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DETERMINATION OF METHYL ETHYL KETONE AND ITS METABOLITES IN URINE USING CAPILLARY GAS CHROMATOGRAPHY

S. KEŽIĆ and A.C. MONSTER*

Coronel Laboratory for Occupational and Environmental Health, University of Amsterdam, Amsterdam (The Netherlands)

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SUMMARY

A method is described for the determination of the concentration of methyl ethyl ketone and its metabolites: 2-butanol, 3-hydroxy-2-butanone and the *meso*- and *d,l*-isomers of 2,3-butanediol in urine. The analytes were isolated from urine by solid-phase extraction and analysed by capillary gas chromatography. The recovery rates were 50–70% for the 2,3-butanediol isomers and 88–96% for the other analytes. The precision of the method ranged from 5 to 12% (S.D.%). The detection limit was 1.0 and 1.4 mg/l for *meso*- and *d,l*-2,3-butanediol, respectively, and ranged from 0.1 to 0.15 mg/l for the other analytes.

INTRODUCTION

Methyl ethyl ketone (2-butanone, MEK) is used extensively in industry because it is easy to produce, is an excellent solvent, has a moderate vapour pressure and can easily be mixed with other solvents. In an occupational setting the primary routes of exposure to MEK are inhalation and skin contact. The maximal acceptable concentration recommended in Dutch Safety regulations for occupational exposure is 200 ppm (590 μg/l).

MEK is readily absorbed by all routes of exposure. It is excreted unchanged in expired air and in urine, as well as in the form of conjugated metabolites in urine. Di Vincenzo et al. [1] characterized the metabolites of MEK in the serum of guinea pigs as 2-butanol, 3-hydroxy-2-butanone and 2,3-butanediol. Dietz and Traiger [2] have found the same metabolites in rats after oral administration of MEK. They reported 2,3-butanediol as the main metabolite in blood, which was present in two isomeric forms: i.e. the *meso* and *d,l* form.

Only one study in the literature reports on the metabolism of MEK in humans.

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Perbellini et al. [3] analysed urine from people occupationally exposed to MEK in order to establish the relationship between the exposure to MEK and the urinary concentration of MEK and its metabolites. They were not able to identify either 2,3-butanediol or 2-butanol, the metabolites reported by Dietz and Traiger [2] and Di Vincenzo et al. [1] in rats and guinea pigs, respectively.

Very few papers in the literature deal with the determination of MEK metabolites. The methods reported in animal studies [1,2] were either qualitative or lacked data for assessing the reliability of the method. These methods included direct injection of deproteinized serum into a gas chromatograph; the concentrations of analytes were much higher than is usually the case after occupational exposure. Perbellini et al. [3] isolated MEK and its metabolites from urine by solvent extraction and thereafter analysed them by gas chromatography (GC). We used the same method for the determination in the urine of workers exposed to MEK. However, we were unable to achieve sufficient extraction of 2,3-butanediol [4].

The purpose of this study was therefore to develop a method to determine the concentrations of MEK and its metabolites in urine from workers occupationally exposed to MEK. For the isolation of analytes from urine a solid-phase extraction technique was applied by using C_{18} reversed-phase columns. Subsequently, the compounds were analysed on a gas chromatograph.

EXPERIMENTAL

Chemicals and reagents

The following chemicals were used: dichloromethane (for chromatography, E. Merck, Amsterdam, The Netherlands); 2-butanone (p.a., Merck); 2-butanol (Merck); 2,3-butanediol (zur Synthese, Merck); (*S,S*)-(+)-2,3-butanediol (zur Synthese, Merck); 1-butanol (Merck); 3-hydroxy-2-butanone (zur Synthese, Merck); methanol (for chromatography, Merck). Solid-phase extraction was accomplished by using an SPE-21 column processor (J.T. Baker, Phillipsburg, NJ, U.S.A.) and 3 ml octadecyl C_{18} disposable columns (J.T. Baker).

Apparatus

A Hewlett-Packard Model 5890 A gas chromatograph (Hewlett-Packard, Amstelveen, The Netherlands) equipped with a flame ionization detector and a Shimadzu CR3A integrator (Shimadzu, Kyoto, Japan) were used. For the determination of 2,3-butanediol isomers a WCOT fused-silica CP WAX 52 CB column (Chrompack, Middelburg, The Netherlands) was used (polyethylene glycol, 10 m$\times$0.53 mm I.D.; 2.0 μm film thickness). For the determination of other analytes we used an HP-U1 fused-silica column (Hewlett-Packard) (cross-linked methyl silicone gum, 25 m$\times$0.2 mm I.D., 0.11 μm film thickness).

Sample preparation

In order to split the metabolite conjugates [4], 0.2 ml of concentrated hydrochloric acid is added to 10 ml of urine. After heating for 30 min at 100°C, the urine is cooled and brought to pH 7 with 3 *M* sodium hydroxide.

Extraction

The reversed-phase extraction columns were conditioned by running through 3 ml of methanol under reduced pressure (10 mmHg). The column was washed with 3 ml of water, the urine sample (6 ml) was transferred to the column, under normal pressure. In order to dry the column before elution a higher reduced pressure (15 mmHg) was applied for 20 min. To avoid evaporation of dichloromethane, the elution was performed with two successive aliquots (200 μl) of dichloromethane. The elution should be done slowly (reduced pressure less than 5 mmHg). After 2 μl of 1-butanol (3 mg/ml) had been added as an internal standard (I.S.), the eluate was gently evaporated in a stream of nitrogen until ca. 0.1 ml of eluate was left.

Chromatography

For the determination of MEK, 2-butanol and 3-hydroxy-2-butanone, an HP-1 methyl silicone gum column was used. The instrument settings were: detector temperature, 250 °C; injector, 220 °C; the column oven temperature was initially 25 °C, then increased to 50 °C at 70 °C/min. The column was kept at 50 °C for 5 min. The carrier gas was nitrogen, at a flow-rate of 0.8 ml/min; the detector make-up gas was also nitrogen, at a flow-rate of 30 ml/min. Splitless injection was used with the activation of a purge vent after 0.20 min. The injection volume was 2 μl.

For the analysis of the 2,3-butanediol isomers a CP WAX 52 CB polyethylene glycol column was used. The initial temperature was 40 °C for 3 min and then increased to 150 °C at 30 °C/min. The detector temperature was 250 °C, and the injector temperature 220 °C. The carrier gas was nitrogen at a flow-rate of 2 ml/min. The injection volume was 1 μl.

Quantification

Calibration curves were prepared by adding a known amount of standard of analytes to urine from a person with no known exposure to MEK, which was analysed according to the standard procedure. At least two different standard concentrations were used and analysed twice. The concentrations of standards were of the levels expected after occupational exposure and were similar to the concentrations reported by recovery assay (see Table II). Peak-area ratios (analyte to I.S.) were calculated for each analyte and plotted versus the concentration in the corresponding standard.

RESULTS AND DISCUSSION

Figs. 1 and 2 show chromatograms of urine from a non-exposed person (A), of the same urine spiked with standards of analytes (B) and of urine from a person occupationally exposed to MEK. The intensity of exposure was ca. 200 ppm. Because of the poor separation and peak tailing of the 2,3-butanediol isomers on the first column (HP-1; methyl silicone gum), these isomers were analysed on a different column (CP WAX 52 CB). On this second column, however, it was not possible to separate 2-butanol and MEK. Since other compounds of interest were also present we preferred to use this separate chromatographic step for 2,3-bu-

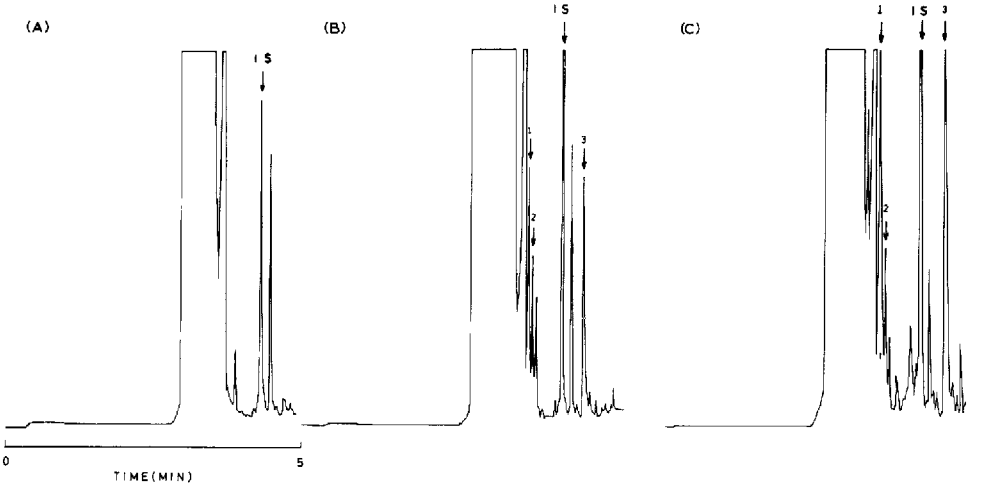

Fig. 1. Gas chromatograms of (A) urine blank, (B) urine blank spiked with standards of MEK (1 mg/l), 2-butanol (0.8 mg/l) and 3-hydroxy-2-butanone (1.5 mg/l), and (C) urine from a person occupationally exposed to MEK. Column, HP-1 (methyl silicone gum). Peaks: 1 = MEK; 2 = 2-butanol; 3 = 3-hydroxy-2-butanone; I.S = 1-butanol (internal standard).

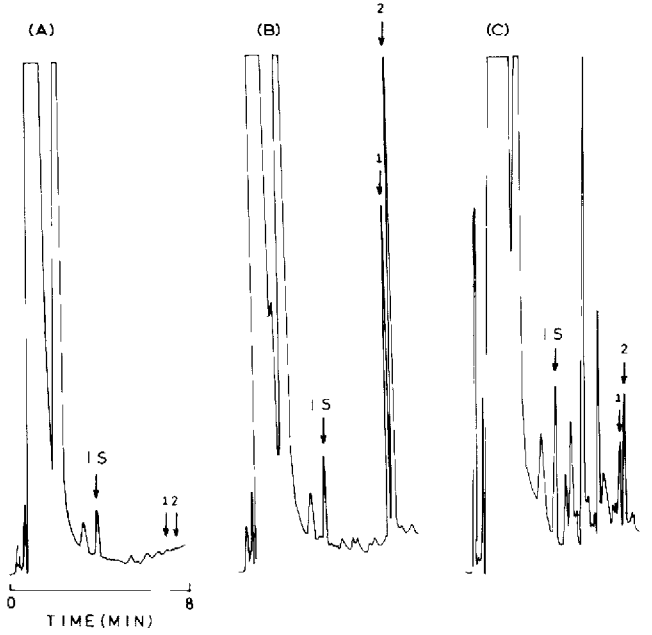

Fig. 2. Gas chromatograms of (A) urine blank, (B) urine blank spiked with standards of *meso*-2,3-butanediol (9.0 mg/l) and *d,l*-2,3-butanediol (6.0 mg/l), and (C) urine from a person occupationally exposed to MEK. Column, CP WAX 52 CB (polyethylene glycol). Peaks: 1 = *d,l*-2,3-butanediol; 2 = *meso*-2,3-butanediol; I.S. = 1-butanol (internal standard).

tanediol isomers rather than to derivatize them, as did Needham et al. [5].

In order to compensate for different volumes of eluates after evaporation and for change in chromatographic conditions, 1-butanol was used as an I.S. 1-Butanol could also be present as a normal constituent of urine but in concentrations much lower (less than 0.1 mg/l) than the concentrations we used (ca. 2 mg/l). However, the concentration of 1-butanol in urine can be increased by occupational exposure to 1-butanol. Therefore, its presence in the workplace air should be checked before it is used as an I.S.

The differences in the chromatograms between urine from exposed and non-exposed persons reveal similar metabolic profiles for MEK to those reported for the guinea pig and the rat [1,2]. The investigated metabolites (2-butanol, 2,3-butanediol and 3-hydroxy-2-butanone) were all present in detectable amounts (Figs. 1 and 2). The reason that Perbellini et al. [3] did not identify either 2,3-butanediol or 2-butanol under similar exposure conditions is probably because of insufficient extraction of those compounds from urine. So far we have analysed about 40 samples from people exposed to MEK. The quantitative relationship between the exposure and biological indicators of exposure (e.g. concentration of MEK and its metabolites in urine) will be published elsewhere [4].

Precision

To estimate the precision of the method, a urine sample (urine from a person with no known exposure to MEK) was spiked with standard of analytes at two different concentrations and repeatedly analysed ($n=8$). These concentrations were at the levels to be expected after occupational exposure. The concentrations of added standard and relative standard deviations of the mean value for each analyte are listed in Table I.

Recovery

Extraction recoveries of analytes were estimated by spiking normal urine samples with standard of analytes at two different concentrations. They were then analysed according to the normal procedure. Separately, the same amount of standard of analytes was added to the eluate of urine (blank sample). In that

TABLE I

ASSAY PRECISION ($n=8$)

Compound	Spiking level (mg/l)	Relative standard deviation (%)
Methyl ethyl ketone	0.67	5.2
	1.33	6.0
2-Butanol	0.51	10.0
	1.02	8.9
3-Hydroxy-2-butanone	0.56	9.5
	1.12	8.8
d,l-2,3-Butanediol	10.0	9.8
	20.0	12.5
meso-2,3-Butanediol	18.3	9.2
	36.5	9.1

TABLE II

ASSAY RECOVERY ($n=8$)

Compound	Amount added (mg/l)	Mean recovery (%)	Relative standard deviation (%)
Methyl ethyl ketone	0.67	85	6.1
	1.33	91	3.8
2-Butanol	0.51	90	5.5
	1.02	88	6.2
3-Hydroxy-2-butanone	0.78	96	4.9
	1.56	95	7.8
d,l-2,3-Butanediol	10.0	66	9.0
	20.0	70	8.6
meso-2,3-Butanediol	18.3	50	6.8
	36.5	59	7.2

way, the final step is identical in both cases, and the difference in the obtained concentrations is due to the extraction recovery. The results are presented in Table II. Despite the low recovery for 2,3-butanediol isomers its uniformity allows good quantification. All other recoveries were satisfactory at the concentration level expected after occupational exposure.

Quantitation limits

The quantitation limits, defined as twice the noise level under the conditions applied, were 0.10 mg/l for MEK, 0.15 mg/l for 3-hydroxy-2-butanone, 0.12 mg/l for 2-butanol, 1.0 mg/l for *d,l*-2,3-butanediol and 1.4 mg/l for *meso*-2,3-butanediol. These limits are all sufficiently low to allow the method to be used for biological monitoring of occupational exposure to MEK.

Application

The method described above could be used for biological monitoring of occupational exposure to MEK. The analytical indicators of the reliability of the method are good and the method enables rapid and simple determination of the concentrations of MEK and its metabolites.

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