Review Article

International Journal of Research in Pharmaceutical Sciences

Published by JK Welfare & Pharmascope Foundation

Journal Home Page: www.ijrps.com

A Review on Chiral Columns/Stationary Phases for HPLC

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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 derivate 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.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i2.2240

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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 enan-

tiomeric couple, only one sample is pharmacologically active (Fan, 2014).

Enantiomers are most commonly formed when there are four separate substitutes in a carbon atom (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. Synephrine l-isomer has 60 times d-isomer's pressor activity. There is only beta-adrenergic 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-ascorbutic; 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 interactions 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; Vander 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 CSP's 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

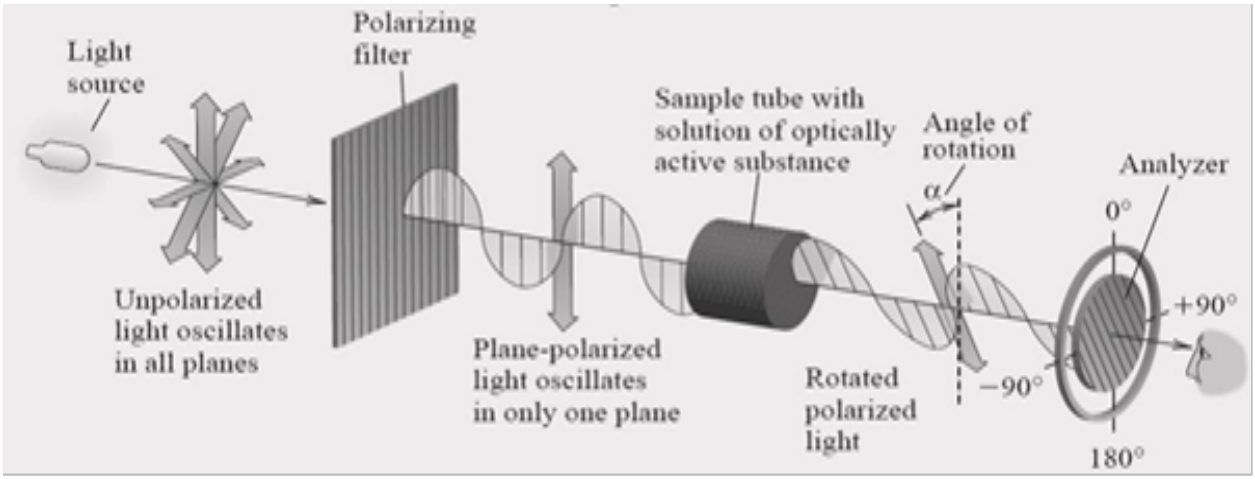

Figure 1: Polarimeter used in determination of opticalactivity (Source: internet)

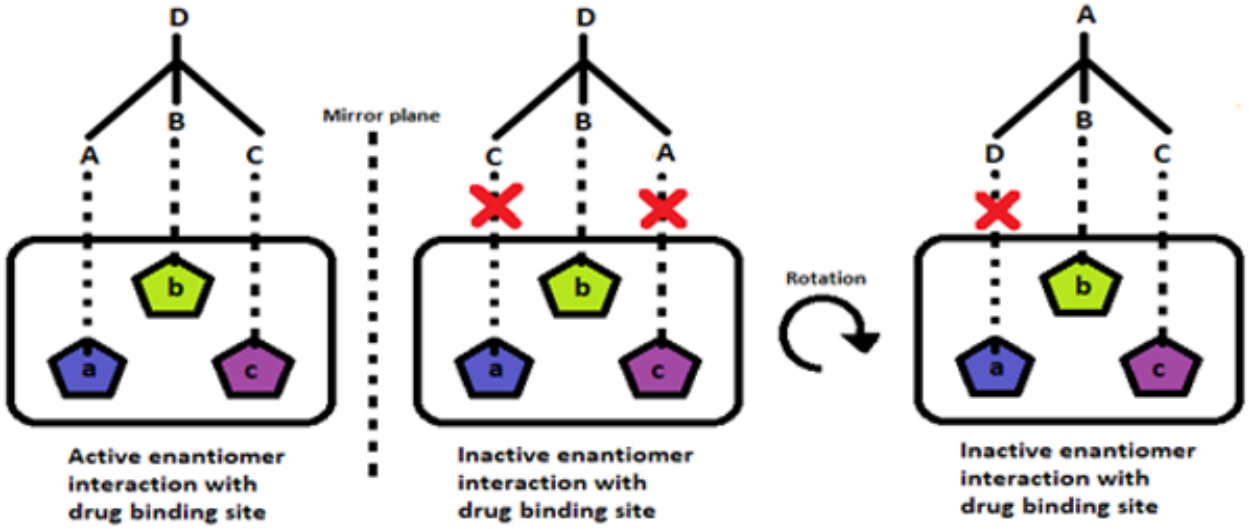

Figure 2: Three point interaction of enantiomer withreceptor

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, sulfinyl and phosphinyl oxygen, and hydroxy and ether oxygen. Steric interactions between large groups may also occur (Xu *et al.*, 2007). Overall interactions possessed by prikle CSPs are shown in the Figure 3 , (Rica, 2015).

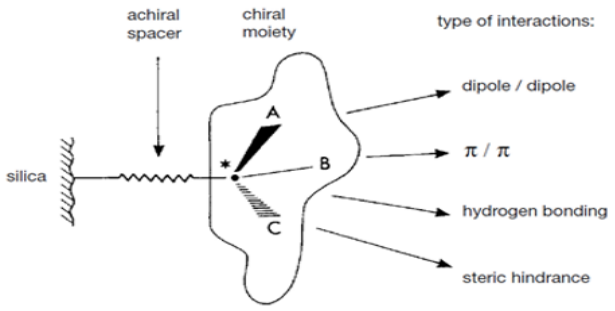

Figure 3: Types of interaction possessed by brush type stationary phase

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

Table 1: chromatographic techniquesused 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.

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 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 derivation. 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, ketones, acids, esters, sulfoxides, phosphin oxides, thiophosphine oxides, phosphine-borane, phosphine selenide, organometallics, beta-lactams, and heterocyclines 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 aminopropyl 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 underivatized. It can also be used to separate aryl propionic acid NSAID enantiomers as well as other drugs (Yang *et al.*, 2008).

ß-Gem 1

β-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 carbinol 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.

Immobilized Polysaccharide Chiral Columns

They 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 (Vander 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, metallocenes, 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 glycopeptide 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 assessesment 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 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.

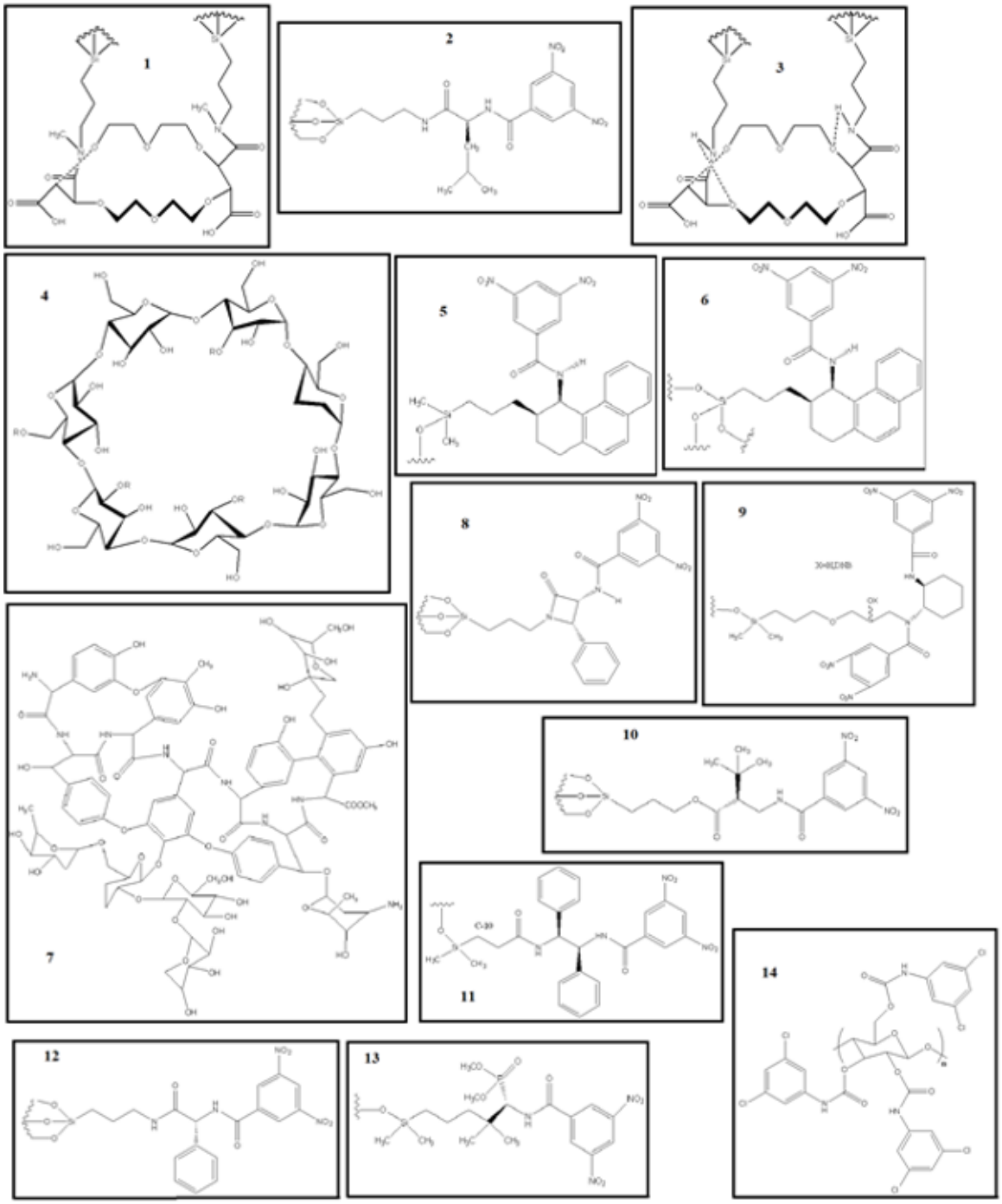

Figure 4: Structures of CSPs widely used separation of isomers (1) Chirosil ME, (2) Leucine, (3) Chirosil, (4) Cyclodextrin, (5) Whelk-O-1, (6) Whelk-O-2, (7) Macrocyclic antibiotic, (8) Prikle 1-J, (9) DACH-DNB, (10) β-Gem 1, (11) UMLO, (12) Phenyl gylicine, (13) Alpha Burke 2, (14) Polysaccharide

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
Alcohols	(+)-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

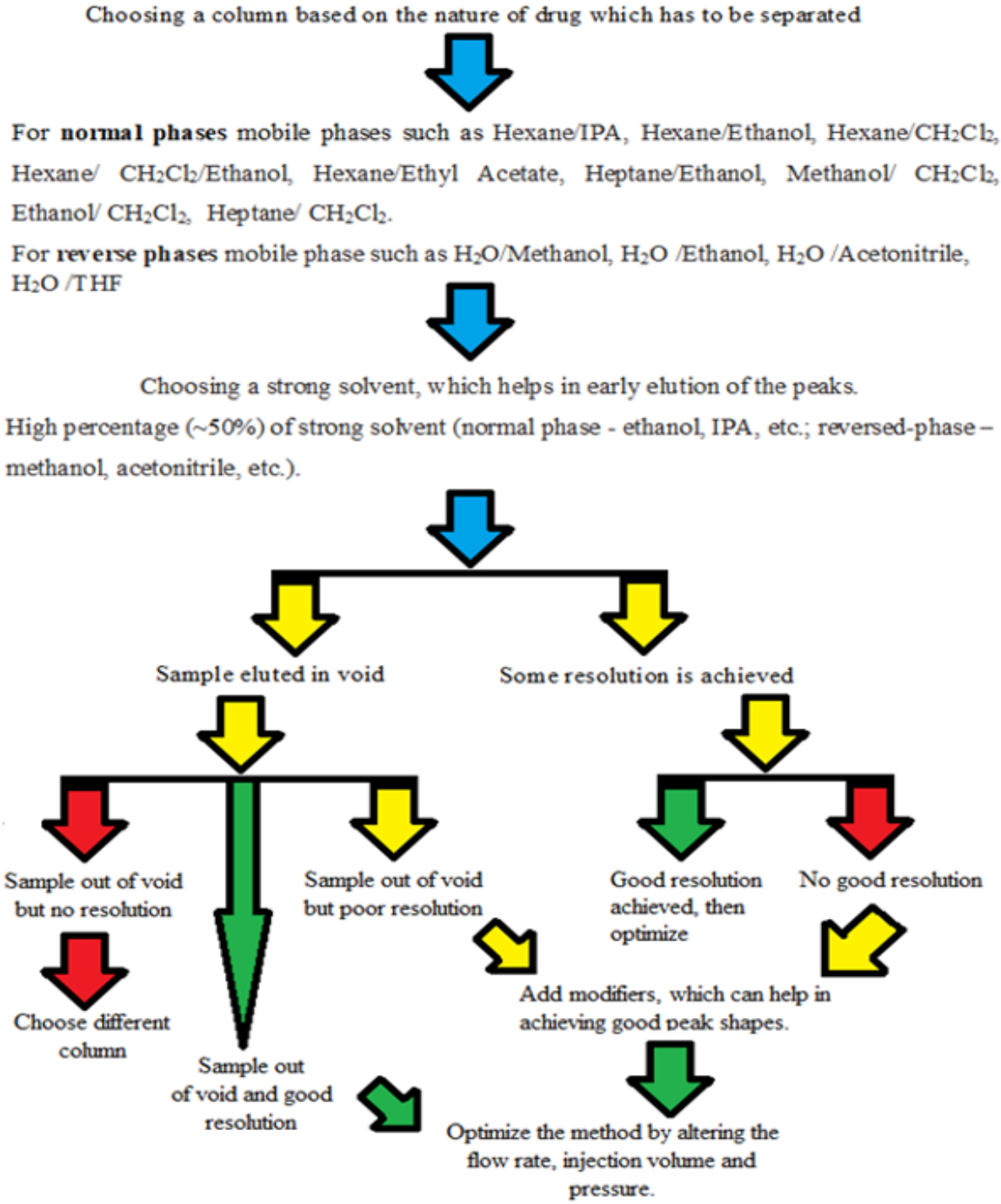

Figure 5: Flow chart of method development using CSPs in HPLC

Table 4: Columns/Chiral stationary phase used forresolution 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) F.R -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) F.R - 1.0 ml/min Detection- UV detector at 215 nm	α = 1.13 k = 2.92
Abscisic acid	Whelk-O 1	M.P - Hexane/IPA and 0.1% Acetic acid (75:25) F.R – 1.5ml/ min Detection - UV detector at 254nm	α = 1.55 k = 2.21
1’ – Acetoxychavicol Acetate	Whelk-O 1	M.P - Hexane and IPA (90:10) F.R – 1.5ml/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) F.R – 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% trimethylamine (40:40:20) F.R – 1.5ml/min Detection - UV detector at 254 nm	α = 1.34 k = 3.50
Azelastine	Whelk-O 2	M.P - Hexane/dichloromethane/ ethanol, 0.1% Triethylamine and 6mM Ammonium acetate (47:47:6) F.R – 2ml/min Detection - UV detector at 254 nm	α = 1.24 k = 8.51

Continued on next page

Table 4 continued

Fluridil	Whelk-O 2	M.P - water and methanol (57:43) F.R – 1.5ml/min Detection - UV detector at 254 nm	α = 1.18 k = 12.9
Homocysteine-Thiolactone HCI	Chirosil	M.P- Methanol/ water + 0.05% TFA (60:40) F.R- 1.5ml/min Detection- UV detector at 240 nm	α = 3.56 k = 0.58
Methionine	Chirosil	M.P- Methanol/ water + 10mM acetic acid (45:55) F.R- 1.0ml/min Detection- UV detector at 210 nm	α = 2.04 k = 1.64
4 nitro phenyl alanine	Chirosil ME	M.P- Methanol + 0.01% Phosphoric acid (60:40) F.R- 1.0ml/min Detection- UV detector at 210 nm	α = 1.51 k = 1.91
DL – Pyridylalanine	Chirosil ME	M.P- Methanol + 0.01% Phosphoric acid (70:30) F.R- 1.0ml/min Detection- UV detector at 210 nm	α = 1.74 k = 2.03
N-(1-Naphthyl)-N'-(1-methylbenzyl) ureaN-(1-Naphthyl)-N'-(1-methylbenzyl) urea	D-Phenyl glycine	M.P – Hexane + ethanol (70:30) F.R- 1.0ml/min Detection- UV detector at 254 nm	α = 1.22 k = 2.37
trans-(R)7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene	β-Gem 1	M.P – Hexane + ethanol (60:40) F.R- 1.0ml/min Detection- UV detector at 254 nm	α = 1.25 k = 3.18

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

Waterer 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 enantio-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).

Chiral stationary phases are very selective and effective for the resolution of enantiomeric mixtures. Drugs contain both effective and non-effective enantiomers in order to obtain a specified action, non-effective racemates have to be separated and eliminated from the drug formulation.

Since the racemates have a closely linked structure there should be a chiral column which can detect the minor changes and separate them effectively, such columns which are used for separation of racemates have been discussed in Table 4 and Figure 6 (Rica, 2015; Layton, 2005).

* α = It is chromatographic system's ability to chemically discriminate between sample components is the selectivity (or separation) factor. It is generally

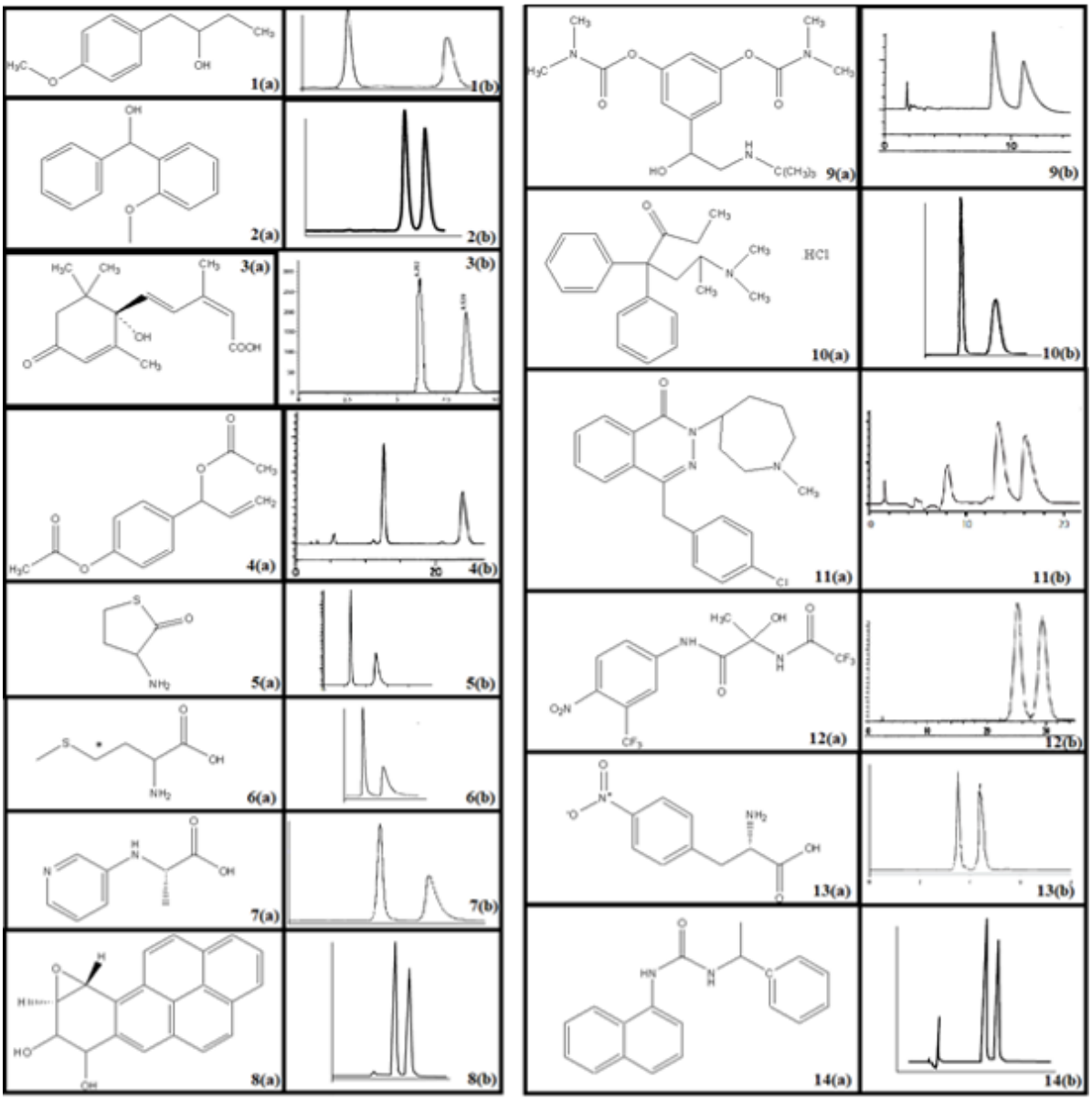

Figure 6: Structures of drugs and theirspectra of- 1(a)&(b): 1-(4-Methoxyphenyl)-2-butanol; 2(a)&(b):2-Methoxyphenyl Phenyl Carbinol; 3(a)&(b):Abscisic acid;4(a) & (b): 1' – Acetoxychavicol Acetate; 5(a)&(b): Homocysteine-ThiolactoneHCI; 6(a)&(b): Methionine; 7(a)&(b):DL – Pyridylalanine; 8(a)&(b): trans-(R)7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene; 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

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 α values suggest good separating power and good separation of each peak between the apex. Nevertheless, α value does not imply the resolution directly (CHROM academy, 2014).

$$\alpha = \frac{k2}{k1} = \frac{tR2 - tR0}{tR1 - tR0}$$

In Table 4 shows,

Mobile phase (M.P.)

Mobile Phase used in a particular ratio to resolute 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 (CHROM academy, 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.

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