Rapid Determination of Benzene Metabolites Phenol and *p*-Cresol in the Urine of Petrol Station Workers by Gas Chromatography

GholamAli KHOSCHSORUR[†] and Walter PETEK

University Surgical Clinic, Department of Clinical Chemistry and Laboratory Medicine, Graz, Austria

The determination of phenol and *p*-cresol in urine has been suggested as a suitable biomarker for the monitoring of benzene exposure of pump workers employed in petrol stations. A method for efficiently extracting a wide variety of drugs from urine for toxicological analysis by gas chromatography is presented. The sensitivity of the method is sufficient to monitor normal levels of phenol and *p*-cresol after exposure to benzene vapors. The detection limit of the method is low enough to measure urinary phenol and *p*-cresol at a concentration of 0.1 mg/l. The overall recoveries of urinary phenol and *p*-cresol relative to the internal standard, DPA, ranged between 95 and 96.5%. Our gas chromatographic method is a rapid, precise and economical one suitable to measure the metabolite benzene also in routine monitoring of phenol and *p*-cresol.

(Received February 14, 2000; Accepted March 27, 2000)

Introduction

Benzene is an important, widely encountered organic chemical and an important component in gasoline, a constituent of engine emissions and tobacco smoke.¹ It is also widely used as a raw material in the chemical industry and in the production of various industrial products, from pharmaceuticals to herbicides. Aside from liver damage, frequent exposure to benzene can cause leukaemia in humans and has a carcinogenic effect on many organs in animals.^{2,3} Great attention has been paid to phenol analysis in environmental samples because of its widespread use in industry and high toxicological impact.⁴ In the literature, it is maintained that phenol and *p*-cresol are produced in the human gut by bacteria from dietary protein.⁵ Abnormal extraction of phenol may result from changes in the intestinal flora caused by the intake of antibiotics, gastrointestinal disorders and liver and kidney diseases.^{5,6} Moreover, phenols have a highly toxic character and it is well known that these substances exhibit properties that are hazardous to human health.⁷

Only high concentrations of phenols or smoke condensate fractions can damage cytochrome P-450 by conversion to cytochrome P-420, whereas lower concentrations exhibit inhibitory effects on monooxygenase activity.⁸ Biological monitoring, which may be used in conjunction with personal monitoring to evaluate the exposure information more accurately, would provide more precise information for risk assessment.⁹ Phenol and *p*-cresol are present in urine as sulfate and glucuronide conjugates. A great variety of analytical methods for the separation, quantitation and identification of benzene metabolites have been used, from distillation and extraction to colorimetry or high-performance liquid

chromatography (HPLC).¹⁰⁻¹² However, these methods are time-consuming, complex and not fast enough for the routine monitoring of individuals with a higher level of phenol, such as pump workers employed in petrol stations. Gas chromatography is most frequently employed for the quantitation of phenols because of its high detection sensitivity. Many authors have developed gas chromatographic methods for the determination of urinary phenol and *p*-cresol.⁸ Gas chromatography is precise, particularly when used with an internal standard.¹³ The present paper discusses a simple method for the determination of both phenol and *p*-cresol in human urine.

Experimental

Twenty-four-hour urine samples collected from 37 petrol station workers were analyzed in order to test our method. The samples were stored in a refrigerator until further analysis. A total of 383 analyses were performed.

Stock solutions

The following standard solutions were used: phenol (2.0 mg/ml) and *p*-cresol (1.5 mg/ml), prepared with water, dipropyl acetic acid (2 mg/ml in distilled water) used as internal standard, hydrochloric acid, diethyl ether, and sodium chloride from Merck (Darmstadt, Germany).

Sample preparation

One milliliter of urine was hydrolyzed in a screw-capped glass tube containing 0.1 ml HCl (conc. 37%) and dipropyl acetic acid (DPA) as an internal standard for 45 min at 90°C in an oven. It was then saturated with sodium chloride (1 g). After cooling, the sample was extracted with approximately 5 – 7 ml diethyl ether. After shaking (3 min) and short centrifugation (5 min by 4600g), the ether phase was transferred to a conical glass tube and evaporated to dryness under a slight stream of nitrogen. The residue was reconstituted with 0.2 ml carbon tetrachloride (CCl₄).

[†] To whom correspondence should be addressed.

E-mail: g.khoschsorur@klinikum-graz.at

Prof. Dr. Walter Petek, head of Department of Clinical Chemistry and Laboratory Medicine, has passed away in the mean time.

Table 1 The reproducibility of phenol and *p*-cresol determination for calibration

Calibration		<i>n</i>	Peak-height ratio group (mean + S.D.)
Sample 1	phenol	9	0.78 + 0.022
	<i>p</i> -cresol	9	0.84 + 0.019
Sample 2	phenol	6	1.35 + 0.073
	<i>p</i> -cresol	6	1.42 + 0.055
Sample 3	phenol	9	2.06 + 0.113
	<i>p</i> -cresol	9	2.21 + 0.121

Sample 1: 5 µl DPA and 0.1 ml HCl were added to a 1 ml volume of urine. After hydrolyzation in an oven, extraction and evaporation, the sample preparation was reconstituted with 1 ml CCl₄.

Sample 2: 5 µl (*c* = 10 µg/ml) phenol, 5 µl (*c* = 7.5 µg/ml) *p*-cresol and 5 µl (*c* = 5 µg/ml) DPA were added to 0.985 ml CCl₄.

Sample 3: The same procedure as in sample 2 was carried out, however, with the standard solution described in sample 1.

Chromatography

For chromatography, we used a gas chromatograph (HP 5890 Series II GC with electronic pressure control), a glass column (1.0 m × 2 mm i.d.) filled with 10% DEGS Chromosorb W AW 80/100 mesh, and a flame ionization detector. The oven temperature was maintained at 120°C, the injection temperature at 230°C, and the detector temperature at 230°C. The hydrogen carrier gas flow rate was 15 ml/min, that of nitrogen 18 ml/min, and that of air 250 ml/min. The injection volume was 0.2 µl.

Results and Discussion

This is a method for efficiently extracting a wide variety of phenol and *p*-cresol from urine for toxicological analysis by gas chromatography. For higher concentration levels, the GC of free phenols on a capillary column is acceptable.

Eadsforth and Coveney¹⁴ reported a recovery of 84% after hydrolysis with hydrochloric acid in a boiling bath for 60 min and extraction with dibutyl ether. Due to the use of an internal standard and improved calculations, our recovery was approximately 95% for phenol and 96.5% for *p*-cresol. Figure 1 presents a chromatogram of the standards contained (phenol and *p*-cresol) and a chromatogram of hydrolyzed human urine. The retention times were 1.86 min for DPA, 3.40 min for *p*-cresol, and 4.55 min for phenol. DPA, used in the preparation, does not interfere with phenol or *p*-cresol, respectively (Fig. 1).

The proposed method provides a rapid procedure for urinary phenol and *p*-cresol determination in petrol station workers. The calibration graph was linear in the range of 0.5 to 20 mg/l for phenol and 0.3 to 15 mg/l for *p*-cresol, with a good correlation coefficient (*r* = 0.99). The detection limit was 0.1 µg/ml and the relative standard deviation less than 3%. The application of the method is illustrated by the analysis of a urine sample collected from a petrol station worker. The phenol and *p*-cresol values were related to the urinary creatinine content.

The calibration graphs for phenol and *p*-cresol in carbon tetrachloride were linear up to at least 0.5 mg/ml. The precision of the method was satisfactory (correlation coefficient, *r*, of 0.99 for both urinary metabolites), with relative standard linearity plots for concentrations that range from 0.5 to 20 mg/l.

Exposure to benzene vapor is a serious health hazard in industry.¹⁵ With new evidence of the risks from benzene exposure associated with neoplasia, the American Conference of Government Industrial Hygienists has proposed to lower the threshold limit for 8 h exposure to 0.1 ppm (parts per million).¹⁵

Fig. 1 Representative chromatograms: (A) baseline CCl₄ (drug-free), (B) standard solution containing DPA (*c* = 5 mg/l), phenol (*c* = 10 mg/l), *p*-cresol (*c* = 7.5 mg/l), (C) human urine sample of phenol (4.05 mg/l) and *p*-cresol (2.75 mg/l).

To test our method, we analyzed 24 h urine samples collected from 37 petrol station workers. We found that 57 out of 383 analyses revealed higher levels of phenol and *p*-cresol, in relation to the creatinine content: 17.28 ± 11.35 for phenol as compared to a normal value of 4.1 – 6.6 mg/g/24 h and 67.17 ± 38.63 for *p*-cresol as compared to 19 – 36 mg/g/24 h (*p* < 0.001) (unpublished).

Wang¹⁶ reported that there is a good correlation between the benzene concentration in alveolar air, the phenol concentration in urine by the end of the work shift and the benzene concentration at the work place. The increased absorption of benzene found in the examined persons indicates a necessity for biological monitoring of this group. This method can also be used to rapidly diagnose acute oral intoxication by phenol or *p*-cresol and to estimate its significance in clinical and forensic toxicology.

Conclusion

The method described above is currently being used in our laboratory for routine clinical monitoring of benzene metabolite. We have found it to be accurate, reproducible, efficient, and adequately sensitive for analysis of human urine samples. Sample preparation is simple, inexpensive and rapid, which is very important in a clinical routine. Because of the purity of the extracts, the column is exposed to minimal strain. This is an ideal method for the determination of phenol and *p*-cresol as an ideal biomarker for benzene metabolite exposure of occupationally exposed persons. This method is specific, available for analysis with non-invasive techniques, sensitive, inexpensive and, most importantly, quantitatively related to the exposure concentrations in petrol station workers.

References

1. G. A. F. Kalf, G. B. Post, and R. Snyder, *Ann. Rev. Pharmacol.*, **1987**, 37, 399.
 2. C. Maltoni, A. Gilliberti, G. Conti, and F. Belpoggi, *Environ. Health Perspect.*, **1989**, 82, 109.
 3. A. Yardley-Jones, D. Anderson, and D. V. Parke, *Br. J. Ind. Med.*, **1991**, 48, 437.
 4. P. Bartak, P. Frnkova, and L. Cap, *J Chromatogr.*, **2000**, 867, 281.
 5. J. Bures, F. Mailer, L. Sobotka, Z. Jergeova, Z. Zadak, and J. Horacek, *Gas-Lek-Cesk*, **1990**, 129, 123.
 6. K. E. Murray and R. F. Adams, *J Chromatogr.*, **1988**, 431, 143.
 7. O. Zuloaga, N. Etxebarria, L. A. Fernandez, and J. M. Madariaga, *J Chromatogr.*, **1999**, 849, 225.
 8. S. M. Dirmikis and A. Darbre, *J. Chromatogr.*, **1974**, 94, 169.
 9. A. Sternitzke and W. Legrum, *Netter: Food-Chem.-Toxicol.*, **1992**, 30, 771.
 10. C.-N. Ong and B.-L. Lee, *J. Chromatogr. B*, **1994**, 660, 1.
 11. A. Astier, *J. Chromatogr.*, **1992**, 573, 318.
 12. P. J. Sabourin, W. E. Bechtold, and R. F. Henderson, *Anal. Biochem.*, **1988**, 170, 316.
 13. M. Veningerova, V. Prachar, J. Uhnak, M. Lukacsova, and T. Trnovec, *J. Chromatogr.*, **1994**, 657, 103.
 14. C. V. Eadsforth and P. C. Coveney, *Analyst*, **1984**, 109, 159.
 15. A. Dasgupta, W. Blackwell, and E. Burns, *J. Chromatogr. B*, **1997**, 689, 415.
 16. Y. H. Wang and X. P. Jin, *Xhung-Hua-Yu-Fang-I-Hsueh-Tsa-Chih.*, **1991**, 25, 265.
-