Chiral Drug Separation

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INTRODUCTION

Consideration of chirality is now an integral part of drug research and development and the regulatory process. There is no choice! Enantiomeric forms of a drug can differ in potency, toxicity, and behavior in biological systems. Enantiomers of all chiral bioactive molecules have to be separated and tested. The Food and Drug Administration (FDA, U.S.A.), and regulatory authorities in Europe, China, and Japan have provided guidelines indicating that preferably only the active enantiomer of a chiral drug should be brought to market.

This entry discusses the main chiral drug separation methods, viz. gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), and some new techniques. The first part presents the concept of molecular chirality and some examples of chiral drugs. The importance of chiral drug separation is briefly discussed. The second part describes the main chiral drug separation techniques and related chiral recognition mechanisms, as well as available chiral selectors. Some typical examples are given, and the pros and cons of chromatographic and capillary electrophoretic techniques are compared. Readers are also referred to specialized review articles for details. The last part summarizes and briefly discusses future development trends in chiral drug separation techniques.

BACKGROUND

What are Chiral Molecules?

A molecule with an asymmetric carbon center has a unique three-dimensional shape and is called chiral, from Greek *cheir* meaning ''hand.'' A chiral molecule and its mirror image are not completely identical: they differ in their ''handedness'' and are not superimposable. Such chiral molecules are called ''enantiomers,'' from Greek *enantios*, meaning ''opposite.'' A human right hand and left hand are enantiomers: they are mirror images, but a ''left'' glove cannot be worn on a right hand. The arrangement of thumb and fingers in three dimensions makes a right hand and a left hand distinctly different from each other. The two different forms of a chiral pair comprise the same number and types of atoms, and they are commonly described as D- and L-isomers with reference to their ability to rotate polarized light. An equimolar mixture of enantiomers is called a ''racemate.''

Fig. 1 depicts 2-butanol. The top two structures are mirror images. Below, ''right-handed'' 2-butanol has been rotated so that the OH group points to the left for comparison with ''left-handed'' 2-butanol. Although the carbon frameworks of the two molecules align, the position of the hydroxyl group is different. In the rotated version of right-handed 2-butanol, in the lower left panel, the hydroxyl group points into the page; in left-handed 2-butanol, in the lower right panel, the hydroxyl group points out from the page. It is impossible to align the two molecules completely without breaking bonds. Left-handed and right-handed forms of a molecule can have profoundly different properties in a biological context. In the food industry, for instance, left-handed limonene smells like lemons, while the right-handed molecule smells like oranges. Different enantiomers can differ widely in their biological properties because chirality is related to the three-dimensional structure, and one form may be more suitable for specific interaction with a biological molecule, such as a receptor, enzyme, etc.

Chiral Drugs

Most synthetic drugs developed in the past were not chiral, though some were. Drugs developed from natural products are largely chiral. Currently, about 40% of the drugs in use are known to be chiral. A report from Technology Catalysts International, Falls Church, VA, states that worldwide sales of chiral drugs in single-enantiomer dosage forms grew at an annual rate of more than 13% to $133 billion in 2000, and that sales could hit $200 billion by 2008.[1] About one-third of all dosage-form drug sales in 2000 were single enantiomers. By geography, the United States is the largest consumer of enantiomeric fine chemicals, contributing 60% of the worldwide total. Drug companies continue

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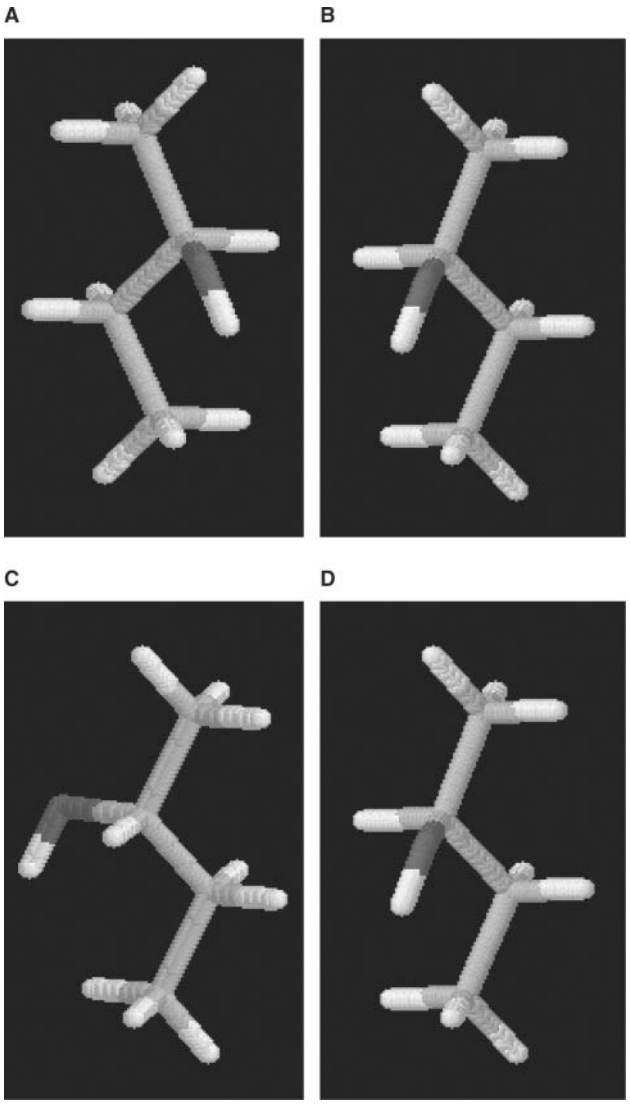

Fig. 1 2-Butanol chiral molecules: (A) right-handed (*R*) 2-butanol, (B) left-handed (*S*) 2-butanol, (C) rotated (*R*) 2-butanol, and (D) (*S*) 2-butanol. *(View this art in color at www.dekker.com.)*

to develop chiral drugs as single enantiomers and use chirality to manage drug life cycles.

Most commercially available drugs are both synthetic and chiral. A large number, however, are still marketed as racemic mixtures. Only about one-third of drugs are administered as pure enantiomers. The respiratory drug montelukast (Merck), the antirheumatoidal infliximab (Centocor), the ophthalmic drug for the treatment of glaucoma latanaprost (Pharmacia), and the prostate hyperplasia agent tamsulosin (Boehringer Ingelheim Pharmacy) are among the best-selling single-enantiomer drugs. The chiral drug (*S*)-(+)-ibuprofen is marketed as fast-acting, and it reaches therapeutic concentrations in blood in 12 vs. 30 min for racemic mixtures. Allegra, an isomer from a metabolite of seldane, is used as an allergy medication. Table 1 lists further examples of chiral drugs and their bioactivities. The enantiomeric forms of a drug can differ markedly in potency, toxicity, and behavior in biological systems.

Importance of Chiral Drug Separation

In the early 1980s analytical chiral separation was a rather difficult task, and preparative synthetic and separation methods were not as advanced as today. Nevertheless, it was clear that chiral drugs should be enantioseparated and that each enantiomer should be used separately. Nowadays, enantiomers are considered distinctly different compounds, as enantiomers of drug substances may have distinct biological interactions and, consequently, profoundly different pharmacological, pharmacokinetic, or toxicological activities.[2] The body is highly chiral selective; it will interact with each racemic drug differently and metabolize each enantiomer by a separate pathway to produce different pharmacological activity. One isomer may thus produce the desired therapeutic activities, while the other may be inactive or produce unwanted side effects (see Table 1). Even when side effects are not serious, the inactive enantiomer must be metabolized and thus represents an unnecessary burden for the organism.

One chiral form of the drug naproxen has 28 times the anti-inflammatory activity of the chiral relative. One isomer of dopamine, used to treat Parkinson's disease, acts on nerve cells to control tremor, while the other is toxic to nerve cells. Racemic mixtures of the drug thalidomide were marketed to pregnant women in the 1960s to counter morning sickness. Therapeutic activity, however, comes exclusively from the (*R*)-(+)-enantiomer. It was discovered only after several hundred birth defects had resulted from administration of thalidomide that the (*S*)-(+)-enantiomer is teratogenic. By then, consideration of chirality has become an integral part of drug research and development and of the regulatory process.

In general, use of the more active isomer of a drug has the following advantages:

- Fewer or diminished side effects, which may result from the unwanted isomeric form.
- Automatically halved dosage for a patient.
- Decreased waste due to decrease in manufacturing of unwanted isomer.
- New commercial opportunities for ''racemic switching:'' a drug previously marketed as a racemate can be redeveloped and introduced as an enantiomerically pure form, possibly useful for extending patent protection of a key product.

Table 1 Examples of chiral drugs and functions

Chiral drugs	Bioactivity
Albuterol	D-isomer may provoke airway constriction; L-isomer avoids side effects
Ethambutol	The (*S*,*S*)-form of ethambutol is a tuberculostatic agent; the (*R*,*R*)-form causes optical neuritis that can lead to blindness
Levodopa	The levodopa (L-dopa) is a Parkinson's disease agent; the D-form causes serious side effects, such as granulocytopenia
Penicillamine	The (*S*)-enantiomer has antiarthritic activity; the (*R*)-form is extremely toxic
Propanolol	Racemic compound is used as drug; however, only the (*S*)-(−)-isomer has the desired β-adrenergic blocking activity
Propoxyphene	α-L-isomer is antitussive (cough); α-D-isomer is analgesic (pain)
Thalidomide	The (*S*)-isomer has the desired antinausea effects; the (*R*)-form is teratogenic and causes fetal abnormalities, such as severely underdeveloped limbs

There are obvious benefits to studying the properties of the enantiomers of a chiral drug molecule with respect to therapeutic efficacy and safety. In view of this, since 1992 the FDA and the European Committee for Proprietary Medicinal Products have required that the properties of each enantiomer be studied separately before decisions are taken to market the drug as one of the enantiomers or as a racemate.[3] This requires powerful means of chiral drug detection and separation. In addition, there is increasing awareness of the need to reevaluate the properties of individual enantiomers of currently marketed racemic drug molecules. The effect has been a significant increase in demand for sensitive chiral analytical and separation methods. At the same time, the number of new chemicals entering development within the pharmaceutical industry has increased significantly. By now most drug companies have clear guidelines recommending that the enantiomers of all chiral bioactive molecules be separated and tested.

The ideal way to obtain pure drug enantiomers would be enantioselective synthesis. This, however, is rarely practical, usually complicated, and almost always expensive. There is little control over which chiral form of a chemical compound will be formed during a typical production process. This lack of control generally results in production of equal amounts of the various possible chiral forms of a compound. Often, therefore, separation of intermediates or final products from a racemic mixture is required. Increasing interest is being paid to development of methods of efficient, high throughput, and sensitive chiral separations, control of chemical synthesis, assessment of enantiomeric purity, and determination of pharmacodynamics.

The various examples of different therapeutic, toxicological, and pharmacokinetic properties of the enantiomers of chiral drugs provide a strong impulse for the development of techniques for chiral drug separation. Enantiomers can differ in absorption, distribution, protein binding, and affinity to the receptor. Such properties have been exploited for the development of powerful techniques for achieving analytical-scale chiral separations, quantifying minor enantiomeric impurities in chiral drugs, and preparing gram to multi-ton amounts of enantiomerically pure chiral drugs. Chromatographic techniques, such as HPLC, GC, thin layer chromatography, and supercritical fluid chromatography have been developed for chiral separations. Capillary zone electrophoresis, capillary gel electrophoresis, electrokinetic chromatography, and capillary electrochromatography have proved powerful alternatives to chromatographic techniques.

CHIRAL DRUG SEPARATION PRINCIPLES AND TECHNIQUES

Principles of Chiral Separation and Chiral Selectors

Principles of chiral separation

Separation of enantiomers has been achieved using GC, HPLC, and CE. Chiral recognition generally

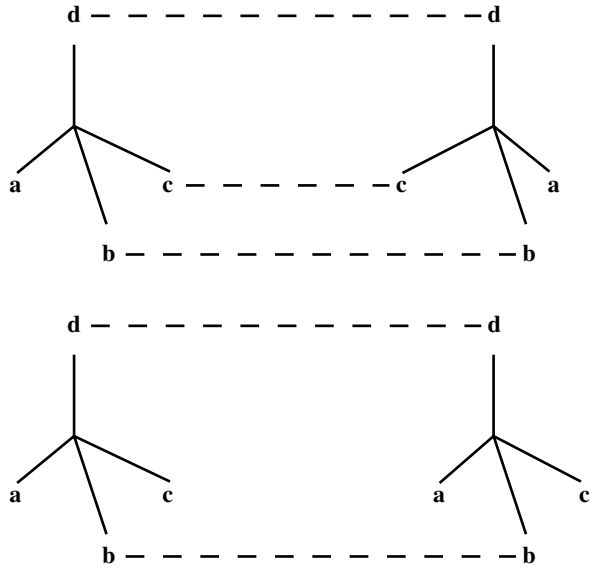

Fig. 2 Three-point interaction model.

depends on a minimum of three simultaneous interactions between the selector and selected—the so-called three-point-interaction rule of Dalgliesh (Fig. 2).[4,5] At least one of these interactions must be stereoselective to form diastereomeric complexes, and thereby enable chiral separation.

The principle task of chiral separation is to create the selectivity essential for separation of stereoselectively different forms of compounds, which may be recognized as such only during the interaction with a chiral selector. This is the separation principle for chromatographic techniques and also for chiral CE. In chromatographic chiral separation, there is a distribution of analyte between two immiscible phases, and these should exhibit different mobilities. Commonly one phase is mobile and the other is stationary. In chiral CE there are not two immiscible phases present but pseudophases at best, or even only one monophasic, homogenous system. Chiral recognition, however, occurs at the molecular level, not on the macroscopic level of the phases. A separation technique therefore must allow the transfer of the molecular level event (in this case chiral recognition) to macroscopic phenomena: different retention times of enantiomers in chromatography and different effective mobilities of enantiomers in electrophoretic techniques.

Immiscibility of phases is a prerequisite in chromatographic separation because pressure as a driving force cannot select a given component from several species in the same phase. Under certain circumstances, however, electrically driven mobility can be selective for one or several species residing in the same phase. So, the immiscibility requirement of the two phases does not apply to chiral CE. In other words, the principal difference between chromatographic techniques and CE is that pressure cannot distinguish between different molecular components in a monophasic system, whereas electrically driven mobility can do this under certain circumstances. The phenomenon responsible for chiral separation is the same in chromatographic and electrophoretic techniques: enantioselective interaction between the analyte enantiomers and a chiral selector.

Chiral selectors

Enantiomers are distinguished on the basis of their interaction with a chiral selector. Development of chiral selectors or chiral stationary phases (CSPs) for GC, HPLC, and CE has rapidly opened a new dimension in the area of chiral drug separation techniques. There are different chiral selectors available for enantiomeric separation of drugs and pharmaceuticals. Finding a suitable chiral selector, whether immobilized on a solid support (GC, HPLC) or added to a running buffer (HPLC, CE), is still often based on trial and error. A few predictions can be made, however, if common structural elements are present. After a selector has been chosen, variables, such as the nature, ionic strength, and pH of buffer, can be varied, as can presence of organic modifiers, temperature, and so on.

Among available selectors, native and derivatized cyclodextrins (CDs) are the most widely used ones at this time. A majority of drug and pharmaceutical applications have been achieved with CDs. Use of CDs as chiral selectors is the subject of a number of reviews (e.g., Refs.[6,7].). Native CDs are cyclic oligosaccharides consisting of six α-CD-, seven β-CD- or eight γ-CD-glucopyranose units. A truncated cone provides a hydrophobic cavity; the exterior surface, surrounded by hydroxyl group, is hydrophilic. Low UV absorbance, low cost, and water solubility are attractive properties of CDs for use as chiral selectors. In addition to CDs, other chiral selectors, such as natural and synthetic chiral surfactants, crown ethers, proteins, oligo- and polysaccharides, macrocyclic antibiotics, and chiral ligands have been applied to chiral separations. Important selectors and some chiral recognition mechanisms are given in Table 2. Some chiral selectors developed thus far can efficiently resolve enantiomers of various important drugs.

Chiral Drug Separation Techniques

The main methods used for chiral drug separation are GC, HPLC, and CE.[2,4,6–25] Other techniques, such as chiral crystallization and enzyme-based kinetic separation, have also attracted attention.[26]

Table 2 A variety of chiral selectors employed for chiral separation and related chiral recognition mechanisms

Chiral selector	Chiral recognition mechanism	Examples	Separated enantiomers
CDs	Chiral recognition is based on inclusion of the bulky hydrophobic group of the analyte into the hydrophobic cavity of the CD and on lateral interactions of the hydroxyl groups, such as hydrogen bonds and dipole–dipole interactions, with the analyte.	Carboxymethylated β-CD, heptakis-*O*-methyl-β-CD, hydroxyethyl-β-CD, mono(6-β-aminoethylamino-6-deoxy)-β-CD, and mono(6-amino-6-deoxy)-β-CD.	Acebutolol, acenocoumarol, carnitine, cathinone, ephedrine, epinephrine, glutethimide, ketotifen, thioridazine, etc.
Macrocyclic antibiotics	The multiple chiral atoms and several functional groups allow multiple interactions with the analytes to enable chiral recognition. The primary interaction is ionic; secondary interactions include hydrogen bonding, dipole–dipole, π–π, hydrophobic interactions, and steric repulsion.	Rifamycin B, rifamycin SV, ristocetin A, teicoplanin, fradiomycin, kanamycin, ansamycins, avoparcin, and vancomycin.	Amephoterin, α-aminoadipic acid, flurbiprofen, fenoprofen, methionine, methotrexate, ketoprofen, and suprofen, etc.
Proteins/ polypeptides	Proteins and peptides are naturally chiral and they often have different qualitative interactions (e.g., different binding sites) and/or quantitative interactions (i.e., different affinity or binding capacity) with the stereoisomers of chiral molecules. Very specific high-affinity binding often occurs, and any sort of intermolecular interactions (e.g., hydrophobic interactions, electrostatic interactions, etc.) may play a role in the binding, which is often reversible.	Bovine serum albumin, human serum albumin, pepsin, lysozyme, avidin, ovomucoid, casein, conalbumin, streptavidin, trypsin, ovoglycoprotein, and β-lactoglobulin.	Benzoin, bunitrolol, epinastine, flurbiprofen, ibuprofen, ketoprofen, leucovorin, pindolol, promethazine, propranolol, trimeputine, warfarin, etc.
Polysaccharides	Hydrogen bonds and dipole–dipole interactions with hydroxyl groups of the sugar molecules are assumed to be the main interactions. In some cases, the helical structure of dextrins might be responsible for chiral recognition.	Heparin, dextran sulfate, dermatan sulfate, streptomycin sulfate, amylose, chondroitin sulfate C, laminaran, dextrin sulfopropyl, and kanamycin sulfate.	Doxylamine, laudanosine, naproxen, oxamniquine, pheniramine, primaquine, timepidium, trimetoquinol, etc.
Chiral surfactants	The chiral separation of analytes is based on their partition coefficients between the chiral micelle phase and the electrolyte bulk phase.	Alkylglucosides, alkylmaltoside, sodium cholate, saponines, sodium dodecyl sulfate, sodium taurocholate.	Ephedrine, fenoldopam, hexobarbital, ketamine, pindolol, timolol, etc.
Chiral crown ethers	Two different diastereomeric inclusion complexes are formed. The primary interactions for complexation are hydrogen bonds between the three amine hydrogens and the oxygens of the macrocyclic ether in a tripod arrangement. Ionic, dipole–dipole interactions, or hydrogen bonds between the carboxylic groups and polar groups of the analytes may act as additional supporting interactions.	18-Crown-6-tetracarboxylic acid, dicyclohexyl-18-crown-6, 18-crown-6-2,3,11,12-tetracarboxylic acid, benzo-monoaza-15-crown-5, and (*R*,*R*)-2,12-bis(hydroxymethyl)-2, 12-dimethyl-18-crown-6.	Aminoglutethimide, aminophenol, baclofen, dopa, ephedrine, mexiletine, noradrenaline, norephedrine, octopamine, primaquine, etc.

(From Refs.[6–8].)

Applications of GC to chiral separation

The first separation of enantiomers was achieved by Gil-Av, Feibush, and Charles-Sigler[9] using capillary GC. Separation of enantiomers using CSPs involves hydrogen bonding, coordination, and inclusion. Typical chiral selectors include modified CDs, derivatized amino acids, and terpene-derived metal coordination compounds. The scope and limitations, applications, and mechanistic considerations of chiral separation by GC have been reviewed by Schurig and Francotte.[10,11]

The main applications of enantiomeric separation by GC concern precise determination of enantiomeric composition of chiral research chemicals, drugs, intermediates, metabolites, pesticides, flavors and fragrances, etc. CHIRBASE, a database of chiral compounds, provides comprehensive structural, experimental, and bibliographic information on successful and unsuccessful chiral separations, and rule sets for each CSP and information about the processes of chiral separations.[27] According to CHIRBASE, an appropriate CSP is available for almost every racemic mixture of compounds ranging form apolar to polar. Some 22,000 separations of enantiomers, involving 5,500 basic chiral compounds and documented in 2,200 publications, have been achieved by GC. This method is particularly suitable for volatile compounds such as inhalation anesthetic agents, e.g., enflurane, isoflurane, desflurane, and racemic α-ionone.

A particularly attractive feature of GC, one that distinguishes it from liquid chromatography methods, is the lack of a sensitive dependence on solvents, modifiers, and gradient elution systems. Prerequisites for the use of GC, however, are volatility, thermostability, and resolvability of the chiral analyte. Obviously, this restricts the utility of the method.

Applications of HPLC to chiral separation

Chromatographic methods have dominated separation of enantiomers during the past several decades, especially HPLC.[4,15–17] Numerous book chapters and review articles deal with the separation of chiral drugs by this method (e.g., Refs.[2,6,12–14].). Chiral HPLC is more versatile than chiral GC for enantiomeric separation because it can separate a wide variety of nonvolatile compounds. It can be used to develop fast and accurate methods of chiral drug separation, and it allows on-line detection and quantitation of both mass and optical rotation of enantiomers when appropriate detection devices are used.

Current chiral separation methods using liquid chromatographic techniques can be divided into two categories: a direct method based on diastereomer formation on CSPs or in mobile phases, and an indirect method based on diastereomer formation by reaction with a homochiral reagent. Direct chiral separation using CSPs is more widely used and predictable in mechanistic terms than methods involving chiral additives in the mobile phase. To date, more than a hundred HPLC CSPs are commercially available. No single CSP can be considered universal; none has the ability to separate all classes of racemic compounds.

Three components should be considered in developing a chiral separation method: analyte, CSP, and mobile phase. The key to successful chiral separation of a particular class of racemates on a given CSP is awareness of possible chiral recognition mechanisms. The enormous increase in recent years in number of groups working on chiral chromatography has led to a rapid and impressive accumulation of data in CHIRBASE.[27]

Some examples of chiral HPLC separations of racemic drugs are the following. Typical chromatograms of the simultaneous determination of isopyramide and its active metabolite, mono-*N*-dealkyldisopyramide, in drug-free human plasma, human plasma spiked with disopyramide and mono-*N*-dealkyldisopyramide, and treated subject plasma are presented in Fig. 3.[4] Chiral HPLC has been used to separate chlorpheniramine and its main monodesmethyl metabolite, verapamil and its metabolite, and tramadol and its metabolite.[15–17]

Applications of CE to chiral separation

CE has become widely popular for enantiomer separation over the past decade as a very powerful complementary or alternative technique to HPLC in pharmaceutical science and industry. Several chiral separation principles successfully applied in HPLC have been transferred to CE. The first chiral separation by CE was by Gassmann, Kuo, and Zare in 1985.[18] This approach offers key advantages such as high efficiency, feasibility of incorporating a large number of chiral selectors that greatly facilitates method development, small amounts of chiral selector and solvents, speed of analysis, low overall cost, and minimal environmental impact. The use of low-UV wavelength (e.g., 200 nm) in CE allows detection of impurities with poor chromophores, which may be difficult or impossible to detect by other methods. CE is suitable for charged and polar compounds for which chromatographic methods are not very strong.

Several comprehensive review articles have appeared in recent years dealing with general aspects and applications of chiral CE separation.[8,19–21] A comprehensive list of more than 280 drugs separate by chiral CE, including the respective chiral selectors and background electrolytes, appears in a review article by Gübitz and Schmid.[21] Chiral CE has also

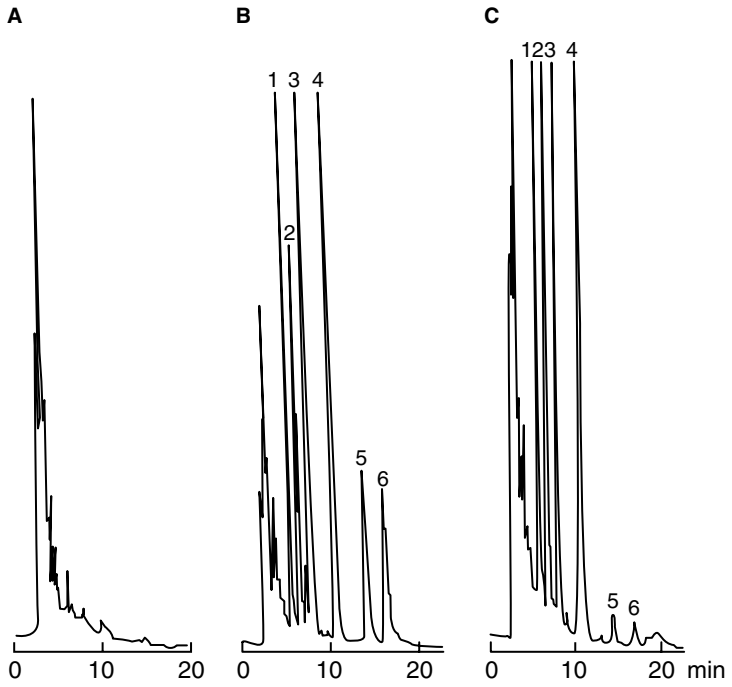

Fig. 3 Chromatograms showing analysis of disopyramide and mono-*N*-dealkyldisopyramide enantiomers in plasma: (A) blank plasma, (B) plasma spiked with 625 ng/ml of disopyramide and mono-*N*-dealkyldisopyramide enantiomers, (C) plasma sample from a healthy volunteer collected 6 hr after administration of 100 mg of Dicorantil. Peak assignments: 1, (*S*)-(+)-disopyramide; 2, (*R*)-(−)-disopyramide; 3, 4, metoprolol; 5, (*S*)-(+)-mono-*N*-dealkyldisopyramide; and 6, (*R*)-(−)-mono-*N*-dealkyldisopyramide. Chromatographic conditions: Chiralpak AD column (250 × 4.6 mm I.D., 10 μm particle size); hexane–ethanol (91 : 9, v/v) mobile phase plus 0.1% diethylamine; 1.2 ml/min flow rate; and detection at 270 nm. (From Ref.[4].)

been the theme of several special issues of *Electrophoresis* and *Journal of Chromatography A*.

Chiral separation in all CE techniques, including capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), and capillary isoelectric focusing (CIEF), relies on enatioselective noncovalent/intermolecular interactions between the analyte and a chiral selector, which may be expressed as effective mobility difference (CZE and CGE), stereoselective shift of the acid–base equilibrium (CIEF), etc. Although the fundamental enantioselection mechanisms in CE are the same as in chromatography, migration is driven by electrophoresis. Enantiomers of a chiral drug compound will have the same charge density, so chiral separation in CE is not commonly based on electrophoretic separation, in which different migration velocities are an effect of different charge densities of analyte components. For enantiomers, both the electroosmotic flow and the electrophoretic mobility of the analyte are equally nonstereoselective. What distinguishes enantiomers in chiral CE is their interaction with a chiral selector.

Chiral separation by CE can be achieved by a direct or an indirect method. Direct separation is the more common approach. The chiral selector is dissolved in the running buffer, where it interacts selectively with the enantiomers to form reversible and transient diastereoisomeric or inclusion complexes of differing effective mobility. In indirect chiral CE separation, the enantiomers form covalent diastereomeric derivatives with a chiral reagent. A chiral selector is unnecessary in this approach because of the different electrophoretic mobilities of the diastereomeric derivatives.

Examples of chiral CE separations of racemic drugs are the following. (*R*)-(−)-ketoprofen has successfully been separated from ketoprofen and detected (Fig. 4).[25] (*S*)-(+)-ketoprofen is the active component. Also, simultaneous chiral separation of a basic drug compound, 2(*R*)-*N*-[1-(6-amino-pyridin-2-ylmethyl) piperidin-4-yl]-2-[(1*R*)-3,3-difluorocyclopentyl]-2-hydroxy-2-phenyl-acetamide, and its chiral acidic intermediate, (*R*,*R*)1-(2,2-difluorocyclopentyl)-phenylacetic acid, has been achieved by CE using a single-isomer CD, octakis (2,3-diacetyl-6-sulfo)-γ-CD (ODAS-γ-CD).[22] Carnitine has been separated using 50 mM DM-β-CD in 20 mM phosphate buffer (pH 4.3) as chiral selector.[23] The separations are done at 30°C in a fused-silica capillary, dynamically coated with triethanolamine present in the background electrolytes.

Similarities and differences in chiral separation by chromatographic and capillary electrophoretic techniques

HPLC remains the dominant technique for chiral separation in industry. CE has become well accepted in academic laboratories. Current GC, HPLC, and CE instruments are automated. Chiral separation in CE relies on a chromatographic separation principle. Nevertheless, there are significant differences, as shown in Table 3, between these techniques. The property of

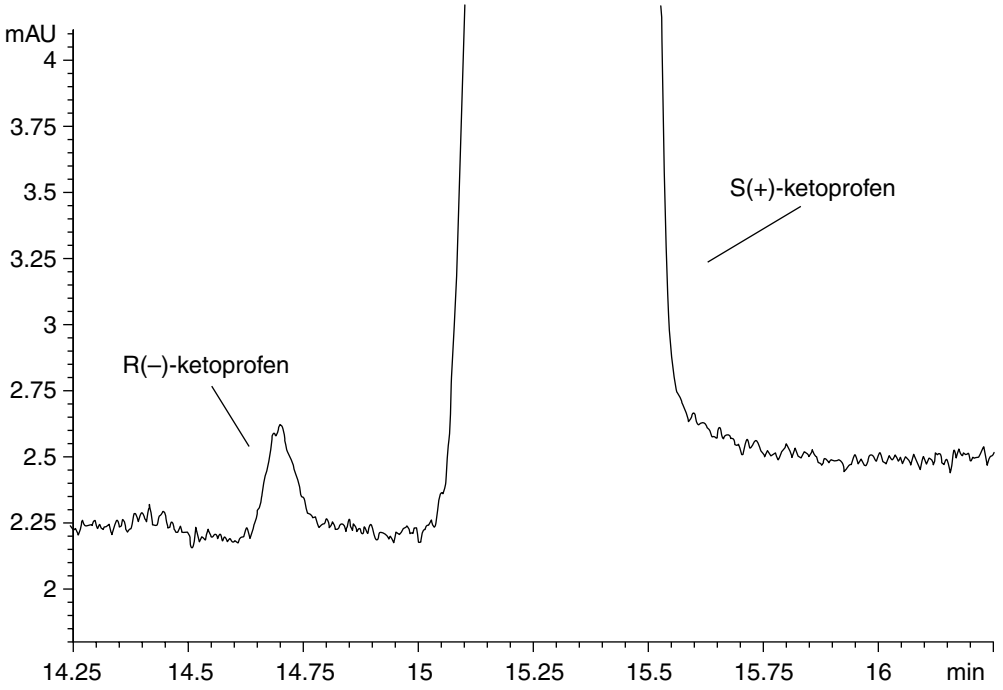

Fig. 4 Detection of the (*R*)-(−)-ketoprofen impurity contained in Enantyum tablets using chiral CE. Conditions: a 20 mM phosphate–20 mM triethanolamine background electrolyte at pH 5.0, with 50 mM of tri-*O*-methyl-β-CD; temperature, 35°C; applied voltage, 20 kV, 8.1 mA. (From Ref.[25].)

electrophoretic mobility in chiral CE in particular, and its ability to be selective for the analytes residing in the same phase, is responsible for all the differences. Another important point is that in chromatographic techniques, except with a chiral mobile phase additive, the analyte is virtually immobile when associated with a chiral selector. By contrast, in CE the analyte selector complex is commonly mobile.

In studies of a chiral drug candidate and its possible metabolites in preclinical and clinical Phase I to Phase III, most of the biological sample matrices, such as urine, plasma, serum, saliva, cerebrospinal fluid, and tissue homogenates, are more compatible with CE than chromatographic techniques. Moreover, it is not possible in GC and difficult in chiral HPLC to achieve the chiral separation of a drug and its phase I and phase II metabolites in a single run. By contrast, this is possible with chiral CE, as in the simultaneous chiral separation of Phase I and Phase II metabolites of chiral antihistaminic drug dimethindene.[24]

In summary, there are significant differences between chiral separations in pressure-driven HPLC and electrically driven CE systems. These differences are advantageous in that they make the techniques complementary. The rules and dependencies observed in one technique, however, are not necessarily applicable to the other.

Recently developed techniques for chiral separation: chiral membranes

HPLC is useful because of its preparative-scale capability. The method is generally slow and labor-intensive, however, requiring specialized engineering approaches for acceptable throughout. In comparison, chiral membrane separation offers significant advantages in simplicity, cost, and throughput.[28]

Polypeptides or modified polypeptides have been tested for use in chiral membrane separation. The partition behavior of optical isomers will largely be influenced by the structure and number of ''recognition sites'' in the polypeptide membrane. Polypeptides could be designed on demand and used to build polypeptide films/membranes.[29] Polypeptide membranes have shown very high enantiomer permeation rates with encouraging selectivity for chiral drug separation.[30] Peptide hydrophobicity, molecular weight, and secondary-structure formation propensity of specially designed peptides will be considered in future work on chiral separation membranes, as polypeptides could be designed to behave like certain proteins in human body and thus to achieve high biomimetic enantioselectivity. This approach would appear to promise very high enantioselectivity and high permeation rates.

Table 3 Differences between HPLC and CE as chiral separation techniques

	HPLC	CE
Instrument, cost, and safety	Expensive columns, consumption of a relatively high amount of buffer solutions, and hazardous organic modifiers.	Simple instrument: no pump, injector valves, and detector cells are required; a minute amount of solvent and an extremely low amount of chiral selector and buffer are required; environmentally friendly and inexpensive.
Chiral selector	Immobilized; great number of commercially available CSPs; a combination of chiral selectors is difficult or at least time-consuming.	Commonly mobile, commercially available chiral selectors are inexpensive, chiral selectors can be mixed in any desired ratio (only limited by the solubility).
Selectivity and efficiency	Chiral separation selectivity may in the best case approach the thermodynamic selectivity of the chiral recognition but will never exceed it; the separation efficiency sometimes is poor.	Separation selectivity may easily exceed the thermodynamic selectivity of the recognition; a chiral separation even in the absence of chiral recognition is, in principle, feasible; high peak efficiency.
Manipulation of mobility terms	Impossible to adjust the selectivity of chiral separation without changing the affinity pattern of the enantiomers toward chiral selector.	Possible to adjust the enantiomer migration order without reverting the affinity pattern between enantiomers of the analyte and a chiral selector.
Separation scale	Semipreparative and preparative scale.	Analytical scale, very small sample volumes.
Reproducibility	Good; high success rate.	Relatively poor and low success rate.
Method development	Relatively slow; changing and conditioning a column is time-consuming.	Rapid; changing a capillary and/or chiral selector takes only few minutes.

CONCLUSIONS

It is necessary to consider the chiral nature of a compound in drug research and development and the drug regulatory process. Enantiomers of all chiral bioactive molecules must be separated and tested. The FDA and regulatory authorities in Europe, China, and Japan have provided guidelines indicating that only the active enantiomer of a chiral drug should be brought to market. Chiral techniques have been developed for the separation and analysis of chiral drugs. Among these, HPLC based on CSPs is widely employed for the assays of drug enantiomers in pharmaceutical preparations and biological fluids. More recently, CE plus chiral selector additives to the running buffer have been used for the same purpose. In both chromatographic and electrophoretic methods, different types of chiral selectors, including CDs, crown ethers, quinine, chiral surfactants, polysaccharides, proteins, and macrocyclic antibiotics, have successfully been used for chiral separation of drugs and drug candidates. In many cases, HPLC and CE are complementary with respect to enantioselectivity. The development of novel separation techniques is an active area of research. Ones showing high throughput and high enantioselectivity are likely to be important to commerce, in view of the increasing trend in marketing single-enantiomer drugs.

ARTICLE OF FURTHER INTEREST

Chromatographic Separation, p. 481.

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