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Gas chromatographic–mass spectrometric analysis of dichlorobenzene isomers in human blood with headspace solid-phase microextraction

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Abstract

Headspace solid-phase microextraction (HS-SPME) was utilized for the determination of three dichlorobenzene isomers (DCBs) in human blood. In the headspace at 30°C, DCBs were absorbed for 15 min by a 100-μm polydimethylsiloxane (PDMS) fiber. They were then analyzed by capillary column gas chromatography–mass spectrometry (GC–MS). By setting the initial column oven temperature at 20°C, the three isomers were resolved at the baseline level. *p*-Xylene-d_{10} was used as the internal standard (I.S.). For quantitation, the molecular ion at *m/z* 146 for each isomer and the molecular ion at *m/z* 116 for I.S. were selected. For day-to-day precision, relative standard deviations in the range 3.2–10.7% were found at blood concentrations of 1.0 and 10 μg/ml. Each compound was detectable at a level of at least 0.02 μg per 1 g of whole blood (by full mass scanning). HS-SPME–GC–MS, when performed at relatively low temperatures, was found to be feasible in toxicological laboratories. Using this method, the plasma levels of one patient who had drunk a pesticide-like material were measured.

Keywords: Dichlorobenzene

1. Introduction

Dichlorobenzene (DCB) isomers are widely used in industrial solvents and insecticides, etc. Their main toxicity is that they can cause damage to the liver and the kidneys [1]. A few cases have been reported where victims ingested insecticides containing *o*-DCB [2–4], although the toxicity was relatively low. Major analytical methods reported to date have included gas chromatography (GC) with electron capture detection and flame ionization detection following liquid–liquid extraction [5–7]. For the most part, these extraction procedures seem to be complicated and inconvenient.

To extract volatile compounds from whole blood, care needs to be taken to purify organic solvents and to prevent them from forming an emulsion. If the extracted sample is analyzed by gas chromatography–mass spectrometry (GC–MS), the extracting solvent may interfere with the identification of these

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volatile compounds through their intact mass spectra. The conventional static headspace GC–MS may perhaps be suitable for detection if a capillary column with a large volume capacity is used. Meanwhile, the headspace solid-phase microextraction (HS-SPME) does not necessitate the use of an extracting solvent or a large capacity column.

Recently, we were faced with a poisoning case where we needed to analyze volatiles in blood. For the GC–MS analysis, we attempted to apply HS-SPME to the sample preparation. In this case, only DCBs were clearly detected. When the samples were stirred at a higher temperature in a manner often used in conventional HS technique, however, the detection had an extremely low reproducibility. Accordingly, further studies on optimization of the conditions for HS-SPME performance were required.

The current study is aimed at optimizing conditions for HS-SPME combined with GC–MS for the detection of DCBs in blood. The applicability of this assay was examined by estimating plasma levels of a patient who had ingested a pesticide-like material.

2. Experimental

2.1. Materials

2.1.1. Reagents

m-, *p*- and *o*-DCBs (of analytical grade) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). As an internal standard, *p*-xylene-d_{10} was obtained from Isotec (Miamisburg, OH, USA). Tetraethylene glycol dimethyl ether (TGDE) used as the solvent was purchased from Aldrich (Milwaukee, WI, USA). The SPME devices and their 100-μm polydimethylsiloxane (PDMS) fiber assemblies were purchased from Supelco (Bellefonte, PA, USA). The whole blood used in this study was provided by an adult male who had not been exposed to any solvents.

2.1.2. Sample solution and internal standard solution

The three DCBs were dissolved together in TGDE to obtain a stock standard solution at a concentration each of 100 μg/ml. The internal standard solution at 0.05% (v/v) was made by diluting *p*-xylene-d_{10} with TGDE.

2.2. Instrumental

GC–MS analyses were performed on a GC–MS QP-5000 system operated in the positive electron impact (EI) mode, with a cryogenic oven temperature device with liquid CO_2 (Shimadzu, Kyoto, Japan), and an XTI^R-capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness; Restek, Bellefonte, PA, USA). The injection port temperature was set at 250°C, and the flow-rate of the helium carrier gas was set at 2.1 ml/min at 100 kPa. The time for the splitless injection was set at 1.0 min. The oven temperature was initially maintained at 20°C for 1 min, then raised to 290°C by 30°C/min, and finally maintained at 290°C for 6 min. The interface temperature was set at 260°C. The ionizing energy was 70 eV. Full scan mass spectra were recorded within the scan range from 33 to 200 amu at a scan cycle of 0.35 s. For quantitative analysis by mass chromatography, the ions at m/z 146 for three isomers and at m/z 116 for *p*-xylene-d_{10} were used.

2.3. Optimization

For optimization of HS-SPME, the factors of temperature and time were investigated. Blood (0.5 g) containing 0.5 μg of each of the DCBs plus 0.5 ml of distilled water was placed into a 12-ml screw cap vial. After sealing the vial with PTFE-coated silicone rubber, 1 μl of 0.05% *p*-xylene-d_{10} was injected into the vial. After incubation for 2 min in an aluminum-block heater, the fiber was passed through the septum, and exposed to the headspace at varying temperatures and for varying periods of time. Finally, the fiber was transferred into the injection port of the GC instrument and held for 3 min.

2.4. Standard curves and reliability

To obtain standard curves, the standard solution containing all the three DCBs at the appropriate concentration was added to 0.50 g of blood in a 12-ml screw cap vial. The added amounts of DCBs were controlled within the range from 0.01 to 2.0

μg, while 0.5 ml of distilled water was also added to the vial. Then, 1 μl of 0.05% *p*-xylene-d_{10} was injected into the sealed vial. After incubation at 30°C for 2 min, the fiber was passed through the septum, and exposed to the headspace at 30°C for 15 min. Finally, the fiber was subjected to GC–MS analysis. The reliability of this assay under the same conditions was evaluated by analyzing two groups of blood samples: 1.0 and 10 μg/g of each DCB.

3. Results

After scanning and recording the full mass spectra from 33 to 200 amu, mass chromatography was performed. A total ion chromatogram and two selected ion chromatograms at *m/z* 116 and *m/z* 146 obtained from the whole blood spiked with DCBs and I.S. are shown in Fig. 1. As seen in Fig. 1 and Table 1, three isomers were individually detected at the baseline level, and all the peaks were characterized by retention time and mass spectra. There were no interfering peaks from the volunteer's blood, although two small unidentified peaks were observed.

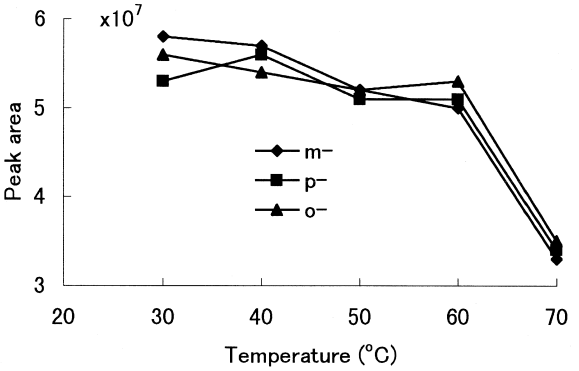

Fig. 2. Examination of optimal temperature for adsorption of DCBs (extraction time: 20 min).

As indicated in Fig. 2, a relatively low temperature proved to be appropriate for adsorption to the fiber (100 μm PDMS). On the other hand, 15 min was enough time to equilibrate the adsorption of the DCBs to the fiber as seen in Fig. 3. Based on the experiments, the sampling conditions for HS-SPME were set at a temperature of 30°C and an exposure time of 15 min. Under these conditions, the respective standard curves were linear as follows: *y*=

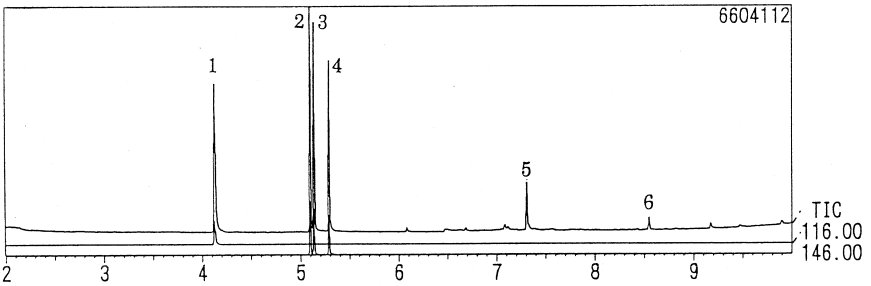

Fig. 1. Total ion chromatogram and selected ion chromatograms obtained from 0.5 ml of whole blood containing 0.5 μg of each of three DCBs and 1 μl of 0.05% internal standard. The details of each peak are summarized in Table 1.

Table 1
Mass spectral data in the peaks indicated in Fig. 1

No.	t_R (min)	Principal ions *m/z* (relative intensity, %)					Compound
1	4.12	116 (45)	98 (100)	82 (12)	54 (20)	42 (25)	*p*-Xylene-d_{10}
2	5.09	148 (60)	146 (100)	111 (38)	75 (36)	50 (36)	*m*-Dichlorobenzene
3	5.13	148 (60)	146 (100)	111 (38)	75 (36)	50 (36)	*p*-Dichlorobenzene
4	5.28	148 (60)	146 (100)	111 (38)	75 (36)	50 (36)	*o*-Dichlorobenzene
5	7.30	101 (8)	85 (15)	75 (21)	57 (100)	45 (67)	Unidentified[a]
6	8.55	180 (7)	123 (12)	97 (82)	57 (100)	41 (48)	Unidentified[a]

[a] Although they were commonly detected in this assay, no reference data for their identification were found.

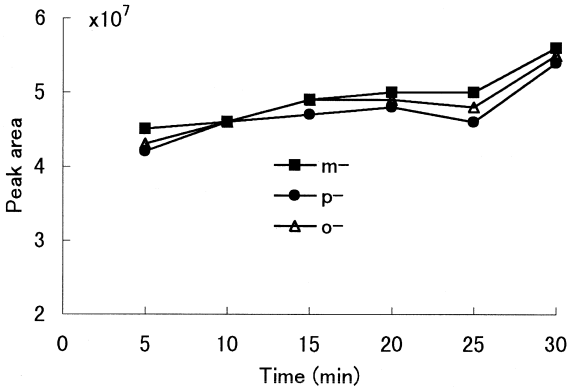

Fig. 3. Examination of optimal time for exposure to headspace containing DCBs at 30°C.

$1.7735x-0.0737$ ($r^2=0.9947$) for *m*-DCB, $y=1.5215x-0.0328$ ($r^2=0.9982$) for *p*-DCB and $y=1.3044x-0.0387$ ($r^2=0.9958$) for *o*-DCB. The lower limit of quantitation for each DCB was 0.02 μg per 1 g of whole blood. The reliability of this assay is expressed as relative standard deviation (RSD) at two concentrations of respective DCB (Table 2).

Table 2
Reliability of blood analysis by this assay (relative standard deviation, RSD, %)

Compound	Within-day ($n=5$)		Day-to-day ($n=4$)	
	1 μg/g	10 μg/g	1 μg/g	10 μg/g
m-Dichlorobenzene	1.53	2.89	9.74	6.12
p-Dichlorobenzene	1.86	3.96	10.10	3.24
o-Dichlorobenzene	2.58	2.21	10.70	3.45

4. Discussion

Since HS-SPME does not require the use of an extracting solvent, a regular capillary column for general drug analysis can be utilized in HS-SPME–GC–MS. When performing GC–MS by conventional static HS, split-injection and specified capillary columns for volatile analysis are required. Although a lower temperature was set in order to obtain symmetrically shaped peaks and good separation, a common column is available for practical analysis as shown in this study. By using *p*-xylene-d_{10} as the internal standard, a satisfactory quantitative analysis can be performed without the interference of any volatile peaks. Usually, to obtain highly reliable results, selected ion monitoring by which only characteristic and abundant ions are detected is used. However, we employed full-mass scanning which can simultaneously provide qualitative and quantitative information. Even by this technique, the quantitative data seem to be satisfactory for forensic purposes. We found the effect of the adsorption was excellent at 30°C. According to the results, a lower temperature is effective not only for the extraction of some volatile compounds, but also for the removal of impurities such as peaks 5 and 6 shown in Fig. 1 (as practical findings).

5. Application

We examined blood samples collected from a 20-year-old male patient who was found by his family in a coma with vomiting about 4 h after he had drunk an unknown liquid, which resembled

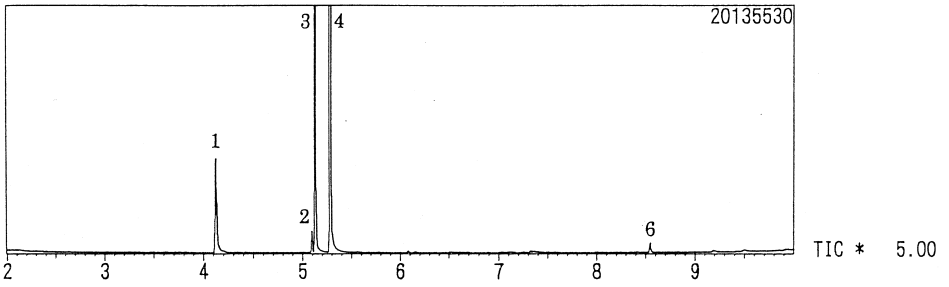

Fig. 4. Total ion chromatogram obtained from the plasma of a patient in an acute poisoning case. The plasma used in this measurement was collected at 4 h after his admission. The peak numbers in this figure correspond to the peaks indicated in Fig. 1.

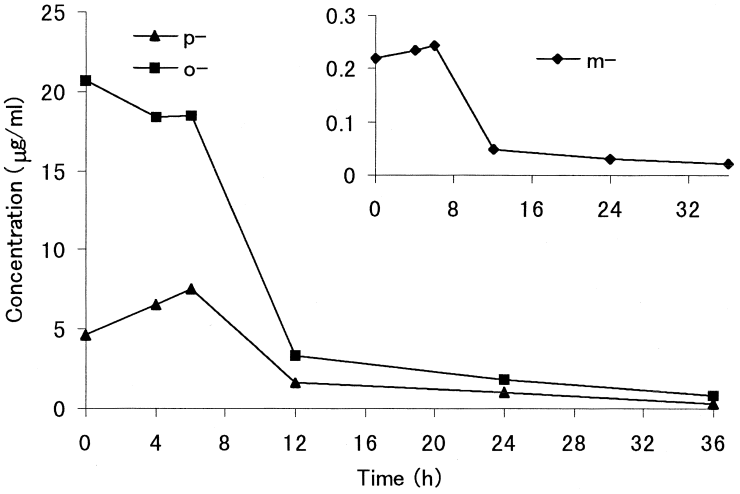

Fig. 5. Plasma concentration–time levels of the three DCB isomers in plasma obtained from a patient in an acute poisoning case.

pesticide. After several hours of preliminary therapies in a local hospital; gastric irrigation and fluid therapy, he was admitted to our hospital. On admission, he was still vomiting with cresolish matter. Accordingly, therapy similar to that given at the first hospital was administered. Furthermore, since oliguria was observed, he was given diuretics and chronic hemodiafiltration therapy. After several hours of therapy, his urinary volume became regulated to a normal level. For chemical analysis, plasma samples were collected at 0, 4, 6, 12, 24 and 36 h.

In toxicological tests, only DCBs were found (by this method). Fig. 4 shows the total ion current chromatogram of the plasma sample at 4 h. In the sample, all the intact mass spectra of three isomers were obtained. The plasma levels versus time are plotted in Fig. 5. On admission, serum concentrations of all DCBs were 20.7 μg/g for the *o*-isomer, 4.6 μg/g for the *p*-isomer (maximum 6.5 μg/g at 4 h after admission) and 0.22 μg/g for the *m*-isomer. By 36 h, the plasma concentrations had decreased to 0.34 μg/g for the *o*-isomer, 0.77 μg/g for the *p*-isomer and 0.02 μg/g for the *m*-isomer.

6. Conclusion

HS-SPME–GC–MS using a common capillary column for drug analysis was used in an attempt to analyze DCB isomers in blood. Under the conditions set in this study, reliable qualitative and quantitative analyses can be simultaneously performed. The applicability of this method was demonstrated for the measurement of plasma levels in an acute poisoning case.

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