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Headspace solid-phase microextraction and gas chromatographic–mass spectrometric screening for volatile hydrocarbons in blood

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Abstract

Optimization for headspace solid-phase microextraction (SPME) was studied with a view to performing gas chromatographic–mass spectrometric (GC–MS) screening of volatile hydrocarbons (VHCs) in blood. Twenty hydrocarbons comprising aliphatic hydrocarbons ranging from *n*-hexane to *n*-tridecane, and aromatic hydrocarbons ranging from benzene to trimethylbenzenes were used in this study. This method can be used for examining a burned body to ascertain whether the victim had been alive or not when the burning incident took place. *n*-Hexane, *n*-heptane and benzene, the main indicators of gasoline components, were found as detectable peaks through the use of cryogenic oven trapping upon SPME injection into a GC–MS instrument. The optimal screening procedure was performed as follows. The analytes in the headspace of 0.2 g of blood mixed with 0.8 ml of water plus 0.2 μg of toluene-d_8 at −5°C were adsorbed to a 100-μm polydimethylsiloxane (PDMS) fiber for 30 min, and measured using the full-mass-scanning GC–MS method. The lower detection limits of all the compounds were 0.01 μg per 1 g of blood. Linearities (r^2) within the range 0.01 to 4 μg per 1 g of blood were only obtained for the aromatic hydrocarbons at between 0.9638 (pseudocumene) and 0.9994 (toluene), but not for aliphatic hydrocarbons at between 0.9392 (*n*-tridecane) and 0.9935 (*n*-hexane). The coefficients of variation at 0.2 μg/g were less than 8.6% (*n*-undecane). In conclusion, this method is feasible for the screening of volatile hydrocarbons from blood in forensic medicine.

Keywords: Volatile hydrocarbons

1. Introduction

Volatile hydrocarbons (VHCs) in blood play a very important role in investigations of a burned body when determining whether the victim had been alive or not when the burning incident took place. They can act as an indicator of the vital signs of the victim when the fire started, or as an environmental factor to indicate which fuel was used for setting the fire around the victim [1]. In order to accomplish this task, simultaneous analysis of numerous kinds of VHC compounds and a convenient isolation procedure are essential.

To detect VHCs in blood, gas chromatography–mass spectrometry (GC–MS) with static headspace sampling is commonly performed. Our previous reports also employed this method and satisfactory results were obtained in which the different types of

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burning cases could be distinguished [2–5]. However, in a general forensic laboratory, many kinds of drug tests need to be carried out quickly. Therefore, techniques which are convenient and economical for common use need to be developed in a universally applicable manner.

It has been demonstrated that the solid-phase microextraction method (SPME) developed by Pawliszyn could be utilized for just such a purpose [6]. The extraction has succeeded in many kinds of analyses either from liquid or from the headspace of samples [7]. However, one of its weaknesses is that the adsorption capacity is more insensitive for highly volatile and low-molecular-mass compounds. An attempt was therefore made to utilize a cooling device to make up for adsorption [8].

In this study, the isolation of a large number of VHCs in blood simultaneously was successfully achieved using a standard SPME assembly and GC–MS analysis under cryogenic oven conditions.

2. Experimental

2.1. Materials

2.1.1. Reagents

The VHCs used for this experiment were all of analytical grade, comprising *n*-hexane, *n*-heptane, *n*-octane, *n*-nonane, *n*-decane, *n*-undecane, *n*-dodecane, *n*-tridecane, benzene, toluene, ethylbenzene, *m*-xylene, *o*-xylene, cumene (isopropylbenzene), propylbenzene, 3-ethyltoluene, mesitylene (1,3,5-trimethylbenzene), 2-ethyltoluene, pseudocumene (1,2,4-trimethylbenzene) and 1,2,3-trimethylbenzene. Toluene-d_8 for use as an internal standard (I.S.) was purchased from ISOTEC (Miamisburg, OH, USA). Tetra(ethylene glycol) dimethyl ether (TEGDE), which was used as a solvent, was purchased from Aldrich (Milwaukee, WI, USA). SPME devices and 100-μm polydimethylsiloxane (PDMS) fiber assemblies were purchased from Supelco (Bellefonte, PA, USA).

2.1.2. Standard solutions

Standard solutions were prepared by dissolving together 20 VHCs in TEGDE at serial concentrations of 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 and 2000 μg/ml. I.S. was added at 500 μg/ml to each solution. These solutions can be kept for at least 6 months in the laboratory.

2.1.3. Blood samples

Whole blood supplied by an adult male volunteer who had not been exposed to any solvent was used in this study. For this study, 10 μl of each standard solution (Section 2.1.2) was added to 5 g of the volunteer's whole blood.

2.1.4. Samples for standard curves

To obtain standard curves, samples comprising 0.2 g of blood were placed in 12-ml screw cap vials containing 0.8 ml VHC-free water. The concentrations of VHCs in the blood samples ranged from 0.002 to 4 μg/g. The sealed vials were subjected to HS-SPME and GC–MS.

2.2. Optimization

For the optimization of HS-SPME, the factors of temperature and time were investigated. Samples comprising 0.2 g of blood containing 1 μg/g or 0.2 μg of each of the VHCs were placed in vials containing 0.8 ml of VHC-free water, then the vials were sealed with a Teflon-coated silicone rubber septum. Two minutes after the vial had been placed in an aluminium-block incubator, a fiber was inserted through the septum into the vial, 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–MS instrument and held for 3 min (sampling for 1 min, purging for 2 min).

2.3. Instrumentation

GC–MS analyses were performed on a GC–MS QP-5000 operated in the positive electron impact (EI) mode, with a cryogenic oven temperature device which was controlled using liquid CO_2 as coolant (Shimadzu, Kyoto, Japan). The GC–MS conditions were as follows: an XTI^R-5 capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness; Restek, Bellefonte, PA, USA); injection port temperature, 250°C; oven temperature, −40 to 290°C (held at −40°C for 1 min, then increased at 30°C/min from −40 to 290°C); helium flow-rate, 2.1 ml/min;

interface temperature, 260°C. SPME sampling was in the splitless mode, the splitter being opened 1 min after the SPME assembly was inserted. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 33 to 200 amu at a 0.35 s cycle.

3. Results

Several chromatograms were obtained under the above experimental conditions, and these are shown in Fig. 1. The identification of VHCs was achieved by their mass spectra and confirming their retention time on the chromatograms tracing their molecular ions and main fragment ions (Fig. 1). As seen in Fig. 2, benzene (9) and *n*-heptane (1), which are highly volatile low-molecular-mass compounds, were trapped and detected as sharp peaks by reducing the initial oven temperature to −40°C. Under the cryogenic oven conditions, *n*-hexane was also observed at an identifiable level.

The optimum temperature for headspace SPME was examined by exposing the fiber to the headspace of the blood sample at varying temperatures for 20 min. The results are shown in Fig. 3, where the peak area measured by tracing the molecular ions of the aromatic hydrocarbons or the main fragment ions of the aliphatic ions are plotted against the different temperatures. The highest efficiencies were observed at −5°C, with the exception of dodecane and tridecane. The largest peak of tridecane was obtained at 60°C, while that of dodecane was found at 15°C. The blood sample could be frozen at −5°C and clotted up to 70°C. In the case of most of the compounds, the highest sensitivity was observed at −5°C (Fig. 3).

The optimum time for exposing the fiber to the headspace at −5°C was examined by measuring the peak area of the VHCs. As seen in Fig. 4, most of the VHC curves reached an equilibrium at 30 to 50 min, although that of propylbenzene continued to decrease.

Based on these experiment results, the sampling conditions for HS-SPME were set at a temperature of −5°C and at an exposure time of 30 min. Under these conditions, the respective standard curve equation of each VHC is shown in Table 1. The lower limit of quantitation was 0.01 μg per 1 g of whole

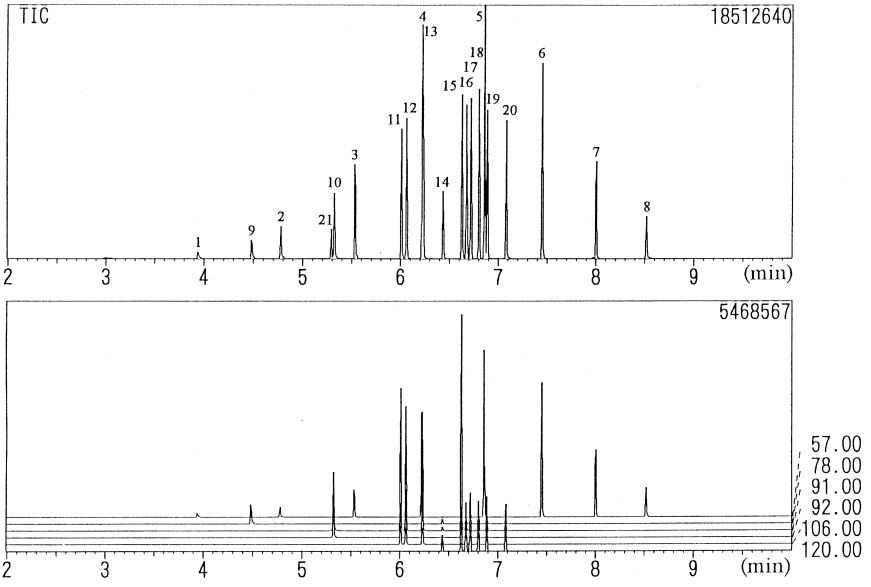

Fig. 1. Total ion chromatogram and selected ion chromatograms obtained from 0.2 g of blood containing 0.2 μg of each of the VHCs and the internal standard. Selected ions: common ions at *m*/*z* 57 for aliphatics, molecular ions and common ion at *m*/*z* 91 for aromatics (numbers correspond to Table 1).

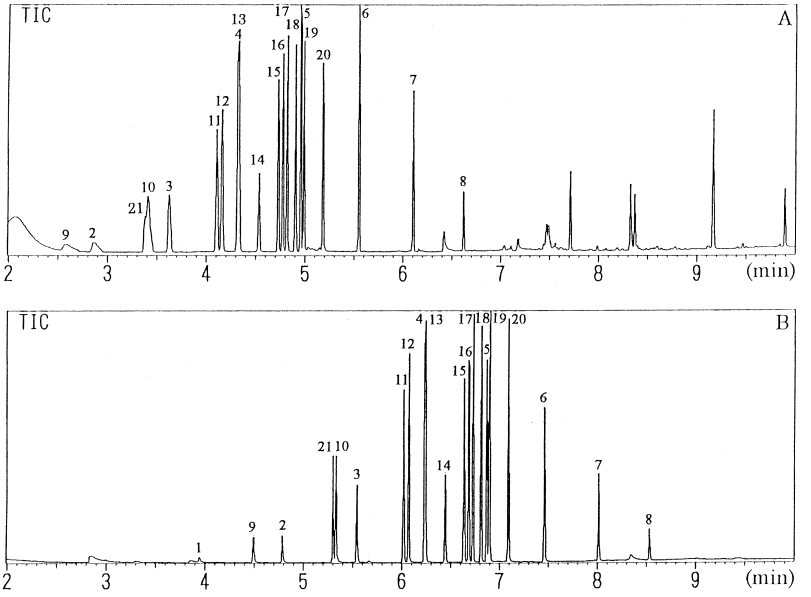

Fig. 2. Comparison of the chromatograms at various initial column temperatures (numbers correspond to Table 1). (A) 20°C; (B) −40°C.

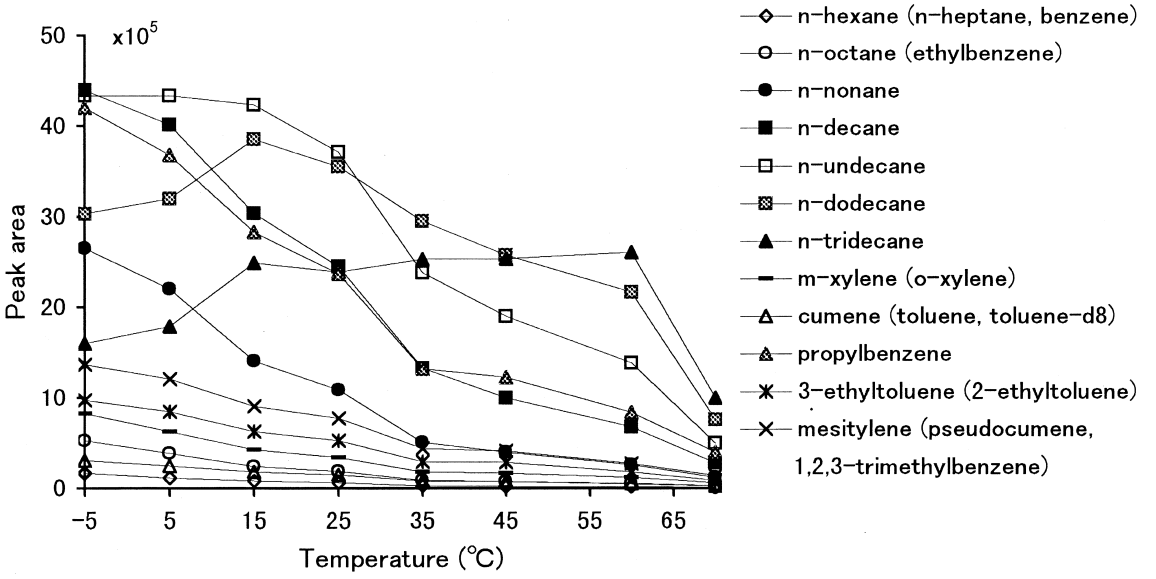

Fig. 3. Determination of optimal temperature for adsorption of VHCs (those of similar tendency are included in parentheses; extraction time, 15 min).

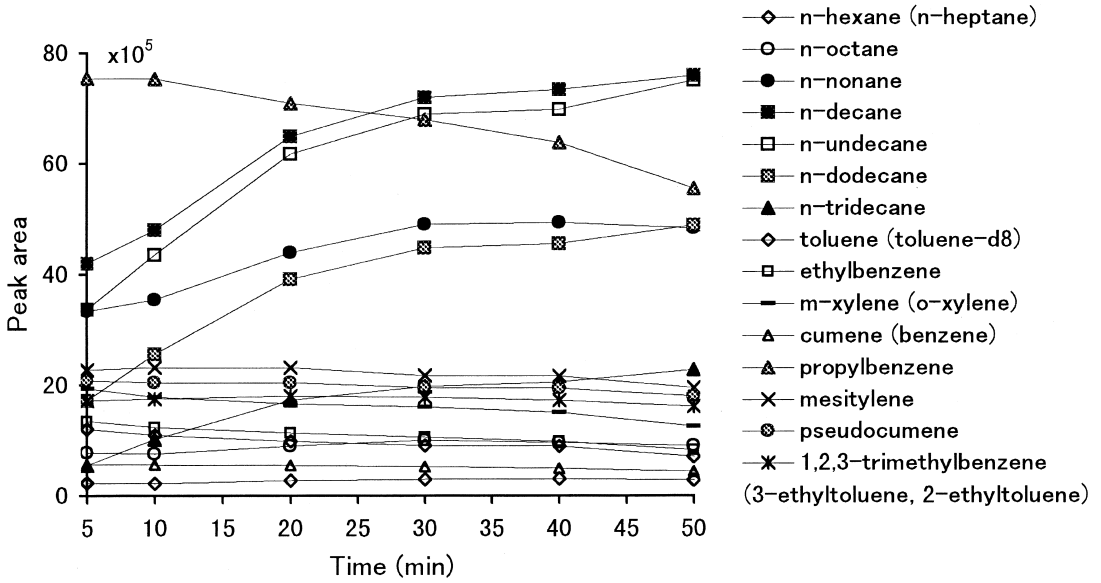

Fig. 4. Determination of optimal time for exposure to headspace containing VHCs at −5°C (those of similar tendency are included in parentheses).

Table 1
Data from the optimization of VHC analysis; linearity and variation

Peak No.	Compound	t_R	Selected ion	Correlation	r^2	C.V.[a]
1	*n*-Hexane	3.94	57	$y = 0.1648x - 0.0006$	0.9935	3.4
2	*n*-Heptane	4.78	57	$y = 0.3090x + 0.0049$	0.9923	5.7
3	*n*-Octane	5.54	57	$y = 0.7347x + 0.0464$	0.9923	6.8
4	*n*-Nonane	6.23	57	$y = 2.8571x + 0.3484$	0.9856	6.5
5	*n*-Decane	6.86	57	$y = 4.2685x + 0.3933$	0.9862	8.2
6.	*n*-Undecane	7.46	57	$y = 3.6256x + 0.0947$	0.9787	8.6
7	*n*-Dodecane	8.01	57	$y = 2.6491x - 0.1062$	0.9556	7.5
8	*n*-Tridecane	8.53	57	$y = 1.2810x - 0.0889$	0.9392	7.6
9	Benzene	4.49	78	$y = 0.7166x + 0.0173$	0.9993	6.7
10	Toluene	5.33	92	$y = 1.0469x + 0.0363$	0.9994	5.6
11	Ethylbenzene	6.01	106	$y = 0.9848x + 0.0574$	0.9960	4.9
12	*m*-Xylene	6.07	106	$y = 1.3272x + 0.1166$	0.9941	4.7
13	*o*-Xylene	6.24	106	$y = 1.2211x + 0.1322$	0.9889	4.7
14	Cumene	6.44	120	$y = 0.4360x + 0.0254$	0.9978	3.1
15	Propylbenzene	6.63	91	$y = 4.7916x + 0.5769$	0.9836	4.1
16	3-Ethyltoluene	6.68	120	$y = 1.0600x + 0.1415$	0.9816	3.3
17	Mesitylene	6.72	120	$y = 1.4590x + 0.2325$	0.9791	2.5
18	2-Ethyltoluene	6.81	120	$y = 1.1019x + 0.1663$	0.9760	3.4
19	Pseudocumene	6.90	120	$y = 1.2997x + 0.2390$	0.9638	3.0
20	1,2,3-Trimethylbenzene	7.09	120	$y = 1.223x + 0.17840$	0.9838	3.7
21	Toluene-d_8	5.29	100			5.4

[a] Coefficient of variation (%); $n = 5$; concentration, 0.2 μg/g.

blood. The reliability of the values obtained by this assay are also expressed as coefficients of variation in Table 1.

4. Discussion

Through the use of cryogenic temperature control of a GC oven with an injection system including pre-column and in-column when highly volatile compounds are being analyzed utilizing conventional headspace GC, a higher sensitivity of detection can be obtained [9]. However, a capillary column suitable for a large injection volume of the headspace sample is necessary. In a forensic laboratory, various kinds of chemical tests using only one piece of GC–MS equipment are required in order to rapidly elucidate various cases. For such practical reasons, changing the capillary column for each analysis is very troublesome. By performing HS-SPME, the same capillary column as that used in regular drug tests can be used for volatile analysis [8]. Peaks of highly volatile compounds can broaden in capillary GC when using the splitless injection mode. By injecting under the cryogenic control of a GC oven, the broader-shaped peaks can be enhanced into sharper peaks (Fig. 2). In this experiment, the broader peaks of *n*-hexane, *n*-heptane and benzene were changed into sharper detectable peaks.

Since the partial pressures of volatile compounds in the headspace are proportional to temperature, the gas concentration in the headspace increases with rising temperature. However, the relative recoveries of analytes in HS-SPME decreased at higher temperature (Fig. 3). During the process of adsorption and release to/from a fiber, temperature plays a very important role. The rate of adsorption increases while the rate of release decreases for VHCs when the temperature is lower. These theories define the results in this experiment [6]. The results of quantitative data suggest that the sensitivity of this method is sufficient for use in practical cases, as described in our previous report [4].

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