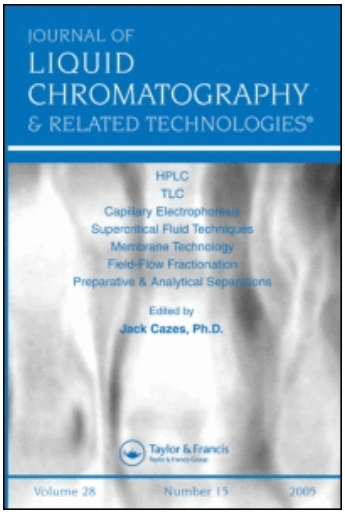

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DIRECT RESOLUTION OF ISAMOLTANE (CGP 361A) AND ENCIPRAZINE (WY 48624) ENANTIOMERS, USING CHIRAL HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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DIRECT RESOLUTION OF ISAMOLTANE (CGP 361A) AND ENCIPRAZINE (WY 48624) ENANTIOMERS, USING CHIRAL HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A chiral high performance liquid chromatography method was developed for the resolution of the enantiomers of two new β-aminoalcohols, isamoltane and enciprazine. The separation of the enantiomers was accomplished, without any derivatization, on a chiral column containing 3,5-dimethylphenylcarbamate of cellulose (Chiralcel OD) as chiral selector.

The effects of diethylamine, organic modifiers (ethanol and 2-propanol) and temperature were studied.

The α and Rs values of isamoltane and enciprazine ranged, respectively, from 1.25 to 2.03 and from 0.80 to 5.16. The optimized conditions were used for a semipreparative chromatographic separation to determine the elution order of the enantiomers.

INTRODUCTION

Over the last few years, optical resolution by high performance liquid chromatography (HPLC) has become increasingly important for the determination of optical purity of pharmaceutical compounds.

Owing to the development of a wide variety of chiral stationary phases (CSPs), many of which are now commercially available, a large number of substrates of different structures and compositions have been made accessible for chiral analysis either at analytical or preparative levels.[1-7]

Indirect methods of chiral separations involves the synthesis of diastereoisomers followed by chromatography on an achiral column, whereas direct methods resolves the molecules as enantiomers utilizing chromatography on chiral CSPs.[8] Direct methods based on CSPs are preferred, because more rapid and suitable for the resolution of racemates; further they allow, in many cases, the recovery of the enantiomers with a high degree of purity.[9-11]

Oxazolidin-2-one derivatives of racemic β-aminoalcohols have been resolved on several CSPs, containing chiral selectors chemically bound to silica gel, the "so-called" Pirkle tipe stationary phases.[12-15]

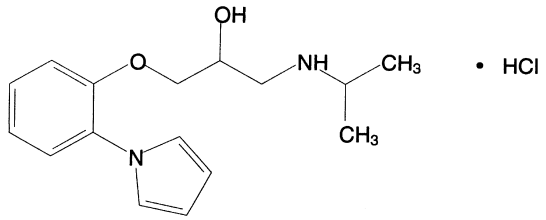
Semipreparative chiral separation of oxazolidin-2-one derivatives of β-aminoalcohols has been obtained on silica-gel columns containing as chiral selector cellulose derivatives.[16]

Chiral separations of β-aminoalcohols have been carried out on swollen microcristalline triacetilcellulose and CSPs consisting of α_1-acid glycoprotein bound to silica gel.[17-18]

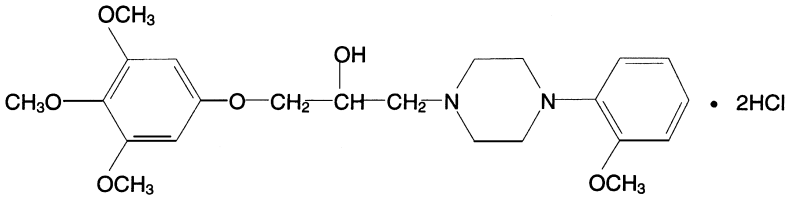
More recently enantioseparation of racemic compounds has been also achieved in capillary electrophoresis (CE) by using cyclodextrins as chiral selectors.[19]

Chiralcel OD column contains cellulose tris(3,5-dimethylpheniylcarbamate), as the chiral selector, coated on macroporous silica gel and has been successfully used in liquid chromatography for chiral separations of a series of amino alcohols with β-adrenergic blocking activity.[20-23].

Isamoltane (CGP 361A) (Figure 1) is a phenoxy propranolamine derivative, possessing β-receptor blocking properties, which are, in terms of potency, about equivalent to those of propranolol and oxprenolol. In animal experiments, however, the drug exerts, at low doses, anxiolytic-like effects and increases positive social contacts. This activity is considerably greater than that



ISAMOLTANE



ENCIPRAZINE

Figure 1. Structures of investigated compounds.

observed for other β-adrenoceptor ligands including propranolol, oxprenolol and cyanopindolol.[24-25] The anxiolytic activity and the β-receptor blocking properties of the compound reside in the (-)-enantiomer, being 71-fold more active than the (+)-enantiomer as anxiolytic.[26]

Enciprazine (WY 48624) (Figure 1) is a non-benzodiazepine anxiolytic drug, with anxiolytic effect comparable with that of diazepines.[27] Based on pre-clinical experimental data, enciprazine appears to have several advantages over benzodiazepines; in fact the side-effects, e.g. abuse potential, interaction with alcohol or sedative/hypnotic drugs, are less than other benzodiazepines, as demonstrated in animal studies.[28] As (phenoxyphenyl-piperazinyl)-propranol derivative, it exhibits a variety of biological activities, such as cardiovascular, hypotensive and local anaesthetic properties.[29]

The present work reports the influence of diethylamine, organic modifiers and temperature, on the resolution of the enantiomers of isamoltane and enciprazine carried out with a Chiralcel OD column. The analytical method was adapted to a semipreparative separation of the enantiomers of the amino alcohols on a semipreparative Chiralcel OD column.

EXPERIMENTAL

Reagents

n-Hexane, ethanol and 2-propanol (HPLC grade) were obtained from C. Erba (Milan, Italy). Diethylamine was obtained from Fluka Chemie (Buchs, Switzerland). 1,3,5-tri-tert.-butylbenzene was purchased from Sigma (St. Louis, MO, USA).

Isamoltane, (CGP 361A), [1-(2-(1-pyrrolyl)-phenoxy)-3-isopropylamino-2-propanol hydrochloride] was obtained from Novartis Farma (Origgio, VA, Italy).

Enciprazine, (WY 48624), [(R,S)-4-(2 methoxyphenyl)-α [(3,4,5-trimethoxyphenoxy) methyl]-1-piperazine ethanol, dihydrochloride] was obtained from Wyeth-Lederle (Aprilia, LT, Italy).

Apparatus

The liquid chromatographic system consisted of a Waters 600 MS pump, a Waters U6K injector with a 2 mL loop, and a Waters 996 programmable multi-wavelength diode array detector operated at 234 and 244 nm. The signal was acquired and processed by the Millenium 2010 software (Waters, Milford, MA, USA).

The analytical and semipreparative columns were Chiralcel OD (250 x 4.6 mm I.D. and 250 x 10 mm I.D. respectively) (Daicel Chemical Industries, Tokyo, Japan).

A thermostat (Lauda RMS, Königs-hofen, Germany) controlled the water temperature, in the column jacket.

Optical rotations were measured on a Perkin Elmer 241 micropolarimeter equipped with a Na lamp operated at 589 nm. The volume of the measuring cell was 1 mL and the length of the optical path 10 cm.

Operating Conditions

Analytical experimental conditions used for isamoltane: mobile phase *n*-hexane-ethanol-diethylamine (98:2:0.5 v/v/v), detector wavelength 244 nm; enciprazine: mobile phase *n*-hexane-2-propanol-diethylamine (50:50:0.5 v/v/v),

detector wavelength 234 nm. Column: Chiralcel OD (250 x 4.6 mm I.D.); flow rate 0.5 mL/min; column temperature: 20°C; volume injected 10 μL. Semipreparative experimental conditions used for isamoltane: mobile phase *n*-hexane-ethanol-diethylamine (98:2:0.5 v/v/v), detector wavelength 280 nm; enciprazine: mobile phase *n*-hexane-ethanol-diethylamine (50:50:0.5 v/v/v), detector wavelength 278 nm; column: Chiralcel OD (250 x 10 mm I.D.), flow rate 2.0 mL/min; column temperature: ambient; volume injected 100 μL. Solvents used as mobile phases were degassed with ultrasonic bath before use.

RESULTS AND DISCUSSION

Organic Modifiers Effect

The compounds, examined in this study, contain amino or hydroxyl groups that might form hydrogen bonds with the stationary phase; therefore the organic modifiers, contained in the mobile phases, affect the retention of the solutes by increasing the solvation of the solutes and by competing for the polar sites in the stationary phase.

We studied the effects of diethylamine, ethanol, and 2-propanol, contained in the mobile phases, on the enantioselectivity (α) and resolution (R_s) factors. The chromatographic parameters decreased with an increase in ethanol or 2-propanol content in the mobile phases. A higher content of diethylamine had a beneficial effect for each amino alcohol examined, since, reduces the hydrogen bonding between the chiral base and the silanol groups of silica gel. In this way the tailing of the peaks and the retention times are reduced significantly, whereas the active sites of the CPS, responsible for the chiral recognition, are more effective.

The chromatographic results relative to isamoltane and enciprazine are presented in Table 1.

The effect of the concentration of ethyl alcohol on retention (k') and resolution (R_s) of isamoltane and enciprazine is showed in Figure 2. The highest resolution and enantioselectivity factors for isamoltane (3.79 and 1.58 respectively) were obtained with a mobile phase consisting of *n*-hexane-ethanol-diethylamine (98:2:0.5 v/v/v/), whereas the lowest values (0.83 and 0.69) were obtained with a mobile phase consisting of *n*-hexane-ethanol-diethylamine (85:15:0.5 v/v/v). Similar behaviour was observed for the enciprazine enantiomers, with resolution (R_s) and enantioselectivity (α) factors ranging from 4.64 to 3.48 and from 1.87 to 1.84 respectively. The enantioselectivity is almost unchanged over the entire range of alcohol concentration for the examined

Table 1

Chromatographic Data for Compounds 1 and 2 on Chiralcel OD*

Compound	First Eluted Enantiomer	k_1'[a]	α[b]	R_S[c]	Eluent[d]
Isamoltane(1)	(-)	3.57	1.58	3.79	A
	(-)	1.46	1.44	2.05	B
	(-)	1.70	1.29	0.97	C
	(-)	0.76	1.43	1.56	D
	(-)	0.53	1.30	0.83	E
	(-)	4.20	1.25	0.80	F
Enciprazine (2)	(-)	1.47	1.87	4.64	G
	(-)	1.05	1.90	4.11	H
	(-)	1.03	1.89	4.04	I
	(-)	0.81	1.90	3.74	L
	(-)	0.68	1.84	3.48	M
	(-)	1.60	2.02	4.70	N
	(-)	1.72	2.03	4.46	O

* Organic modifiers ethanol and 2-propanol.

[a] The capacity factor of the first eluted enantiomer.

[b] The enantioselectivity factor.

[c] The resolution factor.

[d] Eluents employed are: (A) *n*-hexane-ethanol-diethylamine (98:2:0.5) v/v/v); (B) *n*-hexane-ethanol-diethylamine (95:5:0.5 v/v/v); (C) *n*-hexane-ethanol-diethylamine (95:5:0.1 v/v/v); (D) *n*-hexane-ethanol-diethylamine (90:10:0.5 v/v/v); (E)*n*-hexane-ethanol-diethylamine (85:15:0.5 v/v/v); (F)*n*-hexane-2-propanol-diethylamine (95:5:0.5 v/v/v); (G) *n*-hexane-ethanol-diethylamine (70:30:0.5 v/v/v); (H) *n*-hexane-ethanol-diethylamine (60:40:0.5 v/v/v); (I) *n*-hexane-ethanol-diethylamine (60:40:0.1 v/v/v); (L) *n*-hexane-ethanol-diethylamine (50:50:0.5 v/v/v); (M) *n*-hexane-ethanol-diethylamine (40:60:0.5 v/v/v); (N) *n*-hexane-2-propanol-diethylamine (50:50:0.5 v/v/v); (O) *n*-hexane-2-propanol-diethylamine (50:50:0.1 v/v/v).

compounds. This is likely due to the fact that, at constant temperature, the conformation of the polymeric phase, the selective adsorption sites and the selectand/selector associate is not affected by organic modifier concentration in the mobile phase.[30] Chromatograms of the compounds mentioned above are shown in Figure 3.

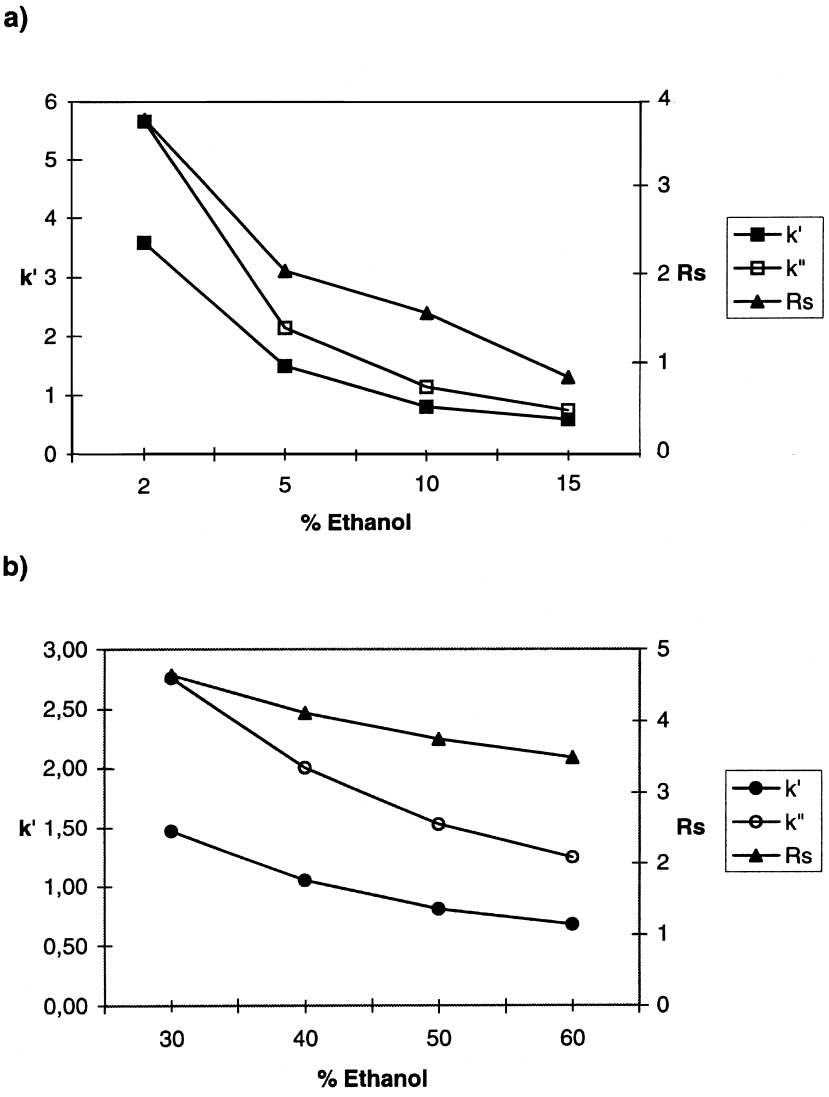


Figure 2. Effect of the ethanol on retention and resolution of isamoltane (a) and enciprazine (b). Column: Chiralcel OD (250 x 4.6 mm I. D.); mobile phase: n-hexane-ethanol-DEA; flow rate 0.5 mL/min; column temperature 20°C; detection wavelength 244 nm (a) and 234 nm (b).

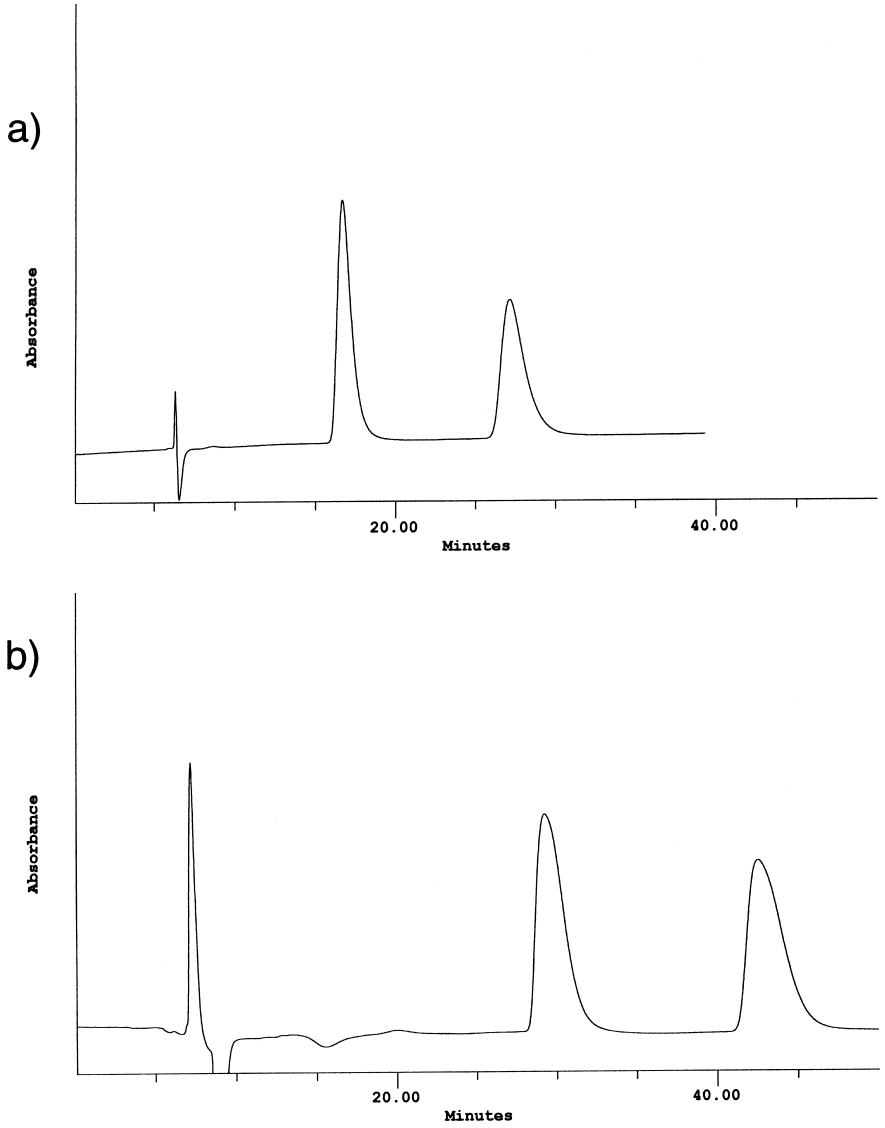


Figure 3. HPLC of isamoltane (a) and enciprazine (b). Column: Chiralcel OD (250 x 4.6 mm I. D.); flow rate 0.5 mL/min; column temperature 20°C; detection wavelength 244nm (a) and 234 (b); mobile phase: n-hexane-ethanol-DEA (98:2:0.5 v/v/v) (a), n-hexane-isopropil alchool-DEA (50:50:0.5 v/v/v) (b).

Table 2

Chromatographic Data for Compounds 1 and 2 on Chiralcel OD Performed by Varying the Temperature

Compound	First Eluted Enantiomer	k_1'[a]	α[b]	R_S[c]	Temperature[d]
Isamoltane (1)	(-)	1.95	1.86	4.26	0
	(-)	1.72	1.68	3.54	10
	(-)	1.59	1.52	2.82	20
	(-)	1.43	1.32	1.67	30
	(-)	1.33	1.26	1.40	40
Enciprazine (2)	(-)	1.67	2.21	4.99	0
	(-)	1.38	2.03	5.16	10
	(-)	1.09	1.91	4.86	20
	(-)	0.81	1.77	4.17	30
	(-)	0.76	1.73	3.88	40

[a] The capacity factor of the first eluted enantiomer.
[b] The enantioselectivity factor.
[c] The resolution factor.
[d] Eluents employed are for isamoltane: *n*-hexane-ethanol-diethylamine (95:5.0.5 v/v/v); for enciprazine: *n*-hexane-ethanol-diethylamine (50:50:0.5 v/v/v).

Temperature Effects

Temperature can affect retention, resolution, and stereoselectivity of chiral compounds examined and can be used as a tool to regulate these parameters.

The distribution coefficients (K') of a solute between the stationary phase and the mobile phase in a chromatographic system is a function of the differences in free energy of the solute in the two phases, according to the Van't Hoff equation: $\ln K' = -\Delta G°/RT = -\Delta H°/RT + \Delta S°/R$. Plots of the natural logarithms of chromatographic retention factors against the reciprocal of absolute temperature are usually linear. Moreover for a given pair of enantiomers, the Van't Hoff equation relates the separation factor (α) to the temperature by this formula: $\ln\alpha = -\Delta_{j,i}\,\Delta G/RT = -\Delta_{j,i}\,\Delta H/RT + \Delta_{j,i}\,\Delta S/R$, where $\Delta_{j,i}\,\Delta G$, $\Delta_{j,i}\,\Delta H$ and $-\Delta_{j,i}\,\Delta S$ are the differences in free energy, enthalpy, and entropy of adsorption between the enantiomer more retained (j) and the less retained one (i), respectively. In this case, a plot of the $\ln\alpha$ vs 1/T will yield a straight line, whose slope and intercept are $-\Delta_{j,i}\,\Delta H/R$ and $-\Delta_{j,i}\,\Delta S/R$ respectively.

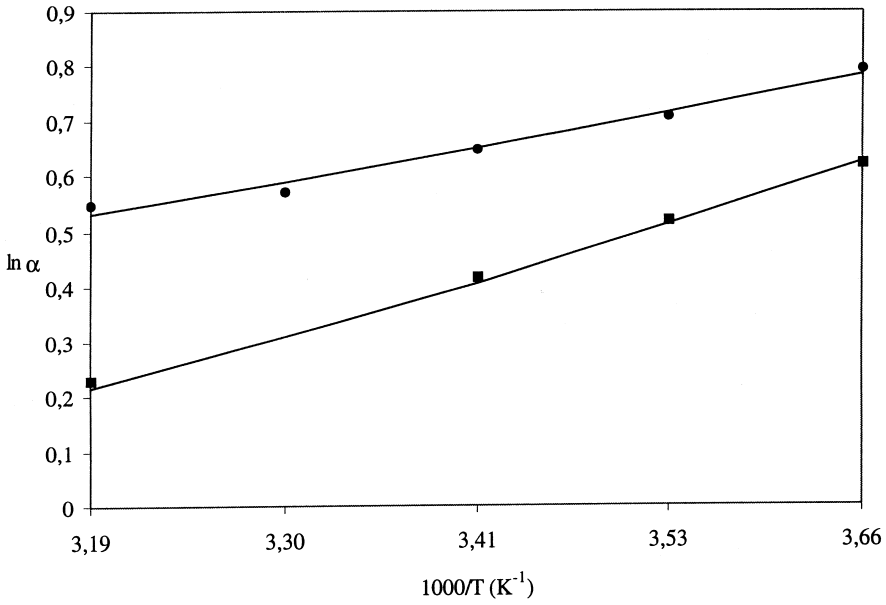


Figure 4. Van't Hoff plots of isamoltane (■) and enciprazine (●). Column: Chiralcel OD (250 x 4.6 mm I. D.); flow rate 0.5 mL/min; detection wavelength 244 nm (■) and 234 nm (●); mobile phase: n-hexane-ethanol-DEA (98:2:0.5 v/v/v)(■), n-hexane-ethanol-DEA (50:50:0.5 v/v/v)(●).

Table 3

Thermodinamic Data for Equilibria Between Compounds 1 and 2 on Chiracel OD Performed by Varying the Temperature

Compound[a]	ΔΔH (cal/mol)	ΔΔS (cal/mol °K)	R[b]
Isamoltane (1)	-7221	-21.33	0.9871
Enciprazine (2)	-4482	-9.87	0.9827

[a] Eluents employed are for isamoltane: *n*-hexane-ethanol-diethylamine (95:5:0.5 v/v/v); for enciprazine: *n*-hexane-ethanol-diethylamine (50:50:0.5 v/v/v).

[b] Linear regression coefficient of the Van't Hoff straight line.

Table 4

Comparison of ΔΔG Obtained by Mathematical Formula and Graphical Method at Different Temperatures

Temperature °K	ΔΔG[a] Isamoltane	ΔΔG[b] Isamoltane	ΔΔG[a] Enciprazine	ΔΔG[b] Enciprazine
273	-1406.16	-1397.91	-1796.84	-1787.49
283	-1218.59	-1184.61	-1663.10	-1688.79
293	-1018.26	-971.31	-1573.69	-1590.09
303	-698.22	-758.01	-1435.96	-1491.39
313	-600.40	-544.71	-1423.96	-1392.69

[a] ΔΔG (J mol^{-1}) calculated by formula: $\Delta G = -RT \ln \alpha$.
[b] ΔΔG (J mol^{-1}) calculated by graphical method.

To observe the effects of the temperature on the chromatographic parameters of the examined compounds, mixtures of *n*-hexane-ethanol-DEA (95:5:0.5 v/v/v and 50:50:0.5 v/v/v) were used for isamoltane and enciprazine respectively.

Void volumes were determined using 1, 3, 5, tri-tert.-butylbenzene.[31] Data were collected in 10°C increments from 0° to 40°C. As it is shown in Table 2, α and R_s values decreased for isamoltane with the increasing of temperature, whereas a maximum of R_s was observed for enciprazine at 10°C.

The Van't Hoff plots are depicted in Figure 4; as one may note these plots are linear and therefore the temperature does not influence the enantioselectivity. In Table 3 are listed the thermodynamic data; the inspection of these reveal that $\Delta_{j,i}\,\Delta H$ and $\Delta_{j,i}\,\Delta S$ have the same signs, indicating that enthalpic and entropic forces exert opposite effects on $\Delta_{j,i}\,\Delta G$ and, hence, on enantioselectivity α, and an enthalpy-driven separations have occurred.

The correlation coefficient values ($r^2 > 0.98$) show that a high linearity was observed and this is in agreement with the previous observation that chiral discrimination mechanism remains essentially constant within the interval of temperatures studied.

In Table 4 are reported the ΔΔG values obtained by the equation $\Delta\Delta G = -RT\ln\alpha$ and those obtained by graphical method. Comparison of these values shows that, within the fairly large experimental errors, there is good agreement.

Semipreparative Analysis

The analytical optimized chromatographic conditions, studied for the compounds mentioned above, were adapted for the preparation of milligram quantities of the pure enantiomers. A semipreparative Chiralcel OD column was operated with eluents *n*-hexane-ethanol-DEA (98:2:0.5 v/v/v and 50:50:0.5 v/v/v) for isamoltane and enciprazine respectively. Owing to the low solubility of enciprazine, a mobile phase containing 50 per cent of ethanol has been preferred.

The semipreparative column was loaded with 100 μL of solutions containing 10 mg/mL of each product. In order to increase the production rate, a scaling up of the sample size injected was carried out by increasing the volume of solution injected.[2] The highest amount of enciprazine injected was 8 mg corresponding to a volume of 800 μL, while the amount of isamoltane injected was 4 mg corresponding to 400 μL; in addition the wavelengths monitored were 278 nm (enciprazine) and 280 nm (isamoltane) to prevent detector saturation. The Figure 5 shows the chromatograms obtained by injecting 1, 4 and 8 mg of enciprazine.

The total amount of enciprazine injected was approximately 112 mg with a recovery of 92 % corresponding to 62.8 mg of the first eluted enantiomer and 40.2 mg of the second one. The enantiomeric purity, checked with an analytical column, of the first eluted enantiomer was 98.3%, while that of the second one was 99.2%. The angle of optical rotation of the more retained enantiomer was, for enciprazine, $[\alpha]_D^{20}$ = +3.0; while the value of the less retained one was $[\alpha]_D^{20}$ = -3.2 (c = 0.5 % in ethanol).

The sample size chosen for isamoltane was 4 mg per injection; approximately an amount of 112 mg was injected in to the column, 24.9 mg of the first eluted enantiomer (optical purity of 87.7%), and 24 mg for the second one, (optical purity of 85.5%) were collected, with a recovery of 43.7%. Measurements of optical rotation of the separated enantiomers gave, for the more retained isomer a value of $[\alpha]_D^{20}$ = +10.4, while the value of the less retained one was $[\alpha]_D^{20}$ = -11.2 (c = 0.5 % in ethanol).

CONCLUSIONS

The analytical and semi-preparative enantiomeric separation of compounds studied in this work, allows investigations of their biological behaviour. In fact the interaction between a single enantiomer and its receptor is an important issue together with the metabolic transformation after administration.

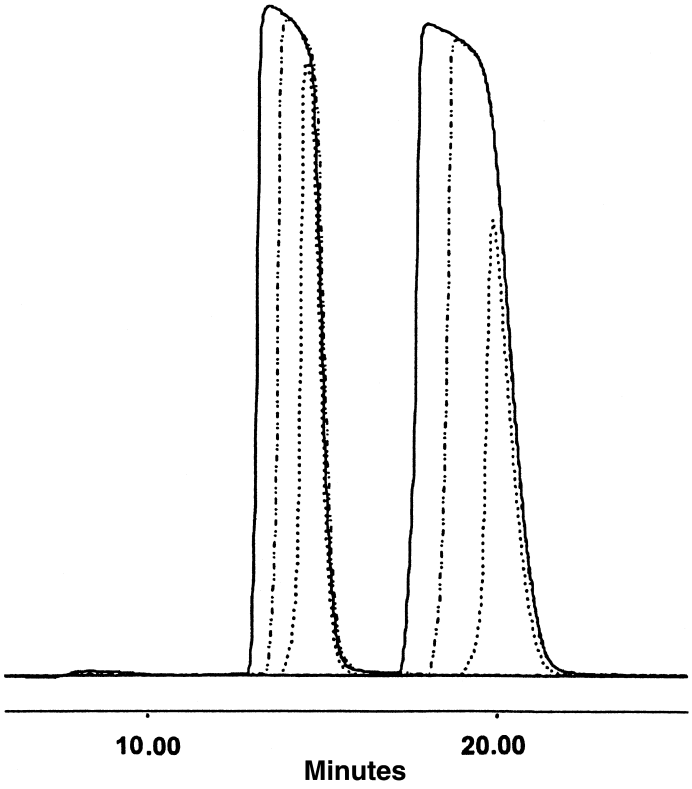


Figure 5. Scaling up for semipreparative separation of enciprazine. Column Chiralcel OD (250 x 10 mm I. D.); mobile phase: n-hexane-ethanol-DEA (50:50:0.5 v/v/v); flow rate 2 mL/min; column temperature: ambient; detection wavelength 278 nm. Injected amounts: 100 µL (- - - - - - - - - - - -), 400µL (— ·· — ·· — ·· — ··), 800 µL (————————) of a solution containing 10 mg/mL in ethanol.

In addition with the increasing interest in enantiomerically pure drug formulations from pharmaceutical industries[32] and regulatory authorities,[33-37] a chromatographic method able to determine the chiral purity is quite suitable for a quality control in bulk product and pharmaceutical formulations.

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