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Reflections on Chiral Chromatographic Analysis

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Prominence of chirality of drugs has been increasingly recognized, and the consequence of using them as racemates or as enantiomers has been frequently discussed in pharmaceutical literature during recent years. This awareness on issues pertinent to chirality in drug design and drug development had increased chiral explorations. This in turn had augmented the need for good chiral analytical tools. Consequently, now a spectrum of enantiospecific analytical methodologies such as physical (includes optical rotation, circular dichroism, nuclear magnetic resonance spectroscopy, infrared spectroscopy, and powder X-ray diffraction), biological (viz. enantiospecific immunoassay and radioreceptor assay) and separation science methods (includes gas chromatography, supercritical fluid chromatography, high performance liquid chromatography and capillary electrophoresis) are available for chiral examination of drugs. In this context, the present article provides a gist of various options available for chiral analysis and examines in detail the direct chiral high performance liquid chromatographic technique, with reference to application in enantiospecific drug analysis.

Over the last two decades interest in pharmacodynamic, pharmacokinetic and toxicological properties of the enantiomers of racemic chiral drugs has increased significantly and become a key issue for both the pharmaceutical industry and regulatory authorities[1-9]. This current awareness is fuelled by the exponential explosion of chiral technology in the area of asymmetric synthesis and enantiospecific analysis, particularly in the area of chromatography. As a consequence of the advances in chiral technology, new drug development is focusing on single stereoisomers and the development of racemic mixtures will require scientific justification. Indeed, several pharmaceuticals currently marketed as racemates are undergoing re-evaluation as single isomer products or chiral switches[10-16]. Irrespective of the decision to develop either a single isomer or racemic drug, in the current regulatory climate there will be a continuing requirement for enantiospecific analysis and thus modern drug development presents a considerable challenge for the pharmaceutical analysts and separation scientists[17,18].

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In pharmaceutical research and development sterochemical analytical methodology may be required to understand enantioselective drug action and disposition, chiral impurity profiling, examine stereochemical stability during formulation and production, evaluate dosage forms, enantiospecific bioavailability and bioequivalence studies of chiral drugs[19,20]. The intent of this article, therefore, is to provide a comprehensive overview of the methods available for the analysis of enantiomers of chiral drugs.

OPTIONS FOR CHIRAL EXAMINATION

Chiral examination generally requires the presence of a chiral environment, which may be provided as plane polarized light, an additional chiral compound or by utilization of the inherent chirality of nature. The majority of the chiral methodologies presented have been examined in detail in literature[21-29].

Physical methods:

Physical methods are the oldest and most frequently used methodologies for the inspection of chiral drug candidates. They include optical rotation, circular dichroism (CD),

nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR) and powder X-ray diffraction (XRD). Optical rotation is comparatively simple and can be used to distinguish between a racemate and its enantiomers as well as between one enantiomer and its mirror image[30]. NMR spectroscopy using chiral shift reagents or chemical solvating agents can be used as an identity test or for the quantification of the enantiomers[31]. It can be used to distinguish between enantiomers as well as the racemate. IR, XRD and melting range can distinguish between a racemate and its enantiomers, but not between one enantiomer and its chiral twin[10].

Biological methods:

The binding of chiral ligands to antibody binding sites may be stereoselective. Thus, if an animal is immunized with an optically pure hapten, the antibodies formed may demonstrate low affinity for the hapten antipode[32]. This forms the basis of chiral immunoassays. Stereoselective immunoassays have been developed to demonstrate the enantioselective disposition of racemic drugs such as ephedrine[33], pentobarbital[34], and warfarin[35]. Though immunoassays may be highly enantioselective, they are susceptible to interferences by drug metabolites, closely related analogues or by endogenous substances in biological fluids[32].

Separation science methods:

The most frequently employed technique in enantiospecific analysis involve the separation science techniques, in particular chromatographic method. Traditionally HPLC, to a lesser extent chiral gas chromatography (GC), chiral supercritical fluid chromatography (SFC) and capillary electrophoresis (CE) have been the method of choice for carrying out chiral separations. A gist on separation technique options GC, SFC and CE is presented before discussing chiral HPLC analysis.

Chiral gas chromatography:

Gas chromatography is the best technique for the separation of volatile, apolar and moderately polar enantiomers. Several less-volatile chiral compounds are also appropriate for GC analysis after suitable derivatisation. GC is also frequently employed to analyse compounds lacking UV or fluorescent activity. The usefulness of GC is well demonstrated by ~20000 chiral separations done with GC, two books[36,37], several reviews[38,39] and a database[40] dealing with chiral GC have been published.

Basically there are three types of chiral stationary phase (CSP) for GC namely CSP based on hydrogen bonding, chiral metal complexes and inclusion effects. Exhaustive account of these types of CSPs and their applications are reported[23]. Research and development of hydrogen bonding chiral stationary phases for GC has been very intense and there are many publications in the field. These are suitable for compounds containing polar functional groups. The CSPs based on metal complexation, on the other hand are suitable for compounds of much lower polarity and consequently higher volatility. The most popular technique is the use of chiral selectors (commonly cyclodextrins) that form inclusion complexes with the analytes. More than two thirds of chiral GC separations were done on CD-based CSPs[40].

Several examples of chiral-selective GC are cited in literature. CD-based CSPs has been employed to separate aminoalcohols such as alprenolol, metoprolol[41] and propranolol[42], NSAIDs such as fenoprofen, flurbiprofen and ibuprofen[43]. Amino acid, baclofene, is also separated by CD-based CSP[44].

Chiral super critical fluid chromatography:

Super critical fluid chromatography (SFC) has been general interest for sometime because of the advantages inherently present in this technique and it is a good technique for enantiomer separation[45-47]. Since super critical fluids have physical properties intermediate between those liquids and gases, they are of interest of use as mobile phases, and SFC is often regarded as a hybrid of GC and LC. The viscosity of such fluids is lower than that of ordinary liquids, which makes possible for rapid diffusion of solutes in the mobile phase. This will, of course, influence the chromatographic flow-rate that can be used in order to achieve a given resolution. Another obvious advantage is the fast evaporation of the mobile phase at atmospheric pressure. Similar to GC, the low viscosity and high permeability of the mobile phase offer fast analysis and high efficiency. Analysis time as short as 2 min have been reported for chiral analysis[48,49]. Consequently, the most promising features of SFC are the possibilities of fast chromatography without loss of resolution, low analysis temperature, and easy interfacing with mass spectrometry and GC detectors.

Almost every type of chiral stationary phase introduced in GC and LC practice has been used in SFC. The mobile phase CSP are relatively weak; therefore, the disturbing silanol effect is more pronounced in SFC, mostly in the case of monomeric CSPs. The decreased silanol effect and higher

loadability explain the advantage of polymeric CSPs over monomeric CSPs. Polymeric CSPs consists of silica particles covered with polymeric chiral molecules. In SFC practice the most frequently used CSPs are derivatised polysaccharide (amylose, cellulose)[50-53]. Chiral separation with SFC using amylose based CSP has been employed for the separation of fenoprofen[50], hexobarbital[50], flurbiprofen and ibuprofen[52]. Chiral separation using cellulose based CSP includes separation of metoprolol and warfarin[50].

Chiral capillary electrophoresis:

Over the past decade there have been major advances in chiral capillary electrophoresis (CE)[54,55]. This technique has been particularly successful in separating ionizable drug enantiomers[56] but it can also separate neutral compounds employing ionised selectors. Basically, CE is a pseudo-chromatographic technique. Partitioning of enantiomers between the chiral selectors and background electrolyte is necessary for chiral recognition. This is the reason why chiral-selective CE is categorized as capillary electrokinetic chromatography (EKC)[57].

In CE a wide spectrum of chiral selectors has been employed. These include cyclodextrin-based selectors, macrocyclic chiral selectors such as vancomycin, ristocetin A, avoparcin, rifampicin B and protein base selectors such as bovine serum albumin (BSA), human serum albumin (HSA)[58]. The application of this technique includes separation of enantiomers of profens (fenoprofen, flurbiprofen, ibuprofen and ketoprofen)[59], anticoagulant (warfarin)[60] and antimalarial agent (primaquine) has been reported by cyclodextrin modified-micellar electrokinetic capillary chromatography[61]. Terbutaline enantiomers separated by rifamycin B chiral selector[62].

A survey[63], showed that only about 20% of pharmaceutical companies used CE for chiral analysis. Although CE can be used for most of the drug assays where HPLC finds application, there is already a massive investment on HPLC systems. The consequence is that, in spite of few limitations, wide spread use of chiral CE in the pharmaceutical industry may depend on finding other areas where CE has a clear-cut advantage over HPLC to justify the purchase of the equipment.

Chiral HPLC:

Two main chromatographic options are available for the resolution of enantiomers: (1) the indirect approach where the drug enantiomers are derivatized with an enantiopure chiral reagent to form a pair of stable or covalent diastereomers; (2) the direct approach involving the formation of transient (unstable) rather than covalent diastereomeric complexes between the drug enantiomers and a chiral selector present either in the mobile or the stationary chromatographic phase.

Indirect method of analysis:

Therapeutic agents often contain chemical functional groups (amino, hydroxyl, epoxy, carbonyl and carboxylic acid) in their structures. They are transformed into covalently bonded diastereomeric derivatives using enantiomerically pure chiral derivatizing agent. The diastereomers formed unlike enantiomers, exhibit different physicochemical properties in an achiral environment and are subsequently separated as a result of differential retention time on a chromatographic column[64-68].

The success of this approach depends on the availability of stable enantiopure chiral derivatizing agent (CDA) and on the presence of a suitable reactive functional group in the chiral drug molecule for covalent formation of diastereomeric derivative. The reaction of a racemic, (R,S)-Drug with an optically and chemically pure chiral derivatising agent (R')-CDA will afford diastereomeric products, (R)-Drug-(R')-CDA + (S)-Drug-(R')- CDA. The scheme may be illustrated as follows: R,S(drug)+R'(CDA)⟶(R)-Drug-(R')-CDA+(S)-Drug-(R')-CDA (diasteriomeric complex). Unlike enantiomers, diastereomers have different physicochemical properties that render them separable on conventional highly efficient a chiral stationary phases.

The major advantage of the indirect technique is that conventional chromatographic mode (reversed-phase HPLC) may be utilized for the separation of the diastereomers. Thus, considerable flexibility in chromatographic conditions is available to achieve the desired resolution and to eliminate interferences from metabolites and endogenous substances. Moreover, the sensitivity of the method can be enhanced by the judicious choice of the CDA and the chromatographic detection system. But this indirect approach to enantiomeric analysis has some potential problems. These include enantiomeric purity of the CDA, racemization of the CDA during derivatisation, racemisation of the analyte during the derivatisation, stereoselective derivatisation (kinetic resolution).

The necessity for high optical purity and stability of the CDA may be illustrated by a consideration of the reaction products from a racemic drug and the R'-enantiomer of a CDA, which is contaminated with its S'-enantiomer: R,S

(Drug)+R'(S') (CDA)——▸(R)-Drug-(R')-CDA+(S)-Drug-(R')-CDA+(R)-Drug-(S')-CDA+(S)-Drug-(S')-CDA (Diastereomeric complex). In this case, an additional pair of diastereoisomers is formed (R)-Drug-(S')-CDA+(S)-Drug-(S')-CDA each of which is the enantiomer of one of the first pair. Thus, the enantiomers (R)-Drug-(R')-CDA+(S)-Drug-(R ')-CDA+(R)-Drug-(S ')-CDA+(S)-Drug-(S')-CDA would coelute in conventional chromatographic systems. Such contamination (or racemisation during the reaction) would lead to analytical error and this would be especially critical when attempting to analyse small quantities of one enantiomer in the presence of a large excess of its antipode. For most pharmacokinetic studies, optical contamination of the CDA of up to 1 % is admissable[69].

Direct method of analysis:

Direct enantiomer resolution on a chiral stationary phase may be necessary when the drug molecule does not contain a functional group suitable for derivatization. In the direct approach transient rather than covalent diastereomeric complexation occurs between the chiral selector/discriminator and the analyte (drug enantiomer). The chiral selector may be present in the mobile phase for use with conventional stationary phase or it may be incorporated into the stationary phase to provide specialized chiral stationary phase (CSP). The use of direct method using CSP offers advantage over indirect HPLC methods in terms of ease and speed of operation. Recently a number of CSPs for HPLC have been described which have been shown to be efficient for the resolution of enantiomeric drugs[24,70-72].

Mechanism of chiral recognition:

Chiral recognition implies the ability of chiral stationary phases to interact differently with mirror-image molecules, leading to their separation. The mechanism of enantiomeric resolution using CSPs is generally attributed to the "three point" interaction model (fig.1.) between the analyte and the chiral selector in the stationary phase[73-76]. Under this model, for chiral recognition, and hence enantiomeric resolution to occur on a CSP one of the enantiomers of the analyte must be involved in three simultaneous interactions, one of which is stereochemically dependent, with complimentary sites on the chiral selector of the CSP. Its antipode may only interact at two such sites. The diastereomeric complexes thus formed will have different energies of interaction and the enantiomer forming the most stable complex will be retained longer by the CSP. The types of interaction involved in the analyte-selector interaction vary depending on the nature of the CSP used. These may include hydrogen bonding, dipole-dipole, π-π, electrostatic, hydrophobic or steric interactions, inclusion complex formation.

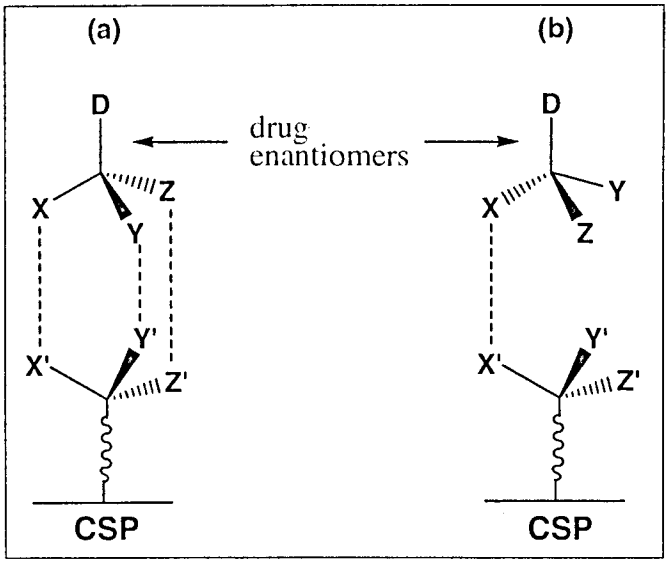

Fig. 1: Illustration of 'three-point interaction' between drug enantiomers and CSP

Enantiomer (a) has the correct configuration of the functional groups (X, Y and Z) for three-point interactions with the complimentary sites (X', Y' and Z') on the CSP, while its mirror image (b) can only interact at one site; (-----) indicates interaction with complimentary sites.

Chiral stationary phases:

Chiral stationary phases can be classified based on the mechanism of their interaction with the analyte. The scheme for the classification of chiral stationary phases proposed by Wainer[77] is presented in Table 1.

Pirkle (Brush-type) CSPs:

Pirkle and co-workers pioneered the development of a variety of CSPs based on charge-transfer complexation and simultaneous hydrogen bonding[21,78-80]. These CSPs fall in the Type I Wainers classification. The Pirkle phases are based on aromatic π-acid (3,5-dinitrobenzoyl ring) and π-basic (naphthalene) derivative. In addition to π-π interaction sites, they have hydrogen-bonding and dipole-dipole interaction sites provided by an amide, urea or ester moiety. Strong three-point interaction, according to Dalgliesh's model, enables enantioseparation. By nuclear magnetic resonance measurements in solution, three interactions are proposed to occur in the more favoured diastereomeric com-

TABLE 1: CLASSIFICATION OF CHIRAL STATIONARY PHASES

Type	Description	Chemistry	Chiral distinction mechanism
I	Brush (Pirkle type) π-acidic or π-basic type	Various chiral selectors ionic or covalent bonding	Solute/CSP complexes formed by attractive interactions; H-bonding, charge transfer (π-π interaction), dipole stacking and steric effects
II	Helical polymers	Cellulose derivatives	H-bonding, dipole-dipole interactions; inclusion complexes also play an important role
III	Cavity	Cyclodextrins, chiral crown ethers, macrocyclic glycopeptide antibiotics	Inclusion complexes, H-bonding; solute enters the cavities within the CSP to form inclusion complexes
IV	Ligand exchange	Coordination complexes to metals	Solute is a part of a diastereomeric metal complex
V	Protein	α-Acid glycoprotein, bovine serum albumin, human serum albumin, ovomucoid	Solute/CSP complexes are based on combinations of hydrophobic interactions and polar interactions

plex between an N-(3,5-dinitrobenzoyl)-α-amino amide and an N-(2-naphthyl)-α-amino ester: a π-donor-acceptor interaction and two hydrogen-bonding interactions[81], as depicted in fig. 2.

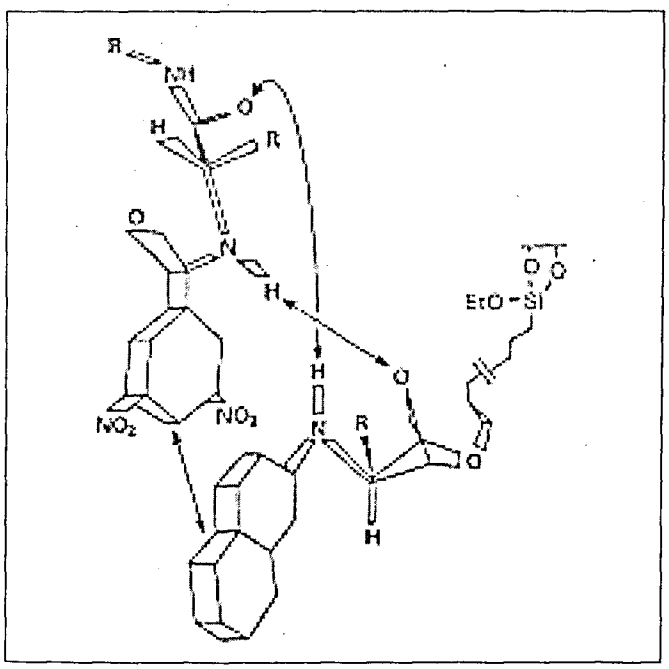

Fig. 2: Chiral recognition model

The more favoured transient-diastereomeric complex between an N-(3,5-dinitrobenzoyl)-α-amino amide and an N-(2-naphthyl)-α-amino ester is depicted. Arrows indicate a π-donor-acceptor interaction and two hydrogen-bonding interactions.

These phases are classified into π-electron-acceptor, π-electron-donor or π-elctron acceptor-donor phase. A number of Pirkle-type CSPs are commercially available[82,83]. They are used most often in the normal phase mode. The ionic form of the DNPBG CSP has been successfully employed to achieve enantioseparation of racemic propranolol after extraction from serum[84]. Pirkle-type CSP has been successfully employed to separate the enantiomers of naproxen and metoprolol[85, 86].

Polysaccharide-based CSPs:

These include a wide range of cellulose and amylose based phases. According to the Wainer classification, they are Type II chiral selector. These polymers have a certain degree of rigidity and assume extended helical conformations. Presumably a chiral cavity or space exists on or within these derivatives, which accounts for their chiral recognition properties. There are different cellulose-based stationary phases, including pure microcrystalline cellulose triacetate, silica coated with cellulose triacetate, cellulose tribenzoate, cellulose tribenzyl ether, cellulose tricinnamate, cellulose triphenyl carbamate- and cellulose tris- (3,5-dimethylIphenyl) carbamate[87]. Another type II stationary phase is amylose-coated silica. Amylose, having predominantly helical structure, is the linear α-D-glucan component of starch. Amylose stationary phases are preferentially chosen to resolve atropisomers with polar substituents[88]. A large number of polysaccharide-based CSPs are commercially available for chiral separation[89]. Chiral recognition mechanism is not well understood but apparently involves

hydrogen bonding and dipole-dipole interaction between the analyte molecule and the ester or carbamate linkage of the CSP. These cellulose-derived CSPs are used in the normal phase. Applications of these CSPs include the direct chiral analysis of β-adrenergic blockers such as metoprolol[90] and celiprolol[91], the calcium channel blocker, felodipine[92] and the anticonvulsant agent, ethotoin[93].

Cavity CSPs:

Another general strategy for chiral discrimination on a stationary phase is creation of chiral cavities, in which stereoselective guest-host interactions govern the separation. The first important consideration for retention and chiral recognition in such stationary phase is the proper fit of the molecule to the chiral cavity in terms of size and shape. Cavity chiral stationary phases includes three categories namely cyclodextrins[94], crown ethers[95] and macrocyclic glycopeptide antibiotics[96].

Cyclodextrins (CyDs):

Majority of pharmaceutical applications were accomplished using cyclodextrins. Cyclodextrins are cyclic oligosaccharides containing α-(1-4) linked D-glucopyranose units. α-, β-, and γ-cyclodextrins, contain 6, 7 and 8 glucopyranose units, respectively. The shape of the cyclodextrins is truncated cone with an inner cavity. The diameter of the cavity is proportional to the number of glucopyranose units. In cyclodextrins, secondary groups (OH-2 and -3) line the mouth of the cavity, and a primary 6-hydroxyl group is at the bottom of the cone. The hydroxyl groups offer chiral binding points, which seem to be essential for enantioselectivity. Apolar glycosidic oxygens make the cavity hydrophobic and ensure inclusion complexing of the hydrophobic parts of analytes. Interactions between the polar region of an analyte and secondary hydroxyls at the mouth of the cavity, combined with the hydrophobic interactions within the cavity, provide a unique two-point fit and lead to chiral recognition.

Selectivity of a cyclodextrin phase is dependent on the size of the analytes and is based on a simple fit-unfit geometrical parameter. α-cyclodextrin includes small aromatic molecules, whereas β-cyclodextrin incorporates both naphthyl groups and substituted phenyl groups. Cyclodextrins, covalently attached to silica gel by Amstrong process[97], offer stable stationary phase. The aqueous compatibility of CyD and its unique molecular structure make the CyD-bonded phase highly promising for use in chiral HPLC of drug. One further advantage of CyD phases is that they are relatively cheaper than the other CSPs. Enantiomers of propranolol, metaprolol, chlorpheniramine, verapamil, hexobarbital, methadone and many more drugs have been separated by using immobilized β-cyclodextrin[98]. However enantiomers of certain drugs, such as chlorcyclizine and bupivacaine, are not effectively resolved, presumably due to the absence of differential interaction with the rim of the CyD cavity.

A number of modified cyclodextrin structures have been developed, by derivatizing the secondary hydroxyl groups, expanding the range of applicable operation modes and eligible analytes. Chiral analogs include (S)-2-hydroxypropyl[99] and (S)- or (R)-I- naphthylehtylcarbamate[100] derivatised cyclodextrins. Several drugs were resolved on derivatised β-CyD phases[101].

Chiral crown ethers:

Stationary phases containing crown ethers all belong to Type III, according to Wainer classification as they form a cavity. Crown ethers can be immobilized on the silica surface to form chiral stationary phases[102]. Similar to cyclodextrins, crown ethers contain oxygen atoms within the cavity. The cyclic structure that contains apolar ethylene groups between oxygen forms hydrophobic inner cavity. Chiral crown ethers of the 18-crown-6-type can separate amino acids as well as primary amines with close neighbourhood of amino function and center of chirality. The interaction occurs between amino protons and the crown ether oxygens.

Macrocyclic glycopeptide antibiotics:

Macrocyclic antibiotics can be immobilized on silica to form Type III chiral stationary phases[103]. Vancomycin and teicoplanin are cyclic glycopeptides with multiple chiral centers and a cup-like inclusion region to which a flapping sugar cover is attached. Similar to protein chiral selectors, the amphoteric cyclic glycopeptides consists of peptide and carbohydrate binding sites by which multimodal possibilities are generated in addition to inclusion complexation.

The 18 chiral centers in the vancomycin molecule offer a complex cyclodextrin-like chiral environment. In contrast a single cavity of cyclodextrins, vancomycin consists of three pockets, resulting in a more complex inclusion of appropriate guest molecules. The attractive forces are π-π interactions, hydrogen bonding, ionic interactions, and dipole stacking. A carboxylic acid and a secondary amine group sit on the rim of the cup and can take part in ionic interactions.

Vancomycin stationary phases operate in reversed and normal phase modes[104]. Teicoplanin has 20 chiral centers, 3 sugar groups, and four-fused rings[105]. There is an acidic group at one end of the peptide cup and a basic one at the other end; both may be involved in ionic interactions. The sugar groups are arranged in three flaps that can be folded to enclose a molecule in the peptide cup. The enantiomers of flurbiprofen, balcofen and devrinol have been separated using Teicoplanin CSPs[106].

Ligand exchange phases:

Davankov and co-workers introduced chiral ligand exchange chromatography[107]. In this CSP an optically active ligand-usually an amino acid, such as L-proline or L-valine is covalently bound to silica support and the resultant phase is treated with a solution of transition metal ion such as copper (Cu^{2+}). The resolution is based on the formation of mixed chelate complexes between the amino acid (fixed ligand), copper ion, and the analyte (mobile ligand) on the stationary phase, in the process each amino acid molecule displaces one of the polymer-bound proline ligand[108]. The differences in stabilities between complexes with R- and S-forms of the analyte lead to the separation of the enantiomers.

The ligand exchange approach is limited to molecules that form chelate complexes with copper or other transition metals. For the separation to be successful the sample molecule must have two polar functional groups with the correct spacing, which can simultaneously act as ligands for the copper ion. For this reason the majority of the studies have been focused on the separation of α-amino acid racemates with their amino and carboxyl functional groups and other similar compounds. The technique, however, has seen only limited application in the analysis of pharmaceuticals[109].

Protein-bound CSPs:

Proteins because they consists of L-amino acids and possess an ordered three-dimensional structure, may bind drugs in a stereoselective manner[110]. This phenomenon has been exploited by Hermansson to develop silica-bound protein CSPs for reversed phase chiral HPLC[111]. Because of their complex nature, however, the mechanism of chiral recognition by proteins is largely unknown. The retention seems to be mainly based on hydrophobic and electrostatic interactions, although hydrogen bonding and charge transfer interactions may also operate.

Several protiens have been employed as CSP, including α_1-acid glycoprotein (enantiopac; chiral-AGP), ovomucoid protein (Ultron ES DVM), human serum albumin (HSA) CSPs and are commercially available[112]. They differ in their chromatographic and enantioselective characteristics because they differ in their size, shape or isoelectric point. These CSPs usually operate under revered-phase mode (phosphate buffer with addition of organic modifiers). As would be expected from proteins, the separation depends very much on parameters such as pH, ionic strength, temperature, the concentration or nature of the modifier. A special advantage of protein CSPs is that they are compatible with the aqueous buffered mobile phases widely used in many biological applications. The major limitation of protein CSPs is their low loading capacity and high cost.

To cite specific applications of α_1-AGP CSP (chiral AGP), it has been employed for analysis of atenolol enantiomers in plasma and urine[113], for pharmacokinetic of study metoprolol enantiomers after administration of the racemate[114]. To avoid interferences by endogenous substances and/or metabolites, it may be necessary to couple the CSP in series with an achiral column. Such a coupled-column approach was employed for the chiral analysis of verapamil and its metabolites[115].

Chiral mobile phases:

In this approach, an enantiomerically pure compound, chiral mobile phase additive (CMPA) is added to the HPLC mobile phase. Enantioresolution, using conventional HPLC stationary phase, occurs as a result of the differential transient diastereomer complex stability and/or differential interaction of these complexes with the stationary phase[116]. With chiral mobile phase additive techniques, there are at least two possible mechanisms; one is that CMPA and the enantiomers may form diastereomers in the mobile phase. Another is that the stationary phase may be coated with the CMPA, resulting in diastereomeric interactions with the enantiomeric pairs during chromatography. It is believed that both mechanisms occur depending on the nature of both the stationary phase and mobile phase used.

Examples of chiral mobile phase additives include ion-pairing agents such as N-benzoxycarbonylglycyl-L-proline[117], cyclodextrins[118] and quinine, and 10-camphosulfonic acid[119]. Limitations of additive approach include detector compatibility. To cite a few example, quinine has been employed as CMPA to separate naproxen enantiomers on a reversed-phase stationary phase[120] and the resolution enan-

tiomers of terfenadine, H_1-receptor antagonist, by reversed-phase high performance liquid chromatography using β-cyclodextrin as a chiral mobile phase additive [121].

CONCLUSIONS

Enantiospecific analytical techniques have made and continue to make, a major impact on our approach to research, development and evaluation of chiral drug candidates. An abundance of methods are available for the analysis of mirror-image molecules. The direct (chiral stationary phases) and indirect (diastereomer formation) chiral HPLC methods are extremely valuable in the separation of enantiomers in pharmaceuticals and biological fluids. The analyst is cautioned not to take single-minded approach of exclusively favouring resolution by either diastereomer formation or chiral stationary phases. These methods have respective strength and weakness and either may be applicable to problem at hand. In the future, these direct and indirect methods could be complimentarily used for pharmaceutical and biomedical applications of enantioseparations using LC techniques.

With the explosion of chiral analytical technology we have a better control on the handedness of drug molecules. It is now easier for chiral examinations of drugs viz. assessing enantiomeric purity, determining the fate of a chiral drug molecule and its metabolites during the *in vivo* and *in vitro* studies. This in turn has contributed to the selection of chiral drug candidates with improved efficacy and safety profiles, hopefully leading to a safer and more efficient drug therapy.

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