Gas-Chromatographic Determination of Ethylene Glycol in Serum

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We describe a new gas-chromatographic procedure for ethylene glycol in serum, determined as the cyclic phenylboronate ester. The method is rapid, requires only 100 µL of serum, and either an OV-1 or an OV-17 column is used. The linear dynamic range extends to at least 5000 mg/L, with a lower limit of detection of 10 mg/L. Analytical recovery of ethylene glycol from serum averaged 97% (95–101%) over the concentration range of 250–5000 mg/L. The method demonstrates good precision, with CVs <2.5%, over the concentration range 500–2000 mg/L.

Additional Keyphrases: ethylene glycol toxicity - phenylboronates - 1,2-diols, 1,3-diols - OV-1, OV-17 - mass spectrometry

Intoxication due to ethylene glycol, although relatively rare, is a major clinical emergency that requires early identification of the toxic agent so that appropriate therapeutic measures can be instituted (1, 2).

The toxicity of ethylene glycol is associated with its metabolism by liver alcohol dehydrogenase to several compounds, including glycolic and oxalic acids (1). Consequently, a moderate to severe metabolic acidosis ensues, along with renal and cerebral organ damage (1, 3). Ethanol is effective as a specific antidote to inhibit the alcohol dehydrogenase-mediated metabolism of ethylene glycol (1, 2). Hemodialysis and forced diuresis may also be used in association with ethanol infusion (1, 2).

Several gas-chromatographic procedures have been proposed for determination of ethylene glycol in serum or blood (4–7). Those which involve the direct determination of underivatized ethylene glycol suffer from low sensitivity and problems associated with tailing (4, 5). Other methods based on derivatization of ethylene glycol are either tedious and suffer from poor precision (6) or do not provide for the use of an internal standard and have only moderate limits of detection (7). We have attempted to analyze serum ethylene glycol by the direct injection of a diluted specimen onto a column of 0.8% THEED on Carpack C 80/100 (Supelco), without success. Although the peak exhibited by ethylene glycol was sharp and non-tailing, a marked "memory effect" was observed; i.e., injection of water just after a serum specimen that contained ethylene glycol resulted in the elution of a substantial ethylene glycol peak. A recent fluorometric procedure based on the Hantzsch condensation reaction after periodate oxidation of ethylene glycol has been reported (8).

This method offers an alternative to some of the problems associated with the determination of ethylene glycol by gas chromatography but is limited by a narrow range of linearity and by interference from above-normal concentrations of triglycerides in serum.

Clearly, this determination continues to present difficult analytical problems. We have therefore developed a gas-chromatographic method based on analysis of the phenylboronate derivative of ethylene glycol on an OV-1 or an OV-17 column. The reaction of alkyl and aryl boronic acid derivatives with 1,2- and 1,3-diols to form cyclic boronate esters is well known (9, 10). The reaction of phenylboronic acid with ethylene glycol proceeds as shown in Figure 1. In a similar fashion, six-membered cyclic boronate derivatives are formed from 1,3-diols. The method described is rapid, sensitive, precise, and demonstrates a wide linear dynamic range.

Materials and Methods

Reagents and Standards

Ethylene glycol (ACS grade) and 1,2-propanediol (propylene glycol) were obtained from Fisher Scientific, Fairlawn, NJ; 1,3-propanediol was a product of Eastman Organic Chemicals, Rochester, NY. Phenylboronic acid and 2,2-dimethoxypropane were products of Aldrich Chemical Co., Milwaukee, WI. Acetonitrile (HPLC grade) was from Waters Associates, Milford, MA. Bovine serum albumin was a product of Sigma Chemical Co., St. Louis, MO.

Ethylene glycol, stock solution, 2000 mg/L, was prepared to also contain 70 g of bovine serum albumin (BSA) per liter. Additional working standards were prepared by diluting the stock standard with 70 g/L BSA to provide ethylene glycol concentrations of 1000 and 500 mg/L.

1,3-Propanediol, the internal standard, 500 mg/L, was prepared in acetonitrile.

Phenylboronic acid, 40 mmol/L, was prepared in 2,2-dimethoxypropane.

Instrumentation

A Perkin-Elmer Model 900 gas chromatograph equipped with dual-flame ionization detectors was used for these studies. Analyses were performed on a 185 cm × 2 mm (i.d.) column of 3% OV-17 on 80/100 mesh Gas Chrom Q (Applied Science Laboratories, State College, PA). Nitrogen was used as the carrier gas at a flow rate of 25 mL/min. The column was operated isothermally at 110 °C. Injector and detector temperatures were 200 and 250 °C, respectively.

Additional experiments were performed with a Perkin-Elmer Model 3920 gas chromatograph using the same size column of 3% OV-1 on 80/100 mesh Supelcoport (Supelco, Bellefonte, PA). Temperature conditions and nitrogen flow rates were as described for the OV-17 column.

For the mass spectral determinations, a Finnigan Model
Results

Figure 2 illustrates the chromatographic response for ethylene glycol phenylboronate and the phenylboronate derivatives of 1,3-propanediol and 1,2-propanediol. The peaks are sharp and symmetrical on both the OV-17 and OV-1 columns, but better resolved on the OV-1 column. On OV-17, ethylene glycol phenylboronate and 1,2-propanediol phenylboronate co-elute from this column at any of the several different temperatures tested. For the method to be applicable for use on either OV-1 or OV-17 columns, 1,3-propanediol was selected as the internal standard.

Figure 3 (left) illustrates the chromatographic response obtained with a typical serum specimen containing no ethylene glycol. In some blank sera (12 of 50), a small peak with a retention time on OV-17 similar to that of ethylene glycol phenylboronate was detected (Figure 3, right). Moreover, this peak was also not resolved from ethylene glycol phenylboronate on the OV-1 column and was not altered by omitting phenylboronic acid from the procedure. It therefore does not represent either ethylene glycol or 1,2-propanediol. In all cases this interference corresponded to <10 mg of apparent ethylene glycol per liter.

The method demonstrates a wide linear dynamic range (Figure 4). For routine use, standards are prepared to contain ethylene glycol at concentrations up to 2000 mg/L. However, as indicated in Figure 4, the response is linear to at least 5000 mg/L.

For analytical recovery studies we used seven pools of human serum, prepared to contain ethylene glycol in concentrations covering the range of 250 to 5000 mg/L. When aqueous ethylene glycol standards were used, the average recovery of ethylene glycol from the serum pools was 85.5% (range 71.7–95.4%). To compensate for this low recovery, we prepared standards to contain 70 g of BSA per liter. With these standards, the mean analytical recovery of ethylene glycol from the serum pools was 97.1% (range 94.8–101.4%).

Precision studies were conducted by performing five repetitive analyses of serum pools containing 500, 1000, and 2000 mg/L ethylene glycol on each of three separate days (i.e., a total of 15 determinations each). The combined within-day

4000 GC/MS instrument was used. The mass spectrometer was operated at an ionizing voltage of 70 eV. The gas chromatograph was equipped with a 122 cm × 2 mm (i.d.) column of 3% OV-101 on Chromosorb W (HP), 100/120 mesh. Helium was used as the carrier gas, at a flow rate of 25 mL/min. The column was operated at 110 °C and the injector at 180 °C.

Procedure

Place a 100-μL aliquot of serum or standard in a 12-mL conical centrifuge tube, and add 200 μL of acetonitrile containing the internal standard, 1,3-propanediol. Vortex-mix and remove the precipitated protein by centrifugation. Mix a 50-μL aliquot of the supernate, which contains the ethylene glycol and the internal standard, with 50 μL of phenylboronic acid in 2,2-dimethoxypropane. Inject between 0.5 and 1.0 μL of the reaction mixture into the gas chromatograph.

For quantitation, plot the peak height ratio (ethylene glycol/1,3-propanediol) for the standards vs the concentration of the standards to provide a calibration curve. Then determine the concentration of ethylene glycol in the patient’s serum by comparing the sample peak height ratio directly with the standard curve.

Fig. 2. Chromatogram of the phenylboronates of ethylene glycol and 1,3-propanediol
Left-hand pattern: OV-1. Right-hand pattern: OV-17. Ethylene glycol phenylboronate 1, 1,2-propanediol phenylboronate 2, 1,3-propanediol phenylboronate 3

Fig. 3. The chromatographic response on OV-17 for sera containing no ethylene glycol
Left: Typical response given by blank sera. Right: Response representative of 25% of the blank sera examined. Peak 1 represents a serum peak with a retention time similar to that of ethylene glycol phenylboronate. Peak 3 represents the internal standard, 1,3-propanediol, present at one-quarter the usually used concentration. The concentration of the serum peak, expressed as apparent ethylene glycol, was <10 mg/L in all cases
and day-to-day precision for the 500, 1000, and 2000 mg/L samples was 2.3, 2.2, and 0.4%, respectively. Comparable results were obtained with either the OV-1 or OV-17 columns.

Discussion

Our method overcomes many of the aforementioned analytical problems in the analysis for ethylene glycol. The phenylboronates form rapidly and demonstrate good chromatographic characteristics. We also investigated the use of n-butylboronic acid for derivatization. However, the n-butylboronate ester of ethylene glycol is not retained on either the OV-1 or the OV-17 column, even at temperatures as low as 70 °C.

Under the conditions of the analyses, the final reaction mixture contains about 80 mL of water per liter. The use of 2,2-dimethoxypropane as the solvent for phenylboronic acid aids in achieving this low water content. In the presence of an equimolar quantity of water, 2,2-dimethoxypropane undergoes solvolysis to form methanol and acetone in a molar ratio of 2:1 (11). Although some phenylboronate derivatives do not form in a aqueous medium (10, 12, 13), the proportion of water in our reaction mixture apparently does not inhibit reaction with the glycols we examined. When the reaction was performed under anhydrous conditions, we saw no increase in absolute peak heights for ethylene glycol phenylboronate.

It is also apparent that phenylboronate formation can occur “on column.” When a sample of ethylene glycol was injected onto the OV-17 column, followed by a separate injection of phenylboronic acid in 2,2-dimethoxypropane, a peak corresponding to the phenylboronate of ethylene glycol was seen. In this regard, we were unable to demonstrate a time dependency for the formation of the phenylboronate. Absolute peak heights obtained immediately after adding phenylboronic acid to a solution of ethylene glycol in acetonitrile did not increase during 30 min. Therefore, the phenylboronate either forms extremely rapidly or at least in part may be formed after injection into the gas chromatograph.

The small amount of water injected onto the column appeared to exert no deleterious effect on column performance. Several hundred injections were made onto the OV-17 column over a period of three months without loss of column effi-

Fig. 4. Linear dynamic range for the gas-chromatographic response of ethylene glycol phenylboronate

"Peak height ratio" is the ratio of the peak height of ethylene glycol to that of the internal standard, 1,3-propanediol. The bars correspond to the range of the peak height ratios obtained on three separate days (i.e., three samples per point on the graph).

Fig. 5. Mass spectra of phenylboronate derivatives

A. Ethylene glycol phenylboronate; B. 1,2-Propanediol phenylboronate; C. 1,3-Propanediol phenylboronate
ciency. It is, of course, possible to dry the sample by evaporation prior to formation of the boronate derivative; however, this not only is less convenient, but results in significantly poorer precision.

That the phenylboronate derivatives were indeed formed under the conditions of the assay is shown by the mass spectra presented in Figure 5. The phenylboronates of ethylene glycol, 1,2-propanediol, and 1,3-propanediol display good mass spectral characteristics with prominent molecular ions observed at m/z 148, 162, and 162, respectively. Corresponding peaks for (M-1)+ represent ions containing 10B rather than 11B (natural abundance, 10B:11B = 1:4.2) and aid in the interpretation of the mass spectra. For ethylene glycol phenylboronate, additional prominent ions correspond to the elimination of CH2O to form an ion with an m/z of 118 (M-30)+ and to the formation of the tropilium ion with an m/z of 91 (Figure 5A). For the phenylboronate of 1,2-propanediol, a prominent ion resulting from the loss of CH3 to produce an ion with an m/z of 147 (M-15)+ is observed (Figure 5B). This ion, as expected, is absent from the mass spectrum representing the phenylboronate of 1,3-propanediol (Figure 5C). Additional ions corresponding to the formation of Ph-B - O+ (m/z = 104) and to the tropilium ion (m/z = 91) are observed in each of the latter two spectra.

The method as described requires only 100 µL of serum, is rapid, and utilizes a gas chromatograph equipped with either an OV-17 or an OV-1 column, both of which are in common use in most toxicology laboratories. The linearity of the method extends to at least 5000 mg/L with a lower limit of detection of 10 mg/L. This lower detection limit is imposed by the presence of a serum peak, corresponding in retention time on OV-1 and OV-17 to that of ethylene glycol phenylboronate, which was observed in 25% of the serum samples examined. This peak can readily be distinguished, however, from that of a possible trace of ethylene glycol by omitting phenylboronic acid from the procedure. The serum peak is unaffected by the omission of phenylboronic acid, whereas ethylene glycol requires the presence of phenylboronic acid for GC detection.

The recovery of ethylene glycol following precipitation of serum proteins with acetonitrile is approximately 85%. Substitution of ethanol for acetonitrile, even in the presence of saturating quantities of MgSO4, did not improve the recovery of ethylene glycol. Therefore, it is necessary to prepare the standards in a solution of 70 g/L albumin to compensate for the apparent protein binding and subsequent loss of ethylene glycol. When standardized in this manner, the recovery of ethylene glycol from pooled serum averaged 97% over the concentration range of 250–5000 mg/L. This recovery was not altered by increasing the albumin concentration to 80 g/L and was decreased only slightly to an average of 96% when the albumin concentration was decreased to 50 g/L.

The method demonstrates good precision, with CVs below 2.5% over the concentration range 500–2000 mg/L. We saw no interference from acetone, ethanol, methanol, isopropanol, lactic acid, or from several different barbiturates or benzodiazepines under the conditions of the determination.

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References