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RAPID DETERMINATION OF ETHYLENE GLYCOL AT TOXIC LEVELS IN SERUM AND URINE

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SUMMARY

The rapid gas chromatographic detection and determination of ethylene glycol in biological fluids is described. Phenylboronic acid in acetone was used for the esterification of glycol. The phenylboronates of ethylene glycol and 1,2-propylene glycol are not separated on a packed column of medium polarity (OV-17), but they can be separated on a non-polar column (OV-101). In both instances 1,3-propylene glycol can be used as an internal standard. The method requires only 100 μl of serum or urine and is suitable for trace analysis in an emergency toxicological laboratory. The utility of the method is demonstrated on two cases of human intoxication with ethylene glycol.

INTRODUCTION

Human intoxication with ethylene glycol occurs in both clinical and forensic toxicology. The toxicity and metabolism of ethylene glycol are well known [1,2], and therapeutic measures must be taken in time to protect organs from severe damage. Therefore, a rapid method for the detection and determination of ethylene glycol in biological fluids should be available in an emergency toxicological laboratory.

The direct determination of glycols by gas chromatography (GC) [3,4] is associated with low sensitivity and reproducibility (peak tailing and ghosting, low response of a flame ionization detector to ethylene glycol), and various derivatization procedures have therefore been used in determinations of glycols [5–8].

Brooks and Watson [9] introduced the use of cyclic boronates of bifunctional compounds in GC. The reaction is selective for bifunctional compounds in which the reactive groups are close enough for ring formation. The OH groups of diethylene glycol are too remote to react. Butylboronates have mostly been studied [10–16]. The cyclic esters of *n*-butylboronic acid are reported to be stable but they must be protected from moisture [10,12,14,16]. Halogen-substituted *n*-bu-

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tylboronic or phenylboronic acids have also been used for the formation of various derivatives and sensitive electron-capture detection [10].

For the rapid esterification of ethylene glycol we used phenylboronic acid in acetone, as this solution is stable for several months, and the retention times of the esters on column commonly used in the laboratory (3% OV-17, 3% OV-101) are convenient. The moisture in biological materials apparently did not affect the hydrolysis of phenylboronates during a period of 48 h. The method is suitable for trace analysis and requires only 100 μl of serum or urine. The application of this method to monitoring the therapy of human intoxication by haemodialysis is described.

EXPERIMENTAL

Chemicals

Analytical-reagent-grade chemicals were used. Acetone was obtained from Lachema (Brno, Czechoslovakia), 1,2-ethanediol and 1,2-propanediol from Reanal (Budapest, Hungary), 1,3-propanediol from Fluka (Buchs, Switerland) and phenylboronic acid from Ventron/Alfa (Danvers, MA, U.S.A.). The chromatographic materials used were 3% SP 2250 on Supelcoport (80–100 mesh) from Supelco (Gland, Switzerland), and the silicone stationary phase OV-101 on Gas-Chrom Q (80–100 mesh) from Applied Science Labs. (State College, PA, U.S.A.).

Instrumentation

A CHROM 61 gas chromatograph (Laboratorní přístroje, Prague, Czechoslovakia) was equipped with a flame ionization detector and a silanized glass column (1.95 m × 2 mm I.D.) packed with 3% SP 2250 on Supelcoport (80–100 mesh) or 3% OV-101 on Gas-Chrom Q (80–100 mesh). The detector output was connected to a TZ 4601 chart recorder (10 mV full scale).

The carrier gas was nitrogen at a flow-rate of 20 ml/min, with hydrogen at 18 ml/min and air 400 ml/min. The injection port and detector temperatures were 200°C and the column temperature was 140°C (3% SP 2250) or 105°C (3% OV-101).

Procedure

A 0.1-ml volume of serum or urine or an aqueous solution of ethylene glycol was mixed with 0.1 ml of acetone containing $2 \cdot 10^{-3}$ mol/l 1,3-propanediol (internal standard) and 0.1 ml of an acetone solution of $5 \cdot 10^{-2}$ mol/l phenylboronic acid. The mixture was shaken briefly, the precipitated proteins were separated by centrifugation and a 0.5-μl aliquot of the supernatant was injected into the gas chromatograph.

Quantitation was performed by comparing the sample peak-height ratio of phenylboronates (1,2-ethanediol/1,3-propanediol) to the standard peak-height ratio of derivatives prepared from aqueous solutions of ethylene glycol in the range $5 \cdot 10^{-4}$ to $5 \cdot 10^{-2}$ mol/l, multiplied by the recovery factor.

RESULTS AND DISCUSSION

The calibration of the method based on aqueous solutions of ethylene glycol was linear up to $5 \cdot 10^{-2}$ mol/l (3104 mg/l) with a limit of detection of 10^{-4} mol/l (6.2 mg/l), as demonstrated in Fig. 1.

The limit of detection was influenced by the background of blank samples. In some blank human and rat sera a small peak with the retention time of ethylene glycol phenylboronate was observed, as described previously [17]. Unfortunately, we found that omitting phenylboronic acid from the procedure does not distinguish this peak from that of underivatized ethylene glycol as was suggested elsewhere [17]. We found that injection of ethylene glycol onto the column on which we had analysed phenylboronates previously resulted in a peak with the same retention time as that of ethylene glycol phenylboronate. As it has been reported that phenylboronates are formed rapidly [10,11,17], we deduced that traces of phenylboronic acid retained in the GC system were sufficient to form on-column derivatives of ethylene glycol. The existence of this small peak in some blank sera determined the limit of detection of the method.

The distinction between negative and positive samples at low levels of ethylene glycol must be performed very carefully. We observed that a small amount of ethylene glycol remained adsorbed on the column and these traces were displaced

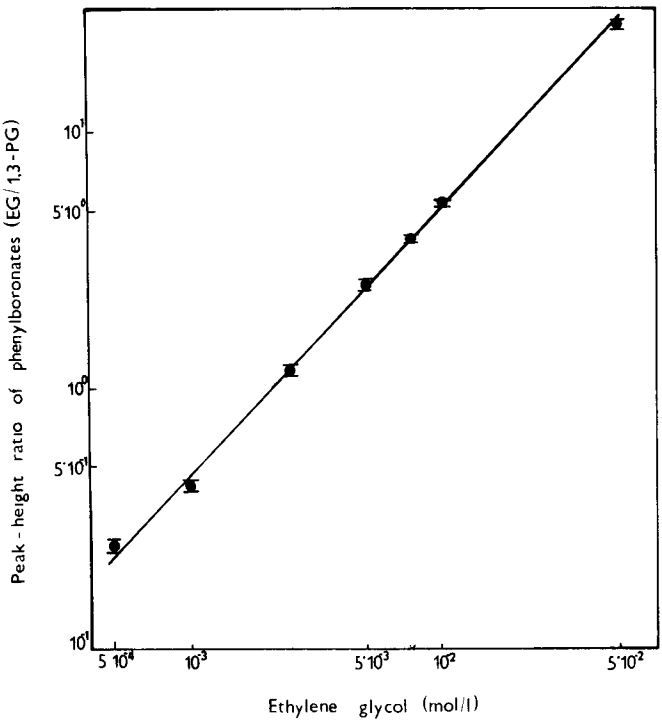

Fig. 1. Linearity of GC determination of ethylene glycol as the phenylboronate. Internal standard, 1,3-propylene glycol; column packing, 3% SP 2250 on Supelcoport; column temperature, 140°C; injector and detector temperatures, 200°C; flow-rates, nitrogen 20 ml/min, hydrogen 18 ml/min and air 400 ml/min.

by subsequent injections of propylene glycol (internal standard) in blank samples. The amount of ethylene glycol adsorbed was lower at higher temperatures of the injector and detector (increasing these temperatures above 200 °C was no longer effective). The amount adsorbed also influenced the reproducibility tests at low levels (Table I).

The water present in the reaction mixtures used has no significant effect on the stability of derivatives during a period of 48 h at laboratory temperature.

The recovery of ethylene glycol was tested in pools of rat negative serum spiked with ethylene glycol at different levels in triplicates (Table II). The average analytical recovery of ethylene glycol from serum was 98.6%.

The phenylboronate of ethylene glycol and 1,2-propylene glycol are not separated on a column packed with SP 2250. If necessary they can be distinguished on an alternative column packed with OV-1 or OV-101 [17]. Under our conditions (3% OV-101) the retention times were 4.42 min for ethylene glycol, 5.00 min for 1,2-propylene glycol and 9.33 min for 1,3-propylene glycol.

The utility of the method described can be demonstrated on two cases of human intoxication with ethylene glycol. A brother and sister, a boy of 8 and a girl of 11 years, ingested by mistake a glass of Fridex antifreeze liquid containing 95% ethylene glycol. After about 6 h haemodialysis was started with continual infusion

TABLE I

REPRODUCIBILITY OF CALIBRATION WITH AQUEOUS SOLUTIONS OF ETHYLENE GLYCOL ($n=5$) AT DIFFERENT TEMPERATURES OF INJECTOR (t_I) AND DETECTOR (t_D)

Column packed with 3% SP 2250 on Supelcoport.

Ethylene glycol concentration (mol/l)	Coefficient of variation (%)	
	$t_I = t_D = 170$ °C	$t_I = t_D = 200$ °C
$5 \cdot 10^{-4}$	19.7	4.2
10^{-3}	5.9	–
$5 \cdot 10^{-3}$	4.7	3.7
10^{-2}	3.3	–
$5 \cdot 10^{-2}$	4.4	2.8

TABLE II

ANALYTICAL RECOVERY OF ETHYLENE GLYCOL FROM SERUM

Ethylene glycol concentration (mol/l)	n	Recovery (%)	
		Range	Mean
$5 \cdot 10^{-4}$	3	100 -108.3	102.8
$5 \cdot 10^{-3}$	3	92.7- 96.9	95.1
$5 \cdot 10^{-2}$	3	95.3-101.0	97.8

TABLE III

LEVELS OF ETHYLENE GLYCOL IN SERUM AND URINE OF INTOXICATED CHILDREN

Sample	Ethylene glycol (mg/l)	
	Boy	Girl
Serum, start of first dialysis	263.3	636.2
Urine, start of dialysis	3071.8	4226.4
Serum, 5 h of dialysis	133.5	317.2
Serum, 5 h after dialysis	132.0	287.5
Serum, start of second dialysis	50.9	–
Serum, end of dialysis	28.3	–

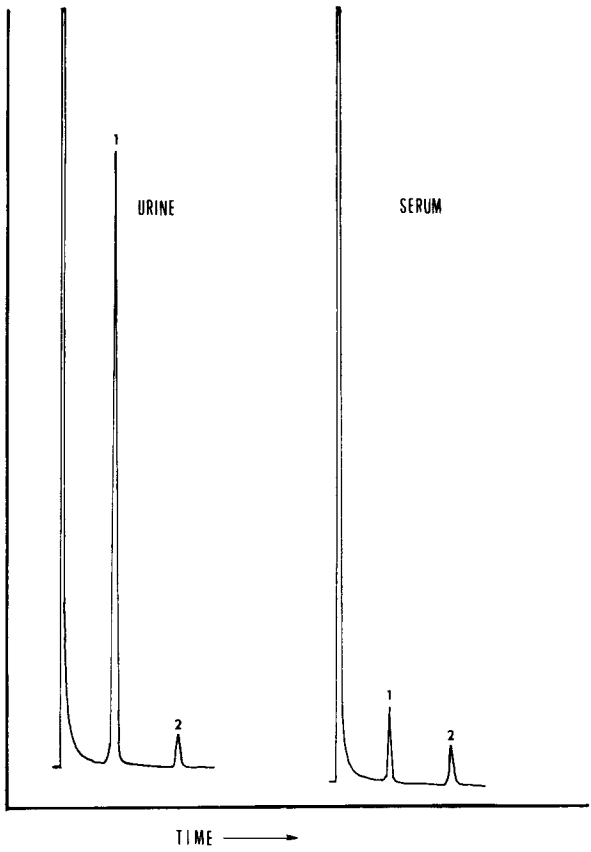

Fig. 2. GC profile of serum and urine of a boy intoxicated by ethylene glycol. GC conditions as in Fig. 1. Peaks: 1 = phenylboronate of ethylene glycol (retention time 2.93 min); 2 = phenylboronate of 1,3-propylene glycol (retention time 6.03 min).

of 50% ethanol to prevent oxidation of ethylene glycol. The kidneys of the boy were much more affected by intoxication, even though the blood level was lower. The boy had to be dialysed every day for several weeks. The levels of ethylene glycol in serum and urine are given in Table III and examples of chromatograms in Fig. 2.

REFERENCES

1 C.L. Winek, D.P. Shingleton and S.P. Shanor, Clin. Toxicol., 13 (1978) 297.
2 R.H. Dreisbach and W.O. Robertson, Handbook of Poisoning, Appleton and Lange, Norwalk, CT, and Los Altos, CA, 1987, p. 175.
3 E. Úlehlová, M. Šikola, Z. Hokeová and B. Chundela, Soudní Lék., 19 (1974) 34.
4 R.O. Bost and I. Sunshine, J. Anal. Toxicol., 4 (1980) 102.
5 W. Vycudilik, Beitr. Gerichtl. Med., 36 (1978) 71.
6 R.L. Peterson and D.O. Rodgerson, Clin. Chem., 20 (1974) 820.
7 P. Verner, F. Pehal, V. Konečná, J. Zeman and J. Hyánek, J. Chromatogr., 416 (1987) 439.
8 J.B. Brooks, M.T. Basta, C.C. Alley, J.S. Holler and E.M. El Kholy, J. Chromatogr., 309 (1984) 269.
9 C.J.W. Brooks and J. Watson, J. Chem. Soc., Chem. Commun., (1967) 952.
10 C.F. Poole and A. Zlatkis, J. Chromatogr., 184 (1980) 99.
11 G.M. Anthony, C.J.W. Brooks, I. Maclean and I. Sangster, J. Chromatogr. Sci., 7 (1969) 623.
12 C.J.W. Brooks and I. Maclean, J. Chromatogr. Sci., 9 (1971) 18.
13 I.M. Mutton, J. Chromatogr., 172 (1979) 435.
14 D.W. Robinson and D.S. Reive, J. Anal. Toxicol., 5 (1981) 69.
15 H.H. McCurdy and E.T. Solomons, J. Anal. Toxicol., 6 (1982) 253.
16 M. Bogusz, J. Bialka, J. Gierz and M. Klys, Z. Rechtsmed., 96 (1986) 23.
17 W.H. Porter and A. Auansakul, Clin. Chem., 28 (1982) 75.