A sensitive method was developed for determination of \( n \)-hexane and cyclohexane in human body fluids by headspace capillary gas chromatography (GC) with cryogenic oven trapping. Whole blood and urine samples containing \( n \)-hexane and cyclohexane were heated in a 7.5 mL vial at 70°C for 15 min, and 5 mL of the headspace vapor was drawn into a glass syringe. All vapor was introduced through an injection port of a GC instrument in the splitless mode into an Rtx-Volatiles middle-bore capillary column at an oven temperature of –40°C for trapping volatile compounds. The oven temperature was programmed to 180°C for GC with flame ionization detection. These conditions gave sharp peaks for both \( n \)-hexane and cyclohexane, a good separation of each peak, and low background impurities for whole blood and urine. The extraction efficiencies of \( n \)-hexane and cyclohexane were 13.2–30.3% for whole blood and 12.7–20.7% for urine. The coefficients of within-day variation in terms of extraction efficiency of both compounds were 5.0–9.5% for whole blood and 3.8–10.8% for urine; those of day-to-day variation for the compounds were not greater than 16.6%. The regression equations for \( n \)-hexane and cyclohexane showed good linearity in the range of 5–500 ng/0.5 mL for whole blood and urine. The detection limits (signal-to-noise ratio = 3) for both compounds were 1.2 and 0.5 ng/0.5 mL for whole blood and urine, respectively. The data on \( n \)-hexane or cyclohexane in rat blood after inhalation of each compound are also presented.

\( n \)-Hexane and cyclohexane are organic solvents commonly used in industry. For workers in the shoe, furniture, rotogravure, chemical, metal, and pharmaceutical industries, exposure to \( n \)-hexane or cyclohexane is a hygienic problem (1–4), because these solvents have toxic effects on the central and peripheral nervous systems (5–7). Accidental death and poisoning by \( n \)-hexane or cyclohexane were occasionally encountered in clinical and forensic science practices (8–10). Analyses of \( n \)-hexane or cyclohexane by gas chromatography (GC) with flame ionization detection (FID) and by mass spectrometry (MS) have been reported (1–4, 11–14). Purge and trap sample concentration followed by capillary GC is probably the most sensitive method for detection of volatile organic compounds from a large volume of water or solid samples (15, 16). However, this technique is not suitable for biological samples, such as blood and tissue homogenates with high protein contents because it causes serious bubbling. Recently, a microcomputer-controlled device for cooling oven temperatures to 0°C or lower has become available for new types of gas chromatographs. This device was originally designed for rapid cooling of oven temperature to reduce time of analysis. In 1997, Watanabe et al. (17) reported a new capillary GC method for sensitive determination of chloroform and methylene chloride in human whole blood by trapping them at cryogenic oven temperature using the above device. As much as 5 mL headspace vapor can be introduced with splitless injection into a medium-bore capillary column without any loss, resulting in a much higher sensitivity. We used the method to determine trichloroethylene, ethyl ether, thinner components, xylenes, cyanide, and ethanol in human body fluids (18–23). In this study, we extended this line of experiments to include \( n \)-hexane and cyclohexane in human whole blood and urine and optimized the conditions to establish an assay method.

**Experimental**

**Materials**

\( n \)-Hexane, cyclohexane, and methanol were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). An Rtx-Volatiles fused-silica capillary column (30 m × 0.32 mm id, film thickness 1.5 μm) was purchased from Restek Corp. (Bellefonte, PA). Other common chemicals used were of analytical reagent grade. Whole blood and urine were obtained from healthy subjects.
Stock solutions (8 μg/mL) n-hexane and cyclohexane were prepared by dissolving them in methanol. To a 7.5 mL screw-cap vial containing 0.5 mL whole blood or urine was added 0.5 mL distilled water containing 80 ng each of the above compounds (either of them as internal standard, IS). The vials were rapidly sealed with a silicone-rubber septum cap and heated at 70°C on an aluminum block heater (Reacti-Therm Heating/Stirring Model; Pierce, Rockford, IL). After vials were heated for 15 min, a 23 gauge needle of a 5 mL glass syringe was passed through the septum. A 5 mL volume of the headspace vapor was drawn into the syringe and injected into the GC port in the splitless mode at an oven temperature of –40°C. For analysis of n-hexane or cyclohexane in whole blood of rats after inhalation of either compound, 80 ng of IS (cyclohexane for n-hexane measurements, and vice versa) was added to the 0.5 mL whole blood obtained at the initial step; the following procedure was the same as described above.

**GC Conditions**

GC analyses were performed on a GC-14B gas chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with FID and with a cryogenic oven temperature device (Shimadzu). An electrically operated solenoid valve introduced liquid carbon dioxide at a rate appropriate to cool the oven to desired temperatures. The GC conditions were: column temperature, –40–180°C (1 min hold at –40°C; 20°C/min from –40–180°C); injection temperature, 240°C; detector temperature, 260°C; and helium flow rate, 2 mL/min. The vapor samples were injected into the splitless mode at a column temperature of –40°C, and the splitter was opened 1 min after completion of the injection. A 0.04 μL aliquot (40 ng each on-column) of methanol solution of the authentic compounds was used for direct GC injection. This solution was prepared from 1 mg/mL stock solutions of both compounds in methanol.

**GC/MS Conditions**

Mass spectra were recorded on a GC-17A gas chromatograph connected to a QP5050A quadrupole mass spectrometer (Shimadzu) with a computer-controlled data analysis system in the positive ion electron impact (PIEI) mode. The MS conditions were: electron energy, 70 eV; accelerating voltage, 1.5 kV; ionization current, 60 μA; and ion source temperature, 200°C. GC separation was made with the above Rtx-Volatiles capillary column. The GC conditions for the GC/MS were the same as those described for the GC–FID.

**Animal Experiments**

Male Wistar rats weighing 270–290 g were obtained from Saitama Experimental Animal Supply (Saitama, Japan). Each animal was put into a glass container (space volume 6.3 L), where n-hexane (20 mg) or cyclohexane (10 mg) had been placed in a corner of the container bottom. After the top of the container was covered, the animal was exposed to each solvent in the container for 30 min. With the rats under pentobarbital anesthesia, the cardiac blood was collected after the inhalation and stored frozen at –40°C until analysis.

**Results and Discussion**

**Analytical Conditions**

Various conditions for the headspace extraction of n-hexane and cyclohexane from whole blood were tested. Heating at 70°C gave more vapor pressure than did heating at 50 and 60°C; this was advantageous for drawing as much as 5 mL headspace vapor. Vials were heated at 70°C for 5–20 min, with maximal extraction of headspace at 15 min. Vials were therefore heated at 70°C for 15 min.

Various initial oven temperatures were tested for trapping n-hexane and cyclohexane vapor (Figure 1). At 20°C, the peaks of both compounds were quite broad and became sharper when the oven temperature was lowered to –40°C. Thus, –40°C initial oven temperature was used for trapping both compounds.

**Validation of the Method**

Figure 2 shows gas chromatograms for nonextracted authentic n-hexane and cyclohexane (40 ng each on-column) dissolved in methanol and for headspace extracts from 0.5 mL whole blood or urine, to which n-hexane and cyclohexane (80 ng each) had been added. Both compounds were well separated from each other and gave sharp peaks under our GC conditions (Figure 2, top panels). The retention times for n-hexane and cyclohexane were 7.3 and 8.5 min, respectively. The backgrounds gave small impurity peaks; no interfering peaks appeared around the test peaks (Figure 2, bottom panels).

The extraction efficiencies and their coefficients of within-day variation measured by the present method for n-hexane and cyclohexane in human whole blood and urine samples are presented in Table 1. The extraction efficiencies were calculated by comparing the peak areas obtained from the extracts of spiked whole blood and urine samples with those obtained from the nonextracted authentic compounds dissolved in methanol. For whole blood, the extraction efficiencies of n-hexane were 14.9–30.3%, and those of cyclohexane were 13.2–22.8%. For urine, those of n-hexane were 13.5–20.7%, and those of cyclohexane were 12.7–15.5%. The coefficients of within-day variation in terms of extraction efficiencies for both compounds in whole blood and urine samples were 5.0–9.5 and 3.8–10.8%, respectively. The coefficients of day-to-day variation for both compounds were 4.4–16.6% for whole blood and 5.6–15.9% for urine (n = 10).

The regression equations for n-hexane and cyclohexane in whole blood and urine were drawn by plotting 9 concentrations with 80 ng IS (for measurements of n-hexane, cyclohexane was used as IS, and vice versa). They showed good linearity in the range of 5–500 ng/0.5 mL for both whole blood and urine. For whole blood, the equations and r values for the curves were: y = 0.0107x + 0.0941 and r = 0.9995 for n-hexane; y = 0.0132x + 0.1357 and r = 0.9997 for cyclohexane. For urine, the equations and r values were: y = 0.0103x + 0.0541 and r = 0.9953 for n-hexane; y = 0.0138x – 0.0330 and...
Figure 1. Headspace capillary GC–FID for n-hexane and cyclohexane as a function of various initial oven temperatures. n-Hexane and cyclohexane (80 ng each) were added to a 7.5 mL vial containing 0.5 mL whole blood and 0.5 mL distilled water. Scales of Y–axes were the same for each panel. Peaks: 1, n-hexane; 2, cyclohexane.

Figure 2. Headspace capillary GC–FID chromatograms with cryogenic oven trapping at –40°C for n-hexane and cyclohexane after headspace extraction from human whole blood and urine. The amounts of the compounds and conditions were the same as in Figure 1. The amount of the authentic compounds directly injected into the gas chromatograph was 40 ng each on-column. Scales of Y–axes were the same for each panel. Key numbers are the same as specified in Figure 1.
$r = 0.9981$ for cyclohexane. The detection limits (signal-to-noise ratio = 3) for both compounds were 1.2 ng/0.5 mL for whole blood and 0.5 ng/0.5 mL for urine. Yasugi et al. (4) reported that the detection limit of cyclohexane in whole blood was 100 ng/mL by headspace GC–FID with a DB-WAX wide-bore capillary column. Nomeir et al. (14) reported that the detection limits of $n$-hexane and cyclohexane obtained by GC–FID with an OV-101 capillary glass column were 50 ng on-column. Thus, the detection limits for $n$-hexane and cyclohexane obtained by the present method are approximately 50–100 times lower than those by the conventional GC methods (4, 14).

**Application of the Method to Rat Blood after Inhalation**

In addition to the above spiked human body fluids, we measured the levels of $n$-hexane or cyclohexane in rat whole blood after inhalation of each compound (Figure 3). With the

![Figure 3. Headspace capillary GC-FID chromatograms with cryogenic oven trapping for $n$-hexane (A) and cyclohexane (B) rat whole blood 30 min after inhalation of each compound (for details of conditions, see text). Key numbers are the same as specified in Figure 1.](image-url)
present method, \( n \)-hexane or cyclohexane was detected in rat whole blood on gas chromatograms.

To confirm that the peaks detected from rat whole blood by this method are \( n \)-hexane and cyclohexane, we recorded PIEI mass spectra for each peak. Molecular peaks at \( m/z \) 86 and 84 for the authentic \( n \)-hexane and cyclohexane, respectively, were observed in the spectra. There were fragment ions at \( m/z \) 57, 43, 41 (base peak), and 29 for \( n \)-hexane, and at \( m/z \) 69, 56 (base peak), 41, and 39 for cyclohexane. These spectra obtained from the extracts of the rat blood were almost identical to those of the authentic compounds, confirming their identities.

The levels of \( n \)-hexane and cyclohexane in rat blood after 30 min of exposure were 63.8–25.1 and 90.3–20.0 ng/0.5 mL, respectively (mean ± SD, \( n = 5 \) for both compounds).

**Conclusion**

To our knowledge, this is the first report on detecting \( n \)-hexane and cyclohexane from biological samples using GC with cryogenic oven trapping. In view of its simplicity, high sensitivity, and excellent quantitativeness, the present method seems useful and should, thus, gain popularity in the fields of environmental, forensic, and clinical toxicology.

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**References**