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Enantiomeric Resolution of Orciprenaline using Liquid Chromatographic Techniques: A Review

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Abstract

The presence of active functional groups amino (-NH2) and hydroxyl groups (-OH) in their simple and unique structure, amino alcohols are very important drugs for the treatment of heart related diseases. Orciprenaline is moderately selective β2-adrenergic receptor agonist and a bronchodilator has importance to use in relaxing the airway muscles and to improve breathing for patients suffering from asthmatic or bronchitis problem. As due to the specific and proper spatial orientation of active groups to react with specific receptors (β2-receptor) enantiomer of β-blockers generally (S)-(−)-enantiomer shows about 50-500 fold higher pharmacological activities as compared to its mirror image. In the current review paper attempts are being done on the enantiomeric resolution of orciprenaline by using liquid chromatographic techniques and also deals with the application of: thin layer chromatography by direct method using specific chiral selector as chiral impregnating reagent, and certain chiral inducing reagents and by applying suitable mobile phases. Direct high-performance liquid chromatography using chiral stationary phase columns and indirect high-performance liquid chromatography using chiral derivatizing reagents for resolution of enantiomers of orciprenaline also incorporated in the present review paper.

Keywords: β-blockers; Chiral Selector; Chiral Derivatizing Reagent; Enantiomeric Resolution; Thin Layer Chromatography; High-Performance Liquid Chromatography

Abbreviations

CSP: Chiral Stationary Phase; LOD: Limit Of Detection; CIR: Chiral Inducing Reagent; CMPA: Chiral Mobile Phase Additives; PDA: Photodiode Array Detector; CC: Cyanuric Chloride; CDRs: Chiral Derivatizing Reagent.

Introduction

β-blockers (e.g. as β-adrenolytic drugs) are amino alcohols which consist of a group of synthetic chiral hydroxyl and amine-containing compounds inhibits adrenaline to bind with nerve receptors and "block" its effects. Orciprenaline is moderately selective β_2-adrenergic receptor agonist and a bronchodilator has importance to use in relaxing the airway muscles and to improve breathing for patients suffering from asthmatic or bronchitis problem. The (S)-(–)-enantiomer of orciprenaline drug (Figure 1) is pharmacologically effective, showing about 50–500 fold higher activities [1]. In most cases orciprenaline is administered as racemic mixtures. The IUPAC name of orciprenaline is [5-[1-hydroxy-2-(propan-2-ylamino)ethyl]benzene-1,3-diol] (Figure 1).

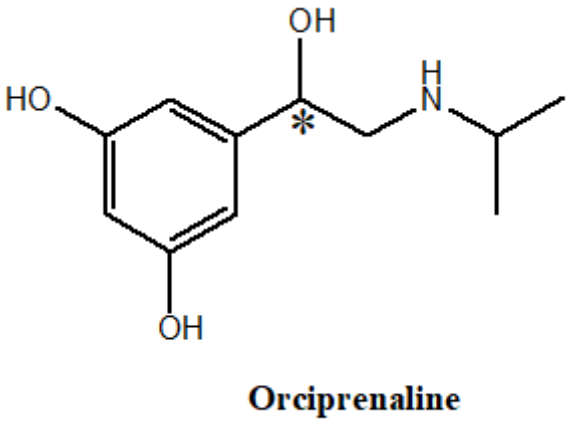

Figure 1: Structure of Metaproterenol (Orciprenaline).

Regulations framed by US-FDA and other such globally renowned agencies have driven and supported the growth of chiral technology internationally with increasing demand and use of chirally pure products in pharmaceutical industries and medical fields. Thus, in the present day scenario issue of enantioresolution becomes very important as well as challenging. This is due to that the enantiomers have different activity in our body having chiral environment therefore such enantiomers are considered as different compounds. Still most of clinical pharmacologists and biologists tend to deal the treatment with enantiomeric mixtures (racemic mixture) of isomers. As a physician, prescribing such a drug under a specific brand name is might be unaware of different physiological/pharmaceutical activity of enantiomers and may make mistake [2], which is not justifiable for human society. Because in many cases, the less reactive or undesired enantiomer can have many side effects or even serious toxic effects. Therefore, efficient and simpler methods of enantiomer resolution are always demanded to control the optical purity of enantiomer or to separate racemic mixtures of the drugs.

From academic and industrial point of view in various fields enantiomeric resolution has immense importance. Two basic strategies "*direct approach*" and "*indirect approach*" have evolved in recent years for enantiomeric resolution. The direct approach is can be employed without any derivatization and separation can be achieved by using specific optically pure chiral selectors: i.e. as chiral impregnating reagent, chiral inducing reagent and chiral mobile phase additives in thin layer chromatography; as chiral stationary phase in high-performance liquid chromatography, to create chiral environment *in situ* formation of transient diastereomers with the enantiomers present in an analyte. Whereas, in indirect method, firstly enantiomers of analyte species are converts in to their diastereomers by their reaction with specific chiral derivatizing reagents and thereafter separation can be achieved in achiral environment by using planar and column chromatographic techniques. Some basic approaches and principles of these chromatographic techniques for enantiomeric resolution of orciprenaline is already reported but no any methods is described for the resolution of enantiomers of orciprenaline using direct and indirect approaches in chromatographic method. Here presented review article is mainly focused on the use of liquid chromatography for the enantiomeric resolution of orciprenaline.

Direct Approach

In direct approach enatiomeric resolution depends on the chiral environment either created by incorporating a suitable chiral selector at an appropriate stage of that approach or produced in the form of that particular chiral stationary phase (CSP). This chiral environment can be produced in TLC and HPLC by using (a) the TLC plate or the column with the specific chiral selector, (b) impregnation of the chiral selector on stationary phase of TLC plate, or (c) addition of the chiral selector to the employed mobile phase. The mentioned material at situation (a) is chiral owing to its own structure (e.g. cellulose) whereas the material mentioned at (b) is prepared by interacting the chiral selector of interest to that particular reactive groups of inert support. In (c) the chiral environment is produced by mixing the chiral selector with mobile phase and separation is achieved by flowing this mixture on TLC plate and through column in HPLC. All the cases includes formation of transient diastereomers occurred by interaction of the chiral selector with enantiomers of analyte of interest. The basic concept behind the separation of these enantiomers is that, the enantiomer which forms more stable diastereomer will be retained more with stationary phase and its elution may be later and the enantiomer forms less stable diastereomer is elutes first. For separation of enentiomers, Martens, et al. [3] applied direct methods which are much important in various types of fields like mechanistic and synthetic study and, pharmaceutical as well as biomedical analysis.

Enantioresolution by TLC

Following methods are used for the separation

Impregnation: In this method an appropriate chiral selector is incorporated with the solid adsorbent without affecting its inert character, owing to application of analyte samples on the TLC plate for their separation is termed as impregnated TLC. There are certain methods employed for impregnating the TLC plate [3]: spray of the solution of the chiral impregnating reagent on the plate; immersion of plain plate or its ascending or descending development into an appropriate solution of

the chiral impregnating reagent; by expose the thin layer to the vapors of the chiral impregnating reagent; and by mixing the chiral selector with the inert support or immersion. The advantages of impregnating reagents have been described in literature for separation of enantiomers by direct method in TLC [4]. The chemical nature of the impregnating reagent the compounds and the kind of the functional groups affect the overall results of resolution, along with the influence of the impregnating reagent on the partition or adsorption. The impregnation methods used also play an important role in the resolution of the enantiomers. This method offers an economical and broad choice of chromatographic conditions for enantiomeric resolution of variety of compounds.

Enantiomeric separation of orciprenaline has been carried out using the (S)-glutamic acid [5], as chiral impregnating reagent and chromatographic data in terms of resolution and limit of detection (LOD) are given in Table 1.

S. No.	CSPs[a]/chiral selectors[b]/CIR[c]	Technique used	Resolution time (t_R/k/run time)	LOD	R_s	Reference
1	(S)-Glutamic acid[c])	i(a)	10 min (run time)	1.4–1.9µg	3.7	[5]
2	(S)-Glutamic acid[b])	i(b)	10 min (run time)	1.4–1.9 µg	2.8	
3	(S)-Glutamic acid[b])	i(c)	10 min (run time)	1.2–1.8 µg	2.1	[6]
4	Sulfobutyl ether β-cyclodextrin [a]	ii	31.7/32.9 (t_{R1}/t_{R2})	NA	0.8	[7]
5	Sulfated-β-cyclodextrin[a]	ii	8.2/8.9 (t_{R1}/t_{R2})	NA	2.03	[8]
6	Methyl-β-cyclodextrin[a]	ii	7.99/8.42 (t_{R1}/t_{R2})	NA	3.95	[9]
7	Cellulose tris(3,5-dimethylphenylcarbamate)[a]	ii	NA	NA	4.68	[10]
8	(R)-2-(5-fluoro-2,4-dinitrophenylamino)-3-(methylthio) propanoic acid	iii	17.72/ 19.74 (t_{R1}/ t_{R2})	12 -13 pg mL^{-1}	9.79	[11]
9	(S)-N-(1-cyclohexylethyl)-5-fluoro-2,4-dinitrobenzenamine	iii	31.39/ 33.86 (t_{R1}/ t_{R2})	12 -13 pg mL^{-1}	10.22	
10	(R)-5-fluoro-2,4-dinitro-n-(1-phenylethyl)benzenamine	iii	29.81/ 31.77 (t_{R1}/ t_{R2})	12 -13 pg mL^{-1}	8.26	
11	*N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)-L-Leu-NH_2	iii	31.81/35.44 (t_{R1}/ t_{R2})	5-7 ng mL^{-1}	8.84	[12]
12	*N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)-L-Val-NH_2,	iii	29.63/ 32.96 (t_{R1}/ t_{R2})	5-7 ng mL^{-1}	8.12	
13	*N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)-L-Met-NH_2	iii	27.22/ 29.92 (t_{R1}/ t_{R2})	5-7 ng mL^{-1}	6.60	
14	*N*-(4-Chloro-6-Methoxy-[1,3,5]triazine-2-yl)-L-Leu-NH_2	iii	30.292/ 33.39 (t_{R1}/ t_{R2})	5-7 ng mL^{-1}	7.55	
15	*N*-(4-Chloro-6-Methoxy-[1,3,5]triazine-2-yl)-L-Val-NH_2,	iii	28.28/ 30.86 (t_{R1}/ t_{R2})	5-7 ng mL^{-1}	6.76	
16	*N*-(4-Chloro-6-Methoxy-[1,3,5]triazine-2-yl)-L-Met-NH_2	iii	25.02/ 27.21 (t_{R1}/ t_{R2})	5-7 ng mL^{-1}	5.36	

CSP, chiral stationary phase; CIR, chiral inducing reagent; tR1 and tR2 are the retention time of first and second eluting isomer, respectively; k1 and k2 , retention factor of first and second eluting isomer; run time, time taken by solvent system to migrate across the TLC plate. i, ii (a to c) and iii represent the techniques used as direct TLC; i(a) chiral impregnation method, i(b) chiral mobile phase additives) and i(c) chiral inducing reagent, direct HPLC and indirect HPLC separation, respectively

Table 1: Literature reports on TLC and HPLC separation (in terms of R_S, LOD and resolution time) of enantiomers Orciprenaline.

Chiral inducing reagent (CIR): When chiral selector mixed with the racemic mixture of the analyte then it induces the chiral environment in medium owing to formation of transient diastereomers of racemic mixture of the analyte, so it is called as chiral inducing reagent. The main advantage of this method over the impregnation is that the chiral selector is required in very less quantity than the quantity of the chiral selector required in impregnation methods. Since, in this method there is no requirement to produce chiral environment on the inert solid support of TLC plate for resolution of enantiomers. Therefore, the TLC is called as achiral TLC and the resolution is known as achiral phase chromatographic resolution.

Literature reported the use of (S)-glutamic acid as CIR and chromatographic run was performed, results obtained were good in terms of resolution [6] as shown in Table 1.

Chiral mobile phase additives (CMPA): The CMPA approach is adopted in TLC, HPLC and CE for enantiomeric separation; for development of chromatogram the chiral selector is mixed with mobile phase system. The column having good compatibility, ruggedness and high efficiency can be used in HPLC separation by mixing the chiral additive in mobile phase. Stereoseletive separation is considered to be achieved in an environment having chiral additive in the mobile phase where one or a combination of the following 'mechanisms' may occur:

- Stereoselective complexation in mobile phase,
- Formation of the transient diastereomeric associates having different distribution properties between the stationary and mobile phase,
- Adsorption of the chiral additives onto the solid stationary phase during the development of chromatogram. Enantiomeric separation of orciprenaline has been carried out using the (S)-glutamic acid [5], as chiral mobile phase additives and chromatographic data in terms of resolution and limit of detection (LOD) are given in Table 1.

Enantiomeric Resolution by HPLC

Direct Approach: HPLC is the most widely used efficient method for enantiomeric resolution. There is no requirement of any derivatization reaction i.e., no covalent bond formation is required between reactive groups of chiral selector and analyte compound. For that, in recent years several chiral columns have been used for enantiomeric resolutions which are commercially available in market as well. Enantiomeric resolutions by HPLC has advantages over TLC as it gives better results in terms of resolution, accuracy, precision and detection limit of the analyte. In this technique the detectors generally used is photodiode array detector (PDA) 2600, offers the detection of analyte molecules up to pico meter level. Several packed columns containing chiral stationary phases, based on chirally pure: amylose, pirkle, cellulose, ovomucoid, monolithic cyclodextrin, glycoprotein and zirconia have been frequently used for enantioseparation. Of the above mentioned chiral stationary phase columns certain are used for enantioresolution of orciprenaline; sulfobutyl ether β-cyclodextrin [7], Sulfated-β-cyclodextrin [8] and Methyl-β-cyclodextrin [9] and Cellulose tris(3,5-dimethylphenylcarbamate) [10], and for comparative study data in terms of resolution time, resolution values and limit of detection are given in Table 1.

Indirect Approach: Herein, first enantiomer converts into their diastereomers by reacting them with chirally pure derivatizing reagent and then diastereomers run through achiral environment for separation, as distereomers have different physical and chemical properties in achiral environment.

In indirect method separation of diastereomeric pair is sometimes simpler and chromatographic conditions can be optimized more easily than the direct method, and often better resolution obtained. For better determination of biological samples highly sensitive detector are useful in this technique. Also the CDRs used for derivatization of sample analyte are generally chosen having good detection limit. For example the Marfey's and Cyanuric chloride (CC) chiral derivatizing reagent (CDRs) because of high molar UV-Vis absorptivity (ε) shows high sensitive detector response to samples and also have good fluorescence quantum yield. Hence, the CDR react with enantiomers of (*R,S*)-mixture is of different rates, therefore derivatization conditions must be optimized to overcome kinetic resolution problem. In regard the structure of analyte sample appropriate reactive group should be present in close proximity to the stereogenic center (chiral center), which is prone a quantitative conversion of analyte with the CDR and without the formation of side products. During transformation of enantiomeric mixture into diastereomers racemization of also the sample and of the CDR must not be there. Resolution of orciprenaline enantiomers have been carried out by transforming them into diastereomers. For that, certain CDRs have been utilized to form diastereomers of orciprenaline prior to their separation by indirect HPLC.

Chromatographic separation data are presented here in terms of "resolution" of diastereomers of orciprenaline formed using following CDRs: (*R*)-2-(5-fluoro-2,4-dinitrophenylamino)-3-(methylthio)propanoic acid, (*S*)-N-(1- cyclohexylethyl)-5-fluoro-2,4-dinitrobenzenamine, and (*R*)-5-fluoro-2,4-dinitro-n-(1-phenylethyl)benzenamine [11]; *N*-(4,6-Dichloro-[1,3,5] triazine-2-yl)-L-Leu-NH_2, *N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)-L-Val-NH_2, *N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)-L-Met-NH_2 , *N*-(4-Chloro-6-Methoxy-[1,3,5]triazine-2-yl)-L-Leu-

NH_2, *N*-(4-Chloro-6-Methoxy-[1,3,5]triazine-2-yl)-L-Val-NH_2, and *N*-(4-Chloro-6-Methoxy-[1,3,5]triazine-2-yl)-L-Met-NH_2 [12].

Conclusion

For quantitative and qualitative analysis of important pharmaceuticals liquid chromatographic techniques by direct and indirect methods are very useful. Direct method is efficient and more suitable for resolution of enantiomers in their pure form where derivatization is not required and therefore it is less time consuming. TLC via direct method is (i) less expensive than compared to HPLC, gas chromatography and capillary electrophoresis that require highly sophisticated and expensive instrumental set-up, more time taking and high running costs, (ii) is good for fast and sensitive resolution, isolation and detection of enantiomers in a lower range than the limits prescribed (1%) by regulatory agencies for pharmaceuticals in industry. HPLC via direct approach is most widely used method for separation of enantiomers this is because of (i) commercial availability of most of CSPs in market, (ii) no requirement of derivatization hence it is less time consuming, and (iii) detection and isolation limit is up to pico meter level. However the chiral columns are of high cost in direct method, whereas in indirect method chiral column is not required therefore being less expensive indirect HPLC is more useful for detection and separation of enantiomers from the enantiomeric mixture.

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