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Simultaneous determination of fluorinated inhalation anesthetics in blood by gas chromatography–mass spectrometry combined with a headspace autosampler

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Abstract

Although the fluorinated inhalation anesthetics, including desflurane, sevoflurane, isoflurane, enflurane, and halothane are commonly used, fatal cases resulting from their abuse or misuse have been reported. To date, gas chromatography (GC) equipped with different kinds of detectors has been utilized to analyze inhalation anesthetics. However, none of them can detect desflurane reliably or analyze all five common anesthetics simultaneously. The purpose of the present work is to further modify the previously developed headspace (HS) GC–MS method for blood isoflurane determination to analyze and distinguish five common clinical inhalation anesthetics, simultaneously. The modified HS-GC–MS method adopts a 60 m×0.25 mm I.D., 0.25 μm film thickness DB-5 capillary column along with an adequate GC temperature program, which gives the five inhalation anesthetics, including isoflurane and its isomer, enflurane, a high resolution. The method also takes both the volatility and the influence of the top space on the obtained concentration into consideration and therefore keeps the sample loss acceptable even for analyzing the highly volatile desflurane. Within a certain concentration range of the calibration standard (about 20–300 μg/ml), this method shows a good linearity with correlation coefficients greater than 0.999. In addition, both within- and between-run precision and accuracy results meet the validation requirements as well as the tested results of practical blood samples of desflurane. In summary, this is a reliable analytical method to simultaneously determine the concentration of five common inhalation anesthetics in blood. Such a method is very practical for both clinical and occupational monitoring, as well as for analytical toxicology. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Desflurane; Sevoflurane; Isoflurane; Enflurane; Halothane

1. Introduction

The current acceptable fluorinated inhalation anesthetics (later referred to as the inhalation anesthetics), including desflurane, sevoflurane, isoflurane, enflurane, and halothane, play an important role in the management of patients undergoing surgery [1,2]. They are clear, colorless, nonflammable, volatile lipophilic liquids administered by vaporizing application in clinical general anesthesia. Among the inhalation anesthetics, halothane is a halogenated

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Table 1
Physical properties of the fluorinated inhalation anesthetics

	Fluorinated anesthetic				
	Desflurane	Sevoflurane	Isoflurane	Enflurane	Halothane
Chemical structure	(*R*,*S*)-Difluromethyl 1,2,2,2-tetrafluoroethyl ether	Fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl)-ethyl ether	(*R*,*S*)-1-Chloro-2,2,2-trifluoroethyl difluoromethyl ether	(*R*,*S*)-2-Chloro-1,1,2-trifluoroethyl difluoromethyl ether	(*R*,*S*)-2-Bromo-2-chloro-1,1,1-trifluoroethane
	$CHF_2OCHFCF_3$	$CH_2FOCH(CF_3)_2$	$CHF_2OCHClCF_3$	CHF_2OCF_2CHFCl	$CF_3CHClBr$
Molecular mass	168.0	200.1	184.5	184.5	197.4
Boiling point (°C)	23.5	58.5	48.5	56.5	50.2
Blood–air partition coefficient (λ)	0.42	0.68	1.38	1.9	2.57
Vapor pressure at 20°C (mmHg)	669	157	240	172	244

Data are from Ref. [9].

hydrocarbon and the other compounds are halogenated ethers. Their chemical structures and important physical characteristics are shown in Table 1. Although inhalation anesthetics are now commonly used, fatal cases due to abuse or misuse have been reported [3–8]. A valid and reliable method for detecting these inhalation anesthetics can not only be applied to the investigation of deaths during anesthesia and potential anesthetic agent abusers, but also to the occupational monitoring of operating room personnel and the therapeutic monitoring of anesthetized patients [9]. Therefore, a method for the assessment of inhalation anesthetics is in critical need.

To date, various extraction methods and different kinds of detectors coupled to gas chromatography (GC) have been utilized to analyze inhalation anesthetic drugs [10–20]. However, none of them can detect desflurane reliably or analyze all five common anesthetics simultaneously. With a favorable blood–gas partition constant, the headspace (HS) extraction technique, a traditional and most widely used technique for detecting volatile compounds, is well suited for analysis of volatile anesthetics [9,10]. Moreover, the mass detector possesses high sensitivity and specificity, which makes it a perfect device for analysis, especially in the aspect of analytical toxicology. Our laboratory has successfully invented a system combining gas chromatography–mass spectrometry and a headspace autosampler (HS-GC–MS) to determine the blood concentration of isoflurane [11]. Since inhalation anesthetics have similar physical properties, we are now interested in how the HS-GC–MS method can be extended to analyze other inhalation anesthetics. The aim of the present study was to develop a method for the detection of five common clinical inhalation anesthetics simultaneously.

With the high volatility and the low blood–gas partition coefficient of inhalation anesthetics, analytical difficulties during the determination of anesthetic concentrations in liquid have been mentioned previously [10]. Any steps in the preparation, storage or transfer of the liquid sample which exposes the liquid to a gas phase will introduce error via partitioning anesthetics from the liquid to the gas phase [10]. Therefore, attention should be paid to the sample collection, storage, and preparation to avoid sample loss by evaporation during analysis. In our previous HS-GC–MS method for isoflurane determination, vacuumed tubes and gas-tight syringes were used to avoid analyte loss during analysis [11]. However, using the previously developed method to determine the desflurane, we always failed to accurately determine the level of this anesthetic in blood. This meant that the previous method should be improved and modified when applied to desflurane determination. Desflurane possesses the highest volatility and the lowest blood–air partition coefficient (0.42 at 37°C) among these five inhalation anesthetics with a vapor pressure of 669 mmHg at 20°C and a boiling point of only 23.5°C which is lower than room temperature (Table 1) (1 mmHg=133.322 Pa). Therefore, preventing desflurane samples from evaporation is much more difficult than with other inhalation anesthetics. In fact, to our knowledge, there is no valid method for blood desflurane determination published in the literature to date. On the

other hand, Saito et al. [12] tried pulse-heating GC–MS combined with a GS-Q GC capillary column (30 m×0.53 mm) to test for halothane, enflurane, isoflurane and sevoflurane altogether, and found that the peaks of isoflurane and its isomer, enflurane, were overlapping. Our previous HS-GC–MS method, with a 30 m×0.25 mm I.D., 0.25 μm film thickness DB-5 capillary column also showed an overlapping peaks for isoflurane and enflurane when testing the five inhalation anesthetics simultaneously. Therefore, fully separating isoflurane and its isomer, enflurane, by chromatography in addition to avoid sample loss by evaporation, is critical when developing a simultaneous analysis method.

The objective of this research is to further modify and extend the previously developed HS-GC–MS method to analyze and distinguish the five common clinical inhalation anesthetics simultaneously. For this purpose, sampling techniques, analysis procedures, as well as the separating efficiency of the capillary column in the present method have been improved from the previous work. The method has also introduced a device for preparation and aliquot of quality control sample to prevent sample loss. We here present a more applicable method for simultaneously determination of fluorinated anesthetics in blood including the highly volatile desflurane.

2. Experimental

2.1. Chemicals and reagents

Reagents of analytical grade, including 1,4-dioxane and ethanol were purchased from J.T. Baker (Philipsbrug, NJ, USA). Triton X-100 came from Sigma (St. Louis, MO, USA). Desflurane, isoflurane, enflurane and halothane with purity >99.9% were obtained by Ohmeda Carlbe (Guayama), and sevoflurane was from Abbott Labs. (North Chicago, IL, USA). Deionized water was prepared in our laboratory by using a Milli-Q Water System (Millipore, Bedford, MA, USA). High-purity helium gas (99.999%) and nitrogen gas (99.995%) were used for GC–MS and the headspace autosampler, respectively. Liquid nitrogen was selected as the coolant for the GC oven.

2.2. Calibration standards and internal standard

To prevent analyte loss during the sample spiking and storage, vacuumed tubes with disposable syringes and gas-tight syringes of various volumes were used to prepare the spiked sample. When preparing the calibration standard solution, five anesthetics were diluted together in a 5-ml vacuumed tube (Becton Dickinson, Franklin Lakes, NJ, USA) containing ethanol before transfering 50 μl to a 5-ml vacuumed tube with a volume of 4950 μl whole blood to give the required working concentration. The calibration standards of the five anesthetics were set as follows: 18.3, 36.6, 73.3, 146.5 and 293.0 μg/ml for desflurane; 19.0, 38.0, 76.0, 152.0 and 304.0 μg/ml for sevoflurane; 18.7, 37.4, 74.8, 149.6 and 299.2 μg/ml for isoflurane; 19.0, 38.9, 75.9, 151.7 and 303.4 μg/ml for enflurane; 23.3, 46.5, 93.0, 186.0 and 372.0 μg/ml for halothane. All procedures were performed on ice and the total spiked volume, stored in a 5-ml vacuumed tube, was set at 5 ml. The internal standard, 1,4-dioxane, was dissolved in deionized water with 1.1 mg/ml Triton X-100 to give a concentration of 2.6 mg/ml [13].

Due to its high volatility, commercial desflurane is often packed in a special container to keep it from evaporating. During the preparation, desflurane was transferred from the special container to a 10-ml vacuumed tube (anticoagulant free) for storage. A pre-cooled (at −20°C) gas-tight syringe (Hamilton, Reno, NV, USA) was used to spike the standard sample of desflurane to prevent it from boiling in the syringe at room temperature. The same problem will not occur with the other anesthetics due their boiling points being higher than room temperature (Table 1).

2.3. Quality control and aliquot procedure

The most important thing while making the quality controls is to avoid anesthetic loss during the preparation and sample aliquot. First, a 100-ml gas-tight syringe with PTFE Luer Lock (Hamilton) was used to take 100 ml of whole blood, and the syringe was then sealed with a septum adapter (Alltech, Deerfield, IL, USA). The remaining gas volume in the gas-tight syringe must be reduced if possible. The gas-tight syringe was chosen because its plunger can go down during the aliquot process, which keeps the

top space from increasing and therefore reduces sample loss by exposure to air. After 10× dilution of the five anesthetics together in a 5-ml vacuumed tube with ethanol, an adequate amount of diluted anesthetic, i.e., 100 μl (50 μl) diluted anesthetic for level II (level I) of quality control was added through the septum adapter into a 100-ml gas-tight syringe with a 100-μl gas-tight syringe. After being well mixed with whole blood, two different concentrations of quality control were prepared and marked as level I and level II. The concentrations of level I and level II quality controls for the five anesthetics were as follows: 73.3 μg/ml and 146.5 μg/ml for desflurane, 76.0 μg/ml and 152.0 μg/ml for sevoflurane, 74.8 μg/ml and 149.6 μg/ml for isoflurane, 75.9 μg/ml and 151.7 μg/ml for enflurane, and 93.0 μg/ml and 186.0 μg/ml for halothane. After replacing the septum adapter with a No. 19 needle, the prepared quality controls were divided into aliquots of 5 ml for each vacuumed tube, with more than 16 tubes of the quality control per batch. These samples were then stored at −20°C before use. Defrosted quality controls were kept on ice prior to testing.

2.4. Sample collection and analysis procedure

To determine the concentration of anesthetics in blood, 5 ml of blood sample was drawn with a 5-ml disposable syringe and then was transferred to 5-ml vacuumed tubes containing anticoagulant (Becton Dickinson), and remained on ice until testing after being fully mixed with anticoagulant. A volume of 1.0 ml of 2.6 mg/ml 1,4-dioxane was placed in a 10-ml headspace vial (Alltech) as an internal standard solution. The vial was sealed immediately with a rubber cap and an aluminum crimp seal. A 100-μl blood sample was injected through the septum into a headspace vial using a 250-μl gas-tight syringe. The sample was then analyzed using the modified HS-GC–MS method. The ratio of the peak area of inhalation anesthetics to that of the internal standard was plotted versus the concentration of inhalation anesthetics in the calibration standard, and linear regression analysis was performed.

2.5. GC–MS equipment and conditions

The GC–MS system (Finnigan MAT GCQ GC–MS) consisted of a gas chromatograph fitted with a DB-5 capillary column (60 m×0.25 mm I.D., 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA), a mass detector, a headspace autosampler (HS850, CE Instruments, Italy) and a computer using the Xcalibur program version 1.0. Helium was chosen as the carrier gas at a flow-rate of 40 cm/s. The split mode was used at a split rate of 1:30. Temperatures of the injection port and mass detector interface were set at 100°C and 275°C, respectively. The temperature gradient of the GC oven was programmed to initiate at 35°C for 3.50 min, then increase to 120°C at a rate of 40°C/min and hold at 120°C for 0.68 min. The completion of a temperature cycle took 6.50 min. For GC oven cooling, the subambient temperature option was turned on. The data were acquired after a 2-min delay in both full scan (40 to 250 u) and selected-ion monitoring (SIM) modes. In the SIM mode, we selected the ions of m/z 51, 69 and 149 for desflurane, m/z 51, 69 and 181 for sevoflurane, m/z 51, 67, 117 and 149 for isoflurane, m/z 51, 67 and 117 for enflurane, m/z 67, 98, 117 and 178 for halothane, and m/z 55 and 88 for 1,4-dioxane. For quantification, the ion of m/z 51 was selected as the quantification ion for desflurane; m/z 51, 69 and 181 for sevoflurane; m/z 51, 67, 117 for isoflurane and enflurane; m/z 67, 98, 117 and 178 for halothane, and m/z 55 and 88 for 1,4-dioxane. An electron impact (EI) ionization mode with an ionization energy of 70 eV was used. As for the conditions of the headspace autosampler, except for the analysis time which was changed to 8 min, all the settings were the same as described in Ref. [11].

3. Validation

3.1. Standard curve and linearity

The therapeutic range of inhalation anesthetics, including sevoflurane, enflurane, and isoflurane, is around 100 μg/ml [10,11,21,22]. Therefore, the concentrations of the calibration standard for the five anesthetics were set as follows: 18.3, 36.6, 73.3, 146.5, and 293.0 μg/ml for desflurane, 19.0, 38.0, 76.0, 152.0, and 304.0 μg/ml for sevoflurane, 18.7, 37.4, 74.8, 149.6, and 299.2 μg/ml for isoflurane, 19.0, 37.9, 75.9, 151.7, and 303.4 μg/ml for enflurane, and 23.3, 46.5, 93.0, 186.0, and 372.0 μg/ml for halothane. Calibration standards were obtained as

previously described in Section 2.2. The calibration curves for the five anesthetics were derived from the average of quintuplicates.

3.2. The limits of detection (LOD) and quantification (LOQ)

To determine the LOD and LOQ, the lowest concentrations of the calibration standard by serial 2× dilution were prepared and analyzed in quintuplicate. The LOD was determined to be the lowest analytical concentration with signal-to-noise (S/N) greater than 3:1, following the rule of chromatography, retention time, and ion ratio matching criteria. The LOQ was defined to meet all of the LOD requirements described above and both the relative standard deviation (RSD) and error from theoretical value (E.T.V.) had to be less than 20% [23]. The calculation of E.T.V. was based on the method proposed by Borenstein et al. [24].

3.3. Within- and between-run precision and accuracy

The within-run precision and accuracy were determined by analyzing quintuplicates ($n=5$) of quality controls at level I and level II concentrations for the five anesthetics. The between-run precision and accuracy were derived from the same spiked aliquot quality controls that were analyzed on 5 different days. Precision and accuracy of method were assessed by the RSDs and E.T.V.s, respectively.

The within-run and between-run precision levels were also estimated by analyzing practical blood samples of desflurane since it is the most volatile anesthetic among the five. The blood samples were collected from a patient, a 78-year-old male, with normal pulmonary function undergoing 7% desflurane induction and high flow anesthesia during cardiac surgery. The patient gave written informed consent for the study, which was approved by the Ethics Committee of the Veterans General Hospital, Taichung, Taiwan. At different time points of 10, 20 and 30 min after induction (I10, I20, I30, respectively), the mixed venous blood was drawn and transferred by syringe to five 5-ml vacuumed tubes containing EDTA and was then analyzed as described previously. The within- and between-run precisions were also determined by analyzing quintuplicates of each sample.

3.4. Aliquot process and storage stability

In order to determine whether the aliquot order resulted in concentration variation, a systematic sample of level II quality controls from the same batch were selected at an interval of five tubes (Nos. 1, 6, 11, and 16) to test their concentration. The E.T.V. was calculated from the obtained concentration, i.e., the E.T.V. stood for the variation between obtained concentrations of samples with various aliquot orders and the spiked concentration. Three of the quality controls with the same spiking were randomly selected to be tested at 1 month after storage at −20°C. The storage stability, the difference between the test result and the spiked concentration, was also evaluated by E.T.V.

3.5. Percentage loss after equilibrium

The partition coefficients (λ) of various anesthetics are different from each other and are affected by temperature. Therefore the degree of liquid phase concentration reduction is associated with the type of anesthetics, temperature, and the volume of gas phase. When a sample is being stored in a 5-ml vacuumed tube, the concentration of liquid (blood) anesthetics will decrease since the anesthetics vaporize into the top space due to the liquid–air partition. The percentage of concentration decrease for various anesthetics stored in a vacuumed tube at 37°C can be further estimated with the partition coefficients. The percentage loss stands for the reduction in percentage of the final sample concentration after equilibrium from the original blood concentration. We then derived a formula from the definition of partition coefficient to evaluate the top space influence over these five inhalation anesthetics (Appendix A). The total volume of a 5-ml tube was about 5.7 ml by averaging the measurements from testing 10 tubes filled with water. This was used for the total volume of a 5-ml vacuumed tube for the following calculation. If the liquid phase took 5 ml (V_L), the gas phase would be 0.7 ml (V_G). The partition coefficients of the five inhalation anesthetics at 37°C are as 0.42, 0.68, 1.38, 1.9, and 2.57 for desflurane, sevoflurane, isoflurane, enflurane, and halothane, respectively

(Table 1). The sample loss could be estimated by using Eqs. (A.7) and (A.9) using the known V_L, V_G, and λ values in the calculations.

4. Results

4.1. Mass spectrum and chromatography

The full scan mass spectra from the GC–MS analysis of desflurane, sevoflurane, enflurane and halothane are shown, respectively, in Fig. 1a–d. The mass spectra of isoflurane and the internal standard, 1,4-dioxane, have been described in Ref. [11]. After the column length and the temperature program of the GC oven were modified from the previous HS-GC–MS method (Section 2.5), the result of the chromatograph in the SIM mode is presented in Fig. 2. The retention times of desflurane, sevoflurane, isoflurane, enflurane, halothane and 1,4-dioxane were 2.82, 3.02, 3.24, 3.36, 3.88, and 5.65 min, respectively. According to the graph, this modified HS-GC–MS method revealed sharp and symmetric peaks and could well distinguish the inhalation anesthetics from each other by the difference in retention time. Even isoflurane and it's isomer enflurane, showed up in high resolution. In addition, the inset of Fig. 2 also gives the chromatography of five anesthetics at concentrations near the detection limits.

4.2. Validation results

The validation results, including linearity, LOQ, and LOD, are summarized in Table 2. The method shows a good linearity with correlation coefficients greater than 0.999 within a certain concentration range of the calibration standard. The LOQs for the five anesthetics were below 10 μg/ml, while the LODs were around 1 μg/ml. The within- and between-run precision and accuracy, assessed by RSD and E.T.V, are presented in Tables 3 and 4, respectively. The results show all RSDs and E.T.V.s of within- and between-run levels were less than 10%. The investigators also applied this method to test mixed venous blood samples obtained from a cardiac surgery patient who underwent 7% desflurane anesthesia. The concentrations of desflurane in blood at different time periods after induction, along with the precision data, are summarized in Table 5. The within-run precision ranged from 2.9 to 6.5% and the between-run values ranged from 6.2 to 9.5%.

4.3. Aliquot process and storage stability

The E.T.V.s of the level II quality control aliquot of desflurane with aliquot order numbers 1, 6, 11 and 16 were 3.6, 2.1, 6.1, and 8.9%, respectively. All the other four inhalation anesthetics revealed similar results, i.e., the E.T.V.s remained below 20% (data not shown). Moreover, the stability evaluation of the frozen samples revealed that after being stored at −20°C for 1 month, all the results derived from level I and level II quality controls of the five anesthetics were still within ±20% of the spiked concentration, i.e., the E.T.V.s were less than 20% (data not shown).

4.4. Percentage loss after equilibrium

With a 5-ml blood sample in a vacuumed tube, the ratio of the final liquid phase concentration (C_L) versus the original concentration (C_T), F, for the five anesthetics were as follows: 0.75 for desflurane, 0.829 for sevoflurane, 0.908 for isoflurane, 0.931 for enflurane, and 0.948 for halothane after equilibrium at 37°C. By Eq. (A.9), the percentages loss of these five anesthetics were 25.0, 17.1, 9.2, 6.9, and 5.2% for desflurane, sevoflurane, isoflurane, enflurane, and halothane, respectively.

5. Discussion

In testing the five inhalation anesthetics simultaneously, this study has overcome two major problems: avoidance of evaporation and distinguishing the five anesthetics via chromatography, with sufficient resolution to distinguish isoflurane from it's isomer, enflurane. The modified HS-GC–MS method for multi-anesthetic detection confronts the highly volatile property of inhalation anesthetics by using spiked samples on ice. Along with the use of vacuumed tubes, gas-tight syringes, as well as sealing containers tightly at every experimental step, we managed to decrease the loss of volatile anesthetics and increase the testing accuracy. In addition, the

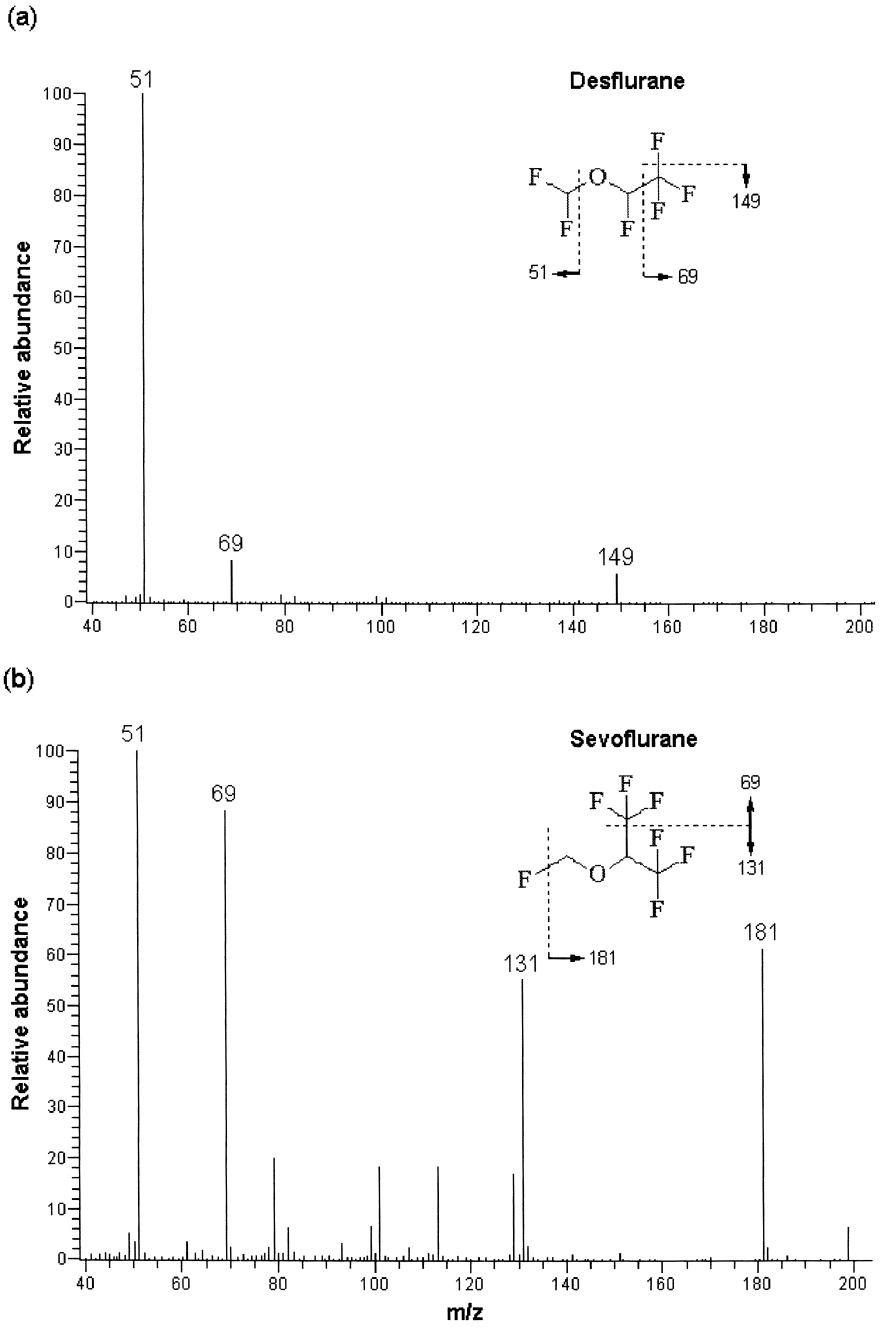

Fig. 1. Mass spectra and fragmentations of (a) desflurane, (b) sevoflurane (c) enflurane, and (d) halothane.

sample loss caused by the top space of the container after the sample being loaded should be taken into consideration, especially for those anesthetics possessing lower blood–air partition coefficients, e.g., desflurane and sevoflurane. One of the modifications from the previous HS-GC–MS method is the column

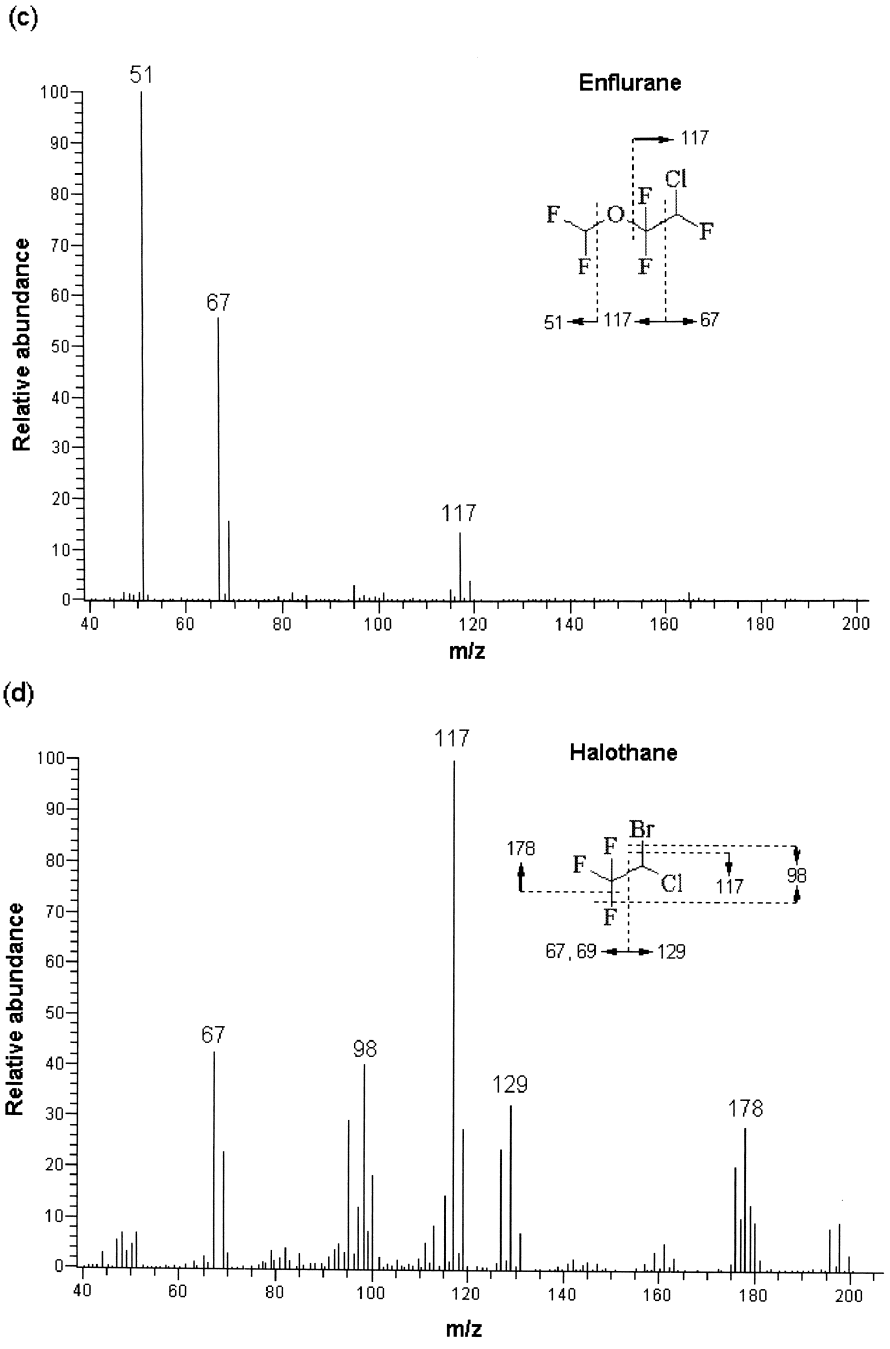

Fig. 1. (*continued*)

length. Using the 60 m×0.25 mm I.D., 0.25 μm DB-5 capillary column can increase the separation efficiency, and the chromatograph has a high enough resolution to distinguish the five inhalation anesthetics, including isoflurane and it's isomer, enflurane (Fig. 2). Moreover, a full temperature cycle

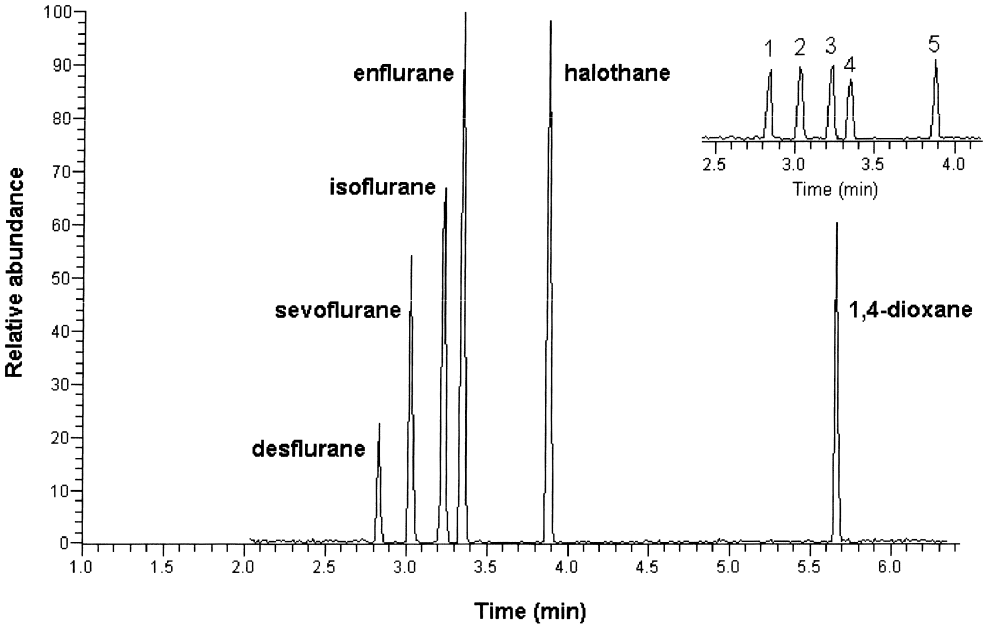

Fig. 2. Total ion chromatogram of a spiked sample tested by the modified HS-GC–MS method using the SIM mode with a concentration of 146.5 μg/ml for desflurane, 152.0 μg/ml for sevoflurane, 149.6 μg/ml for isoflurane, 151.7 μg/ml for enflurane, 186.0 μg/ml for halothane and 2.6 mg/ml for 1,4-dioxane. The inset shows total ion chromatogram at concentrations near detection limits. The number above the peaks represent the following: (1) 9.2 μg/ml for desflurane, (2) 4.8 μg/ml for sevoflurane, (3) 4.7 μg/ml for isoflurane, (4) 2.4 μg/ml for enflurane, and (5) 2.9 μg/ml for halothane.

of the GC oven only takes 6.5 min, and the overall analysis time for a sample, including the oven cooled with liquid nitrogen, is 8 min, which makes it a powerful method for detecting multiple inhalation anesthetics in a short period. The analysis results for all the five anesthetics showed excellent linear relationships ($r>0.999$) within certain concentration ranges of the calibration standard. Both precision and accuracy results below 10%, have met the validation requirements, RSD and E.T.V. values <20% [23]. The results derived from the patient's blood samples of desflurane also confirmed good quality in terms of precision (Table 5), which meant the modified method has improved the sample loss so that even the desflurane sample could be determined accurately. The pharmacological concentrations of inhalation anesthetics range around 100 μg/ml [10,11,21,22], and toxicological concentrations are often higher. The LODs and LOQs obtained in this study ranged from the lowest 0.6 and 2.4 μg/ml, respectively, for enflurane to the highest 2.3 and 9.2 μg/ml, respectively, for desflurane (Table 2), which

Table 2
Validation results of the fluorinated inhalation anesthetics analysis by the modified HS-GC–MS method

Fluorinated anesthetic	Major ions (m/z)	Linearity			LOQ (μg/ml)	LOD (μg/ml)
		Concentration (μg/ml)	Regression line	Correlation coefficient		
Desflurane	51, 69, 149	18.3–293.0	$y=2.61\cdot10^{-3}\chi+0.024$	0.9991	9.2	2.3
Sevoflurane	51, 69, 131, 181	19.0–304.0	$y=9.77\cdot10^{-3}\chi-0.059$	0.9991	4.8	1.2
Isoflurane	51, 67, 117, 149	18.7–299.2	$y=7.84\cdot10^{-3}\chi+0.231$	0.9994	4.7	2.3
Enflurane	51, 67, 117	19.0–303.4	$y=11.6\cdot10^{-3}\chi+0.395$	0.9992	2.4	0.6
Halothane	67, 98, 117, 129, 178	23.3–372.0	$y=8.94\cdot10^{-3}\chi+0.327$	0.9990	2.9	1.5

Table 3
Within-run accuracy and precision analysis of the quality control (QC) sample of the fluorinated inhalation anesthetics by the modified HS-GC–MS method

Volatile anesthetic	Spiked (μg/ml)	Found (μg/ml)	SD[a] (μg/ml)	RSD[b] (%)	E.T.V.[c] (%)
Desflurane	73.3	75.4	7.1	8.4	3.0
	146.5	150.4	5.3	3.5	2.6
Sevoflurane	76.0	75.5	5.9	7.8	0.7
	152.0	148.0	3.0	2.0	2.6
Isoflurane	74.8	75.3	3.5	4.6	0.7
	149.6	156.6	6.4	4.1	4.7
Enflurane	75.9	77.3	3.8	4.9	1.9
	151.7	165.2	3.7	2.2	4.1
Halothane	93.0	93.7	3.9	4.2	0.8
	186.0	197.2	8.2	4.1	6.0

[a] Standard deviation.
[b] Relative standard deviation.
[c] Error from theoretical value.

means that the method is sensitive enough for either clinical monitoring or analytical toxicology of the five inhalation anesthetics.

With a 0.7-ml top space in a 5-ml vacuumed tube after equilibrium at 37°C, the liquid concentration of desflurane in the blood sample decreased by 25%, and the concentration of halothane decreased by 5.2%. The lower the partition coefficient that an anesthetic possesses, the larger the influence the top space will have. For both desflurane and sevoflurane, it is more important to take the sample loss resulting from the top space into consideration. As the partition coefficient decreases with temperature [25], the influence of the top space will decrease λ if all the sample preparation, collection, and operation procedures are performed on ice. However, since there is no literature on the blood–air partition coefficient of inhalation anesthetics at 4°C to date, the percentage loss of inhalation anesthetics at 4°C cannot be estimated.

Since the top space affects the anesthetics of low partition coefficient tremendously, it is critical to control the effect the top space has. Theoretically the problem can be solved by filling the tube as full as

Table 5
Precision of desflurane analysis by the modified HS-GC–MS method in practical samples

Time point	Found (μg/ml)	SD (μg/ml)	RSD (%)
Within-run			
I10	79.2	5.2	6.5
I20	99.3	2.9	2.9
I30	120.7	5.5	4.5
Between-run			
I10	85.1	8.0	9.5
I20	106.7	8.7	8.2
I30	123.8	7.7	6.2

I10, I20 and I30 represent the time points of 10, 20, and 30 min after induction, respectively.

Table 4
Between-run accuracy and precision analysis of the QC sample of the fluorinated inhalation anesthetics by the modified HS-GC–MS method

Volatile anesthetic	Spiked (μg/ml)	Found (μg/ml)	SD (μg/ml)	RSD (%)	E.T.V. (%)
Desflurane	73.3	77.7	7.1	9.2	6.1
	146.5	158.0	12.6	8.0	7.9
Sevoflurane	76.0	70.7	2.8	4.0	7.0
	152.0	153.6	7.9	5.2	1.1
Isoflurane	74.8	76.5	1.2	1.6	2.3
	149.6	158.0	12.3	7.8	5.6
Enflurane	75.9	76.8	3.1	4.0	1.3
	151.7	158.1	7.8	4.9	4.2
Halothane	93.0	91.8	4.2	4.6	1.3
	186.0	201.5	10.5	5.2	8.3

possible during sample spiking or collection to reduce the top space. For example, if a vacuumed tube has a blood sample of 5.5 ml, i.e., $V_L = 5.5$ ml and $V_G = 0.2$ ml, the percentage loss of the five anesthetics from Eqs. (A.7) and (A.9) are, in order as described above, 8, 5.1, 2.6, 1.9, and 1.4%, respectively. It is obvious that the percentage loss can be reduced to below 10% when the tubes are almost completely filled. It can be further decreased if the sample is processed on ice which will reduce λ. However, most commercial 5-ml vacuumed tubes do not have complete vacuums, which causes difficulty in completely filling the tubes. The incomplete mixture and/or insufficient anticoagulant which results from a large blood sample may cause blood clotting, which is another technical problem of testing. Therefore, as some top space is inevitable, the proposed method tries to make each tube an equal volume of 5 ml, including standard, control and sample, following our experience that no blood clot occurred when 5 ml of blood was placed in a 5-ml vacuumed tube. By maintaining an equal top space volume in all of the tubes, the sample loss can be well controlled and easily estimated. The validation results showed that all the RSDs and E.T.V.s, including those of desflurane, could be kept below 10%, which suggests feasibility.

In 1999, Watts et al. [10] used a 1-l bottle to spike the quality control sample, and transferred it into vacuumed tubes with a specific aliquot device. Due to the increasing top space of the bottle during the aliquot process, the sample concentration might decrease along with the aliquot sequence. To solve this problem, we used a 100-ml gas-tight syringe with Teflon Luer Lock (TLL) along with a septum adapter to keep the whole syringe fully sealed. Since the plunger of the syringe goes down as the aliquot process progresses, the aliquot device will keep the top space from increasing. The systematic sample from the same batch of quality controls gave concentrations within ±20% of the spiked concentration, even for desflurane (Section 4.3). It confirms that sample loss during sample aliquot can be well controlled by this method. Moreover, the aliquot quality controls kept stable concentration after storage at −20°C for at least 1 month.

In conclusion, the present work has successfully modified the previously published HS-GC–MS method for isoflurane to analyze multiple common inhalation anesthetics simultaneously. This method can be applied to analytical toxicology in addition to the clinical measurement of blood concentration. The modified HS-GC–MS method has taken the high volatility and low blood–gas partition coefficient of inhalation anesthetics into consideration. With the use of the gas-tight syringe, vacuumed tube, and aliquot device in addition to sealing the container tightly and performing the entire experiment on ice, the method can effectively keep anesthetics from vaporizing. Moreover, the sample loss resulting from the top space can be resolved by controlling equal volume of the standard, quality control and sample. Therefore, it is a valid and reliable method for determination of the five inhalation anesthetics in blood simultaneously.

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Appendix A

According to Lerman et al. [26], the blood–air partition coefficient (λ) is defined as follows:

$$\lambda = C_L/C_G = (W_L/V_L)/(W_G/V_G) \quad \text{(A.1)}$$

$$W_T = W_L + W_G \quad \text{(A.2)}$$

$$\rightarrow \lambda = (W_L/V_L)/[(W_T - W_L)/V_G] \quad \text{(A.3)}$$

From Eq. (A.3):

$$W_L = [\lambda V_L/(V_G + \lambda V_L)] \cdot W_T \quad \text{(A.4)}$$

$$W_T = C_T V_L \text{ and } W_L = C_L V_L \quad \text{(A.5)}$$

From Eqs. (A.4) and (A.5):

$$C_L V_L = [\lambda V_L/(V_G + \lambda V_L)] \cdot C_T V_L$$
$$\rightarrow C_L = [\lambda V_L/(V_G + \lambda V_L)] \cdot C_T \quad \text{(A.6)}$$

C_L is the liquid phase concentration (μg/ml) after

equilibrium; C_G is the gas phase concentration (μg/ml) after equilibrium; V_L stands for the liquid volume (ml) in the vacuumed tube; V_G is the gas volume (ml) in the vacuumed tube; W_L is the total mass of anesthetics in liquid phase (μg); W_G stands for the total mass of the anesthetics in gas phase (μg); C_T is the overall concentration in both liquid and gas phases. Since the anesthetics in the gas phase come from the liquid phase, C_T is also the original concentration in blood.

If:

$$F = [\lambda V_L / (V_G + \lambda V_L)] \quad \text{(A.7)}$$

Then:

$$C_L = FC_T \quad \text{(A.8)}$$

$$\text{Percentage loss} = (1 - F) \cdot 100\% \quad \text{(A.9)}$$

According to the above formulae, given a certain temperature and original blood concentration of anesthetics, C_T (μg/ml), the liquid concentration decreases to C_L (μg/ml) after equilibrium. F refers to the ratio of blood concentration after equilibrium (C_L) versus the original concentration (C_T). Percentage loss means the percentage of blood concentration loss after equilibrium, i.e., $(1-F)\cdot 100\%$.

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