Gas chromatographic determination of methoxyacetic and ethoxyacetic acid in urine

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ABSTRACT Methoxyacetic acid (MAA) and ethoxyacetic acid (EAA), the major metabolites of, respectively, ethylene glycol monomethyl ether and ethylene glycol monoethyl ether and their acetates, are determined by gas chromatography after extraction from urine and methylation using 2-furoic acid (2-FA) as an internal standard. The mean recoveries ($n = 30$) from urine of MAA, EAA, and 2-FA are $31.4 \pm 7.0\%$, $62.5 \pm 13.4\%$, and $58.4 \pm 8.7\%$, respectively. The recoveries decreased ($p < 0.001$), however, as the total amount of acids increased. Standard curves for MAA and EAA in urine are presented. The detection limits of MAA and EAA are 0.15 and 0.07 mg/l. Intra-assay variation for MAA and EAA was $6.0 \pm 2.5\%$ and $6.4 \pm 2.8\%$ and inter-assay variation was $6.2 \pm 2.2\%$ and $8.9 \pm 2.4\%$. When volunteers were exposed to air containing ethylene glycol monoethyl ether (20 mg/m³), urinary concentration of EAA rose significantly one hour after the exposure period ($2.39 \pm 1.03 \text{ v} \leq 0.07 \text{ mg/l}$, $t = 5.2$, $p < 0.005$).

Glycol ethers and their acetates are used widely in industrial work and the toxicological publications relating to them have recently been reviewed by Rowe and Wolf.¹

The industrial exposure to vapours of organic solvents may be estimated by analysis of air samples. Since intoxication can occur as the result not only of inhalation of the vapours but also by skin absorption, analysis of urinary metabolites is a useful complement. Recent studies on the metabolism of ethylene glycol monoalkyl ethers in rats and dogs have shown that their respective alkoxyacetic acids are the major urinary metabolites.²⁻⁵ Moreover, the toxicological properties of methoxyacetic acid (MAA) are remarkably similar to ethylene glycol monomethyl ether (EGME) and the adverse effects of EGME in rats are probably the results of in vivo bioactivation of EGME to MAA.⁶

These findings suggest that the urinary excretion of the alkoxyacetic acids may be a useful indication of human exposure to ethylene glycol ethers. Therefore, we have developed a rapid, selective, and sensitive

method for the determination of MAA and ethoxyacetic acid (EAA), the metabolites of ethylene glycol monomethyl ether and ethylene glycol monoethyl ether and their acetates, in the urine.

Methods

REAGENTS

MAA and EAA were obtained from Merck-Schuchardt (Hohenbrunn, FRG). 2-Furoic acid (EGA-chemie, Steinheim, Albuch, FRG) was used as an internal standard. Spectrograde methylene chloride (CH₂Cl₂) was purchased from Burdick and Jackson (Muskegon, Michigan, USA). All other chemicals used were of analytical grade.

PREPARATION OF THE SAMPLES

Standards or urine samples were adjusted to pH 8-8.5 with a few drops of 5 M KOH before analysis. To 1 ml of sample 50 µg of 2-furoic acid (2-FA) in 100 µl of water was added. The sample was then lyophilised. The dry residue was taken up in 10 µl of concentrated hydrochloric acid and 1 ml of CH₂Cl₂ and mixed vigorously during 2×15 seconds. After this time 500 µl of the organic layer was transferred to a vial and 150 µl of a solution of diazomethane in CH₂Cl₂ was added to esterify the acids. After the excess of diazomethane was removed, the vials were capped

and stored for further analysis.

Diazomethane was prepared immediately before use in a millimole size generator by adding 600 μ l of 20% KOH in methanol to 200 mg of *N*-methyl-*N*-nitroso-*p*-toluenesulphonamide according to Fales *et al.*⁷

GAS CHROMATOGRAPHY

The methylated acids were analysed with a Hewlett-Packard 5890 A gas chromatograph equipped with a flame ionisation detector and a CP WAX 57 CB, WCOT fused silica column (25 m $\times$ 0.33 mm ID, 0.22 μ m film thickness). The oven temperature was programmed from 50° to 187°C at a rate of 12.5°C/min and maintained at 187°C for a further 1.5 minutes. The injector and detector were both kept at 230°C. The injection volume was 5 μ l and the splitting ratio was 10:1. The flow rate of helium carrier gas was 1 ml/min. Retention times of MAA, EAA, and 2-FA were 4.52 $\pm$ 0.02 min, 4.96 $\pm$ 0.02 min, and 8.48 $\pm$ 0.02 min (fig 1).

Fig 1 Gas chromatography of (1) methoxyacetic acid, (2) ethoxyacetic acid, and (3) 2-furoic acid extracted from urine and methylated with diazomethane. Column: CP WAX 57 CB, WCOT fused-silica (25 m $\times$ 0.33 mm ID; 0.22 μ m film thickness); carrier gas: helium (1 ml/min); injection volume 5 μ l; split ratio 10:1; detector: FID. Oven temperature programme is presented in dotted lines.

Results

RECOVERY OF THE EXTRACTION PROCEDURE

MAA and EAA were added to urine in concentrations ranging from 5 to 50 mg/l and analysed as described. Peak areas of the acids and 2-FA were compared with

methylated standards in CH_2Cl_2 .

The mean recoveries ($\pm$ SD) of MAA, EAA, and 2-FA were ($n = 30$) 31.4 $\pm$ 7.0%, 62.5 $\pm$ 13.4%, and 58.4 $\pm$ 8.7%. The recoveries of MAA, EAA, and 2-FA decreased ($F \geq 28$; $p < 0.001$) as the total amount of acids increased. This negative relation may partly explain the large overall variations observed in the recoveries. The recoveries of MAA and EAA, however, are positively correlated ($r \geq 0.86$; $p < 0.001$) with the recovery of 2-FA.

The lower recovery of MAA may be due to the high ratio of partition between water and organic solvents.⁸ The recoveries were optimal when urine was first adjusted to pH 8–8.5 and decreased slowly when the pH was out of this range.

STANDARD CURVE AND DETECTION LIMIT

Standard curves for MAA and EAA in urine were set up in a range of 1–100 mg/l. Data were expressed as the response (area %) relative to the internal standard (fig 2).

Fig 2 Standard curves of (●) methoxyacetic acid and (○) ethoxyacetic acid in urine. Data are means of six determinations $\pm$ SD.

Despite the variations of the recoveries, the standard curves are linear ($r = 0.99$) in the range considered and may be written as:

$$y = 0.32 + 0.541x \text{ for MAA and}$$

$$y = 0.27 + 1.538x \text{ for EAA.}$$

The lower recoveries of MAA and EAA in the higher concentration range are compensated by a lower recovery of the internal standard.

At a signal to noise ratio of 5, the lowest detectable amount on column was 34 pg for MAA and 32 pg for EAA. When extraction recovery, injection volume (5 μ l), and starting volume of urine (1 ml) are taken into account, the procedure is capable of quantitating 0.15 mg/l MAA and 0.07 mg/l EAA.

ANALYTICAL RECOVERY

Urine samples containing MAA and EAA in concentrations ranging from 5 to 50 mg/l were analysed 15 times on three separate days (table 1). Intra-assay variation from MAA and EAA was $6.0 \pm 2.5\%$ and $6.4 \pm 2.8\%$ and inter-assay variation was $6.2 \pm 2.2\%$ and $8.9 \pm 2.4\%$.

Table 1 Analytical recovery of MAA and EAA from urine. (Amounts found are means of 15 determinations $\pm$ SD on three separate days)

Amount added (mg/l)	Amount found (mg/l)
MAA	
5	5.7 ± 1.6
25	25.8 ± 1.6
50	50.2 ± 2.2
EAA	
5	5.5 ± 0.6
25	25.1 ± 3.2
50	49.0 ± 3.9

APPLICATION

To test whether the method is able to detect EAA in urine of man after exposure to ethylene glycol monoethyl ether (EGEE), five healthy male volunteers (ages 24 ± 4 years) were exposed at rest to air containing 20 mg/m^3 EGEE during 4×50 minutes, separated by a 10 minute break. The concentration of EGEE in the air chosen is compatible with the threshold limit value for industrial exposures.⁹ Detailed information about the method used for controlled exposure to organic vapours may be found elsewhere.¹⁰

The volunteers collected their urine immediately before and exactly one hour after the experimental period. The urine samples were analysed for the presence of EAA (table 2).

Before exposure, a small peak eluting at the same retention time as EAA was detected in the chromatogram. We were not able to identify this peak, but assuming it was EAA only, concentrations near or less than the detection limit could be calculated. Only one subject had a higher starting concentration

Table 2 Urinary concentration of EAA before and after exposure to ethylene glycol monoethyl ether (20 mg/m^3)

Subject No	Concentration of EAA in urine (mg/l)	
	Before exposure	After exposure
1	0.41	3.87
2	ND	2.48
3	≤ 0.07	1.86
4	≤ 0.07	1.08
5	≤ 0.07	2.68
Mean $\pm$ SD	—	$2.39 \pm 1.03^*$

* $p < 0.005$.

ND = Not detected.

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(0.41 mg/l). On specific questioning, he stated that he might have been exposed to EGEE during his professional activity some days before the experiment.

One hour after the exposure period, the concentration of EAA in urine was significantly higher than the baseline values ($2.39 \pm 1.03 \text{ v} \leq 0.07 \text{ mg/l}$; $t = 5.2$, $p < 0.005$).

Discussion

Since the identification of alkoxyacetic acids as the major metabolites of ethylene glycol monoalkyl ethers in rats and dogs by several authors²⁻⁵ the urinary excretion of these acids could be used as a biological monitor of exposure to ethylene glycol ethers. Nevertheless, the published methods available for measuring the excretion of alkoxyacetic acids were mostly qualitative. Therefore, we have developed a rapid selective and sensitive method for determining MAA and EAA in the urine.

Before analysis the acetic acids and the internal standard are extracted from the urine. A preceding lyophilisation of the urine sample turned out to be necessary to increase the recovery of the extraction procedure. The lyophilisation was performed at $\text{pH} > 7.5$ to yield quantitatively the potassium salts since losses of the free acids were observed at lower pH values. When the pH was higher than 9, however, recovery of the acetic acids and of the internal standard gradually decreased. This could be due to the limiting quantity of concentrated HCl to acidify the medium just before the extraction. The use of greater quantities of HCl, however, was impossible because a decrease of recovery due to an altered volume ratio between aqueous and organic phases was observed. Taken into account these observations a pH range of 8–8.5 turned out to be optimal.

The recoveries of the acetic acids are acceptable and are positively correlated with the recovery of the internal standard. The recovery of the internal standard compensates for the variation of the absolute recoveries of the individual samples. The standard curves are linear in the range of 1–100 mg/l for both MAA and EAA, and the method can detect concentrations as low as 0.15 mg/l for MAA and 0.07 mg/l for EAA. The day to day variation is small and of the same order of magnitude as the intra-assay variation for both MAA and EAA.

The preliminary results of a study of the excretion of the metabolites in non-exposed and in exposed individuals suggest that EAA could be a specific and sensitive parameter for monitoring workers' exposures to EGEE.

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