Journal of Chromatography B, 878 (2010) 45–50

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Simple assay of plasma sevoflurane and its metabolite hexafluoroisopropanol by headspace GC–MS

Daniel Bourdeaux[a,b,*], Valérie Sautou-Miranda[a,b], Agnès Montagner[a], Sébastien Perbet[c], Jean Michel Constantin[c], Jean-Etienne Bazin[c], Jean Chopineau[a,b]

[a] CHU Clermont-Ferrand, Department of Pharmacy, Hôp G Montpied, F-63003 Clermont-Ferrand, France
[b] Univ. Clermont 1, Faculty of Pharmacy, Laboratory of Clinical Pharmacy and Biotechnics, 28 Place Henri Dunant, F-63001 Clermont-Ferrand, France
[c] CHU Clermont-Ferrand, Surgical Intensive Care Unit, Department of Anesthesiology and Critical Care Medicine, Hôtel-Dieu, F-63000 Clermont-Ferrand, France

ARTICLE INFO

Article history:
Received 25 March 2009
Accepted 11 November 2009
Available online 28 November 2009

Keywords:
Sevoflurane
HFIP
Headspace gas chromatography
Mass spectroscopy
Pharmacokinetic study
Anesthetic conserving device

ABSTRACT

The anesthetic sevoflurane can now be delivered over periods of up to 48 h using a newly developed medical system, the AnaConDa (anesthetic conserving device). Lack of pharmacokinetic data on sevoflurane and its main metabolite (hexafluoroisopropanol, HFIP) in this indication prompted us to develop a headspace GC–MS method to quantify the two substances. The only previously published method for assaying the two substances could not be adapted to our study since it uses expensive and rarely employed system components together with toxic carbon disulfide as a dilution solvent. The method developed is straightforward and uses the relatively non-toxic solvent undecane as dilution solvent and chloroform as internal standard. The method is linear for a concentration range of 1–150 μg/ml, and presents high accuracy and precision. LOD and LOQ are 0.2 and 1 μg/ml, with a short analysis time (7.6 min for a single analysis). The method was applied to determine the plasma levels of sevoflurane and HFIP in six patients under 48-h anesthetic sedation delivered via the AnaConDa system. Average sevoflurane and HFIP concentrations plateaued at 75 and 4 μg/ml, respectively. Sevoflurane quickly tailed off after inhalation was stopped, and HFIP levels remained low.

1. Introduction

Sevoflurane is a halogenated inhalational anesthetic widely used in anesthesiology due to its rapid offset and cardioprotective effects [1]. The use of halogenated agents has rarely been explored as an anesthetic strategy for ICU patients due to technical difficulties with administration. A new medical delivery system, the anesthetic conserving device (or AnaConDa for short), which evaporates and conserves the halogenated vapor in a respiratory filter opens up perspectives for using halogenated agents for ICU patient sedation over periods of several days [2–4]. The time-course patterns of the plasma concentrations of sevoflurane and its main metabolite hexafluoroisopropanol (HFIP) [5] have been determined for anesthetic sedation only over periods of a few hours [6–8]. The pharmacokinetic profile of sevoflurane for sedation periods up to 48 h also remains unknown. The few validated chromatographic assay techniques capable of simultaneously determining sevoflurane and HFIP concentrations are often highly sophisticated and ill-suited to pharmacokinetic studies [9]. This study was designed to develop a simple, rapid technique, coupling headspace gas chromatography and mass spectrometry for the reliable assay of a broad range of sevoflurane and free HFIP concentrations. This broad range-span was designed to make it possible to run pharmacokinetic studies and at the same time highlight any drug accumulation in the blood.

2. Experimental

2.1. Chemicals and reagents

Undecane (dilution solvent), HFIP and chloroform (internal standard) were purchased from Fluka chemicals (USA). Sevoflurane was provided by Abbott Labs (Sevorane®). All analytes and their solvents were kept stored at 4 °C to minimize risk of evaporation when later used.

2.2. Preparation of the calibration standard

Standard plasma solutions were prepared by taking 1 ml of blank plasma (E.F.S. Clermont-Ferrand, France) and adding 20 μl of aqueous solution containing HFIP and 20 μl of solution in undecane containing sevoflurane and chloroform (internal stan-

* Corresponding author at: CHU Clermont-Ferrand, Department of Pharmacy, Hôp G Montpied, F-63003 Clermont-Ferrand, France.
E-mail address: dbourdeaux@chu-clermontferrand.fr (D. Bourdeaux).

1570-0232/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2009.11.018

Table 1
Hadamard matrix, seven factors, eight experiments.

Experiments	X_1	X_2	X_3	X_4	X_5	X_6	X_7
1	1	1	1	−1	1	−1	−1
2	−1	1	1	1	−1	1	−1
3	−1	−1	1	1	1	−1	1
4	1	−1	−1	1	1	1	−1
5	−1	1	−1	−1	1	1	1
6	1	−1	1	−1	−1	1	1
7	1	1	−1	1	−1	−1	1
8	−1	−1	−1	−1	−1	−1	−1

X_n: factors of the experimental design.

dard). These aqueous and undecane solution was concentrated at 50, 250, 1000, 2500 and 7500 μg/ml of sevoflurane and HFIP and 600 μg/ml of chloroform to prepare the five calibration standard points (1, 5, 20, 50 and 150 μg/ml of sevoflurane and HFIP, 12 μg/ml of chloroform as internal standard). The plasma solutions obtained were stored in 20 ml glass headspace tubes (Antélia Lyon, France) screwed hermetically tight with a Teflon-sealed cap. These standards solutions were used to carry out the validation process.

2.3. Equipment setup

The sample to be assayed was introduced into a HS40 headspace system (PerkinElmer, USA) coupled to a gas chromatograph in tandem with a Clarus 500 mass spectrometer (PerkinElmer, USA). The system was run with helium N55 as carrier gas with a flow rate of 20 psi (Saga, France). The column was an Elite VMS (30 m × 1.4 μm × 0.25 mm ID) (PerkinElmer, USA) featuring intermediate polarity. The split rate was set at 20 ml/min.

Optimal operating variables for the method were determined using an experimental design described in Section 2.4.

2.4. Chromatographic and headspace optimization process

Preliminary tests showed that we needed a relatively low temperature plateau (temperature 1: maximum 100 °C) at the beginning of the analysis to retain the sevoflurane on our column (see Section 3.1 for details). We set the duration of this period at 1.5 min. By contrast, if we wanted a short analysis time we had to raise the temperature rapidly to lower the retention time of the other analytes (temperature 2). Finally, a third high temperature plateau was necessary to eliminate the solvent (undecane), which was strongly retained on the column (we set it at 200 °C).

To optimize the chromatographic variables and the headspace procedure, we used an experimental design based on a Hadamard matrix (Table 1) to reduce the number of experiments necessary to evaluate the influence of seven headspace (HS) and gas chromatographic (GC) variables (four HS and three CG) on four decisive characteristics of the method, which we call responses. These are listed below together with their ranges of variation:

- X_1: HS thermostating temperature (65–75 °C).
- X_2: HS thermostating time (10–20 min).
- X_3: HS pressurization time (2–6 min).
- X_4: HS Needle injection times (0.2–0.3 min).
- X_5: GC oven temperature 1 (60–80 °C).
- X_6: GC oven temperature ramp (25–45 °C min^{-1}).
- X_7: GC oven temperature 2 (125–175 °C).

The experiments are summarized in Table 2.

The influence of the variation of these variables was estimated on four responses:

- R_1: sevoflurane and chloroform peak resolution.
- R_2: efficacy for the sevoflurane peak, based on the calculation of the height equivalent to a theoretical plateau.
- R_3: sensitivity, based on the sum of the analyte peak areas.
- R_4: analysis time, based on the retention time of the HFIP.

These experiments were conducted on a standard solution of 20 μg/ml sevoflurane and HFIP and 12 μmol/ml chloroform in blank plasma. We then plotted the cumulated Pareto graphics to evaluate the influence of these variables on each of the four responses.

This permitted us to determine best set of compromise values for these seven variables (see Section 3.1 for details).

2.4.1. Mass spectrometry

After scanning for ions with a mass-to-charge ratio (m/z) between 60 and 400, we determined three single-ion monitoring (SIM) sequences for each target molecule based on the most specific and abundant masses. The m/z ratios adopted for running the three SIM sequences were:

SIM1 (sevoflurane): from 0 to 2.2 min: m/z 79, 131 and 181.
SIM2 (chloroform): from 2.2 to 3.5 min: m/z 83, 85 and 118.
SIM3 (HFIP): from 3.5 to 4.5 min: m/z 79, 99 and 129.

A solvent delay was set from 4.6 to 7.8 min at the retention time of undecane.

For quantitative analysis, all three m/z ions were monitored for each analyte and their ratios were checked with the software. The quantification process was performed using the sum of the three m/z ions.

2.5. Results analysis

The GC/MS system was interfaced with TurboMass GC/MS software (PerkinElmer, USA), and the samples were quantified using

Table 2
Summary of the conditions for each experiment.

Experiments	X_1 (°C)	X_2 (min)	X_3 (min)	X_4 (min)	X_5 (°C)	X_6 (°C min^{-1})	X_7 (°C)
1	75	20	6	0,2	80	25	125
2	65	20	6	0,3	60	45	125
3	65	10	6	0,3	80	25	175
4	75	10	2	0,3	80	45	125
5	65	20	2	0,3	80	45	175
6	75	10	6	0,2	60	45	175
7	75	20	2	0,3	60	25	175
8	65	10	2	0,2	60	25	125

X_1: HS thermostating temperature. X_2: HS thermostating time. X_3: HS pressurization time. X_4: HS injection time. X_5: GC temperature 1 X_6: GC temperature ramp 1. X_7: GC temperature 2.

an internal calibration method based on chloroform as calibration standard. The plasma concentration–time curves were plotted using Excel (Microsoft, USA).

2.6. Sampling technique and matrix selection

Preliminary trials conducted before the study showed that assay repeatability was better on plasma than on total blood. However, as demonstrated by Yang et al. [10], sample handling during centrifugation, combined with residual air in the sampling tubes, is liable to cause analyte losses. We assessed analyte loss by comparing blood samples taken with gas-tight syringes (without residual air) with samples taken in vacuum tubes (with approximately 0.5 ml of residual air for 5 ml of liquid blood). The samples were taken from the venous catheter fitted in the first patient included in the clinical study, using the protocol below. Three samples were collected for each condition.

- 1 ml of whole blood was sampled in a gas-tight syringe (air-free) and the sample was transferred to a headspace tube containing 20 μl of internal standard solution and hermetically sealed.
- 1 ml of whole blood was sampled in a vacuum tube and the sample was transferred to a headspace tube containing 20 μl of internal standard solution and hermetically sealed.
- 1 ml of plasma obtained by centrifugation of whole blood was sampled in a gas-tight syringe and the sample was transferred to a headspace tube containing 20 μl of internal standard solution and hermetically sealed. The syringe was placed directly inside the centrifuge without transferring the contents into a tube, thus avoiding exposure to air. Then 1 ml of plasma was transferred rapidly to the headspace tube.
- 1 ml of plasma obtained by centrifugation of whole blood was sampled in a vacuum tube and the sample was transferred into a headspace tube containing 20 μl of internal standard solution and hermetically sealed.

2.7. Validation

2.7.1. Calibration curve and linearity

Plasma concentrations of sevoflurane used as an anesthetic [6–8] can reach 150 μg/ml for a level of sevoflurane in exhaled air of 2.5%. The sevoflurane level in exhaled air studied under sedation was 1.5%, which makes it reasonable to assume that a calibration standard can be set with an upper bound of 150 μg/ml. The calibration curve was therefore plotted from five calibration standard points (1, 5, 20, 50 and 150 μg/ml of sevoflurane and HFIP, 12 μg/ml of chloroform as internal standard). Linearity was estimated by the coefficient of determination calculated from the average calibration curve ($n = 5$).

2.7.2. Limits of detection (LOD) and quantification (LOQ)

The LOD (concentration giving a signal-to-noise ratio of less than 3) and LOQ (defined as the lowest concentration at which the quantification obtained with precision and repeatability at a factor of less than 20%) were determined.

2.7.3. Precision and accuracy

The precision of the assay method was assessed by repeatability and reproducibility over three concentrations (1, 5 and 150 μg/ml). The coefficients of variation were calculated based on quintuplicate runs for each concentration, analyzed on the same day for repeatability and on different days for reproducibility. Standard deviation from the set-point value was determined for each concentration.

2.7.4. Sample stability

Sample stability in response to freezing was determined on plasma samples at concentrations 1, 5 and 150 μg/ml. At each concentration, assays were run on five samples immediately after preparation (benchmark concentration) and then on five samples 15 days after freezing at −20 °C. A sample was considered stable if its average sevoflurane and HFIP concentrations measured after freezing varied by no more than 10% from the benchmark concentration and if there was no evidence of degradation.

2.8. Application

The method was used to assay plasma sevoflurane and HFIP in six ICU patients presenting no kidney or liver damage and who needed 48-h-plus sedation. The experiment was approved by the local Research Ethics Committee. The patients were placed under sedation for 48 h. A total of 13 samples per patient were taken during sedation and then for 6 h after the anesthetic was withdrawn. We recorded the time-course patterns of plasma sevoflurane and HFIP concentrations.

3. Results

3.1. Experimental plan

Fig. 1 shows the example of the cumulated Pareto diagram on the resolution between sevoflurane and chloroform peaks (R_1). Each bar of the diagram expresses the percentage variation of the R_1 response due to each variable. The percentages are ranked from the highest to the lowest and are cumulated. In this example, we can see that X_5 (GC oven temperature 1), X_4 (HS injection time), and X_2 (HS thermostating time) explain 95% of the variation of R_1 values in the experimental design. Table 3 recapitulates all the Pareto diagrams for the four responses.

This study enabled us to choose the best compromise values for the seven variables to optimize the four responses chosen. This gives the GC and HS variables described below.

3.1.1. Headspace variables

The oven, needle and transfer line temperatures were 95, 110 and 180 °C, respectively. Thermostating time, pressurization time and needle injection time were 20, 3, and 0.03 min, respectively.

3.1.2. Chromatographic variables

The temperature program started with the oven at 60 °C for 1.5 min. The temperature was then ramped up at a rate of 45 °C/min

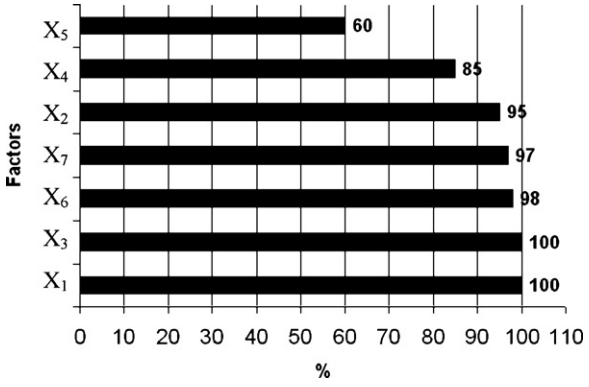

Fig. 1. Example of the Pareto diagram for the R_1 response (sevoflurane and chloroform peak resolution). X_1: HS thermostating temperature. X_2: HS thermostating time. X_3: HS pressurization time. X_4: HS injection time. X_5: GC temperature 1. X_6: GC temperature ramp 1. X_7: GC temperature 2.

Table 3
Summary of the major Pareto coefficients.

	X_1	X_2	X_3	X_4	X_5	X_6	X_7
Sevoflurane and chloroform peak resolution	n.s.	−10%	n.s.	−25%	−60%	n.s.	n.s.
Efficacy for the sevoflurane peak	n.s.	−10%	4%	−47%	−34%	n.s.	n.s.
Sensitivity	46%	15%	−6%	25%	n.s.	n.s.	n.s.
Analyze time	n.s.	n.s.	n.s.	n.s.	−87%	−11%	n.s.

Negatives coefficients: an increase of the factor lead to a decrease of the response; positives factors: an increase of the factor leads to an increase of the response. n.s.: non-significant.

Table 4
Relative standard deviation (RSD) estimation for the quantification of the two matrixes tested: whole blood matrix and plasma matrix.

	Whole blood matrix				Plasma matrix			
	Vacuum tube		Gas-tight syringe		Vacuum tube		Gas-tight syringe	
	Sevoflurane	HFIP	Sevoflurane	HFIP	Sevoflurane	HFIP	Sevoflurane	HFIP
Mean (μg/ml)	91.95	4.42	69.42	3.20	74.48	4.03	68.14	3.55
ETV (μg/ml)	12.62	0.61	14.24	0.61	6.40	0.30	2.08	0.23
RSD (%)	13.73	13.72	20.51	18.94	8.59	7.54	3.05	6.59

The results of the use of the two sample devices tested are shown: mean values ($n = 3$), standard deviation (SD) and RSD.

to 110 °C, and held for 1.5 min. The oven temperature was then increased to 200 °C in a second ramp-up phase at 45 °C/min, and held for a further 1.5 min (7.6 min for the temperature program and 12 min including re-equilibrium time).

3.2. Sampling technique and matrix selection

Table 4 summarizes the results of the sampling study.

The assays on whole blood showed high variability (coefficients of variation reaching 20%), and so they could not be exploited. By contrast, the assays on plasma samples presented coefficients of variation of 8% for sevoflurane and 7.5% for HFIP.

Analyte loss caused by the plasma processing step remained minimal. Sampling in vacuum tubes and then centrifuging the plasma and transferring it to headspace tubes did not cause any significant loss of analytes. The difference in assay results between the two collection methods (gas-tight syringes *versus* vacuum tubes) was approximately 10% (8.5% for sevoflurane and 12% for HFIP), and therefore remained within the bounds of variability obtained with the plasma sample assay.

Following these tests, we elected to carry out assays of plasma samples using the following protocol:

The patient's blood was sampled from a venous catheter directly in a 5 ml vacuum tube, taking extreme care to limit residual air. The blood was then promptly centrifuged for 3 min at 5000 rpm. 1 ml of plasma was recovered and transferred to a 20 ml headspace tube (Antélia, France), and 20 μl of the 600 μg/ml chloroform–undecane solution was added (the final concentration of internal standard being 12 μg/ml). The tube was immediately screwed hermetically tight with a Teflon-sealed cap and frozen at −20 °C until analysis.

As there is binding of the HFIP in the erythrocytes (5) *in vivo* we did not conduct a spiking experiment on whole blood: this binding is impossible to reproduce *in vitro*. For this reason, and after the sampling technique and matrix selection experiments (Section 2.6), we decided to work on the quantification of the plasma phase of the analytes. This represents the biologically active phase when the substances can display toxicity.

3.3. Chromatograms and mass spectra

Fig. 2 presents the chromatograms of the compounds together with the relative proportions of the three ions chosen for each molecule.

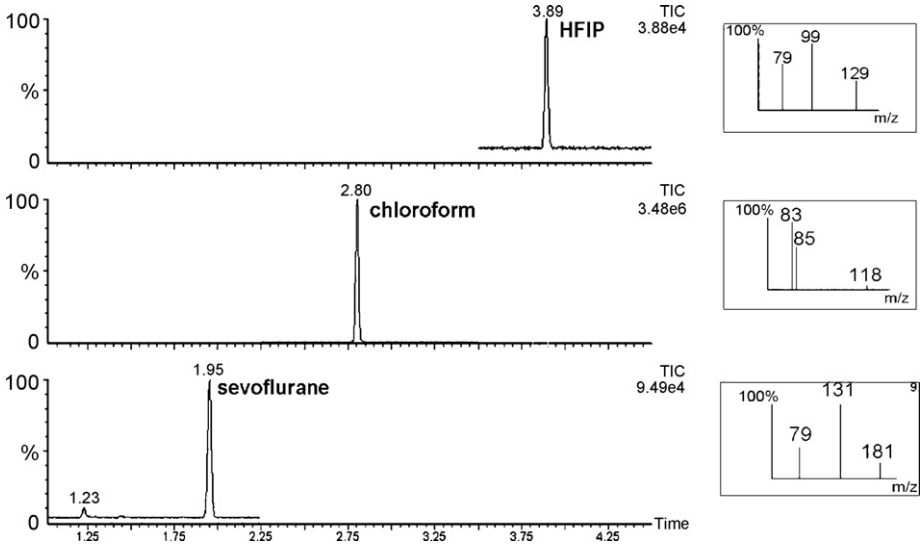

Fig. 2. Chromatograms and mass spectra (SIM mode) of a solution of 1 μg/ml of sevoflurane and HFIP and 12 μg/ml of chloroform.

Table 5
Within run and between run accuracy and precision of the assay method.

Compounds	Spiked (μg/ml)	Between run				Within run			
		Found (μg/ml)	SD (μg/ml)	RSD (%)	ETV (%)	Found (μg/ml)	SD (μg/ml)	RSD (%)	ETV (%)
Sevoflurane	1	0.99	0.07	7.03	0.40	1.05	0.12	11.67	5.44
	20	20.09	1.01	5.04	0.47	18.99	2.09	11.01	5.03
	150	151.50	5.07	3.35	1.00	151.50	5.07	3.35	1.00
HFIP	1	1.11	0.11	9.75	10.80	1.14	0.10	8.72	14.44
	20	19.51	1.62	8.33	2.47	19.13	1.03	5.37	4.35
	150	145.69	7.42	5.09	2.88	150.11	8.82	5.87	0.07

SD: standard deviation, RSD: relative standard deviation, ETV: error from theoretical value.

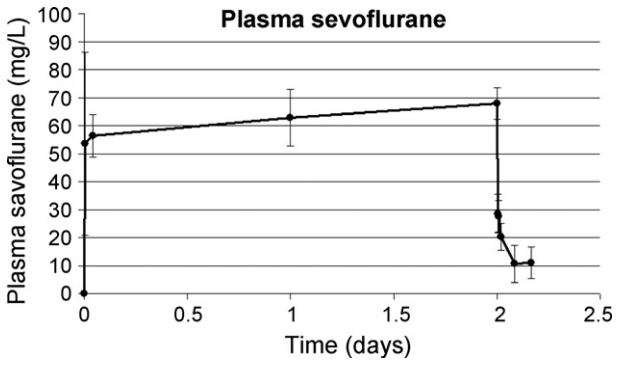

Fig. 3. Time-course patterns of plasma sevoflurane concentrations ($n = 6$ mean ± standard deviation).

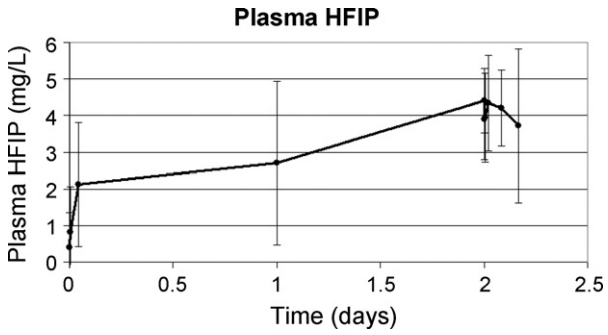

Fig. 4. Time-course patterns of plasma HFIP concentrations ($n = 6$ mean ± standard deviation).

Peak separation was excellent; the retention times of sevoflurane, chloroform and HFIP were 1.95, 2.80 and 3.89, respectively.

3.4. Validation

3.4.1. Linearity

The method was linear for a concentration range from 1 to 150 μg/ml. The calibration plot gave a coefficient of determination r^2 greater than 0.998.

3.4.2. Limits of detection (LOD) and quantification (LOQ)

LOD was 0.2 μg/ml for sevoflurane and HFIP, and LOQ for both compounds was 1 μg/ml.

3.4.3. Precision and accuracy

The precision and accuracy results are reported in Table 5.

3.4.4. Sample stability in response to freezing

Stability in response to freezing was demonstrated by the variation of only 10% between the assays carried out on the day of sample preparation and those performed after 15 days in deep-freeze.

3.5. Application

Figs. 3 and 4 show the average plasma sevoflurane and HFIP concentrations across the six patients studied for 2 days of administration.

4. Discussion

We have developed a simple, rapid, inexpensive method that is well-geared to pharmacokinetic studies as it can be used to assay sevoflurane and its metabolite HFIP simultaneously. Accorsi et al. [9] have also reported a method for simultaneously determining sevoflurane and HFIP levels that presents the advantage of being able to detect small enough quantities to be used for gauging nursing staff exposure to released anesthetic gases [11]. However, the authors employed technology (stir-bar sorptive extraction; SBSE) that is too expensive to make it a viable option for pharmacokinetic studies in anesthesia or sedation management. The system that Accorsi et al. used (coated stir-bar) to concentrate the samples and improve sensitivity relies on expensive, rarely employed components, whereas our technique uses fairly inexpensive consumables.

Our analytical technique also produces results very rapidly (4 min for the HFIP peak) and with a total time of 7.6 min (12 min including the re-equilibrium time); this takes 10.4 min off analysis time compared with Accorsi et al. [9] (18 min without the re-equilibrium time), while maintaining outstanding separation of compounds.

Focusing on HFIP quantification, several authors [9,12] have exploited techniques based on conjugated HFIP deglucuronidation, which is a key step in quantifying total matrix content of HFIP. However, our study is not focused on total HFIP levels. Using sevoflurane over periods greater than those used in anesthesia management may well lead to detoxification of this halogenated agent. At this point, two problems can arise: an increase in plasma sevoflurane levels due to a decrease in sevoflurane metabolism, or an increase in free HFIP due to less HFIP bonding with glucuronic acid, which could generate a risk of reaching toxic concentrations. No increase in free HFIP was observed. Therefore, as demonstrated by Kharash et al. [6], free-form HFIP accounts for 15% of total HFIP. Thus total plasma concentrations of the HFIP metabolite can easily be estimated by extrapolating from the measured levels of free-form HFIP.

In addition, we sought a more suitable sevoflurane dilution solvent than the carbon disulfide used by Accorsi et al. [9]. Carbon disulfide solution presents several drawbacks, from its toxicity (risk phrases: R12 extremely flammable; R23 toxic by inhalation; R24 toxic in contact with skin and R25 toxic if swallowed) [13–17] to its low vaporization point (boiling point: 46 °C; vapor pressure (20 °C): 300 mmHg).

Undecane is a fairly non-toxic solvent (risk phrases: R36 irritating to eyes; R37 irritating to respiratory system and R38 irritating) that will dissolve sevoflurane and the internal standard (chloro-

form). Undecane also possesses a high vaporization point (boiling point: 196 °C; vapor pressure (20 °C): <0.4 mmHg), which offers the advantage of minimizing the potential for headspace saturation during the headspace ramp-up sequences. The experimental design work during the development of our method showed that the optimal temperature for completely vaporizing the analytes was 90 °C. Carbon disulfide will vaporize at this temperature, but not undecane.

Also, the low vapor pressure and high boiling point of undecane help reduce the instability of the calibration solutions by minimizing solvent evaporation.

We also optimized the internal standard selected. Given that the analytes are highly volatile, we opted for a molecule presenting physical properties (and most importantly, vaporizability) that most closely matched those of the target analytes. The step in which the samples are handled before the assay is the most critical step in the method, as it is here that there is a risk of evaporation. Accordingly, we opted for chloroform. Unlike 1,4-dioxane, chosen by Yang et al. [10] (vapor pressure: 27 mmHg; boiling point: 120 °C) and butyl chloride, used by Cunningham et al. [18] (vapor pressure: 101 mmHg; boiling point: 78 °C), chloroform (vapor pressure: 159 mmHg; boiling point: 61.2 °C) shares very similar physical properties with sevoflurane (vapor pressure: 157 mmHg; boiling point: 58.6 °C) and HFIP (vapor pressure: 120 mmHg; boiling point: 58 °C), thus ensuring similar behavior during the phases preceding injection into the chromatography system, and in particular during the headspace temperature and pressure programs.

We elected not to use another halogenated anesthetic such as enflurane, used by Accorsi et al. [9], since the patients in our study were ICU patients who regularly transited through other hospital wards where this gas might have been in use; this would have altered the signal responses of the internal standard during the assays.

The critical point in our method may be the plasma collection and separation protocol, as there is a risk that compounds may evaporate during sampling in vacuum tubes. Our research on the blood-sampling technique did not reproduce the values cited by Yang et al. [10] for the decrease in sevoflurane concentration estimated by a calculation based on the blood-gas partition coefficient (there are no published figures on the blood-gas partition coefficient of HFIP). Yang et al. reported a 17% drop in sevoflurane concentration at equilibrium (at a residual volume of 0.7 ml for 5 ml of liquid). In our study, a small volume of air remained in the vacuum tubes (approximately 0.5 ml for 5 ml of liquid); the differences between the values obtained with the sample taken with the gas-tight syringes (without residual air) and those taken with the vacuum tubes were estimated at 8.5% and 12% for sevoflurane and HFIP, respectively. These figures are comparable to the coefficients of variation values of 8–10% determined when validating the precision of the method. We therefore opted to ignore this effect. There are several possible explanations for this difference. The blood-gas partition coefficient of 0.68 does not match the conditions of our sampling protocol, as it corresponded to a temperature of 37 °C [19]. However, in our sampling and centrifugation procedure, there is a drop in sample temperature, which leads to an increase in the blood-gas partition coefficient and thus reduces the effect of evaporation. Also, 0.68 was a value calculated on whole blood, whereas with our method, the whole blood is centrifuged, so that only plasma is left in contact with the residual air. The literature does not cite any plasma-gas partition coefficients. Finally, we took special care to ensure that the sequence of operations following blood collection was completed as quickly as possible to keep residual air volume to a minimum. Taken together, these measures will tend to minimize the risks of vaporization.

The first test runs using this technique confirmed that it was well-geared to our study. Figs. 3 and 4 show there was no build-up of either sevoflurane or HFIP in the patients' plasma. Sevoflurane concentrations plateaued at an average of 75 μg/ml in about 6 h of inhalation. The figures also highlight how sevoflurane levels quickly tail off (the peak at-plateau concentration was halved in only 5 min after stopping product inhalation), consistent with the speed at which the patients wake up. Free HFIP levels remained low, averaging less than 4 μg/ml. Extrapolating from Kharash et al. [6], 4 μg/ml free HFIP would correspond to a HFIP total plasma concentration of 26.7 μg/ml. There are apparently none of the glucuronidation process accumulation or saturation effects that might have been feared under extended periods of sedation.

5. Conclusion

The technique we have developed for simultaneously determining sevoflurane and HFIP levels is particularly well adapted to gathering the data required for pharmacokinetic modeling. It possesses the necessary degree of sensitivity, while at the same time remaining linear for the high plasma concentrations reached in patients sedated for 48 h periods. Preliminary assays performed with the method have demonstrated that there is no product accumulation in patient plasma. These results, which have been incorporated into a larger-scale clinical trial, are expected to make it possible to describe pharmacokinetic models for sevoflurane and HFIP in this indication.

References

[1] G. Landoni, O. Fochi, G. Torri, Curr. Vasc. Pharmacol. 6 (2008) 108.
[2] P.V. Sackey, C.R. Martling, F. Granath, P.J. Radell, Crit. Care Med. 32 (2004) 2241.
[3] A. Tempia, M.C. Olivei, E. Calza, H. Lambert, L. Scotti, E. Orlando, S. Livigni, E. Guglielmotti, Anesth. Analg. 96 (2003) 1056.
[4] M. Enlund, H. Lambert, L. Wiklund, Acta Anaesthesiol. Scand. 46 (2002) 506.
[5] E.D. Kharasch, A.S. Armstrong, K. Gunn, A. Artru, K. Cox, M.D. Karol, Anesthesiology 82 (1995) 1379.
[6] E.D. Kharasch, M.D. Karol, C. Lanni, R. Sawchuk, Anesthesiology 82 (1995) 1369.
[7] H. Wissing, I. Kuhn, S. Rietbrock, U. Fuhr, Br. J. Anaesth. 84 (2000) 443.
[8] C.C. Lu, C.S. Tsai, S.T. Ho, W.Y. Chen, C.S. Wong, J.J. Wang, O.Y. Hu, C.Y. Lin, Anaesthesia 58 (2003) 951.
[9] A. Accorsi, B. Morrone, M. Benzo, C. Gandini, G.B. Raffi, F.S. Violante, J. Chromatogr. A 1071 (2005) 131.
[10] N.C. Yang, K.L. Hwang, C.H. Shen, H.F. Wang, W.M. Ho, J. Chromatogr. B 759 (2001) 307.
[11] A. Accorsi, A. Barbieri, G.B. Raffi, F.S. Violante, Int. Arch. Occup. Environ. Health 74 (2001) 541.
[12] S.E. Morgan, E.J. Frink, A.J. Gandolfi, Anesthesiology 80 (1994) 201.
[13] C.C. Huang, Acta Neurol. Taiwan 13 (2004) 3.
[14] R.C. Sills, G.J. Harry, W.M. Valentine, D.L. Morgan, Toxicol. Appl. Pharmacol. 207 (2005) 245.
[15] Z.P. Wang, K.Q. Xie, H.Q. Li, Chin. J. Ind. Hyg. Occup. Dis. 23 (2005) 139.
[16] V. Morvai, E. Szakmary, G. Ungvary, J. Toxicol. Environ. Health A 68 (2005) 797.
[17] R. Vermeulen, B.A. Jonsson, C.H. Lindh, H. Kromhout, Int. Arch. Occup. Environ. Health 78 (2005) 663.
[18] D.D. Cunningham, J. Webster, D. Nelson, B. Williamson, J. Chromatogr. B: Biomed. Appl. 668 (1995) 41.
[19] D.P. Strum, E.I. Eger II, Anesth. Analg. 66 (1987) 654.