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# Simple and rapid determination of enflurane in human tissues using gas chromatography and gas chromatography–mass spectrometry

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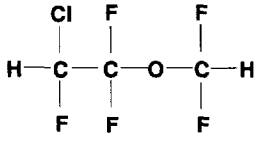
**Abstract**

A simple, rapid and reliable method was devised to determine the levels of enflurane in human tissues, using gas chromatography and gas chromatography–mass spectrometry. 1,4-Dioxane was used as an internal standard (I.S.). Enflurane and the I.S. were extracted from 0.25 g of body tissues using an automatic headspace sampler and 1 ml of headspace gas was injected into the gas chromatograph. Enflurane was analyzed qualitatively by gas chromatography–mass spectrometry and quantitatively by gas chromatography with a flame-ionization detector. The calibration curves in all tissues examined were linear in the concentration range 1–100 μg/0.25 g. The lower limit of detection was 200–300 ng/0.25 g. The accuracy and precision of this method were evaluated at two different concentrations, 1 and 20 μg/0.25 g. The coefficient of variation ranged from 3.4–13.4%. We used this method to determine the presence of enflurane in tissues from an autopsied individual who died suddenly during extirpation of a malignant tumor. © 1997 Elsevier Science B.V.

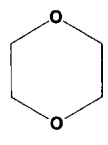
*Keywords:* Enflurane

## 1. Introduction

Enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether, Fig. 1) is a non-flammable volatile general anesthetic agent, which provides rapid induction of and rapid recovery from anesthesia. Although it is widely used, fatal cases due to abuse or misuse have been reported [1–3]. Such being the case, simple and reliable analytical methods are required in clinical and forensic practice.




**Enflurane**




**1,4-Dioxane(I.S.)**

Fig. 1. Molecular structures of enflurane and 1,4-dioxane (I.S.)

Methods to determine enflurane in human blood, include gas chromatography (GC) with electron-capture detector [4], thermal conductivity detector [5,6], flame-ionization detector (FID) [1,2,6] and mass spectrometer (MS) [7,8]. With respect to analysis of enflurane in human solid tissues, we find only one case report in which the concentrations of enflurane in the brain and adipose tissue were stated [1]. Documentation on calibration curves in the tissues and precision data of the method could not be found. A simple, rapid and reliable method has therefore been developed to determine levels of enflurane in various human tissues.

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## 2. Experimental

### 2.1. Reagents

Enflurane was provided by Dainippon Seiyaku (Osaka, Japan). 1,4-Dioxane and distilled water were purchased from Ishizu Seiyaku (Osaka, Japan) and tetra(ethylene glycol) dimethyl ether (tetraglyme) was from Wako (Osaka, Japan). All chemicals used in this study were of analytical-reagent grade.

### 2.2. Biological samples

Whole blood, brain, lung, liver, kidney, spleen, skeletal muscle and adipose tissue were obtained at the time of autopsy. All samples were stored at −20°C until analysis. Drug-free human tissues were used as controls.

### 2.3. Standard solutions of enflurane and the I.S.

Enflurane (10 g, 6.54 ml) was dissolved in 50 ml of tetraglyme to give a concentration of 200 μg/μl. This solution was further diluted to give the required concentrations. 1,4-Dioxane (I.S.) was dissolved in distilled water to give a concentration of 400 ng/μl.

### 2.4. Extraction procedure

The headspace method was used to extract enflurane. Frozen tissue samples (0.25 g) were sliced and immediately put into a headspace glass vial (approximately 10-ml volume) to avoid any decrease in enflurane. To the vial 750 μl of distilled water and 500 μl of I.S. solution (200 μg of 1,4-dioxane) were added, and the vial was immediately sealed with an aluminum cap with a silicone-lined rubber septum. All vials were allowed to equilibrate for 30 min at 55°C and 1 ml of headspace gas was injected into the GC–FID or GC–MS system.

### 2.5. Preparation of calibration curves

From the tissue samples, 5.0 g were accurately weighed and homogenized with 15 ml of distilled water. One gram of homogenate samples equivalent to 0.25 g of tissue, were prepared to contain enflurane at concentrations of 1, 5, 10, 25, 50, 75 and 100 μg/0.25 g, each containing 200 μg of I.S. Duplicate analyses were performed at each concentration. These samples were analyzed as described above. Calibration curves were obtained by plotting the peak-area ratios of enflurane to I.S. versus the amounts of enflurane.

### 2.6. GC conditions

The apparatus used was a Hewlett–Packard 5890 series II gas chromatograph equipped with FID linked to a Hewlett–Packard 7694 headspace sampler. The DB-WAX GC column (30 m×0.53 mm I.D., 1.0-μm film thickness, J&W Scientific, Folsom, CA, USA) was used. Total amount of the headspace gas (1 ml) was injected into GC. The operating temperatures were as follows: oven 35°C (isothermal), injection port and detector 150°C. Helium was used as carrier gas, and the flow-rate was maintained at 20 ml/min. The conditions of the headspace sampler were as follows: temperatures: oven, 55°C; transfer line and sample loop, 100°C; time of events: vial equilibration time, 30 min; pressurization time, 0.13 min; loop fill time, 0.15 min; loop equilibration time, 0.15 min; inject time, 0.20 min; headspace vial pressurization, 124 kPa (18.0 p.s.i.).

### 2.7. GC–MS conditions

GC–MS was carried out on a Hewlett–Packard 5890 series II gas chromatograph interfaced to a Hewlett–Packard 5989A mass spectrometer. The column, HP-WAX (30 m×0.25 mm I.D., 0.25-μm film thickness) coated with cross-linked poly(ethylene glycol) was used. A split injection mode was selected and the ratio was 30:1 with a solvent delay time of 1.0 min. The initial temperature of the column was held at 35°C for 5 min, programmed at 10°C/min to 85°C. Injection port and transfer line temperature were set at 250°C and 280°C, respectively. Helium was used as the carrier gas with a flow-rate of 1.0 ml/min. Ions of $m/z$ 117, 67 and 51 were monitored for qualification of enflurane.

## 3. Results and discussion

### 3.1. Preparation of standard solution

As the therapeutic blood concentration of enflurane was reported to be high, about 100 μg/ml [5,9], a standard solution of enflurane up to 100 μg/μl was necessary to prepare standard samples. As this drug is insoluble in water, Saito et al. [7] used 10% Tween 20 solution, a non-ionic surfactant, to dissolve enflurane. However, the required volume of enflurane was not soluble in 10 or 20% of Tween 20 solution. Gurka et al. [10] used tetraglyme, a non-volatile, water-miscible and oil-miscible solvent. We therefore used this solvent to dissolve enflurane. A high concentration of enflurane was soluble in tetraglyme, and interfering peaks on chromatograms of GC were nil from samples containing enflurane–tetraglyme solution.

### 3.2. Selection of I.S.

Methylene chloride selected as an I.S. by Saito et al. [7] and Ladron de Guevara et al. [11] was tried first. However, the peak-area of methylene chloride on the GC chromatogram was not constant in each sample, due to its extremely high volatility. This problem was overcome by selecting 1,4-dioxane as an I.S., which is widely used for alcohol analysis. As this solvent was less volatile than methylene chloride, a constant peak-area of I.S. was obtained in every sample.

### 3.3. Extraction procedure

A solvent extraction procedure using heptane as extraction solvent was chosen for the analysis of enflurane [4,5,8,12]. Although the extraction of enflurane from human tissues with heptane was easy, a large solvent peak did appear on the chromatogram. Therefore, we selected headspace method for the analysis of enflurane by GC–FID.

### 3.4. Determination of enflurane in human tissues by GC

Gas chromatograms of headspace gas from tissues containing 20 μg enflurane and 200 μg I.S. are shown in Fig. 2. Enflurane and I.S. were clearly separated with retention times of 2.6 min and 5.2 min, respectively. No significant interfering peaks were found on the chromatograms of the drug-free human tissues (Fig. 3.).

The calibration curves were linear in the concentration range 1–100 μg/0.25 g. Correlation coefficients in all tissues tested exceeded 0.99. The slope of the calibration curves in each tissue was similar, but did slightly change from day to day.

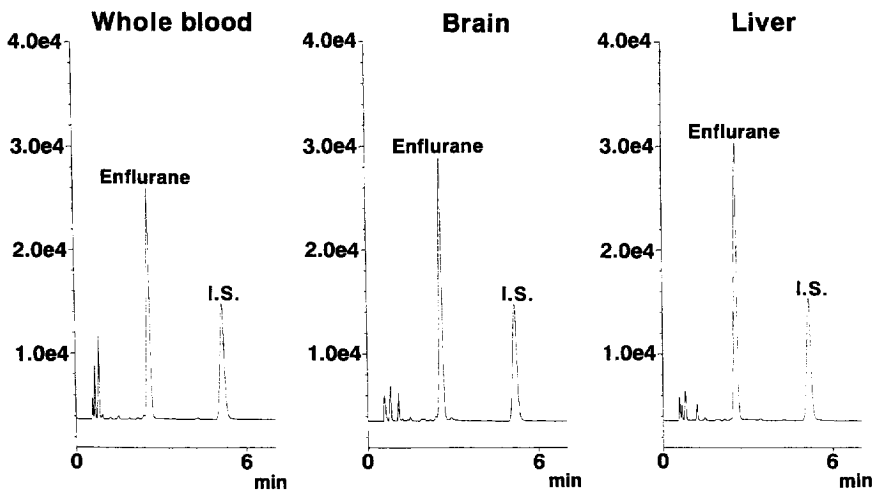


Fig. 2. Headspace gas chromatograms of extracts from human whole blood, brain and liver containing enflurane (20 μg per 0.25 g) and I.S. (200 μg).

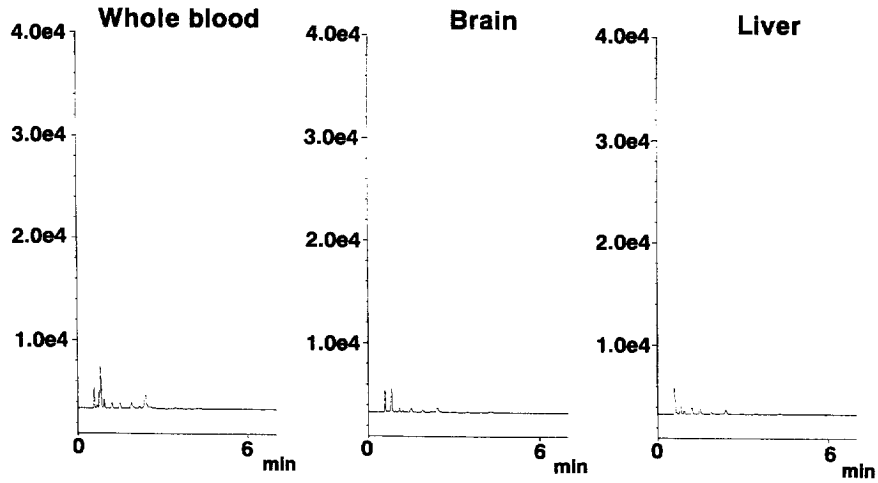


Fig. 3. Headspace gas chromatograms of extracts from drug-free human whole blood, brain and liver.

Therefore, preparation of the calibration curve on the same day of analysis of practical samples is recommended.

The lower limit of detection with a signal-to-noise ratio 2:1 was 200 ng/0.25 g in all samples examined, except for the spleen in which the value was 300 ng/0.25 g. As the therapeutic blood concentration of enflurane is about 100 μg/ml (25 μg/0.25 ml), our method is sufficiently sensitive to determine enflurane, on a practical basis.

The within-day precision of this method in various tissues at the concentrations of 1 and 20 μg/0.25 g is summarized in Table 1 ($n$=6). The coefficients of variation (C.V.) ranged from 3.4–13.4%. The between-day precision in the whole blood at the concentrations of 1 and 20 μg/0.25 g, was also evaluated and similar C.V. values, 7.7 and 8.0%, were obtained ($n$=6).

As the oven temperature of GC is isothermal and only 6 min is required for analysis of one sample, 10 samples/h can be analyzed. Furthermore, enflurane in small amounts of samples (0.25 g each) can be quantitated even with simple equipment (GC–FID). Therefore this simple, rapid method is useful not only for forensic toxicological analysis of enflurane but also for clinical monitoring of this drug.

### *3.5. Identification of enflurane in human tissues by GC–MS*

The mass spectrum of enflurane is shown in Fig. 4. The peak of enflurane was observed with a

Table 1
Within-day precision and accuracy for analysis of enflurane in human tissues

| Human tissue obtained at 12 h after death | Amount determined (mean±S.D.) (μg/0.25 g) | C.V. (%) | Amount determined (mean±S.D.) (μg/0.25 g) | C.V. (%) |
|---|---|---|---|---|
| Whole blood | 0.98±0.08 | 8.1 | 19.54±1.66 | 8.5 |
| Brain | 0.99±0.08 | 8.1 | 20.24±0.96 | 4.8 |
| Lung | 1.01±0.07 | 7.3 | 19.43±2.60 | 13.4 |
| Liver | 1.01±0.04 | 3.8 | 19.95±1.21 | 6.1 |
| Kidney | 0.98±0.06 | 5.9 | 20.15±1.04 | 5.2 |
| Spleen | 1.01±0.08 | 7.5 | 20.12±0.68 | 3.4 |
| Skeletal muscle | 0.98±0.12 | 12.5 | 19.32±2.16 | 11.2 |
| Adipose | 1.03±0.13 | 12.3 | 20.66±1.73 | 8.4 |

Added amount: 1 μg/0.25 g and 20 μg/0.25 g, $n$=6.

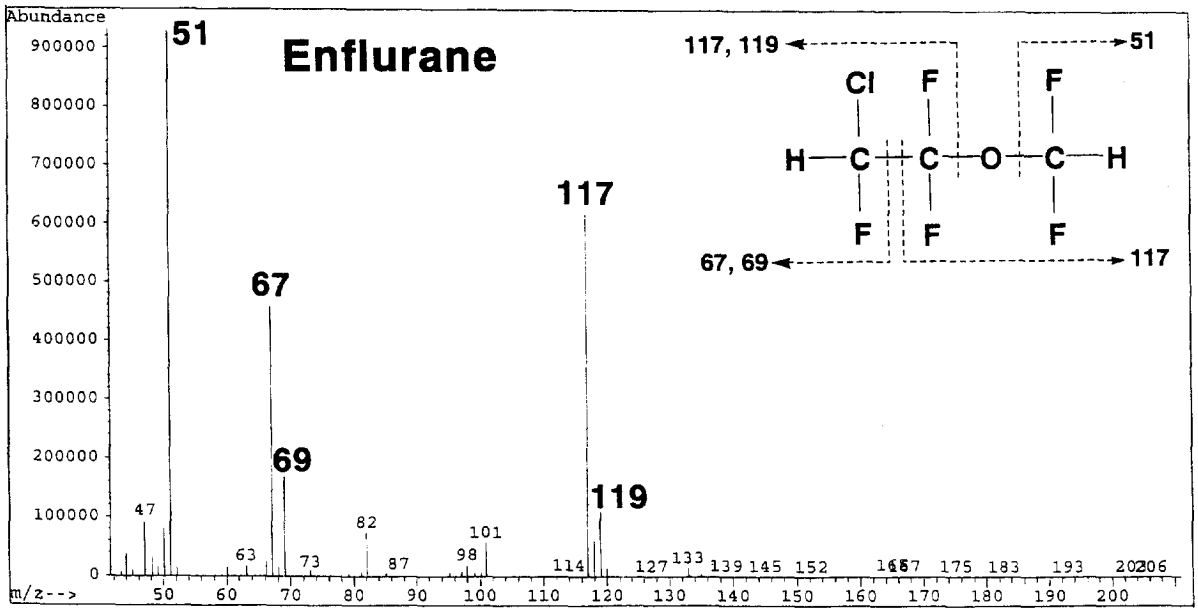


Fig. 4. Mass spectrum of enflurane examined by headspace method.

retention time of 4.2 min on the chromatogram. Characteristic fragment ions of enflurane were observed at $m/z$ 119 ($[CHClF-CF_2]^+$), $m/z$ 117 ($[CHClF-CF_2]^+$ and $[CF_2=O^+-CHF_2]$), $m/z$ 69 and 67 ($[CHClF]^+$) and $m/z$ 51 ($[CHF_2]^+$) as reported by Urich et al. [13]. As mass spectra could not be obtained with a small amount of enflurane (less than 10 μg/0.25 g), a selected ion monitoring (SIM) mode using the above 3 major ions of $m/z$ 117, 67, and 51, was used to confirm the GC peaks. All ions were clearly detected in the all tissues with no interfering peaks. Therefore, this GC–MS method is useful to identify enflurane in forensic practice. Although we chose GC–FID for the quantitation of enflurane because of its simplicity and short analytical time, this GC–MS method can be applicable to quantify enflurane.

## 4. Practical application

A 64-year-old man underwent radical cystectomy. During the surgery of a malignant tumor, cardiac arrest suddenly occurred and he was sent to an emergency hospital. He soon died and forensic autopsy was carried out 12 h after death under the suspicion of malpractice. Findings of pathological disorders were nil. According to the clinical record, the patient had been anesthetized with 1.8% Ethrane® (enflurane) for 3.5 h.

The whole blood sample was analyzed by GC–MS with a SIM mode using 3 monitoring ions of $m/z$ 117, 67 and 51. All ions were clearly detected at 4.2 min, and the presence of enflurane was confirmed. The concentration of enflurane in each tissue obtained by triplicate analyses with GC–FID is shown in Table 2. Miller and Gandolfi [5] reported that the whole blood concentrations of enflurane in 4 anes-

Table 2
Enflurane concentrations in autopsied human tissues

| Sample | Concentration (μg/g) |
|---|---|
| Whole blood | 78.5 |
| Brain | 47.5 |
| Lung | 8.4 (44.8)[a] |
| Liver | 95.9 |
| Kidney | 45.3 |
| Spleen | 65.5 |
| Skeletal muscle | 144.6 |
| Adipose | 397.3 |

[a] The value in parentheses is the concentration of enflurane in the lung where the post-mortem hypostasis presumably occurred.

thetized patients were 44–144 μg/ml. Corall et al. [9] also described the whole blood concentration as being 97 μg/ml when a patient was anesthetized for 2 h with 2.0% enflurane. Therefore, whole blood concentration of 78.5 μg/g in our autopsied patient was a therapeutic level, and overdose by enflurane was ruled out.

The concentrations of enflurane in the skeletal muscle was twice that in the whole blood, and the highest concentration of enflurane was in adipose tissue, the value being 397.3 μg/g.

Jacob et al. [1] reported a fatal case of enflurane intoxication where the concentrations of enflurane in the blood, brain and subcutaneous fat were 130 μg/ml, 350 and 100 μg/g, respectively. Although they found the lowest concentration of enflurane to be in subcutaneous fat, redistribution of this drug was suggested as the victim was autopsied 3.5 days after death. In our case, the highest concentration of enflurane was found in adipose tissue. As enflurane accumulates in the muscle and adipose tissue along with the continuous anesthesia, according to Dripps et al. [14], and as our tissue sample was collected 12 h after death, our data are supposedly close to the actual distribution of enflurane at the time of death.

## 5. Conclusion

We have developed a reliable, simple and rapid method for the determination of enflurane in various human solid tissues, using GC and GC–MS. Enflurane was effectively extracted from only 0.25 g of sample using an automatic headspace sampler, and analyzed qualitatively by GC–MS and quantitatively by GC–FID. The established method offers high sensitivity and reliable reproducibility of the results, and should prove very useful for forensic and clinical measurements of enflurane in human tissues.

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