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Rapid Determination of Ethylene Glycol in Biological Material

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Summary. A rapid and simple modification of a gas chromatographic (AFID) method of ethylene glycol determination, based on derivation with n-butylboronic acid, was developed.

Key words: Ethylene glycol, GC/AFID determination

Zusammenfassung. Es wird eine Modifikation zur schnellen Bestimmung von Glykol mit der Methode der Gaschromatographie mit Hilfe von Veresterung der n-Butylboronsäure dargestellt.

Schlüsselwörter: Äthylenglykol, GC/AFID-Bestimmung

Ethylene glycol poisoning occurs frequently in both clinical and forensic toxicology. Establishing a prompt diagnosis is of critical value in a toxicological ward; therefore, the determination of ethylene glycol in blood should be included in the emergency analyses. Ethylene glycol is usually determined by means of gas chromatography (GC). Several direct methods of GC assay of glycol have been published [1, 2]. These procedures, however, have the disadvantage of rather low reproducibility and sensitivity, mainly due to the adsorption of glycol on the column wall and packing. This may be overcome by the use of special packing [3] or be derived. Various derivation procedures for GC glycol determination have been developed [4–8]. For us, the most suitable procedure for both clinical and forensic purposes was esterification with n-butylboronic acid [7, 8] due to its relative simplicity and sensitivity. The procedures published, however, have some drawbacks: the original method of Robinson et al. [7] requires a long period of drying in a sand bath, whereas in the method of McCurdy et al. [8] the extraction step is introduced. Based on these methods, we have developed a simpler and faster modification, which is more applicable to emergency toxico-

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logical laboratory. Moreover, in the methods published the flame-ionization detector has been used, whereas the response of the alkali-flame ioniziation detector (AFID) has been reported to be higher than that of FID [9].

Material and Methods

Reagents

n-Butylboronic acid, analytical grade, was obtained from Fluka AG, Buchs (Switzerland). Ethylene glycol, 1,3-butanediol (internal standard), anhydrous acetone, and anhydrous sodium sulphate were of analytical grade.

Reagent Derivatives

0.2% n-Butylboronic acid in acetone, containing 1 g/l of 1,3 butanediol. It can be stored up to 4 weeks in a refrigerator.

Procedure

One milliliter of sample (blood or tissue homogenate) was ground with 1.5 g anhydrous Na_2SO_4 in a test tube and 2 ml derivative reagent was added. After 15-s vortexing the sample was centrifuged or filtered, and 1–2 µl was injected on a gas chromatograph without any concentration. The calibration standards were prepared from drug-free samples of appropriate tissue with the addition of ethylene glycol in a concentration range of 0.5 to 5.0 g/l. The chromatographic analysis was carried out on Chrom-5 gas chromatograph (Laboratory Instruments, Prague, Czechoslovakia) equipped with FID/AFID detectors and 3% ÖV-17 column. Nitrogen was used as a carrier gas, and the oven temperature was 90°C. The concentration of ethylene glycol in samples was calculated by means of an internal standard method using 1,3-butanediol as internal standard.

Results

The modification applied, which consists of replacing the sand bath drying or extraction with the rapid dehydration with sodium sulphate, did not alter the analytical results. The chromatographic pictures were identical to those obtained with the methods of Robinson [7] or McCurdy [8]. The minimal concentration of glycol detected was 5 mg/ml. Such sensitivity is sufficient for routine casework; it can be easily enhanced by means of concentration of the sample. The precision of the method was examined in five series using precision standard prepared from autopsy blood and liver homogenate, containing about 2 g/l glycol. Table 1 shows within-day and day-to-day precision. The peak height or peak area ratios showed a linear increase of up to 5 g/l glycol.

Table 1. The precision of ethylene glycol determination

Sample	Precision (Coefficient of variation)	
	Within-day	Day-to-day[a]
Blood	4%	10%
Liver	10%	15%

[a] Data obtained by four different analysts

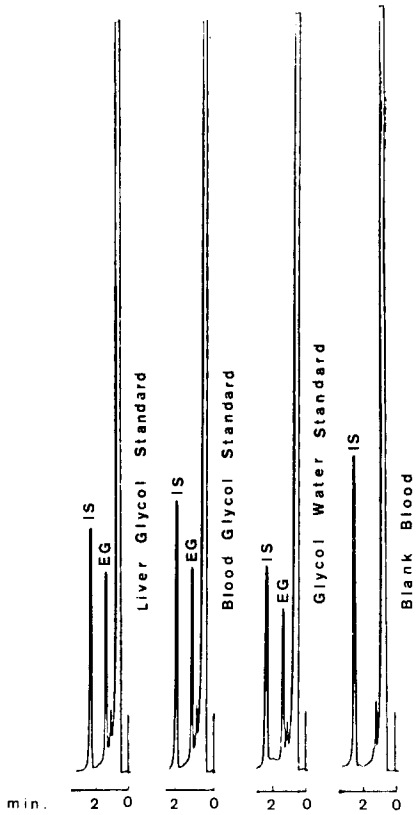

Fig. 1. Chromatograms of blank blood (without glycol), water glycol standard and blood and liver sample with addition of glycol. Conditions as in the text. *EG*, ethylene glycol, *IS*, internal standard

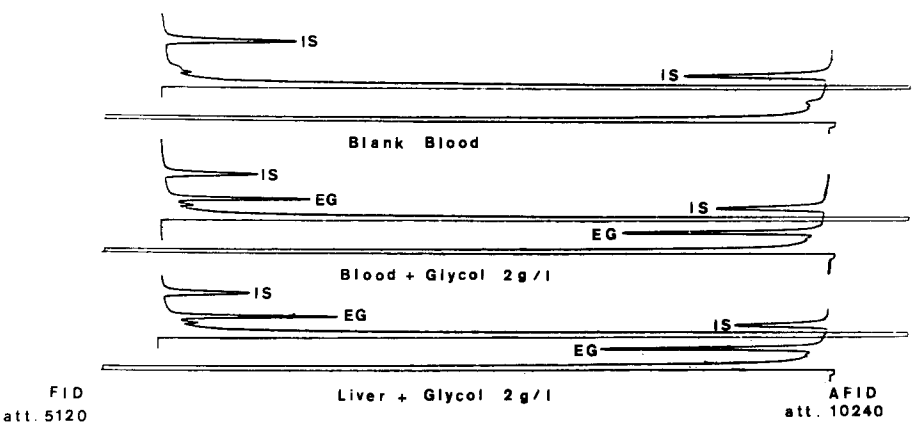

Fig. 2. Comparison of signal response to glycol and internal standard. Column: 3% OV-17, effluent splitter 1:1, detectors FID and AFID (rubidium chloride). Abbreviations as in Fig. 1

The analysis of several blank samples of autopsy blood and liver showed only one endogenous peak at the beginning of the chromatogram (Fig. 1). Therefore, the additional clean-up procedure was not necessary. Figure 2 shows the comparison of AFID and FID response to ethylene glycol and 1,3-butane-

diol butyl boronates. The response ratio AFID : FID was about 3 for ethylene glycol; it is advisable, therefore, to use AFID for glycol detection.

In the literature, there are conflicting statements concerning the influence of water on esterification [7, 8]. In our experience the presence of water distinctly decreases the rate and efficiency of derivation; therefore, the sample must be thoroughly dried with sodium sulphate. The recovery of glycol from blood and liver, calculated against water standards of glycol, averaged 70%.

Conclusion

Gas chromatographic (AFID) determination of ethylene glycol through esterification with n-butyl boronic acid is a simple, sensitive and sufficiently reliable method of detection and quantitation. The modification applied is rapid (about 15 min), extremely simple, and therefore can be recommended for emergency toxiology.

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