SIMULTANEOUS DETERMINATIONS OF URINARY HIPPURIC AND METHYLHIPPURIC ACIDS IN SOLVENT-WORKERS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

高速液体クロマトグラフィ（HPLC）による有机溶剂作業者の尿中馬尿酸およびメチル馬尿酸の同時定量

Determinations of sum of m- and p-MHA in urine would be useful for the evaluation of occupational exposure to xylene, because the mixture of m- and p-xylene are actually used in industry, and the rate of metabolism of m-xylene was reported to be similar to that of p-xylene.¹ In this paper, we describe the method of simultaneous determinations of HA and total MHA (m- and p-isomers) in urine from workers occupationally exposed to the solvents.

Materials and methods

Urine samples used here were those obtained from solvent-workers who visited us for medical examinations and those obtained from workers at the workplace. Urine was injected into HPLC before (direct method) and after extraction by the method of Matsui et al. (indirect method).² Analyses were carried out using DuPont 830 LC equipped with an UV detector (Shimazu SPD-1) and an integrator (Shimazu ITG-4A). The column (4×150 mm) was packed with Lichrosorb RP18 (5 μm). To protect this analytical column we used a line filter and a disposable MPLC guard column (RP-18 cartridge, 4.6×30 mm, Brownlee Labs. Inc.). The guard column was not used when urine extracts were injected. Oven temperature was maintained at 40°C. The mixture of distilled water/methanol/acetic acid (80/20/0.2 by vol.) was used through this investigation to provide a favorable mobile phase. We set the pump pressure at 1,500–2,000 psi to produce the flow rate of 1.5–2 ml/min. To detect HA and MHA eluted from the column, we set the wavelength of UV detector at 272.4 nm, where m- and p-MHA showed the identical molar absorption coefficient. In our HPLC system, m- and p-MHA were not eluted separately, and the peak of m-MHA appeared 19 sec later than that of p-MHA. The peak height of all the mixtures was lower than that of each constituent, namely m-MHA or p-MHA (Fig. 1).

On the contrary, standard m- and p-MHA and their mixture gave the same peak-area (CV=0.2%), which was independent of the ratio of isomers in the mixture. For the calculations of urinary MHA and HA concentrations, we used the mixture of m- and p-MHA, and HA in equal amount as the standard. Determinations of HA and (or) MHA concentrations in urine were also carried out according to the BSC method³ and the method of Sugihara et al.¹

Results and discussion

Urinary HA and MHA concentrations determined with urine extract (HPLC-indirect method)

Fig. 1. High performance liquid chromatograms of the mixture of p- and m-methylhippuric acids. (I) p-MHA : 10 ng, (II) p-MHA : 9 ng, m-MHA : 1 ng, (III) p-MHA : 7 ng, m-MHA : 3 ng, (IV) p-MHA : 5 ng, m-MHA : 5 ng, (V) p-MHA : 3 ng, m-MHA : 7 ng, (VI) p-MHA : 1 ng, m-MHA : 9 ng, (VII) m-MHA : 10 ng.

Fig. 2. Relationship between HA concentrations by the present method (direct method) and those by the BSC method.

Fig. 3. Relationship between urinary MHA (the sum of m- and p-isomers) concentrations determined by the present method and by the method of Sugihara et al.

Fig. 4. Relation of HA and MHA concentrations in urine from 97 printing workers.
were well correlated with those determined by injecting urine without extraction (HPLC-direct method). The correlations of the indirect method (y) vs. the direct method (x) for HA and MHA were \( r = 0.983, n = 137, y = 0.980x + 0.153 \), and \( r = 0.948, n = 72, y = 1.220x + 0.21 \), respectively. These results indicate that determinations of HA and MHA in urine are simplified by direct injection of urine into HPLC. Figure 2 shows the relationship between HA concentrations by the BSC method and those by the HPLC-direct method \( (r = 0.938) \). HA concentrations by the BSC method \( (y) \) were slightly higher than those by the HPLC-direct method \( (x) \) \( (y = 1.158x + 0.536) \), confirming that the HPLC method is more specific than colorimetric determination of urinary HA.\(^{4,21} \) The correlation coefficient of urinary HA concentrations by the method of Sugihara et al. \( (y) \) vs. those by the HPLC-direct method \( (x) \) was 0.984 \( (y = 0.824x + 0.287, n = 67) \). Figure 3 shows the relationship between urinary MHA (sum of m- and p-isomers) concentrations determined by the method of Sugihara et al. \( (y) \) and those by the HPLC-direct method \( (x) \) \( (y = 1.006x + 0.015, n = 72, r = 0.870) \). These results indicate that the HPLC-direct method gives the sum of m- and p-MHA in urine without any pretreatment.

Figure 4 shows the HA and MHA concentrations in urine from 97 printing workers, which are determined by the HPLC-indirect method. The urine was collected between 1:00 and 4:00 p.m. at the workplace. One of them engaged in washing machine with xylene \( (x) \) and the rest were at press work and exposed to toluene \( (\bullet) \). In many of toluene-exposed workers whose urine contained large amounts of HA over 4,000 mg/l, MHA was also detected in their urine (50–200 mg/l). The data might suggest that the solvent (toluene) was contaminated with a small amount of xylene, although we did not analyze the solvent. On the contrary, moderately high levels of MHA (200–400 mg/l) were also found in the urine of the workers who showed slightly high levels of HA (less than 2,000 mg/l). The air inhaled by the workers might have been contaminated not only by toluene but by xylene also, because their workplaces were close to that where xylene was used.

Thus the simultaneous determination of HA and MHA by the present method (HPLC-indirect method) would give indicative data, from which occupational exposure to either toluene or xylene or both are monitored. The relationships between air concentrations of toluene and xylene, and urinary excretion of HA and MHA remain to be further investigated. Of the urine samples examined, the highest levels of HA and MHA were 8,100 mg/l and 700 mg/l, respectively. Miyasaka et al.\(^{3,4} \) reported that urine concentrations of HA and MHA determined by HPLC correlate with air concentrations of the solvents. Based on their figures, the air concentration of toluene corresponding to 8,100 mg/l of HA and that of xylene corresponding to 700 mg/l of MHA are estimated to be about 225 ppm and 15 ppm, respectively.

By the present HPLC methods, 5 samples of urine can be analyzed for an hour if an automated sample injector is used. The limitation and normal values of HA and MHA in the present HPLC methods should be further examined.

References


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Received for publication, December 25, 1981.
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