

## **Enantiomeric separation of betaxolol using liquid chromatographic techniques: A review**

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**ABSTRACT:** Amino alcohols like  $\beta$ -blockers are very important drugs for the treatment of heart related diseases. This is due to presence of active functional groups (-OH and  $\text{NH}_2$ ) in their unique and simple structure. Betaxolol a selective  $\beta_1$ -receptor antagonist is one of the most prescribed drug for treatment of particularly hypertension, and it is also a very strong antiglaucoma agent. (S)-(-)-enantiomer of  $\beta$ -blockers shows about 50-500 fold higher pharmacological activities, due to the specific and proper orientation of groups to react with  $\beta_1$ -receptor. There has been no attempt to review on the enantiomeric separation of betaxolol using chromatographic techniques. This paper deals with the application of: high-performance liquid chromatography by direct method, using chiral stationary phases and, by indirect method using chiral derivatizing reagent and; thin layer chromatography by direct method using chiral selector as chiral impregnating reagent, chiral inducing reagent and chiral mobile phase additives.

**Keywords:**  $\beta$ -blockers, chiral selector, enantiomeric separation, thin layer chromatography, high-performance liquid chromatography, chiral derivatizing reagent

### **INTRODUCTION**

$\beta$ -blockers (also known as  $\beta$ -adrenolytic drugs) consist of a group of synthetic chiral hydroxyl amine-containing compounds with  $\text{pK}_a \sim 9.2$ . They stop adrenaline from binding to nerve receptors and “block” its effects. Betaxolol a potent  $\beta_1$ -receptor blocker used in the treatment of hypertension and glaucoma. It shows greater affinity for  $\beta_1$ -receptor than metoprolol. The (S)-(-)-enantiomer of betaxolol drug (**Figure**) is pharmacologically

effective, showing about 50–500-fold higher activities (Lee and William 1990). In most cases betaxolol is administered as racemic mixtures.

Since the enantiomers have different activity in ‘chiral environment of our body’ therefore these enantiomers are considered different compounds. Many biologists and clinical pharmacologists tend to deal with enantiomeric mixtures of isomers. A

physician, presenting such a drug under a brand name is unaware of different activity of enantiomers and may make mistakes (Ariens 1984). Therefore, many regulatory agencies ask the pharmacologists to present full pharmacokinetics and pharmacological information of both the enantiomers. Because in certain cases, the less reactive or unwanted enantiomer can have side effects or even toxic effects. Therefore, efficient methods of enantioresolution are always required to control the enantiomeric purity or to resolve racemic mixtures and to determine optical purity of the drugs.

Enantiomeric separation has immense importance in various fields from academic and industrial point of view. For enantiomeric separation two basic strategies “*direct approach*” and “*indirect approach*” have evolved in recent years. The direct approach has no require derivatization and separation can be achieved by using chiral selector: as chiral impregnating reagent, chiral mobile phase additives and chiral inducing reagent in thin layer chromatography and; as chiral stationary phase in high-performance liquid chromatography, to produce chiral environment *in situ* formation of transient diastereomers between enantiomers present in analyte and the enantiomer of pure chiral selector. In indirect method, there is first conversion of the enantiomers of analyte species in to daistereomers by reacting them with chiral derivatizing reagents and then separation can be done by chromatography in achiral environment.

In literature review there have been reported some basic approaches and principles of

planar and column chromatography for enantiomeric separation of betaxolol. Nevertheless, there has been no attempt to review on the enantiomeric separation of betaxolol by chromatography using the direct and indirect approaches. The present paper focused on the use of liquid chromatography for the enantiomeric separation of betaxolol.

## DIRECT APPROACH

Enantiomeric separation using direct approach is depends on the chiral environment which is either produced by incorporating a suitable chiral selector at an appropriate stage or created in the form of chiral stationary phase (CSP). This chiral environment can be created in thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) by (a) preparing the TLC plate or the column with the chiral selector, (b) impregnating the chiral selector on stationary phase of TLC, or (c) adding the chiral selector to the mobile phase. The material mentioned at (a) is chiral owing to its own structure (e.g. cellulose) while the material mentioned at (b) is prepared by bonding the chiral selector of interest to reactive groups of inert support. In situation (c) chiral environment is created by mixing the chiral selector and with mobile phase and flow of this mixture prior to separation. In all the cases, transient diastereomers are formed on the surface by the interaction of chiral selector with enantiomers of analyte of interest. The separation of these enantiomers is based on the concept is that, the enantiomer forming most stable

diastereomers will be most retained with stationary phase it means elute later and the enantiomer forming less stable diastereomer elute earlier. Martens and Bhushan (1990) direct methods for separation of enantiomers are important in various fields like synthetic and mechanistic study, pharmaceutical and biomedical analysis.

## **Enantiomeric separation by TLC**

### **Using Impregnation**

The technique in which a suitable chiral selector is incorporated with the adsorbent without covalently affecting its inert character, owing to application of samples on the plate for their separation is termed as impregnated TLC. There are various methods used for impregnation of TLC: spraying the solution of the impregnating reagent on the plate; immersing plain plate or ascending or descending the plate in a normal manner of development into an appropriate solution of the impregnating reagent; exposing the thin layer to the vapors of the impregnating reagent; and mixing the chiral selector with the inert support. In case of immersion there may be peeling of the thin layer, and in spraying method less uniform dispersion of the chiral selector on inert support than in the mixing of chiral selector with inert support or immersion (Bhushan and Martens, 1997).

The overall results of resolution are depends on the kind of the functional groups and chemical nature of the impregnating reagent the compounds being separated, along with the influence of the impregnating

reagent on the partition/or adsorption. The methods used for impregnation also play important role in the resolution of the enantiomers.

This method provides an inexpensive and wide choice of chromatographic conditions for enantiomeric separation of variety of compounds. Enantiomeric separation of betaxolol has been carried out using the L-lysine/L-arginine (Bhushan and Thiong'O, 1998), as chiral impregnating reagent and chromatographic data in terms of resolution and limit of detection (LOD) are given in **Table**.

### **Using chiral inducing reagent (CIR)**

CIR: The chiral selector when mixed with the racemic mixture of the analyte then itself induced the chiral environment in medium prior to formation of transient diastereomers of analyte, so it is called the chiral inducing reagent. The advantages of this method over the impregnation is that the required quantity of the chiral selector is very less than the quantity of the chiral selector required in impregnation methods. Since, in this method no requirement to produce chiral environment on the inert support of TLC for separation of enantiomers. Therefore, the TLC is called as achiral TLC and the separation is known as achiral phase chromatographic separation.

Literature reported the use of (S)-Glutamic acid as CIR and chromatographic run was performed, results obtained were good in terms of  $R_S$  (Bhushan et al. 2015).

## **Enantiomeric separation by HPLC**

It is most widely used method for the enantiomeric separation. Several chiral columns have been used in recent years for enantiomeric separation. These chiral column are commercially available in market. This method gives better result than the TLC method in regard of accuracy, precision and detection limit of the analyte. The detectors generally used in this technique is photodiode array detector (PDA) 2600, make the detection of analyte molecules up to pico meter level. Several columns containing chiral stationary phases, based on: cellulose, amylose, pirkle, ovomucoid, zirconia monolithic cyclodextrin and glycoprotein have been used for enantioseparation.

Of the above stationary phase based columns, certain have been used for enantioseparation of betaxolol; Cellulose tris(3,5-dichlorophenyl-carbamate) (Ates et al. 2013), Cellulose tris(3,5-dimethylphenyl-carbamate), (Peng *et al*, 2010), Amylose tris(3,5-dimethoxyphenyl carbamate), (Cass et al. 1999), and comparative data in terms of resolution time, limit of detection and resolution values, are given in **Table**.

## INDIRECT APPROACH

Chromatographic conditions of indirect method can be optimized more easily than the direct method, therefore, separation of daistereomeric pair via indirect method is sometimes simpler and often better resolution obtained. High sensitive detector response in this technique is providing better determination of biological samples in blood and urine. The CDR used for derivatization of samples are generally chosen with good

limit of detection. For example the Marfey's based CDRs shows high sensitive detector response to samples because of high molar UV-Vis absorptivity ( $\epsilon$ ) and fluorescence quantum yield. The reaction of CDR react with enantiomers of ( $\pm$ )- or the (*R,S*)-mixture is of different rates, therefore derivatization conditions must be optimized.

In the structure of analyte sample suitable reactive group should be in close proximity to the stereogenic center, which is prone a quantitative transformation with the CDR and without forming the side products. Racemization must not be there of the sample and of the CDR in the reaction, which transforms the enantiomeric mixture into daistereomers.

Enantiomers of betaxolol has been separated by converting them into daistereomers. Several CDRs have been used for the formation of daistereomers of betaxolol prior to their separation by HPLC. In **Table** separation data are given in terms of "resolution" of daistereomers of betaxolol formed with following CDRs: ( $-$ )-( $\alpha$ -methoxy-( $\alpha$ -(trifluoromethyl)phenylacetyl chloride (Kim et al. 2001); 1,3-diacetoxy-1-(4-nitrophenyl)-2-propyl isothiocyanate (Péter and Fülöp, 2001); 2,3,4,6-tetra-O-acetyl-b-D-galactopyranosyl isothiocyanate (Ko et al. 2006); 2,3,4,6-tetra-O-acetyl-b-D-glucopyranosyl isothiocyanate (Ko et al. 2006). Marfey's reagent based CDRs; (*R*)-2-(5-fluoro-2,4-dinitrophenylamino)-3-(methylthio)propanoic acid, (*S*)-*N*-(1-cyclohexylethyl)-5-fluoro-2,4-dinitrobenzenamine, (*R*)-5-fluoro-2,4-dinitro-*n*-(1-phenylethyl)benzenamine, have

been used by Bhushan and Nagar (2014) for synthesis of diastereomers of betaxolol and the structures of these diastereomers also optimized by using 09 Rev A. 02 and hybrid density functional B3LYP with 6-31G basis set.

## CONCLUSIONS

The liquid chromatography using direct and indirect methods are very useful for quantitative and qualitative analysis of important pharmaceuticals. Direct method is more suitable for separation of enantiomers in their pure form without any derivatization and is less time consuming. Direct TLC method (i) is less expensive in comparison to HPLC, GC and capillary electrophoresis that require highly expensive experimental set-up and running costs, (ii) is successful for rapid and sensitive resolution, detection and isolation of enantiomers in a range lower than the limits prescribed (1%) for pharmaceuticals in industry. Direct HPLC is widely used method for separation of enantiomers because (i) most of the CSPs are commercially available in market, (ii) detection and isolation limit is up to pico meter level, (iii) no requirement of derivatization hence less time consuming. But the chiral column is of high cost in direct method, while in indirect method no requirement of chiral column so indirect HPLC is less expensive and therefore more useful for detection of enantiomers.

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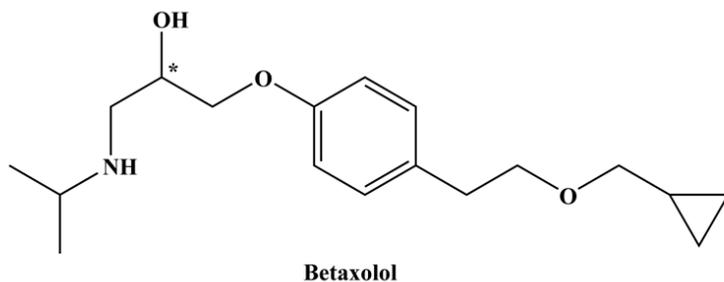
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**Figure**



**Table** Literature reports with present study on chromatographic separation (in terms of:  $R_S$ , LOD and resolution time) of enantiomers of Bel using different CSPs/chiral selectors/CDRs/CIR

S. No.	CSP <sup>a</sup> /CDR <sup>c</sup> /Chiral Selector <sup>b</sup> /CIR <sup>d</sup>	Technique used	Resolution time ( $t_R/k$ /run time)	LOD	$R_S$	Reference
5	Cellulose tris(3,5-dichlorophenyl-carbamate) <sup>a</sup>	i	NA	NA	0.81	23
6	Cellulose tris(3,5-dimethylphenyl-carbamate) <sup>a</sup>	i	15.2/16.9 min ( $t_{R1}/t_{R2}$ )	NA	1.6	24
7	Amylose tris(3,5-dimethoxyphenyl	i	11.8/14.8 min ( $t_{R1}/t_{R2}$ )	10 $\mu\text{g/mL}$	1.54	25
8	carbamate) <sup>a</sup>		31.7/32.9 ( $t_{R1}/t_{R2}$ )			26
9	(-)-( $\alpha$ -methoxy-( $\alpha$ -(trifluoro-methyl)phenylacetyl chloride) <sup>b</sup>	ii	19.8/20.0 ( $k_1/k_2$ )	NA	1.18	Kim 2001)
10	1,3-diacetoxy-1-(4-nitro-phenyl)-2-propyl isothiocyanate <sup>b</sup>	ii	4.67/5.58 ( $k_1/k_2$ )	0.2nmol/mL	1.62	Péter, 2001
	2,3,4,6-tetra-O-acetyl-b-D-galactopyranosyl isothiocyanate <sup>b</sup>	ii	NA	NA	6.32	(Ko, et al, 2006)
13	2,3,4,6-tetra-O-acetyl-b-D-glucopyranosyl isothiocyanate <sup>b</sup>	ii	NA	NA	4.70	(Ko, et al, 2006)
14	(R)-2-(5-fluoro-2,4-dinitrophenylamino)-3-(methylthio)propanoic acid <sup>b</sup>	ii	21.48/23.88 ( $t_{R1}/t_{R2}$ )	12pgmL <sup>-1</sup> and 13pgmL <sup>-1</sup>	11.72	(Bhushan and Nagar 2014)
	(S)-N-(1-cyclohexylethyl)-5-fluoro-2,4-dinitrobenzenamine <sup>b</sup>	ii	34.71/36.92 ( $t_{R1}/t_{R2}$ )	12pgmL <sup>-1</sup> and 13pgmL <sup>-1</sup>	9.65	(Bhushan and Nagar 2014)
	(R)-5-fluoro-2,4-dinitro-n-(1-phenylethyl)benzenamine <sup>b</sup>	ii	33.64/35.63 ( $t_{R1}/t_{R2}$ )	12pgmL <sup>-1</sup> and 13pgmL <sup>-1</sup>	8.70	(Bhushan and Nagar 2014)
14	L-lysine/L-arginine <sup>c</sup>	iii	5.50/6.45 ( $k_1/k_2$ )	NA	2.0	Bhushan and Thiong'O, 1998
18	L-Glutamic acid <sup>d</sup>	iii	10 min (run time)	1.2-1.8 $\mu\text{g}$	2.0	(Bhushan et al. 2015)

CSP, chiral stationary phase; CDR, chiral derivatizing reagent; CIR, chiral inducing reagent;  $t_{R1}$  and  $t_{R2}$  are the retention time of first and second eluting isomer, respectively;  $k_1$  and  $k_2$ , retention factor of first eluting isomer; run time, time taken by solvent system to migrate across the TLC plate. i, ii and iii represent the techniques used as direct HPLC, indirect HPLC and direct TLC separation, respectively.