MEDWIN PUBLISHERS
Committed to Create Value for Researchers

Advances in Pharmacology and Clinical Trials
ISSN: 2474-9214

Chiral Separation- Its Significance in the Pharmacological Treatment of Human Diseases

Mbah CJ*

Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, Nigeria

***Corresponding author:** Chika J Mbah, Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria, Tel: +234-803-659-9955; Email: chika.mbah@unn.edu.ng

Review Article

Volume 9 Issue 3

Received Date: June 04, 2024

Published Date: July 04, 2024

DOI: 10.23880/apct-16000238

Abstract

Chirality plays a pivotal role in the pharmacological activity of many chemical compounds, as the spatial arrangement of enantiomers can significantly influence their biological effects. Separation of chiral drugs is of great importance in the pharmacological treatment of human diseases because it distinguishes the pharmacological active enantiomer of a chiral drug from the other enantiomer that has low or inactive pharmacological activity or undesirable toxic effects. The availability of novel chiral stationary phases makes the separation of enantiomers effective and rapid. This review presents (i) different chiral stationary phases encountered in chiral chromatography and their various interaction mechanisms with chiral drugs, (ii) types of mobile phases involved in the chiral separation, (iii) various analytic techniques utilized and different methods in chiral separation, (iv) some examples of chiral drugs, (v) factors that affect chiral separation and (vi) significance of chiral separation in the treatment of human diseases.

Keywords: Chiral Drugs; Chiral Separation; Chiral Stationary Phase Interaction Mechanisms; Human Disease Therapy

Introduction

Chirality is the spacial orientations differing around a plane of symmetry (axis or stereogenic center) in chemical compounds that possess same molecular formula and structure [1,2]. Molecular dissymmetry in chemical compounds defines the geometric nature of atoms like carbon, sulfur, phosphorous and nitrogen. The absolute conformation of a chiral compound is defined by R,S-configurations where "R" is the abbreviation for rectus (Latin word, meaning right or clockwise), while "S" is the abbreviation for sinister (Latin word, meaning left or counterclockwise) [3].

Chemical substances having same physical and chemical properties and can only be recognized or separated in chiral environment are called enantiomers [4]. They have different 3-dimensional configuration and can be based on (i) carbon-carboxylic acids, esters, aldehydes and ketones (ii) sulfur- sulfinate, sulfoxide, sulfoximide, and sulfonium ion (iii) phosphorus-phosphinate, phosphorus-phosphine, phosphine oxide, and phosphonium ion (iv) nitrogen-amine oxide and ammonium ion. Racemate (50:50 mixture of enantiomers) is optically inactive because the rotation of one molecule exactly cancels the opposite rotation of its enantiomer. The process of isolating enantiomers from a racemic mixture is termed chiral separation [5].

Forces encountered in the chiral separation interaction processes can be attractive as well as repulsive namely: (i) dipole-dipole, (ii) ionic interaction, (iii) hydrogen bonding (either hydrogen donor site or hydrogen acceptor site), (iv) charge-transfer interactions, (v) π−π bonding, (vi) inclusion complexation (vii) steric hindrance [6,7].

A number of chiral stationary phases (CSPs) are employed in chiral separation of chemical compounds into their different enantiomers. They include:

- Polysaccharide-based CSPs-examples are cellulose and amylose carbamates and esters; other derivatives of chitin and chitosan [8,9]. The intermolecular interaction mechanisms involved are dipole–dipole, hydrogen bonding (interactions of the polar chiral drug with carbamate groups on the CSP), π–π (interactions between aromatic groups of the drug and phenyl groups on the CSP), inclusion complexation (depends on the position and strength of substituents on benzene or fused ring drugs; the retention time is typically meta<ortho<para), and steric interactions (as a result of the helical structure of the CSP) [10,11].
- Protein-based CSPs-examples are human serum albumin, bovine serum albumin, α -acid glycoprotein, ovomucoid, and cellobiohydrolase CSPs [12,13]. Due to high variety of different functional groups their interaction mechanisms mainly involve hydrogen bonding, π–π bonding, dipole–dipole, and ionic interactions.
- Immobilized β-cyclodextrin(CD)-based CSPs-examples are native β-CD, phenylcarbamate-β-CD, and chlor-, or methylsubstituted phenylcarbamate-β-CDs [14-17]. The primary interaction mechanism is the inclusion complexation of molecular moieties such as aromatic rings or other hydrophobic groups into the hydrophobic CD-cavity. Secondary interaction mechanisms such as hydrogen bonding, and dipole–dipole or ionic interactions can also be formed [18,19]. Non-inclusion (surface-type) complexes formation is also possible, especially in normal phase and polar organic modes [20].
- Glycopeptide macrocyclic antibiotics-based CSPs-examples are avoparcin, teicoplanin, ristocetin A, vancomycin, and their analogs [21-23]. The interaction mechanisms of these chiral stationary phases with drug molecules include electrostatic, dipole–dipole, hydrogen bonding, hydrophobic, π–π bonding, and steric interactions (repulsion) [24].

Various mobile phases exist for these stationary phases namely:

➢ Mobile phases for glycopeptides macrocycclic stationary phases include:

Normal phase mode composition: polar plus nonpolar, example ethanol or isopropanol and n-heptane or n-hexane, mainly for neutral compounds.

Polar organic mode composition: polar/nonpolar, example methanol or ethanol or acetonitrile or combinations (methanol/acetonitrile), mainly for neutral/polar compounds.

Polar ionic mode composition: methanol/acid/base components, example (a) anhydrous methanol; acid such as anhydrous tetrafluoroacetic acid, acetic acid, formic acid; (b) base such as ammonia, tetraethylamine, diethylamine or volatile ammonium salt (ammonium formate or acetate) mainly for ionizable compounds.

Reversed phase mode (inclusion complexing): organic solvent plus aqueous buffer, example acetonitrile plus tetraethylacetic acid; acetonitrile plus ammonium acetate mainly for all types of compounds [25].

➢ Mobile phase for immobilized β-cyclodextrin(CD)-based CSPs is polar organic phase namely methanol+acetonitrile+anhydrous acetic acid + anhydrous tetraethyl amine. This composition is primarily used for cyclodextrins and cyclodextrin derivatives only.

A number of analytical techniques have been utilized in chiral chromatography and they include gas chromatography (GC) [26]; high performance liquid chromatography (HPLC) [27,28]; micellar electrokinetic chromatography [29,30]; capillary electrophoresis (CE) [31,32]; supercritical fluid chromatography (SFC) [33]; and hyphenated techniques (LC-MS/MS) [34-36]. The CE has the advantages of high separation efficiency, minimal sample consumption, short analysis time, excellent reproducibility. HPLC technique on the contrary, has broad applicability, superior resolution, high sensitivity and selectivity, and is very compatible with range detection techniques. In general, the separation of chiral drugs can be influenced by a number of factors such as buffer type and concentration, pH, organic modifier, flow rate and temperature.

Human disease is a deviation from normal mental, physical and social well-being which will lead to disruption or loss of homeostasis. It can be congenital, degenerative, hereditary, inflammatory, metabolic, and neoplastic etc [37]. Disease diagnosis is usually followed by the best pharmacological treatment options for the patient and one of those options very often entails the use of therapeutic agents.

Discussion

A number of drugs of clinical importance are racemates (chiral drugs) [38]. Some examples of such chiral drugs are: β-blockers (atenolol, bisoprolol, metoprolol, oxprenolol, propranolol, sotalol); antibiotics (D-cycloserin); antivirals (tenoflovir)); statins (atorvastatin); anticoagulants (warfarin); anti-inflammatory agents (fenoprofen, flurbiprofen, ibuprofen, ketoprofen, naproen); anixolytics (oxazepam); narcotics (butorphanol, methadone,); diuretics (bendrofluomethiazide); calcium channel blockers (nicardipine); proton pump inhibitors (omeprazole, lansoprazole); stimulants (amphetamine) [39,40]. However, there is an increasing trend to obtain single enantiomers from

these chiral drugs, a process called chiral switching which is mostly carried out by chromatographic techniques [41]. Other techniques include electrophoresis, crystallization.

Chiral chromatography can be by (i) direct method, involving the use of a chiral stationary phase (CSP) or a chiral mobile phase additive. The chiral stationary phase (CSP) separation will entail an interaction between the CSP and the racemic drug (analyte) to form diastereomeric complexes. One of the complexes has a stronger binding strength than the other, resulting in different retention times for the enantiomeric pair. The chiral mobile-phase additive (CMPA) forms a transient diastereomeric complex with the drug and is followed by resolution of the diastereomeric complexes [42]. Poorly shaped peaks, detection problems, and availability of CMPA limit the use of this procedure. (ii) indirect method, entails using a chiral derivatizing agent. The chiral derivatization is a reaction of the analyte with the chiral derivatizing agent forming diastereomeric derivatives which are then separated on an achiral stationary phase [32].

In general, the choice of a suitable method is controlled by a number of factors namely suitable chiral stationary phase, optimization of chromatographic conditions, assay sensitivity, availability, purity and stability of chiral derivatizing agent(s), ease and efficiency of derivatization, and total analysis time.
Of all the chiral stationary phases, polysaccharide-based CSP is the most commonly and successfully used in chiral chromatography.

It is probably because of the formation of different types of intermolecular interactions (dipole–dipole, hydrogen bonding, inclusion complexation, π–π bonding, etc.) arising from their specific structural properties namely (a) conformational chirality of the helical carbohydrate backbone, (b) the functional groups of the ester and phenylcarbamate substituents attached to the polysaccharide chain, as well as (c) the stereogenic centers of the monosaccharide units, thus making it possible for chiral separation of a large variety of chemical substances of pharmaceutical interest [10].

Glycopeptide macrocyclic antibiotics-based CSP is the next most promising utilized CSP because it has unique structural features and functionalities that allows various interactions (such as those found in polysaccharide-based CSP), between the chiral drug and the stationary phase. Due to these diverse mechanisms in enantioseparation, they can widely be used in normal phase, polar ionic, polar organic, and reversed-phase conditions for the separation of a wide range of chiral drugs with various polarities [43]. The increased efficiency, higher chemoselectivity and enantioselectivity as well as the stability of these CSPs facilitate the analyses of drugs that belong to structurally different classes.

Significance of chiral separation in the pharmacological treatment of human diseases

Chiral drug (mixture of enantiomers) presents difference in pharmacological activity or efficacy as well as adverse or toxic effects. Where one enantiomer is toxic and the other has the desired therapeutic activity, the development of the single enantiomer becomes especially desirable [4]. When both enantiomers are pharmacologically active but differ significantly in their potency, specificity or maximum effect, development of a single enantiomer becomes more important.

Each enantiomer can act uniquely in exhibiting different plasma concentration, bioavailability, and toxicity [35]. The maximum effect of an active enantiomer against any disease state can be verified by determining its maximum plasma concentration. Table 1 below presents some of the single enantiomers utilized in the treatment of human diseases.

Human Illness	Clinical Enantiomers
Nacrolepsy ((extreme sleepiness)	Armodafinil
Depression	Escitalopram
Gastric ulcer	Esomeprazole
Inflammation	Dexibuprofen
Inflammation	Dexketoprofen
Narcolepsy (or attention deficit)	Dexmethylphendate
(hyperactivity disorder)	
Bacterial infection	Levofloxacin
Bacterial infection	Levosalbutamol
Depression	Salmeterol
Depression	Levomilnacipran

Table 1: Treatment of Human diseases with Clinical enantiomers.

Improved therapeutic index, more appropriate dosing frequency, decreased inter-individual variability, and decreased potential for drug-drug interactions are advantages associated with chiral separation [44]. Other advantages of chiral separation include (i) assists to optimize drug design, understand the pharmacodynamics, pharmacokinetics and potential side effects of drugs. (ii) the enantiomeric excess measurement is essential for quality assurance and legal compliance, as well as the integrity and consistency of pharmaceutical formulations. (iii) leads to the manufacture of enantiomerically pure drugs and (iv) provides the required conditions for generating and quantifying the effective enantiomer in biological fluids. Due to variations in the biological activities and pharmacokinetic characteristics of pharmacological enantiomers, the

enantiomeric separation and quantification of chiral drugs have become very significant in the management of human disease states. Enantiomeric quantification is essential for pharmaceutical formulation, quality and uniformity of pharmaceutical dosage forms.

Conclusion

Chirality is defined as non-superimposable mirror-images of chemical compound with the same molecular formula and structure but different spacial orientations around a stereogenic center or plane of symmetry. New analytical and preparative techniques make it easier to develop single enantiomers. Specific and sensitive bioanalytical techniques such as HPLC, micellar electrokinetic chromatography, capillary electrophoresis hyphenated HPLC assist scientists to follow the pharmacokinetics of individual enantiomers following the administration of chiral drug.

Polysaccharide-based CSPs stand out as the most successful and widely used class in chiral chromatography as their versatility extends across various separation modes, permitting for their chromatography. Finally, appropriate dosing frequency, improved therapeutic index, decreased inter-individual variability, and decreased potential for drug-drug interactions make chiral separation of great significance in the pharmacological treatment of human diseases.

References

1. Hutt AJ, Tan SC (1996) Drug chirality and its clinical significance. Drugs 52 (5): 1-12.

2. Jayakumar R, Vadivel R, Ananthi N (2018) Role of chirality in drugs. Organic Medicinal Chem IJ 5(3): 555661.

3. Cahn RS, Ingold SC, Prelog IV (1996) Specfication of molecular chirality. Angew Chem Ind Ed 5(4): 385-415.

4. Fassihi AR (1993) Racemates and enantiomers in drug development. Int J Pharmaceutics 92(1-3): 1-14.

5. Al-Sulaimi S, Kushwah R, Alsibani MA, El Jery A, Aldrdery M, et al. (2023) Emerging developments in separation techniques and analysis of chiral pharmaceuticals. Molecules 28(17): 6175.

6. Nazareth C, Pereira S (2020) A review on chiral stationary phases for separation of chiral drug. Int J Pharm Phytopharmacol Res 10(3): 77-91.

7. Cirilli R, Ferretti R, Gallinella B, Turchetto L, Zanitti L, et al. (2009) Development and validation of an enantioselective and chemoselective HPLC method using a Chiralpak IA column to simultaneously quantify (R)-(+)- and (S)-(-)-lansoprazole enantiomers and related impurities. J Pharm Biomed Anal 50(1): 9-14.

8. Hesse G, Hagel R (1973) Eine vollstandige Recemattennung durch eluitons-chromagographie an cellulose-tri-acetat. Chromatographia 6: 277-280.

9. Okamoto Y, Kawashima M, Hatada K (1984) Chromatographic resolution. 7. Useful chiral packing materials for high-performance liquid chromatographic resolution of enantiomers: Phenylcarbamates of polysaccharides coated on silica gel. J Am Chem Soc 106(18): 5357-5359.

10. Lammerhofer M (2010) Chiral recognition by enantioselective liquid chromatography: Mechanisms and modern chiral stationary phases. J Chromatogr A 1217(6): 814-856.

11. Teixeira J, Tiritan ME, Pinto MMM, Fernandes C (2019) Chiral stationary phases for liquid chromatography: Recent developments. Molecules 24(5): 865.

12. Kalikova K, Riesova M, Tesarova E (2012) Recent chiral selectors for separation in HPLC and CE. Cent Eur J Chem 10: 450-471.

13. Ward KD, Bravenec AD, Ward TJ (2019) Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation. John Wiley & Sons Ltd, Hoboken, NJ, USA, pp: 1-28.

14. Chen X, Zhou Z, Yuan H, Meng (2008) Preparation and chiral recognition of a mono[6A-N-1-(2-hydroxy)- phenylethylimino-6Adeoxy]-β-cyclodextrin HPLC stationary phase. J Chromatogr Sci 46(9): 777-782.

15. Bui CV, Rosenau T, Hettegger H (2021) Polysaccharide- and β-cyclodextrin-based chiral selectors for enantiomer resolution: Recent developments and applications. Molecules 26(14): 4322.

16. Qin Q, Zhang S, Zhang WG, Zhang ZB, Xiong YJ, et al. (2010) The impact of silica gel pore and particle sizes on HPLC column efficiency and resolution for an immobilized, cyclones-train based, chiral stationary phase. J Sep Sci 33(17-18): 2582-2589.

17. Lin C, Fan J, Liu W, Chen X, Ruan L, et al. (2018) Back Cover: A new single-urea-bound 3,5-dimethylphenylcarbamoylated β-cyclodextrin chiral stationary phase and its enhanced separation performance in normal-phase liquid chromatography. Electrophoresis 39(2): 348-355.

18. Scriba GKE (2019) Chiral recognition in separation sciences. Part I: Polysaccharide and cyclodextrin selectors. TrAC Trends Anal Chem 120: 115639.

19. Zhang JH, Xie SM, Yuan LM (2022) Recent progress in the development of chiral stationary phases for high-performance liquid chromatography. J Sep Sci 45(1): 51-77.

20. Dobo M, Adam M, Fiser B, Papp LA, Dombi G, et al. (2023) Enantioseparation and molecular docking study of selected chiral pharmaceuticals on a commercialized phenylcarbamate-β-cyclodextrin column using polar organic mode. Sci Rep 13: 14778.

21. Berkecz R, Tanacs D, Peter A, Ilisz I (2021) Enantioselective liquid chromatographic separations using macrocyclic glycopeptide-based chiral selectors. Molecules 26(11): 3380.

22. Aslani S, Berthod A, Armstrong DW (2023) Macrocyclic antibiotics and cyclofructans. In: (Eds.), Cass QB, et al., Chiral separations and stereochemical elucidation: Fundamentals, Methods, and Applications. John Wiley & Sons Ltd, Hoboken, NJ, USA, pp: 247-272.

23. Ilisz I, Orosz T, Peter A (2019) High-performance liquid chromatography enantioseparations using macrocyclic gly-peptide-based chiral stationary phases: An overview. In Methods in Molecular Biology 1985: 201-237.

24. Jiang H, Li Y, Pelzer M, Cannon MJ, Randlett C, et al. (2008) Determination of molindone enantiomers in human plasma by high-performance liquid chromatography–tandem mass spectrometry using macrocyclic antibiotic chiral stationary phases. J Chromatography A 1129(2): 230-238.

25. Bakhtiar R, Tse FL (2000) High-throughput chiral liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 14(13): 1128-1135.

26. Sidisky LM (2023) Chiral capillary gas chromatography: A highly selective analytical tool. LCGC Int 41(s5): 20-22.

27. Nguyen LA, He H, Pham-Huy C (2006) Chiral drugs: An overview. Int J Biomed Sci 2(2): 85-100.

28. Gales L, Silva AMS, Pinto MMM, Kijjoa A, Fernandes C, et al. (2020) New chiral stationary phases for liquid chromatography based on small molecules: Development, enantioresolution evaluation and chiral recognition mechanisms. Chirality 32(1): 81-97.

29. Peterson AG, Ahuja ES, Foley JP (1996) Enantiomeric separations of basic pharmaceutical drugs by micellar electrokinetic chromatography using a chiral surfactant, N-dodecoxycarbonylvaline. J Chromatography B: Biomed Appl 683(1):15-28.

30. Liu Y, Jann M, Vandenberg C, Eap CB, Shamsi SA (2015) Development of an enantioselective assay for simultaneous separation of venlafaxine and odesmethylvenlafaxine by micellar electrokinetic chromatography-tandem mass spectrometry: Application to the analysis of drug-drug interaction. J Chromatography A 1420: 119-128.

31. Gubitz G, Schmid MG (1997) Chiral separation principles in capillary electrophoresis. J Chromatography A 792(1-2): 179-225.

32. Gubitz G, Schmid MG (2001) Chiral separations by chromatography and electromigration techniques. A review. biopharmaeutics and drug disposition 22(7-8): 291-336.

33. De Klerck K, Parewyck G, Mangelings D, Vander Heyden Y, Dascalu AE, et al. (2012) Enantioselectivity of polysaccharide-based chiral stationary phases in supercritical fluid chromatography using methanol-containing carbon dioxide mobile phases. J Chromatography A 1269: 336-345.

34. Sharma P, Contractor P, Guttikar S, Patel DP, Shrivastav PS (2014) Development of a sensitive and rapid method for the quantification of (S)-(-)- and (R)-(+)- metoprolol in human plasma by chiral LC-ESIMS/MS. J Pharm Anal 4(1): 63-79.

35. Kang W, Lee DJ, Liu KH, Sunwoo YE, Kwon KI, et al. (2005) Analysis of benidipine enantiomers in human plasma by liquid chromatography-mass spectrometry using a macrocyclic antibiotic (vancomycin) chiral stationary phase column. J Chromatography B 814(1): 75-81.

36. Peterson AG, Jiang H, Li Y, Pelzer M, Cannon MJ, et al. (2008) Determination of molindone enantiomers in human plasma by high-performance liquid chromatography–tandem mass spectrometry using macrocyclic antibiotic chiral stationary phases. J Chromatography A 119(2): 230-238.

37. Loscalzo J1, Kohane I, Barabasi AL (2007) Human disease classification in the postgenomic era: a complex systems approach to human pathobiology. Mol Syst Biol 3: 124-128.

38. Millership JS, Fitzpatrick A (1993) Commonly used chiral drugs: a survey. Chirality 5(8): 573-576.

39. Hayball PJ (1996) Chirality and nonsteroidal anti-inflammatory drugs. Drugs 52 (5): 47-58.

40. Katsuki H, Hamada A, Nakamura C, Arimori K, Nakano M (2001) High-performance liquid chromatographic assay for the simultaneous determination of lansoprazole

enantiomers and metabolites in human liver microsomes. J Chromatogr B Biomed Sci Appl 757(1): 127-133.

41. Tucker GT (2000) Chiral switches. The Lancet 355(9209): 1085-1087.

42. Snyder LR, Kirkland JJ, Glajch JL (1997) Practical HPLC Method Development. 2nd(Edn.), John Wiley & Sons Inc, New Jersey, USA, pp: 1-765.

43. Chankvetadze B (1985) Chiral Seperations. Humana Press Inc, Totowa, NJ, USA, pp: 93-126.

44. Rane VP, Shinde DB (2008). Development and validation of chiral LC method for the enantiomeric separation of duloxetine on amylose based stationary phase. J Chromatogr Sci 46(9): 772-776.