International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.2, pp 1584-1594, April-June 2010

Enantioseparation of Chiral Drugs – An Overview

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ABSTRACT: The belief that pure single enantiomers would provide safer and more effective alternatives to racemates, has resulted in a greater stress on the evaluation and development of single isomers as drug products. Almost half of the drugs in use today are chiral. It is well established that the pharmacological activity is mostly restricted to one of the enantiomers and the individual enantiomers of racemic drugs frequently differ in their biological effects. In many cases, the inactive enantiomer shows unwanted side effects or even toxic effects. For pharmacological studies of such drugs, there is, therefore a need for an effective means of separating and quantifying the enantiomers in biological samples. The chiral switch process (racemate to single enantiomer) has resulted in a number of agents being re-marketed as single enantiomer products.
Analytical methods which have been used for enantioseparation, include diastereomeric crystallization, biocatalysis , chromatographic techniques [thin layer chromatography, gas chromatography, super- and sub-critical fluid chromatography, nano-LC, high-performance liquid chromatography] , affinity electrokinetic chromatography, electromigration techniques [capillary electrophoresis (CE: CZE, MEKC, MEEKC) and capillary electrochromatography (CEC)]. The application of nanoparticles or nanostructured materials to enantioseparation has been recently described. Multimodal HPLC screening of polysaccharide-based CSPs enabled the rapid and successful enantioseparation of a large number of chiral drugs.The existing commercial projects have demonstrated that it is often cheaper to produce single enantiomeric drugs with chromatography rather than use traditional technologies such as crystallization and/or asymmetric synthesis. Recent achievements in enantioseparation of chiral drugs have ben reported. The success of chiral separation achieved by CE has been extended to microfabricated electrophoresis devices. Coupling electromigration techniques with MS have already been shown to be means for improving detection sensitivity. Optimization of the separation is still not quite understood and further research in this direction is required.
Keywords: Enantiomers, Chromatography, Capillary electrophoresis, Enantioseparation.

INTRODUCTION

Chiral molecules are constituents of a large proportion of therapeutic agents. The separation of enantiomers is of great interest to the pharmaceutical industry since more than half of pharmaceutically active ingredients are chiral. Chiral compounds exist in two enantiomeric forms, which have identical molecular formula but whose structural arrangement form non-superimposable mirror images. Most bimolecules, for example, enzymes, proteins, hormones, nutrients, sugars, fats, and many others are chiral. In nature, the chirality of a molecule is often as important as its chemical makeup. Moreover, our body recognizes chirality. There can be marked differences between enantiomers in their pharmacological profile [1]. For example, the enantiomers of chiral drugs such as omeprazole, ibuprofen and DOPA exhibit different pharmacological and pharmacokinetic activities because they interact with enzymes and receptors consisting of amino acids and other chiral biomolecules [2].

In many cases, one enantiomer is the active pharmaceutical ingredient while the other can be benign or even toxic. Thalidomide racemate was one of the first drugs recognized to cause birth defects in humans. Now the scientists know that, (-)(S)-thalidomide caused the severe side-effects and thousands of babies were born with missing or abnormal arms, hands, legs, or feet. Nowadays, the regulatory bodies want drugs that are submitted for

approval to be single enantiomers, if it is at all possible. Thus, pharmaceutical firms continue to develop chiral drugs as single enantiomers in a direction to carry out racemic switches [3-5]. This also includes active pharmaceutical ingredients and intermediates, which are used as the framework for single enantiomer drugs. In view of pharmacological studies of such drugs, the separation of enantiomers constitutes a major challenge from the standpoint of efficacy and safety of drugs. Potential advantages of single enantiomer products are: less complex, more selective pharmacodynamic profile, potential for an improved therapeutic index, less complex pharmacokinetic profile, reduced potential for complex drug interactions, and less complex relationship between plasma concentration and effect [5]. The first drug sold as a single enantiomer was the anti-turberculosis drug ethambutol which was sold as a single enantiomer shortly after its discovery in 1961.In fact, the name "ethambutol" refers specifically to the dextrorotatory (+) isomer of 2,2'-(ethylenediimino)-di-1-butanol. Applications of chiral technology to drugs fall into two categories. The first is the retrospective area, which is the attention paid to racemic drugs already being sold and which may be switched to single enantiomers. The second is the prospective arena, which is developing new drugs as single enantiomers from the get go, which had never previously been sold in racemic form. This concerns new chiral drugs in the pipeline, and those to be discovered and commercialized in the future.The separation of two enantiomers present in a racemic mixture or any mixture of enantiomers, is called resolution [6], moreover, it is not a simple procedure.

Techniques used for separation of enantiomers

The main groups of techniques for the separation of enantiomers are shown in Figure 1.

Enantiomer separation methods, with an emphasis on separation by chiral inclusion complexes and crystallization, biological methods, preparative liquid and gas chromatographic methods have been reported. It is not surprising that intensive efforts have been directed world-wide towards the economic production of enantiomerically pure drugs. However, the conventional method of separating the optical isomers of racemic compounds has always been difficult and expensive. Chiral separation can be used to simultaneously produce both enantiomers (dual-isomer recovery) or it can be used in a way that generates only one enantiomer (single-isomer recovery). The former has application to the manufacture of chiral intermediates when both enantiomers have market outlets. The latter has application to the manufacture of either end-use chemicals or intermediates when only one of the enantiomers has a market. In the mode of dual-isomer recovery, the chiral technology selects one of the isomers, leaving the other behind and both are ultimately recovered by conventional means. In the mode of single-isomer recovery, the chiral technology also selects one of the isomers, but in addition it deliberately racemizes the other isomer, and recycles it into the selection process, thus ultimately producing the one wanted isomer. Alternatively, the conventional method of separating the optical isomers of racemic compounds involves the preparation of diastereomeric intermediates which can then be separated from each other by differential crystallization [7], hydrolysis and purification. The resolution of *N*-methylamphetamine (MA) was achieved with the resolution agents *O*,*O*′-dibenzoyl-(2*R*,3*R*)-tartaric acid monohydrate (DBTA) and *O*,*O*′-di-*p*-toluoyl-(2*R*,3*R*)-tartaric acid (DPTTA). After partial diastereomeric salt formation, the unreacted enantiomers were extracted by supercritical fluid extraction [8]. Inclusion complexation of a racemic compound with a chiral host compound, which gives chiral host-chiral guest inclusion compounds, from which the chiral guest can be obtained. When this separation is combined with distillation technique, for example, enantiomer separation can be accomplished by fractional distillation in the presence of a chiral host compound. This represemts a modern and "green" procedure of enantiomer separation [9].

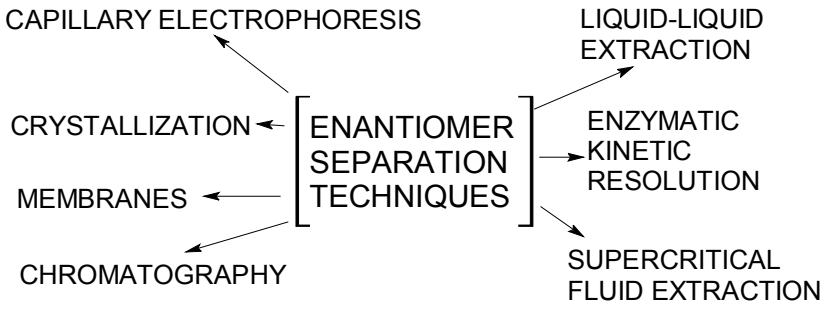

Figure 1. Enantiomer separation methods

Successful techniques such as chiral chromatography, enzyme, and diastereomeric resolution are now complemented by a new technique which meets the demanding criteria of being easy to screen, rapid, and with low capital cost. Membranes extraction technology for enantiomer separation utilizes enantioselective inclusion complexation *via* a chirally selective host molecule coupled to organic solvent nanofiltration for host separation. Then it is recycled, to afford an efficient practical way to resolve diastereomeric mixtures resulting in separation, and resolution, of greater than 95 % ee of racemic mixtures. So, it is a quick and efficient method for enantiomer separation [10]

Chromatography is the most famous way of enantiomers separation. A more promising preparative scale separation technique which has attracted attention recently is to resolve such substances by chromatographic techniques, using chiral adsorbents [chiral stationery phases (CSPs)] which work essentially by a lock-and-key mechanism. Industrial scale separations have been successfully accomplished using simulated moving bed (SMB) processes. If cross-linked, immobilized enzymes are useful organic reagents, then so are enzymes ligated to a solid support and packed into chromatographic columns. Not only proteins, but all biologically derived materials, because of their construction from enantiomerically pure building blocks such as amino acids, and sugars, are suitable as stationary phases in chiral chromatography. The stationary phases find applications in gas chromatography (GC), high performance liquid chromatography (HPLC), and supercritical fluid chromatography (SFC). A number of chiral stationary phases for the separation of enantiomers in drugs and biological compounds have already been developed, and their use is presently widespread [11]. . Recent trends in enantioseparation of chiral drugs have been reported [12]. Micropreparative enantioseparations based on various electromigration techniques in the development and use of chiral drugs was reported [13].

The separation of enantiomers is most readily accomplished by means of chiral chromatography. Chiral chromatography includes the use of gas chromatography (GC), supercritical fluid chromatography (SFC), capillary electrophoresis (CE), and high performance liquid chromatography (HPLC). High performance liquid chromatography is the most widely used method. A broad spectrum of chiral derivatization reagents have been developed for GC, HPLC, and CE [14]. Resolution of enantiomers of chiral drugs has been recently reviewed [15].

Enantiomers are not readily separated by conventional means, such as recrystallization or fractional distillation, since they have the same solubilities, melting points,, boiling points, etc. So, special means are necessary requirement for "resolution" of two enantiomers. One common strategy for resolution is often to take advantage of the circumstance that, while enatiomers have the same solubilities and cannot be readily separated by simple recrystallization, diastereoisomers have different solubilites. The two enantiomers present in a racemic mixtrue can be reacted with a pure enantiomer of a chiral compound (called a resolving agent) which we have on hand (many occur in pure form in nature). This will form a compound with two chiral centers, and will give rise to two different diastereoisomers which can be separated from each other. Following this separation the chiral resolving agent can be removed by through some chemical reaction to give the two separate enantiomers. The chiral resolving agent can also be recovered for re-use. The classification of separation of enantiomers by crystallization into different main categories has been recently reported [16].Gas chromatography, high-performance liquid chromatography, capillary electrophoresis, supercritical fluid chromatography have been developed for chiral separations [17-20].

The selection of the chiral stationary phase is an important consideration in separating enantiomers when using high-performance liquid chromatography, supercritical fluid chromatography, and simulated moving bed chromatography. Types of chiral stationary phases include Pirkle type CSPs, polysaccharide CSPs, cavity CSPs, ligand exchange CSPs, protein bound CSPs [21]. Separations of molecules with multiple chiral centers are more difficult since chiral stationary phases are normally good at separating enantiomers but not at separations of diastereoisomers. Separations of molecules with multiple centers can, however, are achieved by careful selection of operating conditions. One such example is the separation of the various isomers of nadolol (a non-selective beta-blocker used in the treatment of high blood pressure, migraine headaches, and chest pain). This separation was achieved using 0.1% of ethanesulfonic acid in the hexane-ethanol mobile phase with "Chiralpak AD-H" (Chiral Technologies) as a stationary phase [22].

Enantioselective chromatography in recent years is further expanding its methodology, usefulness and multidimensional applications [23.] An overview of develoments in CZE, EKC, and CEC on the progress in electromigration techniques and new methodological developments including new techniques, new chiral selectors as well as new chiral stationary phases for CEC were reported [24,25]. Chromatographic techniques such as thin layer chromatography (TLC), gas chromatography (GC) and

supercritical fluid chromatography (SFC), chiral ligand-exchange capillary electrophoresis have been used for enantiomer separation for more than two decades [26-30]. Supercritical technology enables the use of environmentally green solvents like carbon dioxide in producing pharmaceutical compounds and only a small percentage of an organic solvent is required to solubilize the compound and serve as a cosolvent with the carbon dioxide.Unlike many organic solvents, carbon dioxide is relatively inexpensive, non-toxic and easily recirculated.

Enantioselective HPLC analysis

There are basically two options for chiral HPLC analysis namely direct and indirect approach [31]. The direct chiral high performance liquid chromatographic technique, with reference to application in enantiospecific drug analysis was reported [32]. In the indirect approach, drug enantiomers are derivatized with an enantiopure chiral reagent to form a pair of diastereomers, which may be then separated on a conventional chromatographic column, since diastereomers exhibit different physicochemical properties. In the direct method, transient rather than covalent diastereomeric complexes are formed between the drug enantiomers and a chiral selector present either added to the mobile phase (CMPA) or coated/bonded to the surface of a silica support (CSP). The technique relying on chiral stationary phases (CSPs) are preferred as they offer specific advantages over indirect methods. There is no need to chemically manipulate the analytes, interference with sample matrix, chiral purity of the chiral stationary phase (CSP) does not need be known, fast analysis, method can be readily scaled to commercial production, online coupling with MS or NMR permits structure identification [21]

High-performance liquid chromatography (HPLC) is a powerful tool for the enantioselective separation of chiral drugs [33]. However, the selection of an appropriate chiral stationary phase (CSP) and suitable operating conditions is a bottleneck in method development and a time- and resource-consuming task. Multimodal screening of a small number of CSPs with broad enantiorecognition abilities has been recognized as the best strategy to achieve rapid and reliable separations of chiral compounds [34]. Supercritical fluid chromatography coupled to a hybrid mass spectrometer (Q-Tof2) equipped with electrospray ion source has been used to separate and characterise a wide range of pharmaceutical racemates. Supercritical fluid chromatography (SFC) is best known for chiral separation and, in general, is a superior chromatographic technique for chiral separation than normal phase LC. SFC-MS does not have the problems one would encounter in NPLC-MS [35].

The enantioseparation of reboxetine by HPLC was investigated using chiral stationary phases (CSPs) containing cellulose Tris(3,5-dimethyl phenyl)carbamate on silica gel (Chiralcel OD column) as the chiral selector. Different n-hexane/alcohol mixtures were tested as mobile phase and the best results were obtained by using a mobile phase composed of n-hexane and 2-propanol (80:20, v/v) [36]. Enantiomeric separation of nadolol, a β-blocker with three chiral centers was obtained by high-performance liquid chromatography on a column packed with heptakis (6-azido-6-deoxy-2, 3-di-O-phenyl carbamolyted) β-cyclodextrin bonded chiral stationary phase. Resolution of three stereoisomers of nadolol was obtained with a complete separation of the most active enantiomer, (RSR)-nadolol [37]

Capillary electrophoresis and enantiomer separation

Various methods such as crystallization, biocatalysis, chromatographic methods (HPLC, GC, TLC), capillary electrophoresis, in biological material (urine, serum) for enantioseparation of beta-blockers used in clinical practice (e.g., propranolol, atenolol, metoprolol), have been reported [38]. More recently, electromigration techniques, such as capillary electrophoresis and capillary electrochromatography, have been shown to be powerful alternatives to chromatographic methods [39,40]. Scientists described the application of capillary electrophoresis (CE) to pharmaceutical analysis. The different electrophoretic modes available and their advantages for pharmaceutical analysis are described [41-43].

Capillary Electrophoresis has become an important analytical tool for separation of charged analytes due to the enhanced separation efficiency. In addition, the CE method when used in combination with a micellar pseudostationary phase offered a number of advantages for separation of neutral analytes, called micellar electrokinetic chromatography (MEKC). Scientists have recently employed chiral polymeric surfactants (molecular micelles) for enhanced enantiomeric separations of racemic mixtures using MEKC- Fluorescence anisotropy [44]. The use of Fluorescence anisotropy to probe chiral recognition has been reported [45]. Enantiomer separation of chiral pharmaceuticals by capillary electrochromatography (CEC) has been reported with open-tubular capillaries (o-CEC)[coated with a thin film containing cyclodextrin derivatives, cellulose, proteins, poly-terguride or molecularly imprinted polymers as chiral selectors], with packed capillaries (p-CEC) [typical chiral HPLC stationary phases such as silica-bonded cyclodextrin or cellulose derivatives, proteins, glycoproteins, macrocyclic

antibiotics, quinine-derived and 'Pirkle' selectors, polyacrylamides and molecularly imprinted polymers are used as chiral selectors] or with monolithic capillaries prepared by in situ polymerization into the capillary [46]. Enantioresolution of basic compounds with human serum albumin by means of affinity EKC (AEKC)-partial filling technique depends on the hydrophobicity, polarity, and molar volume of compounds [47].The enantiomeric separation of promethazine and trimeprazine enantiomers by affinity electrokinetic chromatography (AEKC)-partial filling technique using human serum albumin (HSA) as chiral selector [48]. The enantioresolution of donepezil, a centrally acting acetylcholine esterase inhibitor, has been described by a CZE method suitable for applications in pharmaceutical field [49].

The dual cyclodextrin systems (consisting of one highly-sulfated (a-, β-, and γ-HSCD) and one neutral cyclodextrin, i.e. either heptakis (2,3,6-tri-O-methyl)-β-CD (TMCD), heptakis (2,6-di-O-methyl)-β-CD (DMCD) or hydroxypropyl-β-CD (HPCD)) based separation strategy was reported (50). The application of CE for enantioseparation of enantiomers of chiral drugs has attracted increased interest in the last decade [51]. Capillary electrophoretic method has been developed for the enantioselective analysis of amisulpride in pharmaceutical formulations, using beta-cyclodextrin sulfate as the chiral selector [52]. CD-modified microemulsion EKC as a CE technique has been applied to the chiral separation of atropine, scopolamine, ipratropium and homatropine [53]. A comparison between chiral cyclodextrin-modified microemulsion electrokinetic chromatography (CD-MEEKC) and cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) for the enantiomeric separation of esbiothrin was reported [54]. Excellent enantioseparation of profens has been achieved using macrocyclic glycopeptide antibiotic, eremomycin, as a chiral selector in CE [55]. Enantiomeric separation of primaquine, an anti-malarial drug, was achieved by cyclodextrin-modified micellar electrokinetic capillary chromatography [56]. A chiral microemulsion electrokinetic chromatography method has been developed for the enantiomeric separation of 3,4-dihydroxyphenylalanine (dopa), its precursors phenylalanine and tyrosine, and the structurally related substance methyldopa [57]. A capillary zone electrophoresis (CZE) investigation on the enantiomeric separation of lomefloxacin, gatifloxacin, pazufloxacin and ofloxacin was achieved using hydroxypropyl-β-cyclodextrin (HP-β-CD) as the chiral selector [58]. Optical pure (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, a chiral crown ether, was successfully used as a chiral selector for the stereoisomeric separation of numerous real pharmaceutical compounds. The advantages and disadvantages of using chiral crown ether for stereoisomeric separations were also reported with those using sulfated CDs [59].

Hyphenation technologies such as micellar electrokinetic chromatography (MEKC-MS) using molecular micelles and capillary electrochromatography-mass spectrometry (CEC-MS) for a highly efficient separation, and a highly sensitive detection of various classes of enantiomers have been reported [60]. The achievements of enantioseparation of adrenergic drugs and application of liquid chromatography and capillary electrophoresis methods in clinical and pharmaceutical analysis were reported [61]. A capillary electrophoresis method to determine the impurity of etodolac enantiomers, (2-Hydroxypropyl)-β-cyclodextrin (HP-β-CD) allowed determination of 0.2% of (R)-(−)-etodolac in (S)-(+)-etodolac and method validation showed adequate linearity over the required range [62].

The feasibility of using a new and more versatile polymeric chiral surfactant, i.e., poly(sodium *N*-undecenoxy carbonyl-L-leucinate was investigated for simultaneous enantioseparation and detection of eight structurally similar β-blockers with tandem UV and MS detection and the CMEKC−ESI-MS method was more suitable as a routine procedure for high-throughput separation of β-blockers with high sensitivity [63]. Immobilized stationary phases represent a new perspective for analytical as well as preparative scale separation of chiral species. The new immobilized chiral stationary phase CHIRALPAK IA has proven to be useful for the chiral separations of propranolol, metoprolol, guaifenesin, and α-tetralol enantiomers [64].

Simulated moving bed for chiral separation of drugs

In chromatography, the simulated moving bed (SMB) technique is a variant of high performance liquid chromatography. Although chiral HPLC is being a standard technique, but it is a non-continuous process and has high solvent consumption and low productivity. To overcome this problem, SMB technology has been utilized to pharmaceuticals chemicals, particularly to enantiomer separation. S-bupivacanine with the pharmacological activity of epidural anaesthesia was isolated and separated from R-bupivacanine by SMB chromatography [65]. The new CSP was found to be efficient for the enantioseparation of chiral drugs [66]. Simulated counter-current moving bed systems have been applied to enantioresolution trials on a series of racemic non-steroidal antiflammatory drugs (NSADs), anti-

asthmatics and β-blocker drugs [67]. Concurrently, study on integrated processes involving both adsorption and crystallisation is in progress to resolve more difficult drugs. The enantiomeric resolution of the racemic mixture of α-ethyl-2-oxo-1-pyrrolidineacetamide was carried out by simulated SMB, using at least three columns filled with chiral stationary phase [68].Tramadol is a monoamine uptake inhibitor and centrally-acting analgesic, used for treating moderate to severe pain and separation of its enantiomers was creported [69].

Nano-chiral technology and enantioseparation

Chiral, nanoscale science and technology was reviewed relating to nanotechnology in the service of asymmetric synthesis, chiral separations, and analysis [70]. Nano-chiral technology describes the nanoscale approaches to chiral technology such as chiral separation and detection and enantiomeric analysis [71, 72]. Researchers demonstrated enantioselectivity determination for systems of *cinchona* alkaloid carbamates and N-blocked amino acids using HPLC-MS and dynamic titration technique [73-75]. Scientists have carried out chiral separations using an antibody-based nanotube membrane. These membranes are based on alumina films that have cylindrical pores with monodisperse nanoscopic diameters of size 20 nanometers. Further, Silica nanotubes were chemically synthesized within the pores of these films, and an antibody that selectively binds one of the enantiomers of the drug was attached to the inner walls of the silica nanotubes. These membranes selectively transported the enantiomer that specifically binds to the antibody, relative to the enantiomer that has lower affinity for the antibody [76]. New LC separation techniques and applications include a chiral separation based on single walled carbon nanotubes conjugated with bovine serum albumin [77]. Scientists studied the potentiality of nano-liquid chromatography (nano-LC) for the enantiomeric resolution of both basic and acidic compounds of pharmaceutical interest using a vancomycin modified silica stationary phase [78].

Chiral and achiral ionic liquid and enantioseparation

A chiral ionic liquid S-[3-(chloro-2-hydro xypropyl)trimethylammonium] [bis ((trifluoro methyl)sulfonyl)amide] can be successfully used both as co-electrolyte and as a chiral selector for CE to achieve chiral separation. A variety of pharmaceutical products including atenolol, propranolol, warfarin, indoprofen, ketoprofen, ibuprofen and flurbiprofen, were successfully and baseline separated with the use of this chiral ionic liquid as electrolyte. However, additional chiral selector(s) are needed to provide the three-point interactions needed for some chiral separations [79]. In micellar electrokinetic chromatography separation of acidic analytes with intelligently designed synthetic chiral ionic liquids, the comparison of chiral separation of anionic and cationic surfactants demonstrated that the electrostatic interaction between the acidic analyte and cationic micelle plays a profound role in enantioseparation [80].

Researchers proposed a method for the separation of the two enantiomers of ofloxacin enantiomers. The mechanism of chiral discrimination was based on the stabilities of the copper(II) binary complexes and their ternary diastereomeric complexes with amino acids formed in solution and stationary phase [81]. Two new types of negatively charged sulfate and sulfonated groups for polysaccharide CSPs were utilized as chiral stationary phases for capillary electrochromatography and capillary electro chromatography–mass spectrometry and CEC–MS demonstrated excellent durability as well as excellent reproducibility of retention time and enantioselectivity [82]. Recently, researchers introduced a new approach to chiral separation and analysis of amino acids by chiral complexation and electrospray high-field asymmetric waveform ion mobility spectrometry coupled to mass spectrometry (ESI-FAIMS-MS), to the separation of the drug compound terbutaline. Terbutaline enantiomers complexed with metal ions and an amino acid formed diastereomeric complexes of the type $[M^{II}(L\text{-}Ref)_2((+)/(-)\text{-}A)\text{-}H]^+$, where M^{II} is a divalent metal ion, L-Ref is an amino acid in its L-form, and A is the terbutaline analyte, and were separable by Field Asymmetric Ion Mobility Spectrometry-Tandem Mass Spectrometry (FAIMS) [83]. Using an achiral ionic liquid, 1-butyl-3-methylimidazolium chlorine ([BMIM] Cl), as an additive and beta-cyclodextrin (beta-CD) as a chiral selector, the enantiomers of chlorpheniramine, the precursor of chloramphenicol and of loxacin were separated by capillary zone electrophoresis. The results suggested that there are synergistic effects of [BMIM] Cl as an additive for the enantiomeric separations as well as it can provide a new method for the separation of chiral drugs which are hard separable under common electrophoresis conditions [84].

Kinetic resolution-catalyzed by enzymes

Enzymes have competed well with chemical methods for resolution. In kinetic resolution-catalyzed by lipases, only one enantiomer of a chiral reactant fit to the active site properly and is able to undergo the reaction while the second enantiomer is left unreacted and in enantiomerically pure form [85]. Dynamic kinetic resolution is a powerful tool to transform a racemic mixture into one enantiomer. This strategy overcomes the limitation of the maximum 50% yield in a kinetic resolution by combining it with an *in situ* racemization

of the substrate. Recently, the coupling of enzymes and transition metals for dynamic kinetic resolution of a variety of molecules has attracted considerable attention and a deeper understanding of the compatibility of these two catalysts has been achieved [86]. Enzymatic methods used for the enantiomeric separation of *rac*-ibuprofen were reported [87]. An enzymic membrane reactor consisted of a lipase immobilized polymeric membrane, an organic phase dissolving ester and an aqueous phase was reported for the optical resolution of racemic ibuprofen ester [88].

Gas chromatographic–mass spectrometric method and enantioseparation

Separation of the enantiomers of ibuprofen was achived by a gas chromatographic–mass spectrometric method using selected ion ionization and tandem mass spectrometry on a chiral capillary column [88]. In drug testing, the presence of methamphetamine in urine is generally confirmed by a gas chromatography-mass spectrometry (GC-MS) method. Derivatization of the compound to a perfluoroalkylamide, prior to confirmation, typically yields better chromatographic separation 89. GC peaks of (R)-(-)- and (S)-(+)-isomers of amphetamine, 3,4- methylenedioxyamphetamine (MDA), N-methyl-MDA (MDMA), and N-ethyl-MDA (MDEA) were resolved [90].

CONCLUSIONS AND PERSPECTIVES

Drugs with chiral center represent a significant portion of the pharmaceuticals on the market. A better understanding of the stereochemical issues of racemic drugs will assist their clinical use and important aspect of chiral analysis deals with the separation of stereoisomers, of chiral compounds such as chiral drugs. Chiral resolution is an important tool in the production of optically active drugs. Isomer specific pharmaceuticals often exhibit increased potency, higher bioavailability and reduced side effects when compared to racemic pharmaceutical compounds. The pharmacological implications of chirality in drugs and the firm legislation in this regard have led to the development of efficient enantioselective technologies to obtain enantiomerically pure compounds. In this regard, pharmacists, as the drug experts, should be aware of the clinical implications of chirality. Experts are of the opinion that development of pure enantiomers is currently more economically feasible and existing commercial projects have demonstrated that it is often cheaper to produce single-enantiomer drugs with chromatography than with traditional technologies such as crystallization or asymmetric synthesis. A continued interest in the commercial marketing of individual enantiomers will undoubtedly impact our clinical practice.

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