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**- A REVIEW**

# IMPORTANCE OF CHIRALITY AND CHIRAL CHROMATOGRAPHY IN PHARMACEUTICAL INDUSTRY : A DETAILED STUDY

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## ABSTRACT

A large percentage of commercial and investigational pharmaceutical compounds are enantiomers, and many of them show significant enantioselective differences in their pharmacokinetics and pharmacodynamics. The importance of chirality of drugs has been increasingly recognized, and the consequences of using them as racemates or as enantiomers have been frequently discussed in the pharmaceutical literature during recent years. With increasing evidence of problems related to stereoselectivity in drug action, enantioselective analysis by chromatographic methods has become the focus of intensive research of separation scientists. 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. This paper presents the importance of chirality in pharmaceutical substances and basic consideration in method development for chiral compounds by High Performance Liquid Chromatoraphy (HPLC).

**Key words**: Enantiomer, Chirality, HPLC, Chiral Stationary Phase.

## INTRODUCTION

Chirality now plays a major role in the development of new pharmaceuticals. Driven by both regulatory and therapeutic rationales, one-third of all marketed drugs are now sold in single isomeric form. The strategy at many companies is to focus on the development of patentable single isomers of previously marketed racemic drugs. Referred to as racemic switching, moving to the enantiomerically pure drug has many advantages, including increased clinical efficacy, decreased metabolic burden, and fewer side effects. Molecules that relate to each other where an object and its mirror image are not superimposable are called chiral (from Greek word cheiro, meaning “hand”); that is, they are like a pair of hands. These molecules also are called enantiomers. Major differences in biological activity have been observed in chiral molecules. The difference in spatial arrangements of atoms in a molecule (that is, its stereochemistry) can influence its pharmacological, metabolic, or toxicological activity. This is why regulatory requirements in the pharmaceutical industry demand detailed investigations of chiral molecules. Before initiating method development, it is important to develop a basic understanding of stereochemistry[1-3]. This paper presents the

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importance of chirality in pharmaceutical substances and basic consideration in method development for chiral compounds.

**Determine the chirality of the molecule**

First the investigator must take a close look at the molecule that is to be resolved and then answer the following question: Is there a stereogenic center ? The simplest example of this is an asymmetric carbon with four different substituents. As simple as it might sound, this process appears cumbersome when you are looking at many carbon atoms in a molecular structure. The simplest way to counter this problem is to number all carbon atoms in the structure and look at each of them in turn to see if they are asymmetric or not. If no asymmetric carbon is found, look at the plane of symmetry of the whole molecule and other atoms, such as sulfur and nitrogen, which also can confer chirality. If one asymmetric carbon or stereogenic center is found, the investigator can expect two enantiomers. For two stereogenic centers, the number of enantiomers is four. It should be clear that this number grows rapidly as the number of asymmetric centers increases; the 2 *n* rule applies, except for fused rings, where *n* is the number of asymmetric centers.

**Stereoisomerism**

Molecules that are isomeric but have a different spatial arrangement are called stereoisomers. Symmetry classifies stereoisomer as either enantiomers, as defined previously, or diastereomers. Stereoisomerism can result from a variety of sources besides the single chiral carbon (stereogenic or chiral center) mentioned previously - that is, a chiral atom that is a tetrahedral atom with four different substituents. Detailed discussion on these topics can be found in several books and review articles[4-5]. It is not necessary for a molecule to have a chiral carbon to exist in enantiomeric forms, but it is necessary that the molecule, as a whole, be chiral. There are two simple molecular sources of chirality: molecules having a stereogenic center and those having a stereogenic axis. Stereoisomerism is possible in molecules that have one or more centers of chirality, helicity, planar–axial–torsional chirality, or topological asymmetry. The amounts of energy necessary to convert given stereoisomers into their isomeric forms can be used for their classification. Stereoisomers with low-energy barriers to this conversion are termed conformational isomers, whereas highenergy-barrier conversions are described as configurational isomers. Diastereomers differ in energy content and, thus, in every physical and chemical property; however, the differences can be so minute as to be nearly indistinguishable.

A large percentage of commercial and investigational pharmaceutical compounds are enantiomers, and many of them show significant enantioselective differences in their pharmacokinetics and pharmacodynamics. The importance of chirality of drugs has been increasingly recognized, and the consequences of using them as racemates or as enantiomers have been frequently discussed in the pharmaceutical literature during recent years. With increasing evidence of problems related to stereoselectivity in drug action, enantioselective analysis by chromatographic methods has become the focus of intensive research of separation scientists. Most of the pharmaceutical and pharmacological studies of stereoselectivity of chiral drugs before the mid eighties involved pre-column derivatization of the enantiomers with chiral reagents, forming diastereomers.

**Importantance of chirality**

Most of the molecules of importance to living systems are chiral, e.g. amino acids, sugars, proteins and nucleic acids. An interesting feature of these chiral biomolecules is that in nature they usually exist in only one of the two possible enantiomeric forms. When a chemist synthesizes a chiral molecule in an achiral environment using achiral starting materials, an equal mixture of the two possible enantiomers (i.e. a racemic mixture) is produced. In order to make just one enantiomer, some enantioenriched starting material,

reagent, catalyst, or template must be present in the reaction medium. Often times, only a single enantiomer of a chiral molecule is desired, as is the case when the target molecule is a chiral drug that will be used in living systems. Drug molecules can be likened to tiny keys that fit into locks in the body and elicit a particular biological response. Since the 'locks' in living organisms are chiral, and exist in only one of the two possible enantiomeric forms, only one enantiomer of the 'key' molecule should be used (the mirror image of our car key will not start our car). In general, the use of both enantiomers in a racemic formulation of a chiral drug may be wasteful, and sometimes even introduces extraneous material that may lead to undesired side effects or adverse reactions. The importance of chirality has been appreciated and addressed by the pharmaceutical industry for decades. As technologies for measuring and making enantiopure materials have improved, the production of enantiopure pharmaceuticals has become commonplace, with many of the top selling drugs in the world now being sold in enantiopure form. Consequently, the subject of chirality and the pharmaceutical industry is a topic of considerable recent interest and importance[6-8].

**Importance of 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. 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 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. 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, 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 11, 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. 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 successful enantioseparation of a particular class of racemates on a given CSP is the understanding of the possible chiral recognition mechanisms.

Enantiomers are two chemically identical molecular species, which differ from each other as nonsuperposable mirror images. The most simple and vivid model for enantiomeric structures is the two hands, left and right. Enantiomers, in addition to diastereomers and cis-trans-isomers, are thus a special case of stereoisomers. The chirality (handedness) of enantiomeric molecules is caused by the presence of one or more chirality elements (chirality axis, chirality plane, or chirality centre, e.g., asymmetric carbon atom) in their structure. The chirality sense and optical activity of the enantiomers are determined by their absolute configuration, i.e., the spatial arrangement of the atoms in the molecule. In contrast to their conformation, the configuration of enantiomers cannot be changed without a change in the connectivity of constituent atoms. Designation of the configuration of enantiomers should be made in accordance with the Cahn-Ingold-Prelog R, S-system. The Delta-Lambda designations for enantiomers of octahedral complexes and the D, L Fischer-Rosanoff designations for amino acids and sugars are also in use. Conventional chemical synthesis, in contrast to asymmetric synthesis, deals mostly with the transformations of achiral compounds. If these reactions result in the formation of a chirality element in the molecule, the reaction product appears to be an equivalent mixture of a pair of enantiomers, a racemate, which is optically inactive. Racemates are also formed through racemisation of chiral compounds. Racemates crystallise in the form of a racemic compound or, less frequently, as a conglomerate. Separation of the enantiomers comprising the racemate, ie., the resolution of the racemate, is a common problem in stereochemical research as well as in the preparation of biologically active compounds, in particular, drugs. The problem is that in contrast to diastereomers and all other types of isomeric species, enantiomers, in an achiral environment, display identical physical and chemical properties. One approach to separate enantiomers, sometimes referred to as indirect enantiomeric resolution, involves the coupling of the enantiomers with an auxiliary chiral reagent to convert them into diastereomers. The diastereomers can then be separated by any achiral separation technique.

Nowadays, direct separation methods are commonly used in which the enantiomers are placed in an chiral environment. As a matter of principle, only chiral selectors or chiral irradiation (e.g., a polarized light beam which consists of two chiral circular-polarised components) can distinguish between two enantiomers. Chiral selectors can be an appropriate chiral molecule or a chiral surface (e.g., a chiral seed crystal). Due to the enantioselectivity (a special case of stereoselectivity) of the interaction with the two enantiomers, the chiral selector either transforms the enantiomers at a different rate into new chemical entities (kinetic enantioselectivity) or forms labile molecular adducts of differing stability with the enantiomers

(thermodynamic enantioselectivity). Enzymic selective transformation of L-enantiomers of racemic D, L-amino acids is a typical example of a kinetically enantioselective process (kinetic resolution). Enantioselective (chiral) chromatography does not modify the enantiomeric species to be separated and thus represents an example of a thermodynamically enantioselective process. Direct enantiomeric resolutions are only feasible in chromatographic systems which contain an appropriate chiral selector. The latter can be incorporated into the stationary phase (chiral stationary phase) or be permanently bonded to or coated onto the surface of the column packing material (chiral bonded and chiral coated stationary phases). In all these cases it is appropriate to refer to the chromatographic column as an enantioselective (chiral) column. Enantioselective chromatography can also be performed on achiral chromatographic columns using the required chiral selector as a chiral mobile phase or a chiral mobile phase additive. Combinations of several chiral selectors in the mobile phase as well as mobile and stationary phases are also feasible. In the case of chiral stationary phases, the enantiomer that forms the more stable association with the chiral selector will be the more strongly retained species of the racemate. The enantioselectivity of the chiral chromatographic system is then expressed as the ratio of the retention factors of the two enantiomers. This ratio may approach the value of the thermodynamic enantioselectivity of the association of the chiral selector with the enantiomers. This situation occurs when the association with the chiral selector governs the retention of the enantiomers in the chromatographic system and other, nonselective types of solute-sorbent interactions are negligible. On the other hand, a chiral mobile phase reduces the retention of the solute enantiomer, which forms a stronger association with the chiral selector. Here again, the limit for the enantioselectivity of the chiral chromatographic system is set by the enantioselectivity of the selector-solute association (in the mobile phase). However, in the majority of chiral mobile phase systems, the chiral selector as well as its associates with the solute enantiomers are distributed between the mobile and stationary phases.

The effective enantioselectivity of the chromatographic system will therefore be proportional to the ratio of the enantioselectivities of the association processes in the stationary and mobile phases**.** Interaction of the chiral selector of the system with the enantiomers of the solute results in the formation of two labile diastereomers. These differ in their thermodynamic stability, provided that at least three active points of the selector participate in the interaction with corresponding sites of the solute molecule. This three-point interaction rule is generally valid for enantioselective chromatography, with the extension to the rule, stating that one of the required interactions may be mediated by the adsorption of the two components of the interacting pair onto the sorbent surface. Because of the multiplicity and complexity of the interactions of the enantiomers to be separated with the chiral selector, sorbent surface and other components of the chromatographic system, the total enantioselectivity can depend strongly on the composition, pH and temperature of the mobile phase. Therefore, in papers on enantioselective chromatography, it is important to define these parameters. Enantioselective chromatography and capillary electrophoresis are extensively employed in the analysis of the enantiomeric composition (enantiomeric excess, optical purity) of chiral compounds. Liquid and supercritical fluid chromatography are also used for the isolation of chiral compounds from racemic mixtures on a preparative scale. Enantioselective separations have been realised in all possible separation techniques, including gas chromatography, column liquid chromatography, thin-layer chromatography, supercritical fluid chromatography, as well as electromigration methods, counter current liquid chromatography and liquid-liquid extractions. Numerous review papers and special monographs[9-13] describe the technical details as well as the achievements and potential of these important modern separation techniques.

**Basic considerations in method development for chiral compounds**

Cost considerations, availability of equipment, and know-how play important roles in the selection process for an appropriate method. Paper chromatography (PC) and thin-layer chromatography (TLC) have been used where cost considerations outweigh other factors. PC is used very rarely these days; however,

TLC can be a very useful qualitative technique that entails minimal costs. It can also provide good indications as to which HPLC method would be most suitable for resolving enantiomers. Of course, it can also be used as an independent technique with limitations of resolution and low precision. Commonly used methods for separation of enantiomers today can be classified broadly into the following four categories:

- Gas chromatography (GC)
- High-performance liquid chromatography (HPLC)
- Supercritical fluid chromatography (SFC)
- Capillary electrophoresis (CE)

Since HPLC methods are generally favored for a variety of reasons, some basic information on selecting a suitable method for HPLC has been included in this chapter. A basic understanding of chiral discrimination by various chiral stationary phases (CSPs) has been provided to help with method development. A strategy for fast method development is also provided in this chapter.

## General terms related to chirality

### Chirality

The geometric property of a rigid object (or spatial arrangement of points or atoms) of being non superimposable on its mirror image; such an object has no symmetry elements of the second kind. If the object is superimposable on its mirror image the object is described as being achiral.

### Diastereoisomerism

Stereoisomerism other than enantiomerism and cis-trans isomerism. Diastereoisomers (or diastereomers) are stereoisomers not related as mirror images. Diastereoisomers are characterised by differences in physical properties, and by differences in chemical behaviour towards achiral as well as chiral reagents.

### Enantiomer

One of a pair of molecular entities, which are mirror images of each other and non-superposable.

### Stereoisomers

Isomers that possess identical constitution but which differ in the arrangement of their atoms in space.

## Terms related to the separation process

### Chiral additive

The chiral selector, which has been added as a component of a mobile phase or electrophoretic medium.

### Chiral mobile phase

A mobile phase containing a chiral selector.

### Chiral selector

The chiral component of the separation system capable of interacting enantioselectively with the enantiomers to be separated.

**Chiral stationary phase**

A stationary phase, which incorporates a chiral selector. If not a constituent of the stationary phase as a whole, the chiral selector can be chemically bonded to (chiral bonded stationary phase) or immobilized onto the surface of a solid support or column wall (chiral coated stationaryphase), or simply dissolved in the liquid stationary phase.

**Enantioselective chromatography (electrophoresis)**

The separation of enantiomeric species due to the enantioselectivity of their interaction with the chiral selector (s) of a chromatographic (electrophoretic) system. Also called Chiral chromatography (electrophoresis).

**Enantioselective column**

A chromatographic column containing a chiral stationary phase. Also called a chiral column.

**Enantioselectivity (in chiral separations)**

The preferential interaction with the chiral selector of one enantiomer over the other.

**Enantioselectivity of a chromatographic (electrophoretic) system**

The ratio of the retention factors of two solute enantiomers in a chiral chromatographic (electrophoretic) system.

**Terms related to the chiral purity of the sample**

**Diastereoisomer excess/Diastereoisomeric excess**

This is defined by analogy with enantiomer excess, as $D_1 - D_2$ [and the percent diastereoisomer excess as 100 $(D_1 - D_2)$], w here the mole fractions of the two diastereoisomers in a mixture or the fractional yields of two diastereoisomers formed in a reaction are $D_1$ and $D_2$ ($D_1 + D_2 = 1$). The term is not applicable, if more than two diastereoisomers are present. Frequently this term is abbreviated to d.e.

**Optical purity**

The ratio of the observed optical rotation of a sample consisting of a mixture of enantiomers to the optical rotation of one pure enantiomer.

**Separation of chiral compounds by HPLC**

There are basically two approaches to the separation of an enantiomer pair by HPLC. In the indirect approach used rarely, the enantiomers can be converted into covalent, diastereomeric compounds by a reaction with a chiral reagent, and these diastereomers typically are separated on a routine, achiral stationary phase. In the direct approach which is often called chiral HPLC, the enantiomers or their derivatives are passed through a column containing a chiral stationary phase (CSP). Two different types of selectors can be distinguished: a chiral additive in the mobile phase or a chiral stationary phase. Of these approaches, chiral stationary phases are more commonly used for separations of enantiomers.

**Mechanism for chiral separations by HPLC**

To develop an optimum method, it is important to understand the mechanism of chiral separation. Our understanding of chiral separations with some of the systems is quite good, while it remains poor for protein and cellulose stationary phases. The separation basis with various chiral stationary phases is

discussed below in their respective group; some general comments are included here. A number of chiral recognition models have been proposed to account for optical resolutions by HPLC; these are often based upon the three-point interaction rule advanced by Dalgliesh[14] in 1952. He arrived at this conclusion from paper chromatographic studies of certain aromatic amino acids. He assumed that the hydroxyl groups of the cellulose were hydrogen-bonded to the amino carboxyl groups of the amino acid. A third interaction was caused, according to these views, by the aromatic ring substituents. It led to the postulation that three simultaneously operating interactions between an enantiomer and the stationary phase are needed for chiral discrimination. However, this is not always necessary as steric discrimination also could result from steric interactions. Chiral separations also are possible through reversible diastereomeric association between an enantiomeric solute and a chiral environment that is introduced into the column. Because chromatographic resolutions are possible under a variety of conditions, it might be concluded that the necessary difference in association can be obtained by many types of molecular inter actions. The association, which may be expressed quantitatively as an equilibrium constant, will be a function of the magnitudes of the binding as well as the repulsive interactions involved. The latter are usually steric, although dipole-dipole repulsions also could occur, whereas various kinds of binding interactions can operate. These include hydrogen bonding, electrostatic and dipole-dipole attractions, charge–transfer interaction, and hydrophobic interaction (in aqueous systems). CSPs, where steric fit is of primary importance, include those based upon inclusion phenomena, such as cyclodextrin and crown ether phases. It is possible to construct chiral cavities for the preferential inclusion of only one enantiomer. Molecular imprinting techniques are very interesting in this respect[15]. The idea is to create rigid chiral cavities in a polymer network in such a way that only one of two enantiomers will find the environment acceptable.

**Chiral stationary phases (CSPs)**

The term chiral stationary phase does not necessarily mean that the stationary phase itself is chiral (although in practice it usually is) but that the stationary phase is used to separate chiral substances. Two substances can only be separated if their standard energy of distribution differ, which means that their standard enthalpies and/or their standard entropies of distribution also differ. In general, the standard enthalpy reflects the net difference in the interactive forces on the molecule in the two phases (polar, dispersive and ionic interactive forces) whereas the standard entropy reflects their spatial disposition and, thus, their probability and proximity of interaction. Thus, for any chiral separation the stationary phase is chosen such that the spatial arrangement of its composite atoms results in the probability or proximity of interaction differing significantly between the two enantiomers to be separated. In practice this usually mans that the stationary phase itself is also chiral and, in fact, the first chiral separations in gas chromatography were achieved by using and enantiomer of an amino acid as the stationary phase.

Chiral Stationary Phases (CSP's) may be classified according to their interaction mechanism with the solute. A scheme for classification was first proposed by Irving Wainer[16].

**Type I CSP's** are those which differentiate enantiomers by the formation of complexes based on attractive interactions. These may be hydrogen bonds, p-p interactions, dipole stacking.

**Type II CSP's** are those which involve a combination of attractive interactions and inclusion complexes to produce a separation. Most type II phases are based on cellulose derivatives.

**Type III CSP's** rely on the solute entering into chiral cavities to form inclusion complexes. The classic inclusion complex column is the cyclodextrin type of column developed by Armstrong[17]. Other CSP's in this class are crown ethers and helical polymers such as poly (triphenylmethyl methacrylate).

**Type IV CSP's** separate by means of diastereomeric metal complexes. This technique is also known as Chiral Ligand Exchange Chromatography (CLEC) and was developed by Davankov[18].

**Type V CSP's** are proteins where separations rely on a combination of hydrophobic and polar interactions.

CSP may be classified as according to chemical type-

1. Brush type (Pirkle)
2. Cellulose
3. Cyclodextrin
4. Macrocyclic Antibiotics
5. Protein
6. Ligand exchange
7. Crown ethers

## Commonly detectors used in chiral chromatography

Chiral chromatography is highly dependent on the column, which has seen many recent improvements, and the detector. However following types of commercial chiral detectors are available. Therefore only the concepts as there relate to liquid chromatography detectors are highlighted here.

### Polarimeter-1 (PLR-1)

Normal light waves vibrate in many planes; however plane polarized light is generated when normal light is passed through an optical polarizing filter. This effect results in a light beam emerging that vibrates in a single plane (linearly polarized). A compound is optically active if linearly polarized light is rotated when passing through it. The degree of rotation is dependent on both the concentration of a chiral compound and its molecular structure. The specific rotation of the molecule, not the absorption characteristics, is what determines the signal strength using the polarimeter. The Polarimeter-1 uses a diode laser at 670 nm as the light source.

### Polarimeter-2 (PLR-2)

The Polarimeter-2 detector is similar in design and function to the Polarimeter-1, with the exception of a light emitting diode (LED) at 426 nm being the light source and having a second polarizing filter present in-line after the sample. The choice of the blue wavelength is based on the plain curve, The normal behaviour of optical rotation dispersion (the dependence of rotational strength of optically active molecules on the wavelength of light used for the measurements) in the absence of chromophores or in spectral regions that are distant from absorption bands. The angle of rotation, as a function of wavelength, is greatest at shorter wavelengths Therefore, to optimize the chiral response in a molecule, lower source wavelengths yield stronger responses.

### Optical rotary dispersion (ord) detector

The ORD detector is similar in design and function to the Polarimeter-1; however the light source for this detector is a Xe-Hg lamp, which is readily available and utilizes the strong line emissions of Hg at 365 nm, which can be tuned to cover a spectral range of 350 to 900 nm, if required. This detector utilizes the lowest wavelength of the polarimeters (365 nm vs. 426 and 670 nm) and therefore one would expect that this

detector would give the strongest signal, based on Drude's equation. However, the analog signals collected from these detectors were dependent on the gain set for each detector.

**The Detectors: Circular Dichroism (CD)**

When an optically active compound preferentially absorbs right or left circularly polarized light, the difference between the right and left absorbances [A(r) - A(l)] (often a very small value) is recorded as the CD signal. As with UV absorbance, the CD signal is wavelength dependent. A molecule should have a chromophore with absorption in the range of 200 to 420 nm to have strong CD signal.

**Future trends**

With the move to green chemistry gaining more momentum every day, the "clean" technique of Chiral HPLC continues to grow in popularity. Once considered too costly to be practical in many laboratories, Chiral technologies are becoming cheaper and more effective than ever. With pharmaceutical companies building up their drug candidate pipeline, the need for faster analysis and higher column resolving power led to the development of chiral columns packed with 3-µm particles. To address the need for robust and stable stationary phases to carry out challenging separations, immobilized chiral columns have also been developed. People are buying more SFC and fewer chiral columns. Recent weakness in the chiral column business has been attributed to the benefits of SFC, which is far more gentle on the chiral stationary phase. More and more prep applications are being developed in SFC instead of HPLC. With the introduction of more and more sources of chiral phases, the costs are beginning to decline. Recently, there has been a trend in having immobilized chiral stationary phases (CSPs). Previously, most chiral stationary phases were coated which meant that there were limitations in the types of solvents that could be used. With the newer immobilized phases, widely varying solvent polarities can be used to develop and optimize chiral methods. There has also been a trend in the use of smaller particles analogous to other modes of HPLC. Although sub-two micron CSPs are not yet widely available, 3-µm columns are and they allow faster separations with good resolution. Another trend has been in the use of supercritical fluid chromatography for the separations of chiral compounds. This trend does put some stress on CSPs because supercritical fluids can play havoc on some of the coated phases. Another trend is the development of newer wide-range phases that allow most enantiomeric separations to occur on just a few CSPs. Only a few years ago, users had to purchase a dozen or so expensive chiral columns in order to find one that might do the job. Although the market for biological drugs is growing rapidly, the traditional small-molecule drugs are entering clinical trials in much greater numbers than biologics. Clearly, use of chiral chromatography, especially for preparative separations, will continue to grow. Furthermore, chromatography, being the fastest route to market, will be viewed as part of drug production development in the future.

A recent poster by Z. Wang[19] at Merck presented at Pittcon 2008 showed chiral screening on four simultaneous chiral stationary phases on a 5 min. gradient with a total run time of 9 min. including column equilibrations. This could change chiral screening for the entire pharmaceutical industry because now there is a greater than 90% likelihood to get a separation using the main CSPs but with only one SFC instrument using three UV detectors and one Photo Diode Array detector. Chiral HPLC will continue to grow at a good rate as it has done in the recent past. More and more applications will be incorporated into production of pharmaceuticals and chemicals such as herbicides, pesticide, and others.

Many compounds in the pharmaceutical discovery stage have chiral centers. Since the regulatory requirements demand proof of the enantiomeric purity of chiral drugs, there is a bright future for the use of HPLC and SFC for analytical purposes. In addition, chiral SFC has found a niche in the preparation and purification of chiral pharmaceuticals; preparative chiral HPLC should be around for a long time to come. I think an area for future development would be a single CSP that would suffice for 90-95% of all chiral compounds. Thus, users would have to purchase a single column instead of a half a dozen columns to perform method development.

Rational and timely selection of drug candidates for further development is of major concern for pharmaceutical companies. Use of chiral chromatography for rapid compound screening and compound purification, followed by biology analysis of the purified enantiomers, will allow customers to rapidly eliminate racemic compounds with undesirable characteristics. Preparative and semi-preparative separations for pre-clinical and early clinical development will continue to be the fast growing area. More chiral compounds moving from development to production and from branded drugs to generics. This means more analysis in QA/QC. Pharmaceutical enantiomers of basic drugs for drug candidates and finished goods both for analytical and preparative purposes. The single biggest application would be the analytical separation and preparative purification of chiral pharmaceuticals. One of the major obstacles is lack of understanding of chromatography, in general, by synthetic chemists. Consequently, efforts have to be made to convince the researchers that chromatography is a highly scalable technique and as such can be a cost-effective commercially-visible drug production.

HPLC is an old-fashioned wasteful way to purify chiral compounds and the new word for all chemical processes is sustainability. SFC is a much more sustainable technology that is not only better in terms of productivity, but in a hydrocarbon-constrained and $CO_2$ neutral world facing water and food shortages and a surfeit of waste $CO_2$ - why not reuse the waste $CO_2$ for SFC use or even supercritical synthesis ?

The lack of new and different stationary phases presents a limitation. The industry is in need of a different chiral approach. Currently it is expensive to do HPLC-Chiral especially for hard to separate compounds. Although some strides have been made in CSP development, it is often a matter of "trial and error" in terns of choosing the optimum phase. Often, chiral method development consists of a bank of columns in a column selector valving system set up with various mobile phase combinations. The chiral compound (s) is repeatedly injected into each of the columns with different combinations of solvents until the best combo is found. This screening approach is rather archaic and it seems like a set of experiments more scientifically based could be developed. The new generic phases that have come out onto the market are making it more affordable for scientists outside of Big Pharma to experiment with chiral separations. The introduction of lower cost stationary phases to compete with previously sole source suppliers. The development of the new immobilized phases that cover a very wide range of chiral compounds with only three stationary phases.

## CONCLUSION

Chiral chromatography has become a preferred method for rapidly accessing enantiopure compounds in the pharmaceutical industry, largely owing to the speed with which a chromatographic method can be developed and executed as well as the comparatively small labour requirements of the chromatographic approach. As Woodward predicted, the use of preparative chromatography within the field

of organic synthesis can be expected to increase as the technique becomes more familiar to synthetic chemists. Accounting for the growing development of chiral drugs as racemate and single enantiomer worldwide, it is primordial to promote the chiral separation and its development because this operation plays a key role not only in pharmaceutical industry but also in clinical therapeutics. Nowadays, many drugs are still used as racemates with their side-effects, this problem is probably due not only to the difficulty in chiral separation technique but also to the production costs. If a new separation method for chiral drugs will be developed with its large application scale and low cost, the number of racemic drugs could diminish significantly. The direct production of a single drug enantiomer by asymmetric synthesis is useful when its other antipode is found toxic or entirely inactive. However, for the drug discovery process, the obtention of a racemate could give triple informations about the drug to be explored i.e. informations about the racemate and also about the two single enantiomers. Theoretically, the use of a single isomer is ideal, but practically, the decision must be taken after long clinical observation between racemate and single enantiomer actions. In some therapeutic cases, the use of a racemate is more helpful than that of each single isomer because of the complementary effects of each other. Therefore, preparative and analytical HPLC are very useful at the experimental step of drug discovery and also are an invaluable tool for the searcher.

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