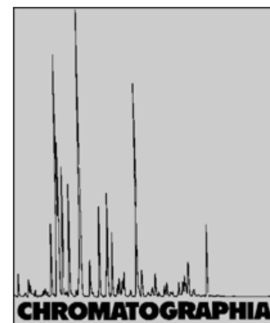
Sensitive Determination of Styrene and Related Compounds in Human Body Fluids by Headspace Capillary Gas Chromatography with Cryogenic Oven Trapping



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Key Words

Gas chromatography
Cryogenic oven trapping
Headspace sampling
Blood and urine samples
Styrene, toluene and ethylbenzene

Summary

A simple and sensitive method is presented for determination of styrene, toluene, ethylbenzene, isopropylbenzene and *n*-propylbenzene in human body fluids by capillary gas chromatography (GC) with cryogenic oven trapping. After heating a blood or urine sample containing each compound and *p*-diethylbenzene (internal standard, IS) in a 7.0-mL vial at 60 °C for 20 min, 5 mL of headspace vapor was drawn into a glass syringe and injected into a GC. All vapor was introduced into an Rtx-Volatile middle bore capillary column in splitless mode at oven temperature of 20 °C to trap entire analytes, and the oven temperature then programmed to 280 °C for GC measurements by flame ionization detection. The present conditions gave sharp peaks of each compound and IS, and low background noises for whole blood or urine samples.

Introduction

Styrene is an important intermediate in chemical syntheses and widely used in the manufactures of plastics, synthetic rubber, resin and fiber glass. The effects of styrene have been studied on factory workers [1–13]. Cases of death from accidental styrene poisoning in a factory have been reported [14]. In addition to acute toxic effects of styrene vapour, such as eye and nose irritation and depression of the central nervous system, chronic oncogenic properties are well known [1–13]. Styrene [15, 16], ethylbenzene and toluene [17–19] are regarded as important compounds causing so-called, sick building syndrome.

There are many reports of styrene analyses by GC [20–25]. However, many of them deal with the presence of styrene in foods due to its migration from the food-contact packaging [20, 21] or in cigarette smoke [22]. Analyses of styrene in mammalian samples are very few [23–25] but include styrene in mouse blood [24] and human hair [25]. Headspace extraction and GC with flame ionization detection (FID) for determination of styrene are common; the use of packed columns gives low sensitivity [20, 23], and the purge and trap method is sensitive but not suitable for biological samples of high protein content [21].

Recently, we have developed a new GC method for sensitive determination of some volatile organic compounds (VOCs) in human whole blood or urine by trapping them at cryogenic oven temperature for headspace samples [26–30]. Here, we have applied this method to determinations of styrene and related compounds in human whole blood and urine.

Experimental

Materials

Styrene, ethylbenzene, isopropylbenzene and *n*-propylbenzene were from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan); toluene, methanol, sodium sulfate, ammonium sulfate and *p*-diethylbenzene from Wako Pure Chemical Industries (Osaka, Japan). Other common chemicals used were of the highest purity commercially available. An Rtx-Volatile fused silica middle bore capillary column (30 × 0.32 mm i. d., 1.5 μm film) was from Restek Corp. (Bellefonte, PA, USA). Human whole blood was from a healthy volunteer.

Procedure

Stock solutions (100 $\mu g\,mL^{-1}$ or 500 $\mu g\,mL^{-1}$) of styrene, toluene, ethylbenzene,

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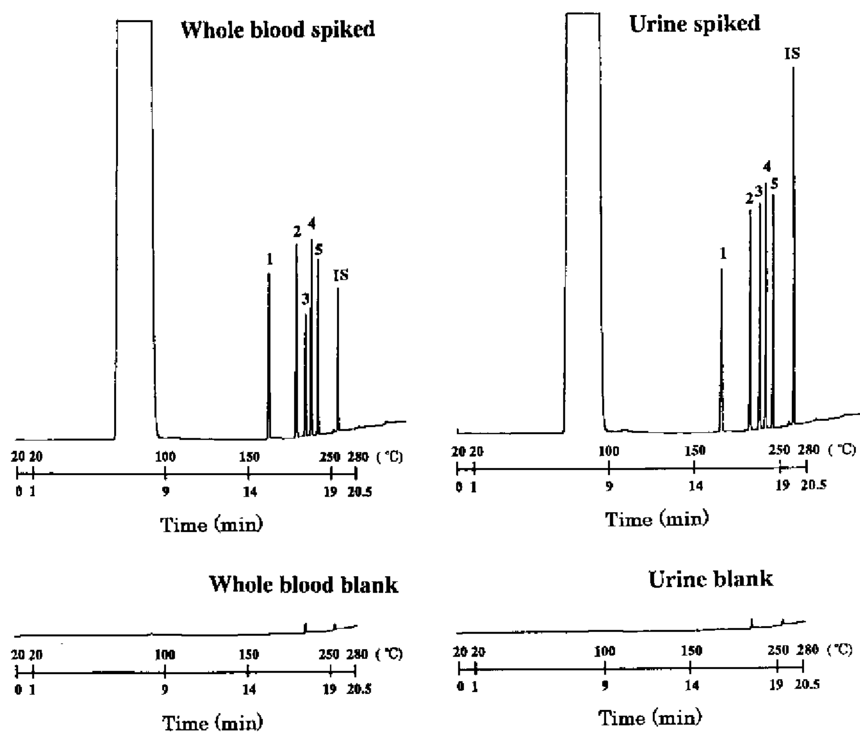

Figure 1. Headspace capillary GC chromatograms with oven trapping at 20 °C for **1** = toluene, **2** = ethylbenzene, **3** = styrene, **4** = isopropylbenzene, **5** = *n*-propylbenzene and **IS** = *p*-diethylbenzene in human whole blood (top left panel) and urine (top right panel). One microgram each of six compounds was spiked in 0.5 mL whole blood or urine. Bottom panels show blank samples.

isopropylbenzene, *n*-propylbenzene and *p*-diethylbenzene (IS) were prepared by dissolving them in methanol. To a 7.0 mL vial containing 0.5 g sodium sulfate, were added 0.5 mL distilled water and 0.5 mL whole blood or urine containing various amounts of each compound and 1 μg of IS. The vial was rapidly sealed with a silicone-septum cap and placed on an aluminum block heater. After heating the vial at 60 °C for 20 min, 5 mL headspace vapor was drawn into a gas tight syringe (5-mL volume) with a 23 G needle and injected into the GC port in splitless mode at 20 °C in ca. 5 s.

GC Conditions

GC analysis was carried out on an HP6890 series gas chromatograph equipped with FID and with a cryogenic oven temperature device (Hewlett-Packard, Palo Alto, CA, USA). An electrically operated solenoid valve introduced liquid carbon dioxide at a rate appropriate to cool the oven to the desired temperature. GC conditions were: column temperature, 20–280 °C (1 min hold at 20 °C, 10 °C min^{-1} from 20 to 150 °C, and 20 °C min^{-1} from 150–280 °C); injection temperature, 250 °C; detection temperature: 240 °C; and helium flow-rate: 3 mL min^{-1}. The vapor was injected in splitless mode, and the splitter was opened 1 min after completion of injection. For GC quantitation, the peak areas of each compound were used.

Animal Experiments

Male Sprague-Dawley rats, weighing ca. 200 g, were used. Each animal was put into a glass container (volume 1839 cm^3), where gauze soaked with 10 mL chloroform in a 50 mL beaker and gauze soaked with 3 mL each of the five compounds except IS in another 50 mL beaker had been placed in a corner of the container bottom. After covering the top of the container until the animal fell into deep anaesthesia by inhalation of vapor of chloroform and the five compounds, the time required was 3–5 min. Under anaesthesia, the animal was rapidly subjected to laparotomy, and about 5 mL arterial blood was drawn from the abdominal aorta in the presence of EDTA-2Na, resulting in death of the animals due to exsanguination. The tube containing the blood was tightly capped, and analysis by GC made on the same day as the samplings.

Results and Discussion

Optimization of Conditions

Various conditions for the headspace extraction of the five compounds and IS from whole blood were tested. Vials were heated to 45, 55, 60 and 80 °C for 10, 15, 20 and 30 min; it was found that optimum extraction into the headspace was attained at 60 °C for 20 min. A Rtx-Volatiles capillary column was used in this study because it gave excellent symmetrical peaks for some VOCs such as chloroform and methylene chloride, and good separation from impurity peaks in previous study [26].

Various initial oven temperatures (80, 60, 40, 20 and 0 °C) for trapping each compound vapor were tested. At 80 °C, peaks were relatively broad and became sharper upon lowering the oven temperature to 20 °C. Peaks at 0 °C were almost the same as those at 20 °C. Therefore, we selected 20 °C as initial oven temperature.

Various salts were reported to be effective for increasing extraction efficiencies of various VOCs [31, 32]. We added 0.5 g sodium chloride, sodium sulfate and ammonium sulfate to 0.5 mL whole blood in the presence of 1 μg each of the five compounds. Peaks were highest with 0.5 g sodium sulfate and this was adopted in our method.

Reliability of Method

Figure 1 shows gas chromatograms for styrene, toluene, ethylbenzene, isopropylbenzene, *n*-propylbenzene and *p*-diethylbenzene (IS) spiked in human whole blood or urine (2 μg mL^{-1} each) in a vial (top left and right panels) and for extracts in the absence of each compound and IS in a vial (bottom panels). Retention times for toluene, ethylbenzene, styrene, isopropylbenzene, *n*-propylbenzene and IS were 15.2, 16.9, 17.4, 17.7, 18.1 and 19.4 min, respectively, at 20 °C. There were two small peaks visible in the blank chromatograms, but gave no problems.

Table I shows extraction efficiencies of styrene and related compounds in whole blood or urine at 2 and 10 μg mL^{-1} (n = 10 for each). Urine gave much higher efficiencies than whole blood. This is not surprising because whole blood contains many proteins and cellular components on which styrene and related compounds adsorb very easily.

Table I. Extraction efficiencies of styrene, and related compounds and IS in human whole blood and urine for measurements by GC with cryogenic oven trapping[a].

	Mean ± SD %					
	Styrene	Toluene	Ethylbenzene	Isopropylbenzene	*n*-Propylbenzene	*p*-Diethylbenzene
Whole blood (n = 10)						
2 µg (mL) ^{1}	4.56 ± 0.59	10.6 ± 1.31	7.08 ± 1.22	5.16 ± 1.16	3.92 ± 0.88	1.66 ± 0.17
10 µg (mL) ^{1}	2.66 ± 0.58	6.01 ± 1.23	4.0 ± 0.98	2.70 ± 0.81	2.24 ± 0.64	–
Urine (n = 10)						
2 µg (mL) ^{1}	16.0 ± 2.83	16.4 ± 2.80	16.3 ± 2.70	17.0 ± 2.94	15.0 ± 2.75	13.7 ± 1.32
10 µg (mL) ^{1}	15.8 ± 1.95	17.4 ± 3.08	16.5 ± 2.01	16.2 ± 1.95	15.7 ± 1.87	–

[a] Each compound (1 µg or 5 µg each) and IS (1 µg) added to 0.5 ml human whole blood or urine samples. Extraction efficiencies calculated by comparing peak areas obtained from extracts of spiked whole blood or urine samples with those obtained from non-extracted authentic compounds dissolved in methanol.

Table II. Coefficients of intra-day and inter-day variations for styrene and related compounds spiked in human whole blood and urine.

		Coefficient of variation, %[a]				
		Styrene	Toluene	Ethylbenzene	Isopropylbenzene	*n*-Propylbenzene
Whole blood (*n* = 5)						
2 µg (mL) ^{1}	Intra-day	5.33	6.12	9.57	13.2	13.6
	Inter-day	11.0	6.96	6.99	10.4	11.2
10 µg (mL) ^{1}	Intra-day	6.15	9.59	8.74	10.2	13.8
	Inter-day	13.0	9.92	13.3	10.7	14.1
Urine (*n* = 5)						
2 µg (mL) ^{1}	Intra-day	3.51	7.13	6.25	3.62	3.56
	Inter-day	6.23	3.12	4.80	2.65	4.65
10 µg (mL) ^{1}	Intra-day	8.54	7.98	7.78	7.76	7.62
	Inter-day	4.45	5.88	5.82	5.96	8.48

[a] Coefficients of variation obtained after analysis with each calibration curve for each compound added to whole blood or urine.

Table III. Calibration curves for styrene and related compounds in human whole blood and urine.

	Whole blood		Urine	
	Equation[a]	correlation coefficient (r)	Equation[a]	correlation Coefficient (r)
Styrene	$y = 1.69\,x - 0.021$	$r = 0.994$	$y = 0.68\,x - 0.114$	$r = 0.991$
Toluene	$y = 3.75\,x - 0.145$	$r = 0.994$	$y = 0.68\,x - 0.100$	$r = 0.990$
Ethylbenzene	$y = 2.36\,x + 0.106$	$r = 0.990$	$y = 0.70\,x - 0.119$	$r = 0.990$
Isopropylbenzene	$y = 1.58\,x + 0.285$	$r = 0.985$	$y = 0.68\,x - 0.115$	$r = 0.991$
n-Propylbenzene	$y = 1.25\,x + 0.254$	$r = 0.986$	$y = 0.63\,x - 0.111$	$r = 0.991$

[a] Data subjected to linear regression analysis of peak area ratios (y) of each compound to IS (1 µg per vial) against spiked concentrations (x). Seven plots with different concentrations (0.1 – 10 µg mL^{-1}) of each compound were used.

The coefficients of intra-day and inter-day variations for styrene and related compounds in whole blood and urine (n = 5 each) are shown in Table II. The majority of values were < 10% and the largest value was 14.1%.

Each calibration curve for styrene and related compounds in human whole blood and urine was drawn by plotting 7 concentrations of each *versus* 2 µg mL^{-1} IS. It was linear in the range 0.1 – 10 µg mL^{-1}. The equations and r values for the curves are in Table III. Detection limits (signal-to-noise ratio = 3) were estimated to be 5 ng mL^{-1} for whole blood and 2.5 ng mL^{-1} for urine. This means that blood styrene in factory workers exposed to air containing styrene below the threshold limit (100 ppm in Japan and Britain) can be measured by the present method, because their concentrations are reported to be 0.01 – 1.0 µg mL^{-1} blood [12, 33]. However, styrene concentrations in many kinds of foods are several nanograms or less g^{-1} – except for cinnamon [34] – which are too low to be measured by our GC-FID method. If the cryogenic oven trapping GC is connected to MS detection, the sensitivity will probably be enhanced ca. 100-fold, enabling measurements of such low styrene concentrations in foods.

Animal Experiments

Separate experiments were made with 4 rats. A chromatogram of five compounds in rat whole blood after inhalation is shown in Figure 2. The mean concentrations were 1.67 ± 0.82 µg mL^{-1} for styrene, 4.36 ± 1.74 µg mL^{-1} for toluene, 1.15 ± 0.39 µg mL^{-1} for ethylbenzene, 0.67 ± 0.42 µg mL^{-1} for *n*-isopropylbenzene and 0.88 ± 0.66 µg mL^{-1} for *n*-propylbenzene (means ± SD, n = 4).

Conclusions

This is the first report dealing with GC with cryogenic oven trapping for styrene and related compounds in biological samples.

It gave sensitivities 10 – 50 times higher than by conventional headspace GC [20 – 24]. In addition to the above high sensitivity, much better separation of compounds

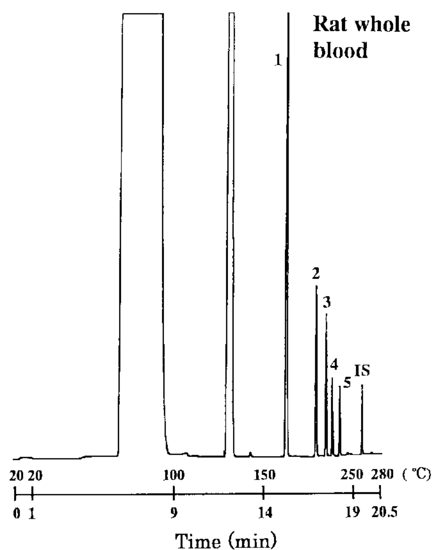

Figure 2. Headspace capillary GC with cryogenic oven trapping obtained from 0.5 mL rat whole blood 3–5 min after inhalation of chloroform and five compounds. Key numbers are as specified in Figure 1. Front large peak due to methanol used as vehicle to dissolve IS; second peak due to chloroform for anaesthesia of animals.

can be also achieved, probably because VOCs are trapped at cyrogenic oven temperatures in a quite narrow zone of the entrance of a capillary column.

The present method for analysis of styrene and related compounds in biological samples is recommended for wide use in forensic and environmental toxicology because it is simple and requires no special GC operations – in addition to the above high sensitivity and high resolution.

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