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Reliable gas chromatographic–mass spectrometric method combined with a headspace autosampler for isoflurane determination in blood

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Abstract

Isoflurane is a nonflammable, liquid, volatile inhalation anesthetic administered by vaporizing. Although it is now commonly used, fatal cases resulting from its abuse or misuse have been reported. A combined system of a gas chromatograph–mass spectrometer and a headspace autosampler is therefore proposed for the detection of blood isoflurane. This analytic method showed sharp and well separated peaks, and revealed a good linear relationship ($r=0.9994$) with a function of $y = 7.3768x - 0.0222$ at concentrations between 18.7 and 299.2 μg/ml. The limits of detection and quantitation of this method were 1.2 and 4.7 μg/ml, respectively. The within- and between-run precision for spiked samples, assessed by the coefficient of variations, ranged from 1.7 to 10.0% and from 4.1 to 12.8%, respectively. The within- and between-run accuracy, assessed by errors from theoretical values, were 2.2–7.8% and 2.4–9.6%, respectively. In addition, practical sample analysis showed a good applicability, with a within-run precision rate of 5.6 to 7.7% and a between-run precision rate of 5.2–10.6%. In summary, the present work presents a valid alternative for blood isoflurane analysis.

Keywords: Isoflurane

1. Introduction

Isoflurane, a nonflammable liquid administered by vaporizing, is a volatile inhalation anesthetic. It is a clear, colorless, stable liquid with the chemical formula of (*R,S*)-1-chloro-2,2,2-trifluoroethyl difluoromethyl ether ($CHF_2OCHClCF_3$) and is similar to its isomer, enflurane, in several physical properties [1,2]. Although isoflurane is now widely used, fatal cases resulting from its abuse or misuse have been reported [3–6]. The investigation of deaths during anesthesia and of potential anesthetic agent abusers, the occupational monitoring of operating room personnel, and the therapeutic monitoring of anesthetized patients all require a justifiable analytic method for inhalation anesthetics [7]. Therefore, it is critical to develop a simple, rapid, reliable method for the assessment of plasma/blood levels of isoflurane.

In the early years, a solvent extraction method using *n*-heptane to extract volatile anesthetic drugs from blood was used in some laboratories [8,9]. However, the large amount of solvent consumption or a narrow linear range has limited the use of this

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method. Recently, gas chromatography (GC) equipped with different kinds of detectors has been utilized to identify volatile anesthetic drugs, including isoflurane, enflurane, halothane, etc. [10–14]. Over the past few decades, the well-developed headspace autosampler combined with GC has conveniently been used to determine the concentrations of volatile anesthetic drugs in humans [10,15,16]. Moreover, the headspace analysis shows obvious advantages in extending column life and preventing injector contamination.

The mass detector possesses high sensitivity and specificity. In addition to the full scan spectra, it is also possible to use a selected ion monitoring (SIM) mode to increase the sensitivity and selectivity in target analyses. We present a simple, rapid and reliable analytical method to determine the concentration of isoflurane in blood employing a GC–MS with a headspace autosampler, headspace GC–MS (HS-GC–MS).

2. Experimental

2.1. Chemicals and reagents

Reagents of analytical grade, including 1,4-dioxane, ethanol and ethyl acetate, were purchased from J.T. Baker (Philipsburg, NJ, USA). Triton X-100 was from Sigma (St. Louis, MO, USA). Isoflurane (purity>99.9%) was obtained by Ohmeda Carlbe (Guayama). Deionized water was prepared in our laboratory by using a Milli-Q water system (Millipore, Bedford, MA, USA). High purity of helium gas (99.999%) and nitrogen gas (99.995%) were used for the GC–MS and the headspace autosampler, respectively.

2.2. Stock solution and internal standard

Although not discussed in the literature, our preliminary data showed that the concentration of isoflurane decreased rapidly when the spiked sample was exposed to air. Therefore, vacuumed tubes with disposable syringes and gas-tight syringes of various volumes were used for preparing the stock solution. In addition to sealing containers tightly at every experimental step, investigators must minimize the top space volume in vacuumed tubes and microcentrifuge tubes used for the storage of samples to as little as possible to prevent sample loss. When preparing spiked samples and calibration standard solution, isoflurane was diluted properly in ethanol before being transferred to 5-ml vacuumed tubes filled with a proper volume of whole blood to give the required working concentrations (299.2, 149.6, 74.8, 37.4 and 18.7 μg/ml). All procedures were performed on ice. The spiked blood solutions were divided into aliquots in microcentrifuge tubes using disposable syringes, and the aliquot samples were used immediately or stored in a freezer (−20°C) before use during the 5-day experimental course. Each aliquot sample was used only once. The internal standard (I.S.), 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 [17].

2.3. Sample treatment and analysis procedure

In a 10-ml headspace vial, a 100-μl blood sample and 1.0 ml of 2.6 mg/ml I.S. solution were pooled. The vials were sealed immediately with a rubber cap and an aluminum crimp seal. These samples were then analyzed using the HS-GC–MS method. The ratio of the peak area of isoflurane to that of the I.S. was plotted versus the concentration of isoflurane in the calibration standard, and linear regression analysis was performed.

2.4. GC–MS equipment and conditions

The GC–MS system consisted of a gas chromatograph (Finnigan MAT GCQ™) fitted with a DB-5 capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA), a mass detector (GCQ™), a headspace autosampler (HS850, CE instruments) and a computer (Gateway 2000) using the GCQ data system 2.11 version. 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 and 275°C, respectively. The temperature gradient of the GC oven was programmed to initiate at 35°C for 1.40 min, then rise to 80°C at a rate of 40°C/min and hold at 80°C for 2 min. It took 4.53 min for the completion of a

temperature cycle. The data were acquired after a 0.5-min delay in both full scan (50–250 u) and SIM mode. In the SIM mode, the ions of m/z 51, 67, 117 (isoflurane) and 55, 88 (1,4-dioxane) were selected and used for the quantification. An electron impact (EI) ionization mode with the ionization energy of 70 eV was used.

The conditions of the headspace autosampler were set as follows: the temperatures of the oven and syringe were set at 55 and 120°C, respectively; 10-ml headspace vials (Alltech) and a 2.5-ml gas-tight syringe (Hamilton) were used. The equilibration time was set for 30 min, the upper gas phase was pulled and pushed three times, and 1.5 ml of the vapor was drawn before the injection of 1.0 ml into the GC for analysis. N_2 was selected as the flushing gas, and the analysis time was set at 6 min.

3. Validation

3.1. Standard curve and linearity

The preliminary data showed that the whole blood concentration of isoflurane in anesthetized patients who received 2% isoflurane anesthesia was approximately 100 μg/ml. Therefore, concentrations between 18.7 and 299.2 μg/ml (299.2, 149.6, 74.8, 37.4 and 18.7 μg/ml) were set as calibration standards to test the linearity of the method and to serve as a daily reference. Calibration standards were obtained as previously described (Section 2.2). The calibration curve for isoflurane was derived from the average of quintuplicates.

3.2. Limits of detection (LOD) and quantitation (LOQ)

To determine the LOD and LOQ, the concentrations of serial 2× dilutions from the calibration standard (18.7 μg/ml) were prepared and analyzed in quintuplicates. The LOD was determined to be the lowest analytic concentration with signal-to-noise (S/N) greater than 3, 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 coefficients of variation (C.V.) and errors from theoretical value (E.T.V.) had to be less than 20% [18].

3.3. Precision and accuracy of within- and between-run

The within-run precision and accuracy were determined by analyzing quintuplicates ($n=5$) of four different spiked samples at concentrations of 299.2, 149.6, 74.8 and 37.4 μg/ml. The between-run precision and accuracy were derived from the same spiked samples that were prepared and analyzed on five different days. Precision and accuracy of method were assessed by the C.V. and E.T.V. values, respectively. The calculation of E.T.V. was based on the method described by Borenstein et al. [19].

The within- and between-run precision levels were also estimated by blood samples which were collected for this experiment from a patient, a 79-year-old male, with normal pulmonary function undergoing 2% isoflurane induction and low flow anesthesia during the 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. After the anesthesia was stable, the mixed venous blood was collected at different time points, including preoperatively (Pre-OP) and 5, 30 and 60 min after sternotomy (S5, S30 and S60, respectively). The blood samples were drawn by syringe and transferred into 5-ml vacuum tubes containing EDTA as an anticoagulant. The vacuum tubes were filled with blood as completely as possible. After mixing, each blood sample was divided into portions which were placed into micro-centrifuge tubes using disposable syringes and then analyzed as described previously. The within- and between-run precisions were also determined by analyzing quintuplicates of each sample.

4. Results

4.1. Chromatogram and mass spectrum

The full scan mass spectrum from the GC–MS analysis for isoflurane and the I.S. solution (1,4-dioxane) are shown, respectively, in Fig. 1a and b. There were four major ion peaks including m/z 51

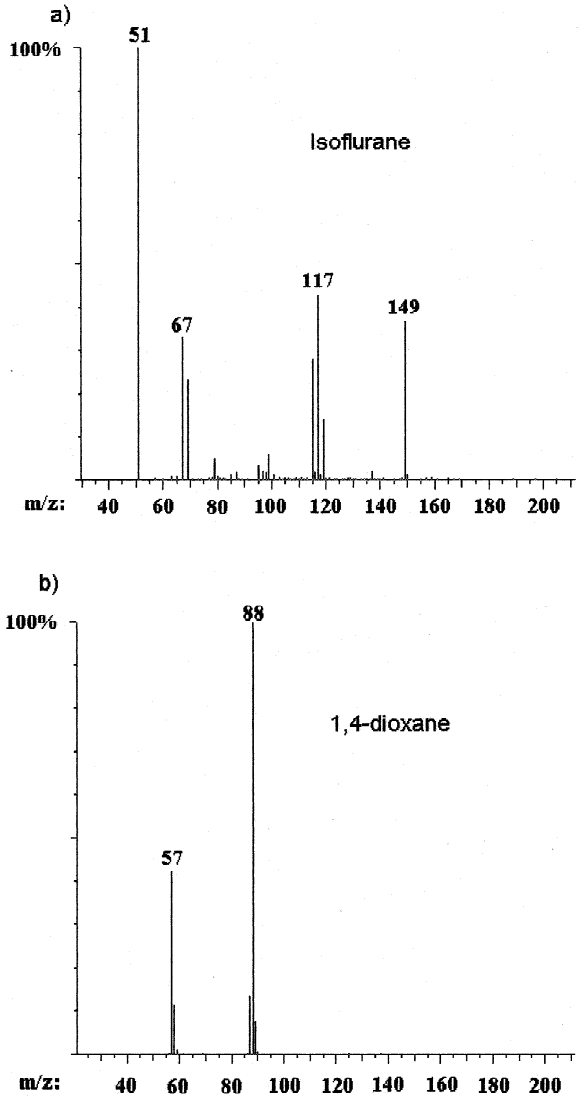

Fig. 1. Mass spectra using the full scan mode for (a) isoflurane and (b) 1,4-dioxane.

(100%), 67 (33%), 117 (43%), and 149 (37%) of isoflurane and two of 1,4-dioxane, m/z 57 (42%) and 88 (100%) in the mass spectrum. The numbers in the parentheses stands for the percentage of each ion (i.e., the ion ratio). The authors therefore selected the ions of m/z: 51, 67 and 117 for isoflurane, and m/z: 57 and 88 for 1,4-dioxane for the SIM mode. Presented in Fig. 2 is the total ion chromatogram of 149.6 μg/ml isoflurane with the I.S. using an SIM

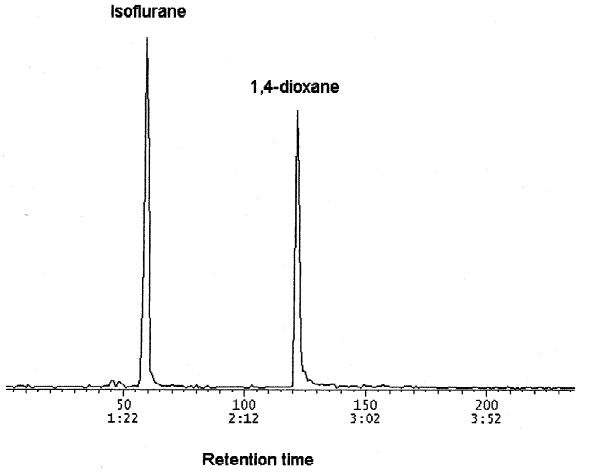

Fig. 2. Total ion chromatogram of a spiked sample examined by the HS-GC–MS method using the SIM mode with a concentration of 149.6 μg/ml of isoflurane and 2.6 mg/ml of 1,4-dioxane. The retention time is presented as min:s.

mode. The retention time of isoflurane and 1,4-dioxane were 1:30 and 2:34 (min:s), respectively. According to the graph, this method could easily distinguish the test compound from the I.S. by the difference in retention times.

This method reveals a good linear relationship ($r = 0.9994$) from the standard curve with a function of $y = 7.3768x - 0.0222$. Table 1 summarizes the precision and accuracy of the spiked samples. With

Table 1
Accuracy and precision of the isoflurane analysis in spiked samples by the HS-GC–MS method

Spiked (μg/ml)	Found (μg/ml)	S.D.[a] (μg/ml)	C.V.[b] (%)	E.T.V.[c] (%)
Within-run ($n=5$)				
37.4	39.0	3.9	10.0	4.3
74.8	73.2	2.9	4.0	2.2
149.6	161.2	10.4	6.3	7.8
299.2	317.4	5.4	1.7	6.1
Between-run ($n=5$)				
37.4	40.4	5.1	12.8	8.0
74.8	67.6	4.4	6.5	9.6
149.6	159.8	10.2	6.4	6.8
299.2	292.0	12.0	4.1	2.4

[a] Standard deviation.
[b] Coefficients of variation.
[c] Errors from theoretical value.

Table 2
Summary of the other validation results for isoflurane analysis by the HS-GC–MS method

Parameter	Result
Linear range (μg/ml)	4.7–2394
Linearity ($n=5$) (μg/ml)	18.7–299.2 ($y = 7.3768x - 0.0222$; $r = 0.9994$)
LOD of the method ($n=5$) (μg/ml)	1.2
LOQ of the method ($n=5$) (μg/ml)	4.7 (C.V. = 10.0%; E.T.V. = 19.8%)

four different isoflurane concentrations of 299.2, 149.6, 74.8 and 37.4 μg/ml, the within-run precision, assessed by the C.V., ranged from 1.7 to 10%, and the between-run precision ranged from 4.1 to 12.8%. The within-run accuracy, assessed by the E.T.V. ranged from 2.2 to 7.8% and the between-run values were from 2.4 to 9.6%. The other validation results are summarized in Table 2. The values of LOD and LOQ were 1.2 μg/ml and 4.7 μg/ml (C.V. = 10.0%; E.T.V. = 19.8%), respectively, and the method also had a wide linear range from 4.7 to 2394 μg/ml.

The investigators then applied this method to test mixed venous blood samples obtained from a cardiac surgery patient who underwent 2% isoflurane anesthesia. The concentrations of isoflurane in blood from different surgical time points, including Pre-OP, S5, S30 and S60, along with the precision data, are summarized in Table 3. The within-run precision ranged from 5.6 to 7.6%, and the between-run values ranged from 5.2 to 10.6%.

Table 3
Precision of isoflurane analysis by the HS-GC–MS method in practical samples[a]

Time points	Found (μg/ml)	S.D. (μg/ml)	C.V. (%)
Within-run ($n=5$)			
Pre-OP	87.7	6.7	7.6
S5	103.0	7.9	7.7
S30	91.9	5.2	5.6
S60	93.1	6.12	6.6
Between-run ($n=5$)			
Pre-OP	85.2	7.1	8.4
S5	104.9	11.1	10.6
S30	89.9	6.2	6.9
S60	89.3	4.7	5.2

[a] Pre-OP, S5, S30 and S60 represent the time points prior to the operation and 5, 30 and 60 min after sternotomy, respectively.

5. Discussion

As can be observed in Fig. 1, the major ions in the mass ion spectra of isoflurane and 1,4-dioxane were, respectively, m/z 51, 67, 117 and 149, and m/z 55 and 88, which were consistent with the studies of Saito et al. [20] and Song et al. [21]. This study indicates that the proposed method has a good linearity and a wide linear range. In addition, both precision and accuracy results meet the validation requirements, i.e., C.V. and E.T.V. values of $<20\%$. The results derived from the patient's blood samples confirmed good quality in terms of precision. This method also considers the highly volatile property of isoflurane by having both the stock solution and the I.S. prepared on ice, along with the use of vacuumed tubes and gas-tight syringes, and therefore decreases the loss of isoflurane and increases the accuracy. This has not been previously discussed in the literature.

Similar results of a maximal blood concentration of around 70 μg/ml enflurane were obtained by Imbenotte et al. [22] after anesthesia with 0.75% halothane and 1.5% enflurane for 1 h. Corall et al. [23] also described the whole blood concentration as being 97 μg/ml when a patient was anesthetized for 2 h with 2.0% enflurane. According to the results in Table 3, the cardiac surgery patient in the present study who underwent 2% isoflurane anesthesia had a concentration of around 90 μg/ml in mixed venous blood. This result is comparable to the previously mentioned studies. Kuhlmann et al. [3] investigated

two deaths involving isoflurane abuse and determined the concentrations of isoflurane in the blood as 9.9 and 45.9 μg/ml. With an LOD of 1.2 μg/ml and an LOQ of 4.7 μg/ml, our proposed method is also applicable to analytical toxicology. Moreover, due to similarities in the physical properties of inhalation anesthetics, this method can be extended to the measurement of other volatile inhalation anesthetics, such as halothane, enflurane, etc.

In conclusion, we have developed a GC–MS combined with a headspace autosampler, which is simple, rapid, accurate and precise for determining the blood concentration of isoflurane. It can be applied in analytical toxicology in addition to its use in the clinical measurement of blood concentration. This method has now been successfully set up for routine tests and research work on the determination of the blood concentration of anesthetics.

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