Journal of Chromatography, 362 (1986) 425–429
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 713

Note

Determination of cresols in urine by high-performance liquid chromatography

MASAHIRO YOSHIKAWA*, YASUKO TAGUCHI and KEIICHI ARASHIDANI
Division of Occupational Hygiene, School of Medical Technology, University of Occupational and Environmental Health, Japan, Iseigaoka 1-1, Yahatanishi-ku, Kitakyushu-shi, Fukuoka 807 (Japan)
and
YASUSHI KODAMA
Department of Environmental Health, School of Medicine, University of Occupational and Environmental Health, Japan, Iseigaoka 1-1, Yahatanishi-ku, Kitakyushu-shi, Fukuoka 807 (Japan)
(First received January 3rd, 1986; revised manuscript received April 4th, 1986)

Toluene is one of the most popular industrial organic solvents. A small amount of toluene absorbed into the blood is oxidized at the aromatic ring, and excreted in urine as cresols[1–3]. Determination of cresols in urine is important for estimating the degree of toluene exposure[3–7], and various methods based on gas chromatography[7–11] have been described. In 1979, a high-performance liquid chromatographic (HPLC) method for the determination of urinary phenol and *p*-cresol was reported by Angerer[9]. Cresol isomers, however, cannot be determined separately by this method. No method of separating cresol isomers by HPLC has yet been reported. Therefore, we have tried to find the most suitable conditions for the separation of cresol isomers by using the inclusion compound β-cyclodextrin (β-CD).

This report describes an HPLC method for the complete separation and accurate determination of cresol isomers, and the application of the method to the determination of *p*-cresol in normal urine.

EXPERIMENTAL

Materials

Cresol isomers were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Acetonitrile, β-CD and other chemicals were obtained from Wako (Osaka, Japan). Acetonitrile was of HPLC grade and other chemicals were of analytical reagent grade. LiChrosorb RP 18, 5 μm beads (Merck, Darmstadt, F.R.G.), was used as a column packing material.

For separation of cresol isomers and phenol, equal amounts of cresol isomers and phenol were dissolved in distilled water as a standard solution.

Apparatus

A Hitachi Model 635 liquid chromatograph equipped with a variable-wavelength UV detector (Japan Spectroscopic) and a Hitachi Model 163 gas chromatograph equipped with a flame ionization detector were used.

0021-9673/86/$03.50

Liquid chromatographic conditions

LiChrosorb RP 18 was packed into an analytical stainless-steel column (150 × 4 mm I.D.) and a pre-column (10 × 4 mm I.D.) by the slurry solvent packing method. The mobile phase was a mixture of 15 ml of acetonitrile, 85 ml of distilled water and 2.5 g of β-CD, and was degassed by sonication for 10 min before use. The flow-rate and column temperature were 1.0 ml/min and 30°C, respectively, and the detector wavelength was set at 270 nm. Some chromatographic parameters, such as retention time and peak area, were printed out via a computing integrator (Shimadzu Model C-RIA, Japan).

Extraction and concentration

Urine samples were prepared as follows: 2 ml of concentrated hydrochloric acid was added to 5 ml of urine in a glass-stoppered tube and heated at 100°C for 1 h. After cooling to room temperature, the hydrolysed sample was extracted with 4.0 ml of isopropyl ether by shaking vigorously for 1 min. The 3.0 ml of organic phase were transferred into another tube, and an equal volume of 0.05 *N* methanolic sodium hydroxide solution was added. The mixed solution was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.5 ml of distilled water, and 5 μl were injected into the column.

RESULTS AND DISCUSSION

Separation of cresol isomers

For separation of cresol isomers, we tried to find the optimal conditions. Fig. 1 shows the relationship between the amount of β-CD added to 100 ml of the mobile phase (15%, v/v, acetonitrile aqueous solution) and the retention times of phenol and cresol isomers.

The retention times of the four standard compounds decreased for all samples as the amount of β-CD was increased. Those of *m*-cresol and *p*-cresol were particularly affected. When more than 1.5 g of β-CD was added to the mobile phase, cresol isomers were separated completely. Therefore, in consideration of relative peak separation, retention time and the content of each cresol isomer in urine, the most suitable amount to be added to the mobile phase is 2.5 g of β-CD.

Concentration of methanolic sodium hydroxide solution

Table I shows the relationship between the concentration of methanolic sodium hydroxide solution and recoveries of cresol isomers. The recoveries of cresol isomers were *ca.* 100% over a concentration range 0.01–0.1 *N*. Therefore, the most suitable concentration of methanolic sodium hydroxide solution is 0.05 *N*.

Chromatogram of urine extract

Fig. 2 shows the chromatograms obtained from (A) a mixture of four standard compounds and (B) an extract from a normal urine. The peaks of phenol and cresols were identified by comparing the retention times with those of standard phenol and cresols. The retention times of peak 1 and peak 2 in Fig. 2 corresponded to those given by authentic phenol and *p*-cresol, respectively. *p*-Cresol was detected, but *o*- and *m*-cresol were not detected from extracts of the normal urine. The analysis time

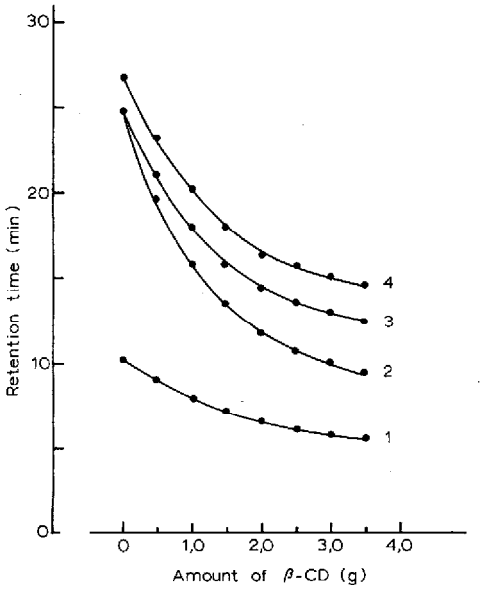

Fig. 1. Relationship between amount of β-CD added to 100 ml of mobile phase and retention times of phenol and cresols. Chromatographic conditions: analytical column, LiChrosorb RP 18, 5 μm, (150 × 4 mm I.D.); pre-column, LiChrosorb RP 18, 10 μm, (10 × 4 mm I.D.); mobile phase, acetonitrile–water (15:85, v/v); flow-rate, 1.0 ml/min; column temperature, ambient. Samples: 1 = phenol; 2 = *p*-cresol; 3 = *m*-cresol; 4 = *o*-cresol.

TABLE I

RELATIONSHIP BETWEEN CONCENTRATION OF METHANOLIC SODIUM HYDROXIDE SOLUTION AND RECOVERIES OF CRESOL ISOMERS

Concentration (N)	*Added (μg)*	*Recovery (%)*		
		o-Cresol	*m-Cresol*	*p-Cresol*
0.001	10	80.8	87.6	86.2
	100	75.9	83.8	85.7
	500	20.0	28.8	28.1
0.005	10	98.7	99.4	99.0
	100	96.1	100.5	98.2
	500	79.4	86.3	86.8
0.01	10	99.4	98.8	100.4
	100	99.4	101.6	100.5
	500	99.9	99.1	98.9
0.05	10	98.6	98.0	99.5
	100	100.2	101.7	100.4
	500	100.7	100.2	99.2
0.1	10	97.0	99.5	98.5
	100	102.7	101.7	103.6
	500	99.6	101.9	98.6

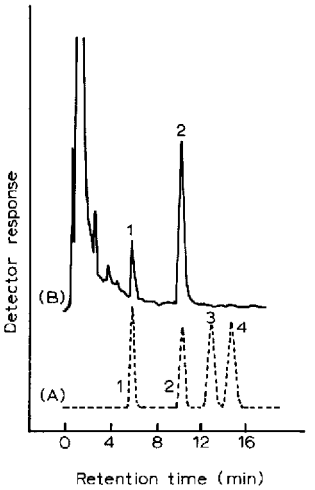

Fig. 2. High-performance liquid chromatograms of (A) a mixture of four compounds and (B) normal urine. Chromatographic conditions: analytical column, LiChrosorb RP 18, 5 μm, (150 × 4 mm I.D.); pre-column, LiChrosorb RP 18, 10 μm, (10 × 4 mm I.D.); mobile phase, 2.5 g β-CD in acetonitrile–water (15:85, v/v); flow-rate, 1.0 ml/min; column temperature, 30°C; detector, UV detector (270 nm). Peaks: 1 = phenol; 2 = *p*-cresol; 3 = *m*-cresol; 4 = *o*-cresol.

was less than 16 min. Detection limits (signal-to-noise ratio = 2) of cresol isomers were *ca.* 15 ng.

Recovery

When the amount of *p*-cresol in urine is more than 200 times that of *o*- and *m*-cresol, the separation and determination of *o*- and *m*-cresol by this method were

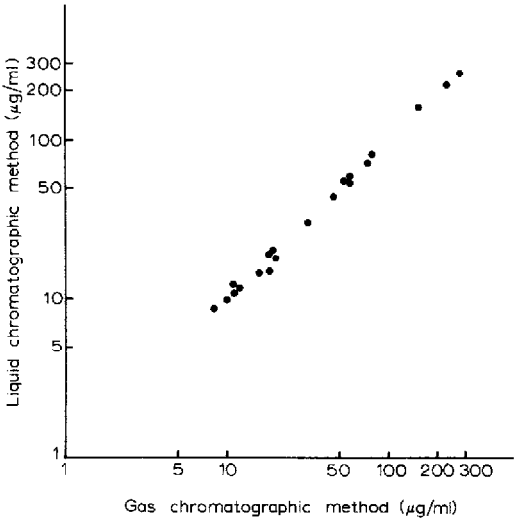

Fig. 3. Relationship between *p*-cresol values obtained by the gas chromatographic method and the present method.

tested by using standard solutions that consisted of different ratios of *p*-cresol. In this case, recoveries of *o*- and *m*-cresol ranged from 96.7 to 102.2%. The result indicates that the method is applicable to urine when the concentration of *p*-cresol is more than 200 times that of *o*- and *m*-cresol. In order to apply the method to urine samples, known amounts of cresol isomers (A) *p*-cresol, 10 μg/ml; *o,m*-cresol, 1 μg/ml; (B) *p*-cresol, 100 μg/ml; *o-m*-cresol, 10 μg/ml) were added to urine samples and the recoveries of cresol isomers were measured. The recoveries (mean ± S.D.) of cresol isomers by the present method were: (A) 102.1 ± 3.3% for *o*-cresol, 102.7 ± 2.5% for *m*-cresol, 103.2 ± 3.3% for *p*-cresol, and (B) 100.8 ± 1.2% for *o*-cresol, 101.4 ± 2.5% for *m*-cresol, 97.7 ± 2.0% for *p*-cresol, respectively.

Reliability

The reliability of the results was examined by comparison with those obtained by a gas chromatographic method[11]. Fig. 3 shows that the values for *p*-cresol from 20 normal urine samples were in good agreement, with a correlation coefficient of 0.998.

CONCLUSION

By addition of β-CD to the mobile phase, cresol isomers were separated completely on a reversed-phase column and monitored by a UV detector. Cresol isomers in urine were determined separately when the concentration of *p*-cresol was more than 200 times that of *o*- and *m*-cresol. The analysis time was less than 16 min, which is shorter than that of Angerer's method. Recoveries of cresol isomers by the present method ranged from 97.7 to 103.2%.

We conclude that the present method has advantages over previous HPLC methods for the determination of cresol isomers in urine.

REFERENCES

1 S. Laham, *Med. Surg.*, 39 (1970) 61.
2 O. M. Bakke and R. R. Scheline, *Toxicol. Appl. Pharmacol.*, 16 (1970) 691.
3 J. Angerer, *Int. Arch. Occup. Environ. Health*, 43 (1979) 63.
4 P. Paffli, H. Savolainen and P. L. Kaliomaki, *Scand. J. Work Environ. Health*, 43 (1979) 286.
5 W. Woiwode and K. Drysch, *Br. J. Ind. Med.*, 38 (1981) 194.
6 P. Apostoli, F. Brugnone, L. Perbellini, V. Cocheo, M. L. Bellomo and R. Silvestri, *Int. Arch. Occup. Environ. Health*, 50 (1982) 153.
7 K. Hasegawa, S. Shiojima, A. Koizumi and M. Ikeda, *Int. Arch. Occup. Environ. Health*, 52 (1983) 197.
8 W. Woiwode, R. Wodarz, K. Drysch and H. Weichardt, *Arch. Toxicol.*, 43 (1979) 93.
9 J. Angerer, *Int. Arch. Occup. Environ. Health*, 42 (1979) 93.
10 M. Makita, S. Yamamoto, A. Katoh and Y. Takashita, *J. Chromatogr.*, 147 (1978) 456.
11 T. Kawai and S. Horiguchi, *Jpn. J. Ind. Health*, 22 (1980) 276.