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Jpn. J. Forensic Toxicol. Vol.16, No.1, 1998

原　　報

SENSITIVE DETERMINATION OF ETHYL ETHER IN WHOLE BLOOD BY CAPILLARY GAS CHROMATOGRAPHY WITH CRYOGENIC TRAPPING

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Received December 9, 1997
Accepted December 18, 1997

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エチルエーテルの高感度定量

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Summary

A new and sensitive method for measurements of ethyl ether in whole blood by capillary gas chromatography (GC) with cryogenic trapping is presented. After heating a body fluid sample (1 ml) containing ethyl ether and propyl ether (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 −50 ℃ of oven temperature to trap the entire analytes, and the oven temperature was programmed up to 260 ℃. The present conditions gave sharp peaks for both ethyl ether and propyl ether (IS), and low background noises for whole blood samples. As much as 53.9 and 38.3 % of ethyl ether 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 whole blood. The detection limit was as low as about 10 ng/ml whole blood.

Key words: Ethyl ether; Propyl ether; Gas chromatography; Cryogenic trapping; Headspace method

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 0915−9606/98/$08.00

Introduction

Ethyl ether is widely used as an extraction solvent or a reagent in industries and laboratories. Many years ago, ethyl ether was the ideal first-choice anesthetic, although it is rarely used today. Death cases due to ethyl ether poisoning were reported [1]. Its exposure for industrial workers is also a problem from a hygienic point of view. Analysis of ethyl ether by gas chromatography (GC) with mass spectrometry (MS) was reported [2]. In the present paper, we report a new method for determination of ethyl ether by capillary GC with the conventional flame ionization detection (FID), in which cryogenic trapping is employed for headspace samples; it gave high sensitivity and good separation.

Experimental

Materials

Ethyl ether was of reagent grade purchased from Wako Pure Chemical Industries, Ltd. (Osaka), and propyl ether from Tokyo Kasei Organic Chemicals(Tokyo). Human blood was obtained from healthy subjects.

Procedure

Stock solutions (500 μg/ml) of ethyl ether or IS were prepared by dissolving them in methanol. To a 7.0-ml screw-cap vial containing 1.0 ml of whole blood, was added 10μl of methanolic solution containing 5 μg or less of ethyl ether and IS. The vial was rapidly sealed with a silicone-septum cap and heated at 60 ℃ on an aluminum block heater. After heating it for 15 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 −50 ℃ of oven temperature.

GC conditions

GC analyses were carried out on a Shimadzu GC-14A gas chromatograph equipped with FID and with a cryogenic oven temperature device with liquid CO_2. 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 −50 to 260 ℃ (1 min hold at −50 ℃, 10 ℃/min from −50 to 100 ℃, 20 ℃/ min from 100 to 260 ℃); injection temperature 230 ℃; detection temperature 260 ℃; and helium flow rate 3 ml/min. The vapor samples were injected in the splitless mode and the splitter was opened 1min after completion of the injection.

Results and discussion

We tested various initial oven temperatures for trapping ethyl ether and IS vapor as shown in Fig.1. At 0 ℃, the peaks of both compounds were low and broad especially for ethyl ether. They became higher and sharper by lowering the oven temperature down to −50 ℃; thus we adopted the initial oven temperature of −50 ℃

Figure 2 shows gas chromatograms for non-extracted authentic ethyl ether and IS (5 μg on-column) and for headspace extract from 1.0 ml whole blood, to which 5 μg each of ethyl ether and IS had been added. Both compounds were well separated and gave sharp peaks. The

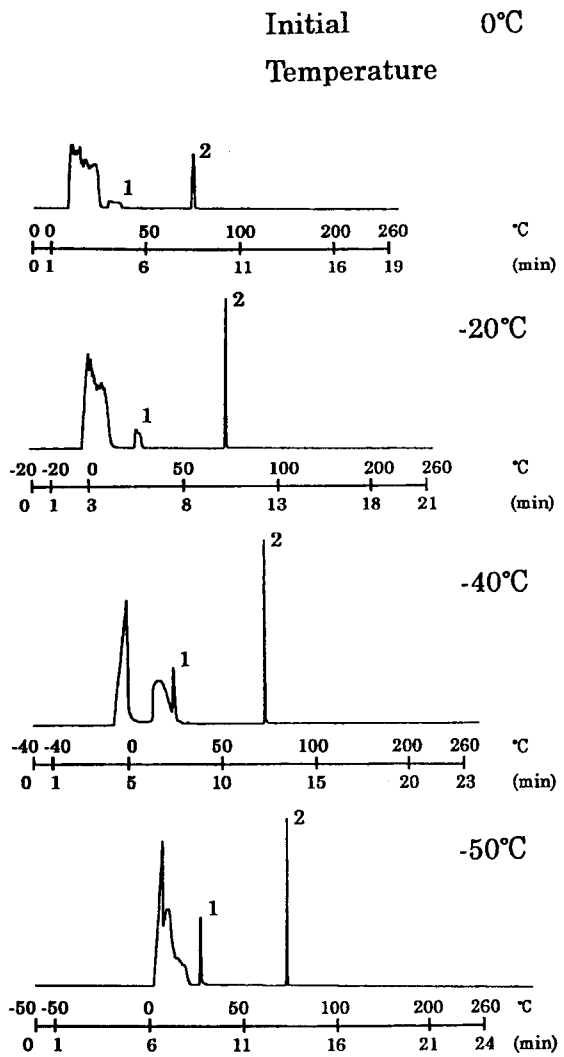

Fig. 1. Headspace capillary GC for ethyl ether (1) and propyl ether (IS) (2) as a function of various initial oven temperatures. Five micrograms of each compound were added to 1.0 ml human whole blood for headspace extraction.

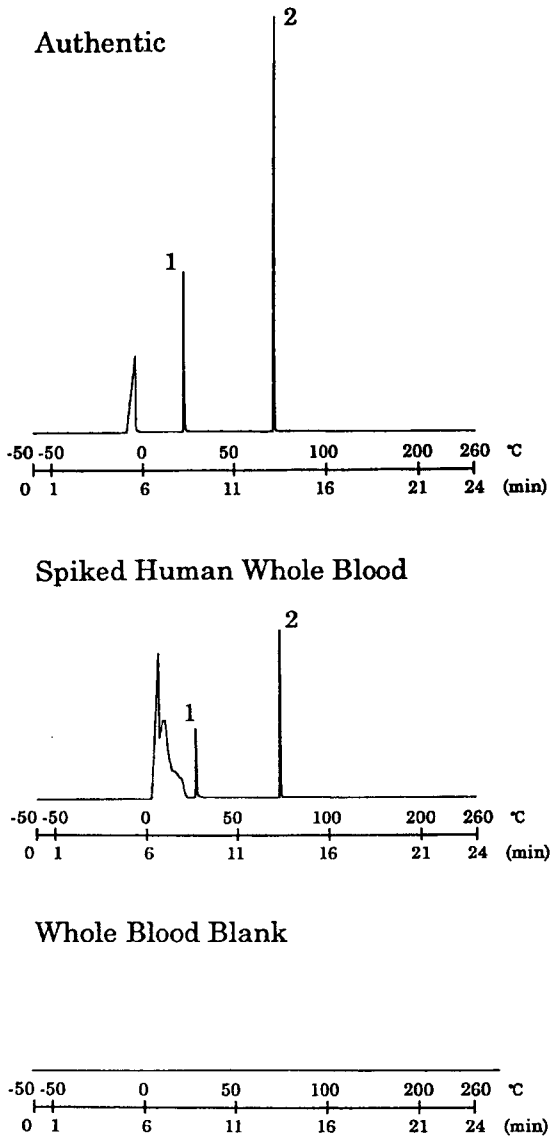

Fig. 2. Capillary GC chromatograms with cryogenic trapping at −50 ℃ for the authentic ethyl ether (1) and propyl ether as IS (2) with direct injection (top panel), for whole blood spiked with 5 μg of each compound in 1.0 ml (middle panel) and for whole blood in the absence of the compounds (bottom panel).

background of whole blood not spiked with the compounds was clean (Fig. 2, bottom panel).

The net recovery of ethyl ether and IS was determined. Peak areas of blood 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 compounds. It was 53.9±4.49 % (mean±SD, n=5, CV=8.32 %) for ethyl ether and 38.3±2.92 % (n=5, CV=7.63 %) for propyl ether (IS) in whole blood.

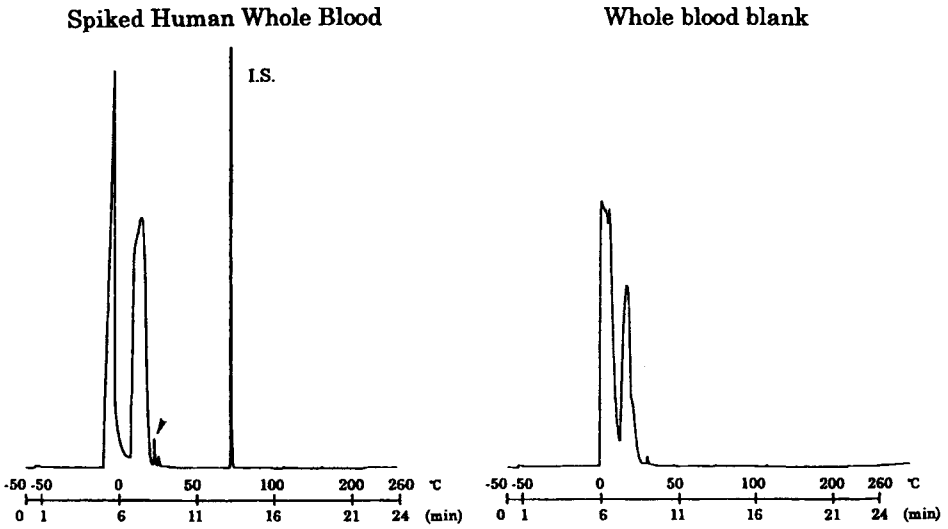

Fig. 3. Headspace capillary GC with cryogenic trapping at −50 ℃ for human whole blood (1.0 ml) in the presence and absence of 0.05 μg of ethyl ether and 5 μg of propyl ether (IS). The arrow shows the peak of 0.05 μlg propyl ether.

A calibration curve for ethyl ether in human whole blood was drawn by plotting five concentrations against 5 μg of IS. It was linear in the range of 0.5−5.0 μg/ml. The equation and r value for the curve were: $y=0.613x-0.0667$ and $r=0.985$.

Figure 3 shows gas chromatograms obtained from headspace extracts of human whole blood in the presence and absence of 0.05 μg of ethyl ether and 5 μg of propyl ether (IS). As can be seen in the figure, 0.05 μg of ethyl ether gave a peak with a signal-to-noise ratio of more than 10. The detection limit (signal-to-noise ratio=3) of ethyl ether in whole blood was estimated to be 10 ng/ml.

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 our previous studies, we reported methods for sensitive determination of chloroform, methylene chloride [3] and trichloroethylene [4] in human body fluids by capillary GC with cryogenic trapping using the above devise. In this study, we have extended this line of experiments to ethyl ether in human whole blood.

The minimum detectable concentration of ethyl ether in lung tissue given by GC/MS with a middle-bore capillary column and with split injection was reported to be about 1 μg/ml [2, 5]. The present GC-FID method with cryogenic trapping gave a much lower detection limit (10 ng/ml). Applicability of large volumes of gas and high net recoveries from whole blood (53.9 %) contributed to such high sensitivity.

This method with cryogenic trapping is recommendable for use in forensic and

environmental toxicology, because it is simple and requires no special GC operation, and gives high sensitivity and good separation of test peaks.

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