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Preparative enantiomer separation of enflurane and isoflurane by inclusion gas chromatography

Volker Schurig* and Heiko Grosenick

Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen (Germany)

ABSTRACT

The dilution of cyclodextrin derivatives in polysiloxanes, previously applied in analytical gas chromatographic enantiomer separation, proved useful also for packed columns. The preparative enantiomer separation of the inhalation anaesthetics enflurane and isoflurane in high chemical purity (≥99.5%) and enantiomeric purity (≥99.9 and 99.4%, respectively) was achieved by preparative gas chromatography using a 4 m × 7 mm I.D. column packed with octakis(2,6-di-O-*n*-pentyl-3-O-butanoyl)-γ-cyclodextrin dissolved in SE-54 (10%, w/w) and coated on Chromosorb P AW DMCS (20.3%, w/w). This approach represents the first preparative racemate resolution of both anaesthetics by chromatography.

INTRODUCTION

Whereas chiral high-resolution capillary gas chromatography is a well established tool in contemporary enantiomer analysis, its (semi)-preparative version is far less developed. This is not surprising in view of the interdependence between analysis time, column loading and selectivity (α) in preparative chromatography. In contrast to LC, the enantioselectivity is usually low, and frequently marginal, in GC owing to the use of elevated working temperatures and apolar mobile phases. Low separation factors (α) are generally not detrimental to enantiomer analysis owing to the high efficiency of capillary columns in GC. However, separation factors of at least $\alpha > 1.3$ are an obvious prerequisite for any useful preparative enantiomer separation.

It should be noted that in the first uses of chiral stationary phases (CSPs) in GC, packed columns were initially involved, *i.e.*, for hydrogen-bonding CSP [1], complexation-type CSP [2,3] and inclusion-type CSP [4], and significant advances of (semi)preparative enantiomer separations by GC have previously been reported. Thus, on a 2 m × 1 mm I.D. column, packed with 5% N-TFA–L-valine–L-valine cyclohexyl ester on Chromosorb W, the enantiomers of N-TFA–alanine *tert.*-butyl ester were separated and subsequently characterized by their optical rotatory dispersion curves [1]. In complexation gas chromatography, (semi)preparative enantiomer separations have been reported for spiroketals (including pheromones) on nickel(II) bis[6-(heptafluorobutanoyl)-(5*S*)-carvonate] [5,6] and for an aromatic oxirane on manganese(II) bis-[3-(heptafluorobutanoyl)-(1*R*)-camphorate] [7]. Likewise, the invertomers of 1-chloro-2,2-dimethylaziridine were separated on a preparative scale on nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] and chiroptical data, absolute configurations and the inversion barrier were readily determined [8]. Following the observation of very large separation factors for *cis*- and *trans*-pinane on native α-cyclodextrin, dissolved in aqueous formamide and impregnated on Celite [4], the preparative enantiomer separation of camphene was subsequently achieved on a packed column [9]. Because of dehydration of

* Corresponding author.

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the stationary phase by the dry carrier gas, the retention times fluctuated and the enantioselectivity and resolution decreased rapidly with time. It was therefore necessary to use a mobile phase saturated with water vapour as proposed previously [10]. These "wetted" cyclodextrin columns had the disadvantage of a limited temperature range and a low efficiency.

With the advent of alkylated/acylated cyclodextrins [11,12], preferentially used as diluted CSPs in polysiloxanes such as OV-1701 (5% cyanopropyl–7% phenylmethylpolysiloxane) [13], numerous classes of compounds have been separated into enantiomers by GC. However, only occasionally have large separation factors been observed, *e.g.*, for halogenated compounds such as methyl 2-chloropropionate [14,15]. To this end, we [16] and others [17] recently used cyclodextrin derivatives in polysiloxane solution for the gas chromatographic (semi)preparative enantiomer separation of selected chiral compounds exhibiting large separation factors. The samples studied were the chiral inhalation anaesthetics enflurane and isoflurane.

EXPERIMENTAL

Materials

Enflurane and isoflurane were obtained from a local hospital. γ-Cyclodextrin was a courtesy from the Consortium für Elektrochemische Industrie, Munich, Germany.

Synthesis of octakis(2,6-di-O-n-pentyl-3-O-butanoyl)-γ-cyclodextrin

A 3-g amount of octakis(2,6-di-O-*n*-pentyl-3-O-butanoyl)-γ-cyclodextrin, introduced by König *et al.* [18], was synthesized according to their procedure except that the acylation was accomplished in triethylamine at 50°C. The product was carefully purified by column chromatography over silica gel with ethyl acetate–light petroleum (b.p. 60–90°C) (from 1:10 to 1:3, v/v).

Preparation of the packing

A 22.95-g amount of polysiloxane SE-54 (1% vinyl–5% phenylmethylpolysiloxane) (WGA, Düsseldorf, Germany) was dissolved in 600 ml of dry chloroform under reflux in a round-bottomed flask and 2.55 g of octakis(2,6-di-O-*n*-pentyl-3-O-butanoyl)-γ-cyclodextrin were added to the solution. A 100-g amount of Chromosorb P AW DMCS (80–100 mesh ≙ 0.18–0.15 mm) (Alltech, Arlington Heights, IL, USA) was poured into the mixture. The slurry was manually agitated until it became homogeneous. The solvent was carefully evaporated using a rotary evaporator at 40°C and a moderate vacuum for several hours followed by drying at 70°C and 0.004 Torr (1 Torr = 133.322 Pa) for 10 h. The Chromosorb prepared in this way is coated with 20.3% (w/w) of stationary phase. The CSP consists of 10.0% (w/w) cyclodextrin derivative in SE-54.

Preparation of the packed column

Three packed columns made of glass were prepared (for dimensions, see Table I). The Chromosorb was filled into the columns under a slight vacuum. Subsequently an ultrasonic bath was applied for 1 minute to achieve a dense, homogeneous bed.

Chromatography

Columns A and B were installed in a Fractovap 2101 gas chromatograph (Fisons, Mainz, Germany) and column C in a 5300 Mega gas chromatograph (Fisons) adapted to preparative GC. Helium (99.996%) was used as the carrier gas. The injector and flame ionization detector temperatures were 150°C. Enflurane and isoflurane were injected on-column. The tightness of the syringe, septum and column connections was carefully checked at the highest applied pressure of 4 bar (gauge) in order to avoid loss of sample during the injection. For preparative applications the Mega gas chromatograph was equipped with a post-column splitting device (1:1500) distributing the eluate to the detector and into a heated transfer line. Outside the oven a cooling trap (Fig. 1) was attached to the transfer line. The samples were separated from the carrier gas via condensation with liquid nitrogen as cooling medium. The traps were operated manually. Their design permits the easy collection of sample with an ordinary syringe, thus avoiding rinsing the traps with solvents. In the case of repetitive injections,

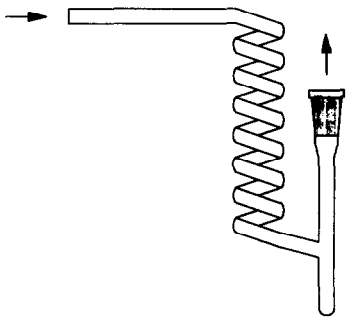

Fig. 1. Cooling trap made of glass (6 mm O.D., 3 mm I.D.). Drying tubes filled with dry calcium chloride are connected at the ground joints.

during the trapping of one enantiomer the trap containing the other enantiomer was sealed with a rubber septum at the inlet and with a rubber stopper at the drying tube outlet and was stored in the liquid nitrogen bath. Hence the highly volatile material remained in the solid state and no moisture entered the traps. After the desired number of repetitive injections the traps were allowed to warm up in another bath (−40°C). The liquid samples were collected with a syringe and sealed in ampoules. The amount of the samples after this transfer was used to determine the yield of recovered material.

The isolated enantiomers were characterized by ^{1}H NMR spectroscopy and capillary GC. The optical rotations of enflurane were determined in solution with limited reproducibility owing to the low specific rotation:

Enflurane, first peak:

$[\alpha]_D^{20} = -4.6 \pm 0.7$ ($c = 1$, *n*-hexane)

$[\alpha]_{365}^{20} = -15.0 \pm 0.6$ ($c = 1$, *n*-hexane)

Enflurane, second peak:

$[\alpha]_D^{20} = +4.6 \pm 0.7$ ($c = 1$, *n*-hexane)

$[\alpha]_{365}^{20} = +15.0 \pm 0.6$ ($c = 1$, *n*-hexane)

From the sign of the measured optical rotations the absolute configuration of enflurane enantiomers was assigned according to ref. 19: first peak (*R*)-(−)-enflurane, second peak (*S*)-(+)-enflurane. The absolute configuration of the enantiomers of isoflurane could not be assigned.

RESULTS AND DISCUSSION

Inhalation anaesthetics

Enflurane ($C^*HFCl-CF_2-O-CHF_2$) and isoflurane ($CF_3-C^*HCl-O-CHF_2$) are important human inhalation anaesthetics. Commercial preparations for clinical use provide both enflurane and isoflurane as racemic mixtures. As enantiomers can have different pharmacological and toxicological properties, access to the antipodes in sufficient amounts and in high chemical and enantiomeric purity is of great interest in order to screen enantioselective effects *in vitro* and *in vivo*. Depending on the outcome of such studies it cannot be excluded that enantiomerically pure inhalation anaesthetics will be introduced for clinical use in the future.

Because of the lack of suitable functionalities, the classical racemate resolution via diastereomeric salts is not feasible. Pearson [19] described multi-step procedures for the synthesis of enflurane and isoflurane enantiomers involving a resolution step via diastereomers of a suitable intermediate. The direct racemate resolution of enflurane was attempted via one-batch molecular inclusion into (−)-brucin [8% enantiomeric excess (ee)] [19] and into tri-*o*-thymotide (TOT, 38% *ee*) [20].

Analytical enantiomer separation

The analytical GC enantiomer separation of enflurane and isoflurane has been achieved on α- and γ-cyclodextrin derivatives [21] and on Chirasil-β-Dex [20]. We now found that enflurane shows an exceedingly high enantioselectivity ($\alpha = 2.16$ at 24°C) on octakis(2,6-di-O-*n*-pentyl-3-O-butanoyl)-γ-cyclodextrin [18] diluted in OV-1701 (40%, w/w) [13] (Fig. 2). At elevated temperatures and/or higher carrier gas velocities, enantiomer separation of enflurane and isoflurane can even be achieved within less than 25 s with a highly increased detection sensitivity (Fig. 3), although the enantioselectivity of isoflurane was less pronounced ($\alpha = 1.36$ at 24°C). The analytical enantiomer separation of the inhalation anaesthetic halothane CF_3-C^*HClBr was achieved at −14°C with $\alpha = 1.025$ (Fig. 4).

The high enantioselectivity invoked in the

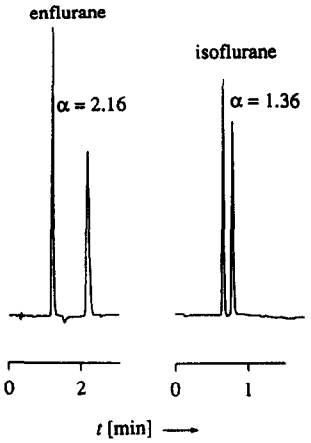

Fig. 2. Analytical gas chromatographic enantiomer separation involving high separation factors α of enflurane and isoflurane. Fused-silica capillary column (6 m × 0.25 mm I.D.) coated with octakis(2,6-di-O-*n*-pentyl-3-O-butanoyl)-γ-cyclodextrin in OV-1701 (40%, w/w), film thickness 0.25 μm. For enflurane, 24°C, 45 cm/s hydrogen; for isoflurane, 24°C, 45 cm/s hydrogen.

interaction of enflurane and octakis(2,6-di-O-*n*-pentyl-3-O-butanoyl)-γ-cyclodextrin is surprising and cannot readily be rationalized by enantioselective non-bonding Van der Waals interactions between the small chiral methyl group and the large cavity of the γ-cyclodextrin derivative. Dipole–dipole interactions which extend much more into space are more likely forces determining the observed high enantioselectivity.

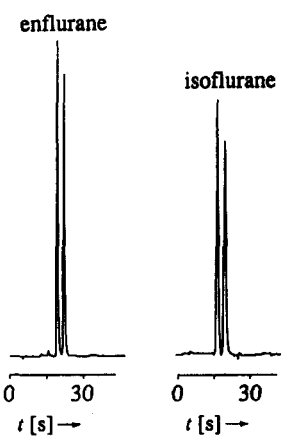

Fig. 3. Rapid analytical gas chromatographic enantiomer separation of enflurane and isoflurane (column as in Fig. 2). For enflurane, 60°C, 54 cm/s hydrogen; for isoflurane, 26°C, 100 cm/s hydrogen.

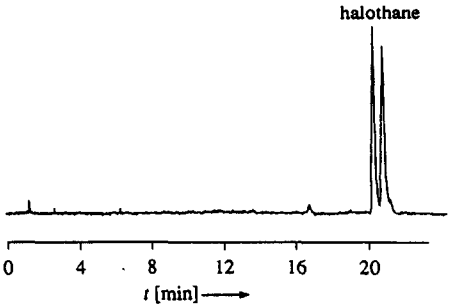

Fig. 4. Analytical gas chromatographic enantiomer separation of halothane. Fused-silica capillary column (25 m × 0.25 mm I.D.) coated with octakis(2,6-di-O-*n*-pentyl-3-O-butanoyl)-γ-cyclodextrin in OV-1701 (20%, w/w), film thickness 0.25 μm, −14°C, 33 cm/s hydrogen.

The occurrence of separation factors $\alpha > 1.3$ prompted us to utilize packed columns for the preparative enantiomer separation of enflurane and isoflurane.

Transfer to preparative-scale separations

A 3-g batch of the chiral selector octakis(2,6-di-O-*n*-pentyl-3-O-butanoyl)-γ-cyclodextrin, introduced by König *et al.* [18], was synthesized and special care was exercised to isolate a uniform product. Regioselective 2,6-dialkylation often leads to the formation of under- and over-alkylated derivatives [23], giving rise to a mixture of products after 3-acylation. In our hands (see Experimental), a highly pure product was obtained, as proved by supercritical fluid chromatography [22], by ionspray MS [23] (Fig. 5) and by ^{1}H NMR (a well resolved doublet for the anomeric hydrogen is an indication of a uniform and symmetric derivative).

Octakis(2,6 - di-O-*n*-pentyl -3 -O-butanoyl)-γ-cyclodextrin was diluted in the apolar polysiloxane SE-54 (10%, w/w). A high dilution was chosen in order to guarantee efficient coating of the support, leading to columns with high plate numbers (systematic studies of the effect of the selector concentration on performance represent an important topic for the future). The polar polysiloxane OV-1701, introduced previously as a versatile solvent for derivatized cyclodextrins in analytical enantiomer separations [13], was replaced with the gum phase SE-54, which is commercially available at a low cost. It dissolves

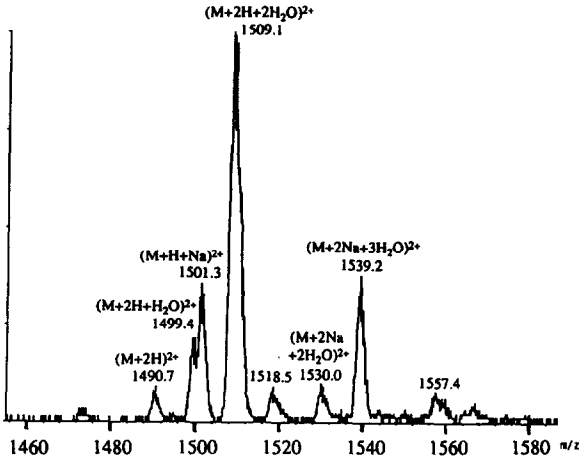

Fig. 5. Ionspray mass spectrum of synthesized octakis(2,6-di-O-*n*-pentyl-3-O-butanoyl)-γ-cyclodextrin (M_r = 2980). Determined for doubly charged protonated and cationized quasi-molecular ions (the natural levels of sodium impurities are sufficient to produce Na^+-containing clusters). Singly charged molecular ion not visible because of the high mass range. The absence of peaks 35 u lower indicates the absence of derivatives devoid of one *n*-pentyl or butanoyl group (M_r = 2910).

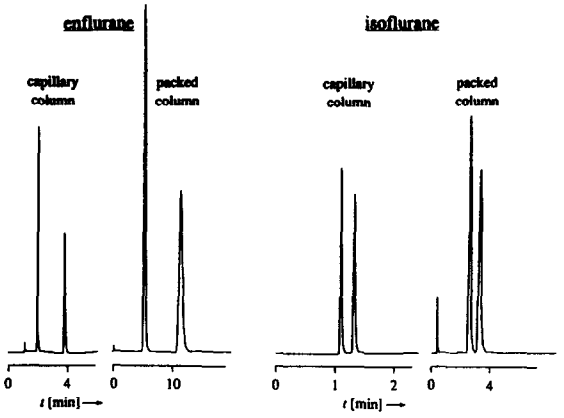

Fig. 6. Capillary *vs.* packed-column gas chromatographic enantiomer separation of enflurane and isoflurane on 10% (w/w) octakis(2,6-di-O-*n*-pentyl-3-O-butanoyl)-γ-cyclodextrin in SE-54 at 25°C. Capillary column (10 m × 0.25 mm I.D., film thickness 0.25 μm); packed column A.

octakis(2,6-di-O-*n*-pentyl-3-O-butanoyl)-γ-cyclodextrin readily, forming a clear and colourless mixture. This CSP was used to prepare an analytical capillary column and three packed columns (with different column dimensions and support materials) as shown in Table I.

First, we checked how the separation factor α is influenced by the change from 40% (w/w) in OV-1701 (*cf.*, Fig. 2) to 10% in SE-54 in the analytical mode of operation (Fig. 6). At 25°C α for enflurane changed from 2.16 to 2.15 and that for isoflurane from 1.36 to 1.32. Obviously the expected decrease in α with decreasing selector concentration [24] is compensated for by an increase in α caused by the weaker interaction with the apolar polysiloxane. Next, the packed column A was prepared, which showed not only the same elution order for enflurane and isoflurane enantiomers but also comparable separation factors α at the given concentration (10%, w/w), *i.e.*, α = 2.17 (25°C) for enflurane and α = 1.31 (25°C) for isoflurane. According to Fig. 6, the peak shape and plate number are favourable, resulting in a separation number *SN* = 5 for enflurane and *SN* = 0.9 for isoflurane (25°C). The results demonstrate that enantiomer separations performed on cyclodextrin derivatives diluted in a polysiloxane in high-resolution capillaries [13] can readily be transferred to packed columns. For column A, Chromosorb W AW DMCS was used because it represents a support type with small surface area and low loadability. An overloading experiment showed that a baseline separation was achieved with up to 0.4 μl (0.6 mg) of enflurane.

TABLE I

PACKED COLUMNS USED IN THIS STUDY

Column	Length × I.D.	Support material	Loading (%, w/w)	Amount of stationary phase (cyclodextrin derivative) (g)
A	2.3 m × 3 mm	4.2 g Chromosorb W AW DMCS	8.8	0.41 (0.041)
B	2.3 m × 3 mm	10 g Chromosorb P AW DMCS	22.1	2.84 (0.284)
C	4.0 m × 7 mm	95 g Chromosorb P AW DMCS	20.3	25.50 (2.55)

Column B was prepared using Chromosorb P AW DMCS, which permits the loading with liquid stationary phase up to 30%. As a safety margin a 22% loading was chosen and an increased capacity factor $k' = 5.74$ at the same temperature of column A due to a decrease in the phase ratio β by the same ratio was observed. This increase in k' is caused by a higher loading with stationary phase and by the higher density of Chromosorb P, both increasing the capacity of the CSP. Interestingly, α remained nearly unchanged and the *SN* values even increased slightly. Hence the use of Chromosorb P offers significant advantages over Chromosorb W and the higher loading with CSP has no adverse effect on performance. Finally, a Van Deemter plot was recorded (helium, 25°C, solute *n*-pentane, $k' = 11.8$) and the optimum was found at 8 cm/s.

Column C with the dimension 4 m × 7 mm I.D. designed for preparative separations, was operated near the Van Deemter optimum at 8.4 cm/s (inlet pressure 4 bar helium, gauge). For safety reasons, higher inlet pressures were avoided for the glass columns used. Fig. 7 shows the enantiomer separation of enflurane with increasing amounts injected at 40°C. Owing to overloading of the stationary phase, the peak maxima are shifted to shorter retention times with increasing sample volumes. A baseline separation is achieved with up to 20 μl (30 mg) of racemic enflurane at 40°C. At 26°C a baseline separation for up to 31 μl (47 mg) is possible. Most importantly, a high separation factor is achieved at a low capacity factor, thus decreasing the analysis time considerably. Moreover, the elution time of the second peak can further be reduced by increasing the elution temperature to 50°C (Fig. 8). This temperature programme reduces the preparative enantiomer separation from 100 to 45 min. Hence the speed of analysis is 5–20 times faster than in semi-preparative separations carried out previously by complexation GC [5–7]. The heading flank of the second peak is slightly distorted owing to rapid heating and to manipulations at the trap during sample collection.

The (semi)preparative enantiomer separation

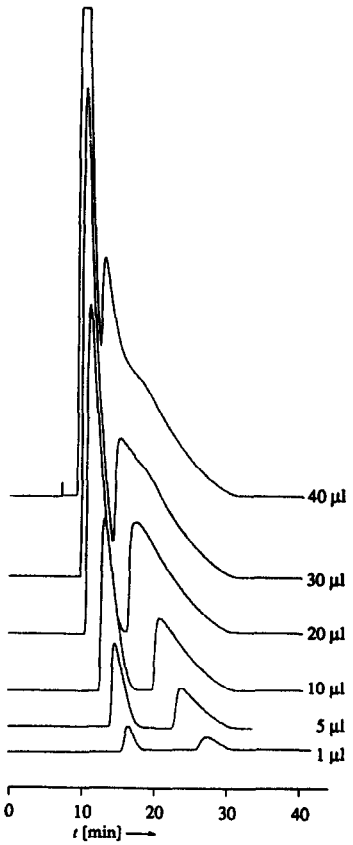

Fig. 7. Preparative gas chromatographic enantiomer separation of enflurane with increasing sample volumes. Column C, 40°C, 8.4 cm/s helium.

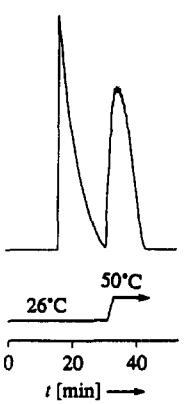

Fig. 8. Temperature-programmed preparative gas chromatographic enantiomer separation of 31 μl (47 mg) of enflurane. Column C, temperature programmed, 8.4 cm/s helium.

of isoflurane was only tested isothermally at 26°C. Because α is smaller than for enflurane, only amounts up to 4 μl (6 mg) of isoflurane can be baseline separated with column C in 28 min. With isothermal runs, repetitive injections in close succession and at short intervals are possible (Fig. 9). This approach might prove useful for automated operation with permanently attached traps and appropriate switching devices.

The recovery of enflurane and isoflurane depends on the trapping device used. The trap depicted in Fig. 1 yielded the best results. This may be due to the low temperature gradient caused by the long pathway along the spiral, thus avoiding the formation of mist [25]. The recoveries were about 80% for enflurane and 60% for isoflurane. In general, repetitive injections are preferred because the handling of larger amounts involves smaller losses than those resulting from a single injection.

The ^{1}H NMR spectra of the isolated enantiomers were identical with those of the racemic samples. Capillary GC with flame ionization detection showed chemical purities of $\geq$99.5% and enantiomeric purities of $ee \geq 99.9\%$ for enflurane and $ee \geq 99.4\%$ for isoflurane (Fig. 10). It was not possible to separate halothane enantiomers (CF_3–C*HClBr) by the method described here.

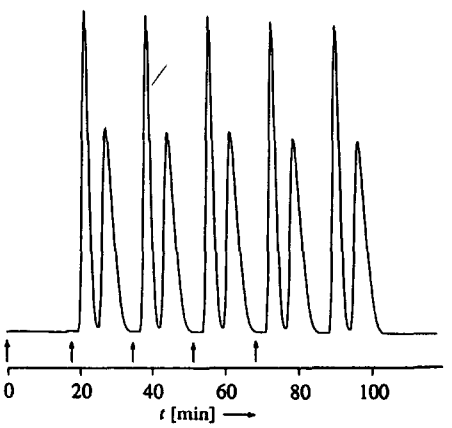

Fig. 9. Preparative gas chromatographic enantiomer separation of isoflurane with repetitive injections of 4 μl during one run. Column C, 26°C, 8.4 cm/s helium.

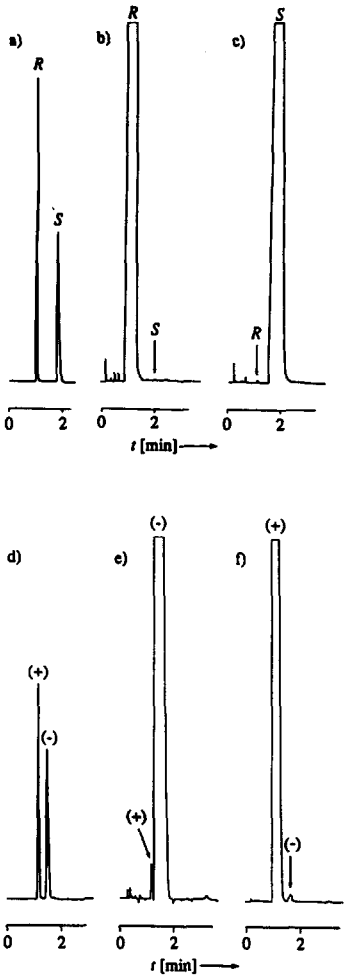

Fig. 10. Gas chromatographic determination of enantiomeric purity of enflurane and isoflurane. Column as in Fig. 2. Enflurane: 25°C, 55 cm/s hydrogen. (a) *rac*-Enflurane; (b) (*R*)-(−)-enflurane, $ee \geq 99.9\%$; (c) (*S*)-(+)-enflurane, $ee \geq 99.9\%$. Isoflurane: 14°C, 42 cm/s hydrogen. (d) *rac*-Isoflurane; (e) (−)-isoflurane, $ee \geq 99.4\%$; (f) (+)-isoflurane, $ee \geq 99.7\%$.

CONCLUSIONS

The preparative enantiomer separation of enflurane and isoflurane with high chemical and enantiomeric purities has been achieved. This approach represents the first complete preparative resolution of both anaesthetics. Thus, up to 47 mg of enflurane can be baseline separated in one run in 45 min and 6 mg of isoflurane in 28 min using a 4 m × 7 mm I.D. column.

As a gas is used as the mobile phase, the recovery of the highly volatile enantiomers in a pure state presents no difficulties and the method thus has important advantages over the recovery of the enantiomers from solvents, educts, reagents and by-products in enantioselective synthesis or in chromatographic methods involving condensed mobile phases.

The preparative column has been used for several months without observable changes in selectivity and efficiency. The production rate can be improved by using larger sample volumes if lower enantiomeric purities are tolerated. Scaling up of the method is straightforward via repetitive injections. In principle, columns of larger dimensions might prove useful in combination with automated recycling techniques. For this purpose, the requirement of large amounts of the CSP could probably be satisfied with a cyclodextrin derivative of lower purity but similar enantioselectivity. Replacement of the carrier gas helium with hydrogen with appropriate safety measures could further decrease separation times.

The high enantioselectivity for enflurane given by a CSP made up of natural D-glucose units implies that similar enantioselective phenomena may also occur *in vivo*.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We thank the Consortium für Elektrochemische Industrie, Munich, Germany, for a generous gift of γ-cyclodextrin.

NOTE ADDED IN PROOF

Independently from our investigations, the preparative enantiomer separation of enflurane and isoflurane ($\alpha \leq 1.5$, $k' < 15$) was reported recently on undiluted octakis(2,6-di-O-*n*-pentyl-3-O-trifluoroacetyl-γ-cyclodextrin (A. Shitangkoon, D.U. Staerk and G. Vigh, *4th International Symposium on Chiral Discrimination, Montreal, September 19–22, 1993*, Abstract number 142).

The assignment of isoflurane in Fig. 10 is as follows: first peak (*S*)-(+), second peak (*R*)-(−) based on recent vibrational circular dichroism studies [P.L. Polavarapu, A.L. Cholli and G. Vernice, *J. Amer. Chem. Soc.*, 114 (1992) 10953].

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