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Short Communication

Gas chromatographic determination of urinary phenol conjugates after acid hydrolysis/extractive acetylation

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ABSTRACT

Phenolic metabolites of inhaled aromatic solvent vapours were liberated by acid hydrolysis of their urinary conjugates. Steam distillation enhanced by salting-out with $MgSO_4$ gave good recoveries. After extractive acetylation, the derivatives of all cresols and xylenols were completely separated on a Se-54 capillary column. The overall recoveries of urinary phenols relative to the internal standard, 3-chlorophenol, were in the range 92–99%.

INTRODUCTION

Biological monitoring of inhaled aromatic solvent vapours can be performed by determination of the corresponding phenolic metabolites in urine samples [1]. The phenol is derived from benzene, the cresols from toluene and the xylenols from xylene isomers. Most of these phenols are present in urine as sulphate and glucuronide conjugates [2], which must be hydrolysed enzymically [3] or with concentrated acids [1,4] prior to determination by gas chromatography (GC). Both an extraction [5–7] and a steam-distillation step [8,9] after hydrolysis result in more or less complete recovery of these analytes owing to their different chemical nature.

This paper describes a simple method for the determination of urinary phenols. The sample is hydrolysed with concentrated sulphuric acid during steam distillation. The condensate is buffered with H_3BO_3–NaOH and acetylated with acetic acid anhydride. The acetate esters are completely extracted into *n*-hexane [6,10,11].

EXPERIMENTAL

Chemicals

The phenol standards, reagents and organic solvents were obtained from Aldrich (Steinheim, Germany); all inorganic chemicals were from Reanal (Budapest, Hungary).

Sample preparation

A 5.00-ml volume of urine sample, 4.00 g of $MgSO_4.xH_2O$, 500 μl of internal standard solution (500 μg of 3-chlorophenol per ml water), and 1.50 ml of concentrated sulphuric acid were placed in a 50-ml Erlenmeyer flask. This boiling flask was equipped with an air-cooled condenser, the end of which was dipped into 1 ml of 2.5 *M* NaOH solution in a 22-ml receiver. This forced the phenols during distillation to be bubbled

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through and trapped in the basic solution. The distillation was carried out with moderate heat applied to the flask. The collection of 1.5–2 ml of distillate required 20–25 min.

The condensate was buffered to pH 8 with 5 ml of 0.59 *M* H_3BO_3 and brought to a volume of 10.00 ml with distilled water. Then 1.00 ml of *n*-hexane containing 1% (v/v) of pyridine and 100 μl of acetic acid anhydride were added, and the collection vessel was vigorously shaken for 3 min. After separation, the upper phase was ready to be injected into the gas chromatograph.

Gas chromatography

Samples were analysed on a Hewlett-Packard Model 5890A gas chromatograph equipped with a split/splitless injection system, a flame ionization detector, an HP Model 3393A integrator and a remote start/stop module. An HP Ultra-Performance fused-silica column (25 m × 0.31 mm I.D., with cross-linked 5% phenylmethylsilicone, film thickness 0.52 μm) was used to separate the phenol acetates. The carrier gas was hydrogen at a head pressure of 40 kPa, and the split vent flow-rate was set at 50 ml/min. The oven temperature started at 60°C and was increased at 8°C/min. The injector and the (flame ionization) detector were held at 250°C and 300°C, respectively. The injection volume was 2 μl.

TABLE I

STEAM-DISTILLATION RECOVERY RELATIVE TO INTERNAL STANDARD, AND STANDARD DEVIATION (S.D.) OF PHENOLS

Recovery from 5 ml of water (pH 2) spiked at 10 mg/l.

Peak No. (Fig. 1)	Compound	Recovery (%) (mean ± S.D., n = 5)
1	Phenol	98.2 ± 0.2
2	2-Methylphenol	95.8 ± 0.6
3	3-Methylphenol	96.0 ± 0.8
4	4-Methylphenol	98.0 ± 0.5
5	2,6-Dimethylphenol	91.9 ± 1.2
6	3-Chlorophenol	(Internal standard)
7	2,5-Dimethylphenol	94.6 ± 1.0
8	2,4-Dimethylphenol	95.4 ± 1.3
9	3,5-Dimethylphenol	98.7 ± 0.5
10	2,3-Dimethylphenol	94.8 ± 1.2
11	3,4-Dimethylphenol	96.7 ± 0.6

RESULTS AND DISCUSSION

In our preliminary investigation we followed the generally accepted method. The urine sample was hydrolysed with concentrated HCl, $HClO_4$ or H_2SO_4 at 100–110°C for 1–2 h, and we obtained very different recoveries with the three acids. Sulphuric acid gave always the highest values. However, when the effect of the H_2SO_4 concentration was investigated, we found that the higher the acid content of the mixture the lower the recovery of some phenol isomers (especially the *para*-isomers). To prevent degradation of these isomers we tried milder conditions, a lower hydrolysis temperature or a shorter reaction time, but the results were even more unsatisfactory.

Finally, we combined the hydrolytic step with steam distillation. The recoveries of different phenols were then the same over a wide range of acid concentrations (1–2 ml of sulphuric acid added to 5 ml of urine sample). The steam-distillation recovery of the phenols relative to the internal standard was very good: 1.5 ml of condensate were enough to reach the maximum values (see Table I). The absolute value of the recovery of the internal standard, 3-chlorophenol, was 96.5%.

Liquid–liquid extraction or solid-phase extraction [6] are often used for the isolation and concentration of phenols. Unfortunately, some very polar phenols could be recovered from aqueous solution only to the extent of 10–40%. On the other hand, acetate esters can be readily formed, and this results in almost quantitative recovery and improved gas chromatographic characteristics (Fig. 1).

In conclusion, it is important to emphasize that the acid hydrolysis must not be performed "off-line" but be combined with steam distillation. Further, the extraction of the phenols from the aqueous distillate should be combined with acetate formation (extractive acetylation), which provides improved gas chromatographic

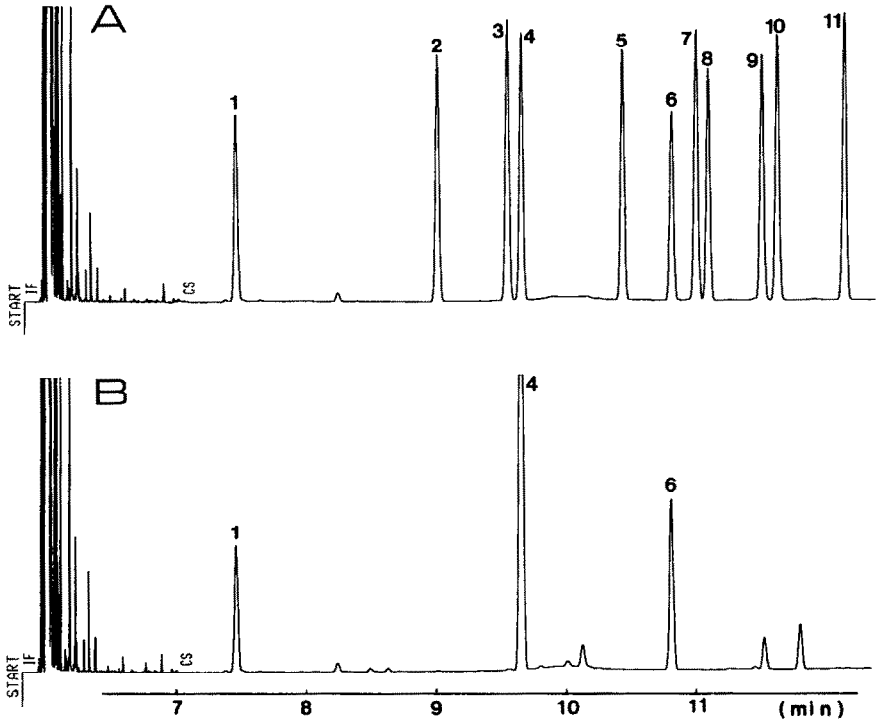

Fig. 1. (A) Chromatogram of acetate esters of phenols (50 mg/l). Numbers as in Table I. (B) Chromatogram of a normal urine sample. Peaks: 1 = phenol, 41 mg/l (retention time = 7.417 min); 4 = *p*-cresol, 87 mg/l (retention time 9.526 min).

characteristics and virtually 100% recovery of the phenols investigated.

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