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Determination of solvent thinner components in human body fluids by capillary gas chromatography with trapping at low oven temperature for headspace samples

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A simple and sensitive method is presented for determination of solvent thinner components in human body fluids by capillary gas chromatography (GC) with a low oven temperature for trapping headspace vapor components. After heating a blood or urine sample containing ethyl acetate, benzene, butan-1-ol, toluene, butyl acetate, isoamyl acetate and ethylbenzene (internal standard) in a 7.5 ml vial at 90 °C for 30 min, 5 ml of headspace vapor were drawn into a glass syringe. All vapor was introduced through an injection port in the splitless mode into a DB-624 medium-bore capillary column at a 5 °C oven temperature for trapping the volatile compounds, and the oven temperature was programmed up to 110 °C for their detection by GC. These conditions gave sharp peaks, a good separation of each peak and low background noise for both whole blood and urine samples. As much as 3.58–55.1 and 3.52–57.9% of the six compounds, which had been added to vials, could be introduced to the GC instrument for whole blood and urine, respectively. The intra-day RSD values in terms of the introduction rate (net recovery) of the six compounds in whole blood and urine samples were ⩽8.1%. The calibration curves showed linearity in the range 0.78–400 ng per 0.5 ml whole blood or urine. The detection limits were 0.5–5 ng per 0.5 ml. The data on toluene in *post mortem* blood in an actual case are also presented.

Keywords: *Solvent abuse; whole blood and urine analysis; headspace analysis; low-temperature oven trap; capillary gas chromatography*

Solvent (thinner) abuse, especially by the young generation, is now causing a serious social problem. Death due to asphyxia secondary to solvent sniffing is frequently encountered in forensic science practice.[1] Exposure to solvent vapor for paint workers is also a problem from a hygienic point of view.[2] Commonly used thinners contain 50–70% toluene, 10–20% ethyl acetate, 5–30% butyl acetate and minor proportions of other solvents; the composition differs according to different purposes or different manufacturers. There have been many reports dealing with analyses of thinner components by gas chromatography (GC) with the headspace method.[3–14] In most of these reports, conventional packed columns, which give relatively low sensitivity and poor separation, were used.[4,5,7,9,10] With wide-bore capillary columns, only a 0.1–0.5 ml volume of the headspace vapor can be injected;[8,12] with medium-bore capillary columns, split injection has to be used.[6,11]

In this paper, we describe a new technique which allows us to introduce as much as 5 ml of the headspace vapor of thinner components with splitless injection into a medium-bore capillary column by use of a low oven temperature. This means that a 10–50 times higher sensitivity can be obtained by this method compared with that by previous methods.[3–14]

Experimental

Materials

Ethyl acetate, benzene, butan-1-ol, toluene, butyl acetate, isoamyl acetate and ethylbenzene as internal standard (IS) were obtained from Wako (Osaka, Japan). A DB-624 fused-silica medium-bore capillary column (30 m × 0.32 mm id, film thickness 1.80 μm) was purchased from J & W Scientific (Folsom, CA, USA). Other chemicals were of analytical-reagent grade. Whole blood and urine were obtained from healthy subjects with their consent, except those obtained in a *post mortem* case. The blood was drawn into a sterilized glass tube containing heparin sodium.

Procedure

Stock standard solutions (20 μg ml^{-1}) of ethyl acetate, benzene, butan-1-ol, toluene, butyl acetate, isoamyl acetate and ethylbenzene (IS) were prepared in acetone. To a 7.5 ml vial containing 0.5 ml of whole blood or urine, which had been spiked with the above standards and IS (100 ng each), 1.5 ml of distilled water and a magnetic stirring bar were added. The vials were rapidly sealed with a silicone-rubber septum cap and heated at 90 °C with stirring on an aluminum block heater (React-Therm Heating/Stirring Model; Pierce, Rockford, IL, USA). After heating for 30 min, a 23 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 a 5 °C oven temperature.

For analysis of thinner components in whole blood from an actual case, 25 μl of the *post mortem* blood was diluted with 475 μl of blank whole blood obtained from healthy subjects, because of the high concentrations of compounds to be determined. After adding 100 ng of IS, the procedure was the same as described above.

GC conditions

GC analyses were carried out on an HP 6890 Series gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) and a GC-14B gas chromatograph (Shimadzu, Kyoto, Japan), both equipped with flame ionization detection (FID) and with a low oven temperature device in which an electrically operated solenoid valve introduces liquid carbon dioxide at a rate appropriate to cooling the oven to desired temperatures. The GC conditions for both instruments were helium flow rate 5 ml min^{-1}, column temperature 5–100 °C (1 min hold at 5 °C,

20 °C min^{-1} from 5 to 100 °C, 3 °C min^{-1} from 100 to 110 °C), injection temperature 200 °C and detector temperature 230 °C. The samples were injected in the splitless mode and the splitter was opened after 2 min.

Case

In June 1996, a 20-year-old male was found dead hanging at his apartment. The *post mortem* interval was estimated to be about 5 h. Plastic bags containing a thinner-like fluid were found in his room. Cardiac blood sample was drawn and stored frozen at −40 °C until assay.

Results

Various conditions for headspace extraction of the present compounds were tested. More than 90% of the maximum levels had already been attained after heating at 80 °C for 15 min. We heated the vials at 90 °C for 30 min to ensure their maximum extraction into the headspace vapor. The effects of salt addition were also checked; neither addition of NaCl nor $(NH_4)_2SO_4$ (0.8 g solid each) to 2.0 ml of solution containing 0.5 ml of whole blood or urine was effective. Therefore, we did not add any salt to the blood sample mixtures.

We tested various oven temperatures (30–0 °C) for trapping headspace components; the maximum peak height or area was obtained at about a 5 °C oven temperature for each compound.

Fig. 1 shows gas chromatograms for non-extracted authentic thinner components and IS (25 ng each on column) dissolved in acetone and for headspace extracts from 0.5 ml of whole blood or urine, to which the six thinner compounds and IS (100 ng each) had been added. All compounds were well separated from each other and gave sharp peaks under our GC conditions (Fig. 1, top). The blank chromatograms gave very small impurity peaks; no interfering peaks appeared around the test peaks (Fig. 1, bottom).

The net recoveries and their intra-day RSDs measured by the present method for six thinner components in whole blood and urine samples from six different individuals are presented in Table 1. The recoveries were calculated by comparing the peak areas obtained from the extracts of the spiked whole blood and urine samples [100 ng (0.5 ml)$^{-1}$] with those obtained from the non-extracted authentic thinner components (100 ng each on-column) dissolved in acetone. The recoveries were 3.58–55.1% for whole blood and 3.52–57.9% for urine. The RSDs were satisfactory and ⩽8.1% for both whole blood and urine samples.

Tables 2 and 3 give data from the calibration curves for thinner components in whole blood and urine samples, respectively. The data were subjected to linear regression analysis of peak area ratios of compound to the IS (100 ng) against the spiking concentrations. All compounds showed good linearity for whole blood and urine samples. The detection limits (signal-to-noise ratio = 3) were 0.5–1.0 ng per 0.5 ml for whole blood and urine, except for butan-1-ol (about 5 ng per 0.5 ml).

Fig. 2 shows the headspace capillary gas chromatogram for an extract of *post mortem* whole blood from a 20-year-old male in an actual case, to which 100 ng ethylbenzene as IS had been added. A large peak of toluene was detected on the chromatogram; its level was 6.23 μg ml^{-1}.

Discussion

Recently, a microcomputer-controlled device for lowering the oven temperature to ⩽0 °C has become available for new types of gas chromatographs. This device was originally designed for rapid cooling of the oven to reduce the time of analysis. In this study, we used it for trapping volatile compounds inside a

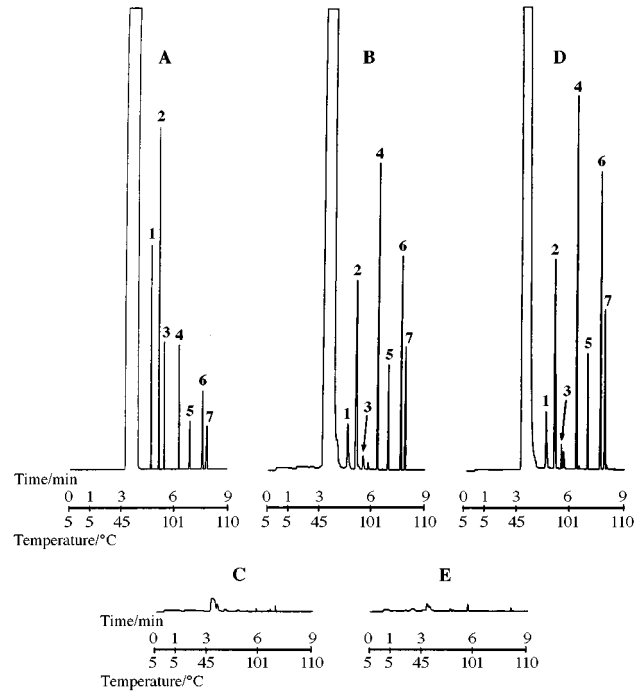

Fig. 1 Headspace capillary GC with low oven temperature for trapping six thinner compounds extracted from human whole blood and urine. The GC analyses were carried out on an HP 6890 Series gas chromatograph. A, the authentic thinner compounds and IS (25 ng each on column) without extraction; B, an extract of whole blood spiked with the six thinner compounds and the IS; C, a blank extract of whole blood; D, an extract of urine spiked with the six thinner compounds and the IS; and E, a blank extract of urine. Peaks: 1, ethyl acetate; 2, benzene; 3, butan-1-ol; 4, toluene; 5, butyl acetate; 6, ethylbenzene (IS); and 7, isoamyl acetate. The mixture of six compounds and IS was added to 0.5 ml of whole blood and urine. The vertical scale in A is not the same as that in B and D. For exact comparison of net recoveries, see Table 1.

Table 1 Net recoveries and RSDs of six thinner components from human whole blood and urine samples measured by the present method. Each compound (100 ng each) was added to 0.5 ml of human whole blood or urine samples. The values are means ± *s* obtained from six individual samples. The recoveries were calculated by comparing the peak area obtained from the extracts of the spiked whole blood and urine samples with that obtained from non-extracted authentic compounds dissolved in acetone

Sample	Ethyl acetate	Benzene	Butan-1-ol	Toluene	Butyl acetate	Isoamyl acetate
Whole blood—						
Mean recovery ± *s* (%)	16.9 ± 1.26	55.1 ± 3.77	3.58 ± 0.23	37.4 ± 2.18	23.2 ± 1.89	23.4 ± 1.19
RSD (%)	7.5	6.8	6.4	5.8	8.1	5.1
Urine—						
Mean recovery ± *s* (%)	18.5 ± 1.49	57.9 ± 1.06	3.52 ± 0.24	42.3 ± 1.03	22.7 ± 0.80	28.5 ± 1.08
RSD (%)	8.1	1.8	6.8	2.4	3.5	3.8

Table 2 Calibration curves for thinner components in human whole blood extracted and measured by the present method

Compound	Equation*	Correlation coefficient (r)	Concentration range/ ng per 0.5 ml	Detection limit/ ng per 0.5 ml
Ethyl acetate	$y = 0.0035x + 0.0064$	0.9994	1.56–400	1.0
Benzene	$y = 0.0188x + 0.0099$	0.9985	1.56–400	0.8
Butan-1-ol	$y = 0.0006x + 0.0026$	0.9981	6.25–400	5.0
Toluene	$y = 0.0123x - 0.0045$	0.9996	0.78–400	0.5
Butyl acetate	$y = 0.0033x + 0.0214$	0.9968	1.56–400	1.0
Isoamyl acetate	$y = 0.0034x - 0.0124$	0.9967	1.56–400	1.0

* The data were subjected to linear regression analysis of peak area ratios (y) of compound to the IS (100 ng) against the spiking concentrations (x). Nine plots with different concentrations of each compound were used.

Table 3 Calibration curves for thinner components in human urine extracted and measured by the present method

Compound	Equation*	Correlation coefficient (r)	Concentration range/ ng per 0.5 ml	Detection limit/ ng per 0.5 ml
Ethyl acetate	$y = 0.0034x + 0.0051$	0.9981	1.56–400	1.0
Benzene	$y = 0.0167x + 0.0245$	0.9992	1.56–400	0.8
Butan-1-ol	$y = 0.0005x + 0.0058$	0.9965	6.25–400	5.0
Toluene	$y = 0.0134x - 0.0043$	0.9997	0.78–400	0.5
Butyl acetate	$y = 0.0035x + 0.0147$	0.9986	1.56–400	1.0
Isoamyl acetate	$y = 0.0047x - 0.0043$	0.9987	1.56–400	1.0

* The data were subjected to linear regression analysis of peak area ratios (y) of compound to the IS (100 ng) against the spiking concentrations (x). Nine plots with different concentrations of each compound were used.

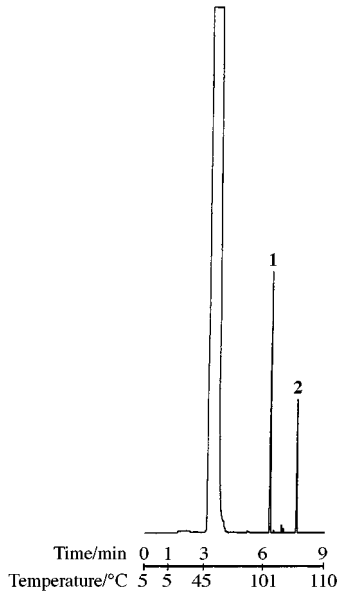

Fig. 2 Headspace capillary GC with low oven temperature for the extracts of *post mortem* whole blood (25 μl) from a 20-year-old male, to which 100 ng of IS had been added. The GC analyses were carried out on an HP 6890 Series gas chromatograph. Peaks: 1, toluene; 2, IS.

medium-bore capillary column at low oven temperature; as much as 5 ml of gas can be injected into the column without any loss. This method is simple and gives much higher sensitivity than published methods.[3–14] This is the first report dealing with GC with low temperature trapping for volatile compounds in biological samples.

Detection limits for toluene in biological samples given by GC–FID with a packed column are about 100 ng ml^{-1}.[4,9,10] Morinaga *et al.*[8] reported a detection limit of 10 ng ml^{-1} for toluene in blood by using GC–MS with a wide-bore capillary column. Hence the detection limits for thinner components obtained by the present method (Tables 2 and 3) are one to two orders of magnitude lower than those by the conventional headspace GC methods.[4,8–10]

We observed net recovery of as much as 55.1% for benzene in blood, whereas only 3.58% of butan-1-ol could be recovered under the same headspace conditions. This large difference in recovery is due to the difference in partitioning of each compound between the gas and liquid phases in a heated vial.[13]

We used ethylbenzene as the IS in this study, as did other investigators.[7] Kato and co-workers[9,10] used *o*-xylene as the IS for GC measurements of thinner components. However, it should be kept in mind that both ethylbenzene or *o*-xylene are normal constituents of gasoline and kerosene.[15]

The present capillary GC method with a low oven temperature can be applied to a number of volatile compounds in biological or environmental samples; this aspect is now under study in our laboratories.

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