted with 392 ml of deionized water.

Trichloracetic acid (TCA) stock solution: 100 g of trichloracetic acid are dissolved in 100 ml of deionized water.

Trichloracetic acid—Isopropanol solution: to 10 ml of the stock solution of TCA add 0.1 ml of isopropanol (Fisher spectral grade) and dilute to 100 ml with deionized water.

Chromatography was carried out using a Varian gas liquid chromatograph Model 2100, equipped with a flame ionization detector. The column was a U-shaped glass tube 4 ft long with a 2-mm internal diameter packed with uncoated Chromosorb 102, 60/80 mesh. The oven temperature was 150 C, injector and detector temperature was 180 C, range 1×10^{-11} and attenuation $\times 32$. Gas flow rates were nitrogen 25 ml/min, hydrogen 30 ml/min and air 100 ml/min.

Methods

Two disposable glass tubes were marked unknown and standard. To each was added 0.5-ml TCA: isopropanol solution and 0.5 ml of either standard or unknown was added to the appropriate tube. The tubes were capped, vortex mixed and centrifuged at 3000 rpm for 5 min.

0.5 μl volumes of the supernatants were injected into the gas chromatograph under the conditions specified above in duplicate. Ethanol and isopropanol peak heights were measured for each injection.

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Calculation of Results

$$\text{mg of ethanol/100 ml unknown} = 100 \times \frac{Eu/Iu}{Es/Is}$$

where Eu/Iu = the average ratio of the ethanol and isopropanol peak heights for the unknown

Es/Is = the average ratio of the ethanol and isopropanol peak heights for the standard

Figure 1 shows a typical chromatogram on Chromosorb 102 of a methanol and ethanol-containing plasma prepared by TCA treatment. The present method has been compared with that used in two outside laboratories where sodium tungstate/sulphuric acid were used as the plasma protein precipitants. The results (Table 1) show good agreement between the two procedures.

TABLE 1—*Test results.*

Ethanol added mg/100 ml of plasma	Ethanol found mg/100 ml of plasma		
	1[a]	2[b]	3[b]
19	21	...	24
38	38	40	39
76	75	80	77
95	94	107	96
110	114	118	114

[a] Present method.
[b] Plasma proteins precipitated with tungstic/sulphuric acid and the supernatants analyzed by gas chromatography in two different laboratories.

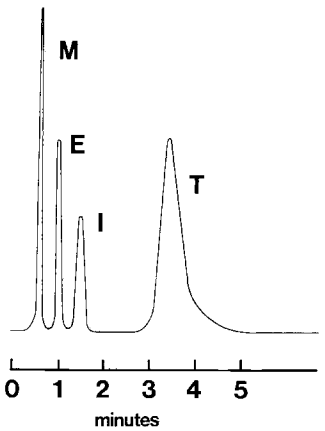

FIG. 1—*Gas chromatogram obtained by injecting 0.5 μl of a standard mixture.*

The within-batch variation (precision) for the procedure was determined by analysis of the same sample 20 times in a single day. The mean value was 114 mg/100 ml with a standard deviation of 4 mg. This degree of variation is partly due to the manual estimation of peak heights [*4*]. When the presence of acetone or isopropanol is suspected in the test specimen a different internal standard, say, isobutanol should be used.

Discussion

In a recent report [*5*] whole blood was diluted 1:1 with aqueous isobutanol and injected directly onto the column. This procedure has the obvious qualities of simplicity and rapidity. However a gas chromatograph with a long injection port is required to avoid on-column injection. In other approaches protein is precipitated using sodium tungstate/sulphuric acid [*6*] or barium hydroxide/zinc sulphate [*7*]. These procedures involve sample dilution on the order of 1:20, and at least three pipettings. Using TCA, dilution is limited to 1:1 and only one pipetting is required.

TCA has previously been used as a precipitating agent [*8,9*], however it has not been generally accepted because of the suggested possibility of alcohol oxidation in the acid environment [*7*]. We have not observed any decomposition of isopropanol (100 mg/100 ml) in 10 percent TCA over a period of one month at 4 C. Methanol, ethanol, and isopropanol in plasma are stable in TCA for 24 h at room temperature.

TCA being volatile gives a peak on Chromosorb 102, but does not affect the separation of volatile organic compounds. Because of the possible variable thermal breakdown of TCA to chloroform [*10*] during chromatography, TCA itself cannot be used as an internal standard. Chloroform and TCA do not separate on this column under the conditions used for ethanol estimations.

Summary

The use of trichloracetic acid as the protein precipitant appears to offer a sensitive, simple, and rapid method of sample preparation for the assay of volatile compounds present in plasma by gas liquid chromatography.

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