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The Significance of Chiral Separation, Determination of Enantiomeric Purity in Drug Design, Safety and Development –An Overview

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ABSTRACT

Chiral separation, as well as the determination of the optical purity of chiral pharmaceuticals, has attracted a great deal of attention from the healthcare and pharmaceutical industries. Most pharmaceutical researches and drug development efforts have been concentrated on the production of enantiomerically pure products because of the increasing demand for such drugs to be administered in a highly optical purified form. Accordingly, chirality is also a major concern in the pharmaceutical industry. When the enantiomers of a drug are administered into a chirally selective living system, these enantiomers frequently exhibit differences in bioavailability, distribution, metabolic and excretion behavior and action. The determination of optical impurity in a drug is very important from the efficacy and safety point of view, and is rapidly becoming one of the key issues in the development of new drugs. Chirality influences drug delivery because a single enantiomer or a non-racemic blend may have improved solubility, dissolution, and stability. 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. To assure patient safety and clinical efficacy, the pharmacological evaluation of stereoisomers is an integral part of new drug development. Analytical methods to determine the enantiomeric purity of new investigational drugs are often attained through a series of generic or screening methodologies. The main methods used for chiral drug separation are GC, HPLC, and CE. Other techniques, such as chiral crystallization and enzyme-based kinetic separation.

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Introduction

Drugs are classified into achiral, racemic and single enantiomer (enantiopure) drugs with one-chiral center or multi-chiral centers. Isomers are compounds with the same molecular formula but different structural formulas. There are two major categories of isomers: constitutional (or structural) isomers and stereoisomers. Constitutional isomers are molecules with the same atomic composition but different bonding arrangements between atoms, stereoisomers are molecules with one or more "chiral" centres that allow the possibility of forms with the same chemical formula but differ in spatial arrangement of atoms. They can be classed as cistrans isomers or optical isomers. An enantiopure drug is a pharmaceutical that is available in one specific purified enantiomeric form.

Chiral separation, as well as the determination of the optical purity of chiral pharmaceuticals, has attracted a great deal of attention from the healthcare and pharmaceutical industries. Most pharmaceutical researches and drug development efforts have been concentrated on the production of enantiomerically pure products because of the increasing demand for such drugs to be administered in a highly optical purified form [1, 2].

Accordingly, chirality is also a major concern in the pharmaceutical industry. When the enantiomers of a drug are administered into a chirally selective living system, these enantiomers frequently exhibit differences in bioavailability, distribution, metabolic and excretion behavior and action. One of the enantiomers is often the more active stereoisomer for a given action (eutomer), while the other, less active one (distomer) may either contribute side-effects, display toxicity or act as an antagonist. The differences in biological properties of enantiomers arise from the differences in protein transport and binding, the kinetics of their metabolism and their stability in the environment.

Single enantiomers can be obtained via (a) the selective synthesis of one enantiomer or (b) the separation of racemic mixtures. The therapeutic action of a chiral drug depends on its stereospecificity, each isomeric form having its own pharmacological effect [3].

The determination of optical impurity in a drug is very important from the efficacy and safety point of view, and is rapidly becoming one of the key issues in the development of new drugs [4,5].

These impurities can be starting materials, intermediates, reaction byproducts, or degradation products [6].

Besides the ethical or environmental reasons for developing single enantiomers, determination of optical impurity represents a real

therapeutic benefit, and, in some cases, has been used as a strategy for extending the patient's life [7].

Regulatory authorities therefore recommend that new chiral drugs should ideally be marketed only in the form of pure enantiomers. Such differences in pharmacological activity necessitate developing adequate methodologies for quality control and analytical methods capable of determining the enantiomeric purity of drugs during chemical and pharmaceutical developments [8].

Developing optically active pure drugs poses a great challenge for researchers and scientists [9].

Drugs that are derived from natural products are usually obtained in the optically active or pure form of a single isomer; only homochiral drugs are safe for humans. However, the drugs that are produced by chemical synthesis are usually a mixture of equal parts of two, four, or more isomers, depending on the number of asymmetric centers. Accordingly, stereoselectivity in chiral drug bioavailability, distribution, interaction with receptor sites, metabolism, and elimination results in differences of isomer activity, ranging from unwanted toxicity to no significance and finally to enhanced activity [10–12].

Currently, the enantiomeric separation of some drugs with multiple stereogenic centers is one of the most difficult tasks for pharmaceutical analysts during method development [13].

To control the enantiomeric purity of starting materials and products, reliable and accurate analytical methods are necessary. At an analytical level, sensitivity and selectivity are important requirements in many fields of academic, industrial and pharmaceutical research. Of all the existing separation methods adapted for analytical purposes, high performance liquid chromatography (HPLC) is the most widespread chiral separation technique in analytical and preparative resolutions and drug discovery.

Chirality – An Emerging Trend in Therapeutics

Stereochemical aspects of drug action have intrigued researchers ever since the introduction of the receptor concept [14].

Today, enantioselectivity is increasingly understood in molecular and atomic detail. As a result, chirality has emerged as an important aspect in the Pharmaceutical Technology. The development of chiral technology has opened a new era in the pharmaceutical field, bringing to the patients an array of drugs that stand a class apart in terms of therapeutic efficacy and safety.

As early as in 1980's and more so since the FDA recognized the importance of single isomer drugs, the pharmaceutical research and development has started focusing on single isomer or chiral drugs. The need arose because of the fact that our body is chiroselective, i.e. the body shows different biological responses to different isomers. Moreover, 2/3rd of the molecules in nature are chiral, hence the best way to interact with nature for a positive outcome would be to use chiral molecules.

Pharmacological Implication of Chirality

Consideration of chirality is now an integral part of drug research and development and the regulatory process. There is no choice! Enantiomeric forms of a drug can differ in potency, toxicity, and behavior in biological systems. Enantiomers of all chiral bioactive molecules have to be separated and tested.

The presence of one stereocenter in the absolute structure of a therapeutic molecule can lead to different situations according to toxicological and pharmacological properties. As the two stereoisomers of the drug have different configurations, their complementary binding sites (receptors, enzymes, etc) are also expected to be different. Thus the stereoisomer (commonly referred to as "eutomer") binding precisely to the target sites could induce the therapeutic activity while the other stereoisomer (distomer) may bind weakly, not at all the relevant site or may bind precisely to other sites that are not the intended targets. In this way, whenever a drug is commercialized as a racemate, the eutomer may be active while the other stereoisomer may have:

- No activity,
- Quantitatively and qualitatively the same activity,
- Qualitatively the same type of activity but lower intensity,
- A completely separate beneficial activity,
- A completely separate adverse activity.

Pharmacogenetic Aspects

Pharmacogenomics refers to the general study of all of the many different genes that determine drug behaviour. Besides environmental factors, genetic factors regulate the fate of drugs in the organism. Some polymorphic enzymes such as some cytochrome P-450 isozymes display stereoselectivity toward chiral substrates or in the formation of chiral metabolites from achiral parent compounds. The pharmacogenetics of metabolism of psychotropic drugs based mainly on the study of the polymorphic enzymes CYP2D6 and CYP2C19, and the knowledge on the pharmacology, metabolism, pharmacokinetics, and pharmacogenetics of antidepressants, antipsychotics, and methadone has been reported [15].

Genetic differences between people contribute to inter-individual differences in the response to many commonly used drugs. Pharmacogenetics primarily uses genetic variation to identify subgroups of patients who may respond differently to a certain medication and comprises of genetic studies on both the pharmacokinetics and pharmacodynamics of treatment response [16].

The pharmacogenetics of drug metabolising enzymes and particular the cytochrome P450 (CYP) enzymes has been in the focus for almost 40 years. Early experiments with debrisoquine and nortriptyline documented that patients fall into different categories: poor, intermediate, extensive and ultrarapid metabolizers. A recent study confirmed an association between CYP2D6-allele/plasma level concentrations of venlafaxine, although it did not find any such association for desvenlafaxine (an antidepressant of the serotonin-norepinephrine reuptake inhibitor). Desvenlafaxine is a synthetic form of the isolated major active metabolite of venlafaxine [17].

The genetically variable CYP450-mediated metabolism of a number of serotonin-active drugs that are often implicated in cases of serotonin toxicity, to assess the impact of pharmacogenetics on drug metabolism, response, interactions and adverse effects has been reported A patient's response to a chiral drug is influenced by their genome, so pharmacogenetics could be used to determine drug sensitivity [18].

Drawbacks of Racemates and Toxicology

In certain instances racemates of drugs have been proven to be extremely harmful. A classical example is that of Thalidomide. The racemate of Thalidomide was marketed as a "morning sickness" drug. The drug when administered to pregnant patients produced

teratogenic effects. Later on it was found that only the (S) isomer produced teratogenic effects and led to phocomelia, whereas later on it was found out that only the (R) isomer of Thalidomide possessed therapeutic activity and was devoid of the teratogenic effects. The R isomer is very useful for the treatment of cancer, AIDS and erythema nodosum leprosum.

The toxicological properties in a pair of enantiomers can be identical or entirely different. They can reside in the pharmacologically active enantiomer or in the inactive one. Some following drugs are marketed as single enantiomer solely because their toxicities reside almost in one of their two enantiomers [19-23].

Chirality in Drug Delivery Formulation

Chirality influences drug delivery because a single enantiomer or a non-racemic blend may have improved solubility, dissolution, and stability [24].

In addition, many available pharmaceutical excipients (cellulose) either naturally occur as single enantiomers or are derivatives of the latter chiral molecules. More attention has been drawn to the influence of chiral excipients on the modification of in vitro release and in vivo disposition of chiral drugs. Chiral excipients have been widely used in pharmaceutical dosage forms.

Potential Advantages of Enantiopure Drugs

Potential advantages of single-enantiomer drugs include: separating unwanted pharmacodynamic side effects from toxic effects in case these reside exclusively in one enantiomer, smaller doses of medication; simpler and more selective pharmacodynamic profile; less complex pharmacokinetic profile; less side-effects because of the elimination of distomers; reduce drug interactions, fewer adverse effects, one form is more prone to adverse drug interactions; reduced metabolic load over the enzymatic system; potential for an improved therapeutic index and less complex relationship between plasma concentration and effect [25-27].

Further, the advantages of enantiopure drugs over racemic drugs have varied, depending on the case, and the biological effects of single enantiomer drugs over their counterpart racemic drugs still remain unclear in some cases.

Enantiopure Drugs Preparation

The separation of enantiomers is called resolution which is essential in order to ensure the safety and efficiency of chiral compounds. Nowadays, the technologies of resolution and asymmetric synthesis have advanced to the point that the cost of making enantiopure material is not so great, and the FDA is expressing a strong preference that all medicinal drugs are sold in enantiopure form. Drug companies develop ways to obtain one enantiomer and produce a medication using only that enantiomer. Three strategies can be applied to obtain single pure isomers: (i) extraction from plants and animal materials (ii) enantio-selective asymmetric synthesis so that only one isomer is formed in the first place [28] or (iii) making a racemate and finding a method for separating the enantiomers (chiral resolution) [29-31].

Among the variety of enantioseparation methods, classical resolution, Simulated Moving Bed technology, chiral chromatography and crystallization are the most dominant methods for the recovery of pure enantiomers [32-35].

Importance of Chiral Separation

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. 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.

Chiral Drug Separation Techniques

The main methods used for chiral drug separation are GC, HPLC, and CE [36].

Other techniques, such as chiral crystallization and enzyme-based kinetic separation, have also attracted attention [37].

Applications of HPLC to Chiral Separation

Chromatographic methods have dominated separation of enantiomers during the past several decades, especially HPLC [36, 38-40].

Numerous book chapters and review articles deal with the separation of chiral drugs by this method. Chiral HPLC is more versatile than chiral GC for enantiomeric separation because it can separate a wide variety of nonvolatile compounds. It can be used to develop fast and accurate methods of chiral drug separation, and it allows on-line detection and quantitation of both mass and optical rotation of enantiomers when appropriate detection devices are used.

Enantioselective HPLC Analysis

There are basically two options for chiral HPLC analysis namely direct and indirect approach. The direct chiral high performance liquid chromatographic technique, with reference to application in enantiospecific drug analysis was reported [41].

In the indirect approach, 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 exhibit different physicochemical properties. In the direct method, transient rather than covalent diastereomeric complexes are formed between the drug enantiomers and a chiral selector present either added to the mobile phase (CMPA) or coated/bonded to the surface of a silica support (CSP). The technique relying on chiral stationary phases (CSPs) are preferred as they offer specific advantages over indirect methods. There is no need to chemically manipulate the analytes, interference with sample matrix, chiral purity of the chiral stationary phase (CSP) does not need be known, fast analysis, method can be readily scaled to commercial production, online coupling with MS or NMR permits structure identification High-performance liquid chromatography (HPLC) is a powerful tool for the enantioselective separation of chiral drugs [42].

However, the selection of an appropriate chiral stationary phase (CSP) and suitable operating conditions is a bottleneck in method development and a time- and resource-consuming task. Multimodal screening of a small number of CSPs with broad enantiorecognition abilities has been recognized as the best strategy to achieve rapid and reliable separations of chiral compounds [43].

Conclusion

Among the chiral analytical techniques currently used to achieve chiral separation of chiral mixtures is the high performance liquid chromatography (HPLC) on chiral stationary phases (CSPs),

which is widely employed and represents one of the most efficient, direct, and facile techniques for the determination of the optical purity and analytical separation of several enantiomeric drugs and pharmaceutical preparations. Currently, the use of HPLC to assess the chiral purity of drugs, their synthetic intermediates, and raw materials has become a routine practice, owing to the commercial availability of a variety of CSPs for the direct separation of enantiomers. The preference of CSPs lies in the inherent advantages of any chromatographic separation, such as the speed of the analysis, the possibility to analyze or purify the enantiomers in complex mixtures, and the reproducibility of the analysis and its flexibility. CSPs have several advantages. They are easily manipulated through synthesis and separate enantiomeric mixtures without the necessity of derivatization. As a consequence, a large number of CSPs are available nowadays, suitable for a variety of different solvents and conditions.

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