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Chromatographic resolution of 1,2-amino alcohols on a chiral stationary phase containing N,N′-(3,5-dinitrobenzoyl)-*trans*-1,2-diaminocyclohexane

Theoretical and practical aspects

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ABSTRACT

Optical resolution of several 1,2-diamino alcohols including some β-adrenergic blocking agents (β-blockers) was obtained by high-performance liquid chromatography on a chiral stationary phase containing 3,5-dinitrobenzoyl derivatives of *trans*-1,2-diaminocyclohexane (DACH-DNB) as chiral selectors. After formation of oxazolidin-2-one derivatives, racemic amino alcohols were completely resolved (α values ranging from 1.14 to 1.55 and R_s from 1.2 to 3.3) on a 250 × 4.0 mm I.D. stainless-steel column. Further, some separations on chiral and achiral, coupled columns are reported: they show diastereo- and enantioselectivity for amino alcohols with more than one chiral centre. The method allows the utilization of both spectrophotometric and spectrofluorimetric detectors; moreover the availability of the (*R*,*R*), (*S*,*S*) selectors makes it possible to evaluate enantiomeric excesses higher than 99.9%. Some separations were also carried out with microbore columns (2.0 mm I.D.), which afforded the same performance.

INTRODUCTION

Many drugs or physiologically important compounds are chiral molecules and the optical isomers of these compounds may differ in their pharmacological activities. In some instances differences in the undesirable side-effects of these enantiomers are important.

One of the major goals in biochemical and pharmaceutical research is the provision of optically pure compounds. Nevertheless, it may be worth noting that 467 of the existing 528 chiral synthetic drugs are marketed as racemates and only the remaining 61 are sold as single enantiomers. Indeed, the production of optically pure compounds either by asymmetric synthesis or by crystallization of diastereoisomeric mixtures is usually expensive. Therefore, rapid and sensitive methods for the

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resolution and quantification of the individual optically pure enantiomers are of great importance; chromatographic methods on both the analytical and preparative scale have become of considerable interest in recent years.

Basically, two different approaches are available for the liquid chromatographic resolution of enantiomers: the formation of non-transient diastereoisomeric derivatives by reaction with a chiral derivatizing agent (CDA) followed by chromatography on a non-chiral column, and the formation of transient diastereoisomers by means of a chiral mobile phase additive (CMPA) or by the use of chiral stationary phases (CSPs) [1].

Direct high-performance liquid chromatographic (HPLC) separation on a column packed with a chiral stationary phase is more rapid and suitable for the resolution of a racemic mixture. Further, in many instances this approach allows the recovery of the enantiomorph with a high degree of optical purity [1–4].

Adrenergic blocking agents (β-blockers) are amino alcohols and are widely used as drugs for the treatment of hypertension and angina pectoris. It is well known that the pharmacological properties of these compounds are mainly due to the (*S*)-(−) isomer. So, for example, the (*S*)-(−)-propranolol is 100 times more active than the (*R*)-(+) enantiomer.

Racemic mixtures of β-blockers have been separated as oxazolidin-2-one derivatives using α_1-acid glycoprotein [5] bonded stationary phase or swollen microcrystalline triacetylcellulose [6] on a semipreparative scale. The same racemic mixtures were also successfully resolved using as chiral selectors (*R*)-(−)-(3,5-dinitrobenzoyl)phenylglycine [7] or monosaccharide derivatives chemically bonded to the stationary phase [8].

Optical resolution of β-blocking agents was also performed without derivatization on columns packed with cellulose triphenylcarbamate derivatives supported on silica gel [9] or α_1-acid glycoprotein bonded stationary phase [10]. Taking into account the wide interest in this type of compound, we have carried out the resolution of several chiral amino alcohols, including the most common β-blockers, by using standard and microbore columns packed with the N,N′-3,5-dinitrobenzoyl derivative of *trans*-1,2-diaminocyclohexane as a chiral selector. The above CSP has been successfully used for the separation of racemic sulphoxides, selenoxides [11], anti-inflammatory agents and some β-blocking agents [12,13].

In this paper we present the results of an HPLC study of the above compounds.

EXPERIMENTAL

Apparatus

Analytical liquid chromatography was performed on a Waters Assoc. (Milford, MA, U.S.A.) chromatograph equipped with a Model U6K injector, two Model M510 solvent delivery systems and a temperature control module (TCM). Different detectors were used, including a Model M490 programmable multi-wavelength detector (Waters Assoc.) and a Model LS-5 luminescence spectrometer (Perkin-Elmer, Norwalk, CT, U.S.A.).

Microbore HPLC was performed with a Carlo Erba (Milan, Italy) System 20 equipped with a micro-UVIS 20 UV–visible detector (2-mm optical path length, 250-nl cell volume, time constant τ = 100 ms) and a Rheodyne Model 8125 5-μl loop injector.

Chromatographic data were collected and processed on a Waters Assoc. Model 840 data and chromatography control station.

^{1}H and ^{13}C NMR spectra were recorded on a Varian (Palo Alto, CA, U.S.A.) XL 300 spectrometer; all resonances are reported in ppm relative to tetramethylsilane (TMS). Mass spectra were recorded with a VG Tritech, (Manchester, U.K.) TS250 mass spectrometer (electron impact 70 eV). IR spectra were recorded as potassium bromide pellets on a Nicolet (Madison, WI, U.S.A.) 5DX Fourier transform (FT)-IR spectrometer.

Chemicals

LiChrosorb Si 100 (5 μm) and HPLC-grade solvents were obtained from Merck (Darmstadt, F.R.G.), 3-glycidoxypropyltrimethoxysilane (GOPTMS) from Janssen (Beerse, Belgium), (1*R*,2*R*)-diaminocyclohexane [(1*R*,2*R*)-DACH], (1*S*,2*S*)-diaminocyclohexane [(1*S*,2*S*)-DACH], 3,5-dinitrobenzoylchloride (DNB-Cl), 1,1'-carbonyldiimidazole (CDI), 20% phosgene solution in toluene, (*R*)- and (*S*)-propranolol (**9**) hydrochloride, (1*R*,2*S*)-ephedrine (**2**), (1*S*,2*R*)-ephedrine (**2**) hydrochloride, (1*R*,2*S*)-norephedrine (**4**) and (1*S*,2*R*)-norephedrine (**4**) hydrochloride from Fluka (Buchs, Switzerland) and (1*R*,2*R*)-ψ-ephedrine (**3**) and (1*S*,2*S*)-ψ-ephedrine (**3**) from Sigma (St. Louis, MO, U.S.A.). Racemic β-blockers were supplied by the following manufacturers: pronethalol (**7**) hydrochloride and atenolol (**14**) from ICI (Macclesfield, U.K.), oxprenolol (**5**) hydrochloride and metoprolol (**8**) tartrate from Ciba-Geigy (Varese, Italy), acebutolol (**12**) hydrochloride from Bayer (Milan, Italy), RBS Pharma (Milan, Italy), SIT (Pavia, Italy) and SPA (Milan, Italy), pindolol (**13**) from Sandoz (Milan, Italy), sotalol (**11**) hydrochloride from Bristol (Latina, Italy) and alprenolol (**1**) hydrochloride from Bik Gulden (Milan, Italy). 1-(2'5'-Dimethoxyphenyl)-2aminoethanol (**10**) was kindly provided by Prof. F. Macchia (Istituto di Chimica Organica, Università di Pisa, Italy). Betaxolol (**6**) was extracted from pharmaceutical preparations. Other chemicals were of analytical-reagent grade and used as received.

Fig. 1 shows the stuctures of the racemic compounds used.

Preparation of CSP

The CSP was prepared as shown in Fig. 2 [14].

Preparation of oxazolidin-2-one derivatives

Cyclization with 1,1-carbonyldiimidazole (CDI). A 1.1-mmol amount of CDI and 0.5 ml of anhydrous pyridine were added to a solution of 1.0 mmol of racemic amino alcohol in 10 ml of anhydrous tetrahydrofuran and then refluxed for 1 h. The mixture was diluted with diethyl ether (30 ml) and the organic layer was washed with acidified water (3 × 20 ml), dried over magnesium sulphate and evaporated to dryness under reduced pressure (*ca.* 23 mmHg). The residue was chromatographed on a silica gel column and afforded the desired products in 80% yield (not optimized).

Cyclization with phosgene [15]. A stirred mixture of amino alcohol (5 mmol), 10% sodium hydroxide solution (15 ml) and diethyl ether (25 ml) (dichloromethane for acebutolol derivatization) was cooled to 0°C and 20% phosgene solution (5 ml) in toluene was added dropwise. The mixture was stirred for 1 h, then the organic layer was collected, dried over magnesium sulphate and evaporated to dryness under reduced pressure (*ca.* 23 mmHg).

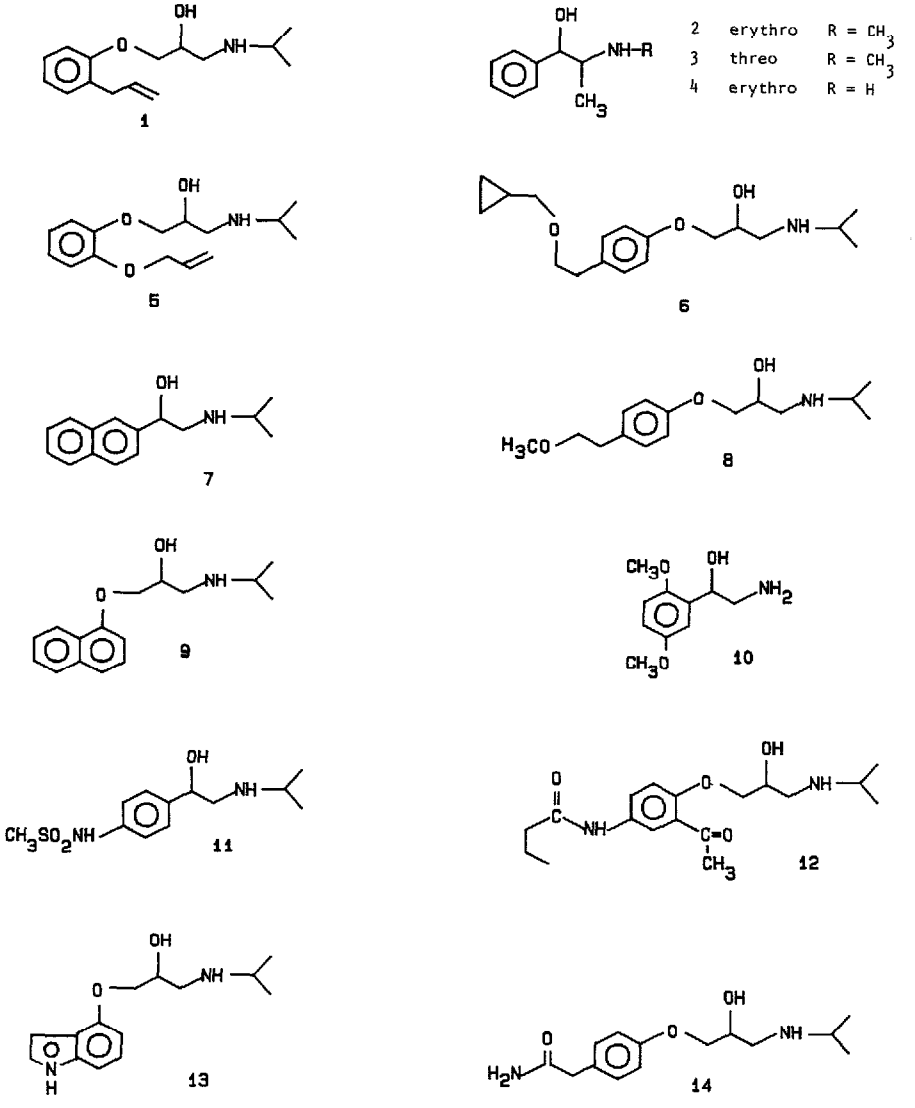

Fig. 1. Structures of amino alcohols resolved as oxazolidin-2-ones on DACH-DNB CSP.

Racemic mixtures of oxazolidin-2-ones were recrystallized from ethyl acetate–*n*-hexane giving pure products in 90% yield (not optimized), and the non-racemic mixtures were purified by filtration on a small column and eluted with a solvent mixture compatible with the substrate polarity.

The physico-chemical characterization of the above derivatives was realized on the basis of ^{1}H NMR and ^{13}C NMR (noise decoupling) signals and DEPT, mass and FT-IR spectra and the results were in agreement with the proposed structures.

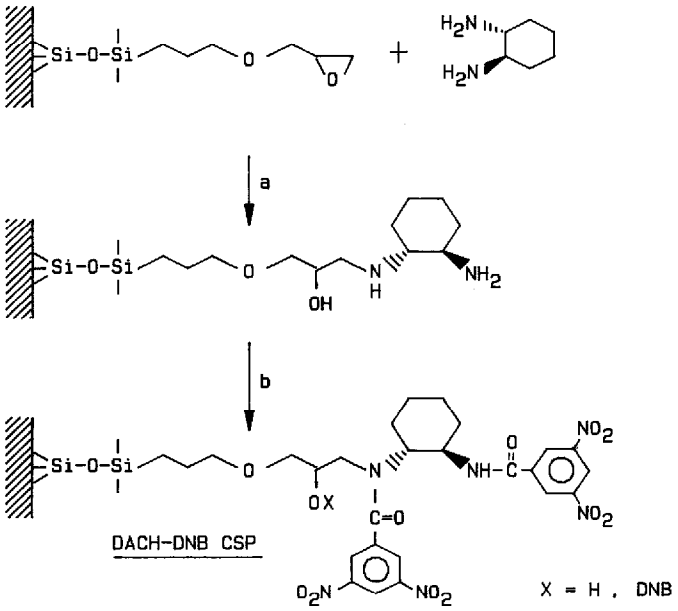

Fig. 2. Synthetic pathway for the preparation of DACH-DNB CSP. (a) (*R,R*)-(−)-diaminocyclohexane, dimethylformamide, 96 h, room temperature; (b) dinitrobenzoyl chloride, tetrahydrofuran, triethylamine, 2 h reflux.

Treatment of samples extracted from whole blood

In this procedure, as already described [7], phosgene was utilized as the derivatizing agent.

Column packing and column evaluation

The stainless-steel column (250 × 4 mm I.D. or 150 × 2.0 mm I.D.) was packed with LiChrosorb Si 100–DACH-DNB (5 μm) using a slurry packing procedure improved with respect to that already described [12]. Grafted silica (3.300 or 0.700 g) was dispersed in chloroform–isopropanol (50:50, v/v) (60 and 10 ml, respectively) and then treated ultrasonically for 5 min. The slurry obtained was packed with a Haskel DSTV-122 pump using *n*-hexane as pressurizing agent (8000 p.s.i., 15 min).

n-Hexane–isopropanol (99:1, v/v) was the eluent used in the evaluation of the kinetic performance of the chiral columns of different inner diameter and *n*-hexane was the solvent for the test mixture (naphthalene and anthracene).

Dimensionless parameters for the two columns such as reduced plate height (h), reduced velocity (v), flow resistance parameter (φ) and separation impedance (E), were calculated according to Bristow and Knox [16] (column of 4.0 mm I.D., $h_{min} = 2.17$, $v_{opt} = 2.01$, $\varphi = 632$, $E = 2976$; column of 2.0 mm I.D., $h_{min} = 2.23$, $v_{opt} = 1.61$, $\varphi = 620$, $E = 3083$; for naphthalene, $k' = 0.50$).

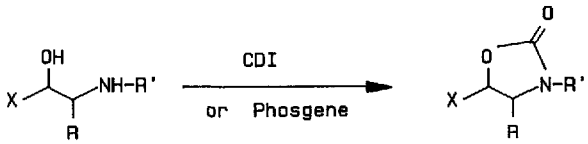

Fig. 3. Scheme of the derivatization procedures.

Chromatographic conditions

Racemic oxazolidin-2-ones were eluted with different mobile phases of increasing polarity (see Table I) at a flow-rate of 2.0 ml/min at 25°C (column 250 × 4.0 mm I.D.). UV absorbances were recorded at 254 nm unless stated otherwise.

RESULTS AND DISCUSSION

The racemic amino alcohols were analysed by HPLC as oxazolidin-2-one derivatives, which reduced the polarities of the analytes and made possible elution with solvents of medium polarity. Moreover, the derivatization (Fig. 3) enhances the

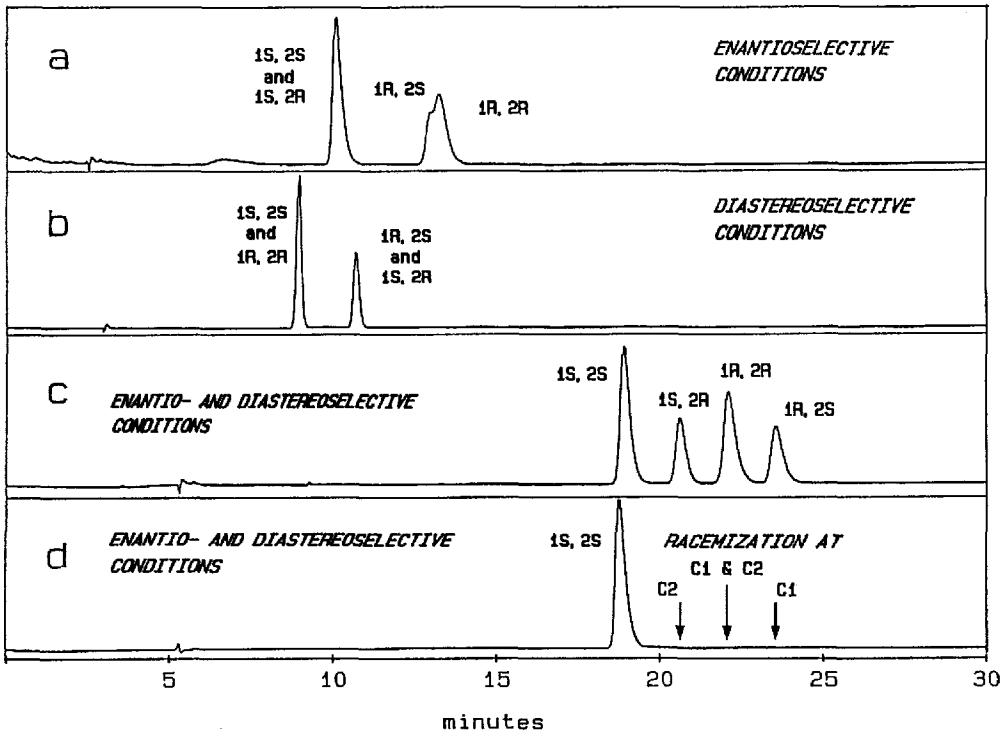

Fig. 4. Analysis of a mixture of racemic ψ-ephedrine and racemic ephedrine. (a) Chiral column, (*R,R*)-DACH-DNB (250 × 4 mm I.D.); (b) achiral column, LiChrosorb Si 60 (250 × 4 mm I.D.); (c) chiral and achiral columns connected in series; (d) coupled columns, racemization test for (1*S*,2*S*)-ψ-ephedrine. Detector, UV at 260 nm; flow-rate, 1.0 ml/min; other chromatographic conditions as in Table I.

recognition ability of the CSP because of the considerable and favourable conformational rigidity of the cyclization products.

The derivatization methods employed (CDI or phosgene, see Experimental) proceed without appreciable racemization of the substrates. Thus, mixtures having a well defined enantiomeric ratio (e.r.) do not exhibit variations of their enantiomeric composition after derivatization and analysis by means of the chiral column; *e.g.*, a commercial sample of (*S*)-propranolol (**9**) (Fluka; labelled e.r.: *S*:*R* = 99.5:0.5) derivatized as reported above and chromatographed on a DACH-DNB column showed an e.r. of *S*:*R* = 99.474:0.526 as revealed by digital electronic integration (mean of five measurements).

The stereochemical stability of both chiral centres of compounds such as **2** and **3** during the derivatization reaction was checked for the oxazolidin-2-one derivatives of (1*R*,2*S*)-ephedrine and (1*S*,2*S*)-ψ-ephedrine by ^{1}H NMR spectroscopy[a] and by HPLC on tandem columns (chiral DACH-DNB column plus achiral silica gel column) (Fig. 4). The reported results clearly indicate that whereas the chiral DACH-DNB column shows a very high enantioselectivity (Fig. 4a), the achiral silica column shows a good diastereoselectivity (Fig. 4b). Hence the connection of the two columns in series allows both enantioselectivity and diastereoselectivity values to be obtained that are suitable for the total separation of the two diastereoisomeric pairs, corresponding to compounds **2** and **3**, into four isolated stereoisomers (Fig. 4c),

Fig. 4d shows the results obtained using the same two columns in series for the analysis of the oxazolidin-2-one derived from the derivatization reaction of (1*S*,2*S*)-ψ-ephedrine. Also in this instance no peak assignable to racemization products was detected.

The chromatographic results for the amino alcohols examined are summarized in Table I; in addition, some examples showing different values of the resolution factor (R_s) are reported in Fig. 5. It can be assumed that the observed high chromatographic resolution values depend both on the great efficiency of the column and on its high enantioselectivity, as the kinetics and thermodynamics of the process are in an optimum state under the selected chromatographic conditions.

The low values of h_{min} show that the introduction of polar functional groups on the silica surface does not significantly influence the ability of the chiral support to establish rapid interactions with solutes (a necessary condition for obtaining highly efficient chiral columns); moreover, the low flow resistance observed guarantees adequate reproducibility of the original performance of silica gel.

With regard to thermodynamic aspects of the stereoselective molecular recognition process, DACH-DNB CSP is characterized by the precence of aromatic groups capable of establishing π–π donor–acceptor interactions with analytes containing π-basic aromatic portions: both π-acid (dinitrobenzoyl) and π-basic group in the

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[a] ^{1}H NMR spectra of (1*R*,2*S*)-ephedrine (**2**) oxazolidin-2-one and (1*S*,2*S*)-ψ-ephedrine (**3**) oxazolidin-2-one did not show evidence of any contamination derived from partial racemization during the derivatization process. ^{1}H NMR of (1*R*,2*S*)-ephedrine (**2**) oxazolidin-2-one (300 MHz, $CDCl_3$): δ 0.781 (3H, d, J = 6.3 Hz, CH_3CH), 2.877 (3H, s, CH_3N), 3.900–4.200 (1H, m, CH_3CH), 5.585 (1H, d, J = 8.1 Hz, C_6H_5CH, 7.200–7.400 ppm (5H, m, C_6H_5). ^{1}H NMR of (1*S*,2*S*)-ψ-ephedrine (**3**) oxazolidin-2-one (300 MHz, $CDCl_3$): δ 1.362 (3H, d, J = 6.3 Hz, CH_3CH), 2.804 (3H, s, CH_3N), 3.500–3.600 (1H, m, CH_3CH), 4.901 (1H, d, J = 7.8 Hz, C_6H_5CH, 7.341–7.500 ppm (5H, m, C_6H_5).

TABLE I

CHROMATOGRAPHIC RESULTS

CSP, (*R,R*)-DACH-DNB–LiChrosorb Si 100, 5 μm, (250 × 4.0 mm I.D.); flow-rate, 2.0 ml/min; temperature, 25°C; detector, UV, 254 nm. k' = Capacity factor; α = separation factor; R_s = resolution.

No.	Compound	k'	α	R_s	Eluent
1	Alprenolol	3.64	1.51	3.3	CH_2Cl_2–*n*-hexane (80:20)
2	Ephedrine	3.00	1.38	2.6	CH_2Cl_2–*n*-hexane–2-propanol (89:19:1)
3	ψ-Ephedrine	3.00	1.40	2.7	
4	Norephedrine	2.04	1.14	1.2	CH_2Cl_2–CH_3OH (99:1)
5	Betaxolol	1.21	1.39	2.8	
6	Oxprenolol	1.21	1.45	3.2	
7	Pronethalol	1.34	1.38	3.0	
8	Metoprolol	1.48	1.39	2.4	
9	Propranolol	1.92	1.40	3.2	
10	1-(2',5'-Dimethoxyphenyl)-2-aminoethanol	2.34	1.21	1.4	
11	Sotalol	5.35	1.55	3.3	
12	Acebutolol	5.56	1.24	1.7	
13	Pindolol	1.00	1.40	2.6	CH_2Cl_2–CH_3OH (90:10)
14	Atenolol	1.51	1.26	1.7	

diastereoisomeric CSP–solute complex are spatially locked in close parallel planes and disposed in such a way as to make simultaneously possible further interactions necessary for chiral recognition (mainly hydrogen-bond and/or dipole–dipole interactions).

The chiral recognition is expected to be more operative if the number of allowed conformations for both analytes and the CSP is reduced; as a consequence, derivatization of racemic solutes leading to a rigid cyclic system was found to produce the highest degree of enantioselectivity (*e.g.*, simple N- or N-,O-acylation products of racemic propranolol were not resolved on DACH-DNB CSP).

Compounds **1, 5, 6, 8, 9, 12, 13** and **14** are structurally similar, having the same propanolamine side-chain and differing only in the aromatic moiety; the highest selectivities were obtained with solutes containing either good π-basic aromatic rings (propranolol and pindolol) or aromatic rings bearing non-polar substituents (oxprenolol, alprenolol, betaxolol, metoprolol). Acebutolol and atenolol, with a polar amide group, distant from the chiral centre, gave lower resolutions, probably because of the strong, non-stereospecific interactions between this group and CSP. Addition of a second stereogenic centre (compounds **2**, **3** and **4**) or direct connection of the aromatic portion to the alcoholic chiral carbon (compounds **7**, **10** and **11**) did not result in significant variations of α values. Finally, the low degree of stereoselectivity and peak tailing shown by the oxazolidin-2-ones derived from N-unsubstituted amino alcohols (compounds **4** and **10** gave the lowest α and R_s values) can be attributed to strong hydrogen bonding between the analytes and CSP amide groups.

Precision of analysis and practical considerations

The high speed of analysis, the high values of enantioselectivity and, therefore, of

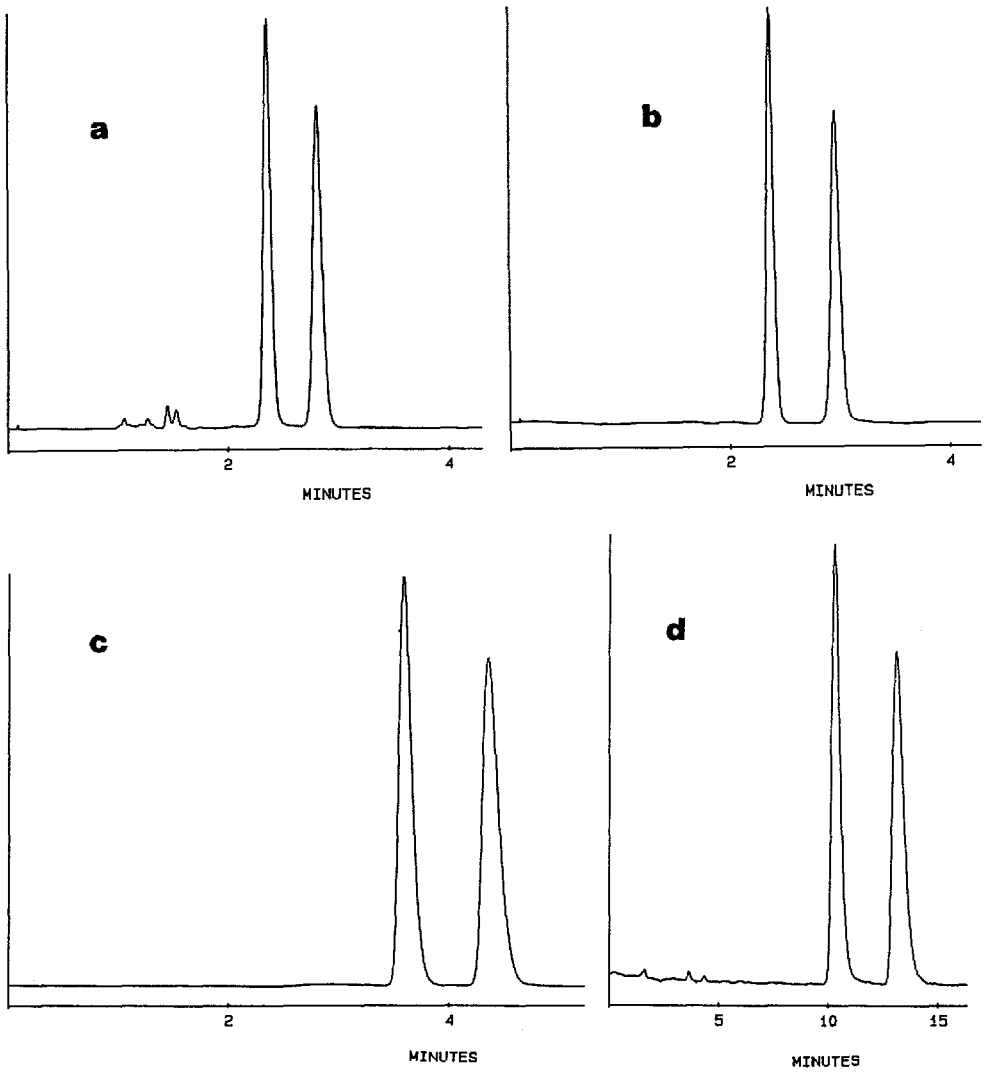

Fig. 5. Resolutions of racemic β-blockers as oxazolidin-2-one derivative. (a) Betaxolol; (b) oxprenolol; (c) metoprolol; (d) sotalol. Detector, UV at 275 nm; other chromatographic conditions as in Table I.

the chromatographic resolution allow the enantiomeric excess (e.e.) to be determined very precisely and accurately also if only very small amounts of product are available. With regard to this point, samples of propranolol (as the oxazolidin-2-one) at different enantiomeric excesses, (prepared *ad hoc* by mixing known amounts of the pure enantiomers) were analysed on a microbore column of 2.0 mm I.D.

Fig. 6a and b show the results obtained by injecting the same sample of (*S*)-propranolol as the oxazolidin-2-one (e.e. 99%) on two columns of DACH-DNB of the same size but of opposite chirality. The reversal of the order of peak elution for enantiomerically enriched selectands by inverting the chirality of the selector is a useful

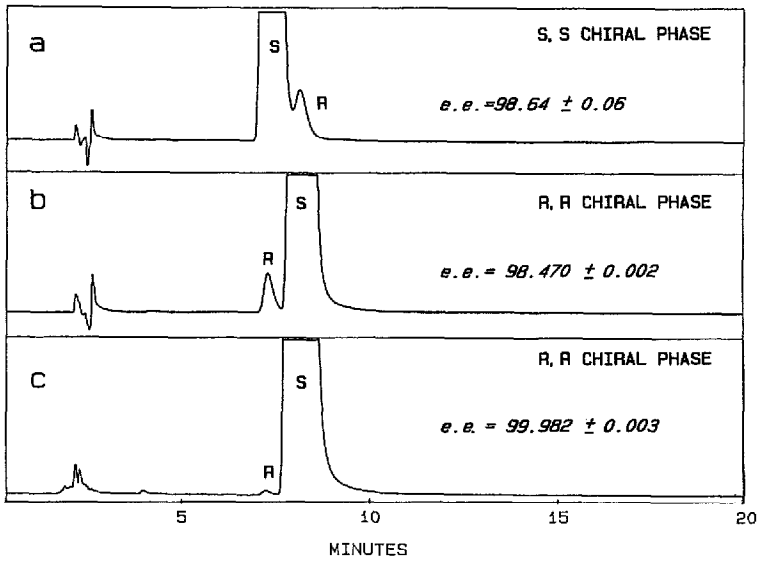

Fig. 6. Enantiomeric trace analysis of (*S*)-propanolol. Columns, 150 × 2 mm I.D. packed with (a) (*S,S*)-DACH-DNB and (b and c) (*R,R*)-DACH-DNB CSP; eluent, *n*-hexane–2-propanol–methanol (40:30:30, v/v/v); flow-rate, 200 μl/min; detector, UV at 230 nm; k'_1 = 2.79; α = 1.20.

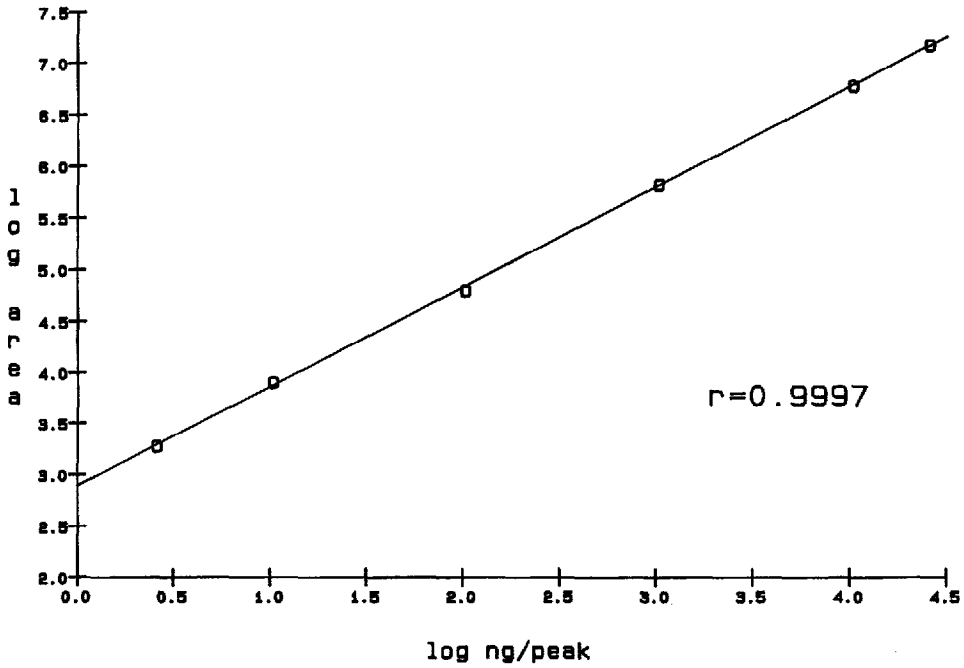

Fig. 7. Variation of peak area (UV detection) as a function of sample size for racemic propranolol. The limit of detection was 1.0 ng for each peak, with a signal-to-noise ratio of 3. Chromatographic conditions as in Fig. 6.

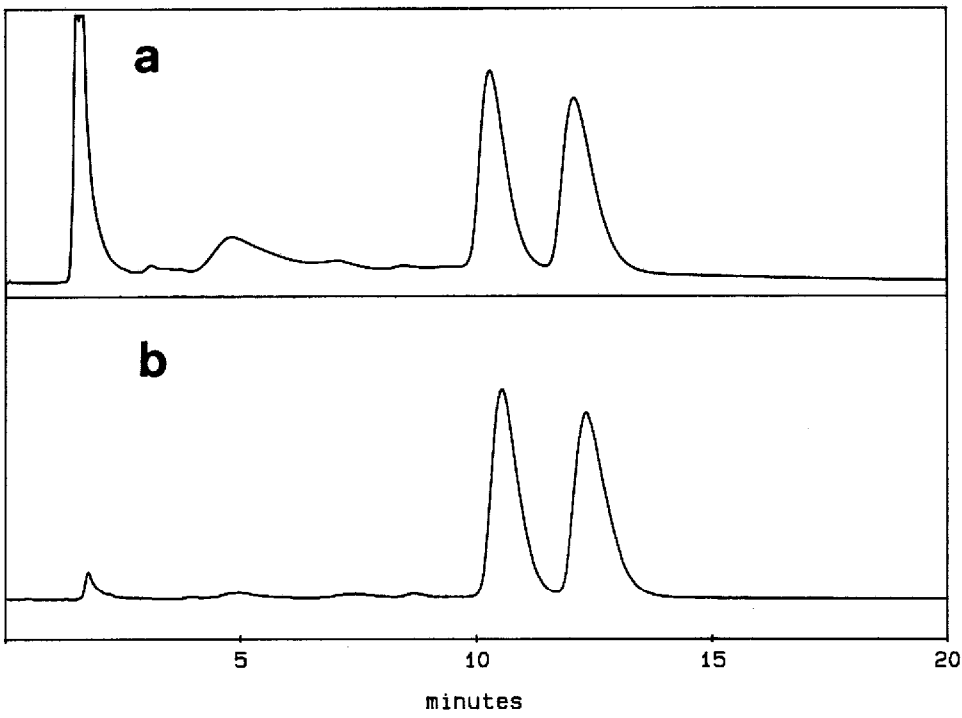

Fig. 8. Comparison between (a) UV (275 nm) and (b) fluorimetric (λ_{ex} = 275 nm, λ_{em} = 300 nm) detection for racemic atenolol extracted from human plasma. Eluent, *n*-hexane–2-propanol–methanol (40:30:30, v/v/v); k'_1 = 4.98; α = 1.21.

diagnostic tool for verifying an enantiomer separation but it may also have an important role in quantitative enantiomer analysis. In the above example, the reversal of the chirality of the selector allows the deficient enantiomer [(*R*)-propranolol oxazolidin-2-one] to be eluted as the peak preceding that of the main component. The digital electronic integration is easy, accurate and precise in the separation reported in Fig. 6b, where the deficient enantiomer is eluted first (e.e. = 98.470 ± 0.002).

Systematic errors in chromatographic enantiomer analysis may be recognized when the e.e. determination is performed on two phases of opposite chirality [17]. Thus, if a correct integration method (tangents method) is applied (Fig. 6a), a value closer to the real one can be obtained also if the precision of the measurement is low. Thus, this technique should be employed for the evaluation of trace amounts, especially when the deficient enantiomer is eluted first.

Fig. 6c shows the determination of the e.e. of another sample of (*S*)-propranolol oxazolidin-2-one. The value obtained was 99.982 ± 0.003 for the (*R*)-antipode as revealed by digital electronic integration carried out on five measurements of both enantiomeric peaks; in our particular case the detection response was linear over four orders of magnitude (Fig. 7).

Finally, we wish to point out that the above method can be successfully applied to the determination of β-blockers in blood plasma. Fig. 8 shows the results obtained;

both detection methods (spectrophotometric and spectrofluorimetric) gave fairly good results but the spectrofluorimetric detection proved to be more selective and sensitive.

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