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 ± 7·0%, 62·5 ± 13·4%, and 58·4 ± 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 ± 2·5% and 6·4 ± 2·8% and inter-assay variation was 6·2 ± 2·2% and 8·9 ± 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 ± 1·03 v ≤ 0·07 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 alkoxycetic 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 alkoxycetic 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

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and stored for further analysis.

Diazomethane was prepared immediately before use in a millimole size generator by adding 600 µl 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 x 0.33 mm ID; 0.22 µm film thickness). The oven temperature was programmed from 50°C 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 µl 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 ± 0.02 min, 4.96 ± 0.02 min, and 8.48 ± 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 x 0.33 mm ID; 0.22 µm film thickness); carrier gas: helium (1 ml/min); injection volume 5 µl; 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 CH₂Cl₂.

The mean recoveries (±SD) of MAA, EAA, and 2-FA were (n = 30) 31.4 ± 7.0%, 62.5 ± 13.4%, and 58.4 ± 8.7%. The recoveries of MAA, EAA, and 2-FA decreased (F > 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 ≥ 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).

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

\[ y = 0.32 + 0.541x \] for MAA and

\[ y = 0.27 + 1.538x \] 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 µl), 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 ± 2·5% and 6·4 ± 2·8% and inter-assay variation was 6·2 ± 2·2% and 8·9 ± 2·4%.

<table>
<thead>
<tr>
<th>Subject No</th>
<th>Concentration of EAA in urine (mg/l)</th>
<th>Before exposure</th>
<th>After exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0·41</td>
<td>ND</td>
<td>3·87</td>
</tr>
<tr>
<td>2</td>
<td>0·6</td>
<td>2·48</td>
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<tr>
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<td>1·86</td>
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<td>4</td>
<td>≤0·07</td>
<td>1·08</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>≤0·07</td>
<td>2·68</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>2·39 ± 1·03*</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Since the identification of alkoxycrylic 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 alkoxycrylic 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 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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Requests for reprints to: D Groeseneke, Laboratorium voor Arbeidshygiène en Toxикologie, Provisorium I, Minderbroedersstraat 17, B-3000 Leuven, Belgium.

References

9 Threshold limit values for chemical substances in the work environment. Adapted by the American Conference of Governmental Industrial Hygienists for 1984–5.

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