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Review Article

PHARMACEUTICAL REVIEW AND ITS IMPORTANCE OF CHIRAL CHROMATOGRAPHY

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ABSTRACT

The separation of chiral compounds has been of great interest because the majority of bioorganic molecules are chiral. Living organisms, for example, are composed of chiral biomolecules such as amino acids, sugars, proteins and nucleic acids. In nature these biomolecules exist in only one of the two possible enantiomeric forms, e.g., amino acids in the L-form and sugars in the D-form in our body. In this article we had an attempt to review about chiral chromatography and its pharmaceutical importance.

INTRODUCTION

The separation of chiral compounds has been of great interest because the majority of bioorganic molecules are chiral. Living organisms, for example, are composed of chiral biomolecules such as amino acids, sugars, proteins and nucleic acids. In nature these biomolecules exist in only one of the two possible enantiomeric forms, e.g., amino acids in the L-form and sugars in the D-form in our body.

Chromatography

Some materials appear homogenous, but are actually a combination of substances. For example, green plants contain a mixture of different pigments. In addition, the black ink in the pens that are used in this experiment is a mixture of different coloured materials. In many instances, we can separate these materials by dissolving them in an appropriate liquid and allowing them to move through an absorbent matrix, like paper. Chromatography is a method used by scientists for separating organic and inorganic compounds so that they can be analyzed and studied. By analyzing a compound, a scientist can figure out what makes up that compound. Chromatography is a great physical method for observing mixtures and solvents.

The word chromatography means "colour writing" which is a way that a chemist can test liquid mixtures. While studying the colouring materials in plant life, a Russian botanist invented chromatography in 1903. His name was M.S. T.S.wett. Chromatography is such an important technique that two nobel prizes have been awarded to chromatographers. Over 60% of chemical analysis worldwide is currently done with chromatography or a variation there on. Chromatography is used in many different ways. Some people use chromatography to find out what is in a solid or a liquid. It is also used to determine what unknown substances are. The Police, F.B.I., and other detectives use chromatography when trying to solve a crime. It is also used to determine the presence of cocaine in urine, alcohol in blood, and lead in water. Chromatography is used by many different people in many different ways. Chromatography is based on differential migration. The solutes in a mobile phase go through a stationary phase. Solutes with a greater affinity for the mobile phase will spend more time in this phase than the solutes that prefer the stationary phase. As the solutes move through the stationary phase they separate. This is called chromatographic development.

Pharmacologically active compounds (PACs) are widely regarded as emerging contaminants and many of them possess at least one stereogenic centre. The aim of this paper is to introduce the subject of chirality within PACs and its implications in

environmental contamination. The paper describes contemporary techniques utilized in the analysis of chiral contaminants and provides a critical review of the methods employed and results gained in the field.

Phenomenon of chirality

Enantiomers are molecular entities which are non-super imposable mirror images. The chirality (handedness) of enantiomeric molecules is caused by the presence of one or more chiral elements (chirality axis, chirality plane, or chirality centre, e.g., asymmetric carbon atom) in the structure. The chirality and optical activity of the enantiomers is determined by their absolute configuration, i.e., the spacial arrangement of the atoms in the molecule.

Nomenclature

IUPAC approved naming system is the Cahn-Ingold and Prelog designation for four- and six-coordinate stereogenic centres, pre-fixed with R or S, when discussing molecules with planar chirality . Dextro and Levo (+/_) may also be used to describe enantiomers where the absolute configuration may not be known or to describe the rotation of light under prescribed conditions, although the prefixes ‘d’ and ‘l’ are discouraged.

Chirality in pharmaceuticals

Two enantiomers of the same compound, despite having the same physical and chemical properties, show different interactions with other chiral molecules due to differences in spacial arrangement of the atoms and therefore binding affinity. This phenomenon is particularly significant in biological interactions as all proteins, enzymes and carbohydrates are chiral . Thus organisms might respond uniquely to each enantiomer, a phenomenon discovered by Pasteur in 1857 . This is of particular importance in the case of chemicals such as PACs and pesticides, which are designed to illicit biological action

Chiral Separation

The separation of chiral compounds has been of great interest because the majority of bioorganic molecules are chiral. Living organisms, for example, are composed of chiral biomolecules such as amino acids, sugars, proteins and nucleic acids. In nature these biomolecules exist in only one of the two possible enantiomeric forms, e.g., amino acids in the L-form and sugars in the D-form in our body. Because of chirality, living organisms show different biological responses to one of a pair of enantiomers in drugs, pesticides, or waste compounds, etc.

Chirality is a major concern in the modern pharmaceutical industry. This interestcan 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. The body being amazingly chiral selective, will interact with each racemic drug differently and metabolize each enantiomer by a separate pathway to produce different pharmacological activity. Thus, one isomer may produce the desired therapeutic activities, while the other may be inactive or, in worst cases, produce unwanted effects. Consider the tragic case of the racemic drug of n-phthalyl-glutamic acid imide that was marketed in the 1960’s as the sedative Thalidomide. Its therapeutic acitivity resided exclusively in the R-(+)-enantiomer. It was discovered only after several hundred births of malformed infants that the S-(+)-enantiomer was teratogenic.

The U.S. Food and Drug Administration(U.S. F.D.A), in 1992, issued a guideline that for chiral drugs only its therapeutically active isomer be brought to market, and that each enantiomer of the drug should be studied separately for its pharmacological and metabolic pathways. In addition, a rigorous justification is required for market approval of a racemate of chiral drugs. Presently, a majority of commercially available drugs are both synthetic and chiral. However, a large number of chiral drugs are still marketed as racemic mixtures. Nevertheless, to avoid the possible undesirable effects of a chiral drug, it is imperative that only the pure, therapeutically active form be prepared and marketed. Hence there is a great need to develop the technology for analysis and separation of racemic drugs.

Chiral compounds are also utilized for asymmetric synthesis, i.e., for the preparation of pure optically active compounds. They are also used in studies for determining reaction mechanisms, as well as reaction pathways. Chiral compounds are also important in the agrochemical industries. Current methods of enantiomeric analysis include such non-chromatographic techniques as polarimetry, nuclear magnetic resonance, isotopic dilution, calorimetry, and enzyme techniques. The disadvantages of these techniques are the need for pure samples, and no separation of enantiomers are involved. Quantitation, which does not require pure samples, and separation of enantiomers can be done simultaneously by either gas chromatography (GC) or high

performance liquid chromatography (HPLC). Chiral HPLC has proven to be one of the best methods for the direct separation and analysis of enatiomers. It is more versatile than chiral GC, because it can separate a wide variety of nonvolatile compounds. It provides fast and accurate methods for chiral separation, and allows on-line detection and quantitation of both mass and optical rotation of enantiomers if appropriate detection devices are used. Current chiral HPLC methods are either direct, which utilizes chiral stationary phases (CSPs) and chiral additives in the mobile phase, or indirect, which involves derivatization of samples. Direct chiral separations using CSPs are more widely used and are more predictable, in mechanistic terms, than those using chiral additives in the mobile phase.

To date nearly a hundred HPLC CSPs have been developed and are commercially available. However, there is no single CSP that can be considered universal, i.e., has the ability to separate all classes of racemic compounds. Choosing the right CSP for the enantioseparation of a chiral compound is difficult. The decision relies mostly on empirical data. Most chiral separations achieved on CSPs, however, were obtained based upon the accumulated trial-and-error knowledge of the analyst, intuition, and often simply by chance. An alternative way of choosing a CSP is by using predictive empirical rules that have been developed based on empirical structures. Neither scheme of choosing a right CSP offers a guarantee for a successful enantiomeric separation.

Although enantioseparation is hoped to be achieved by knowing the chemistry of the racemic analytes and the CSP sometimes, however, it does not work because the interactions of the mobile phase with both the racemic analyte and CSP have to be considered. All three components, analyte, CSP, and mobile phase, must be taken into consideration when developing a chiral separation method. The key, therefore, to a successfull enantioseparation of a particular class of racemates on a given CSP is the understanding of the possible chiral recognition mechanisms.

PRINCPLES OF CHIRAL CHROMATOGRAPHY

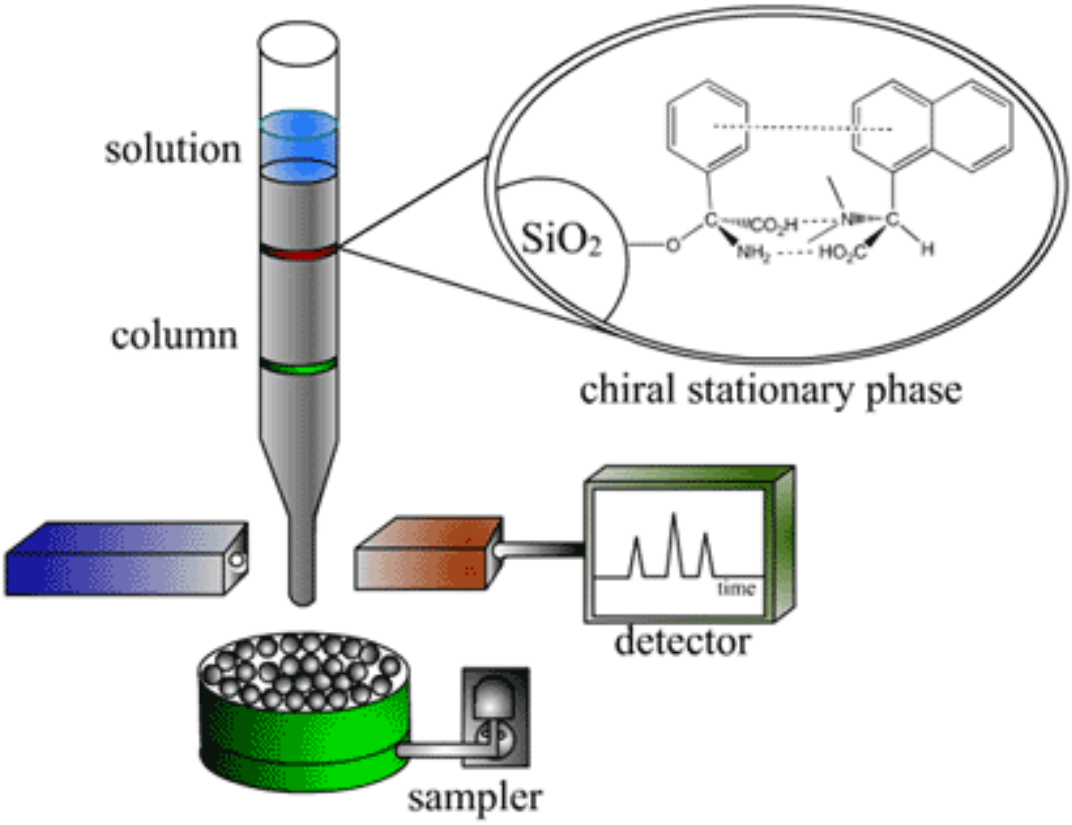

Fig. 1

Separation of optical isomers can be done by using chiral stationary phases. Different principle operates for different type of stationary phases and different samples.

The two enantiomers of the same analyte compound differ in affinity of the single enantiomer to stationary phase and they exit the column (CHIRALCEL*, CHIRACEL* OA, CHIRALPAK*IA, CHIRALPAK*AD) at different times. The chiral stationary phase can be prepared by attaching a suitable chiral compound to the surface of an achiral support such as silicagel.

Chiral stationary phases

Several chiral stationary phases (CSPs) have been developed and successfully used in drug discovery, a major driving force behind chiral chromatography. A brief introduction to generic chiral mechanisms and detailed discussion of chiral stationary phases reported to be used in environmental analysis of chiral pharmaceuticals is given below. There are many comprehensive reviews, the most up to date include. Chiral stationary phases comprise a chiral molecule bonded to a support, usually silica. Chromatography columns rely on a substrate to approach the stationary phase either under a force propelling, or pulling, it to the stationary phase surface or through arbitrary collision with it. In chiral chromatography one of the analyte's enantiomers is then preferentially bound to the station-ary phase due to the spatial arrangement of the atoms; the other enantiomer may not bind at all or, more likely, bind more weakly than its counterpart. This weaker bound enantiomer will have a shorter transient relationship with the stationary phase and therefore pass through the column more rapidly, under the force of the mobile phase. This separates the enantiomers in time and allows for stereoselective analysis. This transient diastereomeric relationship can be described by the equilibrium

Importance of enantiomeric separation

This technique is used for the separation of many enantiomers of drugs like:-

- Albuterol (anti-asthmatic inhalant)
  D-albuterol may actually cause airway constriction
  Levalbuterol (L-albuterol) avoids side effects
- Allegra (allergy medication)
  Single enantiomer of Seldane that avoids life-threatening heart disorders of Seldane.
- Fluoxetine (depression medication)
  R-Fluoxetine – improved efficacy; minimizes side effects, i.e. anxiety and sexual dysfunction. Other indications (eating disorders)
  S-Fluoxetine – use for treatment of migraines

It also usefull in case of thalidomide because, thalidomide as a chiral centere which allows for two slightly different isomers (differing only by the arrangements of their functional groups).The two enantiomers are classified as either "S" / "R". The R enantiomer is a relatively safe drugs with sedative attributes, while the S enantiomer can devastating effect. The dangerous out come of thaliodomide in the blood is that it recimises creating equal amount of both enantiomers, even ois only one kind was initialy present.

INSTRUMENTATION

Fig. 2: Chiral coloumns

Fig.3: Instrumentation of HPLC

Chiral liquid chromatography coupled with mass spectrometry is the method of choice in the analysis of chiral drugs. Examples of other detectors such as Electron Capture Detectors, UV detectors or circular dichroism (CD) detectors are in the minority even when expanding the search to related fields. Detection should be nonstereoselective. However, enantioselective ion suppression or enhancement caused by the matrix and their physical separation in time.

CLASSIFICATION OF CHIRAL STATIONARY PHASES (CSP)

POLYMER-BASED CARBOHYDRATES

Chiral polysaccharide derivatives, i.e. amylose and cellulose, coated on a silica support Enantiomers form H-bonds with carbamate links between side chains and polysaccharide backbone steric restrictions at polysaccharide backbone may prevent access of one of enantiomers to H-bonding site can be used with normal phase HPLC, SFC, RP-HPLC

Limitations: Not compatible with a wide range of solvents other than alcohols

Available columns: i.e. Chiralpak AD, AD-RH, AS, AS-RH, and Chiralcel OD, OD-RH, OJ, OJ-RH, etc. from Chiral Technologies, Inc. Chiralpak IA and IB…same chiral selectors as AD and OD, respectively, but these are immobilized on the silica; more robust and has much greater solvent compatibilities

Pirkle or Brush-type Phases: (Donor-Acceptor)

Small chiral molecules bonded to silica

More specific applications; strong 3-point interactions through 3 classes:

- π-donor phases
- π-acceptor phases
- Mixed donor-acceptor phases

Binding sites are π-basic or π-acidic aromatic rings (π-π interactions), acidic and basic sites (H-bonding), and steric interaction

Separation occurs through preferential binding of one enantiomer to CSP

Mostly used with normal phase HPLC, SFC. May get less resolution with RP-HPLC; compatible with a broad range of solvents

Limitations: only works with aromatic compounds

Available columns: Whelk-O 1, Whelk-O 2, ULMO, DACH-DNB (mixed phases), α-Burke 2, β-Gem 1 (π-acceptor phases),

Naphthylleucine (π-donor phases), from Regis Technologies, Inc.

Cyclodextrin CSPs

Alpha, beta and gamma-cyclodextrins bond to silica and form chiral cavities 3-point interactions by:

- Opening of cyclodextrin cavity contains hydroxyls for H-bonding with polar groups of analyte
- Hydrophobic portion of analyte fits into non-polar cavity (inclusion complexes)

One enantiomer will be able to better fit in the cavity than the other used in RP-HPLC and polar organic mode

Limitations: analyte must have hydrophobic or aromatic group to "fit" into cavity

Available columns: Cyclobond (α-, β-, and γ-cyclodextrins) from Astec, Inc. ORpak CDA (α), ORpak CDB (β), ORpak CDC (γ) from JM Sciences

Chirobiotic Phases

Macrocyclicglycopeptides linked to silica Contain a large number of chiral centers together with cavities for analytes to enter and interact

Potential interactions:

- π-π complexes, H-bonding, ionic interactions
- Inclusion complexation, steric interactions

Capable of running in RP-HPLC, normal phase, polar organic, and polar ionic modes

Available columns: Chirobiotic V and V2 (Vancomycin), Chirobiotic T and T2 (Teicoplanin), Chirobiotic R (Ristocetin A) from Astec

Protein-based CSPs

Natural proteins bonded to a silica matrix Proteins contain large numbers of chiral centers and interact strongly with small chiral analytes through: Hydrophobic and electrostatic interactions, H-bonding

Limitations: Requires aqueous based conditions in RP-HPLC Analyte must have ionizable groups such as amine or acid.

Not suited for preparative applications due to low sample capacity

Available columns:

Chiral AGP (α-glycoprotein) from ChromTech & HAS (human serum albumin) from ChromTec

APPLICATIONS OF CHIRAL CHROMATOGRAPHY

The process have been applied to a wide variety of natural products such as nucleic acid, urine, serum, carbohydrates, lipids, amino acids, bile acids, and manufactured products such as pharmaceuticals, pesticides, herbicides, surfactants and antioxidants.Mainly used for the separation of barbiturates. Resolution of Single enantiomers simplify the human toxicology profile and can have numerous benefits such as reduced side effects and/or reduced dosage requirements. It is used in industry primarily for separation of chiral molecules like Serine, Soman, Glyceraldehydes, Phosphours, Sulfarmetal , Cobalt , Enkephalins. This technique is more important in case of drugs, because many drugs shows different activities with different enantiomers (shown in table 1).

Table 1. Examples of drugs showing different activities with different enantiomers (Indra , 2004).

Drug	Enantiomer	Activity
Thalidomide	S- Enantiomer R- Enantiomer	Teratogen Sedative
Ethambutol	(S,S)-Enantiomer (R,R)- Enantiomer	Tuberculostatic Blindness
Penicillamine	(S)- Enantiomer (R)- Enantiomer	Antiarthritic Mutagen
Asparagine	(S)- Enantiomer (R)- Enantiomer	Bitter Sweet
Carvone	(S)- Enantiomer (R)- Enantiomer	Caraway Spearmint

Thalidomide

The use of thalidomide led to a tragedy in the 1960sin Europe. The drug was prescribed to pregnants womens to counter morning sickness. Studies later suggested that this effects were caused by the S-enantiomer and that the R-enantiomer contained the desired therapeutic activity. More recently, studies have concluded that both enantiomers of thalidomide are unstable and spontaneously epimerize to form the racemate in in-vivo in humans. The in- studies demonstrated the hydrolysis products 5-hydroxy-thalidomide and 5'-hydroxy-thalidomide while in-vivo only the 5'-hydroxy metabolite was found, in low concentrations, in plasma samples from eight healthy male volunteers who had received thalidomide orally(Meyring et-al. , 2002).

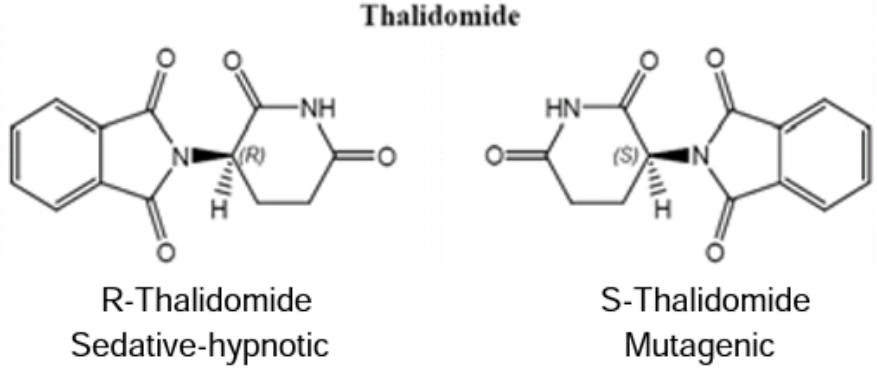

Fig. 4

Amlodipin

Amlodipine exhibit chirality, i.e, it exist as two isomers. Moreover, the receptor binding studies have shown that it the S(-)isomer of amlodipine that has L-type calcium channel blocking activity. The R(-)isomer exhibits a 1000-fold weaker calcium channel blocking activity. Thus, the antihypertensive and antianginal activity of amlodipine can be attributed only to S (-) amlodipine, racemic amlodipine contains R(+) and S(+)isomer in 1:1 ratio, purifying the pharmacologically active S(-)isomer can reduce the doseof racemic amlodipine.

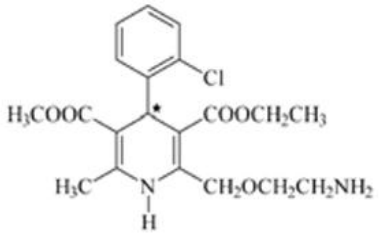

Fig. 5

Albuterol

Albuterol is leading bronchodialator, an adrenoreceptor agonist that can increase bronchial airway diameter without increasing heart rate. The bronchodialator activity resides in (R)-albuterol. (S)-albuterol, however, is not inert, as it indirectly have proinflammatory effects.There are pharmacokinetic differences between the enantiomer with (S)-albuterol being cleared more slowly. The (S)-enantiomer tends to accumulate in preference to the therapeutically effective (R)-enantiomer. These pharmacokinetic and pharmacodynamics differences provided the basis for the chiral switch patent of albuterol to levoalbuterol, (R)-albuterol, which has the same bronchodilator activity as racemic albuterol, but has a superior side effect profile(Nowak, 2003).

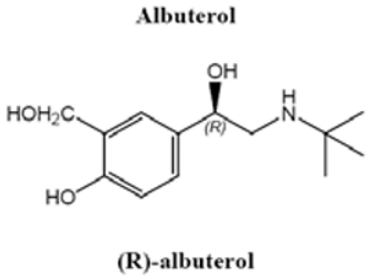

Fig. 6

COMPARISON OF CHIRAL HPLC & CLASSICAL COLUMN CHROMATOGRAPHY

Parameter	Classical column chromatography	Chiral HPLC
Stationary phase particle size	Large	small
Column size	Large	small
Particle operating pressure	Low	high
principle	adsorption	Vary with stationary phase & sample used
Scale of operation	Preparative scale	Analytical & preparative scale
cost	Low- few hundreds	High- few lakhs
Sample load	Low- medium	Low – very low(µg)
Type of column used	Ordinary columns	Chiral columns
Applications	Separation of amino acids, dyes, alkaloids etc	Separation of natural products(nucleic acid, proteins etc), barbiturates etc.

INSTITUTIONS PERFORMING CHIRAL CHROMATOGRAPHY

- Analytical development laboratory Hyderabad
- Institute of science and technology Jawaharlal Nehru technology university, Hyderabad
- Research and development,magafenepharma(p), Nasik
- Daicel chiral technologies (india), Hyderabad.
- Laboratory of govt. chemist Queens Road ,Teddington,Middle sex TW11 OLY U.K
- Laboratory of element-organic chemistry Nankai university 300071,Tianjin P.R china.
- LPD lab services company.

ADVANTAGES OF CHIRAL CHROMATOGRAPHY

- Toxicity of various drugs can be reduced
- Less amount of solvents is required
- Direct sepration of enantiomers achived by changing CPS"s
- This technique is used to eliminate teratogenic effect of S (-) Thalidomide
- Used for the sepration of S(+) barbiturates because they does not produce any pharmacological effect
- This usefull for the sepration of pharmacologicaly inactive
- R(-) Citalopram

LIMITATIONS OF CHIRAL CHROMATOGRPAHY

- Chiral silica gel is much more expensive than standard silicagel
- Need for expensive column with chiral stationary phase
- It requires expensive equipments
- Maintenance of equipment is difficult

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CONCLUSION

Chiral chromatography gives a new hope for various extraction processes in pharmaceutical industry. From this review, we concluded that chiral chromatography is a widely used extraction technique of 21st century. From this study we came to know about several applications of chiral chromatography in pharmaceutical industry such as separation of chiral molecules like Serine, Soman, Glyceraldehydes etc. Also used for the separation of barbiturates .Resolution of Single enantiomers simplify the human toxicology profile and can have numerous benefits such as reduced side effects and/or reduced dosage requirements.

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