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Simultaneous determination of ethylene glycol, propylene glycol, 1,3-butylene glycol and 2,3-butylene glycol in human serum and urine by wide-bore column gas chromatography

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ABSTRACT

A method has been developed for the separation and measurement of ethylene glycol and three other glycols (propylene glycol, 1,3-butylene glycol and 2,3-butylene glycol) in biological samples by wide-bore column gas chromatography with a flame ionization detector. The method used 1,3-propylene glycol (1,3-propanediol) as an internal standard. The method was linear at least from 2 to 1000 μg/ml, with a detection limit of 1 μg/ml. Analytical recoveries were 89–98% for the different concentrations. Precision studies showed coefficients of variation of 1.5–7.7% for the different concentrations. The assay was applied to the analysis of biological samples from two patients who had ingested ethylene glycol and/or other glycols in a suicide attempt.

INTRODUCTION

Ethylene glycol causes severe poisoning in humans. Toxicity results from tissue damage due to the precipitation of calcium oxalate crystals and from severe acidosis due to aldehyde glycolate production [1]. Ethylene glycol is widely available in antifreeze and windshield washer formulations. Other glycols, such as propylene glycol (1,2-propanediol), 1,2-butylene glycol and 2,3-butylene glycol, are used for the same purposes. However, the toxicity of these products is different from that of ethylene glycol. For example, only high doses of propylene glycol can induce acidosis and central nervous depression when ingested [2]. The toxicity of 1,3-butylene glycol is intermediate between those of propylene glycol and ethylene glycol. Moderate doses of 1,3-buty-

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lene glycol may cause serious adverse reactions, ranging from hemolysis to narcosis, and respiratory and cardiac paralysis [3]. Thus, if glycols can be identified in serum as soon as possible, appropriate therapeutic measures can be taken to prevent severe organ damage or death [3,4].

Treatment of ethylene glycol intoxication consists of bicarbonate administration to correct the metabolic acidosis, ethanol or 4-methylpyrazole (4-MP) infusion to competitively inhibit the metabolism of ethylene glycol, and haemodialysis or peritoneal dialysis to increase the removal of ethylene glycol [1,5].

Methods for the determination of glycol in plasma or serum include packed [6,7], wide-bore [8] or capillary [9,10] column gas chromatography (GC), high-performance liquid chromatography [11], gas chromatography–mass spectrometry [12] and enzymatic assay [13]. Most of these methods permit only the measurement of serum ethylene glycol levels, with the exception of the technique described by Moffat *et al.* [7] or Smith [9], using packed and capillary column GC.

This paper describes a selective and sensitive method designed to separate and determine ethylene glycol and three other glycols (propylene glycol, 1,3-butylene glycol and 2,3-butylene glycol) involving protein denaturation and phenylboronate derivatization, followed by wide-bore column gas chromatography. However, phenylboronate derivatization does not measure diethylene glycol, which occurs more often than butylene glycol in toxicology. The method features a simple and reliable sample work-up and a rapid, accurate and reproducible GC method. This assay is applicable to both toxicological research and emergency analysis, particularly in identifying and determining glycols present in unknown substances and thus confirming or excluding the presence of ethylene glycol.

EXPERIMENTAL

Chemicals

Ethylene glycol (EG), propylene glycol (PG), 1,3-butylene glycol (1,3-BG) and 2,3-butylene glycol (2,3-BG) were purchased from Sigma (Saint Quentin Fallavier, France). The internal standard (I.S.) 1,3-propanediol and phenylboronic acid were obtained from Aldrich (Brussels, Belgium). Acetonitrile and methanol were produced from Merck (Darmstadt, Germany). All other chemicals were of analytical-reagent grade and were used as received.

Standard solutions

Stock solutions (5 g/l) of EG, PG, 1,3-BG and 2,3-BG were prepared in methanol. The working solutions in methanol were obtained by diluting stock solutions 1:100. All solutions were stored at 4°C for up to three months. A stock solution of the I.S. (1 g/l) was prepared in methanol, and a working solution (50 mg/l) was prepared in acetonitrile and kept at 4°C for up to three months at least.

Serum standards (calibration standards) were prepared at concentrations of 2, 5, 10, 20, 50 and 100 μg/ml by adding 0.5 ml of drug-free human serum to the residue obtained by evaporations of 20, 50, 100, 200, 500 and 1000 μl of working solution. A 500-μl volume of the I.S. working solution (25 μg) was used for internal standardization.

Instrumentation and chromatographic conditions

The determination was carried out using a system consisting of a Varian 3300 gas chromatograph (Varian, Les Ulis, France) equipped with a direct column injector and a flame ionization detector. The detector output was connected to a Servotrace 8560 recorder (Sefram, Paris, France).

The column was a wide-bore DB5 column (10 m × 530 μm I.D.) (Chomoptic, Montpellier, France) with a film thickness of 1.5 μm.

The instrumental conditions for calibration and assays were as follows: nitrogen, hydrogen and air flow-rates were 3, 20 and 300 ml/min, respectively; the injector port temperature was 250°C; the detector temperature was 300°C.

The column was temperature-programmed from 70 to 250 °C as follows: the initial temperature (70°C) was held for 30 s, then the temperature was increased to 120°C at 20°C/min, from 120 to 150°C at 5°C/min, and from 150 to 250°C at 20°C/min. The temperature was held at 250°C for 30 s.

Extraction procedure

Calibration graph. Serum standards (0.5 ml) were pipetted into a 5-ml screw-capped tube containing 500 μl (25 μg) of the I.S. The tubes were shaken for 30 s on a Vortex-type mixer and centrifuged at 4000 *g* for 5 min to sediment the protein precipitate. An aliquot of the supernatant (500 μl) was transferred to a conical tube, and 250 μl of phenylboronic acid solution in methanol (80 m*M*; stability two months at 4°C) were added. All tubes were mixed and they were incubated at room temperature for 15 min. After incubation, 0.1–0.2 g of sodium sulphate were added to dry the organic layer. A small aliquot (0.5 μl) of this organic layer was injected into the gas chromatograph.

Samples. Serum or urine samples (500 μl) were treated as described above. If necessary, these samples were diluted 1:5 with water and with blank serum, respectively.

Quantification, recovery and precision. The calibration graphs were obtained by linear regression of peak-area ratios of the compounds to the I.S. plotted against concentration. The linearity was assessed between 0 and 1000 μg/ml. The extraction recovery was calculated by comparing the measured values for spiked serum samples with those for standard aqueous solutions at two concentrations (5 and 50 μg/ml). The intra-assay precision was assessed at three concentrations (20, 50 and 100 μg/ml) for ten determinations. The inter-assay precision was determined once at the three concentrations in ten replicates.

Biological samples

Blood samples were collected in tubes without anticoagulant and centrifuged at 2000 *g* for 10 min to separate the serum. Serum was stored at −20°C, until analysis. Total urine was collected, and an aliquot was frozen at −20°C.

Clinical application

Serum samples were obtained from two subjects in order to determine the concentrations of glycol by GC. The first (patient 1) was a 43-year-old white male with chronic alcoholism who had ingested an unknown amount of ethylene glycol solution. He had been admitted to the intensive care and serial serum samples were obtained from admission to 66 h after admission. The second (patient 2) was a 30-year-old white male who had ingested 0.5 l of an unknown mixture in a suicide attempt. He was admitted to the intensive care unit some time (*ca.* 20 h) after ingestion. Serial serum samples were obtained from admission to 32 h after admission. The patient was oliguric, and peritoneal dialysis was performed.

Patients 1 and 2 were treated by intravenous infusion of 4-MP at a total dose of 2500 mg and 1200 mg, respectively.

RESULTS AND DISCUSSION

Fig. 1 shows the separation and determination of glycols (EG, PG, 1,3-BG and 2,3-BG) in human biological fluids using 1,3-propylene glycol (1,3-propanediol) as the I.S. In the chromatograms obtained after extraction of 0.5 ml of blank serum, no additional peaks that could interfere with the determination of EG, other tested glycols and the I.S. were present (Fig. 1a). In serum, the retention times for EG, PG, 2,3-BG, I.S. and 1,3-BG were 5.4, 5.7, 6.1, 7.8 and 8.4 min, respectively (Fig. 1b). Chromatograms obtained by analysis of urine samples had a similar appearance (Fig. 1c). All glycols and the I.S. were well separated. As previously described, the derivatization procedure gave symmetric peaks with no evidence of tailing.

The retention times were shorter than those described by Moffat *et al.* [7]. The resolution of our method was better than that reported by Peterson and Rodgerson [14] or Cheung and Lin [15] who used packed column GC. This resolution was similar to that reported by Moffat *et al.* [7] and Smith [9], who used packed or capillary column GC. However, the method described by Moffat *et al.* does not use a derivatization procedure. Our method permits the rapid and efficient separation of EG and three other glycols. (PG, 1,3-BG and 2,3-BG). In contrast to capillary columns, which drastically lose their resolution power after injection of the boronic acid, the resolution power of the wide-bore column was not affected during the experiments.

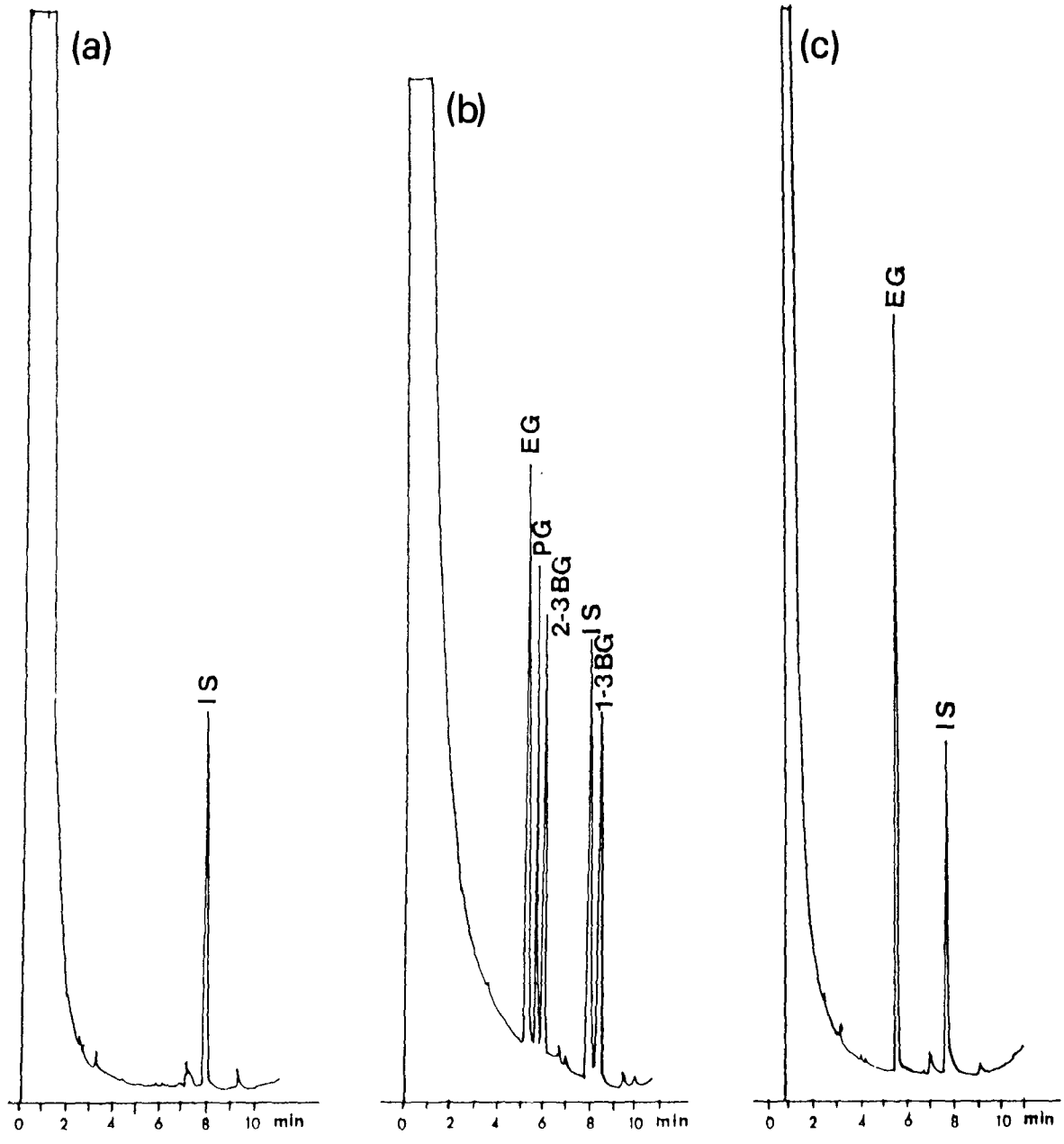

Fig. 1. Gas chromatographic profiles of (a) extracted blank serum with internal standard, (b) spiked human serum containing 100 μg/ml EG, PG, 1,3-BG and 2,3-BG and 25 μg/ml of internal standard, and (c) spiked human urine containing 200 μg/ml EG and 25 μg/ml internal standard. Peaks: EG = ethylene glycol; PG = propylene glycol; 1,3-BG = 1,3-butylene glycol; 2,3-BG = 2,3-butylene glycol; I.S. = internal standard.

Propylene glycol (1,2-propanediol) is normally used as the internal standard to quantify ethylene glycol in serum. We used 1,3-propanediol, to permit the simultaneous determination of ethylene glycol, propylene glycol and butylene glycols. This compound was chosen according to the work of Legatt and Tisdell [16].

The calibration graphs for these glycols in each biological fluid exhibited excellent linearity over the concentration range 2–1000 μg/ml. In each of

TABLE I

REPRODUCIBILITY OF THE GAS CHROMATOGRAPHIC METHOD

EG = ethylene glycol; PG = propylene glycol; 1,3-BG = 1,3-butylene glycol; 2,3-BG = 2,3-butylene glycol; n = number of determinations.

Concentration (μg/ml)	Sample	R.S.D. (%)			
		EG	PG	1,3-BG	2,3-BG
Intra-assay (n = 10)					
20	Serum	2.7	3.4	2.8	2.6
	Urine	3.1	3.0	2.5	3.2
50	Serum	2.9	1.6	3.9	3.1
	Urine	2.6	2.0	2.2	2.1
100	Serum	1.9	1.5	4.9	5.3
	Urine	2.4	2.3	3.6	3.8
Inter-assay (n = 10)					
20	Serum	4.5	4.0	2.8	7.7
	Urine	3.2	4.2	2.1	4.2
50	Serum	4.0	1.9	3.0	4.0
	Urine	5.1	2.5	3.4	2.8
100	Serum	7.1	1.7	3.4	3.6
	Urine	5.2	2.5	4.2	3.3

the biological fluids, the slopes for EG (y = 0.014x + 0.004, r = 0.9997), PG (y = 0.010x + 0.003, r = 0.9996), 1,3-BG (y = 0.008x + 0.002, r = 0.9998) and for 2,3-BG (y = 0.009x + 0.005, r = 0.9997) were similar. The calibration graphs had the same slope regardless of whether serum or urine was used.

By requiring a signal-to-noise ratio of greater than 5:1, the minimum detectable concentration of ethylene glycol was 0.5 μg/ml with a relative standard deviation (R.S.D.) of 15%. Balikova and Kohliak [6] reported a minimum detectable concentration of 6 μg/ml using packed column GC. The sensitivity of our method is the same as that of capillary column GC [9]. We found similar minimum detectable concentrations to that of EG for PG, 1,3-BG and 2,3-BG with R.S.D. of 12, 18 and 15%, respectively. For BG, Moffat *et al.* [7] described a minimum detectable concentration of 50 μg/ml with an R.S.D. of 4.5%.

The accuracy was 1.3, 2.0, 1.8 and 2.1% for 10 μg/ml EG, PG, 1,3-BG and 2,3-BG, respectively.

The intra- and inter-assay precision data for ethylene glycol and other glycols in serum and urine are summerized in Table I. The R.S.D. values (always less than 10%) demonstrate that the reproducibility of the method is good.

Analytical relative recoveries of ethylene glycol and other glycols in serum and urine are summarized in Table II. For ethylene glycol, these values are in agreement with those reported in the literature. Recoveries of 72 and 86% were reported by Peterson and Rodgerson [14] and Smith [9], who used dibenzoate and butylboronic acid derivatization, respectively. Balikova and Kohliak [6] reported analytical recoveries greater than 95% using phenylboronic acid. Unlike these authors [6,9], we did not detect the existence of a small interference peak, which could represent a reagent peak.

TABLE II

RECOVERY OF THE ANALYTICAL METHOD

EG = ethylene glycol; PG = propylene glycol; 1,3-BG = 1,3-butylene glycol; 2,3-BG = 2,3-butylene glycol; n = number of determinations.

Concentration (μg/ml)	Sample	Recovery (mean ± S.D., n = 5) (%)			
		EG	PG	1,3-BG	2,3-BG
5	Serum	90 ± 3.2	95 ± 5.0	91 ± 6.5	89 ± 2.1
	Urine	98 ± 0.5	97 ± 1.0	89 ± 3.0	92 ± 4.8
50	Serum	94 ± 2.4	90 ± 2.0	95 ± 5.1	89 ± 3.0
	Urine	92 ± 1.8	93 ± 2.6	90 ± 2.3	93 ± 2.1

The assay was shown to be selective, without interference from endogenous material (Fig. 1a) or from the other solvents studied, such as methanol, ethanol or acetone. Under our conditions, alle these solvents were rapidly eluted. However, as the possible interference of TRIS, an organic base frequently used to treat metabolic acidosis, has been reported [17], we tested this compound. In agreement with Smith and Raw [18], we concluded that wide-bore GC analysis of glycols in serum and urine was not susceptible to TRIS interference.

The GC procedure described above was used for the assay of serum samples obtained from two subjects who had ingested ethylene glycol, or ethylene glycol and propylene glycol in a suicide attempt. The concentrations of glycols were determined in serum collected regularly during the time-course of the poisoning. In both cases, the time-course of the serum ethylene glycol concentration can be described by a bi-exponential curve (Fig. 2a and b). For these two cases treated with 4-MP, elimination half-lives were 10.5 and 18 h for patients 1 and 2, respectively. These values are similar to those reported with ethanol therapy [19].

For patient 2, there was a great discrepancy between the kinetics of ethylene glycol and propylene glycol (Fig. 2b). The time-course of serum propylene glycol showed an increase of concentration from 2.5 to 12.5 h after admission into the intensive care unit. This first kinetic phase may have been due to prolonged absorption, which was not terminated at the time of the patient's admission. After this first phase, the level of propylene glycol remained constant between 12.5 and 17.5 h after admission. The late phase was a very fast elimination when the excretion of ethylene glycol was practically complete.

For patient 2, ethylene and propylene glycols excretion was determined 2 and 4 h after admission into the intensive care unit (Table III). Amounts of excreted ethylene glycol in the urine were 713 and 541 μg/ml, respectively. These values proved that the kidneys contribute greatly to the removal of ethylene glycol from serum in humans as previously reported [20]. Because of the

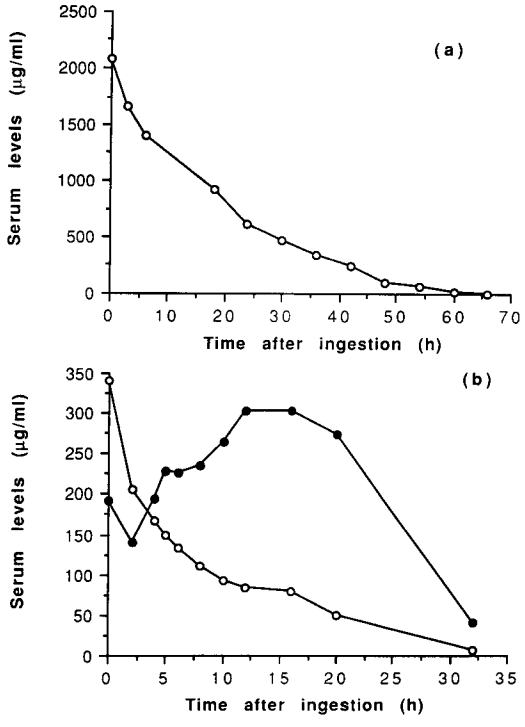

Fig. 2. Time-courses of (○) ethylene glycol and (●) propylene glycol serum levels in (a) patient 1 and (b) patient 2. For patient 1, ethylene glycol serum levels up to 1000 μg/ml were measured after dilution in blank serum.

prolonged absorption of propylene glycol, amounts of this substance excreted in urine are small during the same time (Table III). As patient 2 developed an organic renal failure, peritoneal dialysis was performed. The amounts of ethylene and propylene glycol excreted during dialysis were similar. These results showed that the kidneys were more efficient than peritoneal dialysis at removing ethylene glycol from serum. However, with propylene glycol, the efficiency of the kidneys was similar to that of peritoneal dialysis (Table III).

CONCLUSION

The GC assay described here showed good reproducibility, accuracy and selectivity. This method allows the simultaneous specific identification and precise quantification of ethylene gly-

TABLE III

URINARY AND DIALYSIS EXCRETIONS OF ETHYLENE AND PROPYLENE GLYCOL DURING ACUTE POISONING OF SUBJECT 2

EG = ethylene glycol; PG = propylene glycol.

Time after admission (h)	Urine output (ml)	EG (μg/ml)	PG (μg/ml)	Dialysis bath (ml)	EG (μg/ml)	PG (μg/ml)
2	40	713.0	29.5	6000	35.3	9.8
4	25	541.0	89.0	6000	1.3	4.1
6	–[a]			6000	14.0	27.6
8	–[a]			6000	26.7	48.0
10	–[a]			6000	2.0	12.5
12	–[a]			6000	19.3	51.7

[a] Organic renal failure.

col and three other glycols, which have very different toxicities. This assay has the advantage of being a relatively simple and convenient procedure, which can be used for therapeutic monitoring and diagnosis of glycol poisoning, as in the two cases described in this paper.

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