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Quantitative Determination of Styrene in Blood and Mandelic Acid in Urine of the Occupationally Styrene-exposed Workers

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The concentration of styrene in blood of the occupationally styrene-exposed workers was checked by gas chromatographic headspace analysis. Mandelic acd in urine, that is a major metabolite of styrene, and hippuric acid were also analyzed by high performance liquid chromatography. For the biological momitoring of styrene-exposed workers, the routine method of the quantitative determination of styrene and its metabolites in the biological samples were studied.

Key words: Styrene, Mandelic acid, Hippuric acid, Quantitative determination, Biological monitoring

INTRODUCTION

Styrene is widely used in making polystyrene plastics, protective coatings, styrenated polyesters, copolymer resins, and as a chemical intermediate. In the occupational environment, absorption of styrene occurs mainly through inhalation and, for a minor part, by skin contact with the liquid form (Dutkiewicz *et al.*, 1968). This latter route of entry is important beacause it can be excluded for the evaluation of the exposure by the air index. Usually, each worker's air index around the working area is used for the evaluation of the styrene exposure. But the absorption rate of styrene can be different by individual worker's physical condition, sex, age, and racial difference, etc. So, the biological monitoring of each worker is needed (Droz *et al.*, 1983).

Especially, the concentration of styrene in blood is a specific indicator of styrene exposure. No styrene is present in blood of unexposed subjects. But blood has been much less investigated previously than expired air because of analytical difficulties. Blood sample should be analyzed within 24 hours or as soon as possible to avoid the sample loss (Wigaeus *et al.*, 1983). Brugnone analyzed styrene blood with 10 ml of blood sample by the specfic designed cryogenic trap to condense the sample vapor and then applied to gas chromatography (Brugnone *et al.*, 1989). But sampling blood over 10 ml is not easy and the sample preparation procedure is rather difficult and time consuming. In the industrial field, because of lots of sample numbers and the limitation of sample storage time, a routine analytical method should be concerned especially focused on the reproducibility and convenience of analysis. The determination of blood styrene concentration was studied by gas chromatographic headspace method without any cryogenic trap.

Urine analysis is the indirect method of styrene exposure. The metabolic pathway for styrene is shown in Fig. 1. About 85% of styrene is excreted in urine as mandelic acid and 10% as phenylglyoxylic acid (Engstrom *et al.*, 1978). Hippuric acid is a minor metabolite of styrene too. But hippuric acid is a common urinary constituent that is produced from the metabolism of certain acidic foods such as fruits and the preservative sodium benzoate. This variable intake results in wide range of normal values of hippuric acid from 0.5 to 1.5 g/l (Cohr *et al.*, 1979). Mandelic acid is not present in urine of unexposed subjects. Ethanol intake and exposure to some other chemicals decreased excretion of mandelic acid (Cerny at al., 1990). In spite of indirect indicator of styrene exposure, urine analysis was selected in the present study because of its non-invasive nature in sampling with minimal burden on workers. Matsui extracted hippuric acid and methyl hippuric acid in urine with ethyl acetate and analyzed by HPLC (Matsui *et al.*, 1977). Inoue (Inoue *et al.*, 1991) analyzed mandelic acid and other meta-

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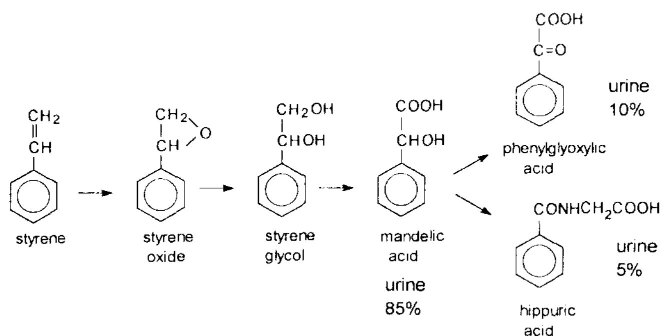

Fig. 1. Metabolic pathway of styrene.

bolites in urine by connecting two HPLC columns. In the present study, a simple high performance liquid chromatographic procedure is described as a routine analysis method of mandelic acid, the main metabolite of styrene, and hippuric acid in urine for styrene exposed workers.

The purpose of this paper is to establish the routine method for the quantitative determination of styrene and its metabolites in biological samples.

MATERIALS AND METHOD

Reagents and Instruments

Styrene stabilised with 20 ppm 4-*tert*-butyl-pyrocatechol as a standard and isobutanol as an internal standard were purchased from Merck Co. Sodium citrate, dextrose, citric acid were purchased from Bunseki Co. Hewlett Packard HP5890 series II gas chromatograph equipped with HP19395A headspace sampler was used.

Mandelic acid and hippuric acid were purchased from Sigma Co. A HPLC system used was Waters 600E solvent delivery system with column oven and the detector was UV detector Waters model 484. The centrifuge was Hanil MF550.

Determination of Styrene in Blood

Blood samples were collected at the end of shift of the workweek in vacutainers containing EDTA as an anticoagulant. The vacutainer was filled completely with blood to avoid contact with the upper air layer. 1 ml of blood was taken to the headspace vial, and diluted with 1 ml of acidium citricum dextrose solution. After isobutanol solution as an internal standard was added, the headspace vial was capped with gastighed septum and aluminium cap. Capped headspace vial was stirred for 5 minutes in a rotator at room temperature. In headspace sampler, blood sample was pre-heated at 70℃ for 20 minutes and 1 ml of headspace gas was injected by sample loop to gas chromatography. For the reproducibility test, five samples at

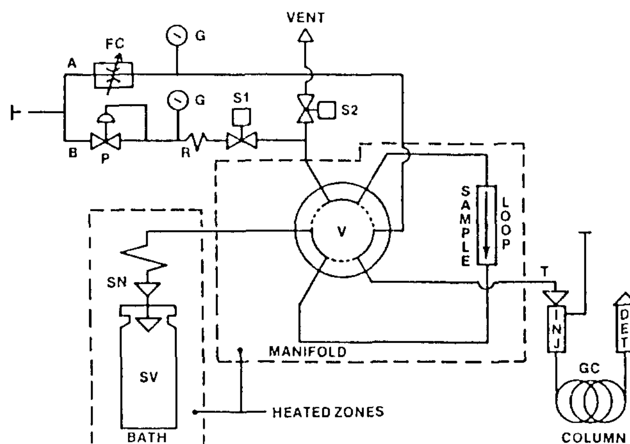

Fig. 2. The valve diagram of headspace injection system.

each three concentration of 0.15, 0.40, 0.80 mg/l were analyzed.

The condition of gas chromatographic headspace sampler for analysis of blood styrene was as follows. The bath temperature of headspace sampler was 70℃ and valve temperature was 75℃. The column temperature of gas chromatography was 75℃. Flame ionization detector was used and the detector temperature was 180℃, and the injector temperature was 170℃, respectively. Gas chromatographic column was a stainless steel column packed with 10% Carbowax 20M (polyethylene glycol, 6ft×1/8 in od.)

Determination of Mandelic acid and Hippuric acid in Urine

After the workshift, urine samples were collected in polyethylene bottles. Twenty aliquot of deionized water was added to the urine and centrifuged for 10 minites at 3000 rpm. 5 μL of the supernatant was injected to HPLC. The condition of HPLC was following. Column was C-18 Supelcosil (25 cm×4.6 mm, 5 μm) with the same kind of guard column purchased from Supelco Co. The column temperature was varied from 25 to 55℃. UV detector was used at the wavelength of 225 nm. Mobile phase was water : methanol : acetic acid (85 : 15 : 0.5, v/v) and the flow rate was 1 ml /min.

For the recovery test, 5 urine and 5 water samples were prepared by adding mandelic acid to make final concentration of 0.2, 0.4, 0.8, 1.2, 2 g/l, respectively. Recovery rate was calculated by the peak areas obtained from urine samples over the peak areas from water samples.

RESULTS AND DISCUSSION

The valve diagram of headspace injection system is shown in Fig. 2. After the carrier gas enters the hea-

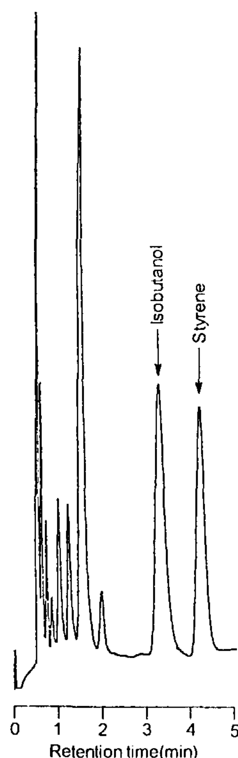

Fig. 3. Gas chromatogram of 1 ml headspace air of styrene-exposed worker's blood. Column; 10% Carbowax 20 M (polyethylene glycol, 6ft×1/8in od.) Column temperature; 75℃, injector temperature; 170℃, detector temperature; 180℃, nitrogen flow rate; 30 ml/min.

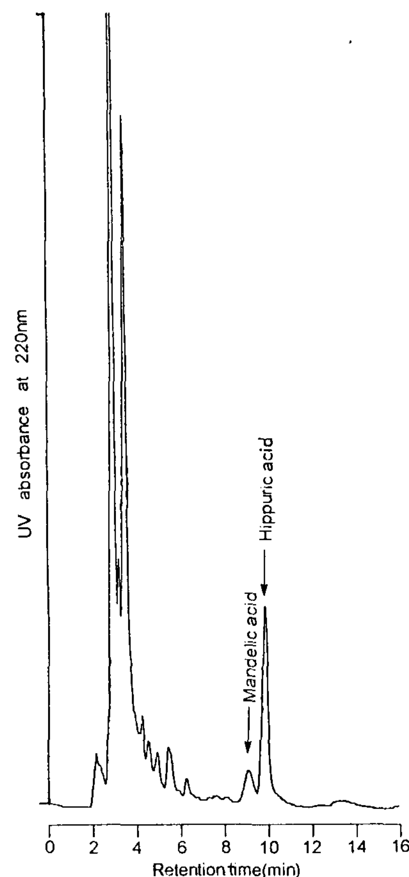

Fig. 4. HPLC chromatogram of a urine sample. Column; C-18 Supelcosil (25 cm×4.6 mm, 5 μm), mobile phase; water : methanol : acetic acid (85 : 15 : 0.5 ,v/v), detector; UV at λ= 225 nm, column temperature; 45℃.

Table I. Reproducibility for the determination of styrene in blood

conc. of styrene (mg/l)	ratio of styrene/isobutanol (Mean value, n=5)	coefficient of variation*
0.15	0.3355	4.6%
0.40	0.9644	2.9%
0.80	1.9768	3.5%

*This factor was calculated by (s.t.d./mean)×100.

dspace injection system, it splits into two pathways. The path A goes to the flow controller, and it regulates the flow of carrier gas through the heated transfer line to the gas chromatograph. The path B goes to headspace vial to pressurize the vial as the solenoid valve controlls. Valve V swiches the sample loop from the headspace sampling circuit to the injection line. A 1 ml sample loop is filled with headspace gas during the sampling step, and injected automatically with the carrier gas.

Gas chromatogram of 1 ml headspace air of blood for styrene exposed worker is shown in Fig. 3. To concentrate the headspace air, 10 ml headspace vial was used for 1 ml blood sample. A stainless packed column was preferred to cappillary column because of its large capacity of sample volume. Styrene peak was eluted at 4.388 min. followed after isobutanol peak as an internal standard at 3.422 min. at the column temperature 75℃, with isothermal procedure. Acetone used as a solvent of isobutanol stock solution and ethanol used as a disinfectant eluted at 1.595 min. and 0.580 min. respectively.

To get standard curve, an internal standard method was used. The control blood samples were spiked with styrene standard solution at the concentration of 0.05, 0.1, 0.2, 0.4, 0.8, 1.2 mg/l, respectively. As an internal standard, 0.5 ml of 0.05 mg/l of isobutanol solution was added to each blood samples. A regression was calculated from the each ratio of the area of styrene and isobutanol at five concentrations. Regression curve was got with r^2=0.9976. The detection limit was 0.01 mg/l. The reproducibility of analysis was shown in Table I. The coefficient variation for five process at each three concentration was from 2.9 to 4.6%.

Fig. 4 shows the HPLC chromatogram of urine sample. Mandelic acid peak eluted at 9.48 min. and hip-

puric acid at 10.20 min. at a column temperature 45℃. Phenylglyoxylic acid was eluted at 3.48 min. overlapped with other peak. Mandelic acid and hippuric acid were separated partially when using acetonitrile as an organic modifier of the mobile phase instead of using methanol. Also, the concentration of acetic acid was affected the peak resolution. Below 0.5% (v/v) acetic acid concentration, peak tailing was increased and the separation was partial. Another point of study was column temperature. Mandelic acid and hippuric acid peaks were seperated partially at column temperature 25℃. According to increasing column temperature, it went better until 45℃. Over 45℃, the separation of mandelic acid and hippuric acid was rather difficult.

The mean rate of recovery test for five sets of urine and water was 98% with 4.0% s.t.d.

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