Journal of Chromatography, 496 (1989) 209–213
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam — Printed in The Netherlands

CHROMBIO 4942

Note

Gas chromatographic determination of 2,3-butanediol isomers in urine

S KEŽIĆ, S KUIPER and A C MONSTER*

Coronel Laboratory for Occupational and Environmental Health, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam (The Netherlands)

(First received April 19th, 1989, revised manuscript received July 6th, 1989)

Recent studies have revealed that 2,3-butanediol is involved in the metabolic pathway of ethanol [1,2], acetone [3,4] and methyl ethyl ketone [5]. In humans, 2,3-butanediol is one of the main known metabolites of methyl ethyl ketone [5,6]. However, isolation of such a polar compound from urine followed by gas chromatography (GC) is generally known to be troublesome and to lack good recovery and precision. The method described here effectively deals with both problems in a single-step procedure.

EXPERIMENTAL

Chemicals and reagents

The following chemicals were used: dichloromethane (GC–spectrophotometric quality, J.T. Baker, Deventer, The Netherlands), (*S,S*)-(+)-2,3-butanediol (zur Synthese, Merck, Amsterdam, The Netherlands), meso-2,3-butanediol (zur Synthese, Merck), 1,2-butanediol (zur Synthese, Merck), sodium sulphate (Analar, BDH, Poole, U.K) and naphthalene (BDH)

Apparatus

A Hewlett-Packard Model 5890 A gas chromatograph (Hewlett-Packard Nederland, Amstelveen, The Netherlands) equipped with a flame ionization detector and a Shimadzu CR3A integrator (Shimadzu, Kyoto, Japan) was used.

0378-4347/89/$03 50

An HP-U1 fused-silica column (Hewlett-Packard) (cross-linked methyl silicone gum, 25 m×0.2 mm I.D., 0 11 μm film thickness) was used. The instrument settings were: detector temperature, 250°C; injector, 250°C, the column oven temperature was initially 100°C for 1 min, then increased to 150°C at 30°C/min. The carrier gas was hydrogen The column head pressure was 80 kPa. The detector make up gas was nitrogen at a flow-rate of 30 ml/min.

Sample preparation

Urine (5 ml) saturated with sodium sulphate was pipetted into a 10-ml tube fitted with a screw-cap coated with PTFE. To each sample, 10 μl of 2.0 g/l 1,2-butanediol were added as an internal standard. After the addition of 1 ml of dichloromethane containing 50 mg/l naphthalene and 5 0 g/l *p*-bromophenylboric acid, the tubes were vortex-mixed for a few seconds and then centrifuged for 10 min at 2200 *g* A 1-μl aliquot of the dichloromethane layer was injected into the gas chromatograph.

RESULTS

Fig. 1 shows the chromatograms of a blank urine sample, of the same urine spiked with standard of 2,3-butanediol isomers and of urine from a person exposed to methyl ethyl ketone for 30 min. The intensity of exposure was 200 ppm.

Recovery

Extraction recoveries of the analytes were estimated by spiking normal urine samples with standards of analytes prepared in water. Standards added to dichloromethane containing the same amount of naphthalene and *p*-bromophenylboric acid as used in the normal procedure were taken as 100% recovery Naphthalene was used as an internal standard for the calculation of all analytes, including 1,2-butanediol. The recovery data are presented in Table I The overall recoveries were difficult to determine because of the presence of the derivatization agent that remained either in the injector or on top of the column (see Discussion) We assume, however, that the reaction of butanediols with *p*-bromophenylboric acid is quantitative, because the reaction took place instantaneously and an increase in the time of reaction did not increase the amount of derivatives. Since the recovery of 1,2-butanediol differed from the recoveries of the other analytes it serves only as an indicator of the efficiency of the derivatization and extraction Naphthalene is used to compensate for differences in the injecting volumes and chromatographic conditions.

Precision

To estimate the precision of the method, two urine samples with different concentrations of analytes were repeatedly ($n=8$) analysed. The concentra-

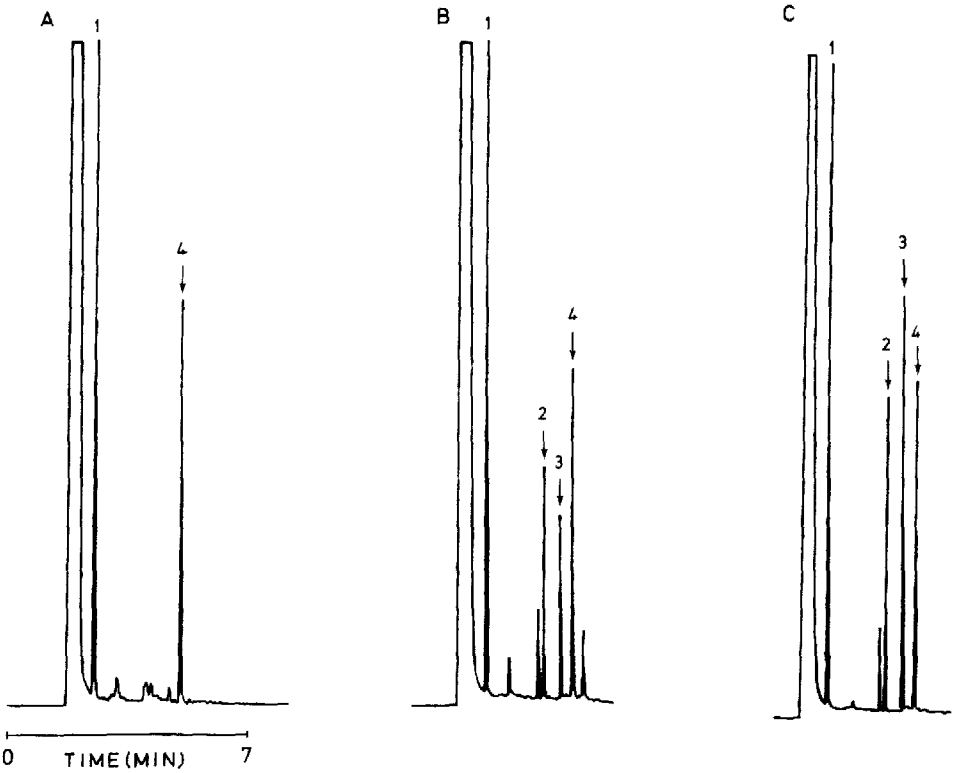

Fig 1 Gas chromatograms of (A) urine blank, (B) urine blank spiked with standards of meso-2,3-butanediol (9 1 mg/l) and *d,l*-2,3-butanediol (3 3 mg/l) and (C) urine from a person exposed to methyl ethyl ketone Peaks 1 = naphthalene (internal standard), 2 = *p*-bromophenylboronate of *d,l*-2,3-butanediol, 3 = *p*-bromophenylboronate of meso-2,3-butanediol, 4 = *p*-bromophenylboronate of 1,2-butanediol (internal standard)

TABLE I

ASSAY RECOVERY ($n=7$)

Compound	Spiking level (mg/l)	Mean recovery (%)	Relative standard deviation (%)
d,l-2,3-Butanediol	3 3	101	3 1
	10 0	102	4 0
Meso-2,3-butanediol	9 1	76	2 8
	27 5	76	7 7
1,2-Butanediol	28 0	93	5 5

TABLE II

ASSAY PRECISION ($n=8$)

Compound	Spiking level (mg/l)	Relative standard deviation (%)
d,l-2,3-Butanediol	2 8	4 3
	14 9	2 6
Meso-2,3-butanediol	9 1	5 5
	41 7	6 4
1,2-Butanediol	28 0	4 5

tions of analytes and corresponding relative standard deviations from the mean values are presented in Table II.

Limit of detection

The limit of the detection, defined as twice the noise level, was below 0.1 mg/l. In twenty normal urine samples (laboratory personnel) we found mean concentrations for the *d,l*-isomer of 2,3-butanediol of 0.2 mg/l (S.D.=0.1 mg/l) and of 0 3 mg/l for the meso isomer of 2,3-butanediol (S.D.=0 2 mg/l). These values are higher than the detection limit of the method. However, if necessary, the sensitivity of the method can be improved by evaporating the dichloromethane extract Interference from endogenous urinary compounds seems to be of minor importance.

DISCUSSION

We present a simple method for the determination of 2,3-butanediol isomers in urine. Derivatization of diols before their extraction makes the extraction step more efficient than the procedures presented in the literature, where diols were first extracted to organic solvents and then derivatized [7–9]. Evaporation of the sample to dryness before derivatization with *p*-bromophenylboric acid as suggested by Needham et al [7] proved not to be necessary The reaction took place instantaneously in urine Besides shortening the time of analyses, our procedure improved the recovery and precision. Methods that include only mixing of urine or serum with organic solvent before derivatization [8,9], or use direct injection of undiluted sample [10], lack any clean-up. Such procedures have the further disadvantage of injecting water into the column. In our method, clean-up, concentration and derivatization of sample are achieved in one step. In contrast to this method, we obtained large differences

in extraction recoveries using the solid-phase extraction technique [6], depending on the batch of the solid-phase extraction columns.

Like other groups [7,8], we encountered the problem that unreacted *p*-bromophenylboric acid remains in the injector or on top of the column. This phenomenon can be shown by injecting underivatized diols, resulting in the same chromatographic pattern given by derivatized diols. However, the presence of unreacted *p*-bromophenylboric acid had no influence on the accuracy of the determination. The injection of blank urine extracts did not lead to falsely increased results.

By using 2,3-butanediol as a parameter in human research studies, for instance after exposure to methyl ethyl ketone, one must be aware that 2,3-butanediol is found in considerable amounts in undistilled alcoholic beverages [11]. We have analysed urine from persons after consumption of pure alcohol diluted with water ($n=4$) and from persons after consumption of wine or beer ($n=5$). The amounts of ingested alcohol were comparable in both groups (20–40 g). After ingestion of pure alcohol, the concentration of 2,3-butanediol isomers was low (<0.5 mg/l), in contrast to the concentrations in urine after consumption of beer or wine (>5 mg/l).

ACKNOWLEDGEMENT

This study was supported by a grant from the Directorate General of Labour, Ministry of Social Affairs and Employment, The Netherlands.

REFERENCES

1 D D Rutstein, R L Veech, R J Nickerson, M E Felver, A A Vernon, L L Needham, P Kishore and S B Thacker, Lancet, ii (1983) 534
2 F J Lopes-Soriano and J M Argiles, Med Hypotheses, 20 (1986) 179
3 J P Casazza, M E Felver and R L Veech, J Biol Chem , 259 (1984) 179
4 J Peinado, F J Lopez-Soriano and J M Argiles, Biochem Arch , 2 (1986) 159
5 J Liira, V Riihimaeki and P Pfaeffli, Int Arch Occup Environ Health, 60 (1988) 195
6 S Kežić and A C Monster, J Chromatogr , 428 (1988) 275
7 L L Needham, R M Hill, Jr , D L Orti, M E Felver and J A Liddle, J Chromatogr , 233 (1982) 9
8 M Balikova and J Kohlicek, J Chromatogr , 434 (1988) 469
9 W H Porter and A Aunasakul, Clin Chem , 28 (1982) 75
10 F K Dietz and G J Traiger, Toxicology, 14 (1979) 209
11 U Sadaham, Hakkogaku Kaishi, 64 (1986) 161