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Short communication

Sensitive determination of four general anaesthetics in human whole blood by capillary gas chromatography with cryogenic oven trapping

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Abstract

Four general anaesthetics, sevoflurane, isoflurane, enflurane and halothane, in human whole blood, have been found measurable with very high sensitivity by capillary gas chromatography–flame ionization detection (GC–FID) with cryogenic oven trapping upon injection of headspace (HS) vapor sample. To a 7-ml vial, containing 0.48 ml of distilled water and 20 μl of internal standard solution (5 μg), a 0.5-ml of whole blood sample spiked with or without anaesthetics, was added, and the mixture was heated at 55°C for 15 min. A measure of 10 ml HS vapor was injected into the GC in the splitless mode at −40°C oven temperature, which was programmed up to 250°C. All four peaks were clearly separated; no impurity peaks were found among their peaks. Their extraction efficiencies were about 10%. The calibration curves showed good linearity in the range of 0.5–20 μg/ml; their detection limits were 10–100 ng/ml, which are almost comparable to those by previous reports. The coefficients of intra-day and day-to-day variations were 6.5–9.8 and 7.3–17.2%, respectively. Isoflurane or enflurane was also measured from whole blood samples in which three volunteers inhaled each compound.

Keywords: Cryogenic oven trapping; Enflurane; Halothane; Isoflurane; Sevoflurane

1. Introduction

Sevoflurane, isoflurane, enflurane and halothane are halogenated inhalation anaesthetics and they are widely used as general anaesthetics. Their abusers are frequently reported in operating room personnel; some intoxication cases are fatal [1–9]. Thus, sensitive determination of these anaesthetics should be necessary.

For analyses of these anaesthetics, gas chromatography (GC)–flame ionization detection (FID) [7,10–12] or –electron capture detection (ECD) [13], gas chromatography/mass spectrometry (GC–MS) [14–20] or GC–Fourier transformation infrared spectro-

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scopy (FTIR) [21,22] were applied. For trapping these volatile compounds, Purge-and-trap method [21,22], headspace method [10–13,16,17,19], pulse-heating [15] and solid-phase microextraction (SPME) [20] were applied.

Recently, a microcomputer-controlled device for lowering oven temperature below 0°C has become available for new types of GC instruments. This device was originally designed for rapid cooling of a GC oven to reduce the time for analysis. We have applied it for trapping volatile compounds inside a capillary column, and named this technique cryogenic oven trapping. It can allow 5–10 ml of headspace vapor to be introduced into a medium bore capillary column without any loss. This method has enabled a lot of volatile compounds, i.e. chloroform, methylene chloride, trichloroethylene, ethanol, xylenes and cyanide to be measured with extremely high sensitivity [23–28].

In this paper, we have established a simple and very sensitive determination method for these four general anaesthetics in human whole blood.

2. Experimental

2.1. Materials

Halothane was purchased from Takeda Chem. Ind. Ltd. (Osaka, Japan); enflurane, isoflurane and sevoflurane from Dainippon Pharmaceutical Co. Ltd. (Tokyo, Japan); Triton X-100 from Wako Pure Chemicals (Osaka, Japan). The chemical structures of these compounds were shown in Fig. 1. An Rtx®-Volatile fused-silica capillary column (30 m× 0.32 mm I.D., film thickness 1.5 μm) was obtained from Restek (Bellefonte, PA, USA). Amber vials (4 ml), screw-cap vials (7 ml) and silicone septa were obtained from Supelco (Bellefonte, PA, USA). Other common chemicals were of analytical grade. Human whole blood samples were obtained from healthy volunteers.

2.2. Standards

All stock solutions were dissolved in ice-cold 10% Triton-X 100 solution. To a 4-ml amber vial containing ice-cold 10% Triton-X solution, 240 mg of each anaesthetic compound (about 128–159 μl) was added up to 4.8 ml in order to reduce the headspace as little as possible, and the vial was tightly sealed immediately. The mixture was vortexed for 1 min, and the mixture was used as a 50-mg/ml stock solution. The 50-mg/ml stock solutions were used within 1 week after the preparation. For spiking anaesthetics to whole blood samples, 1-mg/ml solutions were made as follows. In measuring enflurane, isoflurane and sevoflurane, to a 4-ml amber vial containing ice-cold 10% Triton-X solution, 50-mg/ml stock solutions of enflurane, isoflurane and sevoflurane (96 μl each) were added up to 4.8 ml; the mixture was vortexed for 1 min immediately after sealing. The internal standard (IS) solution (1-mg/ml halothane) was made separately. These 1-mg/ml solutions were prepared on every day of experiments.

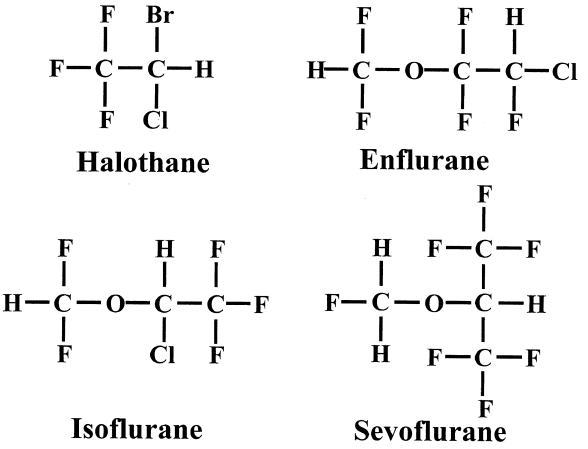

Fig. 1. Chemical structures of four general anaesthetics.

2.3. Procedure

To a 7-ml screw-cap vial containing 0.48 ml of distilled water, 20 μl of I.S. solution (5 μg/vial halothane or isoflurane in 10% Triton-X 100 solution, respectively), 0.5 ml of whole blood with or without anaesthetics was added. Halothane was used as the I.S. for analysis of sevoflurane, isoflurane and enflurane, and isoflurane was used as I.S. for analysis of halothane. When halothane was used as I.S., to spike anaesthetics (10 μg/ml) to a blood sample, 12 μl of 1 mg/ml solution containing enflurane, iso-

flurane and sevoflurane was added to 1.2 ml of human whole blood in a 1.5-ml eppendorf tube, and transferred a 0.5-ml aliquot to the above 7-ml vial immediately after mixing. The vial was rapidly sealed with a Teflon-lined silicon septum cap and then put on an aluminum block heater (Reacti-Therm Heating/Stirring modal, Pierce, Rockford, IL, USA) after vortexing for 30 s. After heating at 55°C for 15 min, a 23-gauge needle of a glass syringe (20-ml volume) was inserted into the GC port in the splitless mode at −40°C of the oven temperature.

2.4. GC conditions

GC analyses were carried out on a Shimadzu GC-14B gas chromatograph equipped with a flame ionization detector (FID) and with a cryogenic oven temperature device (Shimadzu Corp., Kyoto, Japan). The GC conditions were: column used Rtx®-Volatiles (30 m×0.32 mm I.D., film thickness 1.5 μm); column temperature, −40–70°C (1 min hold at −40°C, 10°C/min from −40 to 70°C, 20°C/min from 70 to 250°C, and 4 min hold at 250°C); injection temperature 150°C; detection temperature 250°C; and helium flow-rate 2 ml/min. The headspace sample was injected in the splitless mode, and the splitter was opened 1 min after completion of the injection. For GC quantitation, the peak area of each compound was measured.

2.5. Administration of anaesthetics

Three healthy male volunteers (53, 38 and 24 years-old) inhaled enflurane or isoflurane. A measure of 2 ml of each compound in a 10-ml beaker was put in a plastic bag (about 700 ml); the volunteers inhaled air containing each compound with the bag for 1 min. Immediately after inhalation, 7 ml of blood was taken. The whole blood samples were kept in an ice bath, and they were analyzed on the same day of inhalation.

3. Results and discussion

3.1. Analytical conditions

Various conditions for the HS extraction of the anaesthetics from whole blood were tested. We heated the vials at 50–70°C for 5–30 min; it was found that optimal extraction into the HS was attained at 15 min and 55°C (data not shown).

We have tested various initial oven temperatures for trapping the anaesthetics vapor (Fig. 2). At 20°C, the peaks of the compounds were quite broad and became shaper upon lowering the oven temperature to −40°C. We considered the ability of the cryogenic oven device and −40°C was adopted for trapping the compounds.

3.2. Reliability of the method

Fig. 3 shows gas chromatograms for authentic four anaesthetics (500 ng each) dissolved in 0.2 μl of dichloromethane and for headspace extracts from 0.5 ml of human whole blood in the presence (5 μg

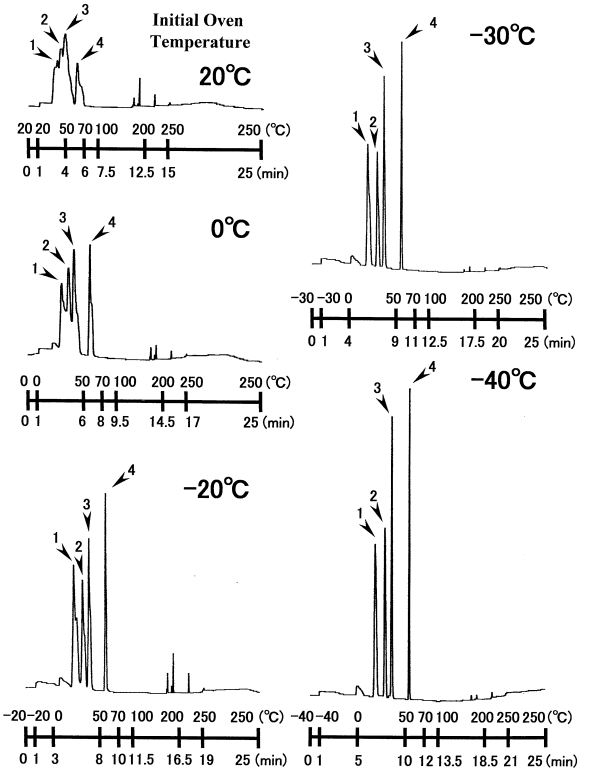

Fig. 2. Headspace capillary GC chromatograms for sevoflurane (1), isoflurane (2), enflurane (3) and halothane (4) in whole blood as a function of various initial oven temperatures. A measure of 5 μg of each compound were added to 0.5 ml of human whole blood for headspace extraction.

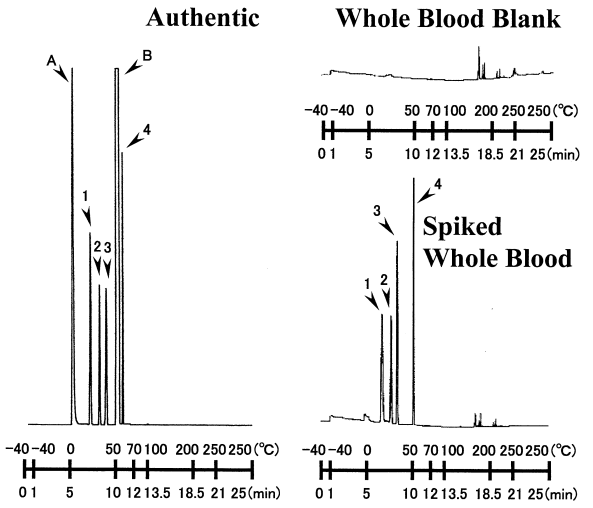

Fig. 3. Capillary GC chromatograms with cryogenic oven trapping at −40°C for the authentic sevoflurane (1), isoflurane (2), enflurane (3) and halothane (4), dissolved in 0.2 μl of dichloromethane (B) with direct injection (500 ng each, left panel), and for whole blood spiked with 5 μg of each compound in 0.5 ml (lower right panel), and for whole blood in the absence of the compounds (upper left panel). The peak A represents that of methanol containing in dichloromethane as preservative.

each) or absence of the compounds. The retention times for sevoflurane, isoflurane, enflurane and halothane were 7.0, 8.0, 8.8 and 10.6 min, respectively. The backgrounds gave very small impurity peaks; no interfering peaks were found around the compound peaks (Fig. 3, right panels).

The extraction efficiencies of the anaesthetics were determined by external calibration. Peaks areas of whole blood spiked with known amounts (5 or 1 μg) of the compounds (after cryogenic trapping of the HS prior to GC analysis) were compared with peak areas obtained by direct injection of the authentic compounds into GC. The extraction efficiencies for four anaesthetics were shown in Table 1.

Calibration curves for four anaesthetics were drawn by plotting six different concentrations using 5 μg of I.S. They gave good linearity in the range of 0.5–20 μg/ml whole blood. The equations and r values for the curves were: $y=0.101\ x+0.00301$ and 0.999 for sevoflurane; $y=0.0632\ x+0.0114$ and 0.999 for isoflurane; $y=0.0692\ x+0.0486$ and 0.998 for enflurane; $y=0.116\ x+0.0575$ and 0.989 for halothane, respectively. Their detection limits (signal-to-noise ratio=3) were estimated to be 10 ng/ml for enflurane, 20 ng/ml for halothane, and 100 ng/ml for isoflurane and sevoflurane, respectively. In the previous reports, their detection limits were about 0.2 μg/ml whole blood for sevoflurane, isoflurane, enfluraene and halothane [15], 22 ng/ml serum for isoflurane [16], 9.4 ng/ml water for halothane [17] and 200–300 ng/0.25 g tissue for enflurane [18] by GC–MS, and they were 50 ng/ml blood for isoflurane [21] and 10–700 ng/ml blood for sevoflurane, isoflurane, enflurane and halothane [22] by GC–FTIR. In the combination of GC–MS and SPME, its detection limits were about 30 pg/ml for isoflurane and halothane, but 10 ml of urine was used in this method. Thus our method is almost comparable to those by GC–MS or GC–FTIR, and it can obtain high sensitivity without expensive apparatus like as a purge-and-trap concentrator or a Curie point pyrolizer.

To check reproducibility of the present method, we have added 1 or 5 μg of anaesthetics and 5 μg of I.S. to 0.5 ml of whole blood and determined them with each calibration curve. The determined concentrations, coefficients of intra-day and day-to-day variations for each compound were summarized in Table 2. As shown in Table 2, the coefficients of intra-day and day-to-day variations for four compounds were 6.5–9.8 and 7.3–17.2%, respectively.

Table 1
The extraction efficiencies and their coefficients of variation (C.V. values) for sevoflurane, isoflurane, enflurane and halothane determined with spiked whole blood samples

Compound	Concentration added			
	2 μg/ml		10 μg/ml	
	Extraction efficiencies (%)[a]	C.V. (%)	Extraction efficiencies (%)[a]	C.V. (%)
Sevoflurane	15.3	9.3	11.7	8.8
Isoflurane	15.2	10.9	9.1	8.6
Enflurane	9.9	9.6	8.3	9.5
Halothane	12.3	11.7	10.6	10.7

[a] The values were means of five experiments.

Table 2
The determined concentrations and intra-day and day-to-day variations for sevoflurane, isoflurane, enflurane and halothane, spiked in human whole blood samples

Compound	Concentration added							
	Intra-day				Day-to-day			
	2 μg/ml		10 μg/ml		2 μg/ml		10 μg/ml	
	Determined concentrations (mean±SD, %)[a]	C.V. (%)	Determined concentrations (mean±SD, %)[a]	C.V. (%)	Determined concentrations (mean±SD, %)[a]	C.V. (%)	Determined concentrations (mean±SD, %)[a]	C.V. (%)
Sevoflurane	2.08±0.16	7.8	10.1±0.69	6.9	2.47±0.33	13.5	10.7±1.27	12.6
Isoflurane	2.20±0.22	9.9	10.2±0.67	6.5	2.14±0.21	10.0	10.0±1.15	11.5
Enflurane	2.32±0.21	9.1	10.5±0.82	7.8	2.44±0.42	17.2	10.6±1.03	9.8
Halothane	2.19±0.22	9.8	10.3±0.69	6.7	1.99±0.25	12.5	10.9±0.79	7.3

[a] The values were means of six experiments.

3.3. Actual measurements of isoflurane and enflurane

Three healthy volunteers inhaled isoflurane or enflurane for one min; their blood samples taken immediately after inhalation were analyzed. Fig. 4 shows the GC chromatograms of the blood taken from Subject 2, who inhaled isoflurane (left panel) or enflurane (fight panel), respectively. The results of the levels of two compounds of blood samples from three subjects were summarized in Table 3. Therapeutic concentrations of these anaesthetics are about 95 μg/ml for enflurane [29], 22–84 μg/ml for halothane [29], and about 72 μg/ml for sevoflurane [30]; therefore, these anaesthetics can be monitored using very small amount of whole blood by our method.

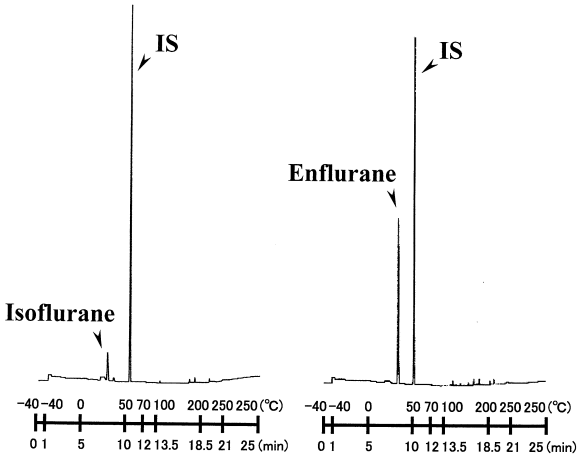

Fig. 4. Capillary GC chromatograms with cryogenic oven trapping for whole blood samples of Subject 2, who inhaled isoflurane (left panel) and enflurane (right panel) for one min. Halothane was used as I.S.

4. Conclusion

To our knowledge, this is the first report for detecting four general anaesthetics, i.e. sevoflurane, isoflurane, enflurane and hanothane from whole blood using capillary GC with cryogenic oven trapping. Our method may be applicable in the fields of forensic and clinical toxicology; at least it could be useful in pharmacokinetic target analysis, because

Table 3
Concentrations of isoflurane and enflurane in whole blood samples obtained from three volunteers after their inhalation (1 min)[a]

Subject	Compound	Concentrations of the compound (μg/ml)
1	Isoflurane	1.47
	Enflurane	1.98
2	Isoflurane	2.12
	Enflurane	8.23
3	Isoflurane	0.65
	Enfluarne	7.02

[a] The values were means of three experiments.

of its simplicity, sensitivity and very low background noises.

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References

[1] J.D. Spencer, F.O. Raasch, F.A. Trefny, J. Am. Med. Assoc. 235 (1976) 1034.
[2] P. Berman, M. Tattersall, Lancet i (1982) 340.
[3] M. Bass, J. Am. Med. Assoc. 251 (1984) 604.
[4] M. Yamashita, A. Matsuki, T. Oyama, Can. Anaest. Soc. J. 31 (1984) 76.
[5] B. Jacob, C. Heller, T. Daldrup, K.F. Bürrig, J. Barz, W. Bonte, J. Forensic Sci. 34 (1989) 1408.
[6] F.B. Walker, R.A. Morano, J. Forensic Sci. 35 (1990) 197.
[7] J.J. Kuhlman, J. Magluilo Jr., B. Levine, M.L. Smith, J. Forensic Sci. 38 (1993) 968.
[8] H. Lofti, S. Clement, J. Debord, A. Brosset, D. Dumont, G. Lâchatre, Bull. Int. Assoc. Forensic Toxicol. 24 (3) (1994) 34.
[9] K. Pihlainen, I. Ojanperä, Forensic Sci. Int. 97 (1998) 117.
[10] J. Flynn, J.D. O'Keeffe, W.S. Wren, I.M. Shanahan, Analyst 114 (1989) 1207.
[11] J. Flynn, S. Masud, J.D. O'Keeffe, W.S. Wren, I.M. Shanahan, Analyst 114 (1989) 1211.
[12] M.T. Watts, M. Escarzaga, C.H. Williams, J. Chromatogr. 577 (1992) 289.
[13] P.J. Streete, M. Ruprah, J.D. Ramsey, R.J. Flanagan, Analyst 117 (1992) 1111.
[14] K. Miyano, Y. Tanifuji, T. Obata, Biomed. Chromatogr. 7 (1993) 116.
[15] K. Saito, T. Takayasu, J. Nishigami, T. Kondo, M. Ohtsuji, Z. Lin, T. Ohshima, J. Anal. Toxicol. 19 (1995) 115.
[16] K. Maruyama, A. Takatsu, T. Obata, Biomed. Chromatogr. 9 (1995) 179.
[17] F. St-Germain, O. Mamer, J. Brunet, B. Vachon, R. Tardif, T. Abribat, C. Des Rosiers, J. Montgomery, Anal. Chem. 67 (1995) 4536.
[18] H. Ise, K. Kudo, N. Jinfuchi, T. Imamura, N. Ikeda, J. Chromatogr. B 698 (1997) 97.
[19] R. Schmidt, H.G. Wahl, H. Häberle, H.-J. Dieterich, V. Schurig, Chirarity 11 (1999) 206.
[20] D. Poli, E. Bergamaschi, P. Manini, R. Andreoli, A. Mutti, J. Chromatogr. B 732 (1999) 115.
[21] I. Ojanperä, R. Hyppölä, E. Vuori, Forensic Sci. Int. 80 (1996) 201.
[22] I. Ojanperä, K. Pihlainen, E. Vuori, J. Anal. Toxicol. 22 (1998) 290.
[23] K. Watanabe, H. Seno, A. Ishii, O. Suzuki, T. Kumazawa, Anal. Chem. 69 (1997) 5178.
[24] K. Watanabe, H. Seno, A. Ishii, T. Kumazawa, H. Hattori, O. Suzuki, Jpn. J. Forensic Toxicol. 16 (1998) 69.
[25] A. Ishii, H. Seno, K. Watanabe-Suzuki, O. Suzuki, T. Kumazawa, Anal. Chem. 70 (1998) 4873.
[26] H. Hattori, M. Iwai, S. Kurono, T. Yamada, K. Watanabe-Suzuki, A. Ishii, H. Seno, O. Suzuki, J. Chromatogr. B 718 (1998) 285.
[27] K. Watanabe-Suzuki, H. Seno, A. Ishii, T. Kumazawa, O. Suzuki, J. Chromatogr. B 727 (1999) 89.
[28] K. Watanabe-Suzuki, H. Seno, A. Ishii, T. Kumazawa, O. Suzuki, Jpn. J. Forensic Toxicol. 17 (1999) 61.
[29] A.C. Moffat, J.V. Jackson, M.S. Moss, B. Widdop (Eds.), Clarke's Isolation and Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986.
[30] K. Fujii, M. Morio, H. Kikuchi, S. Kawachi, Y. Taira, T. Inoue, T. Maekawa, M. Yamamoto, K. Ikeda, K. Mori, Masui (in Japanese) 35 (1986) S373.