Determination of Ethylene Glycol in Postmortem Blood by Capillary Gas Chromatography

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Abstract

A simple procedure for the determination of ethylene glycol in blood by capillary gas chromatography was developed. The proteins are precipitated by the addition of perchloric acid which includes the internal standard 1,2-butanediol. The extract is neutralized and the solution is directly injected. The assay is linear and the precision, expressed as coefficient of variation, is 4–11% (within run). The detection limit is about 0.05 g/L. The method also seems applicable for the determination of ethylene glycol in urine.

Introduction

Poisoning by ethylene glycol is usually confirmed by demonstrating the presence of the diol in the blood. Chromatographic and spectrophotometric procedures have been developed for this purpose (1–12). Since these published methods are either non-specific or have other drawbacks such as nonlinearity, a procedure that uses capillary gas chromatography was developed. This procedure has been in use for the last couple of years and has been found satisfactory.

Experimental

Chemicals. The reagents were of analytical grade except for butanediol, which was synthetic grade. They all came from Merck A.G. A ready-to-use aqueous phosphate buffer (pH 7, 1M) containing 6.65 g potassium dihydrogen phosphate and 12.62 g disodium hydrogen phosphate in 100 mL of water (Merck, Cat. no 9464) was used for adjustment of pH. The concentration of the internal standard was 50 mg butanediol per 100 mL of perchloric acid (0.4M).

Gas chromatographic conditions. The samples were analyzed on a Hewlett-Packard gas chromatograph model 5880A equipped with a flame ionization detector. For chromatography a fused-silica capillary column, 25 m × 0.32 mm, with Chrompack CP Wax 57-CB of 0.20-µm film thickness (Chrompack) was used. Temperature conditions were, injector, 250°C; oven, 110°C, 6°/min to 130°C, held for 0.5 min, 30°/min to 250°C, held for 2 min. Helium carrier gas flow was 3 mL/min, and the split ratio was 1:10.

Procedure. To 0.2 g blood, 0.6 mL of the internal standard solution was added. After mixing and centrifugation the supernatant was decanted into another centrifuge tube. Fifty microliters of phosphate buffer (pH 7) was added and the sample was neutralized with a 2M potassium hydroxide solution (50 µL). The solution was refrigerated and centrifuged. One microliter of the supernatant was injected into the gas chromatograph.

Results and Discussion

Representative chromatograms of blank blood with the internal standard (1,2-butanediol) with and without ethylene glycol are shown in Figure 1. Precipitation of the proteins with perchloric acid results in a very clean extract, and no interfering peaks have so far been observed. However, impurities tend to deteriorate the liner and the column after repeated injection of blood extracts. Therefore, it was found necessary to clean the liner after about 10 injections and to increase the column temperature to 250°C during each run.

A standard curve plot was shown to be linear in the range tested, 0.5 to 5 g/L (0.8–81 mmol/L). The linear regression of 6 points at 5 levels (0.25, 0.50, 1.0, 2.0, and 4.0 g/L) was $y = 0.3526x - 0.0036$ ($r = 0.9999$). The detection limit of the method was found to be 0.05 g/L, which could be attained by injecting larger volumes of the extract. Repeated injections of blood extracts gave coefficients of variation (CV) of 11% at 0.5 g/L and 4% at 5 g/L.

In fatal cases the level of ethylene glycol in postmortem blood usually falls in the range 0.1 – 3.0 g/L. In one case with a protracted survival time, ethylene glycol intoxication being diagnosed at a late stage and no ethylene glycol being found by conventional gas chromatography according to Bost and Sunshine (1), we were able to detect 0.05 g of ethylene glycol per liter in serum from a blood sample taken 24 hours after ingestion of wine containing 41% ethylene glycol.

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Our experience so far is that extracts of urine prepared in the same manner are also quite clean and have detectable peaks of ethylene glycol.

References


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