IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
July 2020 Vol.:18, Issue:4

Chiral High Performance Liquid Chromatography: Review

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
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Submission: 20 June 2020
Accepted: 27 June 2020
Published: 30 July 2020

Keywords: Chiral separation, techniques, types of chiral stationary phases and applications.

ABSTRACT

Chiral separation also called as chiral resolution is procedure used to separates the two isomers of racemic compounds. Many chiral stationary phases have been manufactured for the separation of enantiomers.

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INTRODUCTION:

Chiral HPLC is by far one of the most powerful and sensitive analytical techniques used today for resolving enantiomers (1). Chiral HPLC is more versatile than chiral GC for enantiomeric separation because it can separate a wide variety of non-volatile compounds. It can be used to develop fast and accurate methods of chiral drug separation, and it allows on-line detection and quantification of both mass and optical rotation of enantiomers when appropriate detection devices are used (2).

In case of enantiomers, these have no chemical or physical differences apart from being three-dimensional mirror images.

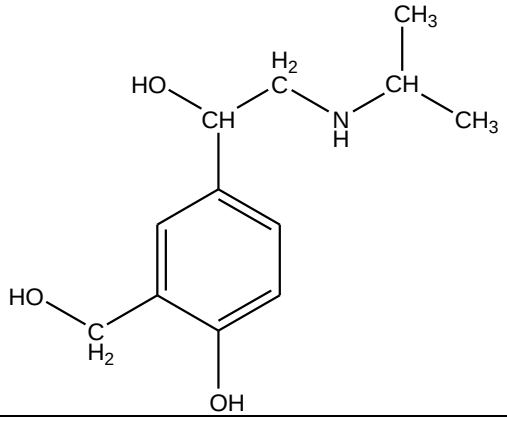
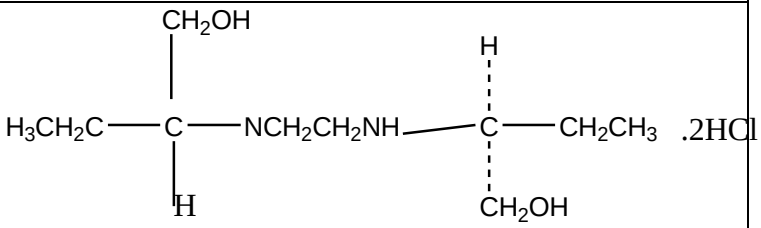
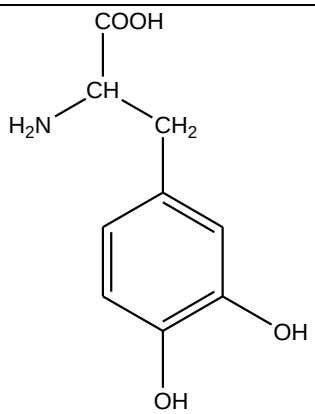
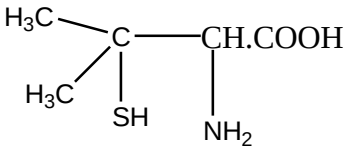
Many of the drugs are available in its racemic mixture from after synthesis. Only one of the enantiomer is having therapeutic activity and other enatiomer is either inactive or having adverse reactions. It is very important to separate them, separation of the enantiomers comprising the racemate, *i.e.* the resolution of racemate, is a common problem in stereochemical research as well as in the preparation of biologically active compounds, in particular drugs. Chiral chromatography made feasible to separates these racemic mixtures.

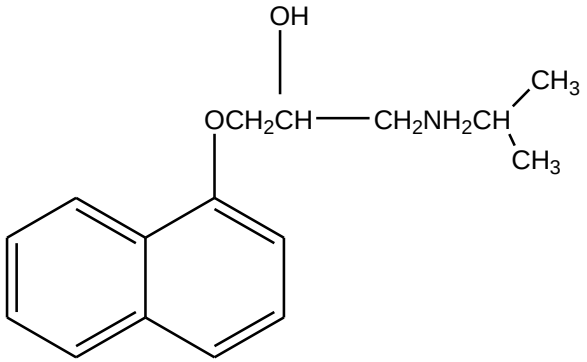
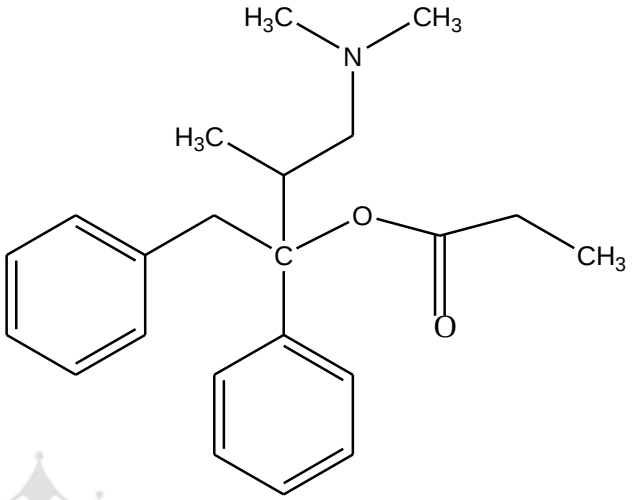
IMPORTANCE OF CHIRAL SEPRATION:

Chirality is a major concern in the modern pharmaceutical industry. This interest can be attributed largely to a heightened awareness that enantiomers of a racemic drug may have different pharmacological activities, as well as different pharmacokinetic and pharmacodynamic effects.

One isomer may thus produce the desired therapeutic activities, while the other may be inactive or produce unwanted effects.

Table No. 1: Examples of chiral drugs and functions (1)

Sr. No.	Chiral drugs	Bioactivity	Structure
1.	Albuterol	D-isomer may provoke airway constriction; L-isomer avoids side effects	
p2.	Ethambutol	The (S, S)-form of ethambutol is a tubercluostatic agent; the (R,R)-form causes optical neuritis that can leads to blindness	
3.	Levodopa	The levodopa (L-dopa) is a Parkinson's disease agent; the D-form causes serious side effects, such as granulocytopenia	
4.	Penicillamine	The (S)-isomer has antiarthritic activity; the (R)-form is extremely toxic	

5.	Propranolol	Racemic compound is used as drug; however only the (S)-(-)isomer has the desired β-adrenergic blocking activity	
6.	Propoxyphene	α-L-isomer is antitussive; α-D-isomer is analgesics	
7.	Thalidomide	The (S) isomer has the desired antinausea effects; the (R)-form is teratogenic and causes fetal abnormalities, such as severely underdeveloped limbs	

TECHNIQUES USED FOR SEPARATION OF ENANTIOMERS: (3)

Enantiomer separation methods, with an emphasis on separation by chiral inclusion complexes and crystallization, biological methods, preparative liquid and gas chromatographic methods have been reported.

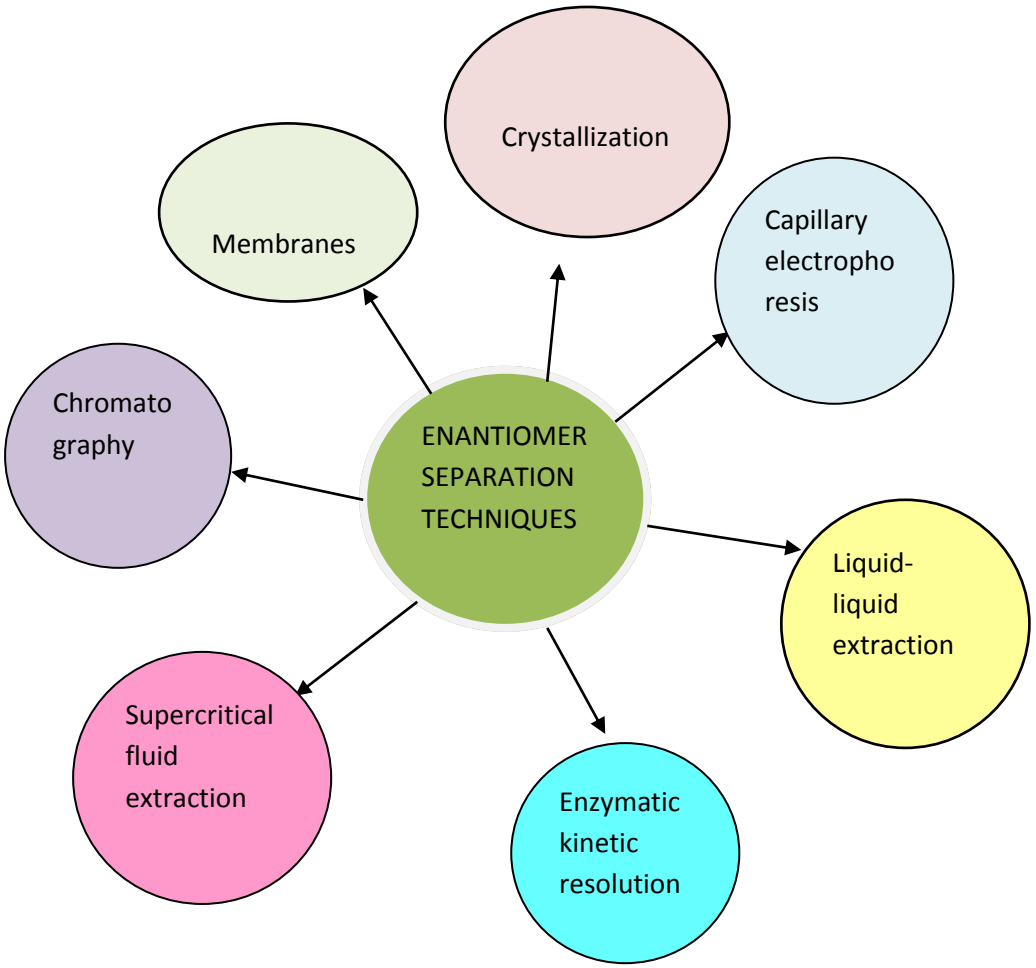


Figure No. 1: Enantiomer separation methods

ENANTIOSELETIVE HPLC ANALYSIS: (3),(1)

There are basically two options for chiral HPLC analysis namely direct and indirect approach.

Direct method:

It is based on diastereomer formation on CSPs or in mobile phase. In that transient rather than covalent diastereomeric complexes are formed between the drug enantiomers and a chiral selector present either add to the mobile phase or coated/bonded to the surface of silica support (CSP). The technique relying on chiral stationary phases are preferred as they offer specific advantages over indirect methods.

Indirect method:

In this drug enantiomers are derivatized with an enantiopure chiral reagent to form a pair of diastereomers, which may be then separated on a conventional chromatographic column since diastereomers exhibits different physicochemical properties.

CLASSIFICATION OF CHIRAL STATIONARY PHASES (CSPs):(4)(9)(13)(15)

Type 1 or "Pirkle" or Brush type CSPs:

Principle:

In that analyte-CSP complex is forms by attractive repulsive interactions, mainly by π electron donor-acceptor mechanism.

Recently, Pirkle has developed a hybrid column with both π-acid (3,5-dinitrobenzoyl) and π-base (naphthyl) groups. It can resolve a variety of compounds, including underivatized carboxylic acid. Mobile phase used for Type 1 CSPs usually contains a non-polar organic solvent with various amounts of polar modifiers e.g. hexane and 2-propanol.

It separates wide spectrum of racemic compounds such as alkyl and aryl carbinols, aryl substituted hydantoins, lactam, succinimides, phthalides, sulfoxides sulfides, amides and imide.

Type 2 Derivatized Cellulose and Related CSPs:

Principle:

It exemplified by derivatized cellulose, involves attractive interactions followed by inclusion into chiral cavities.

The polysaccharides amylase and cellulose are naturally occurring, optically-active, linear (cellulose) and helical (amylose) polymers comprising hundreds to thousands of D+(+)-glucose units joined by α(1→4) glycosidic (amylase) bonds or β(1→4) glycosidic (cellulose) bonds.

The long polysaccharides chain form rope-like bundles held together via multiple hydrogen bonds between proximate hydroxyl groups.

Type 2 CSPs of derivatized cellulose and amylose developed by Daicel Chemical Industries, Japan, are reported to separate a wide range of racemic compounds.

Table No. 2: Type 2 Chiral Columns

Sr. No.	Polysaccharide derivatives
1.	Cellulose triacetate (coated on silica gel)
2.	Cellulose tribenzoate (coated on silica gel)
3.	Cellulose trisphenylcarbamate (coated on silica gel)
4.	Cellulose tris(3,5-dimethyl-carbamate) (coated on silica gel)
5.	Cellulose tris(4-chlorophenyl carbamate) (coated on silica gel)
6.	Cellulose tris(4-methyl phenyl carbamate) (coated on silica gel)
7.	Cellulose tris(4-methyl benzoate) (coated on silica gel)
8.	Cellulose tricinnamite(coated on silica gel)
9.	Amylose tris(3,5-dimethylphenylcarbamate) (coated on silica gel)
10.	Amylose tris[(S)-phenylethylcarbamate] (coated on silica gel)

Type 3 CSPs: Inclusion:

Principle:

Inclusion are achieved through a mechanism by which the guest molecule in included in to the cavity of a host molecule. The exterior of the host molecule generally possesses functional groups that act as steric barrier or interact with the guest molecule in a fashion that induce enantio-selectivity.

Cyclodextrins (CDs): Cyclodextrins comprise D-(+)-glucose residues bonded through α (1→4) glycosidic linkage. The chair configuration of glucose makes the toroid bucket narrower at one end. Derivation of the 2 and 3 position hydroxyl groups affects selectivity.

Crown ethers: Crown ether are heteroatomic macromolecules with repeating units of (-X-C2H4-) where X, the heteroatom, is commonly oxygen but may also be a sulfur or nitrogen atom. Especially 18-crown-6 ethers can complex inorganic cations and alkyl ammonium compounds. The inclusion based on hydrogen bonding between the hydrogen of ammonium group and heteroatom of the crown ether. Additional electrostatic interaction occurs between the nitrogen and crown ether's oxygen lone pair electrons.

Type 4 Ligand exchange CSPs:

Principle:

It involves the formation of reversible coordination complexes between a bidentate analyte, a divalent metal ion, and a chiral ligand immobilized in stationary phase. The metal ion is a transition metal, usually $Cu^{+2,}$ and the chiral ligand is an amino acid e.g. proline. The stability of such complexes is highly depends on the transition metal used. Cu^{+2} complexes are generally the most stable and are preferred in LEC application.

Davankov and Kurganov were first to indicate that cross-linked resins with fixed ligands, (R)-N, N-dibenzyl-1, 2-propane-diamine in the form of Cu^{+2} complexes for alanine, serine and leucine.

Type 5 Proteins CSPs:

Principle:

Protein contains a large number of chiral center centers and many other sites that contribute to the general retention process.

CSPs based on bovine serum albumin (BSA), human serum albumin (HAS), α1-acid glycoprotein (AGP), ovomucoid from chicken egg whites (OMCHI), avidin (AVI), cellobiohydrolase I (CBH I) and pepsin are now commercially available.

A number of examples of drugs such as alprenolol, atenolol, bupivacaine, chlorthalidone, disopyramide, ephedrine, ethotoin, felodipine, fenoprofen, hexobarbital, metoprolol, pheniramine and verapamil have been resolved on chiral AGP.

Enantiomer separation of tryptophan, warfarin, and ibuprofen on HAS column prepared by N-(4-carboxycyclohexyl-methyl) maleimide (SMCC) and succinimidyl iodoacetate (SIA) method.

Table No. 3 APPLICATIONS: (6-15)

Sr. No.	Drug name	Type of mode	Stationary phase	Mobile phase	Flow rate	Temperature	λmax
1.	Atorvastatin [Separation of (S, S)-Atorvastatin from (R, R)-Atorvastatin]	Normal phase chromatographic separation	Chiralpak AD-H (250 mm x 4.6 mm ID) Column	n-hexane:ethanol :trifluoroacetic acid 85:15:0.1 (v/v/v)	1 ml /min	30 °C	246 nm
2.	Enantiomeric separation of Ramelton (determination of (R) isomer)	Normal phase HPLC method	Chiralpak AD-H (250 mm x 4.6 mm 5µm) (Amylose-based)	n-hexane:ethanol :methanesufonic acid (900:100:0.1) (v/v/v)	1ml/ min	25 °C	220 nm
3.	Zaltoprofen	Reverse-phase chiral HPLC	Chiralcel OJ-RH (150 mm x 4.6 mm, 5µm)	Sodium perchlorate (Ph2.5): methanol (20:80)(v/v)	0.6 ml/min	_	220 nm
4.	Omeprazole	_	Chiralpak AD	Hexane:ethanol (40:60)(v/v)	0.7 ml/min	_	302 nm
5.	Imazalil in orange juice	_	ChiralDex	Isopropanol:phosphate buffer solution (1:9) (v/v)	1 ml/mi	_	225 nm
6.	Levocetirizine	_	Chiralcel (250 mm x 4.6 mm 10, µ m)	$NaClO_4$: Acetonitrile (60:40)(v/v)	0.4 ml/min	15 °C	230 nm
7.	Alogliptin benzoate	_	Lux cellulose (250 mm x 4.6 mm 5µm)	ethanol:diethyl amine (100:0.5)(v/v)	1 ml/min	_	230 nm
8.	Chiral separation of thiazide diuretics						
	Butizide	Reversed phase mode	Chiralcel-OD-RH	Acetonitrile: water (40:60)	0.35 ml/min	_	_
	Cicletanine	Reversed phase mode	Chiralcel-OD-RH	Acetonitrile: water (40:60)	0.35 ml/min	_	_
	Polythiazide	Reversed	Chiralcel-	Acetonitrile:	0.35	_	_

		phase mode	OD-RH	water (35:65)	ml/min		
	Bendroflumethiazide	Reversed phase mode	Chiralcel-OD-RH	Acetonitrile:water(35:65)	0.35 ml/min	_	_
	Chlorthalidone	Reversed phase mode	Chiralcel-OD-RH	Acetonitrile:water(35:65)	0.35 ml/min	_	_
	Mefruside	Reversed phase mode	Chiralcel-OD-RH	Acetonitrile:water(35:65)	0.35 ml/min	_	_

CONCLUSION:

Drugs with chiral center show the very important place in pharmaceutical market. A best understanding of stereochemical issues of racemic drugs will assist their clinical use and important aspects of chiral analysis deals with the separation of stereoisomer of chiral compounds such as chiral drugs. Chiral HPLC based on the use of chiral stationary phases they are widely employed for the assay of drugs, enantiomers in pharmaceutical preparation. Different types of chiral stationary phase like Cyclodextrins, crown ethers, proteins have widely used for chiral separation of drugs and drugs candidates.

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