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原　　報

SENSITIVE DETECTION OF ETHANOL IN HUMAN BREATH BY HEADSPACE CAPILLARY GAS CHROMATOGRAPHY WITH CRYOGENIC OVEN TRAPPING

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低温オーブン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呼気中エタノールの高感度検出

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Summary

A sensitive method for determination of ethanol in human breath by headspace capillary gas chromatography (GC) with cryogenic oven trapping is presented. After sampling the breath expired from healthy subjects, who drank common beer, 5 ml of the gas was drawn into a glass syringe. All vapor was introduced into an Rtx-BAC2 wide-bore capillary column in the splittless mode at −60℃ of oven temperature to trap the entire analyte, and the oven temperature was programmed up to 240℃ for GC measurements with flame ionization detection. The present conditions gave sharp peaks of ethanol, and low background noises for breath samples. The external calibration curves showed linearity in the range of 25−125 ng on column. The detection limit was estimated to be about 3 ng on column. The concentrations of ethanol in breath expired from ten healthy subjects, who drank 200 ml beer 30 and 60 min before the samplings, were 30.0±18.1 and 15.8±6.72 ng/ml gas, respectively.

Key words: Ethanol; Alcohol; Breath; Gas chromatography ;Cryogenic oven trapping; Low temperature

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Introduction

Analysis of ethanol in breath is useful to prove drinking, because it is non-invasive and reflects blood ethanol concentrations. Since ethanol concentration in breath expired is about two-thousand times as low as that in blood with drinking, more than 500 ml of gas should be used for its analyses by chromatography (GC) or GC/mass spectrometry(MS) [1−3]. Recently, we have established a new analytical method for volatile compounds in body fluids by capillary GC with the conventional flame ionization (FID), in which cryogenic oven trapping is employed for headspace samples [4−6]. Our method gives high sensitivity with very sharp peaks and low background noises, and requires no special GC operation. This paper provides data for measurement of ethanol in human breath by this method.

Experimental

Materials

Ethanol and methanol were obtained from Wako Pure Chemical Industries (Osaka); an Rtx-BAC2 fused silica wide-bore capillary column (30 m×0.53 mm i.d., film thickness 2.0 μm) from Restek Corp. (Bellefonte, PA, USA). Human breath gas was obtained from healthy volunteers.

Procedure

Healthy subjects of both sexes drank 200 ml of common beer (about 5% ethanol) and then washed their mouths well; the breath expired from them was collected in plastic bags 30 or 60 min after the drinking. Immediately after the samplings, 5 ml each of the gas was drawn into a glass syringe, and injected into GC port in the splittless mode at −60℃ of oven temperature. External calibration was made for quantitation of breath ethanol by injecting different amounts (25−125 ng, 4 concentrations) of ethanol dissolved in methanol into GC directly, without use of any internal standard.

GC conditions

GC analysis was carried out on an HP 6890 series gas chromatograph equipped with FID and a cryogenic oven temperature device (Hewlett−Packard Co., Palo Alto, CA, USA). An electrically operated solenoid valve introduces liquid carbon dioxide at a rate appropriate to cool the oven to a desired temperature. The GC conditions were: column temperature −60 to 240℃ (1 min hold at −60℃, 10℃/ min from −60 to 40℃, 10 min hold at 40℃, and 20℃/min from 40 to 240℃); injection temperature 200℃; detection temperature 240℃; and helium flow rate 3 ml/ min. The vapor was injected in the splitless mode, and the splitter was opened 1

min after completion of the injection.

Results

We tested various initial oven temperatures at −20, −30, −40, −50 and −60℃ for trapping ethanol vapor. At −20℃, the peak of ethanol was broad and overlapped an impurity peak; with lowering the initial oven temperature down to −60℃, the ethanol peak became sharper and more separated from the impurity peak. Thus we adopted −60℃ of initial oven temperature.

We tested various capillary GC columns, such as DB-WAX, GS-Q, Rtx-Volatiles and Rtx-BAC2. The Rtx-BAC2 column gave excellent symmetrical peaks of ethanol and good separation from impurity peaks. For other columns, remarkable tailing, very early appearance and missing of ethanol peaks were observed. Thus, we have used the Rtx-BAC2 column in this study.

External calibration curve was drawn by plotting 4 concentrations of ethanol dissolved in methanol into GC directly. It was linear in the range of 25−125 ng on column. The equation and the r value for the curve were: $y = 395x - 4.60$ and $r = 0.989$. The detection limit was estimated to be about 3 ng on column.

Figure 1 shows chromatograms for ethanol in breath of a healthy subject, who drank 200 ml of beer 30 and 60 min before the gas samplings. The concentrations for each subject are presented in Table 1. The mean concentrations were 30.0±18.1 and 15.8±6.72 ng/ml gas (mean±SD, n=10) 30 and 60 min after drinking, respectively.

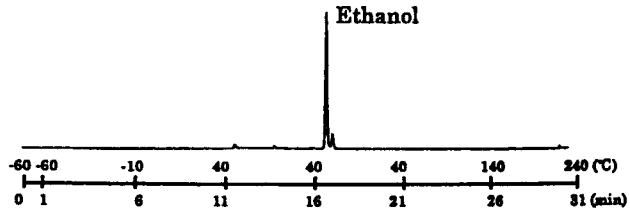
Discussion

In the present study, we have established a detailed procedure for a sensitive headspace GC-FID method for analysis of ethanol in human breath using cryogenic oven trapping.

Recently, a microcomputer-controlled device for lowering oven temperature below 0℃ has become available for new types of gas chromatographs. This device was originally designed for rapid cooling of an oven to reduce the time for analysis. In this study, we have used it for trapping ethanol gas inside a capillary column at cryogenic oven temperature; as much as 5 ml of breath gas can be injected into the column without any loss, causing much higher sensitivity. This is the first report dealing with GC with cryogenic oven trapping for ethanol in breath.

We have measured ethanol concentrations in breath expired from healthy subjects, who drank 200 ml of beer (Table 1). It is widely accepted that breath ethanol concentrations reflect blood ethanol levels; thus the breath is useful as a non-invasive material for detection of

(A) 30 min after drinking



(B) 60 min after drinking

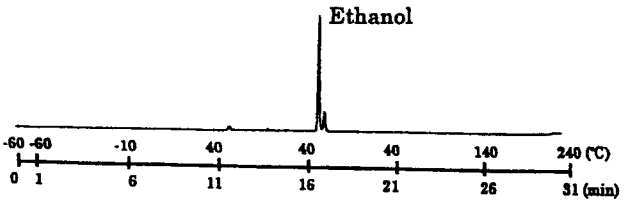


Fig. 1. Capillary GC chromatograms with cryogenic oven trapping for ethanol in breath of a healthy volunteer (individual No.5), who drank 200 ml beer 30 or 60 min before the samplings of the breath.

Table 1. Concentrations of ethanol in breath expired from subjects 30 and 60 min after drinking 200 ml of beer

Individual No.* (sex, age)	Concentration (ng/ml gas)	
	30 min after drinking	60 min after drinking
No.1 (F,29)	30.8	19.6
No.2 (M,23)	9.62	6.23
No.3 (F,23)	73.5	18.0
No.4 (F,24)	24.8	13.0
No.5 (F,20)	34.0	29.0
No.6 (M,24)	18.5	8.94
No.7 (M,35)	20.2	10.4
No.8 (F,22)	45.2	21.8
No.9 (M,23)	19.9	16.3
No.10 (M,36)	23.9	14.9
Mean± SD	30.0±18.1	15.8±6.72

ethanol without blood collection [1−3]. The 5-ml volume of the breath gas was sufficient to give intense peaks of ethanol (Fig.1). Our method is useful for measurements of ethanol in breath expired from subjects who have drunk relatively small amounts of alcohol.

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