Simultaneous Determination of Urinary Creatinine and Metabolites of Aromatic Organic Solvents by Automated High Performance Liquid Chromatography

Key words: Biological monitoring—Urinary metabolites—Creatinine—High performance liquid chromatography—Toluene—Xylene—Styrene—Organic solvents

Concentrations of urinary metabolites derived from organic solvents have been used for biological monitoring of workers exposed to organic solvents as described by the biological exposure indices (BEI) of ACGIH. In order to correct the metabolite concentrations for the dilution of urine, expression of the results per gram of creatinine has been widely used for urinary biological monitoring.

Previously, we established methods for the determination of concentrations of urinary metabolites of organic solvents by HPLC. An attempt was made in the present study to determine the concentrations of urinary metabolites and creatinine simultaneously by HPLC.

Hippuric acid (HA), o-, m- and p-methylhippuric acids (MHAs), and mandelic acid (MA) were obtained from Tokyo Kasei Co., Tokyo, and creatinine was purchased from Wako Pure Chemical Industries, Ltd., Tokyo. These compounds were dissolved in a mobile phase to prepare standard solutions. Sodium 1-decanesulfonate for ion-pair chromatography was obtained from Tokyo Kasei Co.

Urine specimens were collected from workers exposed to a vapor of a mixture of the solvents which were toluene, xylenes and ethylbenzene and diluted 20- to 100-fold with distilled water or mobile phase. The diluted samples were centrifuged at 2,000 rpm for 5 min, and 10 μl quantities of the supernatants obtained were used for HPLC.

Automated HPLC components (Toyo Soda Co., Tokyo, Japan) were employed as described previously. A stainless-steel column (Ø4.6 mm × L 150 mm), packed with octadecyl-silanized silica gel (TSK gel, ODS-80 TM, 5 μm, Toyo Soda Co.) and with a jacket attached for temperature control, was used throughout the investigation. The flow rate was 0.7 ml/min, producing a pressure of 100 kg/cm². The column temperature was 25°C. The effluents were monitored at a wave length of 225 nm. The monitor attached to the HPLC was a UV-visual detector (UV8000, Toyo Soda Co.) of diffraction grating with variable wave length (190 nm – 600 nm). For separation of urinary creatinine and organic acids, a mixed solution of [20 mM KH₂PO₄, (pH 3.3) containing 3 mM sodium 1-decanesulfonate]/[CH₃CN] (85/15) was used as a mobile phase.

The chromatograms of authentic compounds showed that HA, o-MHA, m-MHA, MA and creatinine added to urine were well separated from each other (Fig. 1A). The chromatograms of urine specimens obtained from workers
immediately after exposure to toluene, xylenes and ethylbenzene are shown in Fig. 1B. MA, HA, o-, m- and p-MHAs and creatinine were also well separated. Linear relationships existed between the peak heights and the concentrations of MA, HA, o-MHA, m-MHA and creatinine were 1.0, 1.1, 1.0, 1.0 and 2.3 mg/ml, respectively after dilution of the samples 100 fold with water, and 10 μl was used for HPLC.

B: Urine specimens of workers exposed to 42 ppm of toluene, 8 ppm of o-xylene, 30 ppm of m-xylene, 12 ppm of p-xylene and 22 ppm of ethylbenzene for 8 hours. Concentrations of MA, HA, o-MHA, m or p-MHAs and creatine were 0.52, 0.65, 0.17, 0.72 and 0.67 mg/ml, respectively (the dilution of urine specimens was the same as in A)

Fig. 1. High performance liquid chromatograms of MA, HA, o-, m- and p-MHAs and creatinine.

A: Normal urine with authentic samples of four organic acids and creatine. Concentrations of MA, HA, o-MHA, m-MHA and creatinine were 1.0, 1.1, 1.0, 1.0 and 2.3 mg/ml, respectively after dilution of the samples 100 fold with water, and 10 μl was used for HPLC.

B: Urine specimens of workers exposed to 42 ppm of toluene, 8 ppm of o-xylene, 30 ppm of m-xylene, 12 ppm of p-xylene and 22 ppm of ethylbenzene for 8 hours. Concentrations of MA, HA, o-MHA, m or p-MHAs and creatine were 0.52, 0.65, 0.17, 0.72 and 0.67 mg/ml, respectively (the dilution of urine specimens was the same as in A)

immediately after exposure to toluene, xylenes and ethylbenzene are shown in Fig. 1B. MA, HA, o-MHA, m-MHA and creatinine were also well separated.

Linear relationships existed between the peak heights and the concentrations of MA, HA, (o- and m- or p-) MHAs and creatinine.

The recoveries of MA, HA, o-MHA, m-MHA and creatinine, when added at a concentration of 1.0 mg/ml each to urine and diluted 100-fold with distilled water, were 101.0±5.2, 101.8±1.8, 100.8±3.7, 105.0±1.6, and 101.7±2.2%, respectively, when 5 urine specimens were used. Thus, the recoveries of these compounds were uniformly high.

Variation coefficients (100×SD/mean) were calculated from the values of organic compounds in serial determinations by the automated HPLC. The concentration of MA, HA, o-MHA, m-MHA and creatinine added to a normal urine specimen was 1.0 mg/ml. Thereafter, the specimen was diluted 100-fold with distilled water. The variation coefficients for the determination of MA, HA, o-MHA, m-MHA and creatinine added to the urine were 2.4, 1.6, 1.5, 2.0, and 2.0%, respectively, when 5 urine specimens were used.
The creatinine concentration in the urine \((n=20)\) was determined by the present method and by Jaffe's colorimetry by means of an automated analyzer (Type 726, Hitachi). A linear relationship was obtained between the creatinine concentration determined by HPLC and that determined by colorimetry with a regression equation, \(y=0.016+1.01x\) (correlation coefficient = 0.997), where \(y=\) creatinine concentration (g/l) determined by colorimetry, and \(x=\) creatinine concentration (g/l) determined by HPLC.

The newly established method allowed us to determine simultaneously the concentrations of MA, HA, \(o\)-MHA, \(m\)-MHA and creatinine in urine with reasonable accuracy and precision. The new HPLC method has potential usefulness for the study of the urinary monitoring of organic solvent exposure.

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