A Review on Chiral Columns/Stationary Phases for HPLC

Suraj Koorpet R, Akshay N, Nishanth G, Chandan R S, Anand Kumar Tengli*

Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Sri Shivaramathreeshwara Nagar, Mysuru-570015, Karnataka, India

Article History:
Received on: 26.09.2019
Revised on: 20.12.2019
Accepted on: 25.12.2019

Keywords:
Chiral separation, enantiomer, HPLC, Chiral stationary phases (CSPs), Chiral derivatization reagents (CDRs), Chiral mobile phase additive (CMPA)

ABSTRACT

The chiral separation of pharmaceutical molecules and their precursors is one of the important areas of application of HPLC in pharmaceutical analysis for obtaining the enantiomerically pure drug. The latter procedures include the use of so-called chiral selectors to recognize and isolate the enantiomer enantio-selectively. The direct approaches, i.e., those who do not derive the compound of interest before separation, are addressed in detail since they are now the most common approaches. The role of stereochemistry in medicinal products is being given greater emphasis on medical practice. For physicians to make a conscious choice about the use of single-enantiomered medicinal products, basic knowledge is required. For a few treatments, single-enantiomer formulations can provide more selectivity than a combination of enantiomers in their biological purposes, enhanced therapeutic indexes and/or better pharmacokinetics. This highlights the possible biological and pharmacological variations between the two drug enantiomers and underlines the clinical experience of individual enantiomers. Particular emphases have been put on chiral separation by HPLC on chiral stationary phases (CSPs). Chiral derivatization reagents (CDRs) are optically pure reagent on reaction with drugs forms a pair of diastereoisomers that can be separated on conventional achiral phase. In the Chiral mobile phase additive (CMPA) method, the stationary phase is achiral and the chiral selector is dissolved during the mobile period. Interaction with the analyte’s enantiomer leads to the formation of transient diastereomeric complexes that are separated by their affinity towards mobile/stationary phase. Separation mechanisms and method development for chiral molecules using these phases are discussed in this review.

*Corresponding Author
Name: Anand Kumar Tengli
Phone: +91 9886658520
Email: anandkumartengli@jssuni.edu.in

ISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v11i2.2240

INTRODUCTION

In drug discovery, molecular chirality is a basic factor, it is very essential to comprehend and define biological goals and to design efficient pharmaceutical agents. A molecule's enantiomers connect to each other as an item and its mirror image, which is not super-imposable; they’re called chiral. These have a different optical activity which can be measured using polarimeter shown in Figure 1. Chiral separation now includes a significant area of relevant analytical chemistry for a broad range of science experts. It has been acknowledged that many enantiomeric types of drugs have distinct physiological and therapeutic impacts. Very often, in an enantiomeric couple, only one sample is pharmacologically active (Fan, 2014).
Anand Kumar Tengli et al., Int. J. Res. Pharm. Sci., 2020, 11(2), 2466-2480

Figure 1: Polarimeter used in determination of optical activity (Source: internet)

Figure 2: Three point interaction of enantiomer with receptor

(asymmetric carbon atom, chiral center or stereogenic carbon). A molecule is said to be chiral when it has at least one asymmetric carbon. Carbon is not the sole molecule capable of acting as a source of asymmetry. Chiral molecules such as omeprazole, cyclophosphamide and methaqualone can sometimes be formed by arsenic, phosphorus and nitrogen. There is optical interference in chiral molecules, and enantiomers are sometimes called optical isomers (Nguyen et al., 2006).

The significant stereo chemical stimulation emerged from unfortunate birth defects caused by one of the Thalidomide enantiomers. This drug was produced and marketed as an N-phthalylglutamic acid imide racemic mixture. The required physiological activity, however, was found to be residual only in the R-(+)-isomer and the respective S-(-) enantiomer which was teratogenic and caused serious foetal malformation (Vargesson, 2015). Amphetamine has d-isomer, a potent stimulant of the CNS, and the effect of l-isomer is small. Propoxyphene α-l is antitussive; analgesic is α-d. As a vasoconstrictor, epinephrine l-isomer is ten times more active than d-isomer. Sympathomimetic l-isomer has 60 times d-isomer’s pressor activity. There is only betaadrenergic blocking activity in the propanolol S(-) isomer. Warfarin isomer S(-) is five times more effective than isomer R(+). D-isomer of ascorbic acid is anti-ascorbic; it is not active in l-isomer (Millership and Fitzpatrick, 1993; Ahuja, 2007). Albuterol, D-albuterol may cause constriction of airways, and L-albuterol prevents side effects. Seldane single enantiomer (allergy medication) that prevents life-threatening Seldane heart disorders. Fluoxetine, R-Fluoxetine (antidepressant) –improved efficacy that minimizes the side effects such as anxiety and sexual dysfunction. Other indications (eating disorders) S-Fluoxetine for use in migraine therapy (Prabhu et al., 2016). A chiral drug’s enantiomers differ in their interac-
tions with the body’s chiral environments such as enzymes, proteins, receptors, etc. Such changes may result in different biological activities, including pharmacology, metabolism, pharmacokinetics, immune response, toxicity, etc. In addition, the two enantiomers can be identified by biological systems as two different substances, and therefore their contact with each other leads to different responses (Nguyen et al., 2006).

The explanation for drug receptor chiral identification is a three-point drug interaction with the receptor site suggested by Easson and Stedman. Figure 2 illustrates the difference between two enantiomers of a drug interacting with its receptor (Nguyen et al., 2006). In this situation, one enantiomer is active biologically while the other is not. The active enantiomer compound substituents labeled A, B, and C will interact to their respective regions of the receptor’s binding site, which is labeled a, b, and c to match Aa, Bb, Cc. In this scenario, a powerful biological effect can be created by this acceptable activity. The inactive enantiomer, on the other hand, cannot bind to its receptor in the similar manner when it rotates in space.

It is possible to acquire pure enantiomers either by racemic resolution or by asymmetric synthesis. Asymmetric synthesis is only useful when very large quantities are required, but the time required to develop such synthesis may make it inappropriate for the small quantities required during the early stages of the drug discovery process. The technique of racemic resolution involves enzymatic resolution, diastereomer formation to be separated by crystallization or standard chromatography and direct chromatographic separation of enantiomers using a stationary chiral phase (Layton, 2005; Berthod, 2006; Rica, 2015).

Several approaches were used for the isolation of enantiomers like GC, HPLC, TLC, SCFC, etc. The most common way to get restricted quantities of simple enantiomers has become the enantioselective liquid chromatography, especially during drug discovery (Layton, 2005; Heyden, 2005).

HPLC may be used for separating chiral compounds either directly with chiral stationary phases (CSPs) or chiral mobile phase additives (CMPA) or indirectly with chiral derivative reagents (CDR) (Averkiev et al., 2011; Yu et al., 2013). Table 1 summarizes the features of these three chromatographical techniques. Large types of new polymers are currently being created in liquid chromatography as chiral stationary phases (CSPs). For enantiomeric separation, some new chiral derivative reagents and new chiral mobile phase additives have also been effectively used (Fan, 2014).

CHIRAL SEPARATION METHODS

Chiral Stationary Phases (CSPs)

The most widely used CSPs in enantiomer separation are discussed below and their chemical structures are given in the Figure 4.

Brush type / Pirkle-type Chiral Columns

The stationary phases of Pirkle chiral generally fall into three classes: donors of π-electron acceptor/π-electron, acceptors of π-electron, and donors of π-electron. Chiral recognition occurs at binding sites with the Pirkle phases. Significant binding sites are known as, acidic sites, aromatic rings or steric interaction sites or basic sites that are π-basic and π-acidic. Aromatic rings are possible sources for interactions between π and π. Acidic sites supply hydrogen for possible intermolecular attachments with hydrogen; Amido proton (N-H) is often hydrogen from amide, carbamate, urea, and amine. The formation of hydrogen bonds may also include simple sites such as π-electrons, sulfanyl and phosphinyl oxygen, and hydroxy and ether oxygen. Steric interactions between large groups may also occur (Xu et al., 2007). Overall interactions possessed by pirkle CSPs are shown in the Figure 3 (Rica, 2015).

Figure 3: Types of interaction possessed by brush type stationary phase

Whelk-O 1

Whelk-O 1 is useful for the isolation of underivatized enantiomers in a number of families, including epoxides, amides, esters, ethers, ureas, carbamates, aziridines, phosphonates, alcohols, carboxylic acids, ketones, aldehydes, and NSAIDs. In this step, donor π-electrons/acceptors π-electrons demonstrate a surprising degree of generalization. The significant versatility found in column Whelk-O 1 compares favorably with chiral stationary phases taken from a polysaccharide.

Furthermore, since Whelk-O 1 is covalently attached to the support, the phase is compatible with all the commonly used mobile phases, including aqueous
### Table 1: chromatographic techniques used for enantiomeric separation - Pros and Cons

| Method                        | Pros                                                                 | Cons                                                                 |
|-------------------------------|----------------------------------------------------------------------|----------------------------------------------------------------------|
| Chiral derivatization reagents (CDR) | 1. Less expensive columns can be used, i.e. traditional columns.  
  2. Versatile because the HPLC may be used with various achiral columns and conditions in mobile phases; several forms of chemical derivatization are available and the reagent’s price may be lower than the chiral column’s value.  
  3. Similar selectivities may be performed. | 1. Long-term assessment involving sample preparation and derivatization chemistry verification.  
  2. Inconvenience, particularly in preparatory chromatography, when inversion of derivatization is necessary to recover pure enantiomers  
  3. Synthesis of non-commercially accessible pure derivative reagent;  
  4. Biased outcomes of enantiomeric structure owing to partial racemisation or unequal reaction rates of the derivative agent. |
| Chiral stationary phases (CSPs) | 1. It is possible to select a hefty amount of commercially accessible chiral columns.  
  2. Convenience, particularly in preparatory chromatography  
  3. Easy operation. | 1. Some chiral columns that are commercially available are expensive.  
  2. Sometimes only one particular chiral column is capable of distinguishing Enantiomers.  
  3. It is hard to select CSPs to effectively resolve particular enantiomers. |
| Chiral mobile phase additives (CMPA) | 1. It is feasible to use less costly standard LC columns.  
  2. A broad range of possible additives are accessible.  
  3. Different chiral selectivities can be acquired. | 1. Many chiral additives are expensive and sometimes synthesized.  
  2. The mode of operation is complex.  
  3. Inconvenient for preparatory applications since the chiral additive must be removed from the enantiomeric solutes. |

systems that have a distinct advantage over coated chiral stationary polysaccharide-derived phases. Other benefits include longevity of the column, excellent performance, ability to invert elution order and excellent preparative capacity (Xu et al., 2007).

**Whelk-O 2**

Whelk-O 2 is the covalent trifunctional version of Whelk-O 1. While the Whelk-O 2 has the same chiral selector, it has a trifunctional relation to the silica support. In the majority of cases, enantioselectivity remains the same as the Whelk-O 1. Whelk-O 2 has been designed to improve the stationary phase’s resistance to hydrolysis while using solid organic additives, including trifluoroacetic acid. The Whelk-O 2 is suitable as the product is bound to spherical Kromasil silica (10 μm, 100Å), for preparatory separations. This allows the analyst to develop new methods on analytical columns and transfer the same into the preparatory column for bulk separations (Armstrongbo and Zhang, 2001).

**Alpha Burke 2**

The chiral stationary phase of the α-acceptor is used in particular in the HPLC segregation of β-blocker enantiomers. A class of essential cardiovascular medicines is used with specific enantiomers. The α-Burke two was specially designed to distinguish β-blockers without any chemical derivatization. It also addresses the enantiomers of many drugs isolated on CSPs of the α-acceptor Pirkle form (Tang et al., 2004).
DACH-DNB
A wide range of racemate groups including selenoxides, phosphine oxides, amides, alcohols, acids, esters, sulfoxides, phosphin oxides, thiophosphine oxides, phosphine-borane, phosphine selenide, organometallics, beta-lactams, and heterocycles are resolved using DACH-DNB CSP.

Leucine
The leucine CSP is bound in a covalent manner with 5 µm for aminopropyl silica. For the inverting elution order, columns derived from either L or D-leucine are available. This step demonstrates increased enantioselectivity of different compound groups, including benzodiazepines.

Phenyl glycine
Phenyl glycine is covalently bonded to 5 µm amino-propyl silica, a chiral phase of π-acceptor. Phenyl glycine columns are available for inverting elution order in both L-and D-configurations. This CSP resolve a wide variety of compounds including: aryl-substituted hydantoins, aryl-substituted cyclic sulfoxides, analogs of α-indanol and α-tetralol and bi-β-naphthol and its analogs.

Pirkle 1-J
The Pirkle 1-J has an odd β-lactam structure that affects its molecular identification properties substantially. It is important for the explicit isolation of β-blocker enantiomers that are underderivatized. It can also be used to separate aryl propionic acid NSAID enantiomers as well as other drugs (Yang et al., 2008).

ß-Gem 1
ß-Gem1 is a stationary π-acceptor chiral phase which is formed by binding the chiral selector covalently to 5 µm silica via an ester bond. This chiral step greatly exceeds its commonly used counterpart, Phenyl glycine, in many situations. Anilide derivatives of chiral carboxylic acids, including NSAIDs, can also be separated

ULMO
ULMO CSP can separate the enantiomers of many racemate groups and is particularly suitable for the separation of aryl carbinal enantiomers. Available in inverting elution order forms (R,R) and (S,S).

Polysaccharide CSPs columns
Chiral columns are rugged polysaccharide phases suitable for a wide range of chiral compounds. Unique, proprietary, phase coverage provides excellent peak shape and improved resolution versus leading chiral phases. High resolution greatly improves preparative loading, leading to greater productivity and higher purity separations. Combined with attractive pricing and rapid delivery, Reflect chiral columns deliver the performance and productivity you expect. They are compatible with almost any organic solvent, thereby widening the range of mobile phase options to enhance separations in the most challenging applications. Reflect immobilized phases are available in a range of particle sizes, allowing analytical scale separations up through large scale preparative separations.

Coated Polysaccharide Chiral Columns
Coated amylose and cellulose chiral columns are high-performance polysaccharide chiral phases suitable for a wide range of chiral compounds in normal phase HPLC and SFC modes. Unique, proprietary, phase coverage provides excellent peak shape and improved resolution versus leading chiral phases. This high resolution greatly improves preparative loading, leading to greater productivity and higher purity separations. Reflect coated phases are available in a range of particle sizes, allowing analytical scale separations up through large scale preparative separations (Ali and Aboul-Enein, 2007; Peng et al., 2009).

Additional Coated Polysaccharide Phases
The polysaccharide coated chiral columns were manufactured using an extremely highly pure silica gel to coat the validated chiral selector. Columns can be used in normal phase, reverse phase and polar organic solvent conditions, and are packed and checked at high pressures, for use in both SFC and HPLC mode (Heyden, 2005; Moldoveanu and David, 2013, 2017).

Crown-Ether Chiral CSPs
Chirosil
RCA (+) and SCA (-) are proven chiral stationary phases for the separation of amino acids and compounds containing primary amines. Excellent durability due to covalent bonding
Available in both enantiomeric forms [RCA (+) and SCA (-)], which allows for the inversion of peak elution order
Columns are stable to 5,000 psi (~345 bar)
Fast delivery—all sizes, anywhere in the world
Columns are available in 5 and 10 µm particle sizes and analytical and preparative dimensions.
As a chiral selector bound to a silica support, the ChiroSil-RCA (+) and SCA (-) are (+) or (-)-(18-Crown)-6-tetracarboxylic acid. The ChiroSil CSP is designed to be used in liquid chromatography (HPLC) (Hirose et al., 2005; Conrad et al., 2005).

**Chirosil ME**

Chirosil ME RCA (+) and SCA (-) have (+) or (-)-(18-Crown)-6-tetracarboxylic acid as a chiral selector, which is bonded to the silica support. These are for amino acid chiral analysis and have increased capacity factor (k). In general, capacity factors on Chirosil ME are greater than on standard Chirosil, while the separation factor (α) and resolution are greater on standard Chirosil than on Chirosil ME (Berkecz et al., 2008).

**Cyclodextrin**

Cyclodextrin cavity contains H-bonding hydroxyls with polar analyte groups; the hydrophobic analyte section matches up into the non-polar cavity. Relevant research activities were mainly focused on developing and immobilizing new functional CD derivatives through more widely used linkers (ether, amino, urea linkages) as well as exploring new linkages such as triazole (Wang et al., 2007). Under reverse-phase conditions, chiral identification of CD CSPs is assumed to be driven by the inclusion complexation between the analyte’s hydrophobic mobility and the relatively non-polar interior of the CD cavity. It is used primarily to isolate amino acids, amines, alcohols, amides, ether, metalloenes, lactones and polycyclic aromatic hydrocarbons (Lai et al., 2011).

**Macrocyclic antibiotics**

The macrocyclic antibiotics include numerous chiral centers, functionalities that allow bonding to silica (using pre-derivatized silica with reactive groups) and the ability to offer π-π interactions, ionic interactions, hydrogen bonding, and inclusion/complexation. Some antibiotics of glycotopeptide type, including rifamycin(s), vancomycin (18 chiral centers), avoparcin, ristocetin, glycopeptide A-40, and teicoplanin (23 chiral centers), have been used to render stationary phases. Macrocyclic antibiotics used as a stationary binding step also include a thiopeptide group of thiostrepton as the parent compound (17 chiral centers). Glycopeptide antibiotics have different groups of antibiotics (phenolic-OH type, eNH2, eCOOH, and carbohydrates). Several groups have a zwitterionic potential and are likely to play a major part during their chiral identification in the association with analysts. Macrocyclic antibiotic columns are used both in the normal and in reversed phases in a similar way to other chiral column (Armstrong et al., 1994; Zhang, 2004).

**Chiral Derivatization Reagents (CDRs)**

The indirect derivative technique with some suitable reagent for chiral tagging is a successful way to separate most enantiomers, particularly if CSPs are not feasible. CDRs are optically pure reagent on reaction with drugs forms a pair of diastereoisomers, which can be separated on conventional achiral phase. High optical purity derivative reagents provide the most accurate results. The reaction conditions should be mild enough to avoid the chiral materials from being racemized or epimerized. The by-products of derivatives should also not, if any, conflict with the analysis. Greater attention is needed, particularly if the analyte has more than one functional group able to respond to the derivatives.

Ideal characteristics of CDRs include a high purity of enantiomeric content and a rapid reaction to the molecules to be studied quantitatively or at least reproducible. They bear chromophores to detect sensitive UV and fluorescence, leading to reaction-separated products that are easily decomposable when used over and over with simple non-chiral reagents. A few examples of the most widely used CDRs are given in Table 2.

The derivatives should be isolated from excess reagents or other by-products and tested directly, i.e., injected without isolation or additional purification into the HPLC columns. For sensitive UV or fluorescence detection, the reagents should carry chromophores that detect trace amounts of material in body fluids in particular (Averkiev et al., 2011).

**Critical parameters during chiral derivatization**

**Glassware for derivatization**

Vials with a volume of 0.1-10.0 mL handle sample plus solvent and reagent in usually chromatographically used amounts. The vials must be safe for extreme temperatures, with rubber septum stoppers or Teflon lined disks, vials supplied with open-center screw caps can be sealed. While a Teflon coating is usually very inert, certain specimens and reagents will melt it.

**Deactivation of glassware**

Because the laboratory glassware surface is slightly acidic, certain analytes—especially amines and certain pesticides—can be adsorbed. These reductions can be important in low-level experiments. Glassware used in low-level assessment is usually silanized to prevent sample loss through adsorption. Silanization blocks the polar Si-OH groups on the surface of the glass by chemically adding to the...
Figure 4: Structures of CSPs widely used separation of isomers (1) Chirosil ME, (2) Leucine, (3) Chirosil, (4) Cyclodextrin, (5) Whelk-O-1, (6) Whelk-O-2, (7) Macrocyclic antibiotic, (8) Prikle 1-1, (9) DACH-DNB, (10) β-Gem 1, (11) UMLO, (12) Phenyl glycine, (13) Alpha Burke 2, (14) Polysaccharide.
### Table 2: Examples for Chiral Derivative Agents

| Class of compound | CDRs example |
|-------------------|--------------|
| Amino acids       | BOC-L-cysteine |
|                   | 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate |
|                   | Nα-(2,4-Dinitro-5-fluorophenyl)-L-alaninamide |
|                   | 2,3,4-Tri-O-acetyl-α-D-arabinopyranosyl isothiocyanate |
|                   | (+)-1-(9-Fluorenyl)ethyl chloroformate solution |
|                   | Nα-(2,4-Dinitro-5-fluorophenyl)-L-valinamide |
|                   | N-Isobutyryl-L-cysteine |
| Alcohol           | (+)-Camphanic chloride |
|                   | (-)-Camphanic chloride |
|                   | N-(7-Nitro-4-benzofurazanyl)-L-prolyl chloride |
| Acids             | R(+)-1-(1-Naphthyl)ethylamine |
| Diamine           | (R)-2,8-Dimethyl-5,11-methano-dibenzo(b,f)(1,5)diazocine |
| Amines            | (+)-1-(9-Fluorenyl)ethyl chloroformate solution |
|                   | (-)-1-(9-Fluorenyl)ethyl chloroformate solution |
|                   | Nα-(2,4-Dinitro-5-fluorophenyl)-L-valinamide |

### Table 3: List of modifiers used for method development using CSPs in HPLC

| Analyte           | Modifier                                                                 |
|-------------------|--------------------------------------------------------------------------|
| Acid/Acid salt    | Acetic acid (0.1-0.4%)                                                   |
|                   | Ammonium acetate (0.01-0.1M)                                             |
|                   | Trifluoroacetic acid (0.1-0.5%)                                          |
| Amine/Amine salt  | Triethylamine (0.1-0.5%)                                                 |
|                   | Diethylamine (0.1-0.5%)                                                  |
|                   | Ammonium acetate (0.01-0.1M)                                             |
| Bi-functional     | Ammonium acetate/Triethylamine or Diethylamine                           |
|                   | Acetic acid / Triethylamine or Diethylamine                              |
|                   | Triethylamine or Diethylamine / Trifluoroacetic acid                     |
Choosing a column based on the nature of drug which has to be separated

For **normal phases** mobile phases such as Hexane/IPA, Hexane/Ethanol, Hexane/CH₂Cl₂, Hexane/CH₂Cl₂/Ethanol, Hexane/Ethyl Acetate, Heptane/Ethanol, Methanol/CH₂Cl₂, Ethanol/CH₂Cl₂, Heptane/CH₂Cl₂.

For **reverse phases** mobile phase such as H₂O/Methanol, H₂O/Ethanol, H₂O/Acetonitrile, H₂O/THF

Choosing a strong solvent, which helps in early elution of the peaks.

High percentage (~50%) of strong solvent (normal phase - ethanol, IPA, etc.; reversed-phase - methanol, acetonitrile, etc.).

- Sample eluted in void
- Sample out of void but no resolution
  - Choose different column
  - Sample out of void and good resolution
- Some resolution is achieved
- Sample out of void but poor resolution
  - Add modifiers, which can help in achieving good peak shapes
- Good resolution achieved, then optimize
- No good resolution
  - Optimize the method by altering the flow rate, injection volume and pressure

Figure 5: Flow chart of method development using CSPs in HPLC
Table 4: Columns/Chiral stationary phase used for resolution of drugs.

| Drug                          | CSP          | Mobile phase, flow rate and detection | Spectral information |
|-------------------------------|--------------|---------------------------------------|----------------------|
| 1-(4-Methoxyphenyl)-2-butanol | UMLO         | M.P- n-Heptane and 1,2-Dimethoxyethane (98.5:1.5) FR -1.0 ml/min Detection - UV detector at 254 nm | α = 1.49 k = 2.04 |
| 2-Methoxyphenyl Phenyl Carbinol | UMLO         | M.P - Heptane and IPA (99:1) FR - 1.0 ml/min Detection - UV detector at 215 nm | α = 1.13 k = 2.92 |
| Abscisic acid                 | Whelk-O1     | M.P - Hexane/IPA and 0.1% Acetic acid (75:25) FR - 1.5 ml/min Detection - UV detector at 254 nm | α = 1.55 k = 2.21 |
| 1’-Ace toxychavicol Acetate   | Whelk-O1     | M.P - Hexane and IPA (90:10) FR - 1.5 ml/min Detection - UV detector at 254 nm | α = 2.05 k = 5.94 |
| Bambuterol                    | α-Burke 2    | M.P - Hexane/methylene chloride/ethanol+20mM ammonium acetate (40:40:20) FR - 1.5 ml/min Detection - UV detector at 254 nm | α = 1.35 k = 3.74 |
| Methadone Hydrochloride       | α-Burke 2    | M.P -hexane/ethanol and 0.1% trimethy lamine (40:40:20) FR - 1.5 ml/min Detection - UV detector at 254 nm | α = 1.34 k = 3.50 |
| Azelastine                    | Whelk-O2     | M.P - Hexane/dichloromethane/ethanol, 0.1% Triethylamine and 6mM Ammonium acetate (47:47:6) FR - 2ml/min Detection - UV detector at 254 nm | α = 1.24 k = 8.51 |

Continued on next page
| Compound                        | Mobile Phase                          | Resolution | Retention Factor | Detection | Detection Wavelength | Remarks |
|--------------------------------|---------------------------------------|------------|------------------|-----------|----------------------|---------|
| Fluridil Whelk-O2              | M.P - water and methanol (57:43)      | $\alpha = 1.18$ | $k = 12.9$       | UV detector at 254 nm |
| Homocysteine-Thiolactone HCl   | M.P - Methanol/water + 0.05% TFA (60:40) | $\alpha = 3.56$ | $k = 0.58$       | UV detector at 240 nm |
| Methionine Chirosil            | M.P - Methanol/water + 10mM acetic acid (45:55) | $\alpha = 2.04$ | $k = 1.64$       | UV detector at 210 nm |
| 4-nitrophenylalanine ChirosilME| M.P - Methanol + 0.01% Phosphoric acid (60:40) | $\alpha = 1.51$ | $k = 1.91$       | UV detector at 210 nm |
| DL-Pyridylalanine ChirosilME   | M.P - Methanol + 0.01% Phosphoric acid (70:30) | $\alpha = 1.74$ | $k = 2.03$       | UV detector at 210 nm |
| N-(1-Naphthyl)-N'-(1-methylbenzyl)urea (R)-Gem 1 | M.P - Hexane + ethanol (60:40) | $\alpha = 1.22$ | $k = 2.37$       | UV detector at 254 nm |
| N-(1-Naphthyl)-N'-(1-methylbenzyl)urea (R)-Gem 2 | M.P - Hexane + ethanol (60:40) | $\alpha = 1.25$ | $k = 3.18$       | UV detector at 254 nm |

2476 © International Journal of Research in Pharmaceutical Sciences
surface a nonadsorptive silicone coating, essentially "derivatizing" the glass.

The glassware is treated with a solution of 5-10% dimethyldichlorosilane (DMDCS) in toluene for 30 minutes in the most common silanization process. Signification losses are substantially reduced with a small amount (often less than 1%) of alcohol, such as butanol, added to the solvent.

Sample handling

Teflon-tipped plunging syringes are more suited for transferring volatile reagents than conventional syringes with all-metal plungers. The Teflon plunger tip provides a stronger seal and allows the reagent to be extracted from a closed vial. Each syringe retains a certain reagent in the barrel. If not properly cleaned, a syringe is vulnerable to corrosion and seizing with an all-metal plunger. The finest cleaning is for the plunger to be removed and cleaned and the solvent to be vacuumed through the syringe. Sometimes a seized plunger can be released by soaking in a methanol-filled container.

Reaction time

The time of reaction between compounds varies greatly. Most materials can be derivatized in seconds or minutes at room temperature by reagents described here, while other materials take extended time at high temperatures. The progress of derivatization can be tracked through a periodic chromatography of the aliquots of the reaction mixture for a compound of uncertain reactivity. Heating often increases the yield and/or shortens the time of reaction.

Water

Water can often prevent the reaction in the reaction mixture and/or hydrolyze the derivatives, reducing the derivative yield for analysis. In the reaction mix to contain the water present in the sample, sodium sulfate is added, if necessary. Probable heating or under a stream of dry nitrogen may be used to dry the specimens.

Chiral Mobile Phase Additives (CMPAs)

In the CMPA method, the chiral selector is dissolved during the mobile phase while the stationary phase is achiral. In interaction with the analyte enantiomers, transient diastereomeric complexes are formed. Such complexes vary in their constant formation and/or dispersal between the stationary (achiral) and mobile phases, which contribute to an enantiopolar separation. CSPs are relatively expensive, making the use of a mobile phase chiral selector, the so-called mobile phase additive chiral (CMPA), an appealing choice because of its simplicity and versatility (Yu et al., 2013).

CMPA systems generally find as quite complex the enantio recognition mechanism. Nevertheless, chiral identification is generally considered to involve peculiar interactions due to the simultaneous stereogenic centers of at least three locations in both the chiral selector and the chiral analyte (Berthod, 2006; Inai et al., 2006).

Due to the multiplicity and complexity of interactions among enantiomers and a chiral selector, the stationary phase surface and other chromatographic system components, the total separation efficiency (in particular, chiral-selector concentration and other additives) can depend strongly on composition, the pH and mobile phase temperature (Davankov, 1997).

The specific CMPAs used are ion-pairing, ligand-exchangers, protein-affinity reagents, cyclodextrin inclusion reagents and sulphated β-cyclodextrin (S-β-CD) (Ameyibor and Stewart, 1997, 1998).

Overview of method development using chiral column

The stepwise method development procedure has been discussed in Figure 5 and the modifiers used to achieve good peak shape are given in Table 3.

RESULTS AND DISCUSSION

In recent years, as a patent on a successful drug nears expiration, pharmaceutical companies have sometimes advertised as a new drug (chiral switch) a single stereoisomer of the existing racemic drug, sometimes citing greater efficacy, reduced toxicity, or both. Most medications currently on the market are stereoisomer racemic mixtures. These may be enantiomers, non-superimposable mirror images, and structural isomers, not mirror images, but in either case stereoisomers in bioactivity and pharmacokinetics that differ markedly from each other. The FDA expects the producer of a new racemic mixture to define and describe that single isomer (FDA, 1992; Nguyen et al., 2006). Enantiomeric impurities are now measured routinely to the 0.01% level HPLC can be used to measure an amino acid enantiomeric impurity near the parts-per-million level (Armstrongbo and Zhang, 2001; Zhang, 2004).
Figure 6: Structures of drugs and their spectra of: 1(a) & (b): 1-(4-Methoxyphenyl)-2-butanol; 2(a) & (b): 2-Methoxyphenyl Phenyl Carbinol; 3(a) & (b): Abscisic acid; 4(a) & (b): 1′−Acetoxychavicol Acetate; 5(a) & (b): Homocysteine-Thiolactone HCl; 6(a) & (b): Methionine; 7(a) & (b): DL – Pyridylalanine; 8(a) & (b): trans-(R) 7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene; 9(a) & (b): Bambuterol; 10(a) & (b): Methadone Hydrochloride; 11(a) & (b): Azelastine; 12(a) & (b): Fluridil; 13(a) & (b): 4 nitro phenyl alanine; 14(a) & (b): N-(1-Naphthyl)-N′-(1-methylbenzyl)urea N-(1-Naphthyl)-N′-(1-methylbenzyl) urea.

columns which are used for separation of racemates have been discussed in Table 4 and Figure 6 (Rica, 2015; Layton, 2005).

* $\alpha$ = It is chromatographic system’s ability to chemically discriminate between sample components is the selectivity (or separation) factor. It is generally measured as the ratio of the retention (capacity) factors $k$ of the two peaks in question and can be visualized as the distance between the apexes of the two peaks. High $\alpha$ values suggest good separating power and good separation of each peak between the apex. Nevertheless, $\alpha$ value does not imply the resolution directly (CHROMacademy, 2014).

$$\alpha = \frac{k_2}{k_1} = \frac{t_{R2} - t_{R0}}{t_{R1} - t_{R0}}$$

In Table 4 shows, Mobile phase (M.P.)
Mobile Phase used in a particular ratio to resolve the components in the analyte.

*Flow rate (F.R.)*

It is the rate at which the mobile phase will be set to flow into the HPLC system.

*\( k \) = Retention (or capacity) factor is a means of measuring an analyte’s retention on the chromatographic column. The retention factor is proportional to the analyte’s retention time \( (t_R) \) ratio on the column to a non-retained compound’s retention time \( (t_0) \). The unretained compound has no contact with the stationary phase and elutes with the mobile phase at time \( t_0 \), also known as the ‘hold-up time’ or ‘dead time.’ A high "\( k \)" value means the specimen is strongly retained and has been associated with the stationary phase for a significant amount of time (CHROMacademy, 2014).

**CONCLUSIONS**

The thorough development of single-enantiomer medications aims to provide patients with safer, better tolerated, and more effective narcotics. The physician is responsible for familiarizing himself with the basic characteristics of chiral pharmaceuticals discussed in this article. For general, each enantiomer of a chiral product may have its own specific pharmacological profile, and a drug's single-enantiomer formulation may have different properties than the same drug's racemic formulation. In order to obtain specific enantiomer, there is a need for stationary phases which are more precise and accurate in having interaction with drug enantiomer moiety. Most of the CSPs discussed above have been found to be effective in distinguishing the enantiomers of drugs and it can be inferred that these CSPs can be used to develop new approaches and isolate the enantiomers of other drugs.

**REFERENCES**

Ahuja 2007. A strategy for developing HPLC methods for chiral drugs. *LCGC North America*, 25(11):1112–1128.

Ali, I., Aboul-Enein, H. 2007. Immobilized Polysaccharide CSPs: An Advancement in Enantiomeric Separations. *Current Pharmaceutical Analysis*, 3(1):71–82.

Ameyibor, E., Stewart, J. T. 1997. Resolution and quantitation of pentazocine enantiomers in human serum by reversed-phase high-performance liquid chromatography using sulfated \( \beta \)-cyclodextrin as mobile phase additive and solid-phase extraction. *Journal of Chromatography B: Biomedical Sciences and Applications*, 703(1-2):408–416.

Ameyibor, E., Stewart, J. T. 1998. HPLC determination of ketoprofen enantiomers in human serum using a nonporous octadecylsilane 1.5 µm column with hydroxypropyl \( \beta \)-cyclodextrin as mobile phase additive. *Journal of Pharmaceutical and Biomedical Analysis*, 17(1):161–162.

Armstrong, D. W., Tang, Y., Chen, S., Zhou, Y., Bagwill, C., Chen, J. R. 1994. Macrocyclic Antibiotics as a New Class of Chiral Selectors for Liquid Chromatography. *Analytical Chemistry*, 66(9):1473–1484.

Armstrongbo, D. W., Zhang 2001. Chiral Stationary Phases for HPLC. *Anal. Chem*, 73(19):557–561.

Averkiev, N., Tarasenko, S. A., Lifshits, M. B., Rozhansky, I. 2011. Acoustic conversion of quantum states in semiconductors. *Fizika Nizkikh Temperatur (Kharkov)*, 37(3):251–257.

Berkecz, R., Ilisz, I., Pataj, Z., Fülöp, F., Choi, H. J., Hyun, M. H., Péter, A. 2008. LC Enantioseparation of \( \beta \)-Amino Acids on a Crown Ether-Based Stationary Phase. *Chromatographia*, 68(51):13–18.

Berthod 2006. Chiral Recognition Mechanisms. *Analytical Chemistry*, 78(7):2093–2099.

CHROMacademy 2014. The Theory of HPLC: Chromatographic Parameters, pages 23–23.

Conrad, U., Chankvetadze, B., Scriba, G. K. E. 2005. High performance liquid chromatographic separation of dipeptide and tripeptide enantiomers using a chiral crown ether stationary phase. *Journal of Separation Science*, 28(17):2275–2281.

Davankov, V. A. 1997. The nature of chiral recognition: Is it a three-point interaction? *Chirality*, 9(2):99–102.

Fan, S. S. 2014. Chiral Separation of Cypermethrin Enantiomers by High-Performance Liquid Chromatography. *Advanced Materials Research*, pages 417–422.

FDA 1992. FDA'S policy statement for the development of new stereoisomeric drugs, pages 338–340.

Heyden, V. 2005. 18 Chiral separations, 6:447–498.

Inai, Y., Ousaka, N., Miwa, Y. 2006. Theoretical Comparison between Three-Point and Two-Point Binding Modes for Chiral Discrimination upon the N-Terminal Sequence of 310-Helix. *Polymer Journal*, © International Journal of Research in Pharmaceutical Sciences 2479
Lai, X., Tang, W., Ng, S. C. 2011. Novel β-cyclodextrin chiral stationary phases with different length spacers for normal-phase high-performance liquid chromatography enantioseparation. *Journal of Chromatography A*, 1218(22):3496–3501.

Layton, S. E. 2005.

Millerchip, J. S., Fitzpatrick, A. 1993. Commonly used chiral drugs: A survey. *Chirality*, 5(8):573–576.

Moldoveanu, S., David, V. 2017. Stationary Phases and Columns for Chiral Chromatography. *Selection of the HPLC Method in Chemical Analysis*, pages 363–376.

Moldoveanu, S. C., David, V. 2013. Stationary Phases and Their Performance. *Essentials in Modern HPLC Separations*, pages 191–362.

Nguyen, L. A., He, H., Pham-Huy, C. 2006. Chiral drugs: an overview. *International Journal of biomedical science : IJBS*, 2(2):85–100.

Peng, L., Farkas, T., Jayapaian, S. 2009. Reversed-Phase Chiral Method Development Using Polysaccharide-based Stationary Phases. *Chromatography Today*, pages 26–28.

Prabhu, P., Shinesudev, Kurian, M., Manjusha, Minnu, M. H., ., S. S. 2016. Pharmaceutical Review and Its Importance of Chiral Pharmaceutical Review and Its Importance of Chiral Chromatography. *International Journal of Research in Pharmacy and Chemistry*, 6(3):476–484.

Rica, C. 2015. Review of Chiral Phases and Method Development Strategies SM Technical Guide To Chiral HPLC Separations. *Review of Chiral Phases and Method Development Strategies SM Technical Guide To Chiral HPLC Separations*.

Tang, Q., Song, H., Fu, C., Lei, Y. C., Chen, X. . 2004. Enantioseparation of clenbuterol enantiomers by high-performance liquid chromatography on the chiral stationary phase. *CHINESE JOURNAL OF ANALYTICAL CHEMISTRY*, 32(6):755–758.

Vargesson, N. 2015. Thalidomide-induced teratogenesis: History and mechanisms. *Birth Defects Res C Embryo Today*, 105(2):140–156.

Wang, X., Li, W., Rasmussen, H. 2007. 5 Chiral separations. *Separation Science and Technology*, pages 111–144.

Xu, X., Lan, X. Q., Yang, D., Song, H. 2007. Determination of Optical Purity of Mandelic Acid Enantiomers by HPLC with Pre-column Derivatization. *Journal of Instrumental Analysis*, 26(6):895–895.

Yang, D., Xu, X., Chen, Y. J., Zhang, L., Yao, C. H., Hang, S. 2008. Determination of enantiomeric purity of lactic acid by pre-column derivatization-high performance liquid chromatography. *Chinese Journal of Analysis Laboratory*, 27(4):52–52.

Yu, L., Wang, S., Zeng, S. 2013. Chiral Mobile Phase Additives in HPLC Enantioseparations. *Methods in Molecular Biology*, pages 221–231.

Zhang, B. 2004. Unusual separations with macrocyclic glycopeptide chiral stationary phases and chromatographic analysis and characterization of microbes.