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Quantitative determination of isoflurane enantiomers in blood samples during and after surgery via headspace gas chromatography–mass spectrometry

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Abstract

The quantitative analysis of the chiral volatile anesthetic isoflurane (**1**) for biomedical applications by means of enantioselective gas chromatography (mass sensitive detector, selected ion monitoring) was studied. Two methods for the quantification of the enantiomers in blood samples drawn during and after narcosis were compared. Either the isomeric enflurane (**2**) was selected as an internal standard or a single enantiomer of **1** was used for the standard addition method, an approach referred to as 'enantiomer labeling'. Concentrations up to 0.3 μmol/l of the single enantiomers could be differentiated two days after anesthesia. The presented data imply that the body clearance for (+)-(*S*)-**1** and (−)-(*R*)-**1** proceeds to a measurable degree of enantioselectivity.

Keywords: Enantiomer separation; Isoflurane

1. Introduction

Chiral isoflurane [F_2HC-O-C*HCl-CF_3], 2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro ethane **1**, is among today's most frequently employed inhalation anesthetics and is presently administered as a racemic mixture. Recent in vivo and in vitro studies [1–6] suggest differences in the pharmacological properties of the enantiomers compared to racemic **1**. It therefore has become of great importance to develop a fast, reliable and universal method for the qualitative and quantitative [7] analysis of isoflurane in biological samples that allows (+) and (−) enantiomers to be discriminated and to assess their different pharmacokinetic properties (absorption, distribution, biotransformation, and excretion) as demanded for enantiomers by the FDA [8].

All studies concerned so far with the determination of isoflurane from biological samples fail to distinguish between the single enantiomers. Moreover, not even the possibility that the two enantiomers may be metabolized or retained in the human body to various extents has been investigated. We therefore wanted to provide a simple, quick and straightforward method to monitor and quantify the elimination of the two isoflurane enantiomers during and after anesthesia with racemic **1**. As we reported

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previously [9], the enantiomer separation of the racemic inhalation anesthetics isoflurane **1**, enflurane **2** and even the volatile desflurane **3** can be achieved by headspace GC (cf. Fig. 1) in less than 7 min on a cyclodextrin derivative **4** [10] (cf. Scheme 1) used as chiral stationary phase.

In order to answer the question whether the enantiomers of **1** are metabolized or accumulated in the human body differently we decided to investigate blood samples of patients who had recently undergone surgery under narcosis by **1**. We employed a quantitative methodology similar to the formerly standard-type headspace gas chromatographic determination of ethanol in blood [11,12].

Two different ways to determine the amount of isoflurane enantiomers in blood samples during and after surgery were compared. Quantification was achieved (i) by the addition of an internal standard (in our case the isoflurane isomer enflurane **2**) and (ii) by a standard addition method, referred to as 'enantiomer labeling' [13–15] employing a single enantiomer previously obtained via preparative GC [16]. Both methods should allow the precise determination of the total range of concentrations, extending from mmol/l during the time of narcosis to μmol/l several days after anesthesia.

2. Experimental

2.1. Chemicals

Racemic **1** and **2** were obtained from Pharmacia, Erlangen, Germany. The enantiomerically pure (+)-(*S*)-**1** (*ee*>99.9%) was obtained as described [16] through enantioselective preparative GC. Citrate buffer solution and Ringer solution were purchased from Fresenius AG, Bad Homburg, Germany and Sarstedt, Nümbrecht, Germany, respectively.

2.2. Preparation of the blood specimens

Racemic **1** was used for anesthesia during eye surgery in three patients (2 men and 1 woman). No

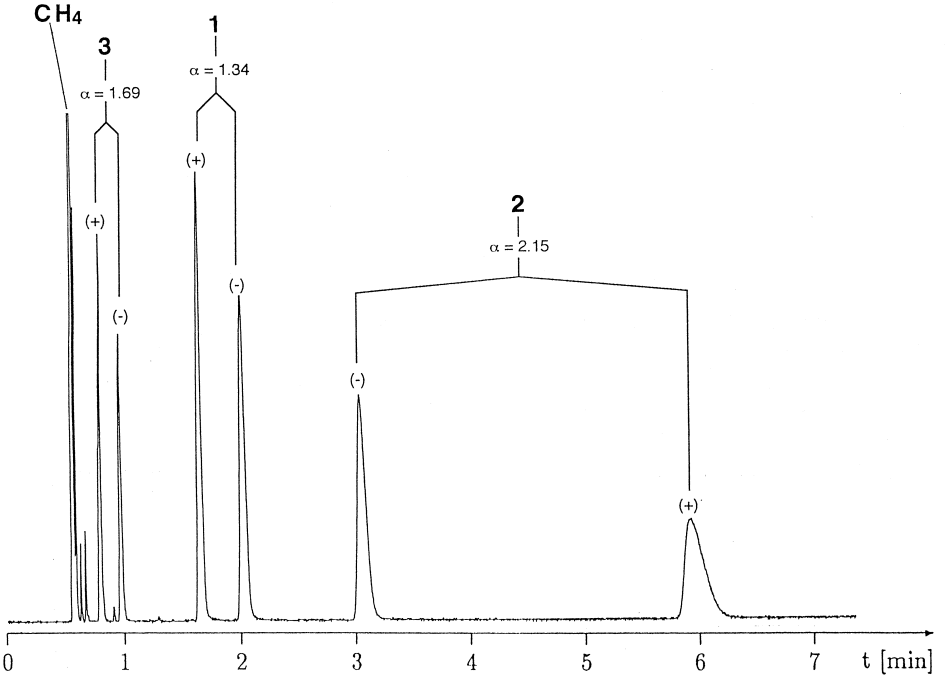
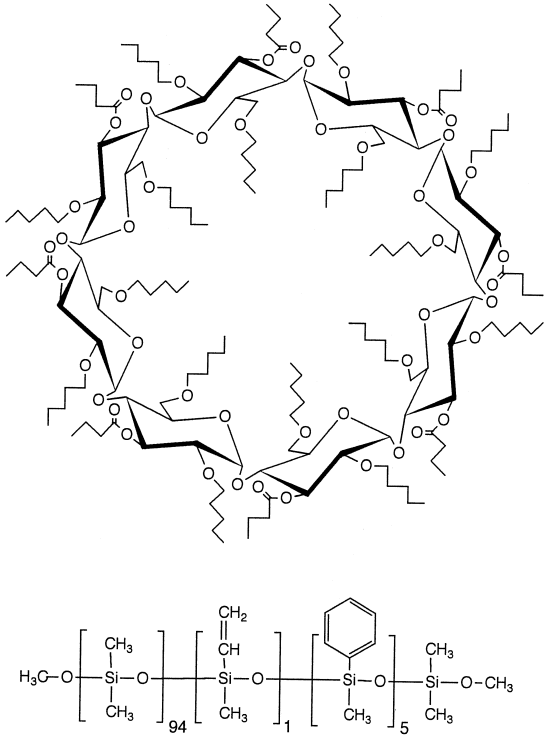


Fig. 1. Simultaneous analytical gas chromatographic enantiomer separation of isoflurane **1**, enflurane **2** and desflurane **3**. Elution order: *S* before *R* for **1** and **3**, *R* before *S* for **2**. Fused silica column (25 m×0.25 mm I.D.) coated with 10% Octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin [10] **4** dissolved in the polysiloxane SE-54 (w:w), film thickness 0.5 μm, $T=30°C$, carrier: He, 1.1 bar, detector: FID.



Scheme 1. Representation of Octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin [10] **4** dissolved in the polysiloxane SE-54.

blood transfusion was necessary during or after surgery. The patients ranged in age from 27 to 64 years. They had not been given barbiturates or any other enzyme inducing drugs and were premedicated with midazolam, 7.5 mg oral, 45–60 min prior to anesthesia. Thiopental, 5–7 mg/kg, and fentanyl, 0.1–0.2 mg/kg iv, were used for induction of anesthesia, and tracheal intubation was performed after muscular relaxation with 0.1 mg/kg rocuronium. Anesthesia was maintained with 70% nitrous oxide in oxygen.

Blood specimens (approximately 4 ml) from the patients were collected before, during surgical operations and after every 12 h for 3 days and were stored immediately after addition of citrate buffer at −20°C in crimp vials sealed with a PTFE-faced silicon septum. The samples were kept at this temperature in order to lyse the blood cells and create a homogeneous liquid phase and to avoid any loss of volatile material or contamination with other VOCs. Written informed consent for the blood collection was obtained from the patients before surgery and collection in every case.

2.3. Standard preparation

Approximately 50 mg of racemic **2** (enflurane) or (+)-(*S*)-**1** (isoflurane) (*ee*>99.9%), respectively, were weighed in a 50 cm^3 glass calibrated flask containing Ringer solution at a temperature of 8°C. After thorough mixing, 10% of this solution was diluted again with a weighed amount of Ringer solution. The final concentration of the stock solutions were 1.179(16) mmol/kg **2**, respectively 0.802(43) mmol/kg (+)-(*S*)-**1**. These stock solutions remained usable for at least 6 months if stored in 5 cm^3 portions at −5°C to −20°C in crimp sealed headspace vials that were filled up to the top. All concentrations were recalculated from mmol/kg to mmol/l blood.

2.4. Sample preparation and quantification

2.4.1. Headspace method

After the samples were thawed at room temperature, a 1 ml volume of blood was quickly transferred to an empty 12 ml headspace glass vial and capped. The exact weight of blood in each headspace vial was determined by weighing the vial before and after filling. Then a small weighed amount of a stock solution containing the internal standard was added with a Hamilton syringe to the vial. In the case of enantiomer labeling, one analysis was performed prior to the addition of the stock solution containing the (+)-(*S*)-enantiomer of isoflurane. All concentrations were recalculated from mmol/kg to mmol/l blood.

For routine analysis, samples and standards were allowed to equilibrate at 27°C for 30 min, and the headspace gas (20–100 μl) was injected with a gas-tight syringe (Hamilton, Bonaduz, Switzerland) into the GC or the GC–MS apparatus. Great care was taken not to introduce any droplets of blood adhering to the surface of the vial or the septum. Each analysis was performed at least three times and after each set of injections the syringe was cleaned by connecting it for several minutes to a vacuum line and by moving the plunger several times up and down to avoid any possible carry-over from previ-

ously analyzed samples. The syringe was checked for residues after this procedure by an injection of 100 μl air.

2.4.2. Cryo focusing

To avoid band broadening of the peaks and thus deviations in the integration of the detected areas by large volume injections a small section of the capillary column was immersed in liquid nitrogen to concentrate the volatile compound ('cryo focusing'). After removal of the cooling trap the sample was promptly volatilized and thus injected.

2.5. Quality control

Quality control specimens were prepared by adding a weighed amount (approximately 50–500 μl) of racemic liquid **1** to a weighed amount (about 1000 ml) of either Ringer solution or outdated blood bank blood/citrate buffer in screw cap septum bottles. These were mixed by agitating and were subsequently refrigerated. 24 Samples of the control specimens were treated as described above for calibration curves using a serial dilution technique (range of concentration 0.1 μmol/l to 10 mmol/l, with 0.989 of correlation coefficient for enantiomer labeling and 0.960 for the internal standardization).

2.6. Gas chromatography

2.6.1. Capillary columns

An untreated fused-silica capillary column (25 m×0.25 mm I.D.) was heated at 200°C in a slow stream of hydrogen for 5 h, and, without further deactivation, coated by the static method with 10% (w/w) Octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin **4** [10] dissolved in the polysiloxane SE-54. The film thickness was 0.5 μm. It should be mentioned that, in principle, similar results should be obtained with commercial columns containing the same chiral selector **4** (Macherey and Nagel, Düren, Germany). However, these columns are not available with a specified film thickness or concentration of the cyclodextrin derivative. An achiral reference column (50 m×0.25 mm I.D.) coated with the pure apolar polysiloxane SE-54 with a film thickness of 0.5 μm was used for comparison of retention times [17].

2.7. Instrumentation

A 5300 Mega gas chromatograph (Thermoseparations, Mainz, Germany) with flame ionization detector was used for the gas chromatographic measurements. The oven was operated isothermally at 35°C, and the detector and injector ports were set at 200°C. Retention times were recorded with a Shimadzu C-R6A integrator. Helium (99.996%, v/v) was used as the carrier gas, and compressed air and hydrogen (99.996%, v/v) were used as flame gases.

2.8. Gas chromatography–mass spectrometry single ion monitoring

The oven was operated isothermally at 27°C by opening the door of the GC (Fractovap 2900/Varian MAT 112S, Thermoseparations, Mainz, Germany). Injector and detector temperature were both 120°C. The carrier gas was hydrogen (99.999%, v/v). Ionization voltage and current were 70 kV and 0.15 mA, respectively. For quantitative analysis, *SIM* experiments were performed at m/z 149 for **1** and m/z 117 for **1** and **2**. Prior to each set of measurements three control injections with racemic **1** were performed. In all cases the deviation from the expected ratio of 50:50 for 24 samples containing **1** was less than ±0.25%. Retention times were recorded with a Shimadzu C-R3A integrator.

2.9. Methods for the quantification of *1*

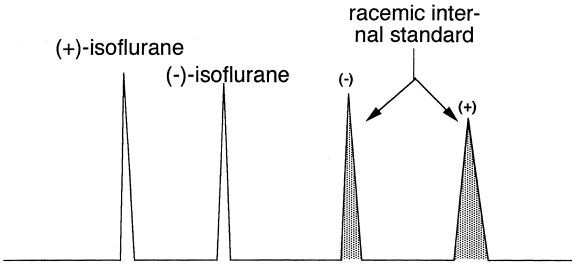
2.9.1. Addition of the internal standard *2*

A small known amount of the standard **2** (with a known relative response factor) was added to the sample (cf. Scheme 2).

The percentage amount of each enantiomer contained in the sample, $P_{\text{isoflurane}}$, is obtained according to Eq. (1) [18]:

$$P_{\text{isoflurane}} = \frac{W_{\text{standard}}}{W_{\text{sample}}} \cdot f_{\text{rr}} \cdot \frac{A_{\text{isoflurane}}}{A_{\text{standard}}} \quad (1)$$

where W_{standard} is the weight of the internal standard added to the blood sample and W_{sample} denotes the weight of the blood sample in the headspace vial. f_{rr}



Scheme 2. Schematic chromatogram for internal standardization. First and second peak: isoflurane enantiomers; third and forth peak: known amount of internal (racemic) standard.

is the dimensionless relative response factor determined in separate measurements with quality standards of known composition, $A_{\text{isoflurane}}$ and A_{standard} are the peak areas obtained for the two compounds. Eq. (1) can be used for quantification of either enantiomer of **1** by either enantiomer of **2**. The total amount of **1** in the sample is given by the sum of the two enantiomers. It should be mentioned that this approach allows a double-check of the measurement, as two standards with exact the same concentration (i.e. the two enflurane enantiomers) are present.

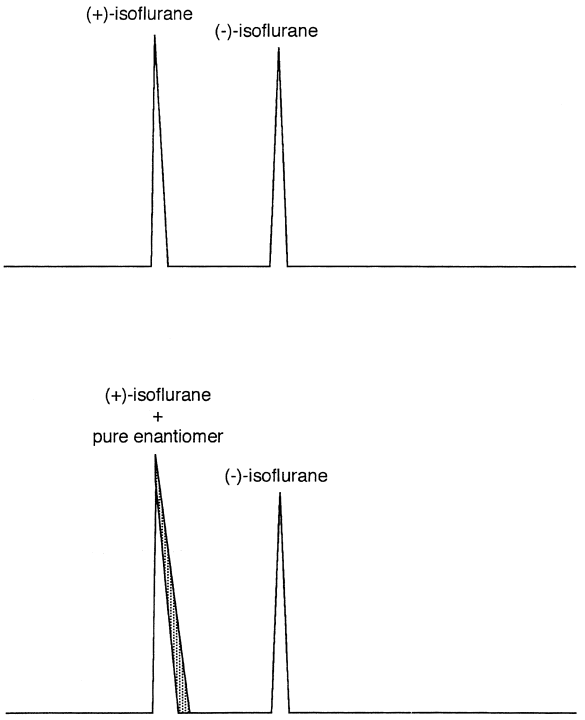
2.9.2. Standard addition method (enantiomer labeling [13–15])

Here an enantiomerically pure single isoflurane enantiomer, obtained previously by enantioselective preparative GC [16], was used as the internal standard (cf. Scheme 3).

The percentage amount of each enantiomer contained in the sample, $P_{\text{isoflurane}}$, prior to the addition of the enantiomerically pure standard, can be calculated via Eq. (2) [18]:

$$P_{\text{isoflurane}} = \frac{W_{\text{standard}}}{W_{\text{sample}}} \cdot \frac{1}{\dfrac{A_{i,2}A_{k,1}}{A_{i,1}A_{k,2}} - 1} \qquad (2)$$

Here W_{standard} is the weight of (+)-(*S*)-**1** added to the blood sample and W_{sample} denotes the weight of the blood sample in the headspace vial, $A_{i,1}$ and $A_{i,2}$ are the peak areas obtained for the first eluted enantiomer in the first (before addition of the standard solution) and second measurement (after addition of the standard solution) and $A_{k,1}$ and $A_{k,2}$ are the peak areas of the second enantiomer in first and second measurement. This equation compensates for all errors possibly caused by different injection volumes in first and second measurement. This approach has the advantage that masses can be determined very precisely ($\pm$0.1 mg) and no corrections for temperature, density of blood, added volume of standard solution and vapor pressures have to be made. Contrary to the method described for internal standardization (cf. Section 2.9.1), the approach of enantiomer labeling can only be used when the racemic analyte can be quantitatively separated by a chiral stationary phase. In addition, the ee^2 of the standard must be known with certainty.



Scheme 3. Schematic chromatogram for enantiomer labeling. Top: first analysis; first and second peak: isoflurane enantiomers. Bottom: second analysis; first peak: isoflurane enantiomer spiked with known amount of pure enantiomer, second peak: second isoflurane enantiomer (non-labeled).

3. Results and discussion

Headspace gas chromatography is particularly suitable for the analysis of gases because most gases

and volatile organic compounds (VOCs) readily enter the headspace. There is a minimum amount of sample handling required, resulting in greater recovery of the gas from the sample. The analysis of blood samples via headspace GC is complicated by the fact that several different VOCs [19–21] may be found in biological material (cf. Fig. 2). The most common VOCs are acetone, ethanol, propan-2-ol, other alcohols and *n*-hexanal [22]. It is also known that biological samples can easily be contaminated on contact by plasticizers from rubber or PVC during storage or transfer of biological specimens [23,24].

During surgery, usually 1.2%, v/v of racemic isoflurane is added to the gaseous mixture of N_2O and O_2 which is inhaled by the patients. Nitrous oxide and isoflurane are distributed very fast through the lungs into the blood and other body compartments. The resulting concentrations of isoflurane **1** in blood are approximately 0.5 mmol/l (resp. ≅ 0.1 g/l) [25,26] during narcosis. This concentration is well in the sensitivity range of an FID detector (cf. Fig. 3).

Several methods for the quantification of volatile inhalation anesthetics in biological fluids have been described. Among the substances used for internal standardization are *n*-hexane [27], **2** in methanol [28], **1** in DMSO [29] as well as sevoflurane and halothane [30]. The standard should meet the following requirements: similar elution time, total separation from all other components, similar concentration, similar vapor pressure, accessibility in sufficient purity. All of the above requirements are met by the isoflurane isomer, enflurane, which has the same sum formula and is nearly as volatile as isoflurane. We have found that fused-silica capillary columns coated with either an 0.5 μm chiral or achiral stationary phase ('thick film') offer several advantages over the packed columns often used for gas chromatographic determination of volatile anesthetics [30]. In particular, as improved resolution of very volatile compounds is obtained at low temperatures, the total analysis time can be reduced to less than 10 min and good peak shapes are obtained for all compounds. Septum injections with cryo focusing (cf. Section 2, Experimental) of up to 0.3 cm^3 of headspace could be performed with no noticeable effect on column efficiency. Hence the sensitivity is at least as good as the one achieved with a packed column.

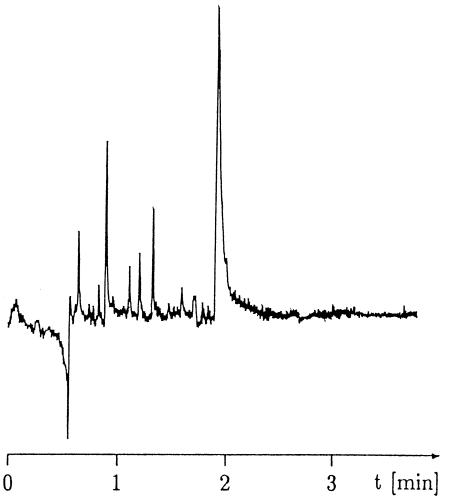


Fig. 2. Headspace analysis of volatile organic compounds (VOCs) present in blood. Sample: 1 ml whole blood donated by M.J., sample temperature 27°C; injected headspace volume 100 μl, column parameters: see Fig. 1.

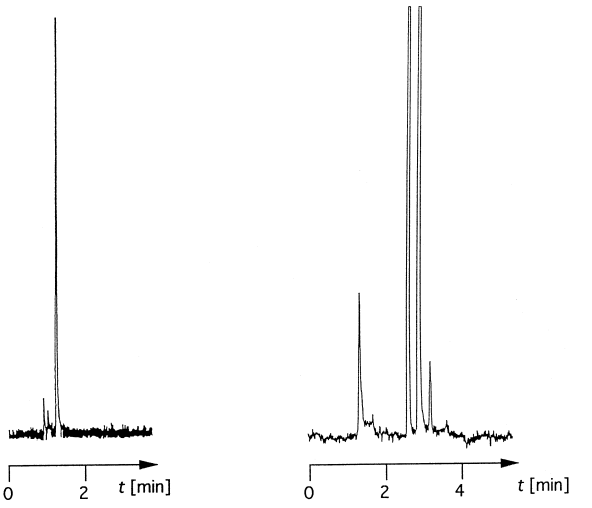


Fig. 3. Headspace analysis of racemic **1** in a blood sample. Sample: 1 ml whole blood at 27°C, collected 30 min after intubation during surgery; injected headspace volume 20 μl each. Left (achiral): fused-silica column (50 m×0.25 mm I.D.), coated with SE-54, film thickness 0.5 μm, carrier gas: He, 2. 1 bar. Right (chiral): see Fig. 1.

However, the quantitative analysis of volatile anesthetics present at low concentrations causes some difficulty where accurate and sensitive assay is required, as VOCs may coelute with the analytes of

interest. For this reason the determination of isoflurane enantiomers was performed by GC–MS-SIM. This technique is only sensitive to a selected mass, thus avoiding problems caused by partial or total coelution of other VOCs. We indeed observed only a flat base-line when injecting large headspace volumes of blood samples containing no inhalation anesthetic during monitoring the appropriate masses. The mass spectrum of **1** shows two characteristic fragment ions, $m/z=149$ and 119, i.e. $[M-Cl]^+$, $[M\text{-}(OCHF_2)]^+$, respectively. By monitoring the ion intensity at $m/z=149$ (in the case of enantiomer labeling) or $m/z=119$ (internal standardization; isoflurane and enflurane have this ion in common) the quantitative analysis of blood samples taken during and after surgery became possible (cf. Fig. 4) over a broad range of concentrations (for a detailed description of both methods cf. Experimental, Section 2.9). Thus coupled GC–MS in the selected ion monitoring mode was used as a reliable method to ensure a sufficient selectivity and sensitivity for the analysis of isoflurane enantiomers.

The results obtained by the two methods of quantification of **1** in blood samples follow the same elimination profile (cf. Fig. 4). While the concentrations obtained during the first day after anesthesia are identical for both methods, large deviations are observed at smaller concentrations.

Incidentally, during the experiments performed on the quantitative determination of **1** in blood samples we could surprisingly also detect small concentrations of racemic **1** in reference blood samples collected from the patients immediately before surgery. It turned out that all plastic syringes stored in the operating theater contained adsorbed racemic isoflurane which was liberated on contact with the blood samples.

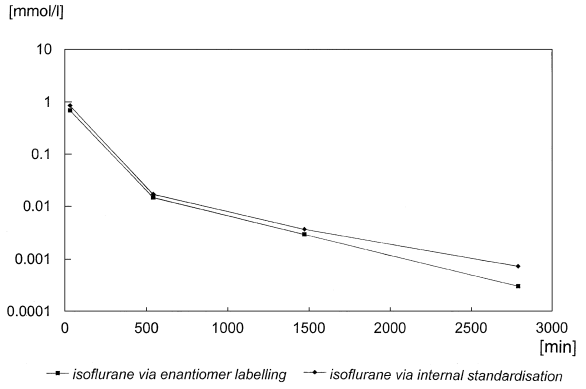


Fig. 4. Washout curve of **1**; ♦: determined via internal standardization; ■: determined via enantiomer labeling.

It should be mentioned that the high volatility and low liquid gas partition coefficient of inhalation anesthetics cause analytical difficulties during the determination of their concentration in liquids. Apart from the non-trivial preparation of liquid standards at concentrations in the therapeutic range (roughly 1 mmol/l) any step in the storage and transfer of the standard which exposes the liquid to a gas phase will introduce error via partitioning of anesthetic from the liquid to the gas phase. Such problems especially exist for any compound generally considered for internal standardization. To provide a simple solution to these analytical problems we have developed a method using a similar approach as for enantiomer labeling [13–15].

3.1. Advantages of enantiomer labeling in comparison to internal standardization

Quantification by enantiomer labeling exploits the fact that enantiomers virtually possess identical chemical and physical properties in an achiral environment and in complex mixtures. The distribution of the added standard (the single enantiomer used as label) will always be the same as for the racemic analyte. The enantiomeric ratio of sample and standard is not influenced by sample manipulations, e.g. dilution, injection, detection, and chemical or physical losses in the absence of specific enantiomer–enantiomer interactions (EE-effect) [31].

For a racemic sample either of the two single enantiomers may be added as the internal standard. Whereas enantiomer labeling of amino acids by addition of an enantiomerically pure D- or L-amino acid maybe complicated by the often encountered racemization [13,15], **1** is a very inert compound that will not be prone to a change of configuration. Thus the division of the original sample in two parts in order to determine the degree of racemization of the original sample is not needed. All samples have to be analyzed twice, i.e., before addition of the enantiomerically pure label and after the addition. Furthermore the enantiomer labeling method presupposes a

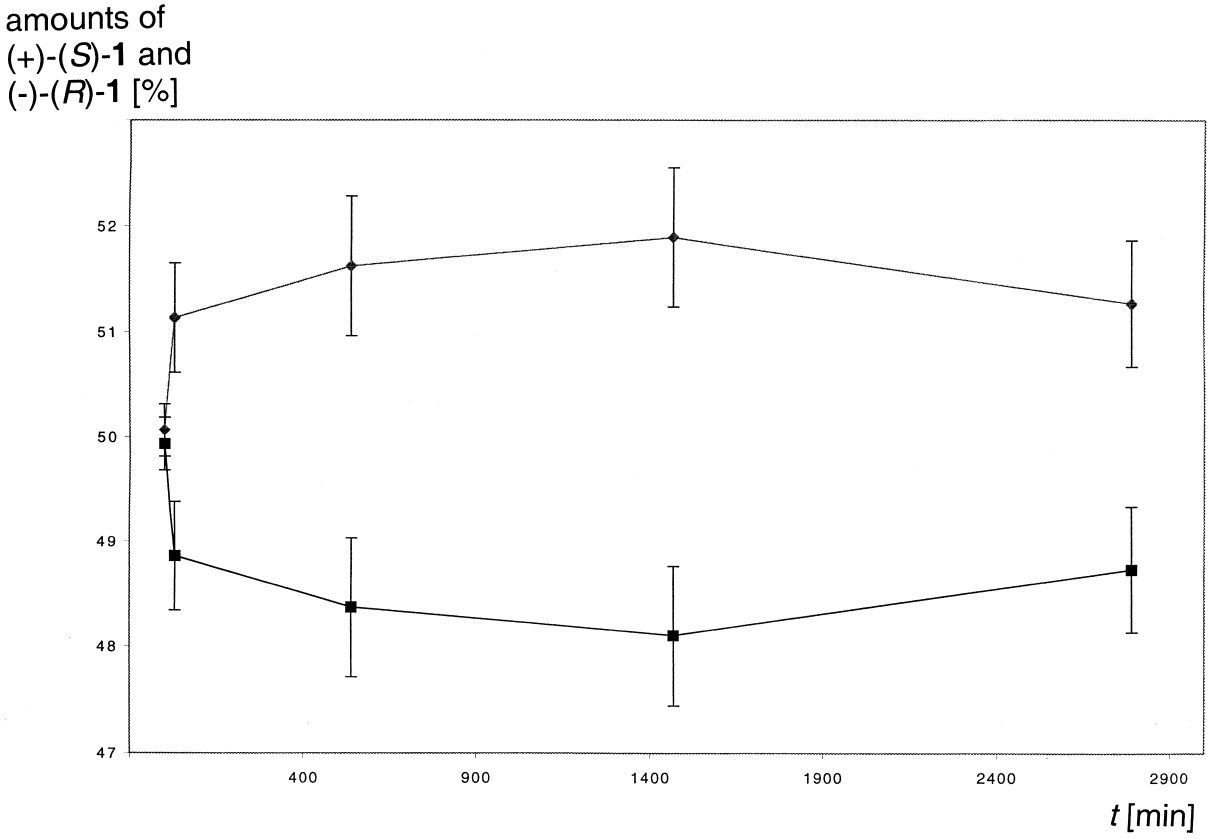


Fig. 5. Enrichment of (+)-*(S)*-**1**, the first eluted enantiomer, in blood samples taken during and after surgery under narcosis with racemic **1**. ♦: (+)-*(S)*-**1**; ■: (−)-*(R)*-**1**.

precise knowledge of the enantiomeric excess, ee[2] of the sample and the standard.

Compared to the results obtained by enantiomer labeling with (+)-(*S*)-**1** the internal standard method proved to be less reliable, since the internal standard enflurane tends to become adsorbed in the biological matrix and its different blood/gas partition coefficient (1.9) [32–34] in comparison to isoflurane (1.4) obviously complicates quantitative determination. Because the added internal standard is a chiral molecule itself, always two peaks of the same area are obtained on the stationary phase for quantification. This allows an important double check of the precision of the measurement, but at the same time introduces significant errors for smaller analyte concentrations (cf. Fig. 4). As can be seen e.g. in Fig. 1, the peak height for both enflurane enantiomers differs for a factor of about two. Thus, at low concentrations, this behavior leads to a small signal-to-noise ratio for the second eluted enantiomer of the internal standard, which renders the integration of this peak suspect.

3.2. Ratio of isoflurane enantiomers in blood samples

When comparing the peak areas obtained by GC–MS-SIM for the single enantiomers of **1** in blood samples taken after surgery a small deviation of the 1:1 ratio expected for the racemate could be detected. This non-negligible enrichment amounted to 49% (*R*): 51% (*S*)-isoflurane ($ee = 2\% \pm 0.6$ *SD* for

[2]The enantiomeric excess for (+)-(*S*)-isoflurane is defined as: $100\%\ ee = S - R/S + R \cdot 100$, where *S* and *R* are the amounts of the isoflurane enantiomers, respectively.

36 measurements) in comparison to our calibration runs before and during the measurements with quality control samples (cf. Experimental, Section 2.5) using outdated blood-bank blood and racemic **1** giving 49.75% (*R*)-: 50.25% (*S*)-isoflurane (ee = 0.5% ± 0.25 *SD* for 24 measurements). Thus the enrichment of (+)-(*S*)-**1** cannot be attributed to a random effect or a systematic error. The maximum difference in the concentration of enantiomers was reached 24 to 48 h post-surgery (cf. Fig. 5). Recently, this result has been confirmed by an independent follow-up study [35].

The observed 'preponderance' of (+)-(*S*)-**1** detected in blood samples after narcosis may be the result of an enantioselective action (up-take, distribution, metabolism or elimination) in one of the five compartments (lung, vessel-rich group, muscle group, fourth compartment, fat group) generally considered for the pharmacokinetics in humans for inhaled anesthetics [36–39]. Since **1** is metabolized to only about 0.2% [40–43], the deviation from the 50:50 ratio of the enantiomers cannot be accounted for by an enantioselective metabolism.

Interestingly, our findings are complemented by several studies confirming different biological properties of isoflurane enantiomers. The (+)-(*S*)-isoflurane was found to bind more strongly to albumin, but not to myoglobin [44]. The potentially enriched (+)-(*S*)-**1** was also found to be 53% more potent when determining the MAC-values [45] (minimum alveolar concentration necessary to prevent movement in response to a painful stimulus) of (+)-(*S*)- and (−)-(*R*)-**1** enantiomers using Sprague-Dawley rats [46]. Also in a recent reinvestigation the 17% higher value for (−)-(*R*)-**1** was in accordance qualitatively, but the difference was not significant ($P < 0.05$) and was smaller than the published values [47]. It was also observed that awakening (recovery of the righting reflex) after anesthesia by intraperitoneal injection of (+)-(*S*)-**1** took longer than awakening from the same dose of the (−)-(*R*)-enantiomer for mice [3]. Furthermore, Ramig et al. recently observed that (+)-(*S*)-**1** possesses virtually no odor for humans, whereas (−)-(*R*)-**1** 'has a typical ethereal odor' [48], which would be a clear indication for the different response of receptors in the human body to the enantiomers of **1**. These in vivo findings are confirmed by several studies showing more expressed effects for (+)-(*S*)-isoflurane in vitro on neurons [4] or $GABA_A$ receptors [5,6,49]. Nevertheless it has to be mentioned that several reports [6,50–53] exist which fail to identify any stereoselective effect for isoflurane enantiomers. The limited number of investigated samples in our study so far precludes drawing any (statistically) significant conclusion from the obtained results. However, analogous experiments employing a different stationary phase, another equilibration temperature, and a different instrumental setup [55] resulted in a comparable enrichment of (+)-(S)-**1** in blood samples taken from a group of 10 patients. It should be noted that this enrichment should also be observable in other biological material, such as cerebral fluid or urine, and might even be expressed in personnel exposed regularly to volatile inhalation anesthetics.

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References

[1] A.C. Hall, W.R. Lieb, N.P. Franks, Br. J. Pharmacol. 906 (1994) 112.
[2] B. Dickinson, N.P. Franks, W.R. Lieb, Biophys. J. 66 (1994) 2019.
[3] N.P. Franks, W.R. Lieb, Nature 607 (1994) 367.
[4] N.P. Franks, W.R. Lieb, Science 427 (1991) 254.
[5] B.D. Harris, E.J. Moody, A.S. Basile, P. Skolnick, Eur. J. Pharmacol. 269 (1994) 267.
[6] E.J. Moody, B.D. Harris, P. Skolnick, Brain Res. 101 (1993) 615.
[7] M. Juza, H. Jakubetz, V. Schurig, H. Hettesheimer, in: 18th Int. Symp. on Capillary Chromatography, Riva del Garda, Italy, 20th–24th May, 1996.
[8] www.fda.gov/cder/guidance.stereo.htm, 5/1/92.
[9] V. Schurig, H. Grosenick, M. Juza, Recl. Trav. Chim. Pays-Bas 114 (1995) 211.

[10] W.A. König, R. Krebber, P. Mischnick, J. High Resolut. Chromatogr. 12 (1989) 732.
[11] P.J. Streete, M. Ruprah, J.D. Ramsey, R.J. Flanagan, Analyst 117 (1992) 1111.
[12] B.J. Gudzinowicz, M.J. Gudzinowicz, in: Analysis of Drugs 1, Respiratory Gases, Volatile Anesthetics, Ethyl Alkohol, and Related Toxicological Materials, Marcel Dekker, New York, Basel, 1977, p. 38.
[13] N.E. Blair, W.A. Bonner, J. Chromatogr. 198 (1980) 185.
[14] W.A. Bonner, J. Chromatogr. Sci. 11 (1973) 101.
[15] H. Frank, G.J. Nicholson, E. Bayer, J. Chromatogr. 167 (1978) 187.
[16] M. Juza, E. Braun, V. Schurig, J. Chromatogr. A 769 (1997) 199.
[17] V. Schurig, M. Juza, J. Chromatogr. A 757 (1997) 119.
[18] W. Gottwald, in: U. Gruber, W. Klein (Eds.), Die Praxis der Instrumentellen Analytik-GC für Anwender, VCH, Weinheim, Germany, 1996, p. 261.
[19] L. Dunemann, H. Hajimiragha, Anal. Chim. Acta 283 (1993) 199.
[20] P.D. Wagner, P.F. Naumann, R.B. Laravuso, J. Appl. Physiol. 36 (1974) 600.
[21] H.J. Lowe, Anesthesiology 25 (1964) 808.
[22] E.N. Frankel, A.L. Tappel, Lipids 26 (1991) 479.
[23] H.G. Wahl, A. Chrzanowski, N. Ottawa, A. Hoffmann, in: 18th Int. Symp. on Capillary Chromatography, Riva del Garda, Italy 20th–24th May, 1996, p. 988.
[24] H.G. Wahl, D. Luft, T. Risler, H.M. Liebich, Nephrol. Dialys. Transplant. 12 (1997) A6.
[25] J. Flynn, J.D. O'Keefe, W.S. Wren, I.M. Shanahan, Analyst 114 (1989) 1207.
[26] J. Flynn, J.D. O'Keefe, W.S. Wren, I.M. Shanahan, Analyst 114 (1989) 1211.
[27] P.M.M. Dooper, J. Beerens, V.J. Brenninkmeijer, P.G.G. Gerlag, Netherl. J. Med. 33 (1988) 74.
[28] J.J. Kuhlman Jr., J. Magluilo Jr., B. Levine, M.L. Smith, J. Forens. Sci. 38 (1993) 968.
[29] B. Jacob, C. Heller, T. Daldrup, K.F. Bürring, J. Barz, W. Bonte, J. Forens. Sci. 34 (1989) 1408.
[30] J.G. Alvarez, in: R.L. Grob (Ed.), Modern Practice of Gas Chromatography, Clinical Applications of Gas Chromatography, 3rd ed, John Wiley, New York, 1995, p. 659.
[31] W.L. Tsai, K. Herrmann, E. Hug, B. Rohde, A.S. Dreiding, Helv. Chim. Acta 68 (1985) 2238.
[32] J. Lerman, G.A. Gregory, E.I. Eger II, Can. J. Anesth. 31 (1987) 14.
[33] J. Lerman, M.M. Willis, G.A. Gregory, E.I. Eger II, Anesthesiology 59 (1983) 554.
[34] J. Lerman, G.A. Gregory, M.M. Willis, E.I. Eger II, Anesthesiology 61 (1984) 139.
[35] R. Schmidt, Diplomathesis, University of Tübingen, 1997.
[36] N. Yasuda, S.H. Lockhart, E.I. Eger II et al., Anesthesiology 74 (1991) 489.
[37] N. Yasuda, S.H. Lockhart, E.I. Eger II et al., Anesth. Analg. 72 (1991) 316.
[38] R.L. Carpenter, E.I. Eger II, B.H. Johnson, J.D. Unadkat, L.B. Shreiner, Anesth. Analg. 65 (1986) 575.
[39] R.L. Carpenter, E.I. Eger II, B.H. Johnson, J.D. Unadkat, L.B. Shreiner, Anesth. Analg. 66 (1987) 1.
[40] T. Davidova, H. Kikuchi, K. Fujii et al., Anesthesiology 69 (1988) 218.
[41] R.I. Mazze, M.J. Cousins, G.A. Barr, Anesthesiology 40 (1974) 536.
[42] D.A. Holaday, V. Fiserova-Bergerova, I.P. Latto, M.A. Zumbiel, Anesthesiology 43 (1975) 325.
[43] R.M. Jones, P.G. Nay, Anesth. Pharmacol. Rev. 2 (1994) 51.
[44] R. Eckenhoff, A. Shuman, Anesthesiology 76 (1993) 96.
[45] E.I. Eger II, L.J. Saidman, B. Brandstater, Anesthesiology 26 (1965) 756.
[46] G.S. Lysko, J.L. Robinson, R. Casto, R.A. Ferrone, Eur. J. Pharmacol. 263 (1994) 25.
[47] E.I. Eger II, D.D. Koblin, M.J. Laster et al., Anesth. Analg. 85 (1997) 188.
[48] K. Ramig, A. Krishnaswami, L.A. Rozov, Tetrahedron 52 (1996) 319.
[49] M.V. Jones, N.L. Harrison, J. Neurophysiol. 70 (1993) 1339.
[50] Y. Xu, P. Tang, L. Firestone, T.T. Zhang, Biophys. J. 70 (1996) 532.
[51] J.J. Kendig, J.R. Trudell, E.N. Cohen, Anesthesiology 39 (1973) 518.
[52] B.M. Graf, M. Boban, D.F. Stowe, J.P. Kampine, Z.J. Bosnjak, Anesthesiology 81 (1994) 124.
[53] E.J. Moody, B. Harris, P. Hoehne, P. Skolnick, Anesthesiology 81 (1994) 1.
[55] R. Schmidt, H.G. Wahl, H. Häberle, H.-J. Dietrich, V. Schurig, Chirality 11 (1999) 206.