Trade Science Inc.

December 2007 Volume 6 Issue 2

Analytical CHEMISTRY

An Indian Journal

Review

ACAIJ 6(2) 2007 [35-42]

# Enantiomeric drugs in pharmaceutical industry-importance, regulatory requirements and analytical techniques

**G.Sridhar, R.Pramod Kumar, M.K.Srinivasu, Sivaiah Sangaraju, K.B.Chandrasekhar, B.M.Rao***

**Analytical Research, Custom Pharmaceutical Services, Dr.Reddy's Laboratories, Miyapur, Hyderabad-500049, (INDIA)**

**Tel.: 91-40-23045440, Fax : 91 40 23044044**

**E-mail : bmrao@drreddys.com**

*Received: 10th July, 2007 ; Accepted: 15th July, 2007*

## ABSTRACT

The article provides a review on the enantiomeric drugs, its importance from the biological point of view in the pharmaceutical industries. It covers the regulatory requirements and guidelines from the regulatory agencies for enantiomeric pharmaceutical drugs. It also emphasizes on the need of accurate analytical methods and the various commonly used and advanced analytical techniques for the identification and accurate estimation of enantiomeric drugs. 

## KEYWORDS

Enantiomeric drugs;
Regulatory requirements;
Chiral HPLC;
Chiral gas chromatography;
Capillary electrophoresis;
Optical rotatory dispersion;
Circular dichroism.

## INTRODUCTION

Living organisms are composed of chiral bio molecules such as amino acids, sugars, proteins and nucleic acids. In nature these bio molecules exists in only one of the two possible enantiomeric forms. For example, amino acid in L form and sugars in D forms. Because of chirality, living organisms show different biological responses to one of a pair of enantiomers in drugs.

Most of the literature available, deals with the product specific analytical methods for the chiral molecules. This article, focus on enantiomeric drugs, its importance in the pharmaceutical industry, regulatory aspects and various analytical methods by which the enantiomers can be estimated.

## DISCUSSION

### 1. Theory

### 1.1. Enantiomers

Enantiomers are two chemically identical molecular species, which differ from each other as non-superimposible mirror images. Enantiomers, in addition to diastereomers and cis-trans-isomers, are thus a special case of stereoisomers. Enantiomers have identical physical and chemical properties except that they rotate the plane of polarized light in opposite direction and behave differently in chiral environment[1].

### 1.2. What causes chirality?

There are several ways that a molecule can display chirality. Firstly if an atom such as carbon, silicon, nitrogen, phosphorous or sulphur forms a tetrahedral structure with four different substituents then two non-super imposable mirror images will be formed. Butan-2-ol is an example of a chiral molecule with a chiral center.

The second way a molecule can show chirality is when there is a rigid feature in the molecule such as dialkenes, which leads to two non-super imposable mirror images being formed. Allene is an example of a chiral molecule with a chiral axis.

Thirdly, certain molecules can form helical struc-

ture due to steric effects, which have high-energy barriers to inter-conversion for two enantiomers to exist. Helicene is an example of a chiral molecule with a chiral plane.

## 2. Biological importance of enantiomeric drugs

The human body being 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 other may be inactive or in some cases produce unwanted effects. Majority of commercially available drugs are both synthetic and chiral. However a large number of chiral drugs are still marketed as racemic mixtures. To avoid the possible undesirable effects of chiral drug, it is important that a pure, therapeutically active form be prepared and marketed.

Optical isomers of drugs often have greatly different affinities at receptor sites are metabolized at different rates, and have different affinities for tissue and protein binding sites. Despite this knowledge, many drugs are administered as their racemates. Manipulation of the enantiomeric ratio or the use of only one enantiomer of a drug may allow separation of toxicity and efficacy, and this may lead to a significant increase in therapeutic ratio and a more rational approach to therapeutics[2].

Enzymes, which themselves are always chiral; often distinguish between the two enantiomers of a chiral substrate. This can be visualized in everyday terms by imagining the enzymes to have three-dimensional glove shaped cavities, which bind these substrates. If this "glove" is right-handed, then right-handed molecules will fit inside snugly and thus be bound tightly. On the other hand, left-handed molecules won't fit well - just like putting your left hand into a right-handed glove. Although this is an oversimplification of the recognition process (enzyme cavities are not really "glove shaped"), it is a useful illustration of a more general point: chiral objects have different interactions with the two enantiomers of other chiral objects.

Other biological processes may be triggered by only one of the two possible enantiomers of a chiral molecule, often being unresponsive to the other enantiomer. For example, S-carvone ("left-handed") is the flavor of caraway, while R-carvone("right-handed") is the flavor of spearmint. Many chiral drugs must be made with high enantiomeric purity due to toxic activity of the 'wrong' enantiomer. An example of this is thalidomide, which is racemic-that is, it contains both left and right handed isomers in equal amounts. One enantiomer is effective against morning sickness, and the other is teratogenic. It should be noted that the enantiomers are converted to each other *in vivo*. That is, if a human is given D-thalidomide or L-thalidomide, both isomers can be found in the serum. Hence, administering only one enantiomer will not prevent the teratogenic effect in humans.

## 3. Regulatory aspects of enantiomeric drugs

Regulatory agencies throughout the world are currently reviewing the importance of chirality with regard to pharmaceutical products[3]. New guidelines from such agencies have been key drivers for the focus on single enantiomer products in pharmaceutical industries. The scientific and regulatory developments have created the need for a guide for workers in the pharmaceutical and chemical industries seeking information on chiral molecules, processes, and commercially available chiral chemicals. The 'Chiral pool' of readily available, relatively inexpensive chiral compounds has been expanding at a rapid rate as more and more products are produced in large quantities at economical prices. New developments in various technologies for isolating, preparing, and purifying chiral materials have greatly increased the opportunities for utilizing optically pure compounds in commercial applications[3]. Novel techniques for classical resolution, new methodologies for developing selective enzymes for biocatalysis, advances in the application of microorganisms for chemical production, and continued progress in the area of asymmetric synthesis have all contributed to the growth of this field. It is ordinarily sufficient to carry out toxicity studies on the racemate. If toxicity other than that predicted from the pharmacological properties of the drug occurs at relatively low multiples of the exposure planned for clinical trials, the toxicity study where the unexpected toxicity occurred should be repeated with the individual isomers to ascertain whether only one enantiomer was responsible for the toxicity. If toxicity of significant concern can be eliminated by development of single isomer with the desired pharmacological effect, it would in general be desirable to do so. It is essential to determine

the concentration of each isomer and define limits for all isomeric components, impurities, and contaminants on the compound tested pre-clinically that is intended for use in clinical trials. The maximum allowable level of impurity in a stereoisomeric product employed in clinical trials should not exceed that present in the material evaluated in non-clinical toxicity studies[3].

The decision whether to develop a single enantiomer, racemate, or non-racemic mixture(enantiomeric mixture other than racemate) rests with the sponsor and should be based on scientific data relating to quality, safety and efficacy and ultimately to the risk/benefit, assessment of the drug under the proposed conditions of use. Cases where the development of a racemate may be justified include, but are not limited to, the following:

**a.** The enantiomers are configurationally unstable *in vitro* or undergo racemization *in vivo*.

**b.** The enantiomers have similar pharmacokinetic, pharmacodynamic and toxicological properties.

**c.** It is not technically feasible to separate the enantiomers in sufficient quantity and/or with sufficient quality.

The cases where development of a non-racemic mixture may be justified include those where a specific enantiomeric ratio is expected to improve the therapeutic profile. If little difference is observed in activity and disposition of the enantiomers, racemates may be developed. In some situations, development of a single enantiomer is particularly desirable(e.g., where one enantiomer has a toxic or undesirable pharmacologic effect and the other does not). A signal that should trigger further investigation of the properties of the individual enantiomers and their active metabolites is the occurrence at clinical doses of toxicity with the racemate that is not clearly expected from the pharmacology of the drug or the occurrence of any other unexpected pharmacologic effect with the racemate. These signals might be explored in animals but human testing may be essential[3].

It should be appreciated that toxicity or unusual pharmacological properties might reside not in the parent isomer, but in an isomer-specific metabolite. In general, it is more important to evaluate both enantiomers clinically and consider developing only one when both enantiomers are pharmacologically active but differ significantly in potency, specificity, or maximum effect, than when one isomer is essentially inert. Where both enantiomers are fortuitously found to carry desirable but different properties, development of a mixture of the two, not necessarily the racemate, as a fixed combination might be reasonable.

If a racemate is studied, the pharmacokinetics of the two isomers should be studied in phase 1. Potential interconversion should also be examined. Based on phase 1 or 2 pharmacokinetic data in the target population, it should be possible to determine whether an achiral assay or monitoring of just one enantiomer where a fixed ratio is confirmed will be sufficient for pharmacokinetic evaluation.

If a racemate has been marketed and the sponsor wishes to develop the single enantiomer, evaluation should include determination of whether there is significant conversion to the other isomer, and whether the pharmacokinetics of the single isomer are the same as they were for that isomer as part of the racemate.

The Regulatory Agencies, particularly, the US FDA, encourage such work and provide even incentives for developing chiral drugs of existing products in the market. Such requirements have led to a whole new field of new technologies to make chiral forms of existing drugs either from their racemic forms, or through direct synthesis and evaluating their biological properties[5].

TABLE 1 shows the distribution of non-chiral and chiral drugs currently used in the therapy and within the later group the distribution of those, which are administered as single enantiomers and as racemates.

## 4. Analytical approach

### 4.1. Need for the analytical methods

The majority of synthetic drugs developed in the past are not chiral, the ones developed from natural products are largely chiral. Although the different enantiomers (chirals) have the same chemical formula, they

**TABLE 1: Sales distribution of single enantiomer and racemate drugs on the market worldwide as on date**

| World total | Source | Chirality | How sold (Chiral drugs) |
|---|---|---|---|
| Drugs (1850) | Natural and semi synthetic-523<br>Synthetic -1327 | Non chiral-6 Chiral-517<br>Non chiral -799 Chiral-528 | Single enantiomer-509 Racemate-8<br>Single enantiomer-61 Racemate-467 |

differ widely in their biological properties. This is primarily due to the fact that since chirality is related to three- dimensional structures, one form may be more suitable for specific interaction with other biological molecules such as receptors, enzymes, etc. Thus, there is obvious benefit in studying the properties of the two enantiomers of a molecule with respect to their therapeutic efficacy and safety. It is for this reason that the FDA(USA) and the European Committee for Proprietary Medicinal Products, since 1992, require that the properties of each enantiomer in a racemic molecule should be studied separately before decisions are taken to market the drug as one of the enantiomers or as a mixture(racemate). In addition, there is an increasing awareness of the need for re-evaluating the properties of individual enantiomers of currently marketed racemic drug molecules.

Keeping in view of the above requirements, it is clear that there is an increased need of the developing sensitive analytical methods for the separation and accurate determination of enantiomers of chiral drugs in pharmaceutical industry.

### 4.2. Analytical methods

Many physico-chemical methods can be used to provide information about chiral drugs. The following are the different analytical method used:

#### Chiral high performance liquid chromatography

Chiral HPLC may be used to separate mixtures of enantiomers directly without forming diastereoisomeric derivatives. Separations can be effected through the use of chiral mobile phase additives or chiral stationary phases in conjunction with regular (achiral) columns.

#### Chiral mobile phase additives

When an optically pure additive is reacted with a mixture of enantiomers to produce diastereomers, the products can be separated using achiral stationary phases such as a reverse phase or a normal phase HPLC columns. This mode has the advantages like, the use of cheaper conventional HPLC columns and the availability of a wide range of additives and the disadvantages are the limited choice of the detection system and that preparative separations are not possible.

The mobile phase additives can be classified as follows:

#### Metal complexes

The structural requirements of the enantiomers to be separated include the presence of two or more of the chelating functions such as amino acids, hydroxy carboxylic acids and β-amino alcohols. Lepage et al.[6] reported the use of chiral triamines containing a hydrophobic $C_8$ substituent as an additive to mobile phases in the presence of Zn(II) for separation of dansyl amino acids using an octyl column.

#### Ion pair formation

When a chiral counter ion is dissolved in the mobile phase it can be used to separate enantiomers of acids or amines by the formation of the diasteriomeric ion pairs.

#### Uncharged chiral additives

The uncharged chiral additive adsorbs on the achiral stationary phase and produces an in-situ chiral stationary phase. Many diamines such as N-acetyl-L-valine tert-butylamide have been shown to be useful as additives in a non-polar mobile phase using silica columns. β-Cyclodextrins have been used successfully with C18 columns and aqueous buffer systems.

#### Chiral stationary phases

This is the most popular mode of separation for chiral molecules. Chiral stationary phases(CSP) are made by immobilizing single enantiomers onto the stationary phase. Resolution relies on the formation of transient diastereomers on the surface of the column packing. The compound, which forms the most stable diastereomer will be most retained, whereas the opposite enantiomer will form a less stable diastereomer and will elute first. The ability of the analyte and CSP to form transient-diastereomeric complexes utilizing hydrogen bonding, π-π interactions, dipole stacking, inclusion complexing and steric bulk is the driving force behind enantioseparation. The main advantages of this mode are the rapid separations, the reproducibility of the analysis, flexibility and the possibility of preparative separations. The CSPs can be used for the separation of positional isomers and diastereomers, if the separation is not achieved on achiral phases. The disadvantages are high cost, low sample loading and the specificity of the stationary phase for the particular separation in hand. Irving Weiner classified the CSPs into five types ac-

**TABLE 2: Classification of chiral stationary phases based on interactions**

| S.no | Classification | Interactions |
|---|---|---|
| 1 | Type I (Brush type or Pirkle type) | Attractive interactions, Hydrogen bonding, π-π interactions and dipole interactions. |
| 2 | Type II (Polysaccharide derivatives) | Both attractive interactions and inclusion complex formation. |
| 3 | Type III (Crownethers, Cyclodextrins) | Retention via formation of inclusion complexes within chiral cavities |
| 4 | Type IV (Metal complexes) | Ligand exchange mechanisms with metal complexes |
| 5 | Type V (Protein based) | Hydrophobic and polar interactions with bound protein phases. |

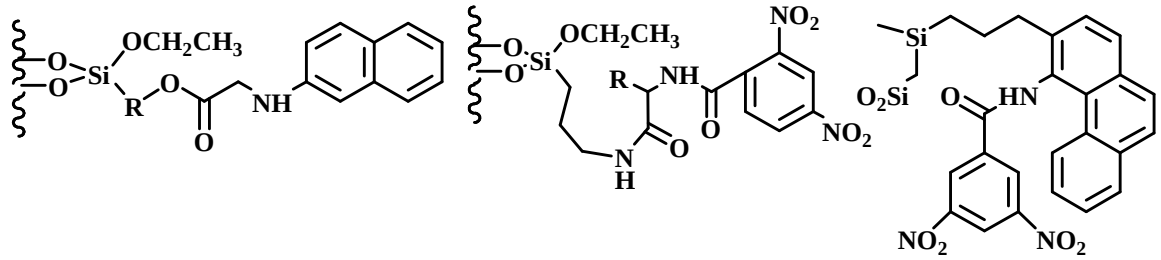

**Figure1 : Type-I chiral stationary phases**

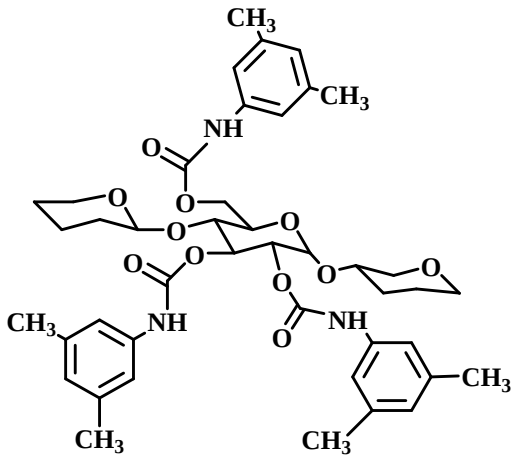

**Figure 2 : Structure of a glucose unit of the amylose based stationary phase, derivatized with dimethylphenylcarbamate**

cording to their interaction mechanism with the solute as shown in TABLE 2 .

Type-I (Brush type or Pirkle type)[7]

The structure of these type of stationary phases is based on single strands of chiral selectors, connected via amidic linkage onto aminopropyl silica as shown in figure 1.

Type-II (Polysaccharide derivatives) illustrated in figure 2.

Type-III (Crownethers, Cyclodextrins)[8]

Type-IV (Metal complexes)

Type-V (Protein based)

## Chiral gas chromatography

Stationary phases modified with chiral agents are available for the separation of enantiomers. In gas chromatography(GC), chiral selectivity is controlled by choice of stationary phase and operating temperature. From a practical point of view, chiral selectivity is achieved by introducing spatially oriented groups into the stationary phase molecules and, as a consequence, an additional entropic component to the standard energy of distribution.

The use of GC for the separation of asymmetric isomers is not as common as LC, but nevertheless there some very effective optically active stationary phases that can be used in GC for the separation of enantiomers.

Some of the more useful GC stationary phases are based on the alpha and beta cyclodextrins. The columns are usually 30 or 60m long 0.25mm I.D. and have an operating temperature range of $30^0$C to $250^0$C. Both the alpha and beta forms are commercially available and both have been used very satisfactorily for the separation of the optical isomers.

The phenyl-methyl-polysiloxane confers onto the column an intermediate level of polarity so the separations are basically enthalpic due to the dispersive and polar interactions that take place largely with the polymer but also entropic resulting from the chiral selectivity of the cyclodextrins. Derivatization of the base cyclo dextrin structure can introduce groups to which only one enantiomer can interact, while the other(s) are partially or wholly entropically hindered from interaction. This increases the differential interaction between the

enantiomers and the stationary phase, thus, increasing the separation ratio and hence the resolution[9].

Recently, it has been found that room-temperature ionic liquids can be used as stable, unusual selectivity stationary phases. They show "dual nature" properties, in that they separate nonpolar compounds as if they are nonpolar stationary phases and separate polar compounds as if they are polar stationary phases. Extending ionic liquids to the realm of chiral separations can be done in two ways: (1) a chiral selector can be dissolved in an achiral ionic liquid, or (2) the ionic liquid itself can be chiral. There is a single precedent for the first approach, but nothing has been reported for the second approach. In this work, we present the first enantiomeric separations using chiral ionic liquid stationary phases in gas chromatography. Compounds that have been separated using these ionic liquid chiral selectors include alcohols, diols, sulfoxides, epoxides, and acetylated amines. Because of the synthetic nature of these chiral selectors, the configuration of the stereogenic center can be controlled and altered for mechanistic studies and reversing enantiomeric retention. Room temperature ionic liquids are low-melting salts that posses a number of interesting properties, such as negligible vapour pressure and high thermal stability. So far, they have mostly been used as alternatives to organic solvents, but have also shown promise as novel catalysts, highly selective transport membranes and stationary phases for gas chromatography[10].

## Optical rotation

This method can be used to distinguish between enantiomers because they rotate the plane of polarized light in opposite directions but in equal amounts.

Polarimetric methods, for the assessment of enantiomeric purity have been limited by the low sensitivity of the commercially available polarimeters, and by the operational difficulties inherent in specialized systems not designed for turnkey operation. A new design for a rugged, sensitive, and easy to operate laser-based polarimeter has been engineered by PDR-Chiral. The system is based on a 670nm diode laser that possesses excellent characteristics as a light source for sensitive polarization measurements, and an innovative modulation scheme that allows the system to function below 25-degree sensitivity level. Applications include the direct detection of chiral materials separated by isocratic or gradient HPLC and SFC (e.g., antibiotics or polysaccharides), and the quantitation of enantiomeric mixtures partially separated using a chiral selective separation system(e.g., amino acids). In the latter case, the bimodal response of the polarimeter allows accurate and precise quantitation of the enantiomeric mixtures under conditions where the chromatographic resolution is less than 0.5, a situation which would be problematic or unworkable for conventional detection approaches. The analytes can be detected directly without derivatization. The detector can also be used with flow injection analysis methodology to measure the specific rotation of pure enantiomers, or to monitor changes in optical activity as a function of pH, solvent, etc.

## Capillary electrophoresis (CE)

Capillary electrophoresis is the ideal analytical tool to assess the purity of enantiomers. With this technology it is simple to construct and modify a chiral environment, which is the key to resolving these complex isomers.

Enantiomers can be resolved by indirect and direct methods using capillary electropherosis. The indirect separation method is based on the reaction, before the analysis, of a racemic mixture with a chiral reagent(R or S) producing a mixture of two diastereoisomers that can be resolved using a non-chiral electrophoretic system. The product of the reaction is a mixture of two stable compounds where relatively strong bonds (covalent) are involved in the process. Although the indirect method is advantageous for separation of enantiomers, it suffers from certain drawbacks like more time consumption, requirement of high pure chiral reagents[11].

In direct separation method, the chiral selector can be added to the background electrolyte, bound to the capillary wall, or included in a gel matrix. It interacts with the two enantiomers during the electrophoretic process, forming labile diastereoisomeric complexes, relatively week bonds like hydrogen, $\pi$-$\pi$ or hydrophobic are involved. The separation of two enantiomers can take place only if the two diastereoisomers formed possess different stability constants, causing the two analytes to move with different velocities. The effective mobility of the most complexed enantiomer is lower than that of its isomer. The use of cyclodextrins for differential host-guest complexation of enantiomers is by

far the most common chiral selector and is the basis of the chiral separation. Highly sulfated cyclodextrins (HSCDs), are a family of three chiral reagents(α, β and γ). Different strategies have been developed for the reduction of analysis time using capillary electrophoresis. These include shortend injection technique, high electric field through a capillary length reduction, external pressure application and capillary dynamically coated to generate an important electrosmotic flow[11].

**Nuclear magnetic resonance spectroscopy (NMR)**

NMR is a useful tool for the determination of enantiomeric purity or enantiomeric composition. This is accomplished by making the NMR signals for the protons of the enantiomers non-equivalent by the use of chiral lanthanide shift reagents, chiral solvating agents or chiral derivatizing agents.

Recently a new NMR tool for the measurement of enantiomeric excess was developed by Jackues Courtieu and coworkers. All NMR methods are based on the nonequivalence of the chemical shifts of the signals, provided by different diastereotopic groups. Enantiotopic groups can be transformed(converted) to diastereotopic by internal or external influence on the investigated molecules. Internal influence is confining by direct chemical transformation of the mixture of enantiomers into mixture of diastereomers by chiral reagent. The molecules of substrate and chiral reagent are linked together by covalent bond[12-13].

Diastereotopity by external influencing may be realised by:

- Chiral solvating solvents and agents
- Chiral lanthanide shift reagents
- Chiral liquid crystal solvents

Chiral solvating solvents and agents forms diastereoisomeric complexes with molecules of mixture of enantiomers. The origin of complex formation is the hydrogen bonding, charge transfer or ionic pair formation. The anisochronity can be distinguished by dipole-dipole or Vander-Vaals type interactions between molecules of chiral reagents and substrate. If substrate tends to associations, then the self induced anisochronity phenomenon may occur. One or more of these factors take place in mechanisms of chiral reagents influence.

The nature of the diastereotopy induction in liquid crystal solvent is entirely different. The cholesteric liquid crystals have a chiral environment. The R and S enantiomers have different ordering properties in these solvents, which imply that their NMR spectra are different. Three type of NMR parameters of R and S species can be different.

- Quadruple coupling constants
- Direct dipole-dipole coupling constants
- Anisotropy of chemical shifts

Maximum difference in values of anisotropic NMR parameters of enantiomers occur for quadrupole couplings, then for dipole-dipole couplings, and finally, for chemical shift anisotropy. All these parameters can't be seen in isotrope solutions, because they canceled by the chaotic motion and reordering in isotrope liquid state[14].

**Optical rotatory dispersion(ORD) and circular dichroism(CD)**

Optical rotatory dispersion(ORD) and circular dichroism(CD) are the two phenomena that results when asymmetrical molecules interact with plane polarized light.

When a molecule is optically active, it absorbs right and left handed circularly polarizes light to different extents. This is called Circular dichroismd(CD). It also has a different refractive index for the two forms of light. This results in the rotation of the plane of polarization of the light. CD measures the differential absorption of left and right circularly polarized light by an optically active compound. The rotation is dependent on the wavelength, so the effect is called Optical Rotatory Dispersion(ORD). ORD measures the change of specific rotation of an optically active compound with the wavelength of the light used. When a molecule exhibits combinations of ORD and CD in the region of absorbance, then the transmitted light is said to be elliptically polarized. These chiroptical methods can be used to identify and/ or quantitate enantiomers.

CD and ORD are phenomena result from the same electronic transitions in a molecule and are related to one another by Kronig-Kramer transform.

Vibrational circular dichroism(VCD) has emerged as a powerful new method for the determination of the absolute configuration of chiral molecules. VCD is the difference in the infrared(IR) absorbance, A, of a molecule for left versus right circularly polarized radiation

Review

during a vibrational transition; namely VCD is ΔA=AL-AR. All the molecules absorb radiation in the IR region where their absorption pattern across the spectrum serves as a rich fingerprint of molecular structure and shape. In addition, all chiral molecules have a VCD spectrum that consists of an even more powerful fingerprint spectrum of the structure and shape of the molecule. The additional power is due to its stereospecific sensitivity. Molecules with opposite absolute configuration, pairs of enantiomers, have the same IR spectrum but opposite VCD spectra. The magnitude of VCD spectrum is 10,000 times smaller than that of the IR spectrum[14]. This approach can be used to monitor process streams to ensure enantiomeric purity of chiral products without the need for chromatographic separation. Figure 1 illustrates the VCD spectrum of (+)- and (-)- R-Camphor

### X-ray crystallography

X-Ray crystallography in the solid state could be used to determine the absolute configuration of molecules and to distinguish conglomerates from racemic compounds.

X-ray crystallography is the only method for determining the "absolute" configuration of a molecule and is the most comprehensive technique available to determine the structure of any molecule at atomic resolution. Results from crystallographic studies provide unambiguous, accurate, and reliable 3-dimensional structural parameters, which are prerequisites for rational drug design and structure-based functional studies.

### Melting point

The melting points may be used in distinguishing individual enantiomers from the racemate. Racemic mixtures, racemic compounds and pseudoracemates can be differentiated from one another on the basis of their melting point behavior, provided, both enantiomers or the racemic drug and at least one pure enantiomer are available[15].

## CONCLUSIONS

From the above, it can be concluded that, the need to develop the active enantiomeric drugs is increasing day by day in the pharmaceutical industry. Even the regulatory agencies are coming out with new guidelines and policies for the enantiomeric drugs. To fulfil these aspects, new analytical technologies for the determination of enantiomers in the chiral drugs are coming out.

## ACKNOWLEDGMENTS

The authors wish to thank the management of Dr. Reddy's group for supporting this work.

## REFERENCES

[1] A.D.Vadim; Pure & Appl.Chem., **69(7)**, 1469-1474 **(1997)**.
[2] K.Williams, E.Lee; Drugs **30(4)**, 333-354 **(1985)**.
[3] 'FDA's policy statement for the development of new stereoisomeric drugs', Publication date, **(1992)**.
[4] 'Guidance for industry-Stereochemical issues in chiral drug development', Ministry of Health, Canada, **(2000)**
[5] M.D.Nair., Chiral Drugs-Opportunities for Indian Pharmaceutical Industry, **(2001)**.
[6] J.Lepage, W.Lindner, G.Davies, B.Karger; Analyt. Chem., **51(3)**, 433 **(1979)**.
[7] W.H.Pirkle, D.W.House; J.Org.Chem., **44**, 1957-1960 **(1979)**.
[8] T.J.Ward, D.W.Armstrong; J.Liq.Chromatography, **9**, 407 **(1986)**.
[9] Reymond P.W.Scott; 'Introduction to Analytical Gas chromatography', Marcel Dekker Inc. New York, NY, **(1981)**
[10] D.Jie, W.Thomas, W.A.Daniel; Anal.Chem., **76**, 6819 -6822 **(2004)**.
[11] Salvatore Fanali; 'An introduction to chiral analysis by Capillary Electrophoresi', BIORAD, Italy, **(1973)**
[12] I.Canet, A.Meddour, J.Courtieu; J.Am.Chem.Soc., **116**, 2155-2156 **(1994)**.
[13] A.Meddour, I.Canet, A.Loewenstein, J.M.Pechine, J.Courtieu; J.Am.Chem.Soc., **116**, 9652-9656 **(1994)**.
[14] R.K.Dukor, L.A.Nafie; 'Encyclopedia of Analytical Chemistry', R.A.Meyers(ed.); John Wiley & Sons: Chichester, UK, 662-676 **(2000)**.
[15] G.Alan Mitchell; J.Pharm.Pharmaceut.Sci., **1**, 8-12 **(1998)**.