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Determination of sevoflurane and its metabolite hexafluoroisopropanol by direct injection of human plasma into gas chromatography–tandem mass spectrometry

Janka Kubincová[a,f], Alexandra Szabóová[a], Peter Podolec[a], Jaroslav Blaško[a], Ladislav Soják[a], Renáta Górová[a], Gabriela Addová[a], Ivan Ostrovský[a], Jozef Višňovský[b], Anton Amann[c,d], Paweł Mochalski[c,d,g], Judith Höfer[c], Arnulf Benzer[c], Darina Behúlová[e], Róbert Kubinec[a,*]

[a] Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina CH-2, SK-84215 Bratislava, Slovakia
[b] Synthcluster s.r.o., Komenského 14, SK-90001 Modra, Slovakia
[c] Univ.-Clinic of Anesthesia, Innsbruck Medical University, Anichstrasse 35, A-6020 Innsbruck, Austria
[d] Breath Research Institute, Austrian Academy of Sciences, Rathausplatz 4, A-6850 Dornbirn, Austria
[e] Department of Laboratory Medicine, University Children's Hospital, Limbová 1, SK-83340 Bratislava, Slovakia
[f] Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University, SK-83232 Bratislava, Slovakia
[g] Institute of Nuclear Physics PAN, Radzikowskiego 152, PL-31342 Kraków, Poland

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ABSTRACT

The developed method for trace analysis of volatile components in plasma allows direct injection of up to 150 samples to the GC–MS/MS system without injector cleaning. This method requires no modification of plasma and the working environment does not interfere with the determination of these analytes. The method allows simultaneous quantification of non-polar sevoflurane and its polar metabolite hexafluoroisopropanol (free, unconjugated form). It is characterized by high repeatability and sensitivity with the detection limit of 0.009 mg L^{-1} for sevoflurane and 0.018 mg L^{-1} for hexafluoroisopropanol and the linear range 0.050–150 mg L^{-1}. The method was used to determine the concentration of sevoflurane and hexafluoroisopropanol in plasma samples of 7 patients undergoing general anesthesia with sevoflurane. The average concentration of sevoflurane and free hexafluoroisopropanol was 57.2 mg L^{-1} and 0.39 mg L^{-1}, respectively. The method can be applied for clinical monitoring, as well as for analytical toxicology.

1. Introduction

Analytical methods allowing the elimination of liquid solvents in the analytical procedures, as well as the shortening of the sample preparation time are constantly being developed [1–3]. Direct aqueous injection (DAI) undoubtedly belongs to this group. In methods involving DAI water samples are directly injected into the gas-chromatographic system without pretreatment [4–6]. The elimination of sample pretreatment and preconcentration reduces the losses of volatile analytes and the risk of sample contamination. The main drawback of this injection technique is that huge amounts of water are introduced into the GC system that generate some analytical problems (e.g. changing the characteristics of stationary phase in capillary columns, or affecting the detectors' response) [7–13].

Plasma is a matrix with its pattern similar to water samples. Apart from water, representing the main component (more than 90%), it also contains a wide range of macromolecules and salts, inducing difficulties during GC analysis with direct sample injection. Problems may arise mainly due to the thermal degradation of the sample in the injector and the subsequent contamination of the gas-chromatographic system by the products of the sample degradation. So far, only few studies dealing with this issue can be found in the literature [14,15].

Direct injection of plasma samples into the GC system was used by Abdel-Rehim et al. [16] for the determination of the anesthetic agent ropivacaine and its metabolite pipecoloxylidide (PPX). Ultrafiltration was used as a pretreatment for the analyzed plasma samples, to obtain a fraction with a molecular weight below the 20,000 amu. A liner with sorbent ATAS "A" was installed in the injector, which had a lifetime of about 20 sample injections. The limit of quantification was 301 pg mL^{-1} for ropivacaine and 325 pg mL^{-1} for its metabolite PPX. [16]. The method is suitable for the determination of substances less volatile than water. A system allowing injection of large volumes of water samples into the GC system and the determination of the non-polar volatile components was presented by Kubinec et al. [12]. Aeppli et al. [15] published the simultaneous determination of polar and non-polar volatiles in

* Corresponding author. Tel.: +421 2 602 96 330; fax: +421 2 602 96 337.
E-mail address: kubinec@fns.uniba.sk (R. Kubinec).

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water using direct injection. However, this method can be applied only when water samples contain trace amounts of non-volatile components. The simultaneous determination of sevoflurane and its metabolite hexafluoroisopropanol in plasma is an analogous problem to simultaneous determination of non-polar and polar volatile components.

Sevoflurane was introduced into clinical practice in 1990 as an inhalation anesthetic agent and today is among the world's most commonly used anesthetics. Due to its low blood–gas partition coefficient and corresponding low solubility in blood, it enables rapid and smooth induction of anesthesia. It is readily excreted from the central nervous system, allowing quick awakening of the patient at the end of anesthesia [17]. Sevoflurane present in blood is rapidly metabolized to its primary metabolite, free fluoride and hexafluoroisopropanol, which are excreted in urine. Hexafluoroisopropanol circulates in blood primarily as a glucuronide conjugate. According to the literature [18], unconjugated hexafluoroisopropanol represents ≤15% of the total concentration of hexafluoroisopropanol.

Several studies are focused on the determination of sevoflurane and hexafluoroisopropanol in biological matrices using headspace technique (HS) combined with the GC system (HS–GC–FID or HS–GC–MS) [18–24]. Unlike sevoflurane, hexafluoroisopropanol is a highly polar substance. Its high solubility in blood induces problems during its determination by headspace methods. The results of headspace methods are affected also by the increased sample temperature during extraction and for the more polar and less volatile analytes are dependent on sample volume in the vial during the sample equilibrium [21,23,24].

The aim of this study was to develop a method for simultaneous quantification of non-polar sevoflurane and its polar metabolite hexafluoroisopropanol with direct injection of human plasma into the GC system without any sample pretreatment.

2. Experimental

2.1. Chemicals and reagents

Sevoflurane and hexafluoroisopropanol were purchased from Sigma–Aldrich (Steinheim, Germany). Analytes were stored at 4 °C to minimize the risk of evaporation. Methanol was purchased from Merck (Darmstadt, Germany).

2.2. Preparation of calibration standards

Standard solutions containing sevoflurane and hexafluoroisopropanol were prepared in methanol at the concentration range of 5–15,000 mg L^{-1}. Calibration standards were prepared by the addition of 15 μL aliquots of standard solution at the concentration range of 0.050–150 mg L^{-1} per analyte to 1.5 mL of blank plasma. This procedure was necessary because methanol has a low boiling point and would immediately evaporate in the injector, while when using the plasma matrix the analytes are released gradually. During the preparation of the calibration standards, the headspace volume was maintained below 20% of the total vial volume. This resulted in lower extent of sevoflurane volatilization.

2.3. GC–MS/MS analysis

Column length, type and thickness of stationary phase, temperature and carrier gas velocity were optimized. Optimization of the GC conditions and column parameters was based on suitable analyte retention for focusation during injection and separation of analytes, and minimalization of the column background. MS parameters were optimized to get the best signal to noise ratio for our analytes.

GC–MS/MS analyses were performed using a Trace GC Ultra gas chromatograph equipped with a split–splitless injector, a TriPlus autosampler and a TSQ Quantum XLS mass spectrometer (Thermo Fisher, Austin, TX, USA). For data processing, Excalibur software from Thermo Scientific was used.

The injector was equipped with a splitless straight liner 105 mm × 5 mm (O.D.) × 3 mm (I.D.) (Thermo Scientific, Italy). The liner was filled with glass wool up to the one-third of its volume. The injection was achieved by introducing 0.5 μL of the sample into the injector operating in the splitless mode with the speed of 1 μL s^{-1}. The split mode was switched on after 0.6 min with the corresponding split flow of 100 mL min^{-1}. The temperatures of the injector and the MS-transfer line were 80 °C and 200 °C, respectively. Compounds were separated on a 60 m × 0.32 mm (I.D.) × 5 μm DB-1 capillary column (Agilent, CA, USA) operating at constant helium flow of 1.5 mL min^{-1}. The column temperature was initially set at 40 °C for 8 min, and then increased at a rate of 30 °C min^{-1} to 200 °C and maintained at 200 °C for 2 min.

Electron energy and electron current were set at 70 eV and 500 μA, respectively. Argon was used as collision gas in GC–MS/MS with pressure of 1.5 Pa in the collision chamber. Ion source temperature was set to 200 °C. Quantitative measurements were performed on the basis of selected reaction monitoring (SRM) of the product ions. The used SRM transitions were m/z 181 → 51, for sevoflurane, with collision energy set to 20 V, and m/z 149 → 101, for hexafluoroisopropanol, with collision energy set to 5 V, with a scan time of 0.2 s each.

2.4. Sampling and application

Blank dipotassium EDTA plasma samples were obtained from Children's University Hospital in Bratislava (Slovakia). Blood samples from 7 consenting patients of Innsbruck Medical University (Austria) were collected during general anesthesia with sevoflurane into dipotassium EDTA tubes and centrifuged to get plasma for analyses. Next, 1.5 mL of the plasma samples were transferred to a 1.8 mL vial with a gas-tight syringe. Samples were stored in a freezer at −20 °C and thawed shortly before analysis.

2.5. Method validation

The elaborated method for the simultaneous determination of sevoflurane and its metabolite hexafluoroisopropanol in plasma was validated following the EURACHEM guidelines [25,26]. Limits of detection (y_D) and quantification (y_Q) were calculated from the mean value of the blank responses ($\bar{x}_b$) and their standard deviation (s_b):

$$y_D = \bar{x}_b + 2ts_b$$

$$y_Q = \bar{x}_b + 10s_b$$

where t is the constant of Student's normal distribution t-test depending on the confidence level (95% confidence level was chosen). The values of $\bar{x}_b$ and s_b were calculated from 10 blank measurements. The concentration values of LOD and LOQ were obtained by a projection of the corresponding signals y_D and y_Q through the calibration dependence $y = f(x)$ onto the concentration axis. Linearity was verified over three concentration orders and confirmed by the Mandel's fitting test. To verify the repeatability, the standard deviations were calculated from five repetitive measurements at four different concentrations.

The linearity of the calibration dependences was examined from samples analyzed at analyte concentration levels of 0.05, 0.1, 0.3, 0.5, 1, 3, 5, 10, 30, 50, 80, 100 and 150 mg L^{-1}.

A memory effect of the method was examined injecting the standard mixture of sevoflurane and hexafluoroisopropanol with concentration of 100 mg L^{-1} and subsequent blank run.

The recovery of both analytes for applied analytical conditions was found to be better than 95%. Further recovery improvement can be achieved by the increase of the splitless time. For example 100% recovery requires an injection with splitless time 10 min.

3. Results and discussion

3.1. Method validation

3.1.1. Linearity

Table 1 shows the values of the slopes and intercepts of calibration dependences, as well as coefficients of determination (F_{calc} and F_{crit}) from the Mandel's fitting test for target analytes. The calibration curves were found to be linear within the whole concentration range of the analytes 0.050–150 mg L^{-1} (see results of the Mandel's fitting test).

3.1.2. Repeatability

Repeatability of the measurements of the chromatographic zones of sevoflurane and hexafluoroisopropanol, expressed by relative standard deviation (RSD), and shown in Table 2, was calculated from the values of signals of the analytes in three independently prepared samples at four concentrations 0.1, 1, 10 and 100 mg L^{-1}. In the given concentration range of 0.1–100 mg L^{-1} RSD values are between 1.0 and 8.5% for sevoflurane and 0.9 and 6% for hexafluoroisopropanol. Higher RSD values for sevoflurane at lower concentrations are most probably caused by the higher volatility of this substance.

Inter-day assay reproducibility was determined using spiked plasma samples at concentrations 0.1, 1, 10 and 100 mg L^{-1}. At each concentration, assays were run on 3 samples immediately after preparation and on 3 samples 7 days after freezing at −20 °C. A sample was considered stable if its average analyte concentrations measured after freezing varied by no more than 10%.

3.1.3. LOD and LOQ

The limits of detection and quantification for sevoflurane and hexafluoroisopropanol are listed in Table 3 along with the mean values of blanks and standard deviations of the blank responses from 10 measurements. The LOD is 0.009 mg L^{-1} for sevoflurane and 0.018 mg L^{-1} for hexafluoroisopropanol and the LOQ is 0.017 mg L^{-1} for sevoflurane and 0.032 mg L^{-1} for hexafluoroisopropanol.

Table 1
Values of slopes (a) and intercepts (b) of the calibration dependences for analytes sevoflurane and hexafluoroisopropanol at the concentration range of 0.050–150 mg L^{-1}, coefficients of determination (R^2), calculated (F_{calc}) and critical (F_{crit}) values of Mandel's fitting test.

	Sevoflurane	Hexafluoroisopropanol
a	12,570	8404
b	138	−866
R^2	0.999	0.997
F_{cal}	3.9	0.2
F_{crit}	9.7	9.7

Table 2
The values of relative standard deviations (RSD) of the areas of chromatographic zones for sevoflurane and hexafluoroisopropanol obtained for three independently prepared samples at 4 concentrations. The repeating number of each sample experiment was 3.

Concentration (mg L^{-1})	RSD (%)	
	Sevoflurane	Hexafluoroisopropanol
0.1	8.5	6.0
1	7.7	4.4
10	2.1	1.1
100	1.0	0.9

Table 3
The mean values of blanks ($\bar{x}_b$), standard deviations of blank responses (s_b), limits of detection (LOD) and quantification (LOQ) for sevoflurane and hexafluoroisopropanol calculated from 10 measurements.

	Sevoflurane	Hexafluoroisopropanol
$\bar{x}_b$	13.99	4.08
s_b	6.48	1.74
LOD (mg L^{-1})	0.009	0.018
LOQ (mg L^{-1})	0.017	0.032

3.1.4. Memory effect

The memory effect was observed only at higher concentrations of sevoflurane and hexafluoroisopropanol. It increased with the number of samples injected to the system. We found that even after 150 sample injections it did not exceed 0.6 respectively 1% of the peak area from the previous sample injection.

3.2. Real samples

The published methods are based on the deglucuronidation of conjugated hexafluoroisopropanol, what is necessary for the quantification of the total hexafluoroisopropanol content in plasma [19,23]. In the present study only free, unconjugated form of hexafluoroisopropanol, which represents about 15% of the total amount of hexafluoroisopropanol is determined [20]. The risk of evaporation and the loss of the target compounds demand particular attention during the blood sampling, storage and processing. This problem was solved by the minimalization of the head-space volume in the sample vial. It was maintained below 20% of the total vial or test-tube volume. Further gas phase reduction was not possible, as some head-space volume was necessary to avoid vial tearing during freezing.

Fig. 1 shows an exemplary MS/MS chromatogram of sample 6 separation. Table 4 shows the concentrations of analytes in plasma sampled in a vial containing EDTA and the relative standard deviations of the measurements. The RSD of sevoflurane in sample 3 was higher than in the other samples because of the turbidity present in that sample, which was not observed in other analyzed samples.

From Fig. 1 it is obvious, that by the described experimental conditions reliable separation of the analyzed components of

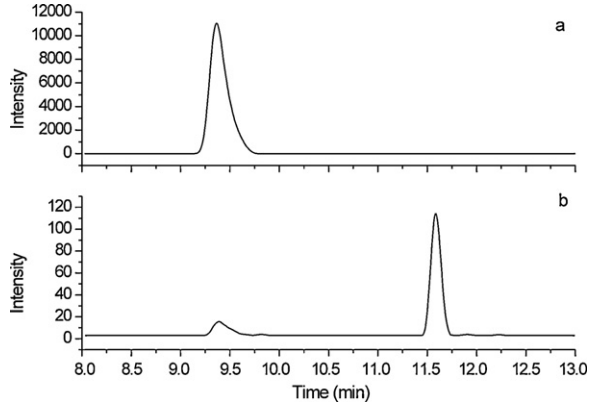

Fig. 1. GC–MS/MS chromatograms of analysis of an EDTA plasma sample 6. (a) Reconstructed ion chromatogram for sevoflurane at SRM m/z 181 → 51. (b) Reconstructed ion chromatogram for HFIP at SRM m/z 149 → 51.

Table 4
Concentrations (c) of sevoflurane and hexafluoroisopropanol in 7 real plasma samples from patients during narcosis and relative standard deviations (RSD) calculated from 3 measurements.

Sample	Sevoflurane		Hexafluoroisopropanol	
	c (mg L^{-1})	RSD (%)	c (mg L^{-1})	RSD (%)
1	54.4	9.0	0.53	6.2
2	19.4	6.4	0.35	8.6
3	85.0	15.4[a]	0.46	7.3
4	87.2	6.0	0.52	6.5
5	63.7	3.4	0.32	8.0
6	20.0	7.3	0.21	10.5
7	70.9	1.2	0.35	1.6

[a] Turbidity in the sample, which was not present in other samples.

the sample was achieved. No degradation products of plasma macromolecules were detected for the applied analytical conditions. The quantification of analytes in real samples is not affected by the coelution of other volatile components of the sample, or components that might arise from the thermal degradation of macromolecules in the liner of injector at 80 °C.

4. Conclusions

A method for the simultaneous determination of non-polar sevoflurane and its polar metabolite hexafluoroisopropanol in plasma was developed. This method allows the direct injection of up to 150 plasma samples into the GC system without the necessity of injector cleaning. No degradation of plasma's macromolecules or polar substances was observed and no substances coeluting with analytes under study were noted. We also observed no reduction in column efficiency, which could be caused by contamination of the column forehead by degradation products. Elaborated analytical method is very rapid and does not require any sample pretreatment. The results of analyses are also not affected by the increased sample temperature during extraction, and in the case of the more polar, less volatile analyte, determined content is not dependent on sample volume in the vial at the time of injection. This method is characterized by high sensitivity as well as the elimination of errors linked to sample manipulation in the laboratory.

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