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Gas chromatographic separation of the enantiomers of volatile fluoroether anesthetics using derivatized cyclodextrin stationary phases. Part I

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ABSTRACT

The capacity factors and chiral selectivity factors were determined as a function of temperature for the enantiomers of desflurane, enflurane and isoflurane on nine commercially available cyclodextrin derivative-coated GC capillary columns. The enantiomers could be separated with the trifluoroacetylcyclodextrin stationary phases which show good chiral selectivities coupled with modest column efficiencies. Very rapid enantiomeric trace determinations of desflurane, isoflurane and enflurane could be achieved with a trifluoroacetyl γ-cylodextrin-coated capillary column.

1. INTRODUCTION

Inhalational anesthetics are used to induce and maintain general anesthesia [1]. Several volatile compounds show anesthetic potential, including cyclopropane, nitrous oxide, halogenated hydrocarbons (*e.g.* chloroform, halothane) and ethers (*e.g.* diethyl ether and various fluoroethers). Although all of these have been used at one time or another in clinical practice, the fluoroether anesthetics are becoming favored owing to their potency, volatility, chemical stability, low toxicity and lack of flammability. The structures of the most commonly used fluoroether anesthetics (desflurane, enflurane and isoflurane) are shown in Fig. 1. Interestingly, they all are chiral compounds.

It has been commonly understood for some time that general anesthetics act on the nervous system by non-specific perturbation [2]. However, in line with the growing awareness of the biological activity differences of the enantiomerically pure drugs [3], stereoselective effects were recently observed when the interactions between the nerve ion channels and the pure enantiomers of isoflurane were studied [4]. Two recent patents suggest that the administration of the pure enantiomers of desflurane and isoflurane is more desirable than that of the racemic mixture [5,6]. Although most fluoroether anesthetics are synthesized as racemic mixtures [7], some progress has been made toward the synthesis of enantiomerically enriched materials [5,6]. Monitoring the enantiomeric purity of these materials therefore became extremely important. The recent, spectacular developments in the fields of chiral separations by capillary gas chromatography [8] allowed for the separation of the enantiomers of

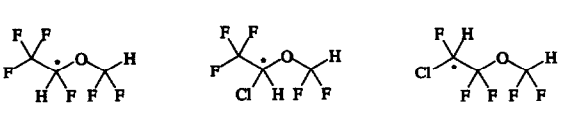

Fig. 1. Structures of desflurane, enflurane and isoflurane.

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isoflurane and enflurane on a 25-m capillary column coated with octakis(6-O-methyl-2,3-di-O-pentyl)-γ-cyclodextrin stationary phase [9].

This paper summarizes the results of a detailed investigation aimed at finding a single commercially available capillary column with sufficiently high chiral selectivity and separation efficiency that would permit the rapid separation of the enantiomers of the three fluoroether anesthetics: desflurane, enflurane and isoflurane. In order to try to elucidate possible correlations between chiral selectivity and the respective structures of the fluoroethers and the cyclodextrin-based stationary phases, the thermodynamic parameters of the enantiomers of all three anesthetics were also determined on all the stationary phases studied. The commercially available derivatized cyclodextrin-based stationary phases studied here included α-, β- and γ-dipentyl-, trifluoroacetyl- and permethyl-(*S*)-2-hydroxypropylcyclodextrins. Dipentylcyclodextrins (DAs) are synthesized [10] by treating a dimethyl sulfoxide solution of the respective cyclodextrin with excess amounts of 1-bromopentane in the presence of sodium hydroxide powder, resulting in a mixture of 2,6-di-O-pentylcyclodextrins. Permethyl-(*S*)-2-hydroxypropylcyclodextrins are then prepared by reacting the aqueous sodium hydroxide solution of the cyclodextrin with (*S*)-propylene oxide, followed by permethylation using an excess amount of methyl iodide [11]. Trifluoroacetylcyclodextrins are made by treating a solution of DAs in tetrahydrofuran with excess amounts of trifluoroacetic anhydride [12]. For most derivatized cyclodextrins, the products are mixtures of isomers and homologues [10–12].

EXPERIMENTAL

Separations were effected on a Hewlett-Packard HP 5890 series II gas chromatograph equipped with a cryostat, a septumless split/splitless injector (Microseal), a flame ionization detector and a Chemstation data collection/analysis unit. Fused-silica capillary columns (30m × 0.25mm I.D.), coated with different cyclodextrin-based stationary phases (0.25 μm film thickness) were obtained from Astec (Whippany, NJ, USA). Three types of cyclodextrin derivatives were studied: (i) DAs (commercial names ADA, BDA and GDA), (ii) permethyl-(*S*)-2-hydroxypropylcyclodextrins (PHs) (commercial names APH, BPH and GPH) and (iii) trifluoroacetylcyclodextrins (TAs) (commercial names ATA, BTA and GTA). The prefixes A, B, and G are used to describe the α-, β- and γ-cyclodextrins, respectively.

Hydrogen was used as the carrier gas at an average linear velocity of 0.5 m/s. Methane was used as an unretained compound. The injector and detector temperatures were maintained at 250°C. All separations were performed isothermally in the −10 to 70°C range as noted in the Results section. All inhalational fluoroether anesthetics were obtained from Anaquest (Murray Hill, NJ, USA), a division of BOC Health Care, and used without further purification. The anesthetics were injected either as *n*-pentane solutions or as air–vapor mixtures.

RESULTS AND DISCUSSION

All three fluoroether anesthetics have similar structures and possess an acidic proton at the chiral center. These acidic protons (the ^{1}H NMR chemical shift values are desflurane C*H*F δ = 5.925 ppm, isoflurane C*H*Cl δ = 6.031 ppm and enflurane C*H*FCl δ = 6.161 ppm) can interact with the suitable functional groups of the derivatized cyclodextrin stationary phases. Hydrogen bonding interactions and/or dipole–dipole interactions are probably the major polar interactions and might play a significant role in the chiral recognition process. Although the derivatized cyclodextrins are mixtures of isomers and homologues, and the thermodynamic parameters are average values representing a range of interactions between the solute and cyclodextrin stationary phases, it was nevertheless suggested [13] that they can be used to infer the processes which result in the eventual chiral separation of the enantiomers. Therefore, the capacity factor (k') and selectivity factor (α) values of the enantiomers of all three fluoroethers were determined as a function of temperature and analyzed as a function of the inverse absolute temperature to yield the enthalpy (ΔH^0) and entropy (ΔS^0) values according to

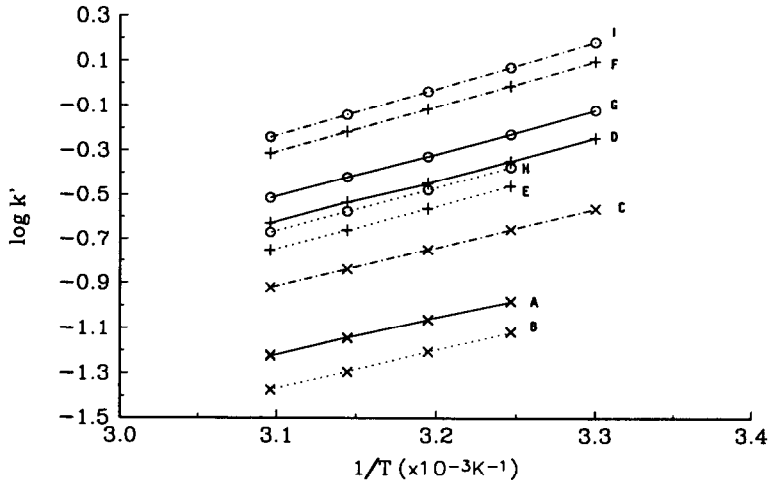

Fig. 2. Log k' *vs.* $1/T$ plots for the three fluoroethers obtained with the DA stationary phase series. A = desflurane–ADA; B = desflurane–BDA; C = desflurane–GDA; D = isoflurane–ADA; E = isoflurane–BDA; F = isoflurane–GDA; G = enflurane–ADA; H = enflurane–BDA; I = enflurane–GDA.

$$\log k' = -\frac{\Delta H^0}{2.3RT} + \frac{\Delta S^0}{2.3R} + \log \beta \qquad (1)$$

where β is the phase ratio and R is the universal gas constant. The log k' *vs.* $1/T$ plots for the more retained enantiomer of the three fluoroethers are shown in Figs. 2, 3 and 4 for the DA, PH and TA stationary phase series, respectively. The calculated enthalpy and entropy values are listed in Table I. All log k' *vs.* $1/T$ plots are linear, with the regression coefficients greater than 0.999. When the enantiomers can be separated, the corresponding $\Delta(\Delta H^0)$ and $\Delta(\Delta S^0)$ values can be calculated from the log α *vs.* $1/T$ plots as

$$\log \alpha = -\frac{\Delta(\Delta H^0)}{2.3RT} + \frac{\Delta(\Delta S^0)}{2.3R} \qquad (2)$$

The log α *vs.* $1/T$ plots for the three fluoroethers are shown in Fig. 5 for the TA stationary phase series, and the $\Delta(\Delta H^0)$ and $\Delta(\Delta S^0)$ values are listed in Table II. All log α *vs.* $1/T$ plots

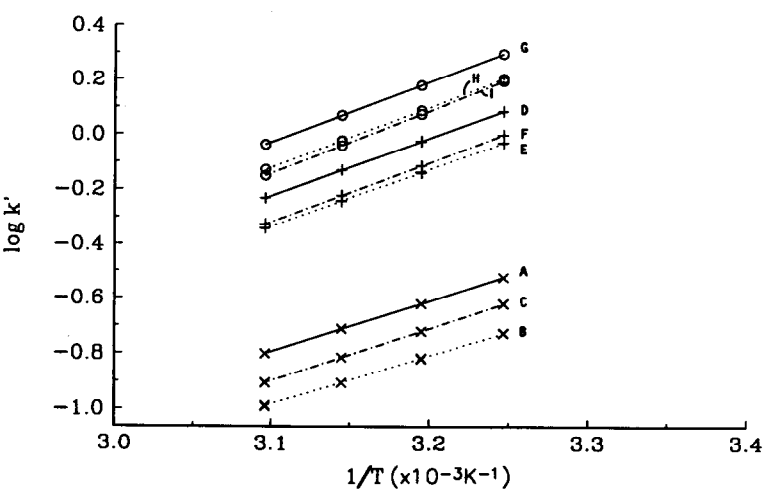

Fig. 3. Log k' *vs.* $1/T$ plots for the three fluoroethers obtained with the PH stationary phase series. A = desflurane–APH; B = desflurane–BPH; C = desflurane–GPH; D = isoflurane–APH; E = isoflurane–BPH; F = isoflurane–GPH; G = enflurane–APH; H = enflurane–BPH; I = enflurane–GPH.

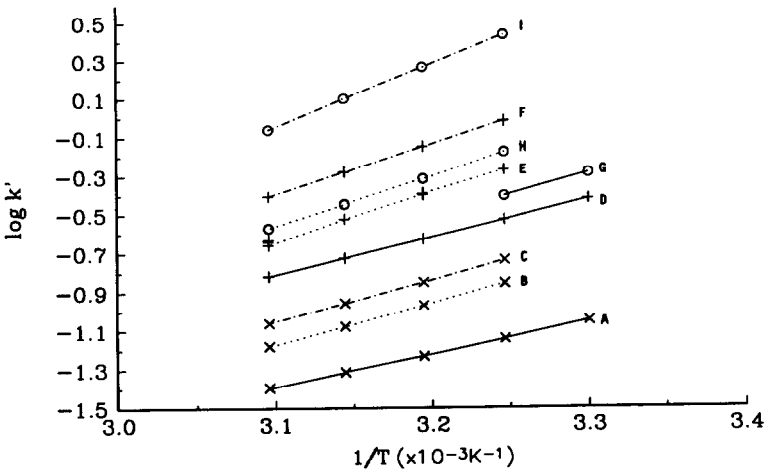

Fig. 4. Log k_2' *vs.* $1/T$ plots for the three fluoroethers obtained with the TA stationary phase series. A = desflurane–ATA; B = desflurane–BTA; C = desflurane–GTA; D = isoflurane–ATA; E = isoflurane–BTA; F = isoflurane–GTA; G = enflurane–ATA; H = enflurane–BTA; I = enflurane–GTA.

were linear with regression coefficients greater than 0.998, except for desflurane ($r^2 = 0.996$).

On the DAs, the enantiomers of the fluoroether anesthetics could not be separated. The ΔS^0 values of the different fluoroethers are the lowest on these stationary phases; they are very similar to each other with a very slight increase (about 1 entropy unit) in the order desflurane < isoflurane < enflurane. The ΔH^0 values of the different fluoroethers increase in the order desflurane < isoflurane < enflurane, following the boiling points of the fluoroethers (23.5, 48.5 and 56.5°C, respectively), and differ from each other by about 1200 cal/mol (1 cal = 4.14 J). When the ring size of the cyclodextrin is changed, the ΔH^0 values increase by about 200–300 cal/mol in the order $\alpha < \beta < \gamma$. However, the ΔS^0 values increase in the order $\alpha < \gamma < \beta$ for all three fluoroether solutes, indicating that the spatial arrangement of the active sites on the dipentyl-β-cyclodextrin is slightly more favorable for simultaneous multiple interactions.

The numerical values of ΔH^0 and ΔS^0 observed on the PHs are very similar to those observed on the DAs, indicating that the strength and specificity of the binding interac-

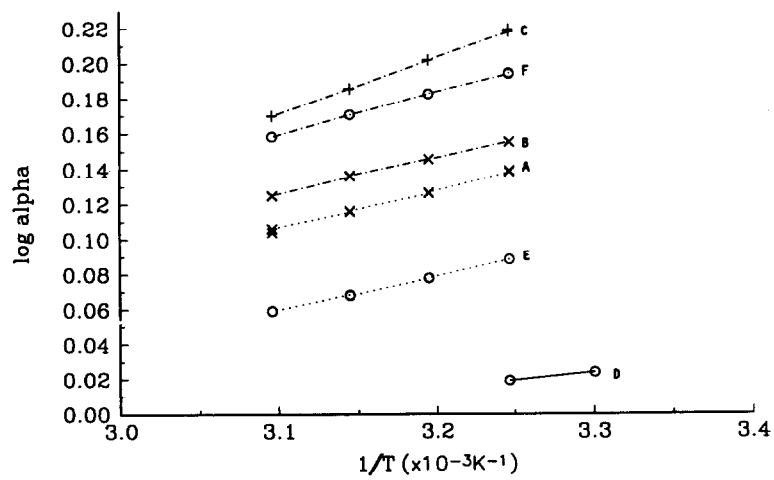

Fig. 5. Log α *vs.* $1/T$ plots for the three fluoroethers obtained with the TA stationary phase series. A = desflurane–BTA; B = desflurane–GTA; C = isoflurane–GTA; D = enflurane–ATA; E = enflurane–BTA; F = enflurane–GTA.

TABLE I

THERMODYNAMIC PARAMETERS FOR THE FLUOROETHERS CALCULATED FROM THE LOG k' *vs.* $1/T$ DATA SHOWN IN FIGS. 2–4

Phase	Desflurane		Isoflurane		Enflurane	
	ΔS^0 [cal/(mol · K)]	ΔH^0 (cal/mol)	ΔS^0 [cal/(mol · K)]	ΔH^0 (cal/mol)	ΔS^0 [cal/(mol · K)]	ΔH^0 (cal/mol)
ADA	−17.31	−7325.78	−18.27	−8514.34	−18.45	−8740.74
BDA	−19.41	−7787.91	−19.78	−8821.93	−19.73	−8923.71
GDA	−18.13	−8039.39	−18.91	−9186.77	−19.37	−9446.12
APH	−18.59	−8360.94	−19.73	−9568.32	−20.42	−10 207.93
BPH	−18.13	−7937.29	−19.69	−9395.60	−20.74	−10 052.97
GPH	−19.87	−8626.56	−20.97	−9825.79	−22.07	−10 447.42
ATA	−19.09	−7644.48	−20.10	−8819.87	−22.02	−9568.55
					−23.26	−9976.77
BTA	−22.30	−8839.96	−27.15	−11 362.59	−25.78	−10 943.74
	−24.91	−9841.49			−28.25	−11 828.34
GTA	−22.02	−8900.42	−23.21	−10 191.46	−33.05	−13 901.46
	−24.26	−9807.53	−26.92	−11 647.75	−35.66	−14 979.78

tions are very similar on both series. Both the ΔH^0 and the ΔS^0 values of the different fluoroethers increase in the order desflurane < isoflurane < enflurane, again following the boiling points. The increase is large from desflurane to isoflurane, but small from isoflurane to enflurane. When the ring size of the hydroxypropylcyclodextrins is changed, the order is $\beta < \alpha < \gamma$, different from those observed on the DAs.

The TAs are capable of chiral differentiation toward the enantiomers of the three fluoroethers, although the extent of this differentiation varies with both the type of the fluoroether and the ring size of the cyclodextrin. The enantiomers of enflurane can be separated on all three TA phases (ATA, BTA and GTA), those of desflurane on BTA and GTA, but those of isoflurane only on GTA. The interactions between the more retained enantiomers of the fluoroethers and the stationary phases (Table I) are much stronger for the TAs than for either the DAs or the PHs, and are mostly due to entropic effects (as indicated by the 2–10 entropy units increase in ΔS^0) and less to enthalpic effects (about 1.2–4 kcal/mol increase in ΔH^0). Both the ΔH^0 and the ΔS^0 values of enflurane increase with the size of the cyclodextrin ring.

The chiral selectivity factors of the fluoroethers (Fig. 5) increase with the increasing size of cyclodextrins: $\alpha < \beta < \gamma$. The $\Delta(\Delta H^0)$ and

TABLE II

THERMODYNAMIC PARAMETERS FOR THE FLUOROETHERS CALCULATED FROM THE LOG α *vs.* $1/T$ DATA SHOWN IN FIG. 5 FOR THE TA STATIONARY PHASE SERIES

Phase	Desflurane		Isoflurane		Enflurane	
	$\Delta(\Delta S^0)$ (cal/mol · K)	$\Delta(\Delta H^0)$ (cal/mol)	$\Delta(\Delta S^0)$ (cal/mol · K)	$\Delta(\Delta H^0)$ (cal/mol)	$\Delta(\Delta S^0)$ (cal/mol · K)	$\Delta(\Delta H^0)$ (cal/mol)
ATA					−1.24	−405.48
BTA	−2.61	−1001.53			−2.47	−884.60
GTA	−2.24	−907.11	−3.75	−1456.29	−2.61	−1078.27

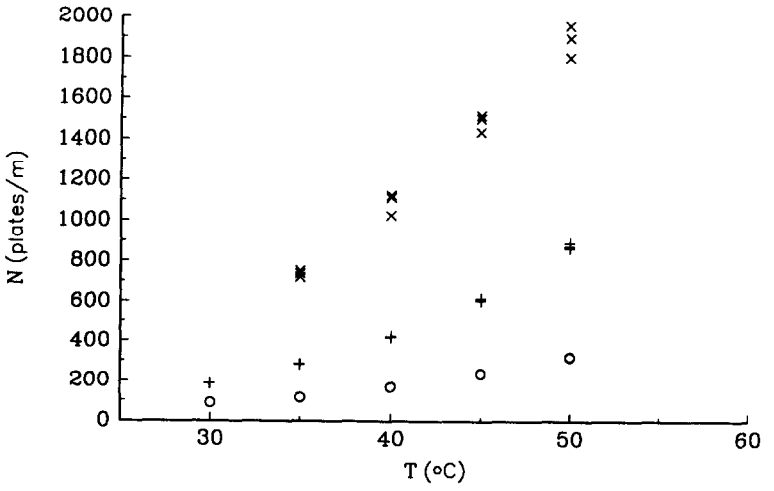

Fig. 6. Column efficiencies (plates/m) as a function of column temperature for the DA stationary phases, determined from the shape of the peak of the more retained enflurane enantiomer. + = ADA; × = BDA; ○ = GDA.

$\Delta(\Delta S^0)$ values increase regularly with the ring size of the cyclodextrin only for enflurane, which has an external stereogenic center (Fig. 1). For desflurane and isoflurane, both of which have internal stereogenic centers, there is no such clear trend: the $\Delta(\Delta H^0)$ and $\Delta(\Delta S^0)$ values for desflurane are highest on BTA and for isoflurane they are highest on GTA. Considering these trends and the fact that the molar volumes for all three solutes are similar, one cannot state that these separations are driven by an inclusion-type discrimination mechanism.

Fig. 6–8 show the column efficiencies (plates/m) for the DA, PH and TA phases, as determined from the shape of the peak of the more retained enflurane enantiomer. All three phases show a strong decrease in efficiency below 50°C. The PH columns are the least efficient and show

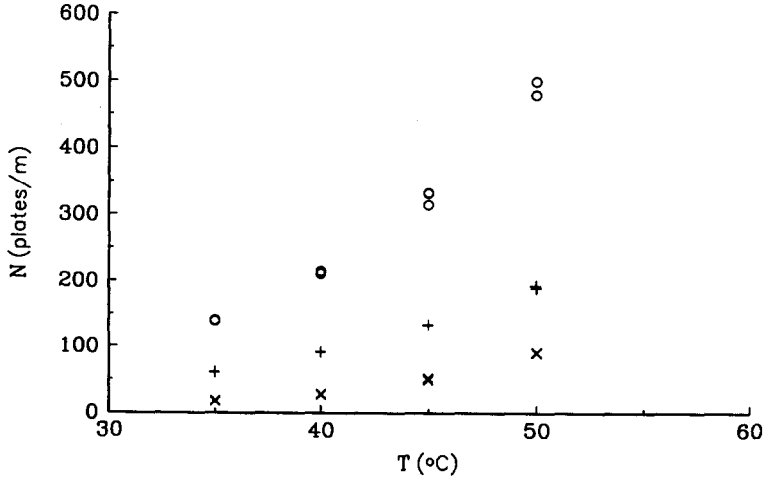

Fig. 7. Column efficiencies (plates/m) as a function of column temperature for the PH stationary phases, determined from the shape of the peak of the more retained enflurane enantiomer. + = APH; × = BPH; ○ = GPH.

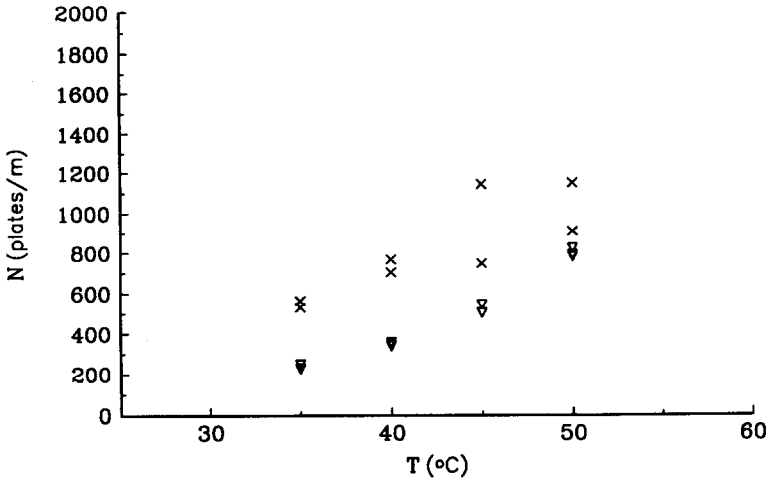

Fig. 8. Column efficiencies (plates/m) as a function of column temperature for the TA stationary phases, determined from the shape of the peak of the more retained enflurane enantiomer. × = BTA; ▽ = GTA.

no chiral selectivity for the fluoroethers; the DA columns are the most efficient, but lack chiral discrimination toward the fluoroethers. The TA columns have modest efficiencies, but their high chiral selectivities permit fast chiral separations for the fluoroethers.

From the selectivity and efficiency studies it can be concluded that the enantiomers of all three fluoroethers can be effectively separated on a single capillary, the GTA-coated column, as shown in Fig. 9. As the selectivity factors for isoflurane and enflurane are large and the capacity factors are small, good trace analyses can be performed very rapidly using short columns. The chromatograms of two enantiomerically almost pure isoflurane samples (99.97 and 98.38% enantiomeric excess, respectively, for the less retained and the more retained enantiomers) are shown in Figs. 10 and 11; the separations were obtained with a 15-m GTA column in less than 2 min.

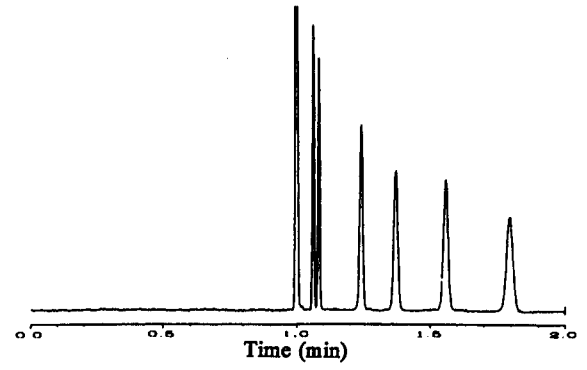

Fig. 9. Separation of the enantiomers of desflurane, isoflurane and enflurane on a 30 m × 0.25 mm I.D. fused-silica capillary, coated with a 0.25-μm thick film of GTA. Conditions: hydrogen carrier gas at a linear flow-rate of 0.5 m/s; 50°C isothermal run.

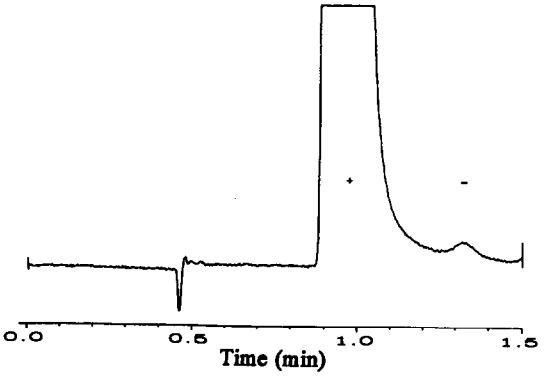

Fig. 10. Separation of the enantiomers of an enriched enflurane sample (99.95% enantiomeric excess for the less retained component) on a 15 m × 0.25 mm I.D. fused-silica capillary, coated with a 0.25-μm thick film of GTA. Conditions: hydrogen carrier gas at a linear flow-rate of 0.5 m/s; 35°C isothermal run.

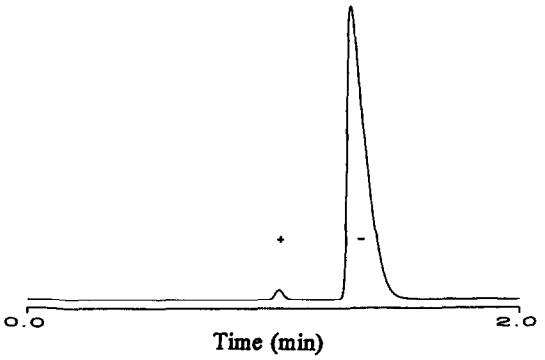

Fig. 11. Separation of the enantiomers of an enriched enflurane sample (96.82% enantiomeric excess for the more retained component) on a 15 m × 0.25 mm I.D. fused-silica capillary, coated with a 0.25-μm thick film of GTA. Conditions: hydrogen carrier gas at a linear flow-rate of 0.5 m/s; 35°C isothermal run.

CONCLUSIONS

A detailed study of commercially available cyclodextrin-based capillary columns, which do not rely on the use of a silicone stabilizing medium, indicates that very rapid enantiomeric trace determinations of desflurane, isoflurane and enflurane can be achieved with a single capillary column coated with the trifluoroacetyl-γ-cyclodextrin phase, opening the way to, among others, meaningful pharmacokinetic studies. Contrary to suggestions in the literature [13], even detailed thermodynamic studies failed to identify unequivocally the separation mechanisms that are responsible for the chiral discrimination.

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REFERENCES

1 V.J. Collins, *Principles of Anesthesiology*, Lea & Febiger, Philadelphia, PA, 2nd ed., 1976.
2 N.P. Franks and W.R. Lieb, *Nature*, 300 (1982) 487.
3 E.J. Ariëns, *Biochem. Pharmacol.*, 37 (1988) 9.
4 N.P. Franks and W.R. Lieb, *Science*, 254 (1991) 427.
5 J.W. Young, *US Pat.*, 5 114 714 (1992).
6 J.W. Young, *US Pat.*, 5 114 715 (1992).
7 D.F. Halpern, *US Pat.*, 4 855 511 (1989).
8 W.A. König, *Gas Chromatographic Enantiomer Separation with Modified Cyclodextrins*, Hüthig, Heidelberg, 1992.
9 J. Meinwald, W.R. Thompson, D.L. Pearson, W.A. König, T. Runge and W. Francke, *Science*, 251 (1991) 560.
10 D.W. Armstrong, W. Li, A.M. Stalcup, H.V. Secor, R.R. Izac and J.I. Seeman, *Anal. Chim. Acta.*, 234 (1990) 365.
11 D.W. Armstrong, W. Li, C.D. Chang and J. Pitha, *Anal. Chem.*, 62 (1990) 914.
12 D.W. Armstrong, W. Li and H.L. Jin, *J. Chromatogr.*, 509 (1990) 303.
13 A. Berthod, W. Li and D.W. Armstrong, *Anal. Chem.*, 64 (1992) 873.