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原　　報

SENSITIVE DETERMINATION OF TRICHLOROETHYLENE IN BLOOD AND URINE BY CAPILLARY GAS CHROMATOGRAPHY WITH CRYOGENIC TRAPPING

Kanako WATANABE[a]*, Hiroshi SENO[a], Akira ISHII[a], Takeshi KUMAZAWA[b], Hideki HATTORI[c] and Osamu SUZUKI[a]

[a] *Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431−31, Japan*
[b] *Department of Legal Medicine, Showa University School of Medicine, 1−5−8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan*
[c] *Department of Legal Medicine, Aichi Medical University, Nagakute-cho, Aichi-gun, Aichi 480−11, Japan*

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低温トラッピングキャピラリーガスクロマトグラフィーによる血中尿中トリクロロエチレンの高感度定量

渡部加奈子[a]，妹尾　洋[a]，石井　晃[a]，熊澤武志[b]，服部秀樹[c]，鈴木　修[a]

[a]浜松医科大学法医学教室　〒431−31 静岡県浜松市半田町3600番地
[b]昭和大学医学部法医学教室　〒142 東京都品川区旗の台１－５－８
[c]愛知医科大学法医学教室　〒480−11 愛知県愛知郡長久手町大字岩作字雁又21番地

Summary

A new and sensitive method for measurements of trichloroethylene in blood and urine by capillary gas chromatography (GC) with cryogenic trapping is presented. After heating a body fluid samples (1 ml) containing trichloroethylene and tetrachloroethylene (internal standard, IS) in a 7.0-ml vial at 55 ℃ for 20 min, 5 ml of the headspace vapor was drawn into a glass syringe. All vapor was introduced into an Rtx-Volatiles middle-bore capillary column in the splitless mode at 0 ℃ of oven temperature to trap the entire analytes, and the oven temperature was programmed up to 270 ℃. The present conditions gave sharp peaks for both trichloroethylene and tetrachloroethylene (IS), and low background noises for whole blood and urine samples. As much as 75.0 and 63.1 % of trichloroethylene and IS, respectively, which had been added to whole blood in a vial, could be introduced into the GC column. The calibration curves showed linearity in the range of 0.5−5.0 μg/ml for both whole blood and urine. The detection limit was as low as about 3 ng/ml of whole blood.

Key words: Trichloroethylene; Tetrachloroethylene; Gas chromatography; Cryogenic trapping; Headspace method

*Correspondence should be addressed to Kanako Watanabe.
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Introduction

Trichloroethylene is widely used as an industrial degreaser, extraction solvent and dry cleaning solvent. Death cases due to trichloroethylene poisoning were reported [1, 2]. The solvent may be abused, because it produces euphoria. Its exposure for industrial workers is also a problem from a hygienic point of view. There have been some reports dealing with analysis of trichloroethylene by gas chromatography (GC) with headspace methods [1–4]; electron capture detection (ECD) was employed to enhance sensitivity [3, 4]. In the present paper, we report a new method for determination of tyrichloroethylene by capillary GC with the conventional flame ionization detection (FID), in which cryogenic trapping is employed for headspace samples; it gave sensitivity comparable to or even higher than that with ECD.

Experimental

Materials

Trichloroethylene and tetrachloroethylene (IS) were of reagent grade purchased from Wako Pure Chemical Industries, Ltd.(Osaka). Human blood and urine were obtained from healthy subjects.

Procedure

Stock solutions (500 μg/ml) of trichloroethylene or IS were prepared by dissolving them in methanol. To a 7.0-ml screw-cap vial containing 1.0 ml of body fluid (whole blood or urine), was added 10 μl of methanolic solution containing 5 μg or less of trichloroethylene and IS. The vial was rapidly sealed with a silicone-septum cap and heated at 55 ℃ on an aluminum block heater. After heating it for 20 min, a 24 G needle of a glass syringe (5-ml volume) 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 0 ℃ of oven temperature.

GC conditions

GC analyses were carried out on an HP 6890 Series gas chromatograph equipped with FID and with a cryogenic oven temperature device with liquid CO_2 (Hewlett-Packard Co., Palo Alto, CA, USA). The GC column used was an Rtx-Volatiles fused silica capillary column (30 m × 0.32 mm i.d., film thickness 1.5 μm, Restek Corporation, Bellefonte, PA, USA). The GC conditions were: column temperature 0 to 270 ℃ (1 min hold at 0 ℃, 10 ℃/min from 0 to 150 ℃, 20 ℃/ min from 150 to 270 ℃); injection temperature 250 ℃; detection temperature 270 ℃; and helium flow rate 3 ml/min. The vapor samples were injected in the splitless mode and the splitter was opened 1 min after completion of the injection.

Results

We tested various initial oven temperatures for trapping trichloroethylene and IS vapor as shown in Fig.1. At 30 ℃, the peaks of both compounds were low and broad especially for trichloroethylene. They became higher and sharper by lowering the oven temperature down to 0 ℃; thus we adopted the initial oven temperature of 0 ℃.

Figure 2 shows gas chromatograms for non-extracted authentic trichloroethylene and IS (5 μg on-column) and for headspace extracts from 1.0 ml whole blood or urine, to which 5 μg each of trichloroethylene and IS had been added. Both compounds were well separated and gave sharp peaks. The backgrounds in the absence of the compounds were clean (Fig. 2, right panels).

The net recovery of trichloroethylene and IS was determined. Peak areas of blood or urine spiked with 5 μg of each compound (after cryogenic trapping of the headspace prior to GC analysis) were compared with peak areas obtained by direct GC injection of the authentic

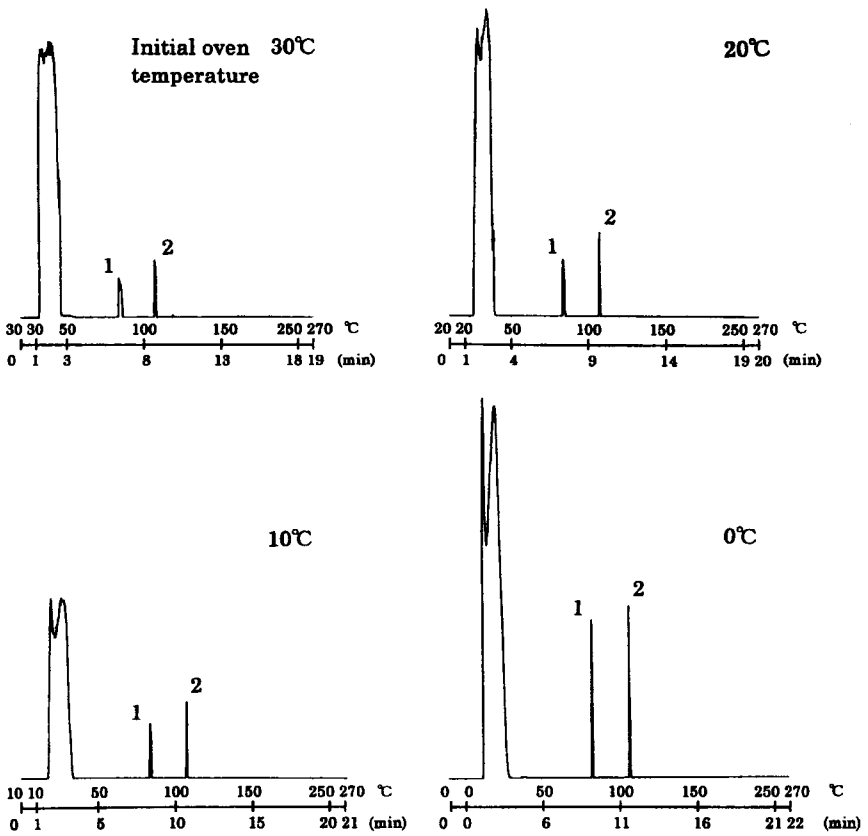

Fig. 1. Headspace capillary GC for trichloroethylene (1) and tetrachloroethylene (IS) (2) as a function of various initial oven temperatures. Five micrograms of each compound were added to 1.0 ml human whole blood for headspace extraction.

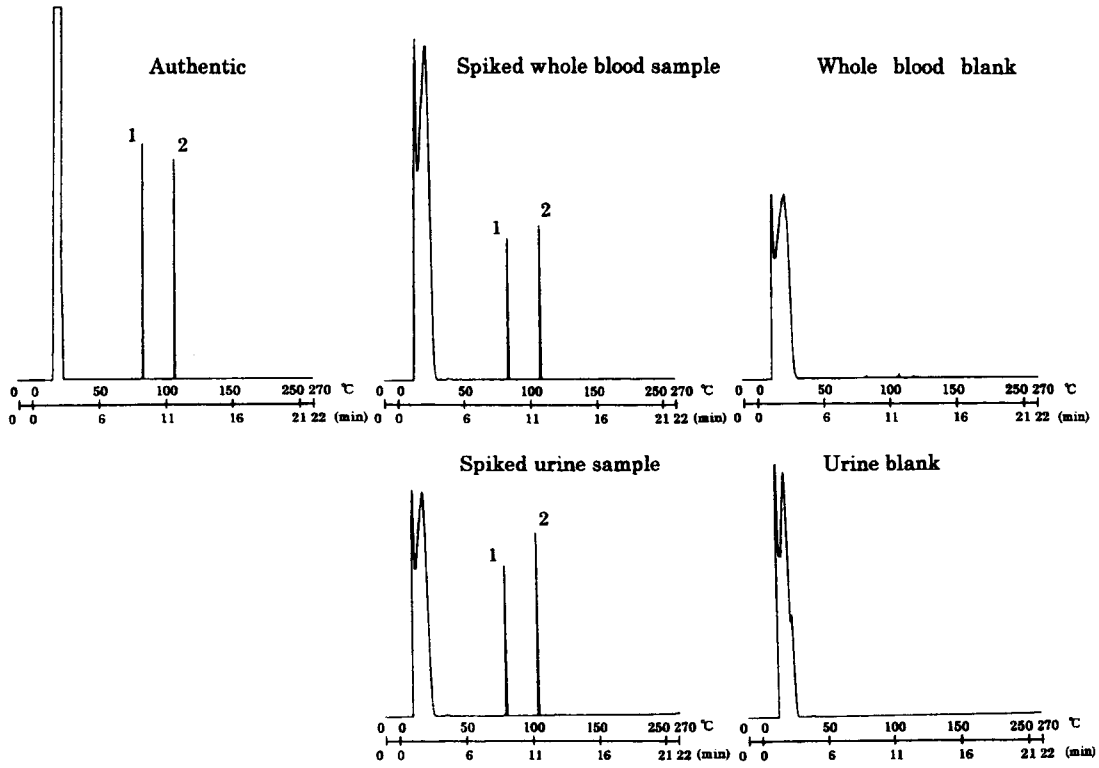

Fig. 2. Capillary GC chromatograms with cryogenic trapping at 0 ℃ for the authentic trichloroethylene (1) and tetrachloroethylene as IS (2) with direct injection (left top panel), for whole blood or urine spiked with 5 μg of each compound in 1.0 ml (middle panels) and for whole blood or urine in the absence of the compounds (right panels).

compounds. It was 75.0±9.48 % (mean±SD, n=6, CV=12.6 %) for trichloroethylene and 63.1±8.39 % (n=6, CV=13.3 %) for tetrachloroethylene (IS) in whole blood; 77.2±9.70 % (n=7, CV=12.5%) for trichloroethylene and 79.5±9.70 % (n=7, CV=12.2 %) for IS in urine.

Calibration curves for trichloroethylene in human whole blood and urine were drawn by plotting six concentrations against 5 μg of IS. They were linear in the range of 0.5−5.0 μg/ml for both whole blood and urine. The equations and r values for the curves were: $y = 0.268x - 0.0202$ and $r = 0.996$ for whole blood; $y = 0.233x - 0.0322$ and $r = 0.996$ for urine.

Figure 3 shows gas chromatograms obtained from headspace extracts of human whole blood in the presence and absence of 0.01 μg of trichloroethylene and 5 μg of tetrachloroethylene (IS). As can be seen in the figure, 0.01 μg of trichloroethylene gave a clean peak with a signal-to-noise ratio of about 10. The detection limit (signal-to-noise ratio=3) of trichloroethylene in whole blood was estimated to be 3 ng/ml.

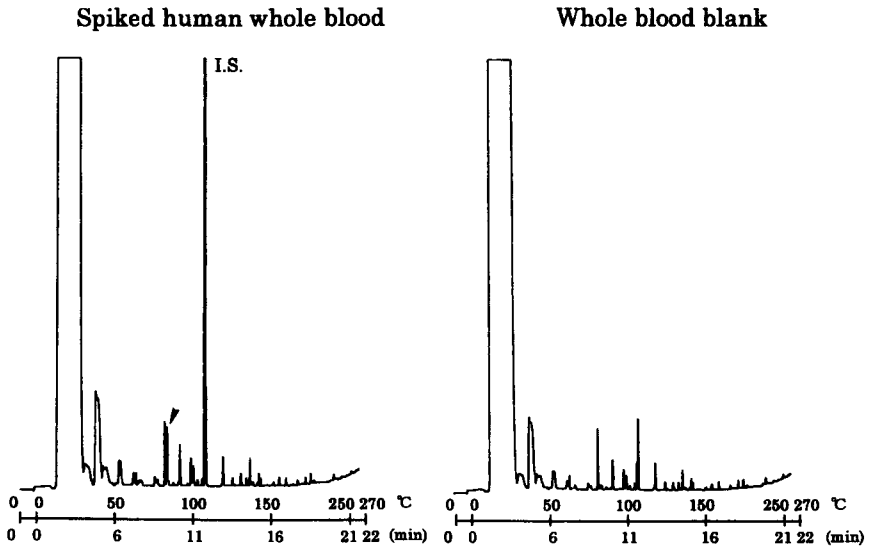

Fig. 3. Headspace capillary GC with cryogenic trapping at 0 ℃ for human whole blood (1.0 ml) in the presence and absence of 0.01 μg of trichloroethylene and 5 μg of tetrachloroethylene (IS). The arrow shows the peak of 0.01 μg trichloroethylene.

Discussion

Purge and trap sample concentration followed by capillary GC is probably the most sensitive technique to detect volatile organic compounds from a large volume of water or solid samples [5]. However, this technique is not suitable for biological samples, such as blood and tissue homogenates, because it causes serious bubbling. Recently, a microcomputer-controlled devise for cooling of oven temperature down to or below 0 ℃ has become available for new types of GC instruments. It was originally designed for rapid cooling of oven temperature to reduce time of analysis. In this study, we have used it for trapping volatile compounds inside a middle-bore capillary column at a low oven temperature; as much as 5-ml gas can be injected into the column without any loss, which results in much higher sensitivity. This method is simple and requires no special GC operations.

The detection limit for trichloroethylene in blood or urine given by GC-ECD with a middle-bore capillary column and with split injection was reported to be about 10 ng/ml [3]. The present GC-FID method with cryogenic trapping gave the detection limit of 3 ng/ml. Applicability of large volumes of gas and high net recoveries from whole blood (75.0 %) and urine (77.2 %) contributed to the high sensitivity.

We have used tetrachloroethylene as IS. This compound is also an object of interest in forensic toxicology, and hygienic and environmental sciences. Tetrachloroethylene can be measured by the present method with trichroloethylene as IS, conversely.

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