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Review

Methods of analysis of chiral non-steroidal anti-inflammatory drugs

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Abstract

Although the analysis of the enantiomers of chiral non-steroidal anti-inflammatory drugs (NSAIDs) has been carried out for over 20 years, there often remains a deficit within the pharmaceutical and medical sciences to address this issue. Hence, despite world-wide therapeutic use of chiral NSAIDs the importance of stereoselectivity in pharmacokinetic, pharmacodynamic and pharmacological activity and disposition has often been ignored. This review presents both the general principles that allow separation of chiral NSAID enantiomers, and discusses both the advantages and disadvantages of the available chromatographic assay methods and procedures used to separately quantify NSAID enantiomers in biological matrices.

Keywords: Reviews; Non-steroidal anti-inflammatory drugs

Contents

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1. Introduction

NSAIDs are a structurally diverse class of therapeutic agents encompassing the salicylates, pyrazoles, oxicams, fenamates, arylacetic acids and arylpropionic acids. In addition, within these classes of NSAIDs a structural feature known as chirality distinguishes certain NSAIDs from all others (Table 1 and Fig. 1).

The vast majority of these chiral NSAID agents are arylalkanoic acids, the most common and simplistic example of this chirality is a sp^3-hybridized

Table 1
Chiral nonsteroidal anti-inflammatory drugs

Arylpropionic acids			
Alminoprofen	Fenoprofen	Ketoprofen	Pranoprofen
Benoxaprofen	Flunoxaprofen	Loxoprofen	Suprofen
Bermoprofen	Flurbiprofen	Miroprofen	Tiaprofenic acid
Carprofen	Ibuprofen	Naproxen	Thioxaprofen
Cicloprofen	Indoprofen	Pirprofen	Ximoprofen
Arylalkanoic acids			
Butibufen	Etodolac	Indobufen	Metbufen
Clindanac	Flobufen	Ketorolac	Sulindac
Non-acidic agents			
Azapropazone	Bumadizone	Oxyphenbutazone	Talnifumate

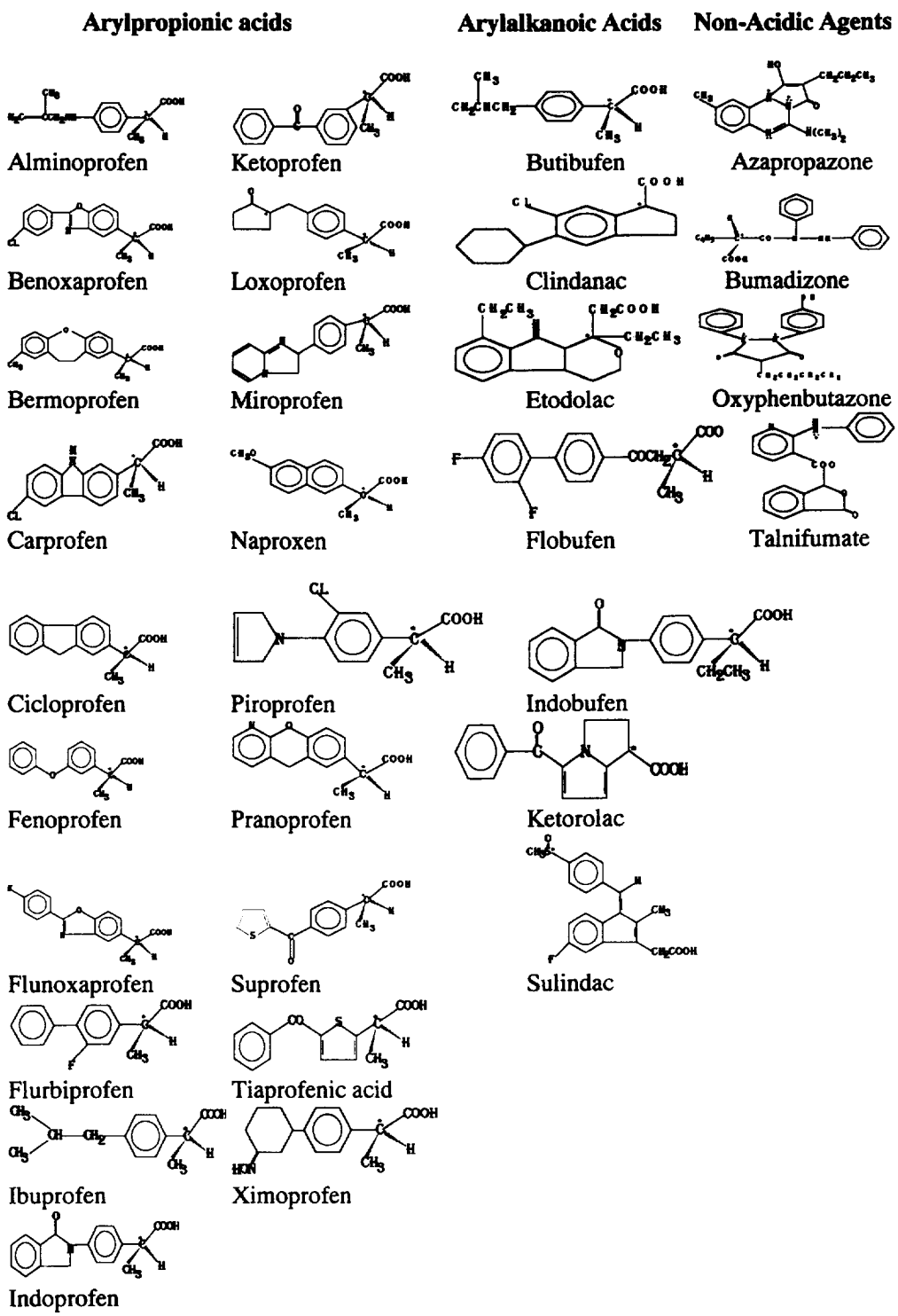


Fig. 1. Chiral nonsteroidal anti-inflammatory drugs. An asterix denotes the chiral centre(s). Sulindac has a chiral sulphur trico-ordinate centre rather than a chiral carbon atom. Ximoprofen and loxoprofen have two chiral centres.

tetrahedral carbon atom to which 4 different substituents are attached. Most of these NSAIDs have the chiral carbon atom within the propionic acid side-chain moiety, although the chiral carbon atom may also be incorporated into a rigid ring structure (i.e., clindanac, etodolac, ketorolac, ximoprofen).

The word chirality comes from the Greek "cheir" meaning hand. Just as we have a right hand that mirrors the left hand, our hands are non-superimposable "enantio" or opposite. A chiral NSAID such as ibuprofen has one chiral carbon and, therefore, exists as a pair of enantiomers.

In the past the relative configuration of enantiomers has been described using the D or (+) and L or (−) nomenclature. Enantiomers have similar physicochemical properties to each other (i.e., melting point, solubility, etc.), however, the direction to which they rotate a plane of polarized light differs. An enantiomer that rotates this light clockwise is said to be dextrorotatory [D or (+)], whereas the enantiomer that rotates light counterclockwise is said to be levorotatory [L or (−)]. The term optical activity refers to rotation of a plane of polarized light and, therefore, enantiomers are often called optical isomers. A racemate is a 50:50 composite of enantiomers that will not rotate light and is denoted as [±]. These descriptors are physical properties that differ between enantiomers and have generally fallen out of use but may still lead to confusion. Optical rotation means little in itself as this can be affected by temperature, solvent, pH, etc.

The Cahn, Ingold and Prelog nomenclature is now employed to assign absolute configuration of the molecular structure of chiral tetrahedral molecules. The stereochemical orientation of ligands in space depends on mass assignments of respective enantiomers and are designated by the descriptors *R* (rectus or clockwise) and *S* (counterclockwise or sinister), and sometimes D and L, generally followed by the rotation of polarized light. The use of D and L comes from the configuration of glyceraldehyde and is still commonly used as a descriptor of amino acids and carbohydrates. The Fischer nomenclature enables elucidation of relative stereochemistry of a pair of compounds of like dissymmetry by a sequence of chemical interconversions. There is no relationship between this system of nomenclature to describe absolute configuration and the ability to rotate polarized light. Additionally, it should be recognized that sulfur, nitrogen, silicon, germanium, arsenic and phosphorus, can all act as chiral centers. Although the chiral carbon atom predominates in pharmaceuticals, sulindac is an example of a racemic NSAID with a chiral sulfur heteroatom.

Furthermore, it is possible for molecules to have more than one chiral centre which complicates the issue of stereochemistry. The racemic NSAIDs loxoprofen and ximoprofen both have two chiral centers. For ximoprofen it is the oxime group which enables 2 geometric isomers to exist for each of the 4 enantiomers of this NSAID. In addition, loxoprofen is an NSAID with two chiral centers and a prochiral carbonyl group. For these NSAIDs, the maximum number of optical isomers is 2^n, where n is the number of chiral carbon atoms in the molecule. Both ximoprofen and loxoprofen have four optical isomers, two pairs of enantiomers (*R*,*S* and *S*,*R* and *R*,*R* and *S*,*S*), and within this group of optical isomers (2*R*,1′*S*) and (2*R*,1′*R*) and (2*S*,1′*R*) and (2*S*,1′*S*) are not mirror images of each other and are termed diastereomers. In addition, because of in vivo metabolism of the prochiral carbonyl group loxoprofen is metabolized into three chiral centres and has a maximum possibility of 23 optical isomers or enantiomers [1–3]. Furthermore, ibuprofen is metabolized in vivo to carboxyibuprofen which introduces a second chiral centre into the molecule; therefore, four stereoisomeric metabolites are possible, that is *RS*-(*R*-ibuprofen, *S*-carboxyibuprofen), *SR*-, *SS* and *RR*-configurations [4,5].

The term diastereomers, can be confusing in that geometric isomers (i.e., E and Z or *cis* and *trans* isomers) are referred to as diastereomers. Sulindac is also a geometric isomer but is marketed as the pure Z isomer. The chiral NSAID ximoprofen exists as geometric isomers as well as optical isomers. Geometrical isomers arise from double bonds or cyclicity. In contrast to optical isomers or enantiomers, diastereomers may differ in their physiochemical properties to one another including solubility, stability and compatibility, etc.

Many of the chiral NSAIDs are marketed as racemates (i.e., equivalent proportions of enantiomers) (Table 1). Naproxen is the only NSAID to be

internationally marketed and used clinically as the pure *S*-(+)-enantiomer. *S*-(+)-ibuprofen (Garbo, Fieberbrunn, Austria) has become available clinically in Austria since 1994, and *S*-(+) ketoprofen has been recently accepted for marketing in Spain (Medarini SA, Badalona, Barcelona). Several other stereochemically pure NSAIDs are currently undergoing various stages of clinical development. The anti-inflammatory activity of NSAIDs has previously been shown to be largely stereospecific for the *S*-enantiomer [6].

The relevance of stereoselective pharmacokinetic and pharmacodynamic disposition of racemic NSAIDs have been extensively recognized in last 20 years. Excellent reviews are available describing stereoselectivity in pharmacological, pharmacodynamic and pharmacokinetic behavior of chiral NSAIDs [6–9]. Some NSAIDs (e.g., fenoprofen, ibuprofen) undergo a unidirectional inversion of the "inactive" *R*-enantiomer to the active *S*-enantiomer. Thus, nonstereospecific assay methods can not interpret the time-course of the individual enantiomers and would be misleading in relating either toxicity or efficacy to the racemic drug concentration. The arylalkanoic acid derivatives etodolac and ketorolac have much lower plasma concentrations of the active *S*-enantiomer than the *R*-enantiomer [8,10,5]. Therefore, any interpretation of effects based on nonstereospecific data can also be misleading for these chiral NSAIDs.

In the absence of the considerations of stereochemistry the growing body of pharmacokinetic and pharmacodynamic consequences of chirality would not have been interpreted correctly. Measuring unresolved plasma or urine drug levels of a racemic NSAIDs may lead to misinterpretation of data and any interpretation relating therapeutic or toxic effects to unresolved drug concentration based on nonstereospecific measurements of racemic NSAIDs may be erroneous. Fortunately, the technology is currently available to separately quantify both the enantiomers of chiral NSAIDs and their respective chiral metabolites in biological matrices.

The purpose of this review is to provide an overview of the methods of analysis of enantiomers of racemic NSAIDs. The advantages and disadvantages of the stereospecific assays and currently available chromatographic methods that can separately quantify chiral NSAIDs will also be discussed.

2. Chromatographic methods of separation of enantiomers

The most commonly used approach to chromatographic resolution of enantiomers has been the formation of diastereoisomers either transiently or covalently. As diastereoisomers have different physico-chemical properties, there is differential retention on a chromatographic column.

Chromatographic enantiospecific resolution of racemic NSAIDs is generally accomplished by two methods. (1) Pre-column derivatization of the enantiomers with a chiral reagent followed by separation of the resulting diastereoisomers on an achiral phase. (2) Temporary formation of diastereoisomers: (A) on a chemically bonded chiral stationary phases (CSP) with an achiral mobile phase, (B) by addition of a chiral mobile phase complexing agent and an achiral stationary phase.

2.1. Direct methods of analysis: chiral stationary phases

A considerable number of CSPs are presently commercially available that have been utilized to separately quantify chiral NSAIDs including: (A) Chiral "brush type" phases, (B) chiral polymer phases and (C) chiral affinity phases.

2.1.1. Chiral "brush type" phases

The application of high-performance liquid chromatography (HPLC) to CSP chromatographic resolution using (*R*)-N-(3,5-dinitrobenzoyl) (DNB) phenylglycine or Pirkle columns to the separation of several 2-arylpropionic acid (2-APA) NSAIDs as secondary or tertiary amides with aromatic substituents but not for the primary amide or for ester derivatives has been described [11]. This involves amide synthesis converting the acid into the acid chloride with thionyl chloride or oxalyl chloride and by reaction with an amine (1-napthyl methylamine). Several other investigators have subsequently re-

ported the separation of several other 2-APA NSAIDs as amide derivatives [12,147–149].

The performance of Pirkle columns are strongly dependent on conformational requirements of the solute and CSP. With respect to chiral NSAIDs three simultaneous interactions take place (Model 1): (1) hydrogen bonding between the hydroxyl moiety of the carboxylic acid group of the NSAID and the carbonyl group of the 3,5-DNB carboxamide in the CSP. (2) Steric interactions between arylalkyl groups of the NSAID and the CSP. (3) A $\pi-\pi$ donor–acceptor interaction between the phenyl ring of the NSAID and the 3,5-DNB group of the analyte and the 3,5-DNB group of the CSP. The three point model considers only the simple case where each point of interaction involves a discrete bond to the asymmetric centre. In the case of amides two or more potential strongest interaction sites are located on the same bond leading to the asymmetric centre. It is also necessary that constraints on conformational freedom within the solute (or as a result of solute–CSP interaction) occur for enantiomeric discrimination. The stronger interaction of the *S*-(+) enantiomer with respect to the *R*-(−) enantiomer when using Pirkle columns has been well defined by the chiral recognition model [15]. However, the validity of this model has been challenged by a proposed model of anti-parallel amide stacking and electron donor acceptor complexes with steric repulsion between their chiral centres and the CSP chiral centre allowing for enantiomer resolution [13].

More recently, resolution of derivatized 2-APAs on columns containing a new series of urea bonded chiral stationary phases consisting of a terminal chiral substituted naphthyl urea attached to silanized silica bearing a long spacer arm ending in a primary amine group (JTB-X) has been accomplished. 3,5-DNB derivatives of 2-APAs have been separated using reversed-phase separations [16]. Ibuprofen had substantially improved separation compared to the traditional Pirkle column. These columns behave in a reciprocal manner to the traditional Pirkle Columns as the nitro groups are a part of the analyte as opposed to the stationary phase. These columns can be used under reversed-phase conditions as opposed to traditional Pirkle columns which are less effective under reversed-phase conditions [16].

Direct separation of several chiral NSAIDs by HPLC with amide and urea derivatives bonded to silica gel as CSPs has also been described [17]. In addition, the use of ergot alkaloid-based stationary phase microbore columns to the separation of several 2-APA enantiomers has increased the choice of resolution techniques available [18].

2.1.2. Chiral polymer phases

Another optically active column employs D-cellulose esters to which various terminal groups are attached. Within this group of phases is the β-cyclodextrin bonded phase column (Cyclobond I). This macrocyclic molecule contains seven glucopyranose units arranged in the shape of a hollow truncated cone, in which the interior cavity is relatively hydrophobic. The exterior faces, on the other hand are hydrophilic. Because of the rigid cavity size of β-cyclodextrin, only "guest" molecules of proper size can form strong β-cyclodextrin inclusion complexes. The use of two 25 cm β-cyclodextrin columns were capable of resolving ketoprofen enantiomers [19]. When optimized pH values and salt concentrations are used with the mobile phase, sufficient separation of the optical isomers of NSAIDs are possible on a cyclodextrin CSP [20]. Additionally, this technology has been applied to the preparative separation of enantiomers of NSAIDs on a β-cyclodextrin CSP [21].

Several silica-supported cellulose columns are now commercially available with the Chiracel OD (tris(3,5-dimethylcarbamate)-cellulose) phase being extremely popular and able to separate several 2-APA NSAIDs as amide derivatives [14]. Direct separation of several 2-APA NSAIDs has been reported on both cellulose and amylose CSPs [22]. Separation of several 2-APA NSAIDs has also accomplished on an experimental tris(4-methylbenzoate) cellulose phase (Bio-Rad RSL) with most separations requiring prior derivatization to benzylamide derivatives [23,24]. The utility of a cellulose derivative bearing simultaneously 3,5-dimethylphenylamino carbonyl and 10-undecenoyl groups for 2-APA resolution has also been described [25].

A recently reported broad-spectrum chiral selector and two of its homologs were incorporated into polymethylhydrosiloxane and immobilized on silica gel to form a CSP. Separation of many chiral NSAID

by HPLC and/or supercritical fluid (SFC) chromatography was accomplished [26].

2.1.3. Chiral affinity phases

Stereoselective HPLC analysis often involves the use of proteins immobilized on a solid support as the chiral selector. Several direct resolutions of underivatized 2-APA NSAIDs using this class of CSPs, based on either bovine serum albumin [27], human serum albumin [28], ovomucoid [29,30], second-generation silica-bound α_1-acid glycoprotein (AGP) [31], avidin [32] and egg yolk riboflavin binding protein (RFBP) [33] are described in the literature.

As the AGP column is based on immobilized human plasma protein on silica particles, the separation power, decreases with an increasing number of plasma samples and may differ from one column to another. Thus, retention times, resolution and separation factors, and the quantification limit may vary to a limited extent depending on the column used. The use of an α_1-AGP column for the resolution of several 2-APA NSAIDs has been reported [34]. The effects of mobile phase composition and temperature on the resolution of several 2-APAs has been determined [35]. The effect of pH, 2-propanol concentration and the content of dimethyloctylamine (DMOA) in the mobile phase, as well as temperature were all analyzed. The separation factor could be improved by reducing the concentration of 2-propanol and lowering the temperature, but the retention increased reciprocally. The higher the content of DMOA the better the enantiomeric resolution and retention [35]. It is postulated that solutes are distributed as ion-pairs with DMOA to the stationary phase and that the bulky DMOA ion-pair of one enantiomer in each pair fits better in the chiral active site of the protein due to sterical reasons [31]. Without the addition of DMOA the resolution was either impossible or unsatisfactory. Interestingly, the Enantiopac® column could not resolve tiaprofenic acid, indoprofen, or carprofen under any of the conditions examined. The Enantiopac® column depends to a high degree on chromatographic conditions such as pH, the ionic strength and content of the organic modifier in the mobile phase and the temperature. Consequently, the range of optimum conditions to avoid interfering peaks from plasma is rather narrow. Owing to temperature dependence of the chiral separation a column thermostat is often necessary. The advantages of the Enantiopac® are associated with the reversed-phase character of the column which allows direct injection of aqueous samples. Dynamic modification of the chiral bonding properties of a Chiral-AGP column by inorganic and organic additives in particular hydrophobic tertiary amines has enabled optimization of enantiospecific resolution of several chiral NSAIDs [36].

The use of bovine serum albumin (BSA) columns to separately quantify 2-APA enantiomers has also been reported [27]. The use of 1-propanol and octanoic acid under reverse phase conditions enhanced the ability to separate ibuprofen.

Application of ovomucoid-conjugated column for the optical resolution of ibuprofen, ketoprofen and flurbiprofen has been demonstrated [29]. The ovomucoid-bonded silica column has been successful in separating the enantiomers of ketoprofen [30]. However, more recent findings suggest that ovomucoid has no appreciable chiral recognition ability, and that the chiral recognition reported previously for ibuprofen and ketoprofen is ascribable to ovoglycoprotein present in the crude ovomucoid preparations [37].

Recently, it has been reported that an avidin-conjugated column exhibited chiral discrimination of ketoprofen enantiomers [38]. This has been followed by the separation of flurbiprofen and ibuprofen by the same research group [39]. An avidin-bonded column for direct separation of ketoprofen enantiomers from plasma by direct injection has subsequently been published [32].

The stereochemical resolution of nine 2-APA NSAIDs on an immobilized human serum albumin (HSA) based HPLC CSP has been reported [28]. The addition of octanoic acid to the mobile phase significantly increased capacity factors. In addition, the resolution of ketorolac was possible on a HSA-based CSP and the potential utility for such columns to probe stereoselectivity of NSAID protein binding was demonstrated [40].

2.1.4. Chiral mobile phase additives

The addition of an optically active molecule to the mobile phase allows for separation on conventional stationary phases. Chiral NSAIDs with a carboxylic acid functional group may be separated with the

addition of an amino-alcohol to the mobile phase. It is assumed that the basic process is an interaction of the chiral counter ion with the enantiomers to give two diastereomeric ion pairs. The stereospecific resolution is due to the different distribution of the diastereomeric ion-pairs between the organic mobile phase and the adsorbing stationary phase.

It is also possible to separate the enantiomers of underivatized chiral acids as ion pairs with quinine or quinidine on normal-phase HPLC [41–43]. In addition, the enantiomers of naproxen have been separated with the chiral additive quinine in the mobile phase using a chiral acetylquinidine–silica stationary phase [44]. Furthermore, some preliminary work with indoprofen enantiomers has allowed separation with 0.5 m*M* quinine acetate in 0.1% pentanol–dichloromethane [45]. Although there are several examples of separation of NSAID enantiomers with chiral mobile phase additives complete validation of assays and application to pharmacokinetic and pharmacological studies remains limited.

Capillary electrophoresis (CE) with its various modes of operation (micellar electrokinetic chromatography, isotachophoresis, capillary zone electrophoresis, etc.) is a versatile and powerful separation technique. The potential advantages of using CE over HPLC are the higher peak efficiency and resolution attained with similar selectivities. The resolving power of CE is possible by the addition of chiral modifiers to the background electrolyte. The application of CE to stereospecific analysis of chiral NSAIDs has grown in recent years. Several linear oligosaccharides have been used as potential chiral discriminators in CE with maltodextrins being effective chiral electrolyte modifiers to perform direct, rapid separations of several 2-APA enantiomers [46]. Several other investigators have demonstrated that cyclodextrins in CE are effective at separating 2-APA NSAIDs [47–50].

2.1.5. Derivatization in direct method

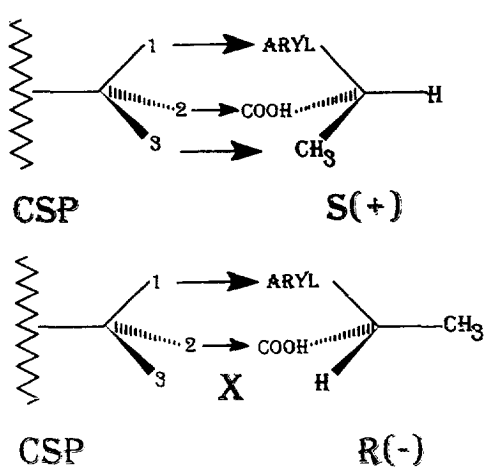
Although some chiral NSAIDs can be separated on CSPs without derivatization, it may be necessary to modify the compound. The objectives of derivatization are three fold: (1) enhancement of ultraviolet/visible or fluorescence detection of the solutes, (2) introduction of an aromatic moiety complementary to the aromatic function in the CSP i.e., π-basicity or acidity and (3) attenuate the polarity of the functional groups of the solute to reduce individual polar interactions for improved resolution.

2.2. Indirect method of analysis: chiral derivatization techniques

The first assays capable of enantiospecific analysis of chiral NSAIDs were published in the early 1970s and employed the indirect approach to resolution. Indirect methods of chiral separation are based on the reaction (derivatization) of a racemate with a homochiral reagent to form a pair of diastereoisomers. These diastereoisomers (unlike enantiomers) have different physiochemical properties in an achiral environment and are subsequently separated using chromatographic techniques.

Optimum separation of diastereoisomers appears to be achieved when the functional group of both enantiomers and resolving agents are adjacent to the chiral centres. With some chiral NSAIDs a carboxylic acid functional group is available for formation of salts or derivatization into the ester or amide functionality (Scheme 1). Formation of diastereoisomers of chiral NSAIDs and subsequent separation of the isomers utilizing gas chromatography (GC), thin-layer chromatography (TLC) or HPLC has been used extensively.

The formation of esters with chiral 2-APA NSAIDs has been accomplished using optically



Scheme 1.

active (*S*)-(+)-2-octanol [58,59] and (1*R*,2*S*,5*R*)-(−)-menthol [53]. However, in virtually, all of other cases, an amide derivative is formed by reaction of the reactive carboxylic acid group of the chiral NSAIDs with a chiral amine such as amphetamine or phenylethylamine, naphthylamine or leucinamide. These reactions take place in the presence of a coupling reagent like 1,1′-carbonyldiimidazole or thionyl chloride or through a mixed anhydride intermediate utilizing a chloroformate derivative such as ethylchloroformate (ECF). Reactions using 1,1′-carbonyldiimidazole usually requires a relatively high temperature and/or a long reaction time [54,55]. The NSAID acid chloride prepared with thionyl chloride has been shown to be ineffective for the preparation of diastereoisomeric tiaprofenic acid–benzeneethanamides [56]. In addition, it has been reported that hydroxylated metabolites of ibuprofen are readily dehydrated and hydrohalogenated with thionyl chloride [57]. Reactions using ECF take place at room temperature in a short period of time. The diastereoisomers of ketoprofen [58], indoprofen [50], fenoprofen [59] and flurbiprofen [60] have been formed at ambient temperature in less than 3 min by utilizing L-leucinamide and ECF. The suitability of ECF/L-leucinamide for separation of 2-APAs was reported to be "generally applicable" as a method [61]. However, this method could not be applied to the analysis of ibuprofen, tiaprofenic acid or etodolac, therefore, some modifications may be necessary for other chiral NSAIDs [62]. When this method is applied to ketorolac it has been reported that significant racemization takes place [63]. Substitution of ECF with the more reactive trichloroethyl chloroformate increased the derivatization efficiency by several fold. Addition of 4-dimethylaminopyridine (DMAP) to the reconstituting solution further improved reaction efficiency of tiaprofenic acid [64]. However, this method was found unsuitable for ibuprofen or etodolac. While the derivatization reaction was successful with etodolac the diastereoisomers could not be resolved on previously described reversed-phase systems [50,58,60], nor when a normal-phase system was employed. A rapid derivatization technique utilizing ECF and (*S*)-(−)-1-(1-naphthyl)ethylamine in plasma capable of separating ibuprofen enantiomers has been reported [65]. However, this method could be applied to ketoprofen and flurbiprofen. In addition, this approach did not appear to derivatize tiaprofenic acid and etodolac enantiomers. The use of (α)-methylbenzylamine to from diastereomeric amide derivatives of 2-APAs which were separated by normal-phase HPLC has been reported [66]. Hence, it must be stressed that a specific assay for a chiral NSAID does not necessarily mean "generalizability" of all structurally similar chiral NSAIDs.

Scheme 1: $RCOOH+R'OH \rightarrow RCOOR'$

Scheme 2: $RCOOH+R'NH_2 \rightarrow RCONR'$

2.3. Advantages and disadvantages

All of these methods of analysis may have certain disadvantages: (a) they take too long, (b) the reaction of the drug with the derivatizing chiral agent is too variable, (c) the cost of the optically active materials is prohibitive and (d) there is a lack of optical purity and/or stability of the chiral derivatizing reagent.

It has been suggested that although the diastereoisomeric approach to analysis commonly provides excellent separations, it contains the inherent risk of undetected bias of the results because of partial racemisation of either reactant or kinetic resolution [11].

Stereochemical conversion of ibuprofen enantiomers during derivatization with ECF has been suggested [67]. This possibility of stereochemical conversion of chiral NSAIDs induced by derivatization methods employing ECF was recently addressed [68] The apparent extent of conversion is inversely related to the concentration of ECF present during the derivatization reaction for all the 2-APAs tested but not for etodolac. However, both enantiomers appear to be inverted to the same extent in the presence of ECF and using the published analytical methods for the drugs studied the absolute error is less than 5%. However, it is necessary that degree of stereochemical conversion be evaluated as part of chiral assay validation process. Subsequently, ECF and 2-ethoxy-1-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) were employed as first-step coupling reagents in acetonitrile and toluene [69]. By utilizing a stereospecific method of ibuprofen analysis [65], the extent of racemization during the derivatization procedure was directly affected by the nature of the derivatizing reagent and by the reaction

solvents. When ECF was employed acetonitrile was found to induced more racemization than toluene. Conversely, when EEDQ was utilized as the first-step coupling agent there was significantly less racemization (>5%) than with ECF, and there was no apparent effect of the solvents employed.

It is, therefore, prudent to avoid any environment that may isomerize the chiral centre of either the drug or derivatizing reagent. This would for example be the use of extreme alkaline or acidic conditions or elevated temperatures. Cooperation of pharmaceutical manufacturers in the provision of pure enantiomers for assay validation would also be beneficial to the analyst.

Several GC and HPLC CSPs are now commercially available. However, the use of these columns has not met with the degree of success that was initially anticipated. The choice of mobile phase compositions is rather limited with CSPs, and the cost of the columns are often prohibitive. Many publications report the applicability of a CSP to resolve a chiral NSAID, however, there are still comparatively few published and validated assays in biological matrices. Furthermore, these columns have limitations, including relatively low thermal stability of the many GC phases and significant racemization at the critical temperature. In the case of HPLC, some columns can only be used with non-aqueous solvents, and pre-column achiral derivatization may be necessary to shorten very long retention times [11,12].

3. Arylpropionic acids

3.1. Alminoprofen

Alminoprofen, (±)-α-methyl-4-[(2-methyl-2-propenyl)aminobenzeneacetic acid, is undergoing clinical trials. Currently there are no published stereospecific assays available in the literature.

3.2. Benoxaprofen

Benoxaprofen, (±)-2-[2-(4-chlorophenyl)benzoxazol-5-yl]propionic acid, is no longer used clinically.

A GC method for the analysis of benoxaprofen using conventional phases required high temperatures and gave poor resolution of the diastereoisomer peaks [70] (see Table 2). The application of HPLC CSP using (*R*)-N-(3,5-DNB)phenylglycine or Pirkle columns to the separation of benoxaprofen has been reported [11]. Subsequently, the use of direct HPLC injection of a benoxoprofen amide derivative on a Pirkle column coupled with mass spectrophotometry has been utilized for equine urinary analysis [12].

A method for determination of benoxaprofen enantiomers derivatized by the method of Björkman [45] has been reported [61,74], although assay validation in biological fluids was not determined. The preparative separation of benoxaprofen enantiomers by HPLC and their application as fluorescent derivatization markers for chiral amines has been published [75]. Another investigator also reports stereospecific resolution of benoxaprofen enantiomers [76]. More recently, resolution of benoxaprofen on an immobilized HSA based HPLC CSP has been described [28], and separation of benoxaprofen has also accomplished on an experimental tris(4-methylbenzoate) cellulose phase (Bio-Rad RSL) [23].

3.3. Bermoprofen

Bermoprofen, (±)-10,11-dihydro-α, 8-dimethyl-11-oxodibenz[*b*,*f*]oxepin-2-acetic acid, is currently under clinical development. No stereospecific assays have been published in the literature.

3.4. Carprofen

Carprofen, (±)-2-(6-chlorocarbazol-2-yl)propionic acid, was separately quantified using 1,1′-carbonyldiimidazole as the coupling agent and (*S*)-(−)-1-phenylethylamine to form 1-phenylethylamide diasteroisomers by HPLC without complete assay validation [66]. A method of separation of carprofen enantiomers as their diasteroisomeric amides with *S*(−)-1-napthen-1-yl)ethylamine by HPLC has also been published [80].

Validation of a previous method [45] in plasma and urine for conjugated and unconjugated carprofen has been reported [61,81]. Additionally, other investigators report the suitability of their assay for carprofen resolution [83] (see Table 3).

Separation of carprofen has also been accomplished on an experimental tris(4-methylbenzoate)

Table 2
Stereospecific chromatographic assays for benoxaprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
1.0	1.0, 0.5	1,1-carbonyldiimidazole, (*S*)-(−)-α-methylbenzylamine	Helium, hydrogen, oxygen	NA	U-shaped glass column (1.02 m×3 mm I.D.) 2% OV17	NR	Plasma, urine	[71]
NR	NR	Thionyl chloride, 1,1''-carbonyldiimidazole, *S*-(−)-α-methylbenzylamine	Isooctane–methylene chloride–methanol (55:44.8:0.2)	308	Hypersil 5-APS (12.5 cm×5 mm I.D.)	NR	NR	[72]
NR	NR	Thionyl chloride, *R*-(+)-α-methylbenzylamine	Dichloromethane–2,2,4-trimethylpentane–methanol (100:100:1.35, v/v)	309	(250×4 mm column of 10 μm Partisil)	NR	Rat and human plasma, rat liver	[73]
NR	2	*R*-(+)-α-methylbenzylamine	Cyclohexane–dichloromethane–tetrahydrofuran (10:10:1, v/v)	309	Zorbax-Sil-7 (250 mm×21.2 mm I.D.)	NR	NR	[75]
NR	NR	ECF, L-leucinamide	Dichloromethane–methanol (100:2, v/v)	Ex 313 Em 365	Zorbax Sil (25×0.46 cm, 5 μm)	*S*-Naproxen	Urine	[74]

Table 3
Stereospecific chromatographic assays for carprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
≅4.0	0.5	1,1'-Carbonyldiimidazole, (*S*)-(−)α-methylbenzylamine	Benzene–ether–methanol (60:35:5)	NR	TLC plates pre-coated with silica gel 60 F-254 (10×20 cm)	NR	Rat blood, bile	[77]
0.6, 0.6, 0.6	0.5, 1.0, 10	1,1'-Carbonyldiimidazole, (*S*)-(−)α-methylbenzylamine	0.75% methanol in dichloromethane	254	(25 cm×4.6 mm I.D. stainless steel column containing 10 μm silica gel)	(*S,R*)-N-(2-Phenethyl)α-methyl (6-chloro-9H-carbaxol-2-yl)acetamide (*S,R*)PMCA and (*R,R*) PMCA	Blood, urine, feces	[78]
0.25	1.0	1,1'-Carbonyldiimidazole, (*S*)-(−)α-methylbenzylamine	50% acetonitrile 0.1% H_3PO_4	Ex 292 Em 360	Ultrasphere C_{18} (5 μm, 4.6 mm×15 cm)	Naproxen	Plasma	[79]
0.04, 0.1	0.1	ECF/L-leucinamide	10 m*M* phosphate buffer pH=6.5–acetonitrile (45:55, v/v)	Ex 285 Em 345	Zorbax 7-ODS (250 mm×21.2 mm)	*S*-Naproxen	Plasma, urine	[81]
0.05, 0.2	0.1	1,1'-Carbonyldiimidazole, (*S*)-(−)-α-methylbenzylamine	Acetonitrile–tetrabutylammonium hydroxide buffer (pH=2.5)	Ex 290 Em 360	Altex Ultrasphere 5-ODS (4.6×250 mm)	*S*-Naproxen	Plasma, urine	[82]
0.125	NR	NR	NR	NR	Chiracel OJ	NR	Cow, horse plasma	[84]

cellulose phase (Bio-Rad RSL) requiring prior derivatization to benzylamide derivatives [23]. In addition, carprofen has been directly resolved via cyclodextrin modified CE [50], a CSP containing egg yolk riboflavin binding protein (RFBP) [33], a polysiloxane-based CSP [26] and by organic and inorganic additives on a Chiral-AGP [36].

3.5. Cicloprofen

Cicloprofen, (±)-α-methylfluorene-2-acetic acid, is no longer used clinically.

The ability to separate the enantiomers of cicloprofen using 1,1′-carbonyldiimidazole as the coupling agent and (*S*)-(−)-1-phenylethylamine to form 1-phenylethylamide diasteroisomers by HPLC has been described but without assay validation [66]. The suitability of ECF/L-leucinamide foe separation of carprofen has been reported [59]. The suitability of other HPLC assays for stereospecific determination of cicloprofen has also been suggested [80] (see Table 4).

A report also indicates the applicability of a GC assay utilizing amphetamine as the derivatizing reagent, however, complete assay validation was not conducted [53].

Moreover, several types of CSPs have demonstrated the ability to separately quantify cicloprofen enantiomers include brush-type CSPs [86] and a polysiloxane-based CSP [27].

3.6. Fenoprofen

Fenoprofen, (±)-α-2-(3-phenoxyphenyl)propionic acid, has been stereospecifically quantified using (*R*)-N-(3,5-DNB)phenylglycine or Pirkle columns [11,86]. Furthermore, the use of direct HPLC injection of fenoprofen amide derivative on a Pirkle column coupled with mass spectrophotometry for equine urinary analysis has been reported [12]. In addition, direct separation of fenoprofen by HPLC with amide and urea derivatives bonded to silica gel has also been demonstrated [17] (see Table 5).

The earlier reported methods of analysis of fenoprofen enantiomers required lengthy sample preparations [54,55]. The originally reported GC assays utilized amphetamine as the derivatizing reagent and these assays also required exposure to high temperatures [55]. GC assays were capable of quantifying fenoprofen enantiomers in plasma and urine, with large amounts of the conjugated *S* and small amounts of the conjugated *R* 4′-hydroxy metabolite quantifiable [54].

Application of the same derivatizing technique as applied to ketoprofen [58] and indoprofen [45] utilizing ECF and L-leucinamide is also applicable to fenoprofen [59]. The wavelength within run was changed from 275 nm for the first 13 min and then switched to 232 nm as some endogenous peaks co-eluted with the internal standard at 232 nm. These interfering peaks observed when analyzing fenoprofen and ketoprofen in plasma, therefore, necessitated a lengthy procedure involving reversed-phase chromatography of the extracted plasma, collection of the drug, derivatization of the enantiomers and normal-phase chromatography of the derivatives was employed [75]. Additionally, calibration curves indicated analysis was stereospecific for the *R*-enantiomer. After alkaline hydrolysis, hydrolyzed conjugates of hydroxy metabolites of the fenoprofen enantiomers were found to presumably elute after the fenoprofen peaks [59]. Furthermore, with a simple extraction of unresolved drug from acidified plasma and subsequent normal-phase HPLC separation of the diastereomeric *S*-1-phenylethylamides derivatives is accomplished within 5 min although this assay has

Table 4
Stereospecific chromatographic assays for cicloprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
<5.7	1.0	Oxalyl chloride, L-leucine	Benzene–diethyl ether–methanol (8:3:1, v/v)	NA	Quanta-gram Q-1-F silica gel (20×20 cm)	NR	Plasma, urine, bile	[85]

Table 5
Stereospecific chromatographic assays for fenoprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
NR	NR	Thionyl chloride, (−)-α-methylbenzylamine	Isooctane–methylene chloride–methanol (75:24:1)	308	Hypersil APS (12.5 cm×5 mm I.D.)	NR	NR	[72]
NR	1.0	1,1′-Carbonyldiimidazole, (S)-(−)-α-methylbenzylamine	Nitrogen, hydrogen, air	NA	(1 m×6 mm O.D.×4 mm I.D.) 3% OV-17	(±)-2-(4-phenoxy phenyl) valeric acid	Plasma, urine	[54]
2.5	0.2	$SOCl_2$, *R*-2-phenylethylamine	Acetonitrile–dichloromethane (4:96)	272	5 μm LiChrosorb Si-60 (250 mm×4 mm I.D.)	Ketoprofen	Plasma	[76]
0.25, 1.0	0.5	ECF/L-leucinamide	0.067 *M* KH_2PO_4–acetonitrile–triethylamine (65:35:02)	232	5 μm Partisil 5 ODS-3 10 cm stainless steel 4.6 mm I.D. octadenyl bonded silica	Ketoprofen	Plasma/ urine	[59]
0.1, 0.1	1.0, 0.5	ECF/L-leucinamide	40% acetonitrile–60 m*M* potassium phosphate buffer pH 6	NR	Beckamn Ultrasphere 5-ODS (25 cm×0.46 cm I.D.)	Ketoprofen and flunoxaprofen	Plasma/ urine	[88]
0.25	0.5	NR	0.5% 2-propanol 5 m*M* DMOA in 20 m*M* phosphate buffer (pH=6.7)	220	Enantiopac (100 mm×4.0 mm I.D.)	*R*-Ibuprofen	Plasma	[36]

not been completely validated for fenoprofen [87]. Additionally, a stereospecific assay utilizing the same derivatization procedure as Mehvar and Jamali [59] was capable of simultaneous detecting of fenoprofen and its 4′ hydroxy metabolite in plasma and urine [88]. A pre-column derivatization method with a chiral derivatizing reagent D-and L-1-(4-dansylaminophenyl)ethylamine (DAPEA) can also resolve fenoprofen enantiomers [89].

Subsequently, the use of an α_1-acid glycoprotein column for the resolution of fenoprofen has been reported with or without organic and inorganic additives [34,35]. More recently, resolution of derivatized fenoprofen 3,5-DNB derivatives on JTB-X columns [16], the use of ergot alkaloid-based stationary phase microbore columns described [18], a polysiloxane-based CSP [26], an immobilized HSA based HPLC CSP [28], an experimental tris(4-methylbenzoate) cellulose phase (Bio-Rad RSL) requiring prior derivatization to the benzylamide derivative [23] and cyclodextrin-modified CE can also be utilized for stereospecific fenoprofen analysis [47].

3.7. Flunoxaprofen

Flunoxaprofen, (±)-2-(4-fluorophenyl)-α-methyl-5-benzoxazoleacetic acid, is a 2-APA NSAID which has been proposed to be marketed and used clinically as the pure *S*-enantiomer. Several stereospecific assays are available to quantify *S*-flunoxaprofen in biological matrices. A method for determination of flunoxaprofen enantiomers derivatized by the method of Björkman [45] has been reported although assay validation in biological fluids was not determined [61] (see Table 6).

3.8. Flurbiprofen

Flurbiprofen, (2-(2-fluoro-4-biphenylyl)-propionic acid, has been stereospecifically chromatographed utilizing a GC method with amphetamine as the derivatizing reagent, however, assay validation was not completed [55]. A pre-column derivatization method with a chiral derivatizing reagent (DAPEA) can also resolve flurbiprofen enantiomers [89].

Furthermore, the utility of both Pirkle columns [86] and cellulose trisphenylcarbamate CSP in separation of a series of derivatized flurbiprofen amides has been reported [14].

The majority of assay methods for flurbiprofen and its metabolites have quantified either total parent drug or total metabolites, however, more recent assays can quantify the enantiomers of flurbiprofen and the enantiomers of its 4′-hydroxyflurbiprofen, 3′-hydroxy-4′-methoxyflurbiprofen and 3′,4-dihydroxyflurbiprofen metabolites [93] (see Table 7). However, this procedure is somewhat lengthy and can not be analyzed in a single isocratic system and requires changes in mobile phase and fluorescent detection for the metabolites.

The ability of bovine serum albumin-silica (Resolvosil®) to separate flurbiprofen has been suggested unfortunately assay validation and sensitivity were not provided [25]. A GC assay capable of resolving flurbiprofen enantiomers [94] is based on a previously reported derivatization procedure [95]. Furthermore, the separation of flurbiprofen enantiomers using an amylose tris(3,5-dimethylphenyl-

Table 6
Stereospecific high-performance liquid chromatographic assays for flunoxaprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
0.05	1	NA	4 m*M* phosphoric acid and 10 m*M* monobasic potassium phosphate in acetonitrile–water (60:40, v/v)	300	25 cm×0.46 cm I.D. LiChrosorb RP-18 (10 μm)	Benoxaprofen	Serum, urine	[90]
0/5	1	*S*(−)-1-Phenylethylamine with 1,1′-carbonyldi-imidazole	Acetonitrile–water (52.5:47.5, v/v)	300	125 mm×4.6 mm I.D. reversed-phase ODS	Benzyl cinnamate	Plasma, urine	[91]

Table 7
Stereospecific chromatographic assays for flurbiprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
0.05	1.0	1,1′-Carbonyldiimidazole, (*S*)-(−)-1-phenylethylamine	Isopropanol–cyclohexane (7:93)	245	LiChrosorb SI 60 (10 μm) (250×6 mm O.D. 3 mm I.D.)	*S*-Suprofen	Rat liver	[66]
0.1, 0.25	0.5	ECF/L-leucinamide	0.067 *M* KH_2PO_4–triethylamine	250	5 μm Partisil 5 ODS-3 10 cm stainless steel 4.6 mm I.D. octadenyl bonded silica	Ketoprofen	Plasma/ urine	[60]
50	0.1	Thionyl chloride, *S*(−)-1-phenylethylamine	0.05 *M* sodium phosphate (pH=7.2)–acetonitrile–tetrahydrofuran (55:25:20, v/v)	Ex 275 Em 315	IBM C_8 Octyl column	2-(2-fluoro-4-buphenyl) acetic acid	Plasma/ urine	[92]
0.1, 0.25	0.5	1,1′-Carbonyldiimidazole, *S*-(α)-methylbenzylamine	NR	245	Beckman Ultrasphere 5 ODS column (25 cm×4.6 mm I.D.)	Ibuprofen	Plasma/ urine	[93]
0.05	0.5	NA	5% 2-propanol and 1 m*M* dimethyloctylamine (v/v) in 20 m*M* phosphate buffer (pH=6.5)	246	α_1-acid glycoprotein column (100 mm×4.0 mm I.D. 5 μm)	*S*-Naproxen	Plasma	[96]
NR	0.1	1-Methyl-3-nitro-1-nitrosuguanidine, diazomethane	Hexane–2-propanol (90:10)	254	Chiralcel OJ (25×4.6 mm I.D.)	NR	Urine	[97]

carbamate)(ADMPC) stationary phase [23], an immobilized human serum albumin based HPLC CSP has been described with or without organic and inorganic additives [28,36], the use of ergot alkaloid-based stationary phase microbore columns [18]; a CSP containing egg yolk RFBP [33]; a polysiloxane-based CSP [26], an experimental tris(4-methylbenzoate) cellulose phase (Bio-Rad RSL) [23,24] and stereospecific analysis utilizing HPLC with amide and urea derivatives bonded to silica gel has been described, however, application to pharmacokinetic studies has not been demonstrated [17].

Lastly, the utility of SFC on a CSP for stereospecific analysis may provide a powerful addition to the family of resolution techniques [98,99], and the arsenal of resolution techniques for flurbiprofen now also includes cyclodextrin-modified CE [50].

3.9. Ibuprofen

Ibuprofen, (±)2-(4-isobutylphenyl)-propionic acid, was initially resolved by formation of diastereoisomers with (−)-1-phenylethylamine and the urinary metabolites were determined to be predominantly of the *S*-configuration [100]. Additionally, stereospecific analysis of α-methylbenzyl-amides derivatives, formed by means of 1,1′-carbonyldiimidazole and L-α-methylbenzylamine has been reported but requires laborious sample preparation and lacks suitable sensitivity for pharmacokinetic studies [96]. This method which uses tedious TLC work up steps was subsequently extended to determine the enantiomeric composition of the two major oxidative metabolites [101].

A pre-column derivatization methods with a chiral agent L-1-(4-dimethylamino-1-naphthyl)ethylamine (DANE) or (DAPEA) separated on a HPLC system with fluorescence detection can stereospecifically quantify ibuprofen [89,102]. Another chromatographic approach is to esterify ibuprofen by reacting the drug with (*S*)-2-octanol at 150°C for 1 h. The diastereoisomers were then separated by normal-phase HPLC on two columns of 5 μm silica, connected in series. However, significant interference from contaminating peaks limited sensitivity to 0.5 mg/l [52].

The ability to separate the enantiomers of ibuprofen using 1,1′-carbonyldiimidazole as the coupling agent and (*S*)-(−)-1-phenylethylamine to form 1-phenylethylamide diasteroisomers by HPLC without assay validation has also been reported [66].

The application of HPLC CSP using (*R*)-N-(3,5-DNB)phenylglycine or Pirkle columns to the separation of ibuprofen has been demonstrated [11,86]. Furthermore, ibuprofen enantiomers were resolved in equine urine on a CSP after chiral derivatization with benzylamine [12]. Optical resolution of the amide derivatives of ibuprofen using reverse and normal-phase HPLC on a N-(*R*)-1-(α-naphthyl)-ethylamine carbonyl-(*S*)-valine methylester CSP have been reported [103]. Direct separation of ibuprofen by HPLC with amide and urea derivatives bonded to silica gel has also been described [17].

A novel assay method utilized to study in vitro plasma protein binding of ibuprofen enantiomers using methane chemical ionization GC–MS monitoring fragment ions has been demonstrated [104]. The use of a GC assay using amphetamine as the derivatizing reagent has also been reported [55]. In addition, the GC–MS assay for the enantiomers of ibuprofen in plasma (bound and unbound) and its metabolites in urine has been reported [105]. This procedure was capable of detecting the hydroxy-metabolites and 3 of the 4 carboxy metabolites.

Furthermore, the ability of direct liquid chromatographic resolution using a chiral α_1-acid glycoprotein column has been demonstrated with or without organic and inorganic additives although assay validation has not been provided [31,34–36,93]. Application of ovomucoid, egg yolk RFBP, and avidin-conjugated columns for the optical resolution of ibuprofen have also been demonstrated [29,33,37,38].

A rapid derivatization technique utilizing ECF and (*S*)-(−)-1-(1-napthen1-1-yl)ethylamine (NEA) in plasma has been validated [63]. The antipode *R*-NEA resulted in appearance of interfering peaks that co-eluted with ibuprofen when used for derivatization. This method which employs a commercially unavailable internal standard may also result in the co-extraction of numerous endogenous compounds when columns with less than the number of theoretical plates are used. HPLC separation utilizing *S*-(−)-NEA to form diasteroisomeric amides has been accomplished [80,87]. The ability to derivatize ibuprofen using L-leucinamide after activation with

either ECF or with 1,1′-carbonyldiimidazole is possible but provides poor chromatographic resolution [81].

The separation of five aromatic amide derivatives of ibuprofen and the metabolites of ibuprofen after derivatization with ECF coupled to 4-methoxyaniline has been accomplished on a Pirkle column [13,107]. The utility of both Pirkle columns and cellulose trisphenylcarbamate CSP in separation of a series of derivatized ibuprofen amides has been demonstrated by other investigators [14]. The use of ergot alkaloid-based stationary phase microbore columns to the separation of ibuprofen enantiomers has also been described [18].

The use of the CSP β-cyclodextrin for the separation of ibuprofen enantiomers in various biological matrices has been shown [20] (see Table 8). The ability of bovine serum albumin-silica (Resolvosil®) to separate ibuprofen has been suggested but unfortunately assay validation and sensitivity were not provided [27]. More recently, resolution of ibuprofen on an immobilized human serum albumin based HPLC CSP has been described [28].

Ibuprofen has also been resolved as the anilide derivative on cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD) column but it could not be resolved without prior derivatization [22]. Separation of ibuprofen has also accomplished on an experimental tris(4-methylbenzoate) cellulose phase (Bio-Rad RSL) requiring prior derivatization to benzylamide derivatives [23,24]. The utility of a cellulose derivative bearing simultaneously 3,5-dimethylphenylamino carbonyl and 10-undecenoyl groups for ibuprofen resolution has been reported [25].

An assay capable of simultaneously quantifying the stereoisomers of the two major metabolites of ibuprofen: hydroxyibuprofen and carboxyibuprofen has also been developed [105]. More recently, resolution of derivatized ibuprofen 3,5-DNB amides on JTB-X columns have been accomplished [16]. With a simple extraction of unresolved drug from acidified plasma and subsequent normal-phase HPLC separation of the diastereomeric *S*-1-phenylethylamides are resolved within 5 min although this assay has not been validated for ibuprofen [87].

An improved assay based on Mehvar et al. [65] using fluorescence detection has been described [112]. However, a late eluting peak necessitated run times of over 60 min under isocratic conditions, therefore, a 6 min flush with 100% acetonitrile every third sample and 9 min equilibration was employed [113].

The use of both a CSP method and a modification of the indirect method of Mehvar et al. [65] has been reported [67]. EEDQ is demonstrated as an alternate coupling reagent however it requires long derivatization time and heating. In the direct method ibuprofen enantiomers were derivatized to *p*-nitrobenzyl ureides and then resolved on a (*R*)-(−)-(1-napthyl)-ethylurea CSP column. The sensitivity of this assay is not superior to other methods. Nor is the method of reacting ibuprofen with (−)-2-[4-(1-aminoethyl)phenyl]-6-methoxy benzoxazole [(−)-APMB] in the presence of 2,2′-dipyridyldisulfide (DPDS) and triphenylphosphine (TPP) at room temperature [101].

More recently, the utility of packed column SFC with a CSP has been suggested which may provide future possibilities for the analysis of chiral NSAIDs [98,114]. Several linear oligosaccharides have been used as potential chiral discriminators in CE to perform direct, rapid separations of ibuprofen enantiomers [46,47,49]. In addition, a polysiloxane-based CSP has demonstrated stereospecific resolution of ibuprofen enantiomers [26].

3.10. Indoprofen

Indoprofen, (2-[4-(2-isoindolinyl-1-one)-phenyl]-propionic acid, is no longer used clinically but was initially chromatographed using a gas liquid method [117]. A highly fluorescent chiral reagent DANE was capable of separation of indoprofen enantiomers although a derivatization time of 3 h was required [102]. In addition, the use of high-performance TLC to resolve the enantiomers of indoprofen by means of their *R*-(+)-1-phenylethylamides has been validated [118].

Recently, resolution of indoprofen on immobilized HSA with or without organic and inorganic additives and egg yolk RFBP CSPs have been reported [28,33,36]. Furthermore, Pirkle-concept CSPs also appear to be effective in the stereospecific resolution of indoprofen [119], and the use of cyclodextrin-modified CE can also resolves indoprofen enantio-

Table 8
Stereospecific chromatographic assays for ibuprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
NR	NR	Thionyl chloride, *R*(+)-α-methylbenzylamine	Nitrogen	NA	U-shaped (5 m×3 mm I.D.) of 1% OV-17	NR	Urine	[4]
1.0	1.0	Thionyl chloride, 1,1′-carbonyldiimidazole, *S*(−)-α-methylbenzylamine	Helium, hydrogen, oxygen	NA	U-shaped (1.5 m×3 mm I.D.) of 3% OV-17	*n*-Tidecanoic acid	Plasma, urine	[95]
0.5	1	*S*(+)-2-octanol	0.05% isopropyl alcohol in heptane	220	Two 5 μm silica (25×0.46 cm)	4-*n*-Pentyl, phenyl, acetic acid	Plasma, synovial fluid	[52]
10	9.0	Thionyl chloride, benzylamine	Hexane–isopropanol–acetonitrile (95:5:1)	254	DNPG 5 μm (4.6 mm×25 cm) and MS	NR	Equine urine	[12]
0.05	1.0	*S*(+)-2-Octanol	NR	NA	GCMS	D14 ibuprofen	Plasma	[103]
0.75	1.0	1,1′-Carbonyldiimidazole, *S*-(+)-amphetamine	Helium, hydrogen, air	NA	Fused-silica capillary (12 m×0.2 m.m. I.D.)	*p*-Methoxy, phenyl, acetic acid	Plasma, urine	[53]
0.1	NR	1,1′-Carbonyldiimidazole, *S*-(−)pheylethylamine	NR	NA	DB-5 capillary column (30 m×0.25 mm)	2-Chloro-biphenyl-propionic acid	Plasma, urine	[105]
1.0	0.5	1-Hydroxybenzotriazole 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride, (*S*)-(−)-1-(1-naphtthyl)-ethylamine (NEA)	Hexane–ethyl-acetate (4:1)	254	Hypersil 10 μm (250×4.5 mm I.D.)	*p*-Chloro-phenoxy-acetic acid	Plasma	[106]
0.1	0.5	Ethylchloroformate, (*S*)-(−)-1-(1-NEA)	Acetonitrile–water–acetic acid–triethyl amine (55:45:0.1:0.02, v/v) pH 4.9	232	Partisil 5-ODS-3 (4.6 mm×10 cm)	(±)-2-(4-Benzoyl-phenyl)-butyric acid	Plasma	[65]
6.25	1.0	Methylene chloride, ECF, 4-methoxyaniline	Hexane–chloroform–2-propanol (18:2:1)	254	Pirkle covalent column (25 cm×4.6 mm I.D.) with α-aminopropyl packing of 5 μm spherical particles with (*R*)-N-(3,5-dinitro benzoyl)phenyl-glycine	α-Phenyl-propionic acid	Plasma, urine	[107]
0.1	0.5, 0.1, 0.1	NA	Acetonitrile–0.1% triethyl ammonium acetate buffer (30:70, v/v) pH=7.5	220	Cyclobond I (250 mm×4.5 mm I.D)	NR	Plasma, urine, dog bile	[20]
0.1, 1.0	0.2	Thionyl chloride, *S*(−)-1-phenylethylamine	Isopropanol–heptane (2.5:97.5)	216	Hibar Lichrosorb 5- Si60 (250 mm×4 mm I.D.)	NR	Total plasma, unbound plasma	[108]

0.1	0.5	NA	0.5% 2-propanol 5 m*M* DMOA in 20 m*M* phosphate buffer (pH=6.7)	220	Enantiopac (100mm×4.0 mm I.D.)	*S*-Ketoprofen	Plasma	[35]
1	1.0	NA	1,2% v/v 2-propanol 1.2 m*M* N,N-dimethyloctylamine (DMOA) in 0.02 *M* sodium dihydrogenphosphate	227	Chiral-AGP (100 mm×4 mm I.D.)	4-Pentyl-phenyl-acetic acid	Plasma	[109]
NR	NR	Thionyl chloride (1*R*,2*S*,5*R*)-(−)-menthol	Hexane–diethyl ether (100:1)	354	2 Whatman Partisil columns 10 μm (2×4.6 mm I.D.×25 cm)	NR	Plasma	[110]
0.25	Rat, 0.25, 0.5	ECF, *R*-(+)-α-phenylethylamine	Acetonitrile–water–acetic acid–tetraethyl amine (46.5: 53.5:0.1:0.03, v/v) pH=4.9	225	Phenomenex C_{18} 5-ODS (10 cm×4.6 mm I.D.)	Fenoprofen	Rat/human plasma	[111]
0.1	0.5	ECF, (*S*)-(−)-1-(1-naphthyl) ethylamine (NEA)acetonitrile	Water–acetic acid–triethylamine (60:40:0.1:0.02, v/v) pH 5.0	Ex 280 Em 320	Partisil 5-ODS-3 RAC II (4.6 mm I.D.×10 cm)	Fenoprofen	Plasma	[112]
NR	0.5	EEDQ or ECF, *S*-NEA	Acetonitrile–water–acetic acid (55:45:0.1:0.02, v/v)	232	Ultrasphere 5-ODS (100×4.6 mm I.D.)	NR	Plasma	[69]
2.5	0.5	EEDQ, *p*-nitrobenzylamine hydrochloride (PNBA)	Hexane–isopropanol (35:5, v/v)	235	CSP (*R*)-(−)-(1-napthyl)ethylurea (100×4.6 mm I.D. 3 μm, aminopropyl silanized silica)	Tidecanoid acid	Dog plasma	[69]
0.4	NR	(−)-APMB, DPDS, TPP	NR	Ex 320 Em 380	Reversed-phase column	NR	Rat plasma	[114]
0.003	0.2	ECF, dexamphetamine	Helium	NA	25 m×0.32 m I.D.	Naproxen	Plasma, synovial fluid	[115]
0.005	0.1	*R*(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol oxalyl chloride	Helium	BA	2.0 m×2 mm I.D. glass column	4-methoxy phenyl acetic acid	Plasma	[116]

Table 9
Stereospecific chromatographic assays for indoprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
NR	NR	ECF/L-leucinamide	Acetonitrile–10 m*M* phosphate buffer, pH 6.5 (38:62)	275	LiChroCart RP-18 7 μm column (250×4 mm)	Indobufen	Plasma	[45]
NR	NR	NA	Chloroform–ethyl acetate (3:1)	270	HPTLC pre-coated Kieselgel 60 F254 plates	NR	Microsomal supernatant	[118]

mers [47]. See Table 9 for stereospecific chromatographic assays of indoprofen.

3.11. Ketoprofen

Determination of ketoprofen, (±)-α-(benzoylphenyl)propionic acid, enantiomers was first accomplished by GC through carbodiimide mediated conversion of 1-phenylethylamides into diastereoisomers [120]. The ability of direct liquid chromatographic resolution using a chiral α_1-acid glycoprotein column with or without organic and inorganic additives has been suggested although assay validation was not provided [31,34,36]. Several types of CSPs have been shown to separately quantify ketoprofen enantiomers include brush-type CSPs [86], and the use of ergot alkaloid-based stationary phase microbore columns [18]. A stereospecific GC assay capable of separating the enantiomers of several NSAIDs including ketoprofen has been described. However, this assay gives no account of precision or sensitivity and requires excessively long sample preparation times [50]. The use of high-performance TLC to resolve the enantiomers of ketoprofen by means of their *R*-(+)-1-phenylethylamides has also been verified [118]. Reversed-phase HPLC isolation and quantification of racemic ketoprofen utilizing a protracted sample clean-up followed by a enantiospecific normal-phase HPLC assay of thionyl chloride mediated derivatized *S*-1-phenylethylamides diastereoisomers of ketoprofen has been described in the literature [76]. A rapid derivatization technique utilizing ECF and (*S*)-(−)-1-(1-naphthyl)ethylamine in plasma is capable of separating ketoprofen enantiomers [65], and a GC assay [94] based on a previously described derivatization procedure [95] is also capable of resolving ketoprofen enantiomers (see Table 10).

An HPLC assay for the enantiomers of indoprofen involved extraction of unchanged drug, conversion to a mixed anhydride with ECF, derivatization with L-leucinamide, extraction of the formed diastereoisomers with an organic solvent, evaporation and injection into the HPLC system [43]. There was a presence of interfering peaks when applying this method to resolution of ketoprofen enantiomers, therefore, the assay was substantially modified [58,121]. Two assays for ketoprofen based on this previous method of analysis used for indoprofen were simultaneously developed. One assay utilized an ion paired system to extract the ketoprofen enantiomers from plasma but involves lengthier sample processing and is less sensitive than the previously reported assay [122]. The other assay is also capable of analysis of the urinary excretion of conjugated ketoprofen enantiomers [58].

The ability of BSA-silica (Resolvosil) to stereospecifically assay ketoprofen has been described unfortunately assay validation and sensitivity were not provided [27], and an immobilized HSA based HPLC CSP [28], an amylose tris(3,5-dimethylphenylcarbamate) (ADMPC) stationary phase [22], an α_1-AGP column [35], an experimental tris(4-methylbenzoate) cellulose phase (Bio-Rad RSL) requiring prior derivatization to benzylamide derivatives [23,24], direct separation of ketoprofen by HPLC with amide and urea derivatives bonded to silica gel [17] and several linear oligosaccharides have also been used as potential chiral discriminators in CE to perform direct, rapid separations of ketoprofen enantiomers [44,45].

Table 10
Stereospecific chromatographic assays for ketoprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
NR	NR	Thionyl chloride, (−)-α-methylbenzylamine	Isooctane–methylene chloride–methanol (55:44.8:0.2)	308	Hypersil APS (12.5 cm×5 mm I.D.)	NR	NR	[72]
0.2	0.2	$SOCl_2$, *R*-2-phenylethylamine	Acetonitrile–dichloro methane (5:95)	254	5 μm LiChrosorb Si-60 (250 mm×4 mm I.D.)	Naproxen	Plasma	[76]
0.25	0.5	ECF, L-leucinamide	Acetonitrile–10 m*M* phosphate buffer, pH 6.5 (38:62)	260	LiChroChart RP-18 7 μM column (250×4 mm I.D)	2-(4-benzoyl phenyl) butyric acid	Plasma	[111,21]
0.05, 0.5	0.5	ECF, L-leucinamide	0.06 *M* KH_2PO_4–acetonitrile–tetraethylamine (64:36:0.02)	275	Partisil 5 ODS-3 10 cm reversed-phase column	Fenoprofen	Urine/ plasma	[59]
0.1	1.0	NA	0.5% 2-propanol 5 m*M* DMOA in 20 m*M* phosphate buffer (pH=6.7)	260	Enantiopac (100 mm×4.0 mm I.D.)	*R*-Ibuprofen	Plasma	[35]
0.15	1.0	*S*-1-phenylethylamine in dicloromethane	Isopropyl alcohol–*n*-heptane (8:92, v/v)	254	SGE glass lined column (250 mm×4 mm I.D.)	*S*-Naproxen	Plasma	[87]
0.5	0.1	ECF, L-leucinamide	0.06 *M* KH_2PO_4–acetonitrile–tetraethylamine (64:36:0.1)	275	Partisil 5 ODS-3 (10 cm×4.6 mm I.D.)	Indoprofen	Rat plasma/ urine	[82]
0.002	0.2	ECF, dexamphetamine	Helium		25 m×0.32 m I.D.	Methyl-sterate	Plasma, synovial fluid	[115]
0.01	1.0	NA	M1 (acetonitrile–water–perchloric acid) 30:70:0.1, v/v M2 (1 m*M* KH_2PO_4 buffer pH=4.2) M3 (THF–water) (7:93, v/v containing 20 m*M* KH_2PO_4)	260	C1 (150×4.6 mm I.D. Inertsil ODS-2 column) C2 (10×4.0 mm I.D. Ultron ES-OVMG) C3 (150×4.6 mm I.D. Ultron ES-OVM)	Ethyl-benzoate	Plasma	[123]
0.01	1.0	NA	Acetonitrile–10 m*M* tetrabutyl ammonium bromide in 1 m*M* KH_2PO_4 (pH=4.3)		Superspher 100 RP 18 4 μm (125×4 mm I.D.)	NR	Liver	[124]
0.05	0.5	NA	Hexane–isopropyl alcohol–trifluoroacetic acid (80:19:9:0.1, v/v/v)	254	Chiralpak AD (250×4.6 mm)	Fenoprofen	Urine/ plasma	[124]

3.12. Loxoprofen

Loxoprofen, 2-[4-(2-oxycyclopentylmethyl)-phenyl]propionate, has two chiral centres in the parent molecule and a prochiral carbonyl group that is metabolized in vivo to an alcohol metabolite which contains three chiral centres. Therefore, eight optically active enantiomers may be present in biological samples [1–3].

A TLC method was first described for the isolation of the parent acid and its *cis* and *trans*-OH metabolites in rat plasma [125]. Both the enantiomers of the parent acid and the major metabolites were determined in plasma of rats using the chiral reagent DANE [126] (see Table 11). This is a highly fluorescent chiral reagent initially used for determining enantiomers of naproxen [104]. Subsequently, a stereospecific assay capable of quantifying the carboxylic acid enantiomers and the two monohydroxy metabolites, *trans* and *cis*-alcohols in urine by column liquid chromatography has been described [1]. Loxoprofen and its metabolites were converted to amides by condensation with DANE and separated on normal-phase column. However, this method was not capable of separating the enantiomers of the cyclopentanol moiety. An assay capable of detecting loxoprofen and its diastereomeric alcohol metabolites in plasma and urine by fluorescence labelling with 4-bromomethyl-6,7-methylenedioxycoumarin has been described [2]. The application of antibody-mediated extraction for the stereospecific determination of the active metabolite in human urine and plasma and quantification of the four isomers of loxoprofen carboxylic acid and enantiomers of the *cis* and *trans* alcohol has been validated [3]. An optical resolution of the carbamate-alkylester derivative of the *trans*-alcohol metabolite of loxoprofen and an analogous compound CS-670 was obtained on a N-(*R*)-1-(α-naphthyl)-ethylamine carbonyl-(*S*)-valine methylester CSP. The propylester derivative exhibited nearly perfect resolution, however, validation in biological matrices has not been provided [103].

3.13. Miroprofen

Miroprofen, 4-imidazo[1,2-a]pyridine-2-yl-α-methylbenzeneacetic acid, is no longer used clinically and no stereospecific assays have been published in the literature.

3.14. Naproxen

Although naproxen, 2-(6-methoxynaphth-2-yl)propionic acid, is marketed as the pure *S*-(+)-enantiomer there are stereospecific assay methods published. Naproxen was first determined by HPLC after acid-catalyzed esterification with (*S*)-(+)-2-octanol to form diastereomeric esters [51] (see Table 12). Pre-column derivatization methods with a chiral agent L-1-(4-dimethylamino-1-naphthyl)ethylamine (DANE) or (DAPEA) to from diasteroisomers separated on a HPLC system with fluorescence detection has been described [89,102,127]. Two highly sensitive, chiral derivatization reagents, 1-(1-anthryl)- and 1-(2-anthryl)ethylamines, have been developed. Condensation of naproxen with these reagents in the presence of carbodiimide formed diastereomeric amides resolved by normal-phase HPLC [128]. A report suggests the ability of a GC assay utilizing amphetamine as the derivatizing reagent is possible [56]. The suitability of ECF/L-leucinamide to separate naproxen was also reported [61].

Several papers dealing with resolution of naproxen

Table 11
Stereospecific chromatographic assays for loxoprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
0.05	1.0	DANE, 1-hydroxy benzotriazole N,N'-dicyclohexyl carbodiimide (DCC)	*n*-Hexane–ethyl acetate (68:32)	Ex 313 Em 420	10 μm Porasil (30 cm×3.9 mm I.D)	NR	Plasma, urine	[1]
0.05	0.2	N,N'-Dicyclohexyl carbodiimide 1-hydroxy benzotriazole, (+)-(*R*)-1-(1-napthyl)ethylamine	*n*-Hexane–ethyl acetate (59:41)	Ex 283 Em 330	ERC-Silica 1282 column (6×250 mm)	3,3',5,5' Tetramethyldine	Plasma	[3]

Table 12
Stereospecific high-performance Liquid Chromatographic Assays for Naproxen.

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
NR	NR	(*S*)-(+)-2-Octanol	Ethylacetate in hexane	332	(25 cm×3 mm I.D.)	NR	NR	[46]
0.01	0.1	1-Ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (WSC), 1-hydroxy benzotriazole, L-1-(4-dimethylamino-1-naphthyl)ethylamine (DANE)	*n*-Hexane–tetrahydrofuran (80:26)	Ex 320 Em 410	μ Porasil column (1 ft×1/4 in. I.D.) Sep-Pak C_{18}	3,5-*tert.*-Butyl-4-hydroxy benz aldehyde O-(methoxycarbonyl methyl) oxime	Rabbit plasma	[7]
0.05	1	1,1′-Carbonyldiimidazole, (*S*)-(−)-1-phenylethylamine	Isopropanol–cyclohexane (7:93)	230	LiChrosorb SI 60 (10 μm) (250×6 mm O.D. 3 mm I.D.)	*R*-Ibuprofen	Rat liver	[67]
0.05	1.0	NA	25 m*M* potassium phosphate buffer (pH=7.0), 0.5% propan-2-ol and 1.5 m*M* DMOA	Ex 330 Em 355	Chiral AGP (100 mm×4 mm I.D.)	NR	Rat urine	[129]

Table 13
Stereospecific high-performance liquid chromatographic assays for pirprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
0.25	0.5	1,1′Carbonyldiimidazole/ *R*(+)-1-methylbenzylamide	*n*-Hexane–dichloromethane (64:36, v/v)	272	CSP (*R*)-N-(3,5-dinitro benzoyl) phenyl glycine (DNBPG)	$C_{14}H_{14}ClNO_2$ butyric acid analogue of the pyrrole derivative	Plasma	[130]
0.1	0.5, 0.1	ECF, L-leucinamide	0.06 *M* KH_2PO_4–acetonitrile–tetraethylamine (64:36:0.1; pH 6.8)	275	Partisil ODS 3.5 μm, Phenomenenex 100 mm×4.6 mm I.D.	Racemic ketoprofen	Rat urine/ plasma	[131]

enantiomers with the chiral additive quinine in the mobile phase have been outlined [41–44]. The application of HPLC CSP using (*R*)-N-(3,5-DNB)phenylglycine or Pirkle columns to the separation of naproxen [11], direct separation of naproxen by HPLC with amide and urea derivatives bonded to silica gel [17], the use of ergot alkaloid-based stationary phase microbore columns [18], an α_1 AGP column [34], BSA-silica (Resolvosil) [27], derivatized naproxen 3,5-dinitrobenzoylamides on JTB-X columns [16], brush-type CSPs [105], a cellulose derivative bearing simultaneously 3,5-dimethylphenylamino carbonyl and 10-undecenoyl groups [26]; direct liquid chromatographic resolution using a chiral α_1-acid glycoprotein column Enantiopac® [31], resolution of naproxen on an immobilized HSA HPLC CSP [28], a polysiloxane-based CSP [26], CE with hydroxypropyl-β-cyclodextrin as a chiral selector [48] and a validated method of determination of the enantiomers of naproxen and 6-O-desmethylnaproxen on the second-generation Chiral-AGP column have been published [129].

3.15. Pirprofen

Pirprofen, (±)-2-[3-chloro-4-(3-pyrrolinyl)-phenyl] propionic acid, was initially resolved by Spahn [61] (using a method of derivatization of Björkman [45]), although assay validation in biological fluids was not determined. A GC assay utilizing amphetamine as the derivatizing reagent [55], and HPLC separation of utilizing *S*-(−)-NEA to form diasteroisomeric amides has been accomplished [80].

Several types of CSPs have been shown to separately quantify pirprofen enantiomers include brush-type CSPs [86]; an experimental tris(4-methylbenzoate) cellulose phase (Bio-Rad RSL) requiring prior derivatization to a benzylamide derivative [23]; an immobilized HSA based HPLC CSP [28] and a polysiloxane-based CSP, however, assay validation was not provided [26].

Pirprofen can spontaneously oxidize to its pyrrole analogue during sample preparation. In the development of an assay for pirprofen a short sample preparation time is desirable. A rapid and convenient assay of pirprofen enantiomers was also capable of determining the enantiomers of the pyrole metabolite without interference [131]. The enantiomers of pirprofen could also be derivatized and separated using a method previously applied to stereospecific analysis of etodolac enantiomers [132]. Although the resolution factor afforded by that method was greater than the published assay [133], the use of the L-leucinamide derivatizing reagent appeared to provide greater sensitivity (see Table 13).

3.16. Pranoprofen

Pranoprofen, (±)α-methyl-5H-[1]benzopyrano-[2,3-*b*]pyridine-7-acetic acid, is used clinically in Japan. Direct separation of pranoprofen by HPLC with amide and urea derivatives bonded to silica gel has been described [17] (see Table 14). The use of a avidin-bonded silica and a bovine serum albumin-bonded CSP have also been shown to resolve pranoprofen [135,136]. In addition, a pre-column derivatization method with a chiral derivatizing reagent (DAPEA) can resolve pranoprofen enantiomers [89].

3.17. Suprofen

Suprofen, (±)-α-methyl-4-(2-thienylcarbonyl)benzeneacetic acid, is no longer used clinically in some countries. The ability to separate the enantiomers of

Table 14
Stereospecific high-performance liquid chromatographic assays for pranoprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
NR	NR	NA	0.5 m*M* Perchloric acid buffer pH 5.0–acetonitrile–water (60:44:50, v/v)	Ex 300 Em 370	Chiracel OD (4.6×250 mm)	NR	Urine	[133]
0.05	0.5	NA	*n*-Hexane–2-propanol–acetic acid (70:40:1)	Ex 250 Em 330	Chiracel OJ (25 cm×0.46 mm I.D.)	Methyl-p-aminobenzoate	Rabbit plasma, urine	[134]

Table 15
Stereospecific assay of suprofen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
0.4	1.0	1-Hydroxybenzotriazole (HOBT), 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (WSCD), *S*-(−)-1-(naphthyl)ethylamine	Helium		Methylsilicone bonded-phase fused-silica capillary column SPB-1 (10 m×0.25 mm I.D.)	(±)-[$^2H_7$] suprofen	Plasma	[138]

suprofen using 1,1′-carbonyldiimidazole as the coupling agent and (*S*)-(−)-1-phenylethylamine to form 1-phenylethylamide diasteroisomers by HPLC has been described, however, assay validation was not reported [64]. The use of high-performance TLC to resolve the enantiomers of suprofen by means of their *R*-(+)-1-phenylethylamides has been described [118]. A β-cyclodextrin column which can separate the positional isomers of suprofen has been reported, however, this assay was not capable of separating suprofen enantiomers [137].

More recently, suprofen has been resolved directly on CSPs. The use of ergot alkaloid-based stationary phase microbore columns [18], an immobilized human serum albumin based HPLC CSP [28], a CSP containing egg yolk RFBP [33], Pirkle-concept CSPs [119], and small-particle (3 μm) underivatized, aminoproylated and octadecylated spherical silicas from the Hypersil range to support cellulose tris(3,5-dimethylphenyl carbamate) have been shown to resolve suprofen [139] (see Table 15). Furthermore, a preliminary report suggests that modified cyclodextrin-CE can also resolve suprofen enantiomers [47].

3.18. Tiaprofenic acid

Tiaprofenic acid, (±)-(5-benzoyl-2-thienyl) propionic acid, was first assayed by a GC method which was not ideal for pharmacokinetic studies due to lengthy run times and heating during sample preparation [50,140] (see Table 16).

Several types of CSPs have been shown to separately quantify tiaprofenic acid enantiomers include brush-type chiral stationary phases [86], immobilized HSA [139], Chiral-AGP column with inorganic and organic modifiers [36], experimental tris(4-methylbenzoate) cellulose phase (Bio-Rad RSL) requiring prior derivatization to a benzylamide derivative [23,24] and polysaccharide tris(phenylcarbamate) derivatives particularly tris(3,5-dimethylphenyl-carbamate)s of cellulose and amylose [23,143,144].

The method for the simultaneous separation, identification and measurement of the enantiomers of racemic tiaprofenic acid achieved on chiral columns containing the 3,5-dimethylphenylcarbamate of cellulose and amylose has also been shown to suitable for the stereospecific analysis of (*RS*)-5-benzoyl-α-methyl-3-thiopheneacetic acid (3-isomer of tiaprofenic acid), together with the achiral impurities 5-benzoyl-2-acetylthiophene, 5-benzoyl-2-ethylthiophene contained in bulk racemic tiaprofenic acid and pharmaceutical formulations [144]. A more recent report has suggested the direct chiral separation of tiaprofenic acid by cyclodextrin-modified CE although complete assay validation in biological matrices and use in pharmacokinetic studies remains limited [50].

The possibility of racemization during the indirect chromatographic resolution of tiaprofenic acid has been suggested [142,45]. Replacement of triethylamine in the second step of the derivatization procedure with N-methylmorpholine was found to significantly reduce the racemization [145]. Another recent report further suggests that racemization appears to be only partial for both enantiomers (<10%) [143].

3.19. Thioxaprofen

There are no published stereospecific assays available in the literature.

Table 16
Stereospecific chromatographic assays for tiaprofenic acid

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
0.25	1.0	1,1'Carbonyldiimidazole, *S*(+)amphetamine	Helium, hydrogen, air	NA	Fused-silica capillary column (12 m×0.2 mm I.D.)	*S*-Naproxen	Plasma, urine, synovial fluid	[140]
0.2	0.5	2,2,2-Trichloro ECF/L-leucinamide	0.06 *M* monopotassium phosphate–acetonitrile–triethylamine (65:35:0.02) pH=6.0	310	Partisil 5 ODS-3 a 10 cm×4.6 mm	(±)-2-(4-benzoyl phenyl) butyric acid	Plasma/urine	[141]
0.01	0.3	NA	Na dihydrogen phosphate–disodium hydrogen phosphate (0.05 *M*, pH 7)–acetonitrile–*n*-octanoic acid (90:10:0.015, v/v)	305	HSA-CSP	Dansyl-sarcosine	Rat and human plasma	[142]
0.1	0.5	NA	Hexane–isopropanol–triflouroacetic acid (90:10:0.1)	310	Chiral Pack AD	*S*-Naproxen	Human plasma	[143]

Table 17
Stereospecific chromatographic assay of clindanac

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
0.5	1.0	2,2-Dichloro ethanol borontrifluoride ethyl ether	N_2	NA	0.5% OV-17 on Chromosorb G in 2.5 mm I.D.×2 m glass tube	Chlorendic acid dicyclohexyl ester	Rat plasma, liver homogenate	[146]
0.25	1.0	1-Ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride, *R*(+)-α-methylbenzylamide	Benzene–acetonitrile (100:13, v/v)	280	TLC plates (20×20 cm) coated with a 0.25 mm	NR	Guinea pig plasma	[147]

3.20. Ximoprofen

Ximoprofen, (±)-2-(4-[3-oxminocyclohexyl]-phenyl)propionic acid, is currently under development, and no reported stereospecific assays are currently available in the literature.

4. Arylalkanoic acids

4.1. Butibufen

Butibufen, α-ethyl-4-(2-methylpropyl)benzeneacetic acid, is currently under development and no stereospecific assays have been published.

4.2. Clindanac

Clindanac, (±)-6-chloro-5-cyclohexyl-1-indancarboxylic acid, has been directly separated by HPLC with amide and urea derivatives bonded to silica gel [17] (see Table 17).

4.3. Etodolac

Etodolac, ((±)-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-acetic acid), was initially stereospecifically chromatographed with utilizing a GC method which employed *S*-(+)-amphetamine to form diastereomers of the drug in the presence of 1,1′-carbonyldiimidazole as the coupling reagent, and which required long incubation times [55,148]. Unfortunately, the extraction procedure required an alkalinisation step to remove endogenous amines from the sample, before acidification and extraction of the etodolac enantiomers could be undertaken. This step hydrolyzed the acyl-glucuronide conjugates of etodolac, and hence overestimated the concentration of unchanged drug.

An indirect HPLC method which is rapid, sensitive and suitable for measurement of etodolac enantiomers and their ester conjugates in plasma and urine was subsequently developed [132] (see Table 18). The previous assay methods for etodolac and its metabolites have quantified either total parent drug or total metabolites, however, a recent assay can also quantify the enantiomers of etodolac and the enantiomers of its phase 1 metabolites 6-hydroxyetodolac, 7-hydroxyetodolac and 8-(1′-hydroxy-ethyl)etodolac in human urine, including the ester conjugates [149]. In addition, a polysiloxane-based CSP has been reported to resolve etodolac enantiomers [26].

4.4. Flobufen

Flobufen, (±)-4-(2′,4′-difluorobiphenyl-4-yl)-4-oxo-2-methylbutanoic acid, is currently under development and no stereospecific assays have been published.

4.5. Indobufen

Indobufen, ((±)2-[4-(1,3-dihydro-1-oxo-2H-isoindol-2-yl)phenyl]propionic acid, has been analyzed by the same method as indoprofen (see Table 19). The method of derivatization with ECF and L-leucinamide has been successful for chiral resolution, however, this method has not been validated for indobufen [45].

4.6. Ketorolac

Ketorolac, ((±)-5-benzoyl-1,2-dihydro-3H-pyrrolo[1,2-*a*]pyrrole-1-carboxylic acid. The earliest report of ketorolac resolution utilized the formation of ester diastereomer esters with (−)-α-phenethyl alcohol followed by separation with normal-phase HPLC [153]. However, partial racemization was evident during transesterification and assay validation in biological matrices was not provided.

A subsequent report describes the development and application of an indirect HPLC method for quantification of ketorolac enantiomers [154]. This method involved the derivatization of ketorolac with ECF/L-leucinamide and subsequent reverse phase chromatography of the diastereomers. This method has the disadvantage of a long chromatography run (up to 35 min per sample). Moreover, this method has been demonstrated to undergo complete racemization [63].

An enantiospecific assay utilizing a coupled achiral–chiral HPLC system with an achiral C_{18} stationary phase and a HSA CSP has been validated and is also capable of detection of the enantiomers of the

Table 18
Stereospecific high-performance liquid chromatographic assays for etodolac

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
0.5	0.5	1,1'-Carbonyldiimidazole, *S*-(+)-amphetamine	Helium (carrier), hydrogen/air	NA	Fused-silica capillary column (12 m×0.2 mm I.D.)	*S*-(+)-Naproxen	Plasma, urine	[148]
0.2	0.5	ECF/D,L(−)-αphenylethylamine	Hexane–ethylacetate–isopropanol (85:15:0.2)	280	Partisil 5 Silica 4.6 mm×25 cm normal-phase column	(±)-2-(4-benzoyl phenyl) Butyric acid	Plasma	[132]
NR	NR	NA	0.05 *M* Phosphate buffer (pH 4.0)–2-propanol (98:2, v/v)	230	BSA-FA 125×4 mm I.D.	Suprofen	Urine	[149]
NR	NR	NA	2-Propanol–*n*-Hexane (90:10), trifluoroacetic acid or 2-propanol–*n*-hexane (95:5), trifluoroacetic acid	230	Chiracel OD (25 mm×4.6 mm I.D.) Pirkle (R) DNBPG	1,3,5-tri-*tert*.-Butylbenzene	Plasma	[150]

Table 19
Stereospecific high-performance liquid chromatographic assays for indobufen

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
0.1, 1.0	0.1–0.2, 0.2	ECF, L-leucinamide	Acetonitrile–10 m*M* phosphate buffer. pH 6.5 (38:62)	275	NR	*S*-Indoprofen	Rat/mouse/human plasma, urine	[151,152]

p-hydroxyketorolac metabolite [156] (see Table 20). In addition the use of a Chiral Pak AD column has also been reported to separately quantify ketorolac enantiomers [63].

4.7. Metbufen

There are no stereospecific assays available for analysis of Metbufen reported in the literature.

4.8. Sulindac

Sulindac, (Z)-5-Fluoro-2-methyl-1-[[4-(methylsulfinyl)phenyl]methylene]-1H-indene-3-acetic acid. Currently, there are no published assays available for determination of the sulfoxide enantiomers. However, it is possible to separate these enantiomers using a Chiracel AD column (Davies et al., unpublished observations).

5. Non acidic agents

5.1. Azapropazone

Currently there are no stereospecific assays published for this NSAID.

5.2. Bumadizone

Bumadizone, α-carboxycaproyl-N,N′-diphenylhydrazine, is currently under development and no stereospecific assays are reported in the literature.

5.3. Oxyphenbutazone

Oxyphenbutazone, [4-butyl-1-(*p*-hydroxyphenyl)-2-phenyl-3,5-pyrazolidinedione. No stereospecific assays have been published in the literature.

5.4. Talnifumate

Talnifumate, 2-[[3-(trifluoromethyl)phenyl]-amino]-3-pyridine carboxylic acid. No reported stereospecific assays are published in the literature.

6. Conclusions

Since the first reported stereospecific aasay methods became available in the early 1970s, a plethora of methods and techniques have been developed for stereospecific analysis of chiral NSAIDs. The indirect stereospecific chromatographic approach predominates in the literature in terms of published and validated assays. Advances in the derivatization process are still possible and an increase in the commercial availability of suitable derivatizing reagents has occurred in recent years. Direct methods are still relatively new and have more limited experience in pharmacokinetic studies but their potential is certainly evident and is becoming increasingly employed. Both of these methods have respective strengths and weaknesses. There still remains a lack of stereospecifc assays published in the literature for several chiral NSAIDs. With the increased use of CE, SFC, GC–MS and HPLC–MS–MS further advances in stereospecific analysis methods and their applications to chiral NSAIDs chromatography can be anticipated.

Table 20
Stereospecific high-performance liquid chromatographic assays for ketorolac

MQ (mg/l)	V (ml)	D R	M P	λ (nm)	Column	I.S.	Specimen	Ref.
0.136	0.1	ECF/D-leucinamide	Acetonitrile–0.067 *M* KH_2PO_4–triethylamine (30:70:0.02, v/v)	280	Partisil 5 ODS-3 10 cm×4.6 mm	Racemic ketoprofen	Rat plasma, urine	[154]
0.05	1.0	Thionyl chloride/(*S*)-1-phenylethylamine	50% acetonitrile and 0.1% triethylamine in 20 m*M* sodium acetate buffer (pH 5.5)	310	Nova-Pak 4 μm, 100×8 mm I.D.	(*S*)-Ketoprofen	Plasma	[10]
0.035	0.5	NA	4% propan-2-ol in 0.1 *M* NaH_2PO_4 pH 5.5	325	Chiral AGP-CSP (100×4 mm I.D. column)	Naproxen	Plasma	[155]
0.01	1	NA	Sodium dihydrogen phosphate–disodium hydrogen phosphate (0.05 *M*, pH 6.9) modified with 13% (v/v) acetonitrile 0.1 m*M* octanoic acid	313	Merck LiChrosopher 100 RP-18 (125×4 mm I.D.), HSA-CSP (150×4.6 mm I.D.)	Naproxen	Plasma, urine	[156]
0.05	0.5	NA	2-Propanol-20 m*M* potassium dihydrogenphosphate (0.5:99.5, v/v)	320	Chiral AGP (100×4.0 mm I.D.)		Plasma	[157]
0.025	0.5	NA	0.5 m*M* ammonium acetate–methanol ethylacetate–isopropanol (50:50:2, v/v)	313	Partisil 5 ODS 3 followed by 5 cm tirt-leucine	Naproxen	Plasma/urine	[63]

7. List of abbreviations

AGP	α_1-Acid glycoprotein
BSA	Bovine serum albumin
CE	Capillary electrophoresis
CSP	Chiral stationary phase
DANE	(*S*)-1-(4-Dimethylaminonaphthalen-1-yl)ethylamine
DAPEA	D-and L-1-(4-Dansylamino-phenyl)ethylamine
DMAP	4-Dimethylaminopyridine
DMOA	Dimethyloctylamine
DNB	Dinitrobenzoyl
D R	Derivatizing reagent
ECF	Ethylchloroformate
EEDQ	2-Ethoxy-1-1-ethoxycarbonyl-1,2-dihydroquinoline
Em	Emission
Ex	Excitation
HSA	Human serum albumin
I.S.	Internal standard
M P	Mobile phase
MQ	Reported minimum quantifiable concentration of reach enantiomer
NA	Not applicable
NR	Not reported
NSAIDs	Nonsteroidal anti-inflammatory drugs
RFBP	Riboflavin binding protein
SFC	Super critical fluid chromatography
TLC	Thin-layer chromatography
2-APA	2-Arylpropionic acid
V	Volume
λ	Wavelength of detection (nm)

References

[1] H. Nagashima, Y. Tanaka and R. Hayashi, J. Chromatogr., 345 (1985) 373.
[2] H. Naganuma and Y. Kawamura, J. Chromatogr., 530 (1990) 387.
[3] W. Takasaki and Y. Tanaka, Chirality, 4 (1992) 308.
[4] C.J.W. Brooks and M.T. Gilbert, J. Chromatogr., 99 (1974) 541.
[5] A.C. Rudy, K.S. Anliker and S.D. Hall, J. Chromatogr., 528 (1990) 395.
[6] A.J. Hutt and J. Caldwell, J. Pharm. Pharmacol., 35 (1983) 693.
[7] A.J. Hutt and J. Caldwell, Clin. Pharmacokin., 9 (1984) 371.
[8] F. Jamali, Eur. J. Drug Metab. Pharmacokin., 13 (1988) 1.
[9] A.M. Evans, Clin. Pharmacol., 42 (1992) 237.
[10] P.J. Hayball, J.G. Tamblyn, Y. Holden and J. Wrobel, Chirality, 4 (1992) 1.
[11] I.W. Wainer and T.D. Doyle, J. Chromatogr., 284 (1984) 117.
[12] J.B. Crowther, T.R. Covey, E.A. Dewey and J.D. Henion, Anal. Chem., 56 (1984) 2921.
[13] D.A. Nicoll-Griffith, J. Chromatogr., 402 (1987) 179.
[14] D.M. McDaniel and B.G. Snider, J. Chromatogr., 404 (1987) 123.
[15] W.H. Pirkle and T.C. Pochapsky, Chem. Rev., 89 (1989) 347.
[16] S.V. Kakodkar and M. Zief, Chirality, 2 (1990) 124.
[17] N. Ôi, H. Kitahara, F. Aoki and N. Kitsu, J. Chromatogr. B, 689 (1995) 195.
[18] L. Castellani, M. Flieger and M. Sinibaldi, J. Liq. Chromatogr., 17 (1994) 3695.
[19] D.W. Armstrong, T.J. Ward, R.D. Armstrong and T.E. Beesley, Science, 232 (1986) 1132.
[20] G. Geisslinger, K. Dietzel, D. Loew, O. Schuster, G. Rau, G. Lachmann and K. Brune, J. Chromatogr., 491 (1989) 139.
[21] G. Farkas, L.H. Irgens, G. Quintero, M.D. Beeson, A. Al-Saeed and G. Vigh, J. Chromatogr., 645 (1993) 67.
[22] Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, N. Inotsume and M. Nakano, Chirality, 1 (1989) 239.
[23] A. Van Overbeke, W. Baeyens, W. Van Den Bossche and C. Dewaele, J. Pharm. Biomed. Anal., 12 (1994) 901.
[24] A. Van Overbeke, W. Baeyens, W. Van Den Bossche and C. Dewaele, J. Pharm. Biomed. Anal., 12 (1994) 911.
[25] L. Oliveros, P. Lopez, C. Minguillon and P. Franco, J. Liq. Chromatogr., 18 (1995) 1521.
[26] G.J. Terfloth, W.H. Pirkle, K.G. Lynam and E.C. Nicolas, J. Chromatogr., 705 (1995) 185.
[27] S. Allenmark and S. Andersson, Chirality, 1 (1989) 154.
[28] T.A. Nocter, G. Felix and I.W. Wainer, Chromatographia, 31 (1991) 55.
[29] T. Miwa, T. Miyakawa and M. Kayano, J. Chromatogr., 408 (1987) 316.
[30] J. Haginaka, T. Murashima, H. Fujima and H. Wada, J. Chromatogr., 620 (1993) 199.
[31] J. Hermansson and M. Ericksson, J. Liq. Chromatogr., 9 (1986) 621.
[32] Y. Oda, N. Asakawa, S. Abe, Y. Yoshida and T. Sato, J. Chromatogr., 572 (1991) 133.
[33] G. Massolini, E. De Lorenzi, M.C. Ponci, C. Gandini, G. Caccialanza and H.L. Monaco, J. Chromatogr. A, 704 (1995) 55.
[34] G. Schill, I. Wainer and S. Barkan, J. Chromatogr., 365 (1986) 73.
[35] S. Mezel-Soglowek, G. Geisslinger and K. Brune, J. Chromatogr., 532 (1990) 295.
[36] J. Hermansson and I. Hermansson, J. Chromatogr. A, 666 (1994) 181.
[37] J. Haginaka, C. Seyama and N. Kanasugi, Anal. Chem., 67 (1995) 2539.
[38] T. Miwa, M. Ichikawa, M. Tsuno, T. Hattori, T. Miyakawa, M. Kayano and Y. Miyake, Chem. Pharm. Bull., 35 (1987) 682.

[39] T. Miwa, T. Miyakawa and Y. Miyake, J. Chromatogr., 457 (1988) 227.
[40] P.J. Hayball, J.W. Holman and R.L. Nation, J. Chromatogr. A, 662 (1994) 128.
[41] C. Pettersson and K. No, J. Chromatogr., 282 (1983) 671.
[42] C. Pettersson, J. Chromatogr., 9 (1986) 269.
[43] C. Pettersson and G. Schill, J. Liq. Chromatogr., 9 (1986) 269.
[44] C. Pettersson and C. Gioeli, J. Chromatogr., 435 (1988) 225.
[45] S. Björkman, J. Chromatogr., 339 (1985) 339.
[46] A. D'Hulst and N. Verbeke, J. Chromatogr., 608 (1992) 275.
[47] S. Fanali and Z. Aturki, J. Chromatogr. A, 694 (1995) 297.
[48] A. Guttman and N. Cooke, J. Chromatogr. A, 685 (1994) 155.
[49] Y.Y. Rawjee, R.L. Williams and G. Vingh, J. Chromatogr. A, 680 (1994) 599.
[50] A.E. Karger, E. Stoll and W. Hänsel, Pharmazie, 49 (1994) 155.
[51] D.M. Johnson, A. Reuter, J.M. Collins and G.F. Thompson, J. Pharm. Sci., 68 (1979) 112.
[52] E.J.D. Lee, K.M. Williams, G.C. Graham, R.O. Day and D. Champion, J. Pharm. Sci., 73 (1984) 1542.
[53] C.Y. Chen, P.H. Lu and C.S. Chen, J. Formos. Med. Ass., 90 (1991) 437.
[54] A. Rubin, M.P. Knadler, P.P.K. Ho, L.D. Bechtol and R.L. Wolen, J. Pharm. Sci., 74 (1985) 82.
[55] N.N. Singh, F.M. Pasutto, R.T. Coutts and F. Jamali, J. Chromatogr., 378 (1986) 125.
[56] N.N. Singh, F. Jamali, F.M. Pasutto, R.T. Coutts and A.S. Russell, J. Chromatogr., 382 (1986) 331.
[57] D.C. Kaiser, G.J. Van Giessen, R.J. Reischer and W.J. Wechter, J. Pharm. Sci., (1976) 269.
[58] R.T. Foster and F. Jamali, J. Chromatogr., 416 (1987) 388.
[59] R. Mehvar and F. Jamali, Pharm. Res., 5 (1988) 53.
[60] B.W. Berry and F. Jamali, Pharm. Res., 5 (1988) 123.
[61] H. Spahn, J. Chromatogr., 423 (1987) 334.
[62] R. Mehvar and F. Jamali, J. Chromatogr., 431 (1988) 228.
[63] M. Vakily, B. Corrigan and F. Jamali, Pharm. Res., 12 (1995) 1652.
[64] R. Mehvar, F. Jamali and F.M. Pasutto, J. Chromatogr., 425 (1988) 493.
[65] R. Mehvar, F. Jamali and F.M. Pasutto, Clin. Chem., 34 (1988) 493.
[66] J.-M. Maître, G. Boss and B. Testa, J. Chromatogr., 299 (1984) 397
[67] H.Y. Ahn, S.T. Wu, G.K. Shiu, T.D. Doyle, C.A. Brunner and W.F. Trafton, Pharm. Res., 8 (1992) S31.
[68] M.R. Wright and F. Jamali, J. Chromatogr., 616 (1993) 59–65.
[69] H.Y. Ahn, G.K. Shiu, W.F. Trafton and T.D. Doyle, J. Chromatogr. A, 653 (1994) 163.
[70] S.H. Wilen, A. Collet and J. Jacques, Tetrahedron, 33 (1977) 2725.
[71] R.J. Bopp, J.F. Nash, A.S. Ridolfo and E.R. Shepard, Drug Metab. Dispos., 7 (1979) 356.
[72] S.W. McKay, D.N.B. Mallen, P.R. Shrubsall, B.P. Swann and W.R.N. Williamson, J. Chromatogr., 170 (1979) 482.
[73] R.G. Simmonds, T.J. Woodage, S.M. Duff and J.N. Green, Eur. J. Drug Metab. Pharmacokin., 5 (1980) 169.
[74] H. Spahn, S. Iwakawa, E.T. Lin and L.Z. Benet, Pharm. Res., 5 (1987) 988.
[75] H. Weber, H. Spahn, E. Mutschler and W. Möhrke, J. Chromatogr., 307 (1984) 145.
[76] B.C. Sallustio, A. Abas, P.J. Hayball, Y.J. Purdie and P.J. Meffin, J. Chromatogr., 374 (1986) 329.
[77] J.M. Kemmerer, F.A. Rubio, R.M. McClain and B.A. Koechlin, J. Pharm. Sci., 68 (1979) 1274.
[78] J.K. Stoltenborg, C.V. Puglisi, F. Rubio and F.M. Vane, J. Pharm. Sci., 70 (1981) 1207.
[79] Y.C. Lee, W.L. Gee, L.Z. Benet and E.T. Lin, Pharm. Res., 3, (1986) 7S.
[80] A J. Hutt, S. Fournel and J. Caldwell, J. Chromatogr., 378 (1986) 409.
[81] H. Spahn, I. Spahn, G. Pflugmann, E. Mutschler and L.Z. Benet, J. Chromatogr., 433 (1988) 331.
[82] S. Iwakawa, T. Suganuma, S.F. Lee, H. Spahn, L.Z. Benet and E.T. Lin, Drug Metab. Dispos., 17 (1989) 414.
[83] E.L. Palylyk and F. Jamali, J. Chromatogr., 568 (1991) 187.
[84] T.A. Graser, J.C. Jordan, M. Karpf and M. Holck, Acta Vet. Scand. Suppl., 87 (1991) 247.
[85] S.J. Lan, K.J. Kripalani, A.V. Dean, P. Egli, L.T. Difazio and E.C. Schreiber, Drug Metab. Dispos., 4 (1976) 330.
[86] W.H. Pirkle and C.J. Welch, J. Liq. Chromatogr., 15 (1992) 1947.
[87] P.J. Hayball, R.L. Nation, F. Bochner and R.K. Le-Leu, J. Chromatogr., 570 (1991) 446.
[88] C. Volland, H. Sun and L.Z. Benet, J. Chromatogr., 534 (1990) 127.
[89] K. Iwaki, T. Bunrin, Y. Kameda and M. Yamazaki, J. Chromatogr. A, 662 (1994) 87.
[90] S. Pedrazzini, W. Zanoboni-Muciacia and A. Forgione, J. Chromatogr., 413 (1987) 338.
[91] S. Pedrazzini, W. Zanoboni-Muciacia, C. Sacchi and A. Forgione, J. Chromatogr., 415 (1987) 214.
[92] W.J. Adams, G.J. VanGiessen and D.G. Kaiser, Pharm. Res. Suppl., (1988) S-229
[93] M.P. Knadler and S.D. Hall, J. Chromatogr., 494 (1989) 173.
[94] B. Blessington, N. Crabb and J. O'Sullivan, J. Chromatogr., 396 (1987) 177.
[95] G.J. Van Giessen and D.G. Kaiser, J. Pharm. Sci., 64 (1975) 798.
[96] G. Giesslinger, S. Menzel-Soglowek, O. Schuster and K. Brune, J. Chromatogr., 573 (1992) 163.
[97] H.Y. Aboul-Enein and S.A. Bakr, J. Liq. Chromatogr., 15 (1992) 1983.
[98] W.H. Wilson, Chirality, 6 (1994) 216.
[99] S.M. Wilkins, D.R. Taylor and R.J. Smith, J. Chromatogr. A, 697 (1995) 587.
[100] S.S. Adams, E.E. Cliffe, B. Lesel and J.S. Nicholson, J. Pharm. Sci., 56 (1967) 1686.
[101] D.G. Kaiser and G.J. Van Giessen, J. Pharm. Sci., 63 (1974) 219.
[102] J. Goto, N. Goto, A. Hikichi, T. Nishimaki and T. Nambara, Anal. Chim. Acta, 120 (1980) 187.

[103] M. Asami and K.-I. Nakamura, Chirality, 7 (1995) 28.
[104] T. Hansen, R. Day, K. Williams, E. Lee, R. Knihinicki and A. Dufield, Clin. Exp. Pharmacol. Physiol., 9 (1985) 82.
[105] M.A. Young, L. Aarons, E.M. Davidson and S. Toon, J. Pharm. Pharmacol., 38 (1986) 60P.
[106] A. Averginos and A.J. Hutt, J. Chromatogr., 415 (1987) 75.
[107] D.A. Nicoll-Griffith, T. Inaba, B.K. Tang and W. Kalow, J. Chromatogr., 428 (1988) 103.
[108] A.M. Evans, R.L. Nation, F. Bochner, L.N. Sansom and A.A. Somogyi, Eur. J. Clin. Pharmacol., 36 (1989) 283.
[109] K.J. Petterson and A. Olsson, J. Chromatogr., 563 (1991) 414.
[110] C.Y. Chen, P.H. Lu and C.S. Chen, J. Formosan Med. Ass., 90 (1991) 437.
[111] M.R. Wright, S. Sattari, D.R. Brocks and F. Jamali, J. Chromatogr., 583 (1992) 259.
[112] C.H. Lemko, G. Caillé and R.T. Foster, J. Chromatogr., 619 (1993) 330.
[113] N. Suzuki, H. Naganuma, J. Kondo and Y. Kawahara, Int. Symp. Mol. Chir., 1994, p. 314.
[114] P. Petersson and K.E. Markides, J. Chromatogr., 584 (1992) 189.
[115] D.S. Jack, R.H. Rumble, N.W. Davies and H.W. Francis, J. Chromatogr., 584 (1992) 189.
[116] M.-J. Zhao, C. Peter, M.-C. Holtz, N. Hugenell, J.-C. Koffel and L. Jung, J. Chromatogr. A, 656 (1994) 441.
[117] G.P. Tosolini, E. Moro, A. Forgione, M. Ranghieri and V. Mandelli, J. Pharm. Sci., 63 (1974) 1072.
[118] V. Rossetti, A. Lombard and M. Buffa, J. Pharm. Biomed. Anal., 4 (1986) 673.
[119] T. Cleveland, J. Chromatogr., 18 (1995) 649.
[120] N. Blazeyic, M. Zinic, T. Kovac, V. Sunjic and F. Kajifez, Acta Pharma. Jugoslav., 25 (1975) 155.
[121] S. Björkman, J. Chromatogr., 414 (1987) 465.
[122] Y. Oda, N. Asakawa, Y. Yoshida and T. Sato, J. Pharm. Biomed. Anal., 10 (1992) 81.
[123] S. Chakir, M.H. Maurice, J. Magdalou, P. Leroy, N. Dubois, F. Lapique, Z. Abdelhamid and A. Nicolas, J. Chromatogr. A, 654 (1994) 61.
[124] R.A. Carr, G. Caille, A.H. Ngoc and R.T. Foster, J. Chromatogr. B, 668 (1995) 175.
[125] K. Matsuda, Y. Tanaka, S. Ushiyama, K. Ohnishi and M. Yamazaki, Biochem. Pharmacol., 33 (1984) 2473.
[126] S. Naruto, Y. Tanaka, R. Hayashi and A. Terada, Chem. Pharm. Bull., 32 (1984) 258.
[127] J. Goto, N. Goto and T. Nambara, J. Chromatogr., 239 (1982) 559.
[128] J. Goto, M. Ito, S. Katsuki, N. Saito and T. Nambara, J. Liq. Chromatogr., 9 (1986) 683.
[129] J.V. Andersen and S.H. Hansen, J. Chromatogr., 577 (1992) 362.
[130] A. Sioufi, D. Colussi, F. Marfil and J.P. Dubois, J. Chromatogr., 414 (1987) 131.
[131] W.T.C. Liang, D.R. Brocks and F. Jamali, J. Chromatogr., 577 (1692) 317.
[132] F. Jamali, R. Mehvar, C. Lemko and O. Eradiri, J. Pharm. Sci., 77 (1988) 963.
[133] N. Arima, J. Pharmacobiodyn., 13 (1990) 733.
[134] T. Nomura, T. Imai and M. Otagiri, Biol. Pharm. Bull., 16 (1993) 298.
[135] J. Haginka and N. Kanasugi, J. Chromatogr. A, 694 (1995) 71.
[136] J. Haginka, T. Murashima and C. Seyama, J. Chromatogr. A, 677 (1994) 229.
[137] F.C. Marziani and R. Sisco, J. Chromatogr., 465 (1989) 422.
[138] Y. Shinohara, N. Kirii, H. Tamaoki, H. Magara and S. Baba, J. Chromatogr., 525 (1990) 93.
[139] S.J. Grieb, S.A. Matlin, A.M. Belenguer and H.J. Ritchie, J. Chromatogr. A, 697 (1995) 271.
[140] N.N. Singh, F. Jamali, F.M. Pasutto, A.S. Russell, R.T. Coutts and K.S. Drader, J. Pharm. Sci., 75 (1986) 439.
[141] R. Mehvar, F. Jamali and F.M. Pasutto, J. Chromatogr., 425 (1988) 135.
[142] N. Muller, F. Lapique, E. Drelon, P. Gillet, C. Monot, B. Poletto and P. Netter, J. Chromatogr., 616 (1993) 261.
[143] M. Vakily and F. Jamali, Pharm. Res., 12 (1995) A1045 S-22.
[144] R. Feretti, B. Gallinella, F. La Torre and C. Villani, J. Chromatogr., 704 (1993) 217.
[145] A.J. Hutt, M.R. Hadley and S.C. Tan, Eur. J. Drug Metab. Pharmacokin., 3 (1994) 241.
[146] S. Kuzuna, N. Matsumoto, T. Kometani and K. Kawai, Japan. J. Pharmacol., 24 (1974) 695.
[147] S. Tamura, S. Kuzana, K. Kawai and S. Kishimoto, J. Pharm. Pharmacol., 33 (1981) 701.
[148] N.N. Singh, F. Jamali, F.M. Pasutto, R.T. Coutts and A.S. Russell, J. Chromatogr., 382 (1986) 331.
[149] U. Becker-Scharfenkamp and G. Blaschke, J. Chromatogr., 621 (1993) 199.
[150] S. Caccamese, Chirality, 5 (1993) 164–167.
[151] M. Strolin-Benedetti, E. Moro, E. Frigerio, M.G. Jannuzzo, R. Roncucci and J. Caldwell, Biochem. Pharmacol., 40 (1990) 1719.
[152] M. Strolin-Benedetti, E. Frigerio, V. Tamassia, G. Noseda and J. Caldwell, Biochem. Pharmacol., 43 (1992) 2032.
[153] A. Guzman, F. Yuste, R.A. Toscana, J.M. Young, A.R. Van Horn and J.M. Muchowski, J. Med. Chem., 29 (1986) 589.
[154] F. Jamali, F.M. Pasutto and C. Lemko, J. Liq. Chromatogr., 12 (1989) 1835.
[155] M.H. Mills, L.E. Mather, X.S. Guand J.L. Huang, J. Chromatogr. B, 658 (1994) 177.
[156] M.J. Diaz-Perez, J.C. Chen, A.-F. Aubry and I.W. Wainer, Chirality, 6 (1994) 283.
[157] D.J. Jones and A.R. Bjorksten, J. Chromatogr. B, 661 (1994) 165.