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Direct chiral separations of third generation β-blockers through high performance liquid chromatography: a review

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Abstract

High performance liquid chromatography with chiral stationary phase (CSPs) is a predominant enantiomeric separation technique, widely used in the separation and analysis of chiral drugs. Wide ranges of chiral selectors of natural and synthetic origin have been studied and are effectively employed in enantioseparation of drugs and chemical compounds in diverse matrixes, including pharmaceuticals and biological fluids using either HPLC or CE. This review covers development and application in direct chiral separation of third generation β-blockers through high performance liquid chromatography using chiral stationary phases.

Keywords: Chiral separations, HPLC, Chiral stationary phases, Third generation β-blockers.

1. Introduction

Historically, isomerism was ignored and chiral drugs were marketed as racemates, leading to tragic consequences[1-3] as well as well-known, everlasting, side effects. However, with the advance of science, it was possible to discover that most drugs exist in the optically active form and that the biological activity differs between the pairs of enantiomers[4].

Enantiomeric drugs have become increasingly important over the last 20-30 years, since about 56% of the drugs currently in use are chiral compounds, and 88% of these chiral synthetic drugs are used therapeutically as racemates[5].

In an attempt to reduce side effects caused by drugs enantiomers, the Food and Drug Administration (FDA) and other worldwide regulatory agencies now recommend that the production of enantiomers should be made using enantioselective synthesis and the development of analytical methods to separate racemates that are of great importance[6,7].

From analytical standpoint, chiral drugs are very intriguing and sometimes neglected due to limitations in correlating physical and biological properties, especially due to limited availability of low cost separation methods. The escalating development of chiral drugs as single enantiomers or racemate demands development of chiral separation methods in order to assure proper patient health care. It is of utmost importance being able to separate and analyze chiral racemic drugs, so that it becomes possible to obtain the optimal treatment for the patient having an adequate therapeutic control[5].

Many pharmaceutical compounds have chiral centers and regulatory requirement demands to prove the enantiomeric purity of chiral drugs[7]. This interest can be attributed essentially to well-known fact that enantiomers of a racemic drug may have different pharmacological activities, as well as different pharmacokinetic and pharmacodynamic profiles[8,9].

The qualitative and/or quantitative enantiomeric analysis can be achieved through detection and/or separation approach. That includes techniques such as polarimetry, NMR, isotopic dilution, calorimetry, enzymatic techniques, high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE)[1,10].

HPLC with chiral stationary phase (CSPs) and CE with chiral additives are the predominant chiral analytical techniques used in the separation and analysis of chiral drugs. Wide ranges of chiral selectors have been studied and are effectively employed in chiral analysis of enantiomers in pharmaceutical preparations and biological fluids using either HPLC or CE.

This review covers developments and applications in direct chiral separations of third generation β-blockers through high performance liquid chromatography using chiral stationary phases.

2. Difference of Enantiomers Activity

Almost half of the drugs in use are chiral. It is well known that the pharmacological activity is restricted, in most cases, to one of the enantiomers and that can differ qualitatively and/or quantitatively amongst antipodes. The distomer (enantiomer with lesser activity) can show undesired side effects and in some cases even antagonistic and/or toxic effects. The enantiomers can differ in absorption, distribution, protein binding and affinity to the receptor. Furthermore, can even sometime follow completely distinct metabolic pathways[8,9,11].

The α_1-receptors cause vasoconstriction, increase of peripheral resistance, and increase of arterial pressure. The α_2 activation cause contraction of smooth muscle relaxation. The activation of the β_1-receptor leads to increases in contractile force and heart rate. Activation of β_2-receptors leads to vascular and nonvascular smooth muscle relaxation[12-15].

The therapeutic effects of β-blockers are normally explained by their capacity to block the β-adrenoceptors, hindering the access of the endogenous agonists' noradrenaline and adrenaline[16]. These drugs can be classified based on their cardio-selectivity, β_1 that act on heart adrenergic receptors and β_2 that act on receptors found on bronchus, peripheral blood vessels, etc.

Most of these drugs, with or without intrinsic sympathomimetic activity can be sub-divided into three generations[17]. The first-generation is composed of nonselective drugs, which blocks β_1- and β_2-adrenergic receptors and includes drugs such as propranolol, timolol, pindolol, nadolol and sotalol. The second-generation is composed of β_1-selective (cardioselective) drugs, and includes atenolol, bisoprolol and metoprolol. The third generation β-blockers provide additional vasodilation effects, which is most effective in rapid reduction of blood pressure. The β-blockers may also have α-receptor blocking effects (labetalol, carvedilol), intrinsic sympathomimetic effects (pindolol), or class III antiarrhythmic effects (sotalol)[18].

In the 1970s and 1980s, another drug development effort aimed at improving the treatment of hypertension led to the conception of β-blockers with vasodilating activity[19]. Because of blockade of both β_1 and β_2 adrenergic receptors as well as other properties, third generation β-blockers possess a more comprehensive antiadrenergic profile than second-generation β_1-selective compounds[19]. Third generation includes bucindolol, carvedilol, labetalol and nebivolol[19], that are discussed further in following section along with their stereoselective discriminative activities.

2.1. Bucindolol

Bucindolol (Figure 1) is a non-cardioselective β-blocker. It is reported to possess weak α_1-blocking activity and direct mild vasodilation activity[19,20]. However, the degree of enantioselective affinity for different sympathomimetic receptors (β_1, β_2, α_1, α_2) is unclear. The myocardial stimulant activity as well as vasodilator capacity of (-)-bucindolol is reported to be greater than (+)-bucindolol[9]. The racemic bucindolol hydrochloride was investigated in the management of hypertension, heart failure, and other cardiac disorders. However, its development was brought to an end. It has been suggested that it may be of benefit in a genetically identifiable subgroup of patients[17,21].

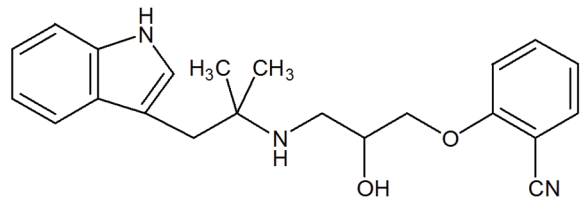

Figure 1. Chemical structure of bucindolol.

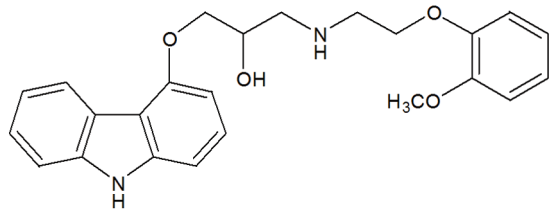

Figure 2. Chemical structure of carvedilol.

2.2. Carvedilol

Carvedilol (Figure 2) is a non-cardioselective β-blocker, which has vasodilating activity due to its α_1-blocking effect. Besides potent antioxidant activity, at higher doses, it also presents calcium-channel blocking activity. Its unique properties plays multiple adrenergic blockade (β_1, β_2 and α_1), as well as its antioxidants and anti-proliferative effect may be important to inhibit the progressive deterioration of ventricular dysfunction and heart failure[17,21]. Carvedilol is reported to have no intrinsic sympathomimetic activity and only weak membrane-stabilizing activity[20]. It is used in the management of hypertension and angina pectoris, and as an adjunct to standard therapy in symptomatic heart failure, besides being used to reduce mortality in patients with left ventricular dysfunction after myocardial infarction[9,21].

The selective action of carvedilol on β_1-receptors is seven to eight fold greater than that on β_2-receptors, and as a result, at low doses (≤6.25 mg twice a day) carvedilol predominantly presents β_1 selective activity. Carvedilol and one of its metabolites have significant antioxidant properties, although in human subjects it is not clear whether blood levels are sufficient to manifest any effect. If these antioxidant properties were, in fact, active in subjects with heart failure, they would be expected to produce an anti-proliferating/growth-inhibiting effect [Bristow, 1998].

In recent years, it was found that the (S)-carvedilol is a potent competitive inhibitor at β_1-adrenoceptors, whereas the R-enantiomer is considerably less potent. In contrast, the enantiomers are essentially equipotent with respect to α_1-adrenoceptor blockade. Thus, the vasodilator and antihypertensive effects of the drug arise due to α_1-adrenoceptor blockade activity attributed to both enantiomers and the β_1-blockade from the S-enantiomer, which prevents reflex tachycardia. This gives the drug utility in congestive heart failure owing to the combination of decreased vascular resistance (α-adrenergic antagonism) and lack of reflex tachycardia (β-blockade)[9].

Some of carvedilol metabolites have β-blocking and vasodilating activity. One metabolite has greater β-blocking activity than carvedilol but all have weaker vasodilating effects than carvedilol. The *o*-dimethyl-*p*-hydroxyl and *m*-hydroxyl metabolites possess β-blocking activity. Its metabolism is stereoselective and plasma concentrations of *R* (+)-carvedilol are about 2 to 3 times higher than *S* (−)-carvedilol. The metabolism of carvedilol is subject to genetic polymorphism with poor metabolizers of debrisoquine having plasma-*R*(+)-carvedilol concentrations 2- to 3–fold higher than extensive metabolizers[19,22].

2.3. Labetalol

Labetalol (Figure 3) is a non-cardioselective β-blocker, which is administered as a combination of four stereoisomers (RR, SR, RS and SS). The RR isomer is mostly responsible for the β-blocking activity where as the SR isomer has predominantly potent α-adrenoceptor blocker activity. Both the RS and SS isomers, on the other hand, display weak antagonistic activities against α and β-receptors. The reported sequential β-blocking activity is RR>SR; RR>RS; RR>SS whereas the reported sequential α-blocking activity is SR>RR; SR>RS; SR>SS[9,21].

According to reports, labetalol possess some intrinsic sympathomimetic and membrane-stabilizing activity. In addition, it has selective α_1-blocking properties that decrease peripheral vascular resistance. The ratio of α- to β-blocking activity has been estimated to be about 1:3 after oral administration and 1:7 via intravenous route. Labetalol is available as hydrochloride and often used to induce hypotension, during surgery. Labetalol decreases blood pressure more rapidly than other β-blockers[21].

2.4. Nebivolol

Nebivolol (Figure 4) is a cardioselective β-blocker that has vasodilating activity, which appears to be due to a direct action on the endothelium, possibly involving nitric oxide release. It lacks intrinsic sympathomimetic and membrane-stabilizing activity. Nebivolol is used in the management of hypertension, and as an adjunct to standard therapy in patients aged 70 years and older with stable chronic heart failure[21]. It has selectivity for β_1-receptors and vasodilatory effect is mediated through the L-arginine nitric oxide pathway. The aromatic hydroxylated and alicyclic oxidized metabolites have the same pharmacologic activity as the parent compound, whereas the N-dealkylated metabolite and glucuronides are inactive[23].

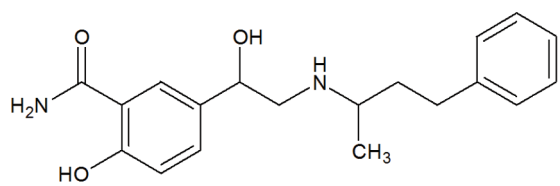

Figure 3. Chemical structure of labetalol.

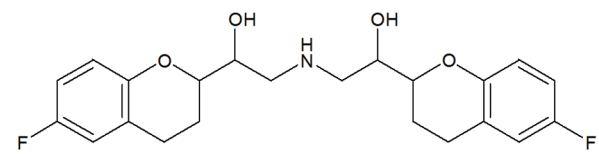

Figure 4. Chemical structure of nebivolol.

The vasodilator properties of nebivolol are attributed to nitric oxide production. The addition of vasodilation to the pharmacologic profile of a β-blocker improves drug tolerability during initiation of therapy and up-titration by afterload reduction-related attenuation of the drop in cardiac output, which is associated with removal of β-adrenergic support of heart rate and contractility. The nitric oxide-potentiating activity of nebivolol might also produce an anti-proliferating effect[20].

Nebivolol, have 4 chiral centers and consequently 16 stereoisomers ($2^4 = 16$). Owing to the presence of a plane of symmetry, some of these isomers are identical and exist in the *meso* form. Accordingly, only ten stereoisomers are present namely, RSSR, SRRS, SRSR (=RSRS), SRRR (=RRRS), RSSS (=SSSR), RRRR, RSRR (RRSR), SRSS (SSRS), SSRR (RRSS), SSSS[24].

The β_1-adrenergic receptor binding activity of nebivolol resides predominantly in the (S,R,R,R)-enantiomer, the (R,S,S,S)-enantiomer have less than one hundredth of the affinity towards β_1-receptor as compared to (S,R,R,R) antipode[25]. Moreover, it is interesting to observe that the (-)-SSSR enantiomer potentiates the hypotensive effect of (+)-RRRS nebivolol enantiomer[26].

3. Analytical Methods

Despite the fact that, at large, β-blockers seem to be structurally similar, analytical methods for their enantiomers determination and quantitation has to be developed individually for each drug due to their different solubility, stability, optic characteristics, and interaction with chiral HPLC phases.

As mentioned earlier, distinct analytical approaches are used for enantiomeric analysis, including polarimetry, NMR, isotopic dilution, calorimetry and enzyme techniques beside modern separation techniques such as HPLC, GC and CE with chiral selectors. This review approaches only on direct chiral HPLC separation using chiral stationary phases (CSPs), and exclusively the method applied in the analysis of third generation β-blockers, namely bucindolol, carvedilol, labetalol and nebivolol listed in Tables 1 to 4. Even though bucindolol is included in this review, it's worth mention that a single analytical method was found in the literature[27], perhaps due to its discontinuation.

Table 1. Enantiomeric separation of bucindolol described in literature using HPLC-CSP.

Analytical system (detection)	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp.	Time of analysis	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Author, year
LC-UV (220 nm)	Chiralpak AD-H (250 x 4.6 mm)	API	Ethanol:diethylamine (100:0.2, v/v)	0.5	Room temperature	15 min	N.I.	N.I.	k1=1.0 k2=1.23	1.23	N.I.	[27]

LC-UV: HPLC with ultraviolet detection; API: active pharmaceutical ingredient; N.I.: not informed.

Table 2. Enantiomeric separation of carvedilol described in literature using HPLC-CSP.

Analytical system (detection)	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp.	Time of analysis	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Ref.
LC-FL Ex: 254, Em: 356nm)	Chiralcel OD (250 x 4.6 mm)	Urine	Acetonitrile:water with (trifluoroacetic acid and diethylamine, 0.05% each), Gradient system	0.3 to 1.0 gradient	25	50	36.7	41.3	-	-	-	[28]
LC-PDA	Chiralcel OD (250 x 4.5 mm)	N.I.	Heptane:isopropanol:diethylamine (80:20:0.1, v/v/v)	0.6	30	-	-	>15	-	-	-	[29]
LC-PDA	Chiralcel OD (250 x 4.5 mm)	N.I.	Heptane:ethanol:diethylamine (80:20:0.1, v/v/v)	0.6	30	-	-	>15	-	-	-	[29]

LC-UV: HPLC with ultraviolet detection; LC-FL: HPLC with fluorescence detection; LC-PDA: HPLC with PDA detection; LC-MS: HPLC with mass spectrometry; API: active pharmaceutical ingredient; N.I.: not informed.

Table 2. Enantiomeric separation of carvedilol described in literature using HPLC-CSP.

Analytical system (detection)	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp.	Time of analysis	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Ref.
LC-UV (220nm)	Chiralcel OD-H (250 x 4.6 mm)	API	Heptane:isopropanol:diethylamine (90:10:0.1, v/v/v)	1.0	N.I.	N.I.	-	-	- -	-	0.00	30
LC-UV (220nm)	Chiralcel OD-H (250 x 4.6 mm)	API	Heptane:ethanol:diethylamine (90:10:0.1, v/v/v)	1.0	N.I.	N.I.	-	-	- -	-	0.00	30
LC-UV (220nm)	Lux Cellulose-I (250 x 4.6 mm, 5 µm)	API	Acetonitrile:diethylamine (100:0.1, v/v)	1	20	-	-	-	k1=7.7 k2=9.64	1.25	-	31
LC-UV (220nm)	Lux Cellulose-2 (250 x 4.6 mm, 5 µm)	API	Acetonitrile:diethylamine:formic acid (100:0.1:0.1, v/v/v)	1	20	-	-	-	k1=2.0 k2=3.11	1.6	-	31
LC-UV (220nm)	Sepapak 2 (250 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-UV (220nm)	Sepapak 2 (250 x 4.6 mm)	API	Methanol:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-UV (220nm)	Sepapak 2 (250 x 4.6 mm)	API	Ethanol:diethylamine:trifluoroacetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-UV (220nm)	Sepapak 3 (250 x 4.6 mm)	API	Heptane:ethanol:diethylamine (90:10:0.1, v/v/v)	1.0	N.I.	N.I.	-	-	- -	-	1.56	30
LC-UV (220nm)	Sepapak 3 (250 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-UV (220nm)	Sepapak 3 (250 x 4.6 mm)	API	Methanol:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-UV (220nm)	Sepapak 3 (250 x 4.6 mm)	API	Ethanol:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-UV (220nm)	Lux® Cellulose-4 (250 x 4.6 mm)	N.I.	CO_2/(Methanol:0.10% Isopropanol:IPA:0.10% trifluoroacetic acid), 80/20 (v/v)	3.0	30	-	-	-	-	-	1.61	33
LC-UV (220nm)	Lux Cellulose-4 (250 x 4.6 mm, 5 µm)	API	Acetonitrile:formic acid (100:0.1, v/v)	1	20	-	-	-	k1=5.1 k2=6.5	1.27	-	31
LC-UV (220nm)	Lux Cellulose-4 (250 x 4.6 mm, 5 µm)	API	Acetonitrile:diethylamine:formic acid (100:0.1:0.1, v/v/v)	1	20	-	-	-	k1=6.9 k2=9.2	1.33	-	31
LC-FL Ex: 284, Em: 343nm)	Chiralpak AD (2.0 x 250 mm)	Whole blood and plasma	Hexane:isopropanol:diethylamine (78:22:1, v/v)	0.3	45	25	10	15	-	-	-	34
LC-FL Ex: 284, Em: 343nm)	Chiralpak AD (250 x 2.0 mm, 5 µm)	Plasma	Hexane:isopropanol:diethylamine (78:22:1, v/v)	0.3	Room temp.	-	-	-	k1=1.2 k2=2.0	1.7	-	34
LC-UV (220nm)	Chiralpak AD-H (250 x 4.6 mm)	API	Ethanol (100, v/v)	0.5	Room temp.	120 min	N.I.	N.I.	N.I.	N.I.	N.I.	35
LC-UV (220nm)	Lux Amylose-2 (150 x 4.6 mm)	N.I.	Heptane:ethanol:diethylamine or triethylamine (80:20:0.1)	1.0	20	-	-	-	k1=4.40	-	2.04	36
LC-UV (220nm)	Lux Amylose-2 (150 x 4.6 mm)	N.I.	Heptane:ethanol:diethylamine or triethylamine (70:30:0.1)	1.0	20	-	-	-	k1=2.21-	-	1.67	36

LC-UV: HPLC with ultraviolet detection; LC-FL: HPLC with fluorescence detection; LC-PDA: HPLC with PDA detection; LC-MS: HPLC with mass spectrometry; API: active pharmaceutical ingredient; N.I.: not informed.

Table 2. Enantiomeric separation of carvedilol described in literature using HPLC-CSP.

Analytical system (detection)	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp.	Time of analysis	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Ref.
LC-PDA	Lux Amylose-2 (250 x 4.6 mm)	N.I.	Heptane:ethanol:diethylamine (80:20:0.1 v/v/v)	1.0	20	-	-	-	k1= 4.40	-	2.04	37
LC-PDA	Lux Amylose-2 (250 x 4.6 mm)	API	Acetonitrile:20 mM borate buffer (pH 9)- (50:50 v/v/v)	0.5	Room temp.	N.I.	N.I.	N.I.	k2 =3.30	N.I.	1.97	38
LC-UV (220nm)	Chiralcel OD-RH (150 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.66	32
LC-UV (220nm)	Chiralcel OD-RH (150 x 4.6 mm)	API	Methanol:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	1.66	32
LC-UV (250nm)	Chiralcel OD-RH (150 x 4.6 mm)	API	Acetonitrile:50 mM/L sodium perchlorate, pH 5.5 (35:65)	0.3	N.I.	35	-	-	-	-	-	39
LC-FL Ex: 285, Em: 355nm)	Chiralcel OD-RH (150 x 4.6 mm)	plasma	50 mM sodium acetate, pH 6.0: acetonitrile (10:90, v/v)	1	35	18	-	-	-	-	-	40
LC-UV (220nm)	Chiralpak AD-RH (150 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-UV (220nm)	Chiralpak AD-RH (150 x 4.6 mm)	API	Methanol:diethylamine:trifluoroacetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-UV (220nm)	Chiralcel OJ-R (150 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-UV (220nm)	Chiralcel OJ-R (150 x 4.6 mm)	API	Methanol:diethylamine:trifluoroacetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-UV (220nm)	Chiralpak AD-H (250 x 4.6 mm, 5 µm)	API	Isopropanol:diethylamine (100:0.1, v/v/v)	1	20	-	-	-	k1=1.29 k2=3.1	2.4	-	31
LC-UV (220nm)	Chiralpak AD-H (250 x 4.6 mm, 5 µm)	API	Isoproponol:diethylamine:formic acid (100:0.1:0.1, v/v/v)	1	20	-	-	-	k1=0.61 k2=0.97	1.58	-	31
LC-UV (220nm)	Chiralpak AS-RH (150 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-UV (220nm)	Chiralpak AS-RH (150 x 4.6 mm)	API	Methanol:diethylamine:trifluoroacetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	N.I.	-	-	- -	-	0.00	32
LC-FL Ex: 284, Em: 343nm)	Phenomenex 3022 (250 x 3.2 mm)	Serum	Hexane:dichloromethane:ethanol (50:35:15, v/v/v), and 0.25% (v/v) trifluoroacetic acid	0.55	N.I.	25	-	-	-	-	-	41
LC-PDA	Chiral AGP (120 x 4.5 mm)	N.I.	Isopropanol:0.02M phosphate buffer, pH 4.6 (10:90, v/v)	0.8	Room temp.	-	-	-	-	1.23	-	42
LC-MS (407 > 100)	Chirobiotic T (250 x 4.6 mm, 5 µm)	Plasma	Methanol:acetic acid:diethylamine (100:0.15:0.05, v/v/v)	1	Room temp.	-	-	-	k1=2.0 k2=2.1	1.1	-	43
LC-UV (241nm)	Imprinted polymer (∑)-propranolol CSP (Ground)	N.I.	Acetonitrile:20 mM phosphate buffer, pH 5.1 (70:30)	-	40	-	53.8	74.5	-	1.4	0.4	44
LC-UV (241nm)	Imprinted polymer (∑)-propranolol CSP (Silica-grafted)	N.I.	Acetonitrile:20 mM phosphate buffer, pH 5.1 (70:30)	-	40	-	34.1	34.1	-	1.0	0.0	44
LC-UV (241nm)	Imprinted polymer (∑)-propranolol CSP (Beads)	N.I.	Acetonitrile:20 mM phosphate buffer, pH 5.1 (70:30)	-	40	-	27.27	38.0	-	1.4	0.1	44

LC-UV: HPLC with ultraviolet detection; LC-FL: HPLC with fluorescence detection; LC-PDA: HPLC with PDA detection; LC-MS: HPLC with mass spectrometry; API: active pharmaceutical ingredient; N.I.: not informed.

Table 3. Enantiomeric separation of labetalol described in literature using HPLC-CSP.

Analytical system	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp. (°C)	Time of analysis (min)	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Ref.
LC-UV (254nm)	Cyclobond-I	API	Acetonitrile:methanol:acetic acid:triethylamine (98:2:0.8:0.6, v/v/v/v)	1.0	25	N.I.	N.I.	N.I.	K1=8.8 k2=9.2 k3=9.5 k4=10.3	α1=1.0 α2=1.0 α3=1.1	N.I.	45
LC-FL Ex: 220, Em: 412nm)	Chirex 3022 (250 x 4.0 mm)	Plasma	Hexane:1,2-dichloroethane:ethanol: trifluoroacetic acid (56:35:9:0.25)	0.6	25	60	N.I.	N.I.	k1=3.7 k2=4.3 k3=5.1 k4=6.3	α1=1.2 α2=1.2 α3=1.2	N.I.	46
LC-FL Ex: 230, Em: 400nm)	α_1-acid glyco-protein (100 x 4 mm)	Plasma	0.02 mM phosphate buffer with 0.015 M tetrabutylammonium phosphate, pH 7.1	0.5	Room temp.	40	N.I.	N.I.	k1=3.9 k2=4.9 k3=6.2 k4=7.7	α1=1.2 α2=1.3 α3=1.2	N.I.	47
LC-UV (225nm)	Teicoplanin chiral column (300 x 4.6 mm)	API	Methanol:acetic acid:triethylamine (100:0.05:0.05, v/v/v/v)	1.0	Room temp.	40	N.I.	N.I.	k1=2.0 k2=2.2 k3=2.83	α1=1.1 α2=1.3	N.I.	48
LC-UV (225nm)	Three Chirex 3022 (50mm x 4.6mm)	API	Hexane:dichloromethane:metanol: trifluoroacetic acid (55:35:9:0.5, v/v/v/v)	1.0	Room temp.	40	N.I.	N.I.	k1=2.0 k2=2.2 k3=2.83	α1=1.1 α2=1.3	N.I.	49
LC-UV (220nm)	Chiralpak AD-H (250 x 4.6 mm)	API	Heptane:ethanol:diethylamine (90:10:0.1, v/v/v)	1.0	N.I.	-	-	-	-	0.75/0.91/1.63	-	30
LC-UV (220nm)	Chiralcel OD-H (250 x 4.6 mm)	API	Heptane:isopropanol:diethylamine (90:10:0.1, v/v/v)	1.0	N.I.	-	-	-	-	0.00	-	30
LC-UV (220nm)	Chiralcel OD-H (250 x 4.6 mm)	API	Heptane:ethanol:diethylamine (90:10:0.1, v/v/v)	1.0	N.I.	-	-	-	-	0.00	-	30
LC-UV (220nm)	Sepapak 3 (250 x 4.6 mm)	API	Heptane:ethanol:diethylamine (90:10:0.1, v/v/v)	1.0	N.I.	-	-	-	-	0.56	-	30
LC-UV (220nm)	Sepapak 2 (250 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	0.00	-	30
LC-UV (220nm)	Sepapak 2 (250 x 4.6 mm)	API	Methanol:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	0.00	-	30
LC-UV (220nm)	Sepapak 2 (250 x 4.6 mm)	API	Ethanol:diethylamine:trifluoroacetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	0.00	-	30
LC-UV (220nm)	Sepapak 3 (250 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	0.00	-	30
LC-UV (220nm)	Sepapak 3 (250 x 4.6 mm)	API	Methanol:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	0.00	-	30
LC-UV (220nm)	Sepapak 3 (250 x 4.6 mm)	API	Ethanol:diethylamine:trifluoroacetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	-	-	30
LC-UV (220nm)	Chiralpak AD-RH (150 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoroac etic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	-	-	30
LC-UV (220nm)	Chiralpak AD-RH (150 x 4.6 mm)	API	Methanol:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	-	-	30
LC-UV (220nm)	Chiralcel OD-RH (150 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	-	-	30
LC-UV (220nm)	Chiralcel OD-RH (150 x 4.6 mm)	API	Methanol:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	-	-	30

LC-UV: HPLC with ultraviolet detection; VWD: variable wavelength detection; LC-FL: HPLC with fluorescence detection; LC-PDA: HPLC with PDA detection; API: active pharmaceutical ingredient; N.I.: not informed.

Table 3. Enantiomeric separation of labetalol described in literature using HPLC-CSP.

Analytical system	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp. (°C)	Time of analysis (min)	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Ref.
LC-UV (220nm)	Chiralpak AS-RH (150 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	-	-	30
LC-UV (220nm)	Chiralpak AS-RH (150 x 4.6 mm)	API	Methanol:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	-	-	30
LC-UV (220nm)	Chiralcel OJ-R (150 x 4.6 mm)	API	Acetonitrile:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	-	-	30
LC-UV (220nm)	Chiralcel OJ-R (150 x 4.6 mm)	API	Methanol:diethylamine:trifluoro acetic acid (100:0.1:0.1, v/v/v)	0.5	N.I.	-	-	-	-	-	-	30
LC-PDA	Chiral-HSA (100 mm x 4.0 mm)	API	0.01 M Phosphate buffer at pH 7.0	0.5	N.I.	-			k1=9.8 k2=12.4	1.26	1.87	50
LC-PDA	Chiral-AGP (100 mm x 4.0 mm)	API	Methanol:0.01M phosphate buffer at pH 7.0, 10:90 (v/v)	0.9	N.I.	-	-	-	k1=22.3 k2=25.2 k3=29.7	1.13/1.18	1.11 1.56	50
LC-PDA	Chiral-AGP (100 mm x 4.0 mm)	API	Methanol:0.01 M phosphate buffer at pH 7.0, 10:90 (v/v), 1 mM dimethyloctylamine	0.9	N.I.	-	-	-	k1=10.5 k2=12.8	1.22	-	50
LC-PDA	Chiral-AGP (100 mm x 4.0 mm)	API	Methanol:0.01 M phosphate buffer at pH 7.0, 10:90 (v/v), 2 mM dimethyloctylamine	0.9	N.I.	-	-	-	k1=8.9 k2=10.3	1.17	-	50
LC-PDA	Chiral-AGP (100 mm x 4.0 mm)	API	Methanol:0.01 M phosphate buffer at pH 7.0, 10:90 (v/v), 3 mM dimethyloctylamine	0.9	N.I.	-	-	-	k1=7.87	-	-	50
LC-PDA	Chiral-AGP (100 mm x 4.0 mm)	API	Methanol:0.01 M phosphate buffer at pH 7.0, 10:90 (v/v), 4 mM dimethyloctylamine	0.9	N.I.	-	-	-	k1=7.26	-	-	50
LC-UV (220nm)	Cellulose with benzoate at the position 2 and 3 and a *meta*-substituted phenylcarbamate at the 6 position	API	Hexane:isopropanol (80:20, v/v)	1.0	N.I.	-	-	-	k1=2.53	-	1.00	51
LC-UV (220nm)	Cellulose with a 3,5-dimethyl-phenylcarbamate ate the 2 and 3 positions and a phenylethylcar-bamate at the 6 position	API	Hexane:isopropanol (80:20) containing 10 mM octanoic acid	1.0	N.I.	-	-	-	k1=2.11	-	1.27	51
LC-UV (220nm)	Cellulose with 3,5-dimethylphe-nylcarbamate at the 2 and 3 positions and a *para*-substituted benzoate at the 6 position	API	Hexane:isopropanol (80:20) containing 10 mM diethylamine	1.0	N.I.	-	-	-	k1=3.50	-	1.00	51

LC-UV: HPLC with ultraviolet detection; VWD: variable wavelength detection; LC-FL: HPLC with fluorescence detection; LC-PDA: HPLC with PDA detection; API: active pharmaceutical ingredient; N.I.: not informed.

Table 3. Enantiomeric separation of labetalol described in literature using HPLC-CSP.

Analytical system	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp. (°C)	Time of analysis (min)	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Ref.
LC- PDA	Labmade - Dinitrophenyl ether substituted β-cyclodextrin	N.I.	Methanol:water:triethylammonium acetate (20:80:0.1), pH 4.1	1.0	Room temp.	-	-	-	k1=8.54	-	1.05	[52]
LC- PDA	Labmade - Dinitrophenyl ether substituted β-cyclodextrin	N.I.	Methanol:water:triethylammonium acetate (25:75:0.1), pH 4.1	1.0	Room temp.	-	-	-	k1=5.34	-	1.03	[52]
LC-PDA	Rifamycin-capped (3-(2-O-β-cyclodextrin)-2-hydroxypropoxy)-propylsilyl-appended silica particles	N.I.	Methanol:water (40:60)	N.I.	Room temp.	-	-	-	k1=1.48	-	1.10	[53]
LC-UV (VWD)	EnantioPac (100 x 4.0 mm)	N.I.	0.32 M ethylene glycol + 0.1 M sodium chloride in 0.02 M phosphate buffer, pH 7.0	0.3	20	-	-	-	k1=27	-	1.69	[54]
LC-UV (VWD)	EnantioPac (100 x 4.0 mm)	N.I.	1.29 M ethylene glycol + 0.1 M sodium chloride in 0.02 M phosphate buffer, pH 7.0	0.3	20	-	-	-	k1=12	-	1.64	[54]
LC-UV (VWD)	EnantioPac (100 x 4.0 mm)	N.I.	0.25 M propylene glycol + 0.1 M sodium chloride in 0.02 M phosphate buffer, pH 7.0	0.3	20	-	-	-	k1=19	-	1.71	[54]
LC-UV (VWD)	EnantioPac (100 x 4.0 mm)	N.I.	1,2 butanediol + 0.1 M sodium chloride in 0.02 M phosphate buffer, pH 7.0	0.3	20	-	-	-	k1=8.4	-	1.27	[54]
LC-UV (VWD)	EnantioPac (100 x 4.0 mm)	N.I.	0.44 M ethanol + 0.1 M sodium chloride in 0.02 M phosphate buffer, pH 7.0	0.3	20	-	-	-	k1=15	-	1.45	[54]
LC-UV (VWD)	EnantioPac (100 x 4.0 mm)	N.I.	1.74 M ethanol + 0.1 M sodium chloride in 0.02 M phosphate buffer, pH 7.0	0.3	20	-	-	-	k1=5.2	-	1.12	[54]
LC-UV (VWD)	EnantioPac (100 x 4.0 mm)	N.I.	0.33 M 2-propanol + 0.1 M sodium chloride in 0.02 M phosphate buffer, pH 7.0	0.3	20	-	-	-	k1=9.7	-	1.19	[54]
LC-UV (VWD)	EnantioPac (100 x 4.0 mm)	N.I.	0.01 M octanoic acid in 0.02 M phosphate buffer, pH 7.0	0.3	20	-	-	-	k1=11.8	-	1.35	[54]
LC-UV (VWD)	EnantioPac (100 x 4.0 mm)	N.I.	0.03 M octanoic acid in 0.02 M phosphate buffer, pH 7.0	0.3	20	-	-	-	k1=9.8	-	1.17	[54]
LC-UV (VWD)	EnantioPac (100 x 4.0 mm)	N.I.	0.05 M octanoic acid in 0.02 M phosphate buffer, pH 7.0	0.3	20	-	-	-	k1=7.4	-	1.14	[54]
LC-FL Ex: 330, Em: 418nm)	Chiral-AGP	Plasma	0.02 M NaH_2PO_4 buffer, 0.1 M tetrabutylammonium hydrogen bromide, pH 7.1	0.7	N.I.	-	-	-	-	-	-	[55]
LC-UV (254nm)	Labmade - cellulose *tris* (3,5-dimethyl-phenylcarba-matecoated on zirconia CSP	API	2-Propanol:n-hexane:ethanolamine (10:90:0.05 v/v)	1.0	25	-	-	-	k1=10	-	1.0	[56]

LC-UV: HPLC with ultraviolet detection; VWD: variable wavelength detection; LC-FL: HPLC with fluorescence detection; LC-PDA: HPLC with PDA detection; API: active pharmaceutical ingredient; N.I.: not informed.

Table 3. Enantiomeric separation of labetalol described in literature using HPLC-CSP.

Analytical system	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp. (°C)	Time of analysis (min)	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Ref.
LC- PDA	Heterosubstituted polysaccharide derivatives 6-(R) Lab CSP	N.I.	Hexane:isopropanol (80:20 v/v)	1.0	N.I.	-	-	-	k1=2.65	-	1.00	[57]
LC- PDA	Heterosubstituted polysaccharide derivatives 6-(S) Lab CSP	N.I.	Hexane:isopropanol (80:20 v/v), octanoic acid 10 mM	1.0	N.I.	-	-	-	k1=1.60	-	1.00	[57]
LC- PDA	Heterosubstituted polysaccharide derivatives 6-(R,S) Lab CSP	N.I.	Hexane:isopropanol (80:20 v/v), diethylamine 10 mM	1.0	N.I.	-	-	-	k1=3.50	-	1.00	[57]
LC-FL Ex: 340, Em: 389nm)	Asahipack ODP 50 (150 x 6 mm)	Plasma	0.05 M diethylamine pH 11.5:acetonitrile (160:840 v/v), 0.036 M sodium chloride	0.9	N.I.	12	-	-	-	-	-	[58]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:acetic acid:triethylamine (100:0.05:0.05 v/v/v)	1.0	Room temp.	-	-	-	k1=1.99 k2=2.20 k3=2.83	1.10/1.29	0.23/0.90	[48]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:acetic acid:triethylamine (100:0.10:0.10 v/v/v)	1.0	Room temp.	-	-	-	k1=1.83 k2=2.0 k3=2.60	1.09/1.30	0.18/0.93	[48]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:acetic acid:triethylamine (100:0.20:0.20 v/v/v)	1.0	Room temp.	-	-	-	k1=1.60 k2=1.71 k3=2.25	1.06/1.31	0.08/0.94	[48]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:acetic acid:triethylamine (100:0.50:0.50 v/v/v)	1.0	Room temp.	-	-	-	k1=1.50 k2=2.04	1.36	1.10/	[48]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:triethylamine (100:0.05 v/v)	1.0	Room temp.	-	-	-	k1=1.10 k2=1.16 k3=1.37	1.15/1.18	0.42/0.69	[48]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:triethylamine (100:0.10 v/v)	1.0	Room temp.	-	-	-	k1=0.67 k2=0.78 k3=0.91	1.16/1.18	0.20/0.46	[48]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:triethylamine (100:0.15 v/v)	1.0	Room temp.	-	-	-	k1=1.10 k2=1.16 k3=1.37	1.15/1.18	0.42/0.69	[48]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:acetic acid:triethylamine (100:0.05:0.05 v/v/v)	1.0	Room temp.	-	-	-	k1=0.99 k2=2.21 k3=2.83	1.10/1.29	0.23/0.90	[48]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:acetic acid:triethylamine (100:0.05:0.10 v/v/v)	1.0	Room temp.	-	-	-	k1=2.17 k2=2.27 k3=2.99 k4=3.26	1.05/1.31/1.09	0.01/0.96/0.18	[48]

LC-UV: HPLC with ultraviolet detection; VWD: variable wavelength detection; LC-FL: HPLC with fluorescence detection; LC-PDA: HPLC with PDA detection; API: active pharmaceutical ingredient; N.I.: not informed.

Table 3. Enantiomeric separation of labetalol described in literature using HPLC-CSP.

Analytical system	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp. (°C)	Time of analysis (min)	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Ref.
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:acetic acid:triethylamine (100:0.05:0.15 v/v/v)	1.0	Room temp.	-	-	-	k1=1.66 k2=2.16 k3=2.46	1.30/1.14	0.91/0.48	[48]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:acetic acid:triethylamine (100:0.05:0.05 v/v/v)	1.0	Room temp.	-	-	-	k1=1.99 k2=2.21 k3=2.83	1.10/1.29	0.23/0.90	[48]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:acetic acid:triethylamine (100:0.10:0.05 v/v/v)	1.0	Room temp.	-	-	-	k1=1.63 k2=1.80 k3=2.37	1.11/1.32	0.18/0.94	[48]
LC-UV (225nm)	Teicoplanin chiral column (4.6 x 300 x 5 µm)	N.I.	Methanol:acetic acid:triethylamine (100:0.15:0.05 v/v/v)	1.0	Room temp.	-	-	-	k1=1.37 k2=1.48 k3=2.01	1.08/1.36	0.08/0.93	[48]
LC-MS (329 > 311)	Chirobiotic V (250 x 4.6 mm)	N.I.	Methanol:acetic acid:diethylamine (100:0.3:0.1 v/v/v)	1.0	24	20	-	-	-	1.15/1.28	-	[59]
LC-UV (220nm)	Lux Cellulose-3 (250 x 4.6 mm)	N.I.	Acetonitrile:100 mM KPF6 + 50 mM phosphate buffer (pH 2) (20:80 v/v)	1.0	20	-	-	-	-	-	1.28/2.10	[38]
LC-PDA (200-300nm)	Labmade - heptakis(6-deoxy-6-azido-phenylcarbamo-ylated)- cyclodex-trin CSP	N.I.	Methanol:water with 1%triethylamine adjusted to pH 4.2 (35:65 v/v)	0.7	N.I.	-	-	-	k1=3.02 k2=3.23	1.07	<0.3	[60]
LC-UV (280nm)	(R)-α-Burke 2	N.I.	Ethanol:dichloromethane (10:90 v/v) with 20 mM ammonium acetate	2.0	N.I.	-	-	-	k1=2.48 k2=2.97	1.20	1.35	[61]
LC-UV (280nm)	(R)-α-Burke 2	N.I.	Ethanol:dichloromethane (10:90 v/v) with 20 mM ammonium acetate	2.0	N.I.	-	-	-	k1=4.44 k2=5.16	1.16	1.14	[61]
LC-UV (280nm)	(R)-α-Burke 2	N.I.	Ethanol:acetonitrile (20:80 v/v) with 12 mM ammonium formate	2.0	N.I.	-	-	-	k1=4.65 k2=5.13	1.10	1.68	[61]
LC-UV (280nm)	(R)-α-Burke 2	N.I.	Ethanol:acetonitrile (20:80 v/v) with 12 mM ammonium formate	2.0	N.I.	-	-	-	k1=7.01 k2=8.06	1.15	0.82	[61]
LC-UV	EnantioPac (100 x 4.0 mm)	N.I.	0.32 M ethylene glycol + 0.1M NaCl in 0.02 M phosphate buffer, pH 7.0	-	N.I.	-	-	-	k1=27	1.69	-	[62]
LC-UV	EnantioPac (100 x 4.0 mm)	N.I.	1.29 M ethylene glycol + 0.1M NaCl in0.02 M phosphate buffer, pH 7.0	-	N.I.	-	-	-	k1=12	1.64	-	[62]
LC-UV	EnantioPac (100 x 4.0 mm)	N.I.	0.25 M 1,2-butanediol + 0.1M NaCl in0.02 M phosphate buffer, pH 7.0	-	N.I.	-	-	-	k1=8.4	1.27	-	[62]
LC-UV	EnantioPac (100 x 4.0 mm)	N.I.	0.44 M ethanol + 0.1M NaCl in0.02 M phosphate buffer, pH 7.0	-	N.I.	-	-	-	k1=15	1.45	-	[62]
LC-UV	EnantioPac (100 x 4.0 mm)	N.I.	1.74 M ethanol + 0.1M NaCl in0.02 M phosphate buffer, pH 7.0	-	N.I.	-	-	-	k1=5.2	1.12	-	[62]

LC-UV: HPLC with ultraviolet detection; VWD: variable wavelength detection; LC-FL: HPLC with fluorescence detection; LC-PDA: HPLC with PDA detection; API: active pharmaceutical ingredient; N.I.: not informed.

Table 3. Enantiomeric separation of labetalol described in literature using HPLC-CSP.

Analytical system	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp. (°C)	Time of analysis (min)	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Ref.
LC-UV	EnantioPac (100 x 4.0 mm)	N.I.	0.32 M ethylene glycol + 0.1M NaCl in0.02 M phosphate buffer, pH 7.0	-	N.I.	-	-	-	k1=25	1.37	-	62
LC-UV	EnantioPac (100 x 4.0 mm)	N.I.	1.29 M ethylene glycol + 0.1M NaCl in0.02 M phosphate buffer, pH 7.0	-	N.I.	-	-	-	k1=14	1.30	-	62
LC-UV	EnantioPac (100 x 4.0 mm)	N.I.	0.25 M 1,2-butanediol + 0.1M NaCl in0.02 M phosphate buffer, pH 7.0	-	N.I.	-	-	-	k1=9.6	1.17	-	62
LC-UV	EnantioPac (100 x 4.0 mm)	N.I.	0.44 M ethanol + 0.1M NaCl in0.02 M phosphate buffer, pH 7.0	-	N.I.	-	-	-	k1=19	1.28	-	62
LC-UV	EnantioPac (100 x 4.0 mm)	N.I.	1.74 M ethanol + 0.1M NaCl in0.02 M phosphate buffer, pH 7.0	-	N.I.	-	-	-	N.S.	N.S.	-	62

LC-UV: HPLC with ultraviolet detection; VWD: variable wavelength detection; LC-FL: HPLC with fluorescence detection; LC-PDA: HPLC with PDA detection; API: active pharmaceutical ingredient; N.I.: not informed.

Table 4. Enantiomeric separation of nebivolol described in literature using HPLC-CSP.

Analytical system	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp. (°C)	Time of analysis (min)	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Ref.
LC-UV (225nm)	3-AmyCoat(amylose-tris-3,5-dimethylphenyl carbamate) (250 x 4.6 mm)	API	n-Heptane:ethanol:diethylamine (85:15:0.1, v/v/v)	3.0	27	12 min	-	-	k1=7.85 k2=10.90	1.39	1.83	63
LC-UV (220nm)	Cellulose Tris (3,5-di-chlorolphenylcarba-mate)	API	Isopropanol	0.50	23	-	-	-	k1=0.46 k2=0.79	1.72	-	64
LC-UV (220nm)	Chiralpak OZ-H	API	CO2:Methanol:Isopropyle amine:trifluroacetic acid, (75:25:0.1:0.1, v/v/v/v)	4.0	N.I.	-	-	-		1.5	-	65
LC-UV (220nm) and optical rota-tion detector	Chiralpak IA (250 x 4.6 mm)	API	n-Hexane:ethanolamine (100:0.1, v/v)	0.2	Room tempera-ture	55	34.7	45.7	-	2.4	1.3	66
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	Methanol	0.5	23 ± 1	-	-	-	k1=1.06 k2=1.95	1.84	0.96	24
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	Methanol	1.0	23 ± 1	-	-	-	k1=1.88 k2=3.21	1.71	0.50	24
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	Methanol	1.5	23 ± 1	-	-	-	k1=2.04 k2=3.38	1.66	0.41	24
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	Ethanol	0.5	23 ± 1	-	33.39	43.88	k1=4.57 k2=6.32	1.38	2.63	24
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	Ethanol	1.0	23 ± 1	-	-	-	k1=4.33 k2=6.06	1.40	2.28	24
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	Ethanol	1.5	23 ± 1	-	-	-	k1=4.18 k2=5.77	1.38	1.40	24
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	1-Propanol	0.5	23 ± 1	-	19.29	25.25	k1=4.22 k2=5.84	1.38	1.71	24

LC-UV: HPLC with ultraviolet detection; LC-MS: HPLC with mass spectrometry; API: active pharmaceutical ingredient; N.I.: not informed.

Table 4. Enantiomeric separation of nebivolol described in literature using HPLC-CSP.

Analytical system	Column	Matrix	Mobile phase	Flow ($mL.min^{-1}$)	Temp. (°C)	Time of analysis (min)	1st peak (min)	2nd peak (min)	Capacity factor (k')	Separation factor (α)	Resolution (Rs)	Ref.
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	1-Propanol	1.0	23 ± 1	-	-	-	k1=2.10 k2=3.03	1.44	1.45	[24]
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	1-Propanol	1.5	23 ± 1	-	-	-	k1=1.09 k2=1.86	1.71	1.21	[24]
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	2-Propanol	0.5	23 ± 1	-	-	-	k1=1.53 k2=6.32	4.13	2.45	[24]
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	2-Propanol	1.0	23 ± 1	-	-	-	k1=1.36 k2=5.96	4.38	2.36	[24]
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	2-propanol	1.5	23 ± 1	-	4.88 13.72	-	k1=1.40 k2=5.85	4.18	2.53	[24]
LC-UV (220nm)	Chiralpak AD (250 x 4.6 x 10 µm)	N.I.	1-Butanol	0.1	23 ± 1	-	-	-	k1=3.16 k2=3.34	1.06	0.22	[24]
LC-UV (220nm)	Chiralpak AD-RH (150 x 4.6 mm)	N.I.	Ethanol	0.5	23 ± 1	-	22.59 30.38	-	k1=5.65 k2=7.94	1.41	1.73	[24]
LC-UV (220nm)	Chiralpak AD-RH (150 x 4.6 mm)	N.I.	Ethanol	1.0	23 ± 1	-	-	-	k1=3.00 k2=4.27	1.42	1.10	[24]
LC-UV (220nm)	Chiralpak AD-RH (150 x 4.6 mm)	N.I.	Ethanol	1.5	23 ± 1	-	-	-	k1=2.60 k2=3.75	1.44	1.15	[24]
LC-UV (220nm)	Chiralpak AD-RH (150 x 4.6 mm)	N.I.	1-Propanol	0.5	23 ± 1	-	-	-	k1=8.36 k2=11.5	1.38	1.76	[24]
LC-UV (220nm)	Chiralpak AD-RH (150 x 4.6 mm)	N.I.	1-Propanol	1.0	23 ± 1	-	-	-	k1=2.61 k2=3.83	1.47	1.20	[24]
LC-UV (220nm)	Chiralpak AD-RH (150 x 4.6 mm)	N.I.	1-Propanol	1.5	23 ± 1	-	13.17 17.45	-	k1=1.10 k2=1.75	1.59	1.10	[24]
LC-UV (225nm)	AmyCoat (150 x 4.6 x 3 µm)	API	n-Heptane:ethanol:diethylamine (85:15:0.1, v/v/v)	3.0	27±1	-	10.91	16.72	NI	1.53	2.0	[67]
LC-MS 406 > 151	Chirobiotic V (250 x 4.6 mm)	Plasma	Methanol:acetic acid:diethylamine (100:0.15:0.05, v/v/v)	1.0	24	17	14.3	15.4	k1=2.6 k2=2.85	**1.1**	-	[68]

LC-UV: HPLC with ultraviolet detection; LC-MS: HPLC with mass spectrometry; API: active pharmaceutical ingredient; N.I.: not informed.

4. Discussion and Conclusions

The high-performance liquid-chromatography with chiral stationary phase (HPLC-CSP) is a predominant technique in the separation, identification or quantification of enantiomers. The technique has been widely applied in the enantiomeric separation and optical purity determination of drugs in raw materials, pharmaceutical preparations and biological fluids, configurational instability studies of drugs (enantiomerization, racemization) and pharmacokinetics studies.

In recent years, numerous stereoselective materials have been studied and often made available in the marketed for the enantiomeric separation of basic, acidic and neutral properties. The most frequently used chiral selectors includes, polysaccharide derivatives, dinitrobenzoyl derivatives, proteins and glycoprotein derivatives, oligosaccharide (cyclodextrin) derivatives and macrocyclic antibiotics derivatives. These CSPs are able to separate enantiomers of large number of chemical and pharmaceutical compounds in normal phase, reverse phase and in polar organic phase. Most

suppliers, of chiral columns, provide customer services and provide help in the development and assembly of tailor-made CSPs, taking into account the specific needs of the customers. Most of these columns are available for analytical (i.d. 2.1-4.6 mm) and semi-preparative (i.d. 10-100 mm) enantiomeric separation applications. The CSPs are prepacked with distinct chiral selector on 3, 5, 10, 20 μm silica.

The enantiomeric separation of carvedilol was successfully achieved on cellulose based[35-37,39,41] and amylose based[29,31,38,42] CSPs, in normal phase mode. The cellulose tris (3, 5-dimethylphenylcarbamate) and cellulose tris (4-chloro-3-methylphenylcarbamate) chiral selector seem to have better enantioselectivity towards carvedilol, especially in polar organic mode[41]. The difference in enantioselectivity between above cited phases is due to structural difference of polysaccharides. In recent years, cellulose and amylose derivatives with chlorine at 3 and/or 4 position of phenylcarbamate has shown to possess wider and better enantiomeric discrimination potential[30].

Typically, mobile phase composed of pure isopropanol or acetonitrile, with acidic or basic modifiers, presented results that are more efficient in the enantiomeric separation of carvedilol. Interestingly, when ethanol and methanol were used as eluents on same CSPs, only partial separations were observed. That can be explained by the fact that, the acetonitrile and isopropanol have greater steric hindrance due to their chemical conformation and molecular weight as compared to lower alcohols (methanol and ethanol), consequently showing less competition with the analytes towards CSPs[69].

Trace levels of strong acid (acetic acid, trifluoroacetic acid, formic acid) or base (diethylamine, triethylamine) in the mobile phase contribute to better selectivity amongst carvedilol enantiomer peaks. According to literature, enantiomeric separation of acidic and basic drugs on polysaccharide based CSPs can be improved by addition of diethylamine and trifluoroacetic acid, respectively. In some cases, simultaneous use of a strong acid and base, at trace level, in the mobile phase can significantly improve selectivity as well as capacity factor of adjacent peaks[41,70]. It is evident that the normal phase mode presented better selectivity and efficiency in enantiomeric separations of carvedilol on cellulose and amylose based CSPs as compared to reverse phase mode[28,33,36,41].

As mentioned earlier, labetalol has two chiral centers and four enantiomers. It is evident that the cellulose and amylose based CSPs shows poor enantioselectivity towards four isomers of labetalol. A partial enantiomeric separation was obtained on amylose tris (3, 5-dimethylphenylcarbamate) CSP, in normal phase, using a mobile phase composed of heptane:ethanol, modified with diethylamine or trimethylamine[36,51,56]. Similar results were obtained in polar organic mode, on cellulose tris (3, 5-dimethylphenylcarbamate) and amylose tris (3, 5-dimethylphenylcarbamate)[31].

According to the literature, the chiral recognition mechanism of polysaccharides based columns is yet to be fully elucidated. It is believed that the chiral discrimination is possible due to the formation of inclusion complexes and polar interactions with CSPs carbamates group[71]. The carbamate group (CSP) interacts with the solute through hydrogen bonding with -NH and C=O and dipole-dipole interaction with the C=O. The number of hydroxyl groups, in drug structure, appears to be important for chiral recognition[72,73].

Baseline separation ($\alpha \geq 1.1$) of all four enantiomers of labetalol were obtained on a β-cyclodextrin based CSP, in normal as well as in polar organic phase[45,46,49,52,53]. Interestingly, the β-cyclodextrin based CSP presented partial enantioselectivity towards other third-generation β-blockers. That can be explained by the fact that, the formation of inclusion complexes depends on the shape, size and geometry of the solute and the diameter of the insertion cavity of β-cyclodextrin. Thus, four enantiomers of labetalol were well separated while carvedilol[41], and nebivolol[52] were partially separated on β-cyclodextrin CSP.

A limited number of protein based CSPs have been studied for enantiomeric separation of the selected third-generation β-blockers. The CSPs derived from bovine serum albumin (BSA) and human α_1-acid glycoprotein (AGP) are commercially available. These CSPs are especially useful in enantioselective pharmacokinetic studies.

Originally, the first generation human α_1-acid glycoprotein (AGP) based CSP was developed by Hermansson and co-workers[74] and CSP were denominated EnantioPac®. All separations obtained on EnantioPac® showed poor selectivity towards labetalol enantiomers[53,54,62]. In recent years, a new generation of α_1-acid glycoprotein based CSP (CHIRAL AGP®) were introduced and presented exceptionally high enantioselectivity towards labetalol enantiomers. All four enantiomers can be separated to baseline, on new CHIRAL AGP®, with excellent selectivity between all peaks ($\alpha \geq 1.1$)[47,50].

Macrocyclic antibiotics represent a relatively new class of chiral selectors applied to enantiomeric separation of drugs with HPLC-CSPs. Since its introduction in 1994[75,76], the macrocyclic antibiotic based CSP present high degree of selectivity for large number of compounds and do not loose enantiomeric separation efficiency. These CSPs have various stereogenic center and functional groups, thus allowing multiple interactions with chiral molecules. The mechanism of separation is based on hydrophobic and dipole-dipole interactions, hydrogen bonding as well as participation of steric repulsion forces[75,76].

The macrocyclic antibiotic based CSP, especially those derived from teicoplanin and vancomycin, are highly efficient in the enantiomeric separation of labetalol[48,59]. All four enantiomers were baseline separated in polar organic mode with trace levels of acetic acid and triethylamine in the mobile phase.

All ten stereoisomers of nebivolol were successfully separated on an amylose tris (3, 5-dimethylphenylcarbamate) based CSP in polar organic mode[24]. The mobile phase was composed of ethanol at a flow-rate of 0.5 ml/min[35].

In conclusion, the commercially available polysaccharide and macrocyclic antibiotic based CSPs has the broadest enantioselectivity for single (bucindolol, carvedilol) and multiple (labetalol, nebivolol) chiral center third-generation β-blockers. There is no universal CSP available on the market for enantiomeric separation of all third-generation β-blockers.

List of commercial chiral stationary phases applied to enantiomeric separation of third generation β-blockers through high performance liquid chromatography

Phenomenex 3022 = Chirex® (S)-ICA and (R)-NEA, LC Column 250 x 4.6 mm
Chiral phase = (S)-indoline-2-carboxylic acid and (R)-1-(α-naphthyl)ethylamine
Amphoteric glycopeptide teicoplanin (Chirobiotic T)
Astec® CYCLOBOND I 2000 is β-cyclodextrin
Human a-acid glycoprotein (AGP) = EnantioPac AGP = CHIRAL AGP = Bovine serum albumin (BSA) EnantioPac BSA = RESOLVOSIL-BSA
Labmade – heptakis (6-deoxy-6-azidophenylcarbamoylated)- cyclodextrin (heptakis-N3-Ph-CD)
Cellulose tris (3, 5-dimethylphenylcarbamate), [CHIRALCEL OD, Sepapak 1, Lux Cellulose I]
Cellulose tris (3-chloro-4-methylphenylcarbamate), [CHIRALCEL OZ, Sepapak II, Lux Cellulose II]
Cellulose tris (4-methylbenzoate), [CHIRALCEL OJ, Sepapak III, Lux Cellulose III]
Cellulose tris (4-chloro-3-methylphenylcarbamate), [CHIRALCEL OX, Sepapak IV, Lux Cellulose IV]
Amylose tris (3, 5-dimethylphenylcarbamate), [Chiralpak AD-H, Lux Amylose-1]
Amylose tris (5-chloro-2-methylphenylcarbamate) [CHIRALPAK AY, Lux Amylose-2]

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