ANNALS OF PHARMACY AND PHARMACEUTICAL SCIENCES
Volume 4 | Issue 1&2 | April & October, 2013 | 36-40

A REVIEW

Advances in resolution of racemic drugs

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ABSTRACT

Chiral separation also called racemic resolution is a procedure used to separate the two isomers of a racemic compound. In pharmaceutical analysis this topic is especially important as it is apparent from the proportion of drugs which are coming on the market are chiral and importance of chiality in many fields of natural and applied science is well established. Thus analytical methods for the chiral separation are paramount to a full understanding of enantioselective drug action and disposition. The body with its numerous homochiral compounds being amazingly chiral selector will interact with each racemic drug differently and metabolize each enantiomer by a separate pathway to generate different pharmacological activity. One isomer may produce the desired therapeutic activities, while other may be inactive or produce toxic effects. This review provides the information to understand the advances taking place in chiral separation. Chiral chromatography including HPLC, column chromatography, capillary electrophoresis and micro-chip capillary electrophoresis are most readily accomplished methods for the enantiomer resolution. HPLC is the most widely used of all the methods because of its simplicity and rapidity. Accounting for the growing development of chiral drugs as racemates and single enantiomers worldwide it is primordial to promote the chiral separation and its development.

Key words : Chirality, Racemic resolution, Electrophoresis, Chiral chromatography

How to cite this paper : Nerkar, Amit G., Tare, Bharati T. and Sawant, Sanjay D. (2013). Advances in resolution of racemic drugs. *Ann. Pharm. & Pharm. Sci.*, **4** (1&2) : 36-40.

Article chronicle : Received : *19.09.2013;* **Accepted :** *29.09.2013*

INTRODUCTION

Chiral separation is a procedure used to separate the two enantiomers of a racemic compound [1] in pharmaceutical industry, two main categories are often applied for resolution:

Traditional method :

In the classical approach the most widely used technique is the resolution by diastereomeric salt formation. In this strategy, an acid-base reaction is involved between a racemic drug and a pure single enantiomer called resolving agent. This reaction leads to the formation of two diastereomeric salts that now have different physical and chemical properties and can be easily separated either by crystallization or by filtration.

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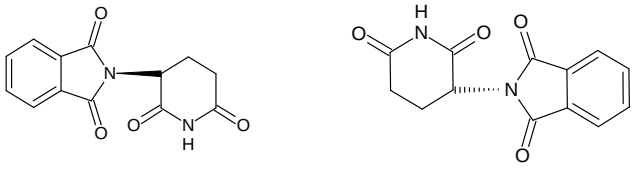
Advanced techniques :

In advanced techniques high- performance liquid chromatography (HPLC) is the method of choice for the enantiomer separation because of its simplicity and rapidity. The indirect HPLC involves derivatisation of samples with a chiral derivatization reagent *i.e.* a pure single enantiomer, resulting the formation of two diastereomers, which can be separated by a classical reversed-phase column. This indirect HPLC method is rarely used in industry, but frequently performed in biological analysis because of its high sensitivity. However, this indirect technique requires a functional group in the analyte e.g. amine, hydroxyl, carboxyl and thiol. A chiral derivating agent (a pure single enantiomer) added in the sample will react with this fuctions to form two diastereomers that can be separated by a classical reversed phase column (C18 or C8). On the other hand, the direct HPLC utilizes the chiral selector

either in chiral stationary phases (CSPs) or in the mobile phase called chiral mobile phase additive(CMPA).Today, the resolution by HPLC using CSPs is one of the most useful technique for estimating both enantiomer composition and obtaining pure enantiomers. The other chromatographic method comprises gas chromatography (GC) and supercritical fluid chromatography. GC has been extensively used for analysis as well as separation of enantiomers working on the same principle as HPLC. The trifluroacetyl derivatives of optically active amino acid have been used for chromatographic resolution of racemic alcohols via the corresponding esters. Paper chromatography can also effect partial resolution; the paper itself (cellulose material) newer techniques introduced in chiral separation. Capillary electrophoresis is often used as fast alternative to high-performance liquid chromatography.

Need to resolve racemic mixture :

Thalidomide was used in the 1950s and 1960s on pregnant women to avoid morning sickness.The (R) enantiomer is effective against the morning sickness but the (S)enantiomer is teratogenic and causes birth defects (Fig. 1).



(R)- Thalidomide (S)- Thalidomide

Fig. 1 : R and S forms of Thalidomide

Top 5 drugs marketed in 2004 (chemical and engineering news vol.78 oct.2004) the active ingredients were chira (Table 1) This shows the increasing intrest of pharmaceutical industries in developing chiral drugs.

Table 1 : Top 5 drugs are chiral compounds in 2004

Sr. No.	Drug	Active ingedient	Form of active ingredient	Therapy class
1.	Lipitor	Atorvastatin	Single enantiomer	Cholesterol reducer
2.	Zocor	Simvastatin	Single enantiomer	Cholesterol reducer
3.	Plavix	Clopidogrel	Single enantiomer	Antithrombotic
4.	Nexium	Esomeprazole	Single enantiomer	Antiulcerent
5.	Zyprexa	Olenzapine	Achiral	Antipsychotic

(Chemical and engineering news vol.78 oct.2004)

Traditional methods of chiral separation :

Traditional methods of chiral separation includes following :

- Mechanical separation-crystallization method
- Resolution through the formation of diastereomers
- Kinetic method of resolution
- Enzymes as resolving agents

Mechanical separation method :

Direct crystallization of an optically active compound from a racemate may be achieved provided the crystals of the form known as conglomerate. In conglomerate, two enantiomorphism crystals are distinguishable visually and can be separated by hand-sorting with the help of magnifying glass and a pair of tweezers. Pasteur in 1848 crystallized sodium ammonium tartrate by slow evaporation of an aqueous solution when the temperature happened to be below 27ºC. He then separated the two types of crystals with distinguishable hemihedral facets. When dissolved in water, they showed optical rotation of opposite directions.

Diastereomeric salt formation method :

The principle is illustrated in resolution of a racemic acid, (±) - A with an optically pure base, (–) - B which combines with racemic acid giving two diastereomeric salts. Being diastereomeric, the two salts differ in properties like solubility, boiling point, adsorption co-efficient. When crystallized from a solvent, one of them can be separate first. After several crystallization another would be available in pure state. Organic acids and bases (amines) are by far most important groups of compounds which are resolved directly by this method. Organic bases used as resolving agents are the naturally occurring alkaloids such as strychnine, ephedrine, cinchonine etc. A large number of racemic acids have been successfully resolved with quinine and brucine alone.

Kinetic method of resolution :

It is based on the principle that one of the diastereomer is formed, destroyed, or transformed selectively by a chemical reaction. This is possible because the activation energies of the two diastereomeric transition states in the reaction of a race mate with a chiral reagent are different and so two enantiomers react at different rates. If a racemic substrate, *i.e.* (±)-A is allowed to react with an optically active reagent, *i.e.* (+)-X the reaction proceeds through two diastereomeric transition states. Because of diastereomeric relationship, the products as well as the transition states are of different free energies, the differences being ΔG in the ground states of the products and $\Delta\Delta G‡$ in the transition states. If the reaction is carried out in such a way that the equilibrium is established between two diastereomers the reaction is called thermodynamically controlled and the product ratio is governed

by G. If the reaction is carried out so that equilibration is completely avoided, the reaction is known to be kinetically controlled.

Enzymes as resolving agents :

Enzymes are usually highly enantiospecific. The chiral reagent is replaced by microorganism or enzymes which are highly stereoselective in their reaction. If the test sample is treated with an appropriate enzyme and no reaction occur as indicated by the absence of any change in optical rotation, the sample is enantiomerically pure. Any enhancement or reduction of the original rotation would mean optical impurity.

Disadvantages of traditional methods of chiral separations :

- Only one of the enantiomeric pair can be obtained, so yields are necessarily less than 50 per cent.
- The separation of material, so obtained is usually incomplete, leading to material with enhanced rather than complete optical purity.
- The optically active materials used to form the diastereomers are expensive and toxic e.g. alkaloids such as brucine, ephedrine and are partially recoverable.
- Regeneration of optically active material from its derivative may itself cause the racemisation of the desired compound, leading to diminution of optical purity.

Advanced techniques in chiral separation :

- HPLC resolution using polysaccharide derivatives as chiral stationary phases.
- Chiral separations by capillary electrophoresis.
- Chiral separations in microfluidic devices.

HPLC resolution using polysaccharide derivatives as CSP :

The chromatographic method of racemic resolution is carried out under 4 different conditions :

- Formation of diastereomeric mixtures and separation by classical chromatography.
- Direct resolution by using chiral adsorbent as stationary phases.
- Separation on achiral solid phase using a mobile chiral liquid phase.
- Resolution using an achiral solid stationary phase modified by chiral reagent.

Chiral stationary phases for HPLC :

The key factor of this technique is selection of suitable CSP for the resolution of a target compound. Polysaccharide-based CSPs are most commonly used for HPLC resolution.

- Cellulose esters
- Cellulose and amylose phenylcarbamates
- Amylose benzylcarbamates

Cellulose esters :

Cellulose triacetate (CTA), one of the oldest CSPs has been prepared by the heterogeneous acetylation of native microcrystalline cellulose in benzene. Besides the acetates, benzoates(OB), 4-methylbenzoate(OJ), and cinnamate(OK) of cellulose coated on silica gel are also commercially avilable.The introduction of a methyl group at the *para* –position of OB results in significant difference inrecognisationabilities of OB and OJ. OJ appears to separate large-size molecules more efficientely thanOB resolves non-aromatic chiral compounds. OK shows a recognisation similar to that of O.J. (Fig. 2).

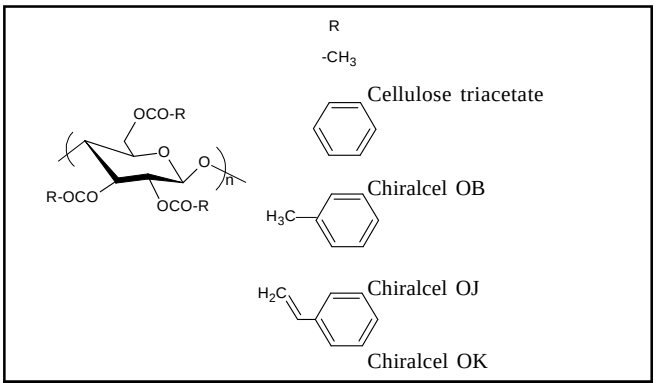


Fig. 2 : Cellulose ester chiral stationary phases

Cellulose and amylose phenylcarbamates :

Cellulose and amylose are easily converted to trisphenylcarbamate derivatives by reacting them with the cooresponding phenyl isocyanates and a variety of phenylcarbamates of cellulose and amylose have been prepared for use as the CSPs for HPLC. The chiral recognisation abilities are significantly influenced by by the sustituents on the phenyl ring.The introduction of an electron-donating alkyl group or an electron withdrawing halogen at the meta and/or para position on phenyl ring often improves chiral recognisation ability of many racemates (Fig.3).

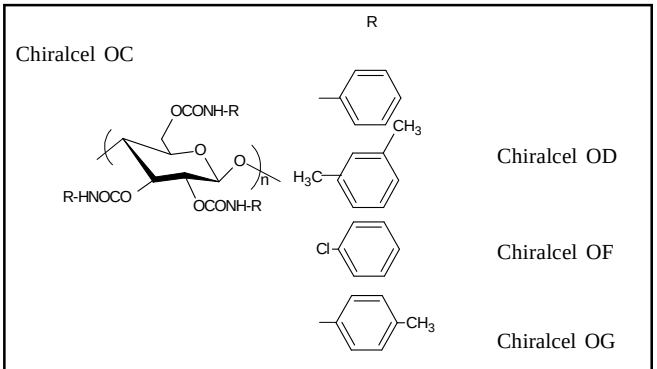


Fig. 3 : Cellulose carbamate chiral stationary phases

Amylose benzylcarbamates :

Amylose benzylcarbamates (Fig. 4) show a characteristic chiral recognisation for many racemates different from those of the phenylcarbamates. Among several benzyl-carbamate derivatives, only 1-phenylethylcarbamate and 1-phenylpropylcarbamate exhibit high chiral recognisation.

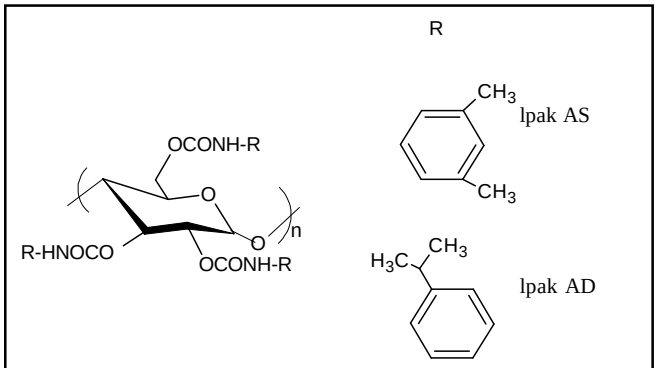


Fig. 4 : Amylose benzylcarbamates chiral stationary phases

Chiral separation by capillary electrophoresis :

Electrophoresis involves the separation of charged species under the influence of an electric field. The rapid development of capillary electrophoresis as an analytical tool for chiral as well as achiral separations has benefited tremendously from extensive knowledge-base generated over decades in the application of classical electrophoretic methods.

Advantages of capillary electrophoresis for chiral separation:

- Small amounts of chiral selector required.
- Ability to rapidly change chiral selector.
- Any chiral molecule which is available commercially pure can be used as chiral selector. Commonly used chiral selectors are shown in Table 2.

Table 2 : Chiral selectors used in capillary electrophoresis

Selector	Analyte structural features	Additive concentration
Cyclodextrin	Hydrophobic and hydrogen bonding groups near stereogenic center	1-5%
Polysaccharide	Hydrophobic and hydrogen bonding groups near stereogenic center	1-10%
Crown ether	Primary amine near stereogenic center	5-10 mm
Ligand exchange	Two heteroatoms near stereogenic center	1-40 mm
Chiral surfactant	Hydrophobic group near stereogenic center	5-160 mm
Protein	Hydrophobic and hydrogen bonding groups near stereogenic center	0.1-1 mm
Macrocyclic antibiotics	Hydrophobic and hydrogen bonding groups near stereogenic center	2-20 mm

Principle :

In capillary electrophoresis the motive force driving the separation is the voltage applied across the column. higher voltage-faster analysis. The migration of buffer ions in capillary column in response to the applied field generates a current that in turn generates heat similar to pressure generated in chromatographic column. Buffer with higher ionic mobility (e.g.Na^+) generates more heat for given voltage.

Sample injection :

In contrast to most chromatographic methods sample introduction in capillary does not use syringe. Sample introduction is accomplished by placing the inlet end of a capillary columin a sample vial and briefly applying a finite pressure called hydrodynamic or Voltage across the column-electrokinetic.

Chiral separation in micro-fluidic devices :

Also called as Microchip capillary electrophoresis (MCE).Separation is performed in intersected channels appearing like microfluidic chips. Main field of application is for analysis of biomolecules like proteins, oligonucleotides and DNA separations.

Principle :

Enantiomers have identical electrophoretic mobilities they can only be separated in a chiral environment. For differentiation of enantiomers by electrophoresis chiral additives are dissolved in the electrolyte as the so called pseudo-stationary phase. Two enantiomers bind to the dissolved selectors to a different extent resulting in different effective mobilities of the enatiomers which enables chiral resolution in CE. The chiral selectivity of system is altered by changing buffer constitution. The flow of fluid through a microfluidic channel can be characterised by the Reynolds number.

$$\mathbf{Re} \; Z \; \frac{\mathbf{LV^{av}}\circ}{\ll}$$

L = most relevant length scale

ρ = density

V_{av} = average velocity of fluid

μ = viscosity

Due to small dimensions of microchannel Reynolds number is less than 100. So, flow is completely laminar and no turbulence occurs.Molecules can be transported in relatively predictable manner through microchannel.

Advantages of MCE :

- Improved separation performance .
- Reduced instrument size reagent consumption.
- Enhanced separation speed and sample throughput.
- Realization of new integrated devices Detectors.

The reduction of size of a potential instrument has explored the feasibility to use microfabricated electrophoresis devices to analyse for extinct or extant life signs in extraterrestrial environments. One of the important application of MCE in chiral separations is the development of methods for chiral high-throughput screening (HTS). While traditionally the main challenge in chiral analysis was to achive chiral separation, the significant improvement in sample through-put in chiral analysis is only a recent demand. One of the reasons for the demand of such chiral HTS system is introduction of combinatorial techniques for the development of asymmetric catalysts. Using these methods, thounds of potential catalyst can be generated per day using time saving parallel synthesis.

Instrumentation and methodology :

The separation principles in chiral MCE are basically the same as is classical CE.The main difference between classical CE and MCE is format of separation device.Conventional CE-performed in fused silica.MCE- accomplished on planar device with micro-fabricated channels (Fig.5).

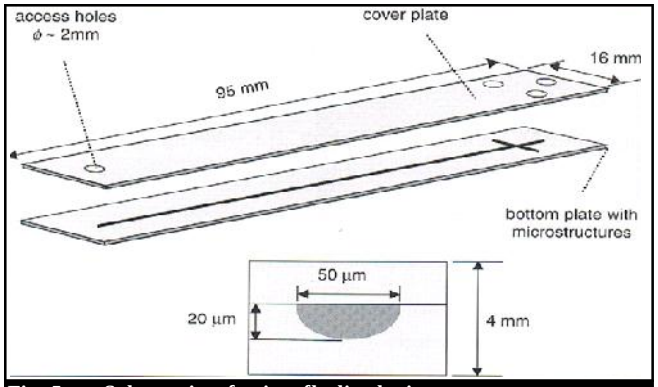


Fig. 5 : Schematic of microfludic device

Detector :

- Detection sensitivity usually also decreases with deceasing amount of analyte injected.
- Highly sensitive detection techniques are required as MCP uses very small sample.
- Most commonly used detection in MCE is florescence detection- due to sensitivity.
- Fluorescein isothiocyanate (FITC) - most popular reagent for florescence labeling in chiral MCE.This compound is suitable for labeling amine functionalities, fluorescence can be excited with the 448-nm line of argon-ion lasers. Fluorescence labeling of amines with FITC results in florescent compounds which are anionic at high pH. The fact ththe derivatives are anionic and by that exhibit an electrophoretic mobility simplifies method development for chiral separations.

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