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Direct high-performance liquid chromatographic resolution of 2-aryl- and 2-heteroarylpropionic acids on a chiral stationary phase containing the N,N′-dinitrobenzoyl derivative of (1*R*,2*R*)-diaminocyclohexane

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ABSTRACT

Racemic mixtures of eight non steroidal anti-inflammatory agents (derivatives of arylpropionic acids) were separated as 1-naphthalenemethylamides by HPLC on a chiral stationary phase containing the N,N′-3,5-dinitrobenzoyl derivative of (1*R*,2*R*)-diaminocyclohexane as chiral selector. Ibuprofen N,N-diethylamide, *n*-hexylamide and benzylamide and naproxen *n*-hexylamide and benzylamide were also resolved with chromatographic separation factors comparable to those obtained for the corresponding 1-naphthalenemethylamides; however, the latter derivatives showed significantly greater capacity factors (k'). The α values ranged from 1.10 to 1.83 and the k' values from 1.88 to 18.40 on a 250 × 4.0 mm I.D. stainless-steel column using *n*-hexane–2-propanol as the mobile phase.

INTRODUCTION

Analytical and preparative chromatographic methods for the separation of enantiomeric drugs are of great importance in the development of new therapeutic principles. The use of chromatographic systems (mainly HPLC and TLC) for monitoring enantiomeric purity has grown considerably in recent years [1–3]; in fact, the enantiomers of drugs may differ not only in their pharmacological properties but also in their side effects [4].

The optical resolution of a racemate may be achieved by reaction of the enantiomers with an enantiomerically pure reagent; the mixture of diastereoisomers is then separated on traditional columns. The formation of diastereoisomeric derivatives can introduce undetected errors arising from optically impure reagents, racemization of the reagents during the derivatization or different rates of formation of the diastereoisomers. These drawbacks can be avoided by means of direct liquid chromatographic separation of enantiomers after the formation of diastereoisomeric derivatives with a chiral selector in the mobile phase or by the use of chiral stationary phases (CSPs).

Arylpropionic acids are an important class of non-steroidal anti-inflammatory agents widely used for the relief of acute and chronic rheumatoid arthritis and osteoarthritis. Owing to a stereogenic

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centre they exist as enantiomers, displaying pharmacokinetic stereoselectivity both *in vitro* and *in vivo*. The (*S*)-enantiomers of this class of compounds have a consistently higher pharmacological activity than (*R*)-enantiomers; further, the (*R*)-(−)-enantiomers of ibuprofen and naproxen were found to be converted *in vivo* into the corresponding (*S*)-(+)-enantiomers [5–8]. However, only naproxen and flunoxaprofen are administered as the resolved (*S*)(+)-enantiomer.

Several chromatographic methods for the resolution of arylpropionic acids have been developed. A series of anti-inflammatory agents were resolved as amides and anilides on a chiral stationary phase composed of (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine or a variant of this chiral selector covalently bonded to the silica [9,10]. The effects of the mobile phase modifiers and four derivatizing reagents were investigated for the separation of enantiomers of ibuprofen and α-methoxyphenylacetic acid on a Pirkle column and on cellulose triphenylcarbamate coated on macroporous silica [11]. A chiral recognition model was proposed, involving interaction between amide derivatives of ibuprofen and the chiral selector contained in a Pirkle column [10]. Enantiomers of ibuprofen in biological fluids were resolved as amide derivatives on a Pirkle column [12,13]. Anionic drugs were separated without any derivatization on a chiral α_1-acid glycoprotein column (Enantiopac), and the effects of mobile phase additives and pH on the chiral resolution were also investigated [14]. Ibuprofen was separated as an amide derivative on a chiral stationary phase obtained by reacting γ-mercaptopropyl silica with quinine [15]. Acetylquinine chemically bonded to silica was used as a chiral selector in reversed-phase chromatography for the separation of naproxen derivatives [16]. Racemic mixtures of arylpropionic acids were resolved as diastereoisomeric derivatives of 1-phenylethylalanine [17] or 1-aminoethyl-4-dimethylaminonaphthalene using a normal-phase system [18]; enantiomers of naproxen [8] and ibuprofen in serum [19] were separated by means of a silica column after derivatization with chiral reagents. Diastereoisomeric derivatives of tiaprofenic acid [20] were resolved on a reversed-phase column after extraction from human plasma and urine. Derivatives of several non steroidal anti-inflammatory agents with optically active amines were separated as diastereoisomers by using TLC [21] or GC methods [22,23].

In this paper we describe the resolution of a series of racemic anti-inflammatory agents as amides using a chiral column containing the N,N′-3,5-dinitrobenzoyl derivative of (*R*,*R*)-1,2-diaminocyclohexane (DACH) as a chiral selector covalently bonded to a siliceous matrix [24,25].

EXPERIMENTAL

Apparatus

Chromatography was performed with a Waters (Milford, MA, USA) HPLC apparatus consisting of two Model 510 delivery systems, a U6K injector and a Model 481 variable-wavelength UV detector; chromatographic data were collected and processed on a Waters Model 840 data station.

Materials

HPLC-grade solvents were purchased from Merck (Darmstadt, Germany), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), N,N-diethylamine, *n*-hexylamine and benzylamine from Fluka (Buchs, Switzerland) and 1-napthalenemethylamine from Aldrich-Chemie (Steinheim, Germany). Racemic pure acids (ketoprofen, flurbiprofen, fenoprofen, tiaprofenic acid, suprofen and ibuprofen) were extracted from pharmaceutical preparations: racemic naproxen and Br-naproxen [26] and the (*S*)-enantiomers [96% enantiomeric excess (e.e.)] were supplied by Zambon (Milan, Italy) and racemic flunoxaprofen and the (*S*)-enantiomer (96% e.e.) by Ravizza (Milan, Italy). Ibuprofen enriched in the (*S*)-enantiomer was obtained as described by Nicoll-Griffith *et al.* [13].

Amide derivatives of α-methylarylacetic acids were prepared by two different procedures, as follows.

Method A. This was used for ibuprofen, ketoprofen, fenoprofen and flurbiprofen 1-naphthalenemethylamides and ibuprofen *n*-hexylamide, benzylamide and N,N′-diethylamide.

In a typical run, ibuprofen (1.03 g, 5 mmol) was dissolved in 1.0 ml of thionyl chloride and refluxed for 2 h; the excess of thionyl chloride was removed under reduced pressure, then 5 ml of dry benzene were added to the residue and the solution was evaporated under reduced pressure; 1.1 g (98%) of acid chloride was obtained as a yellowish oil.

To a solution of the acid chloride (1.1 g, 4.9 mmol) in 10 ml of chloroform, 1-napthalenemethylamine (0.78 g, 4.9 mmol) and triethylamine (0.7 ml, 4.9 mmol) were added dropwise; after 1 h at room temperature, the solution was washed with 1 *M* HCl (2 × 10 ml), 1 *M* NaOH (2 × 10 ml) and brine (2 × 10 ml) and dried over anhydrous sodium sulphate. Filtration and evaporation afforded 1.3 g of 1-naphthalenemethylamide (75%).

Method B. This was used for flunoxaprofen, naproxen, Br-naproxen and tiaprofenic acid 1-naphthalenemethylamide and naproxen benzylamide and *n*-hexylamide.

To a solution of naproxen (0.23 g, 1 mmol) in 5 ml of dry tetrahydrofuran (THF), EEDQ (0.25 g, 1 mmol) was added; the mixture was stirred at room temperature for 2 h and then 1-naphthalenemethylamine (0.15 g, 1 mmol) was added. After 4 h at room temperature, the THF was removed under reduced pressure and the residue dissolved in diethyl ether. Work-up of the crude product (see method A) afforded 0.31 g of the desired amide (85%).

Chiral stationary phase

The preparation of the chiral stationary phase and the evaluation of the kinetic performance of the columns have been reported previously [24,25]. The structure of the CSP is shown in Fig. 1.

For this work a 250 × 4.0 mm I.D. stainless-steel column, packed with (*R,R*)-DACH-DNB CSP (LiChrosorb Si 100, 5 μm), was used. The elution order of ibuprofen, flurbiprofen, flunoxaprofen, naproxen and bromonaproxen derivatives was determined by injection of samples enriched in one enantiomer of known configuration.

RESULTS AND DISCUSSION

The racemic acids were converted into substituted amides as shown in Fig. 2; this manipulation allowed us to include in the structure of the analytes additional interaction sites complementary with the CSP, namely the secondary amide group and the electron-rich naphthalene ring; the latter, a strong UV-absorbing chromophore, is also useful in the enantiomeric trace analysis of solutes (*e.g.*, ibuprofen) with low UV molar absorptivities.

No detectable isomerization was observed during the conversion of naproxen, Br-naproxen, flunoxaprofen and ibuprofen, enantiomerically enriched (96% e.e.), into substituted amides with methods A and B. Chromatographic results are given in Table

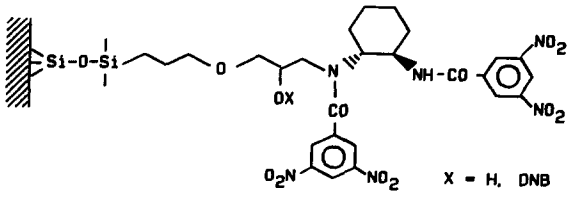
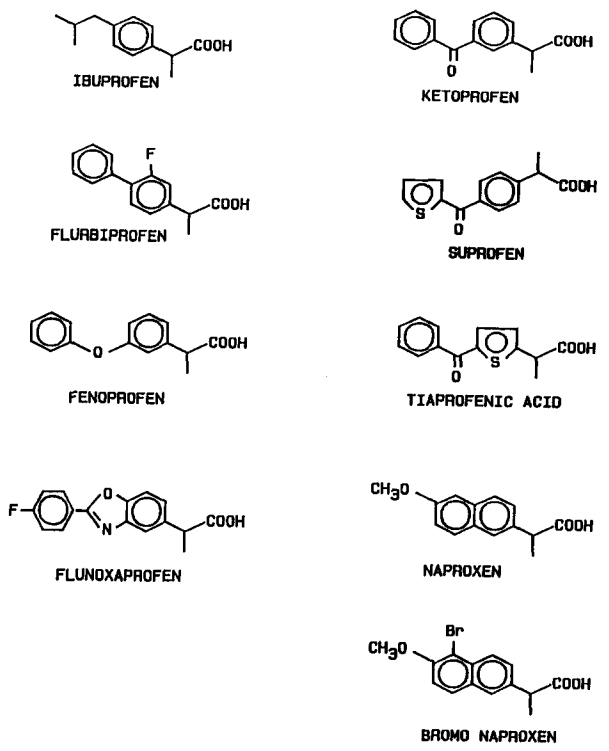


Fig. 1. Structure of the chiral stationary phase.



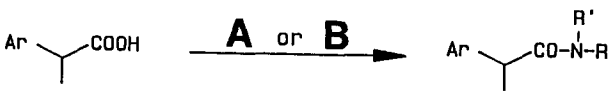


Fig. 2. Structures of racemic acids and derivatization procedures. A = $SOCl_2$, amine, Et_3N. B = EEDQ, amine. R = n-C_6H_{13}, $PhCH_2$, 1-naphthyl-CH_2, R′ = H; R = R′ = ethyl.

TABLE I

CHROMATOGRAPHIC RESULTS

CSP: (*R,R*)-DACH-DNB LiChrosorb Si 100, 5 μm. All compounds were eluted at a flow-rate of 2 ml/min at 25°C. UV detection at 280 nm.

Compound	Acid	Derivative	k'^a	α^b	R_s	2PA[c] (%)	First-eluted isomer
1	Ibuprofen	N,N'-Diethylamide	2.00	1.11	1.0	5	*S*
2	Ibuprofen	*n*-Hexylamide	2.59	1.16	1.2	10	*S*
3	Ibuprofen	Benzylamide	7.21	1.20	1.4	10	*S*
4	Ibuprofen	1-Naphthalenemethylamide	1.88	1.26	1.0	40	*S*
5	Flurbiprofen	1-Naphthalenemethylamide	2.98	1.27	1.2	40	*S*
6	Fenoprofen	1-Naphthalenemethylamide	3.34	1.27	1.2	40	n.d.[d]
7	Flunoxaprofen	1-Naphthalenemethylamide	4.66	1.46	1.9	40	*S*
8	Ketoprofen	1-Naphthalenemethylamide	6.06	1.23	1.1	40	n.d.
9	Suprofen	1-Naphthalenemethylamide	9.88	1.10	0.6	40	n.d.
10	Tiaprofenic acid	1-Naphthalenemethylamide	10.22	1.83	2.7	40	n.d.
11	Naproxen	*n*-Hexylamide	2.22	1.47	1.5	40	*S*
12	Naproxen	Benzylamide	6.37	1.49	2.2	40	*S*
13	Naproxen	1-Naphthalenemethylamide	18.40	1.49	2.0	40	*S*
14	Br-naproxen	1-Naphthalenemethylamide	18.02	1.55	2.1	40	*S*

[a] Capacity factor for the first-eluted enantiomer.
[b] Enantioselectivity factor.
[c] Percentage of 2-propanol in *n*-hexane in the mobile phase.
[d] Not determined.

I. The α-values range from 1.10 to 1.83 using *n*-hexane–2-propanol as the mobile phase; slightly different selectivities and lower retentions were observed using a ternary mobile phase containing both dichloromethane and 2-propanol as polar modifiers (see Table II and Fig. 3). Owing to the efficiency of the chiral column, all the examined compounds were completely resolved; for compound **9**, partial resolution was obtained as a result of low stereoselectivity and pronounced peak tailing.

TABLE II

CHROMATOGRAPHIC RESULTS

CSP: (*R,R*)-DACH-DNB LiChrosorb Si 100, 5 μm. All compounds were eluted at a flow-rate of 2 ml/min at 25°C. UV detection at 280 nm. All compounds as 1-napthalenemethylamide derivatives.

Compound	Acid	k'^a	α^b	R_s	Eluent[c]	First-eluted isomer
4	Ibuprofen	1.76	1.21	1.1	A	*S*
5	Flurbiprofen	2.54	1.41	1.5	A	*S*
7	Flunoxaprofen	2.00	1.34	1.3	B	*S*
10	Tiaprofenic acid	2.92	2.08	3.7	B	n.d.[d]
13	Naproxen	4.11	1.49	2.0	B	*S*

[a] Capacity factor for the first-eluted enantiomer.
[b] Enantioselectivity factor.
[c] A = *n*-hexane–dichloromethane–2-propanol (60:20:20); B = *n*-hexane–dichloromethane–2-propanol (50:25:25).
[d] Not determined.

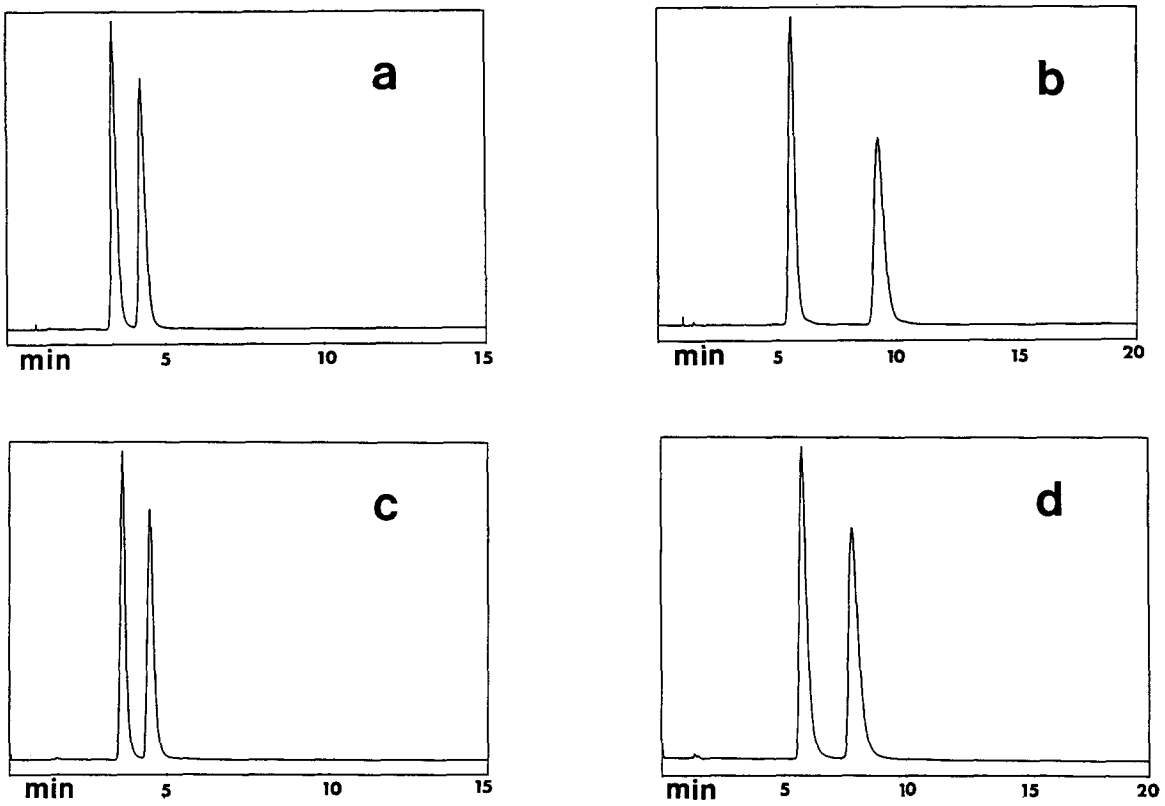


Fig. 3. Resolution of racemic acids as 1-naphthalenemethylamides on (*R,R*)-DACH-DNB CSP. (a) Ibuprofen; (b) tiaprofenic acid; (c) flunoxaprofen; (d) naproxen. UV detection at 280 nm; other chromatographic conditions as in Table II.

Solute structure and stereoselectivity

Both retention and enantioselectivity show a significant dependence on the nature of the aromatic groups arranged around the stereogenic centre of the analytes; in the series of 1-naphthalenemethylamides the capacity factors (k') increase with increasing ability of the acid-derived aryl substituent to act as a π-donor and a similar trend is observed for the α values.

As far as the carboxylic group transformation is concerned, the data in Table I clearly show that the nature of the amine employed has little effect on the chiral recognition ability of the CSP towards different derivatives of the same acid; the chromatographic separation factors for the *n*-hexylamides and benzylamides of ibuprofen and naproxen are comparable to those noted for the corresponding 1-naphthalenemethylamides; however, the latter derivatives show significantly greater capacity factors.

Tertiary amide derivatives of ibuprofen and naproxen are also resolved with similar α values and reduced retentions in comparison with the secondary amide derivatives; these results indicate that both hydrogen bonding (between the N–H amide proton of the secondary amides and the CSP) and dipole stacking (tertiary amides) are operative during the chiral recognition process.

The elution order is constant for all the amides of ibuprofen, flurbiprofen, flunoxaprofen, naproxen and Br-naproxen, the (*R*)-enantiomers being those most retained on the (*R,R*)-DACH-DNB CSP; the same elution order has been reported by several workers for the resolution of amides of ibuprofen, naproxen, benoxaprofen, fenoprofen on a Pirkle (*R*)-phenylglycine-derived CSP [9,11,27]. From the above observations, at least two extreme possible mechanisms of resolution can be outlined: for solutes obtained from amines lacking π-basic groups,

chiral recognition occurs through a π–π interaction between the DNB groups of the CSP and the aryl (or heteroaryl) substituent of the analytes, together with dipole stacking and/or hydrogen bonding of amide dipoles; in the case of amides prepared from amines containing adequate π-basic groups (*i.e.*, 1-naphthalenemethylamine), the dominant π–π interaction is established between the dinitrobenzoyl group of the stationary phase and the aryl group derived from the amine.

These two mechanisms can be operated simultaneously for compounds containing two aryl groups of comparable π-basicity (compounds **3**, **13** and **14**); the high retentions observed for these analytes, especially **13** and **14**, can be accounted for the assumption of multiple interaction sites and modes with the stationary phase.

The broad selectivity of the (*R*,*R*)-DACH-DNB towards different derivatives of the same acid can be exploited to design "flexible" analytical methods capable of detecting a low concentration of a single enantiomer even in complex matrices. For example, the resolution of ibuprofen can be carried out after its conversion into a secondary amide using either amines containing an aromatic group or aliphatic amines: in the first instance, the two enantiomers corresponding to the ibuprofen derivatives are well separated from the impurities arising from the derivatization step, allowing the sample to be injected directly without any work-up. Moreover, the sensitivity of the chromatographic method is greatly enhanced by the additional UV-absorbing group introduced in the analyte structure. On the other hand, the use of aliphatic amines as derivatizing agents results in the formation of secondary amides that are still well resolved on the CSP but show lower capacity factors, which lead to reduced analysis times.

The precision and accuracy of the stereoselective chromatographic methods based on DACH-DNB CSP can also be improved by switching from one enantiomeric form of the chiral selector to the other [both the (*R*,*R*) and the (*S*,*S*) versions of the CSP, as well as the racemic version, have been prepared and evaluated] [25]. This technique is successfully employed when a low level of a single enantiomer has to be determined in the presence of a large excess of the other; by choosing the appropriate chirality of the CSP, the trace isomer can be positioned in the chromatogram before the major constituent, thus allowing more precise and accurate quantification.

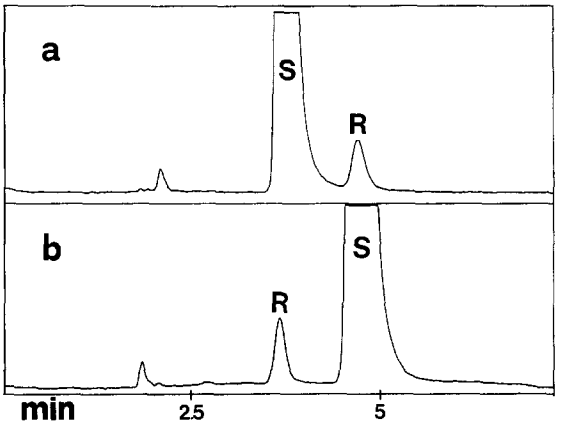


Fig. 4. Optical purity determination on two columns of opposite chirality; (a) (*R*,*R*)-DACH-DNB column; (b) (*S*,*S*)-DACH-DNB column. Sample: (*S*)-flunoxaprofen as 1-naphthalenemethylamide. UV detection at 293 nm; other conditions as in Table II.

An example is shown in Fig. 4: six replicate injections of (*S*)-flunoxaprofen-1-naphthalenemethylamide containing a small amount of the (*R*)-isomer gave a mean value for the trace enantiomer of 2.64 ± 0.03% using the (*S*,*S*) column (minor enantiomer eluting first) and 2.68 ± 0.08% using the (*R*,*R*) column.

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