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Short communication

Simultaneous determination of phenol, cresol, xylenol isomers and naphthols in urine by capillary gas chromatography

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Abstract

An attempt was made to establish a method for the simultaneous determination of urinary concentrations of phenol, *o*-, *p*- and *m*-cresols, 1- and 2-naphthol and xylenol isomers by capillary gas chromatography. Urine samples were extracted after acid hydrolysis of glucuronides and sulfates by solid-phase extraction. The ten substances were separated gas chromatographically using a capillary column (Ultra 2) of cross-linked 5% phenylmethyl silicone. Calibration graphs were linear for 5–100 μg/ml of all the phenols determined. The corresponding detection limits for phenolic compounds varied from 0.1 to 0.2 μg/ml. The relative standard deviations for samples in urine were in the range 2.6–16.6% and the accuracy was in the range 1.4–25%. Recoveries were generally over 80%.

Keywords: Phenol; Cresol; Xylenol; Naphthol

1. Introduction

In environmental analyses, capillary gas chromatography (GC) plays a major role in the detection of urinary metabolites of toluene [1], benzene [2] and ethylbenzene [3]. It is well known that the phenols are metabolites of benzene and its alkyl derivatives [4–6]. Free and conjugated phenols are also present in blood and urine. The co-occurrence of the phenols and cresols has been determined in cigarette smoke and its condensate [7].

In order to analyse phenol, cresols, xylenols and naphthalenols in urine, they must first be separated from the biological carrier. This is usually accomplished by heating a urine sample with a mineral acid or with enzymes [8,9]. The transfer of phenols from the aqueous hydrolysate to an organic solvent is accomplished by extraction with a volatile organic solvent, such as diethyl ether [9–11], dichloromethane [12], methanol [13] or acetonitrile [14]. Highly purified and concentrated isolates for chromatographic analysis can be achieved by selective extraction with appropriate sorbents to yield chromatograms with minimal interferences and improved sensitivity [15]. An efficient extraction method can improve the assay precision and accuracy. The reversed-phases based on octadecylsilane sorbent have been used extensively for the trace enrichment of organics from aqueous matrices in clinical and environmental analyses [14,16]. The GC analysis of phenols with the use of capillary columns was reported previously [12,17]. Determination of the concentration of urinary phenols has been used for biological monitoring of workers exposed to phenols and aromatic hydrocarbons [10]. Urinary excretion of 2,4-xylenol [18,19] and *m*-cresol [7,9,20–22] was used to

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Table 1
Solid-phase extraction of phenols using Baker SPE octadecyl (C_{18}) columns. Each result is the average of three repeated measurements. The concentration of each phenol was 50 μg/ml

Analyte	Recovery (%)	R.S.D. (%)	12% Salt added	
			Recovery (%)	R.S.D. (%)
Phenol	84	4.8	75	6.7
o-Cresol	83	4.4	77	5.4
p-Cresol	97	2.5	87	3.6
2,6-Xylenol	95	2.3	84	2.2
2,5-Xylenol	87	5.2	79	6.6
2,3-Xylenol	96	3.1	86	4.6
3,5-Xylenol	94	3.5	83	4.3
3,4-Xylenol	82	4.1	74	3.2
1-Naphthol	77	5.9	68	7.0
2-Naphthol	74	7.1	71	8.7
Average	87	4.8	78	5.2

monitor human exposure to organic solvents. The present method was applied to the determination of phenols in the breathing zone air and in the urine of coke plant workers [23].

In this paper, the analytical method of separation and its validity for the determination of phenols in urine, particularly the development of solid-phase extraction (SPE) procedures, is discussed.

2. Experimental

2.1. Reagents

All chemicals were of reagent grade quality or better and were used as received without further purification. Phenol (99+%), (CAS registry No. 108-95-2), *o*-cresol (99+%) (95-48-7), *p*-cresol (99+%) (106-44-5), 2,3-dimethylphenol (99%) (526-75-0), 2,5-dimethylphenol (99+%) (95-87-4), 2,6-dimethylphenol (99.8+%) (576-26-1), 3,4-dimethylphenol (99%) (95-65-8), 3,5-dimethylphenol (99+%) (108-68-9), 1-naphthol (99+%) (90-15-3) and 2-naphthol (98%) (135-19-3) were all obtained from Aldrich (Milwaukee, WI, USA). Concentrated hydrochloric acid was obtained from POCH (Gliwice, Poland). Acetonitrile and methanol, of gradient grade, were obtained from Riedel-de Haën (Seelze, Germany). Bakerbond SPE octadecyl C_{18} columns (7020) were from J.T. Baker (Phillipsburg, NJ, USA). Distilled water was used in all analyses.

2.2. Standard solutions

Stock solutions of phenol, *o*-cresol, *p*-cresol, 2,3-, 2,5-, 2,6-, 3,5- and 3,4-xylenol isomers were dissolved in methanol at a concentration of 200 μg/ml. Then, they were diluted again to yield appropriate working solutions for the preparation of the calibration standards. The final concentrations of the phenolic compounds were approximately as follows: 5, 10, 20, 50 and 100 μg/ml. The solutions were kept at 4°C until use.

2.3. Sample preparation

A 0.4-ml volume of concentrated hydrochloric acid was added to 1 ml of urine in a 10-ml glass tube and heated in a water bath at 95°C for 90 min. After cooling to room temperature, the samples were loaded onto SPE columns.

2.4. Procedures for SPE

Standard samples were prepared by adding several phenols to acid-hydrolyzed urine so that the concentration of each of them was 10, 20, 50 and 100 μg/ml. The pH was adjusted to 1.8–2 with hydrochloric acid. In some cases sodium chloride was

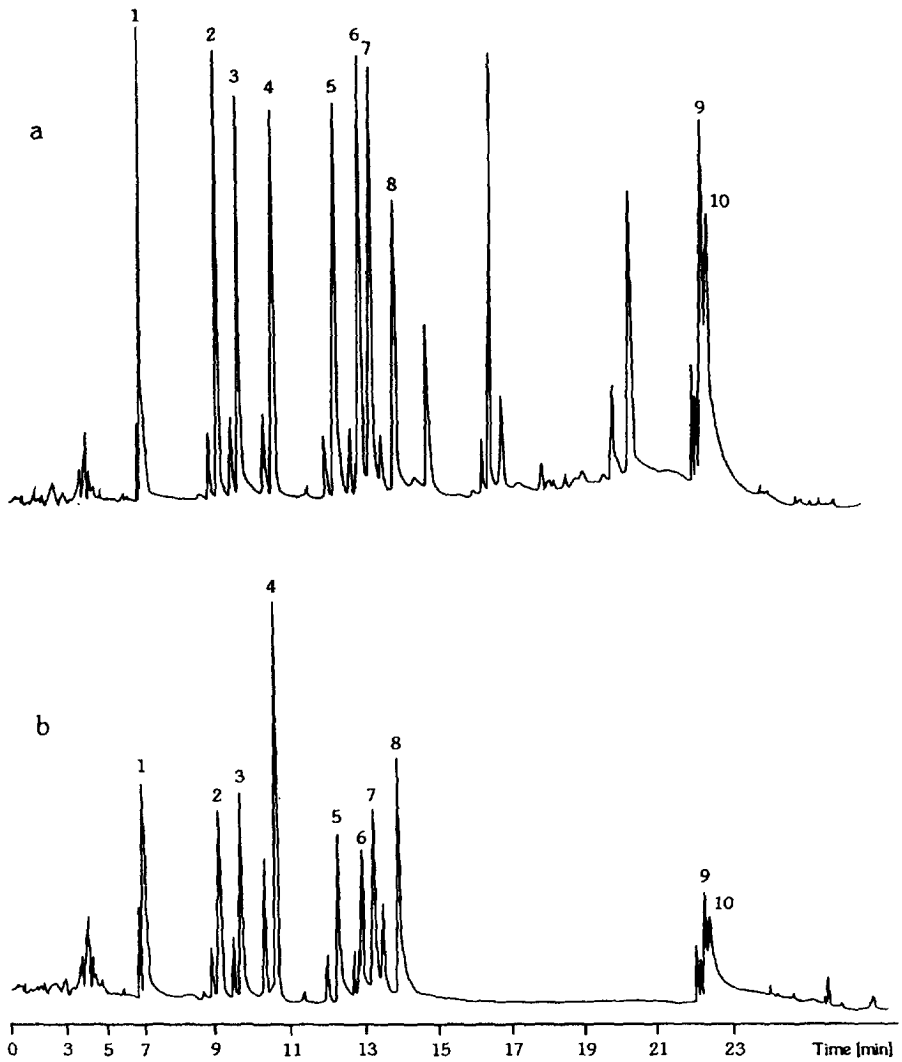

Fig. 1. Chromatograms of (a) the urine of coke plant workers exposed to phenolic compounds and aromatic hydrocarbons and (b) an aqueous standard of 2,6-xylenol (10 μg/ml), 3,4-xylenol (10 μg/ml) and other phenols (5 μg/ml). Peaks: 1=phenol; 2=*o*-cresol; 3=*p*-cresol; 4=2,6-xylenol; 5=2,5-xylenol; 6=2,3-xylenol; 7=3,5-xylenol; 8=3,4-xylenol; 9=1-naphthol and 10=2-naphthol.

added to a concentration of 12% (w/v). A SPE column was conditioned by pumping 5 ml of methanol through the column, followed by 8 ml of distilled water. After washing, the aqueous sample was administered at a flow-rate of 1 ml/min by applying a pressure of 1.3–1.5 kPa. The column was washed again with 5 ml of distilled water and gently aspirated. The phenols were eluted with 1 ml of acetonitrile-methanol (1:10 v/v). The eluate was then collected in a 1.8-ml GC vial. The vial was capped and kept at 0°C before being analysed, to avoid evaporation. A 1-μl aliquot of sample was injected for GC.

2.5. Chromatographic conditions

The chromatographic system consisted of a Hewlett-Packard HP Model 5890 II series with a flame-

ionization detector (FID). A gas chromatograph was equipped with an HP 7673 autosampler/autoinjector and a HP 3396 II series integrator was used. The phenols were separated chromatographically using a capillary column, Ultra 2.

2.6. GC conditions

Capillary column, Ultra 2 (cross-linked 5% phenylmethyl silicone), 25 m×0.32 mm I.D., 0.52 μm film thickness (HP Part No. 19091B-112);

Table 2
Precision and accuracy of the assay of phenolic compounds

Compound	Concentration added (μg/ml)	Concentration (mean±S.D.) (μg/ml)	R.S.D.[a] (%)	Accuracy[b] (%)	Recovery (%)
Phenol	10	8.31±0.59	7.1	−16.9	83
	20	16.23±1.12	6.9	−18.8	81
	50	42.62±2.64	6.2	−14.8	85
	100	86.31±4.31	5.0	−13.7	86
p-Cresol	10	8.88±0.43	5.0	−11.2	88
	20	17.87±0.97	5.4	−10.6	89
	50	48.32±2.28	4.6	−3.4	96
	100	96.76±2.63	2.8	−3.2	96
o-Cresol	10	8.26±0.79	9.6	−17.4	83
	20	17.80±0.83	4.7	−11.0	89
	50	41.02±2.26	5.5	−18.0	82
	100	87.82±3.05	3.5	−12.2	87
2,3-Xylenol	10	8.87±0.80	9.0	−11.3	88
	20	18.64±1.29	6.9	−8.0	93
	50	47.52±2.81	5.9	−4.9	95
	100	98.58±4.02	4.1	−1.4	98
2,5-Xylenol	10	8.46±0.61	7.2	−15.4	84
	20	17.44±0.92	5.2	−12.8	87
	50	42.25±2.41	5.7	−15.5	84
	100	86.17±2.23	2.6	−13.8	86
2,6-Xylenol	10	9.49±0.44	4.6	−5.1	94
	20	19.05±1.09	5.7	−4.7	95
	50	45.77±1.98	4.3	−8.5	91
	100	94.03±3.29	3.5	−6.0	94
3,4-Xylenol	10	8.56±1.34	15.6	−14.4	86
	20	16.88±1.22	7.2	−15.6	84
	50	42.66±2.73	6.4	−14.7	85
	100	86.28±4.26	4.9	−13.7	86
3,5-Xylenol	10	9.42±0.67	6.8	−6.8	94
	20	20.04±1.11	5.5	0.2	100
	50	47.82±1.31	2.7	−4.4	95
	100	95.73±4.06	4.2	−4.2	95
1-Naphthol	10	7.82±0.69	8.8	−21.8	78
	20	15.03±1.26	8.4	−24.8	75
	50	39.73±2.85	7.2	−20.5	79
	100	82.26±3.03	3.7	−17.7	82
2-Naphthol	10	7.70±1.28	16.6	−23.3	77
	20	15.14±1.87	12.4	−24.3	76
	50	37.40±3.05	8.2	−25.2	74
	100	81.44±5.03	6.2	−18.6	81

Analyses were carried out under the experimental conditions described in Section 2.

[a] Relative standard deviation.

[b] Defined as the percentage deviation between the average concentration obtained from the experiment and the theoretical concentration.

injector temperature, 270°C; detector temperature, 280°C; oven temperature, 50°C for 1 min, then increased by 5°C/min to 90°C, by 2°C/min up to 104°C and then by 10°C/min to 250°C; final temperature, 250°C for 1 min; carrier gas, helium at a flow-rate of 2.5 ml/min; injection volume, 1 μl; splitless time, 1 min; split ratio, 1:30.

3. Results and discussion

C_{18} columns were chosen for reversed-phase chromatography with non-polar bonded sorbents, to extract the phenols. Earlier investigations revealed that higher recoveries were obtained by adding salt to the aqueous samples [14,24]. In this particular case, addition of sodium chloride resulted in recoveries that were about 9% lower than those with no salt (Table 1). The results obtained in these investigations are in excellent agreement with the previously published data by Schmidt et al. [13].

Separation of all phenols was achieved using the Ultra 2 column. Representative chromatograms are shown in Fig. 1. Chromatogram (a) was obtained by analysis of the hydrolysed urine sample (1 ml) collected from the workers exposed to phenolic compounds and aromatic hydrocarbons. Chromatogram (b) presents results of the analysis of an aqueous solution. The peaks of interest were well separated from potential interferences. Fig. 1a shows a typical gas chromatogram of the phenols isolated from an acetonitrile concentrate of hydrolysed urine. Phenol, cresols and xylenol isomers were the main metabolites in the urine of workers employed in the distillation of the carbolic oil [6,23].

The precision and accuracy were evaluated by using the samples spiked at concentrations of 10, 20, 50 and 100 μg/ml. The accuracy was expressed as the percentage deviation between the mean concentration value of the six samples and the theoretical concentration. The samples were extracted and subjected to GC analysis. Each concentration was calculated on the basis of peak areas with respect to the calibration graphs. The integrator programme (external standard) was employed. The precision and accuracy of the proposed method are shown in Table 2. A linear relationship was found between the peak areas and the concentrations of phenols for each measurement consisting of six samples spiked at levels of 5, 10, 20, 50 and 100 μg/ml. The parameters of the calibration graphs are given in Table 3. The detection limit was defined as the lowest concentration of each of the phenolic compounds resulting from a signal-to-noise ratio of 3. In our case, the detection limits for phenolic compounds varied from 0.1 to 0.2 μg/ml.

The temperature application programme presented in this paper can be used in the determination of phenols in the extracts of either urine samples or aqueous solutions as well as during the analysis of air [23].

Table 3
Equations of linear calibration graphs

Compound	Equation[a]	r^2
Phenol	$y = 1653.55x$	0.994
o-Cresol	$y = 362.83 + 1260.56x$	0.992
p-Cresol	$y = 1869.01x$	0.994
2,6-Xylenol	$y = 418.95 + 1566.86x$	0.992
2,5-Xylenol	$y = 1642.19x$	0.995
2,3-Xylenol	$y = 1810.17x$	0.994
3,5-Xylenol	$y = 170.91 + 1814.52x$	0.995
3,4-Xylenol	$y = 1660.04x$	0.992
1-Naphthol	$y = 1753.04x$	0.994
2-Naphthol	$y = 2650.03 + 1464.97x$	0.993

[a] y = peak area (arbitrary units); x = concentration (mg/l).

4. Conclusions

An assay involving solid-phase extraction with non-polar bonded octadecyl (C_{18}) sorbents and simultaneous determination of phenols by GC analysis was described. The proposed method may be useful for environmental and toxicological studies of phenols as well as of aqueous solutions and air.

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