Liquid-Chromatographic Determination of Ethylene Glycol in Plasma

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In this novel procedure for determining ethylene glycol in plasma by liquid chromatography, benzoyl esters of ethylene glycol and of benzyl alcohol (used as the internal standard) are prepared directly in plasma. The benzoyl esters, highly ultraviolet-absorbing chromogens, are ideal compounds for analysis by reversed-phase liquid chromatography with methanol/water as the mobile phase. The benzoyl derivative of ethylene glycol is well separated from the derivative of the internal standard and from plasma constituents. The standard curve is linear to 400 mg of ethylene glycol per liter. As little as 10 mg of ethylene glycol per liter of plasma can be measured. Other commonly ingested alcohols do not interfere.

Additional Keyphrases: toxicology · liquid- and gas-chromatography compared

Determination of ethylene glycol (EG) in plasma is useful for the diagnosis and management of patients who are believed to have ingested ethylene glycol, a constituent of antifreeze and of a wide range of other commercial products (1). Colorimetric (2) and fluorometric (3) procedures for determination of EG are based on its oxidation to formaldehyde and are considered nonspecific. Enzymic procedures (4) for determination of EG are also nonspecific. Gas chromatography involving direct injection of diluted plasma on columns used for routine blood-ethanol analysis (5, 6) is apparently the most commonly used technique, but it shows low sensitivity and poor precision because of the nonsymmetrical and tailing peak of EG (5, 7, 8). In attempts to improve the sensitivity, EG has been extracted, and the extract evaporated and converted to the dibenzoyl derivative before gas chromatography (7), but this procedure shows poor precision, attributed by the authors to extraction difficulties. Extraction of EG is also required before preparation of n-butylboronate esters in the recently described gas-chromatographic procedure for the analysis for EG in blood (8). Here we report a sensitive procedure for quantitation of ethylene glycol in plasma, in which benzoyl esters of EG and benzyl alcohol, used as internal standard, are prepared directly in plasma by the Schotten–Baumann reaction. The benzoyl esters are analyzed by liquid chromatography with use of an RP-18 column and methanol/water as the mobile phase.

Materials and Methods

Materials

All reagents were of analytical grade and solvents had been distilled in glass by the supplier (Caledon Laboratories Ltd., Georgetown, Ont., L7C 4R9). De-ionized water was used throughout.

*Stock EG solution*, 10 g/L. Dilute 0.9 mL of ethylene glycol (relative density 1.1) to 100 mL with water. Store this solution at 4 °C. It is stable for at least a month.

*Plasma standard*, 400 mg/L. Dilute 2 mL of stock EG solution to 50 mL with blood-bank plasma. Prepare standards of 200, 100, 50, and 25 mg/L, in plasma, by serial dilution. Divide the plasma standards into 1-mL aliquots and store frozen.

*Stock benzyl alcohol*, 10 g/L. Dilute 1 mL of benzyl alcohol to 100 mL with water. Store at 4 °C, this is stable for at least a month.

Working internal standard. Prepare when required by diluting 1 mL of stock benzyl alcohol solution to 100 mL with water.

Procedures

*Esterification.* To 0.5 mL of plasma (standard or test) in Teflon-lined screw-capped culture tubes, add 0.5 mL of working internal standard and 1 mL of 4 mol/L sodium hydroxide. Cool the tubes in ice water and mix their contents. Drop 50 μL of benzylo chloride onto the liquid surface in each tube, and mix in a rotary mixer for 10 min. To each tube add a drop of 10 g/L solution of glycine, mix, allow to stand for 2–3 min, add about 8 mL of pentane to each tube, mix the contents of the tubes in a rotary mixer for 3–4 min, and centrifuge. Collect the clear pentane layer into correspondingly labeled disposable 16 × 100 mm tubes, taking care not to collect any trace of aqueous phase or emulsion. Evaporate the pentane extract at 45–50 °C and dissolve the residue in 100 μL of methanol. Inject 5 μL of this solution into the liquid chromatograph.

*Chromatography.* We used a pump (Model 750) with a ternary gradient programmer (Model 752), a variable-wavelength detector (Model 786), and an integrator plotter (Model 740), all from Micrometrics Instrument Corp., Norcross, GA 30093. The syringe loading injector (Model 7125) with a 20-μL loop was from Rheodyne Inc., Cotati, CA 94928. We used a 5-μm UltraSphere-ODS column (25 cm × 4.6 mm) from Altex Scientific Inc., Berkeley, CA 94710, protected by a guard column packed with CO: Pell ODS (Whatman Inc., Clifton, NJ 07014). The chromatography was done at room temperature. The flow rate of the mobile phase (methanol/water, 72/28 by vol) was 1.8 mL/min, with an operating pressure of 27.7 MPa (4000 lb/in.2). The effluent was monitored at 237 nm.

Results and Discussion

Figure 1 shows a representative chromatogram of an extract of plasma supplemented with EG. The relation between ratios of areas or peak heights of EG/internal standard and concentration of EG standards is linear from 25 to 400 mg/L, and the curve passes through the origin. The benzoyl derivatives of EG and of benzyl alcohol are stable for at least 24 h at room temperature.

Esterification of alcohols in aqueous medium by the Schotten–Baumann reaction is a well-known technique. As seen in Figure 1, extension of this reaction to benzyolation of EG in plasma produces only a few additional peaks. Analytical recovery of EG from plasma is 40–50% of EG recovery from aqueous standards.

Analysis of plasma supplemented with 100 mg of EG per liter showed a within-batch CV of 12% (n = 10, mean = 99 mg/L) and a between-batch CV of 11% (n = 10, mean = 98 mg/L) during three weeks. Although it has been claimed that
EG in plasma is unstable and cannot be stored (6), we have observed no significant change in EG in the frozen supplemented samples over a period of one month or in samples stored at 4 °C for three days.

It is more economical to use gas chromatography than liquid chromatography because of the high cost of glass-distilled solvents; moreover, benzoyl esters of dihydric alcohols obtained by hydrolysis of meprobamate and mebutamate have been analyzed by gas chromatography with acceptable precision (9). However, when 2-μL aliquots of a methanolic solution of the benzoyl derivative of EG were repeatedly injected onto a gas-chromatographic column (glass, 1.8 m × 4 mm) packed with 3% OV-17 on Gas Chrom Q (100/120 mesh) and run at 260 °C, the resulting EG peak heights were highly variable. In contrast, under the conditions we use, 2-μL aliquots of a methanolic solution of benzyl benzoate on repeated injections produced peaks of the same height. Possibly the poor precision observed by Peterson and Rodgerson in their gas-chromatographic analysis of EG (7) may have been the result of this apparently poor gas-chromatographic behavior of the benzoyl ester of EG.

Primary and secondary amines readily form benzoyl derivatives under the conditions of the Schotten–Baumann reaction. However, pentane, a nonpolar solvent, does not extract amides. Therefore, basic drugs and drugs such as acetaminophen, which has an amide group, do not present interference problems. Similarly, the benzoyl derivative of salicylic acid cannot be extracted because of the high pH. Benzoyl derivatives of methanol, ethanol, and 2-propanol do not interfere, either with EG or with the internal standard. Plasma constituents produce no interfering peaks under the described conditions, and EG at a concentration of 10 mg/L was easily detected.

We conclude that the described procedure is an acceptable alternative to the gas-chromatographic procedure for determination of ethylene glycol in plasma.

References