BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **12**, 309–316 (1998)

Resolution of Enantiomers of Ibuprofen by Liquid Chromatography: A Review

Ravi Bhushan and Jürgen Martens*
FB. Chemie, Universität Oldenburg, Postfach 2503, D-26111 Oldenburg, Germany

Ibuprofen is one of the most effective and widely used non-steroidal analgesic and anti-inflammatory agent. It is marketed as a racemic mixture though it is known that the pharmacological activity resides in the (*S*)-(+)-enantiomer only. Several direct/indirect liquid chromatographic methods involving a variety of chiral/achiral phases along with their possible role in resolution, chiral and achiral agents used for derivatisation have been discussed with special reference to ibuprofen, and mentioning their application to the resolution of other 2-aryl-propionic acids/profens. © **1998 John Wiley & Sons, Ltd.**

INTRODUCTION

It is well known that the enantiomers exhibit different biological, physiological and chemical behaviour and may exert different pharmacodynamic effects. Pharmacokinetic differences have been reported as well. Therefore, there has been a considerable interest in the stereospecific pharmacokinetics, metabolism and clinical pharmacology of chiral drug molecules. The interest has in part been stimulated by the pharmaceutical industry placing new emphasis on the synthesis, isolation and analysis of enantiomerically pure drugs.

Ibuprofen belongs to the general category of 2-arylpropionic acids which are a group of extensively used drugs worldwide with most effective non-steroidal analgesic, antipyretic and anti-inflammatory properties (Fig. 1). Their anti-inflammatory activity is widely applied in relief of a range of general inflammatory conditions and particularly of chronic rheumatoid arthritis and osteoarthritis. These compounds are characterized by a stereogenic centre adjacent to the carboxylic acid moiety.

Ibuprofen is marketed as a racemic mixture (and also other members of the group, with the exception of naproxen, which is commercialized as a pure (*S*)-(+)-enantiomer). Though the pharmacological activity resides in the (*S*)-(+)-enantiomer and the *R*-isomer of ibuprofen or ketoprofen may accumulate as a residue in fatty tissues. There are also reports that anti-inflammatory effect by the *R*-isomer may arise through a unique bio-inversion at its chiral centre; this is a uni-directional process varying for different profens. The inversion of ibuprofen in man is significant in comparison to the other members of the family (like flurbiprofen, tiaprofenic acid, and ketoprofen).

Therefore, the stereoselective determination of the drug enantiomers in plasma or urine etc. is of potential clinical importance. Development of highly efficient chromatographic methods for the analytical determination of enantiomers in biological fluids has made it feasible to determine low levels of each of the enantiomers of a chiral drug.

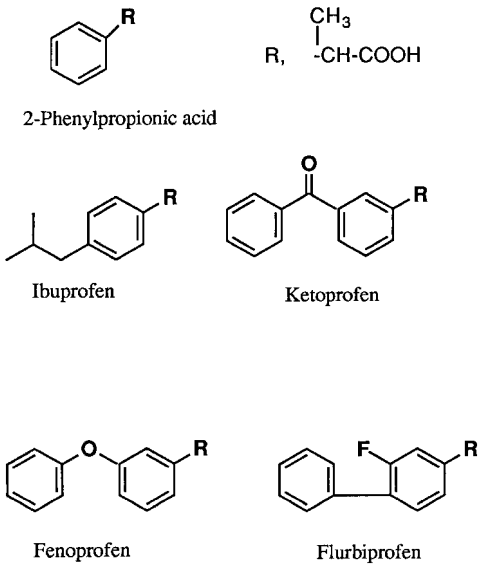

Figure 1. Structures of some 2-arylpropionic acids commonly used as non-steroidal anti-inflammatory drugs.

DETERMINATION OF ENANTIOMERIC PURITY

Curently, following approaches are used to achieve resolution of enantiomers of ibuprofen and related compounds by liquid chromatography:

(1) Direct separation (of the free 2-aryl propionic acids) using chiral HPLC columns.
(2) Pre-column derivatization with optically pure chiral reagents and separation on achiral columns or preparation of derivatives with achiral reagents such as amines or alcohols (to obtain amides or esters) prior to separation on chiral columns.

*Correspondence to: J. Martens, FB. Chemie, Universität Oldenburg, Postfach 2503, D-26111 Oldenburg, Germany.

CCC 0269–3879/98/060309–08 $17.50

Received 8 September 1997
Accepted 19 November 1997

(3) Using achiral HPLC columns with chiral mobile phase.
(4) TLC impregnated with a suitable chiral selector.

HPLC

Table 1 summarizes the direct or derivatized resolution of enantiomers of ibuprofen by HPLC; in most of the cases the methods were applied to other members of the family and were successful. The enantiomers were separated in both the derivatized form and in the underivatized form. In the derivatization approach, the carboxyl group of the enantiomers was converted into ester, an anilide, or an amide group resulting in an improved separation and/or improved detection limits. In other approach, derivatization was carried out with an enantiomerically pure reagent leading to the formation of diastereomers which then were separated either on a chiral column of (*R*)-*N*-(3,5-dinitrobenzoyl)-phenylglycine based silica (Crowther *et al.*, 1984; Wainer and Doyle, 1984) or on an achiral octadecyl silica column (Lau, 1996; Péhourcq *et al.*, 1995; Lemko *et al.*, 1993; Lee, *et al* 1984; Wright *et al.*, 1992; Hutt *et al.*, 1986; Avgerinos and Hutt, 1987; Mehvar *et al.*, 1988; Shimada *et al.*, 1987). In some cases, a non-chiral reagent was used to derivatize the ibuprofen (and other profens) enantiomers followed by their separation on one of the cellulose based stationary phases (Okamoto *et al.*, 1989; Van Overbeke *et al.*, 1995, 1996) or a chiral stationary phase consisting of *R*-1-(1-naphthyl)ethylurea covalently bound to silica through a propyl linkage (Ahn *et al.*, 1994). Enantiomers of the underivatized ibuprofen (and other compounds of the family) have also been separated using the α1-acid glycoprotein stationary phase (Camilleri and Dyke, 1990; De Vries *et al.*, 1994), the human serum albumin stationary phase (Noctor *et al.*, 1991), Cyclobond-I β-cyclodextrin silica phase (Farkas *et al.*, 1993), and an ergot alkaloid based stationary phase (Castellani *et al.*, 1994).

The first commercially available *brush type* phases for HPLC designed by the group of Pirkle (CSP1, DNB-phenylglycine derivatives) and Ôi permitted a separation of ibuprofen (and other 2-aryl propionic acid derivatives) after derivatization of the carboxylic acid moiety which was necessary to meet the requirement for stereoselective interaction. Other phases made available also required a pre-column derivatization with an amine or an alcohol. New creations of Pirkle phases, however, no longer require derivatization of the acid group. Table 2 shows the minimum quantifiable concentrations for each enantiomer of ibuprofen diastereomer obtained under different conditions.

Cyclodextrin based columns. Interesting results were obtained using modified and immobilised cyclodextrins (Dingenen, 1994; Wainer, 1993). Cyclodextrins (CD) are non-reducing cyclic oligoglucose molecules containing 6, 7 or 8 glucose units (α-, β- or γ-cyclodextrins). Their structure resembles a truncated cone with both ends open. The larger opening is rimmed with secondary -OH groups of the glucose units, all rotated to the right, and the smaller opening is rimmed with the more polar primary -OH groups and therefore is relatively hydrophobic, while the outer surface is polar. Cyclodextrins can have molecules into their cavities and form host-guest reversible inclusion complex. The secondary -OH groups can be derivatized to extend the depth of the cavity and/or change the nature of the polar interaction sites.

The commercially available cyclodextrins silica HPLC phases contain CD moieties which are chemically bonded to the silica support via the 3-glycidoxysilane spacer. Hydroxypropylated CD silicas of the desired chirality are produced by first reacting the CD with the appropriate propylene oxide, followed by binding of the modified CD to the glycidoxysilylated silica. Naphthyl-ethyl-carbamoylated CD silica stationary phases are produced by further reacting the CD silica, *in situ*, with (*S*)-(+)- or (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate (Armstrong *et al.*, 1990).

Among the factors that control the enantioseparation process are: (1) differences in the stability/binding constants of the CD complexes, (2) differences in the adsorption of CD complexes on the surface of the stationary phase, and (3) differences in the adsorption of free solute molecules on the CD layer that is adsorbed on the surface (Hilton and Armstrong, 1991). Only those guest molecules which can be fitted into the chiral cavity of the CD, providing an intimate interaction with the inner surface, can form stable inclusion complexes; if the size of the molecule is smaller or larger than the cavity, either a weak or no interaction occurs and hence no separation. The interactions include, hydrophobic and dipole–dipole interactions, inductive effects, and hydrogen bonding.

Separation studies carried out on β- and γ-cyclodextrin silicas, (*S*)- and racemic-2-hydroxypropyl-β-cyclodextrin silicas and the (*S*)-naphthylethyl carbamoylated β-cyclodextrin silica by Beeson and Vigh (1993) showed that there was no enantiomeric resolution for ibuprofen on (*S*)- and racemic-2-hydroxypropyl-β-cyclodextrin silicas while a good chiral separation was observed for the ionic form of ibuprofen on the (*S*)-naphthylethyl carbamoylated β-cyclodextrin silica column and the chiral selectivity was better than on the native β-cyclodextrin silica column; the dissociated profen anions were strongly retained than the non dissociated form in the presence of increased concentration of acetonitrile and this behaviour was exactly opposite to that is observed in a regular reversed-phase system (Karger *et al.*, 1980). Eventually, in pure aqueous eluents the free acids bonded more strongly than the respective anions. On native cyclodextrin silicas operated in the reversed-phase mode, solute retention is attributed to the concerted action of the hydrophobic interactions between the hydrophobic parts of the solute and the hydrophobic interior of the cyclodextrin cavity, and the hydrogen-bonding interactions between the polar functional groups of the solute and the hydroxyl groups of the cyclodextrin (Szejtli, 1982).

Native cyclodextrin and its hydroxypropyl-, methyl- and sulphate derivatives have been used as chiral mobile phase additives (Ameyibor and Stewart, 1997) for the resolution of ibuprofen enantiomers with partial success on hexasilane and octasilane reversed-phase columns. The reason attributed for this is the presence of only one aromatic ring and small size of ibuprofen and thus a strong or tight inclusion complex with the CD cavity was not formed. Problem associated with the use of CDs as chiral mobile phase additives is their poor solubility in organic solvents, addition of urea enhances solubility but increases the viscosity and leads to baseline problems. A chemical modification can overcome these problems and

changes both the chiral selectivities and physical properties of the CD.

Cellulose based columns. Pioneering studies in the development of cellulose columns were performed by Okamoto and his group, and many of these chiral stationary phases have been commercialized by Daicel (*Application Guide for Chiral Column Selection,* Daicel Chemical Industries, Ltd., Tokyo, Japan). Carbamate derivatives and tris-(3,5-dimethylphenyl carbamate) cellulose have proved to be efficient in separating most profen enantiomers; in some cases chiral resolutions could only be obtained after derivatization of the carboxylic acid moiety. The methylbenzoate based celluloses found many applications among the ester derivatives of cellulose. These polymers proved their enantioselective capabilities configured as pure beads offering a much higher loadability. A new experimental cellulose based CSP, called *Tolycellulose* column manufactured by Bio-Rad RSL (Nazareth, Belgium) contains tris-(4-methylbenzoate) cellulose polymer similar to the Chiralcel OJ phase (Daicel, Tokyo, Japan). The former is used under reversed-phase conditions as in it the cellulose layer is covalently bound onto the aminopropylsilanized silica gel (300 Å) rather than being adsorbed onto macroporous (1000 Å) aminopropylsilanized silica gel as is the case for the latter.

Van Overbeke *et al.* (1995) studied the resolution of ibuprofen and other profens on the above mentioned columns (*Tolycellulose* and Chiralcel OJ) and did not obtain resolution for the free acid form on *Tolycellulose* columns, the carboxylic moiety was derivatized with 1-naphthylmethylamine, benzylamine, 2-methylbenzylamine, and benzylalcohol. The derivatives of 1-naphthylmethylamine showed the best results on *Tolycellulose* under reversed phase conditions. Chiralcel OJ column gave better results under normal phase, and in general the feasibility of chiral separations turned out in their favour.

Cellulose shows a helical structure, not affected by derivatization of the -OH groups of the glucose units, with chiral cavities capable of *including* molecules stereoselectively. The three-dimensional structure and shape of the solutes play an important role for a successful resolution since inclusion mechanism is involved. The chiral recognition mechanism depends upon the arrangement of polymer chains or on the actual conformation of the benzoyl groups, and is influenced by the position and number of methyl substituents on the phenyl moieties; any variation in these generates completely different chiral recognition abilities.

The enantioselective properties of the derivatised cellulose e.g. tris-(4-methylbenzoate)cellulose can be considered to involve following aspects: (1) the derivatized cellulose chiral stationary phase (CSP) forms a diastereomeric complex through attractive interaction between the amide or ester portion of the solute and one or more ester moieties of the CSP. The possible modes of interaction include hydrogen bonding between the amide or alcohol hydrogen and the ester carbonyl, π–interaction between the phenyl moieties and dipole–dipole stacking between the amide or alcohol and ester dipoles; stronger the interaction, the tighter the binding of the solute to the CSP and greater the expression of stereochemical differences between the enantiomeric solutes. (2) The positioning of the solute and CSP within this diastereomeric complex through the use of at least two of those possible interactions. (3) The steric fit of the asymmetric portion of the solute in the chiral cavity of the CSP. This determines the relative stability of the diastereomeric complexes and thus the chiral resolution. The difference in energy between the enantiomer-CSP complexes determines the observed stereoselectivity and the capacity factors are related to energy of interaction with the CSP. In recognition to the above model, studies on the resolution of various derivatives of ibuprofen and other profens showed that their amide derivatives get better resolved on ester derivative of cellulose (Van Overbeke *et al.,* 1996).

Sample preparation. Several of the methods reported for derivatization entail extensive sample preparation or lengthy chromatography times associated with lack of sensitivity. One of the approaches consists of extraction of the racemate from acidified plasma followed by conversion to a mixed anhydride with ethylchloroformate and derivatization with L-leucinamide. Péhourcq *et al* (1995) modified the L-leucinamide method by simplifying the extraction of unresolved drug from acidified plasma and rapid reversed-phase HPLC separation on achiral column.

To an aliquot of plasma (0.5 mL) containing ibuprofen, appropriate internal standard solution (0.1 mL from a solution of 0.2 mg/mL) and sulphuric acid (0.2–0.5 mL, 0.6 M) are added. Ibuprofen and internal standard are extracted with dichloromethane (15 mL) or isooctane-isopropanol (3 mL, 95:5 v/v). The organic layer is transferred to a clean tube and evaporated to dryness. The residue is treated with derivatizing reagent by either of the following methods: (a) The residue is reconstituted in 0.1 mL of triethylamine in acetonitrile (50 mM), followed by addition of 0.5 mL of ethylchloroformate solution (60 mM in acetonitrile) and vortexed, and the chiral reagent, 0.5 mL of L-leucinamide is added and after 2 min the reaction is terminated by adding distilled water and aliquots of 10–50 μL are used for injecting into the HPLC system (Péhourcq *et al.,* 1995) or the chiral reagent, 0.25 mL of 10 mM *S*-(−)-1-(1-naphthyl)ethylamine is added followed by 0.25 mL of 1:40 ethanol-amine/acetonitrile, evaporated to dryness and reconstituted in mobile phase to inject into HPLC system (Lau, 1996). (b) To the residue 1 mL of 2-ethoxy-1-ethoxy-carbonyl-1,2-dihydroquinoline (2.4 mg/mL in ethylene chloride) and 5 mL of *p*-nitrobenzylamine hydrochloride (PNBA, 5 mg dissolved in 5 mL of 0.2 M NaOH and then extracted with 5 mL of ethylene chloride) are added (the achiral derivatizing reagent), refluxed for 10 min, diluted to 10 mL with ethylene chloride and then washed with equal volumes of 0.2 M NaOH, 1 M HCl and water. The organic layer is dried, evaporated to dryness, reconstituted with mobile phase for HPLC injection (Ahn *et al.,* 1994).

The majority of methods reported in literature have employed indirect chiral chromatographic methods based on the formation of diastereomeric derivatives prior to analysis. Such an approach may introduce inaccuracies into the determination of enantiomeric ratio due to chiral imupurities in the reagent or to the racemization during the process of derivatization. Direct enantiomeric analysis using enantioselective chiral stationary phases avoids these problems.

Use of achiral agent for derivatization eliminates the possible analytical error from enantiomeric contamina-

Table 1. Summary of resolution of enantiomers of ibuprofen (direct or derivatized) by HPLC along with derivatizing agent, solvent system, column and method of detection

Derivative or the reagent	Solvent system and flow-rate	Column	Detection	Ref.
4-(1-amino ethyl)-*N,N*-dimethyl-1-naphthylamine	Hexane–ethyl acetate, (1:3) or Hexane-THF, (5:1), 1 mL/min	μ-Porasil	Fluorimetric, 395 nm	Goto *et al.,* 1980
2-[4-(L-leucyl) amino phenyl]-, 2-[4-(D-phenyl glycyl) amino phenyl]- and 2-[4-(L-phenylalanyl) amino phenyl]-6-methoxy benzoxazole	Hexane–ethyl acetate–acetic (700:300:1 or 600:400:1), 1.5 mL/min	TSK gel. Silica-60 (5 μm)	Fluorescence detection at 375 nm (excitation at 330 nm)	Kondo *et al.,* 1994
(-)-2-[4-(1-amino ethyl) phenyl]-6-methoxy benzoxazole	Hexane–ethyl acetate–propan-2-ol–acetic acid (900:50:1:1), 1 mL/min	TSK gel. Silica-60 (5 μm)	Fluorescence detection at 375 nm (excitation at 320 nm)	Kondo *et al.,* 1993
	or acetonitrile–water–acetic acid (600:400:1), 1 mL/min	TSK gel. ODS 80TM (5 μm)	at 380 nm (excitation at 320 nm)	
(*R*)-(-)-1-ferrocenylethyl amine or (*S*)-(+)-1-ferrocenylpropyl amine	Acetonitrile–1.5% sodium acetate buffer, pH 5.0, (3:2 or 5:4)	Develosil ODS-5 (5 μm)	Electrochemical at + 0.45 V vs. Ag–AgCl with detection limit of 0.5 pmol	Shimada *et al.,* 1987
S-(-)-(1-Naphthyl) ethylamine	Acetonitrile–H_2O–acetic acid–triethyl amine (2750:2250:5:1), 1 mL/min	Partisil-5 ODS-3 (5 μm)	232 nm	Mehavar *et al.,* 1988
S-(-)-(1-Phenyl) ethyl amine	Propan-2-ol-cyclohexane (3:37), 2 mL/min	LiChrosorb Si-60 (10 μm)	225 nm	Maitre *et al.,* 1984
S-1-(Naphthyl)-ethylamine	Hexane–ethyl acetate (4:1), 3.2 mL/min	Hypersil (10 μm)	254 nm	Avgerinos and Hutt, 1987
S-(-)-1-(1-Naphthyl)-ethylamine	Hexane–ethyl acetate (4:1), 0.8 mL/min	Porasil (10 μm)	254 nm	Hutt and Coldwell, 1986
R(+)-(2-phenyl) ethylamine in presence of ethyl chloroformate	Acetonitrile–H_2O–acetic acid–triethyl amine (4650:5350:10:3, pH 4.9), 1.6 mL/min	HPLC with a pre column of RP material (37–53 μm)	225 nm	Wright *et al.,* 1992
S-(+)-D-Octan-2-ol	Propan-2-ol–hexane 1 mL/min	Ultrasphere Si (5 μm)	220 nm	Lee *et al.,* 1984
S-(-)-1-(1-Naphthyl)-ethylamine	Acetonitrile–H_2O–acetic acid–triethyl amine (3000:2000:5:1), 1.6 mL/min	Partisil-ODS-3 (5 μm)	Fluorescence detection at 320 nm (excitation at 280 nm)	Lemko *et al.,* 1993
L-Leucinamide	0.06 M KH_2PO_4–acetonitrile–triethyl amine (510:490:1), 1.8 mL/min	UltrabaseC18 (5 μm)	225 nm	Péhourcq *et al.,* 1995
S-(-)-1-(1-Naphthyl)-ethyl amine	Water (pH 3.0)–acetonitrile, (33.5:66.5), 1.2 mL/min	Inertsil ODS-2 (5 μm)	Fluorescence detection at 320 nm (excitation at 280 nm)	Lau, 1996
N-(1-Naphthyl)ethyl amine	Hexane–propan-2-ol, (97:3) or 23:2) 2 mL/min	Pirkle type chiral stationary phase of (*R*)-*N*-(3,5-dinitrobenzoyl)	254 nm	Crowther *et al.,* 1984
N-(1-Naphthyl)ethyl amine	Hexane–propan-2-ol (97;3)	phenyl glycine bonded to 5 μm		Wainer and Doyle, 1984
4-chloro aniline, 4-bromo aniline, 4-methoxyaniline, or 1-naphthylamine		spherical silica support		Nicoll-Griffith, 1987
Ethyl chloroformate to give 4-methoxy anilide derivative	Hexane–$CHCl_3$–propan-2-ol (18:2:1)	as above		Nicoll-Griffith *et al.,* 1988
Ethyl chloroformate and anisidine to give amides	Hexane–propan-2-ol (9:1)	as above		Nicoll-Griffith *et al.,* 1993
p-nitro benzyl amine hydrochloride	Hexane–isopropanol (35:5) 1.5 mL/min	*R*-1-(napthyl) ethyl urea, covalently bound to silica through a propyl linkage	235 nm	Ahn *et al.,* 1994

Anilides	Hexane–propan-2–ol (9:1)	Tris-3,5-dimethyl phenyl carbamates of cellulose or amylose	uv	Okamoto *et al.,* 1989
Amides and esters with 1-naphthyl methyl amine/benzyl amine and benzyl alcohol	*n*-Hexane-isopropanol with 0.5% acetic acid in varying ratio, 1 mL/min	Cellulose based chiral stationary phases, containing tris(4-methyl benzoate) cellulose	230 nm	Van Overbeke *et al.,* 1995
Several bi- and tri-cylic amines and alcohols	Methanol-perchlorate buffer(0.1 M, pH 2.0 or 1.5) in varying ratio, 1 mL/min	tris(4-methyl benzoate) cellulose covalently bonded onto γ-amino propyl silica (10 μm)	230 and 254 nm	Van Overbeke *et al.,* 1996
Direct	H_2O or ^2H_2O based phosphate buffers (0.01 M KH_2PO_4 0.1 M KH_2HPO_4 0.5 mL/min	α_1-acid glycoprotein	225 nm	Camilleri *et al.,* 1990
	Sodium dihydrogen phosphate-disodium hydrogen phosphate (pH 6.9) modified with octanoic acid and 18% of acetonitrile	Chiral HPLC column containing human serum albumin immobilized on diol phase	254 nm	Noctor *et al.,* 1991
	1.2% Propan-2-ol and 1.2 mM *N,N*-dimethyloctyl amine in 0.02 M NaH_2PO_4 (adjusted to pH 5.5 with NaOH)	Chiral-AGP (5 μm)	227 nm	Petersson and Olsson, 1991
	0.05 M Potassium acetate–acetonitrile	Microbore column packed with an ergot alkaloid based stationary phase of 5 μm particle size and 100 Å poresize	254 nm	Castellani *et al.,* 1994
	70 mM Citrate–acetonitrile–water with triethylamine	β-cyclo-dextrin silica Cyclobondl (5 μm)	UV	Farkas *et al.,* 1993
	5 mM Citrate buffer–acetonitrile	Cyclodextrin bonded to silica support via 3-glycidoxy silane spacer or Naphthyl ethylcarbamoylated cyclodextrin silica produced by reaction *in situ* with (*S*)-(+)- or (*R*)-(-)-1-(1-naphthyl) ethyl isocyanate	UV	Beeson and Vigh, 1993
	0.01 M phosphate buffer (pH 7.0) modified with 0.001 M dimethyloctyl amine and 2-propanol modified with 0.001 M dimethyloctyl amine as gradient system, 0.9 mL/min	Chiral-AGP, spherical (5 μm) particle, α1-acid-glycoprotein	220 or 245 nm	De Vries *et al.,* 1994
β-cyclodextrin, and its hydroxypropyl-, methyl- and sulphate derivatives as chiral mobile phase additives	5–20 mM cyclodextrin(s) in 0–10% v/v acetonitrile and 90%–100% v/v of 0.1% aq. trifluoro acetic acid (pH adjusted to 4.0 with triethyl amine) 0.8 mL/min	Hexa silane and octa silane reversed-phase columns	265 nm	Ameyibor and Stewart, 1997

Table 2. Minimum quantifiable concentrations (MOC) for each enantiomer of ibuprofen diastereomer

Derivatizing reagent	MQC μg/mL	Ref.
S-(-)-1-(1-naphthyl)ethylamine	0.1	Mehavar, 1988
S-(-)-1-(1-naphthyl)ethylamine	0.1	Lemko, 1993
S-(-)-1-(1-naphthyl)ethylamine	0.1	Lau, 1996
R-(+)-α-phenylethylamine	0.25	Wright, 1992
L-Leucinamide	0.1	Péhourcq, 1995
p-Nitrobenzylamine hydrochloride	2.5	Ahn *et al.,* 1994
S-(-)-1-(1-naphthyl)ethylamine	0.25	Avgerinos and Hutt, 1987
(-)-2-[4-(1-amino ethyl) phenyl]-6-methoxy benzoxazole	10 fmol	Kondo *et al.,* 1993
(*R*)-(-)-1-ferrocenylethyl amine or (*S*)-(+)-1-ferrocenylpropyl amine	0.5 pmol	Shimada *et al.,* 1987
Direct resolution on α1-acid-glycoprotein CSP	0.1 μg	De Vries *et al.,* 1994
Direct resolution on impregnated TLC	0.05 μg	Bhushan, 1996

tion of the reagent and the racemisation, e.g., *p*-nitrobenzylamine hydrochloride, 1-naphthyl-methyl-amine, benzylamine, 2-methyl-benzyl-amine, and benzyl alcohol, and the derivatization accounts for improved resolution or an increased absorbance.

TLC

A little attention has been paid to TLC resolution of ibuprofen/other profens' enantiomers, though in terms of equipment cost and cost of running/maintaining the HPLC equipment it is simpler and less expensive. Various advantages of TLC as a chromatographic technique in general have earlier been described in literature (Martens and Bhushan, 1989, Bhushan and Martens, 1997).

There has been only one report on the direct resolution of (±)-ibuprofen by TLC on silica plates impregnated with L-arginine (Bhushan and Parshad, 1996). L-arginine, the chiral selector, was mixed with the silica gel slurry which was used for making the plates and chromatograms were developed with acetonitrile–methanol–water (5:1:1, v/v); the hR_f values for the resolved (+) and (−) isomers of ibuprofen were 80 and 77, respectively. Arginine having a pI of 10.8 existed as a cation below it and interacted with (±)-ibuprofen to give (+)(−) and (−)(−) diastereomeric salts leading to enantiomeric separation. The method was successful in resolving as little as 0.1 μg of the enantiomeric mixture which is much lower than many of the reported HPLC detection limits.

CONCLUSION

To date there have been a large number of analytical methods using chromatography particularly HPLC for the separation and quantitation of ibuprofen enantiomers in biological samples. Most of them suffer from lengthy sample preparation, presence of endogenous interference, poor sensitivity, expensive chiral stationary phases and lengthy analysis time mainly due to an extensive column flushing procedure. Addition of chiral selector into mobile phase with an achiral column/stationary phase offers certain advantages in terms of flexibility, wide range of possible additives, and often lower cost.

Ibuprofen has a weak chromophore and UV detection of nanogram amounts is difficult, and to detect low levels of ibuprofen in biological samples a moiety with a high UV molar absorptivity or a fluorescent label is added by derivatization. Besides, the objective of derivatization is to introduce functionality complimentary to CSP, and attenuation of the polarity of the functional group of the solute so that individual polar interactions are not of a magnitude to overwhelm the influence of other interactions. On the other hand, derivatization with chiral reagent may introduce inaccuracies into the determination of enantiomeric ratio due to chiral impurities in the derivatizing agent or to racemization during derivatization procedure. Therefore derivatization with achiral reagents followed by application of chiral column is sometimes suggested.

However, TLC can provide the method of direct resolution of its enantiomers and determination of enantiomeric purity in a much simple and less expensive manner. Often HPLC and TLC are unjustly looked as competitive methods, but each of these has its own advantages. Optimizing the particular separation parameter with TLC would be a step leading to a considerable time saving and less cost of materials for an analysis prior to HPLC. With TLC various phase systems can be checked at the same time without expensive apparatus. Also in TLC and HPLC mobile and stationary phases are comparable and a large accordance in the retention mechanism exists.

Ibuprofen is marketed as a racemic mixture and has a huge world market as a powerful non-steroidal analgesic, antipyretic and anti-inflammatory drug. The therapeutic activity lies in the (*S*)-isomer and the (*R*)-isomer may accumulate as a residue in fatty tissues. Drug stereoselective disposition may be an important factor in the individual therapeutic response to a non-steroidal anti-inflammatory drug, therefore, it becomes essential to measure the ibuprofen enantiomers for a proper understanding of stereochemical mechanism of ibuprofen disposition after administration of racemic dose.

Acknowledgements

Thanks are due to Alexander von Humboldt-Stiftung, Bonn, Germany for award of a fellowship, and to the University of Roorkee, Roorkee, India for granting leave of absence to R.B.

REFERENCES

Ahn, H.-Y., Shiu, G. K., Trafton, W. F. and Doyle, T. D. (1994). Resolution of the enantiomers of ibuprofen; comparison study of diastereomeric method and chiral stationary phase method. *J. Chromatogr. B,* **653**, 163–169.

Ameyibor, E. and Stewart, J. T. (1997). Enantiomeric HPLC separation of selected chiral drugs using native and derivatised β-cyclodextrins as chiral mobile phase additives. *J. Liq. Chrom. Rel. Technol.,* **20**, 855–869.

Armstrong, D., Stalcup, A., Hilton, M., Duncan, J., Faulkner Jr, J. and Chang, S. (1990). *Anal. Chem.* **62**, 1610.

Avgerinos, A. and Hutt, A. J. (1987). Determination of enantiomeric composition of ibuprofen in human plasma by HPLC. *J. Chromatogr. Biomed. Appl.* **59**, 75–83.

Beeson, M. D. and Vigh, G. (1993). Examination of the retention behaviour of underivatised profen enantiomers on cyclodextrin silica stationary phases. *J. Chromatogr.* **634**, 197–204.

Bhushan, R. and Martens, J. (1997). Direct resolution of enantiomers by impregnated TLC. *Biomed. Chromatogr.* **11**, 280–285.

Bhushan, R. and Parshad, V. (1996). Resolution of (±) ibuprofen using L-arginine-impregnated thin layer chromatography. *J. Chromatogr. A,* **721**, 369–372.

Camilleri, P. and Dyke, C. (1990). Effect of deuterium oxide (heavy water) on the resolution of the optical isomers of ibuprofen on an α-acid glycoprotein column. *J. Chromatogr.* **518**, 277–281.

Castellani, L., Flieger, M. and Sinibaldi, M. (1994). Enantiomer separation of 2-arylpropionic acids on an ergot alkaloid based stationary phase microbore column application. *J. Liq. Chromatogr.* **17**, 3695–3703.

Crowther, J. B., Covey, T. R., Dewey, E. A. and Henion, J. D. (1984). Liquid chromatographic–mass spectrometric determination of optically active drugs. *Anal. Chem.* **56**, 2921–2926.

De Vries, J. X., Schmitz-Kummer, E. and Siemon, D. (1994). The analysis of ibuprofen enantiomers in human plasma and urine by HPLC on an α 1-acid glycoprotein chiral stationary phase. *J. Liq. Chromatogr.* **17**, 2127–2145.

Dingenen, J. (1994). Polysaccharide phases in enantioseparation. In *A Practical Approach to Chiral Separations by Liquid Chromatography* (Subramanian, G. ed.), VCH Verlagsgesellschaft, Weinheim, Germany.

Farkas, G., Irgens, L. H., Quintero, G. and Besson, M. P. (1993). Displacement chromatography on cyclodextrin silicas IV. Separation of the enantiomers of ibuprofen. *J. Chromatogr.* **645**, 67–74.

Goto, J., Goto, N., Hibichi, A., Nishimaki, T. and Nambara, T. (1980). Sensitive derivatisation reagents for resolution of carboxylic acid enantiomers by high performance liquid chromatography. *Anal. Chim. Acta.* **120**, 187–192.

Hilton, M. L. and Armstrong, D. W. (1991). In *New Trends in Cyclodextrins and Derivatives* (Duchene D. ed.) Chap. 15, p. 536. Editions de Sante, Paris.

Hutt, A. J., Fournel, S. and Coldwell, J. (1986). Application of a racemic compression column to the hplc separation of the enantiomers of some 2-arylpropionic acids as their diastereoisomeric *S*-(−)-1-(1-naphthyl) ethyl amides. *J. Chromatogr. Biomed. Appl.* **51**, 409–418.

Karger, B. L., LePage, J. N. and Tanaka, N. (1980). In *High Performance Liquid Chromatography-Advances and perspectives.* Horváth, S., ed.) pp. 113–204. Academic Press, New York.

Kondo, J., Imaoka, T., Kawasaki, T., Nakanishi, A. and Kawahara, Y. (1993). Fluorescence derivatisation reagent for resolution of carboxylic acid enantiomers by hplc. *J. Chromatogr.* **645**, 75–81.

Kondo, J., Suzuki, M., Imaoka, T., Kawasaki, T., Nakanishi, A. and Kawahara, Y. (1994). 6-Methoxy-2-(4-substituted phenyl) benzoxazoles as fluorescent chiral derivatisation reagents for carboxylic acid enantiomers. *Anal. Sci.* **10**, 17–23.

Lau, Y. Y. (1996). Determination of ibuprofen enantiomers in human plasma by derivatisation and HPLC with fluorescence detection. *J. Liq. Chrom. Rel. Technol.* **19**, 2143–2153.

Lee, E. J. D., Williams, K. M., Grahm, G. G., Day, R. O. and Champion, G. D. (1984). Liquid chromatographic determination and plasma concentration profile of optical isomers of ibuprofen in humans. *J. Pharma. Sci.* **73**, 1542–1544.

Lemko, C. H., Caille, G. and Foster, R. T. (1993). Stereospecific HPLC assay of ibuprofen: improved sensitivity and sample processing efficiency. *J. Chromatogr. Biomed. Appl.* **130**, 330–335.

Maitre, J. M., Boss, G. and Testa, B. (1984). HPLC separation of antiinflammatory 2-aryl propionates:suitability of the method for *in vivo* metabolic studies. *J. Chromatogr.* **299**, 397–403.

Martens, J. and Bhushan, R. (1989). TLC enantiomeric separation of amino acids. *Int. J. Peptide Protein Res.* **34**, 433–444.

Mehvar, R., Jamali, F. and Pasutto, F. M. (1988). Liquid chromatographic assay of ibuprofen enantiomers in plasma. *Clin. Chem.* **34**, 493–496.

Nicoll-Griffith, D. A. (1987). Stereoelectronic model to explain the resolution of enantiomeric ibuprofen amides on the Pirkle chiral stationary phase. *J. Chromatogr.* **402**, 179–187.

Nicoll-Griffith, D. A., Inaba, T., Tang, B. K. and Kalow, W. (1988). Method to determine the enantiomers of ibuprofen from human urine by hplc. *J. Chromatogr. Biomed. Appl.* **72**, 103–112.

Nicoll-Griffith, D. A., Scartaozzi, M. and Chiem, N. (1993). Automated derivatisation and high performance liquid chromatographic analysis of ibuprofen enantiomers. *J. Chromatogr.* **653**, 253259.

Noctor, T. A. G., Felise, G. and Wainer, I. W. (1991). Stereochemical resolution of enantiomeric 2-aryl propionic acid NSAID on human serum albumin based hplc chiral stationary phase. *Chromatographia,* **31**, 55–59.

Okamoto, Y., Aburatani, R., Kaida, Y., Hatada, K., Inotsume, N. and Nakano, M. (1989). Direct chromatographic separation of 2-aryl propionic acid enantiomers using tris-3,5-dimethylphenylcarbamates of cellulose and amylose as chiral stationary phases. *Chirality,* **1**, 239–242.

Péhourcq, F., Lagrange, F., Labat, L. and Bannwarth, B. (1995). Simultaneous measurement of flurbiprofen, ibuprofen and ketoprofen enantiomer concentrations in plasma using L-leucinamide as the chiral coupling component. *J. Liq. Chromatogr.* **18**, 3969–3979.

Peterson, K. J. and Olsson, A. (1991). Liquid chromatographic determination of the enantiomers of ibuprofen in plasma using a chiral AGP column. *J. Chromatogr. Biomed. Appl.* **101**, 414–418.

Shimada, K., Haniuda, E., Oe, T. and Nambara, T. (1987). Ferrocene derivatisation reagents for optical resolution of carboxylic acids by hplc with electrochemical detection. *J. Liq. Chromatogr.* **10**, 3161–3172.

Szejtli, J. (1982). *Cyclodextrin and Their Inclusion Complexes.* Akademiai Kiado, Budapest.

Van Overbeke, A., Baeyens, W. and Dewaele, C. (1995). Comparative study on the enantiomeric separation of several non-steroidal anti-inflammatory drugs on two cellulose based chiral stationary phases. *J. Liq. Chromatogr.* **18**, 2427–2443.

Van Overbeke, A., Baeyens, W. and Dewaele, C. (1996). Enantiomeric separation of six 2-arylpropionic acids after pre-column derivatisation with various amines and alcohols on a cellulose based chiral stationary phase. *Anal. Chim. Acta,* **321**, 245–261.

Wainer, I. W. and Doyle, T. D. (1984). Applications of high performance liquid chromatography, chiral stationary phases to pharmaceutical analysis:structural and conformational effects in the direct enantiomeric resolution of α-methyl aryl acetic acid anti-inflammatory agents. *J. Chromatogr.* **284**, 117–124.

Wainer, I. W. (1993). HPLC chiral stationary phases for the stereochemical resolution of enantiomeric compounds,

the current state of the art. In *Drug Stereochemistry, Analytical Methods and Pharmacology,* Marcel Dekker, New York.

Wright, M. R., Sattari, S., Brocks, D. R. and Jamali, F. (1992). Improved HPLC assay method for the enantiomers of ibuprofen. *J. Chromatogr. Biomed. Appl.* **121**, 259–265.

