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#### **Review Article**

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# Chirality of $\beta_2$ -agonists. An overview of pharmacological activity, stereoselective analysis, and synthesis

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**Abstract:**  $\beta_2$ -Agonists ( $\beta_2$ -adrenergic agonists, bronchodilatants, and sympathomimetic drugs) are a group of drugs that are mainly used in asthma and obstructive pulmonary diseases. In practice, the substances used to contain one or more stereogenic centers in their structure and their enantiomers exhibit different pharmacological properties. In terms of bronchodilatory activity, (R)-enantiomers showed higher activity. The investigation of stereoselectivity in action and disposition of chiral drugs together with the preparation of pure enantiomer drugs calls for efficient stereoselective analytical methods. The overview focuses on the stereoselectivity in pharmacodynamics and pharmacokinetics of β<sub>2</sub>-agonists and summarizes the stereoselective analytical methods for the enantioseparation of racemic beta-agonists (HPLC, LC-MS, GC, TLC, CE). Some methods of the stereoselective synthesis for  $\beta_2$ -agonists preparation are also presented.

**Keywords:** 2-agonists, stereoisomers, pharmacological activity, stereoselective analysis, stereoselective synthesis

#### 1 Introduction

 $\beta_2$ -Agonists (sympathomimetic drugs, adrenergic drugs, and adrenomimetic drugs) produce effects similar to the stimulation of sympathetic nerves. They induce stimulation of the sympathetic nervous system either by direct

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Čižmáriková Ružena, Horáková Renáta: Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University in Bratislava, Odbojárov 10, 832 32 Bratislava, Slovakia action on adrenergic receptors or by indirect action, causing flushing of endogenous catecholamines, with subsequent limitation of their backflushing. Natural transmitters are noradrenaline and, to a lesser extent, adrenaline. Isoprenaline (isoproterenol) was prepared as one of the first synthetic sympathomimetic amines, which is structurally related to adrenaline and acts almost exclusively on  $\beta\text{-adrenergic}$  receptors.

A key role in inducing  $\beta$ -responses is considered the activation of adenylyl cyclase with subsequent increased production of cyclic adenosine monophosphate (AMP). The mechanism of cAMP's action consists of activating protein kinases. Activated protein kinases transfer gamma-phosphate from AMP to various proteins, phosphorylating them by binding them to serine or threonine, while changing their activity. Binding to the  $\beta_1$ -receptors of these substances is manifested by narrowing the peripheral vessels in the skin and mucous membranes, acceleration of heart activity, and increase in blood pressure. Binding to the  $\beta_2$ -receptors is associated with bronchodilation, uterine relaxation, and skeletal muscle glycogenolysis [1–3].

The presented overview focuses on stereochemical aspects of  $\beta_2$ -agonists which are nowadays, along with corticoids, one of the most effective drugs in asthma therapy. In the case of  $\beta_2$ -agonists, bronchospasm is being released in contrast to corticosteroids, in the case of which the inflammation process is suppressed in the asthma therapy [4,5].

According to the action of the bronchodilatory effect, they are divided into  $\beta_2$ -agonists that are as follows:

short-acting – salbutamol, levosalbutamol, terbutaline, pirbuterol, procaterol, fenoterol

long-acting – salmeterol, formoterol, bambuterol, mabuterol, clenbuterol;

ultra-long-acting – indacaterol, olodaterol, vilanterol, carmoterol, abediterol [6,7].

In addition to these  $\beta_2$ -agonists, naturally occurring  $\beta$ -agonists such as adrenaline (epinephrine), the suprarenal gland hormone, and ephedrine, an alkaloid, are

Table 1: Chemical IUPAC names of selected  $\beta_2$ -agonists

Albuterol	4-[2-( <i>tert</i> -Butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol
Abediterol	5-{(1R)-2-[6-(2,2-Difluoro-2-phenylethoxy)hexylamino]-1-hydroxy-ethyl}-8-hydroxy-1H-quinolin-2-one
Bambuterol	5-[2-(tert-Butylamino)-1-hydroxyethyl]benzene-1,3-diyl-bis(dimethylcarbamate)
Carmoterol	8-Hydroxy-5-{(1R)-1-hydroxy-2-[[(2R)-1-(4-methoxyphenyl)propan-2-yl]amino]ethyl}-1H-quinolin-2-one
Clenbuterol	1-(4-Amino-3,5-dichlorophenyl)-2-( <i>tert</i> -butylamino)ethanol
Fenoterol	5-{1-Hydroxy-2-[1-(4-hydroxyphenyl)propan-2-ylamino]ethyl}benzene-1,3-diol
Formoterol	N-{2-Hydroxy-5-[1-hydroxy-2-[1-(4-methoxyphenyl)propan-2-ylamino]-ethyl]phenyl}formamide
Indacaterol	5-[(1R)-2-[(5,6-Dietyl-2,3-dihydro-1H-inden-2-yl)amino]-1-hydroxyethyl]-8-hydroxy-1H-quinolin-2-one
Isoxsuprine	4-[1-Hydroxy-2-(1-phenoxypropan-2-ylamino)propyl]phenol
Mabuterol	1-[4-Amino-3-chloro-5-(trifluoromethyl)phenyl]-2-(tert-butylamino)-ethanol
Olodaterol	6-Hydroxy-8-{(1R)-1-hydroxy-2-[1-(4-methoxyphenyl)-2-methylpropan-2-ylamino]ethyl}-3,4-dihydro-2H-1,4-
	benzoxazin-3-one
Orciprenaline	5-[1-Hydroxy-2-(propan-2-ylamino)ethyl]benzene-1,3-diol
Ritodrine	4-{2-[1-Hydroxy-1-(4-hydroxyphenyl)propan-2-ylamino]ethyl}phenol
Salmeterol	2-(Hydroxymethyl)-4-{1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino]-ethyl}phenol
Vilanterol	4-{(1R)-2-[6-[2-[(2,6-dichlorophenyl)methoxy]ethoxy]hexylamino]-1-hydroxyethyl}-2-(hydroxymethyl)phenol
Trantinterol	2-[4-Amino-3-chloro-5-(trifluoromethyl)phenyl]-2-(tert-butylamino)-ethanol
Terbutaline	5-[2-(tert-Butylamino)-1-hydroxyethyl]benzene-1,3-diol

also used in the treatment of asthma [8]. The overview focuses on  $\beta_2$ -agonists from the aspect of their stereogenic configuration, especially with regard to their optically active stereoisomers. Chemical IUPAC names of presented  $\beta_2$ -agonists are listed in Table 1.

Chemically,  $\beta_2$ -agonists represent a single group with a side aminoethanol chain. The aromatic nucleus is substituted with hydroxyl groups that are either in the 3,4-positions (isoprenaline and hexoprenaline) or in the 3,5-positions (fenoterol, reproterol, orciprenaline, and terbutaline). For some  $\beta_2$ -agonists, the phenolic OH group is substituted with hydroxymethyl (salbutamol and salmeterol).

There is one stereocenter in the structure of these drugs, with two optically active forms of (R)-(-) and (S)-(+) in individual drugs that differ in pharmacological and pharmacokinetic properties.

The differences in the structure of the two enantiomers can be seen in the orciprenaline structure (Figure 1).

In the case of multiple stereocenters, several stereoisomers can be distinguished, *e.g.*, ritodrine or fenoterol

Figure 1: Two stereoisomers of orciprenaline.

with two stereocenters form four stereoisomers, and they cannot all be nonsuperimposable mirror images of each other (Figure 2). These stereoisomers (diastereoisomers) differ in their physicochemical properties unlike enantiomers.

The mechanism of action of the individual enantiomers is explained through a stronger binding to the  $\beta_2$ -receptor upon the interaction of the more efficient isomer at the receptor sites under precisely defined stereochemical conditions. By using the X-ray structural analysis, the interpretation of the Easson–Stedman hypothesis [9] was confirmed in many  $\beta_2$ -agonists. The crucial binding sites are mainly linked to three functional groups, such as the amino group, the hydroxyl group of the side chain, and the substituted aromatic nucleus.

Mesecar and Koshland in 2000 proposed a fourpoint model that is suitable for the receptor binding site [10,11].

The receptor to which  $\beta_2$ -agonists bind is a protein macromolecule composed of seven transmembrane helixes with three intracellular and three extracellular loops. A schematic structural model of the  $\beta$ -adrenoreceptor, highlighting the agonist and antagonist binding regions, is shown in Figure 3. The  $\beta$ -adrenoreceptor agonists bind to a hydrophobic pocket or active site located approximately 30–40% into the depth of the receptor. This location corresponds to the predicted location of the several amino acid residues that are critical in the binding of adrenaline [12,13]. Aspartate 306 in helix 3 forms a salt bridge with the amine of adrenaline. Serine 204 and 205 in helix 5 interact with the two hydroxyl groups of an aromatic ring.

Figure 2: Chemical structures of ritodrine stereoisomers.

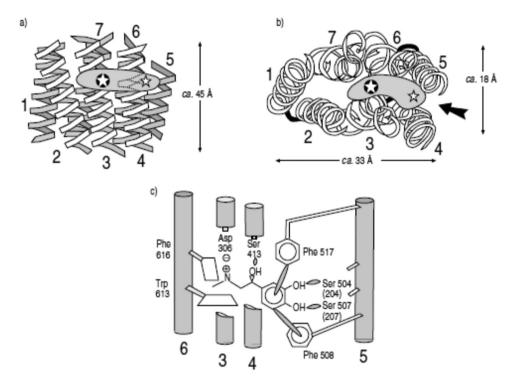


Figure 3: Schematic diagram of the β-adrenoreceptor and its binding domain for agonist ( $\alpha$ ) and antagonist ( $\alpha$ ). Helices are individually numbered, and the approximate dimension of the receptor is marked in Angstroms ( $\alpha$ ). (a) (Side-view) It shows a cut-away view of the seven-transmembrane  $\alpha$ -helices of the β-adrenoreceptor seen from the plane of the membrane lipid bilayer. (b) (Top view) It shows the same beta-adrenoreceptor viewed from the extracellular space. (c) It shows an expanded detail of the ligand-binding site for agonists formed by helices 3, 4, 5, and 6, during binding of adrenaline. Reproduced with the permission of the © ERS 2020: European Respiratory Journal 7(3):569–78; Published 1 March 1994 [12].

Phenylalanine 517 and 508 interact with the aromatic nucleus of adrenaline through van der Waals forces. In terms of efficacy, the hydroxyl group on the stereogenic carbon in the side chain is important. It forms a hydrogen bond with serine 413 on helix 4.

Binding interactions to individual amino acids in the  $\beta_2$ -receptor were also described in the case of noradrenaline and other  $\beta_2$ -agonists [14,15].

A deeper study of bindings on the  $\beta$ -receptor enabled the resolution of the  $\beta_2$ -receptor's by X-ray structural analysis [16,17]. Molecular interactions between fenoterol stereoisomers and derivatives and the  $\beta_2$ -adrenergic receptor binding site were studied by

docking and molecular dynamics simulations [18–20]. Docking studies indicate that the hydroxyl group at the first chiral center of the ligand creates hydrogen bonds with Asp113 or/and Asn312 in the case of (R, \*') stereoisomers mainly. Molecular dynamics simulations confirm the existence of the stereoselective effects accompanying the ligand–receptor interactions, namely, different stereoisomers exhibit diverse conformational behaviors and distances between characteristic ligand–receptor atom–atom pairs. Stereochemistry of fenoprofen molecule also affects the coupling of the receptor to different G proteins. In a rat cardiomyocyte contractility model, (R,R')-fenoprofen was shown to selectively

Figure 4: Chemical structures of more active isomers of fenoterol.

activate Gs protein signaling while the (S,R')-isomer activated both Gi and Gs protein. The overall data demonstrate that the chirality at the two chiral centers of the fenoprofen molecule influences the magnitude of binding affinity, thermodynamics of local interactions within the binding site, and the global mechanism of  $\beta_2$ adrenergic receptor activation. The marketed product is the racemic mixture of the (R,R';S,S')-fenoterol (Figure 4) which was selected after initial development studies, demonstrating that this racemic mixture was 9-20-fold more active than the (R,S';S,R')-fenoterol racemate [20].

#### 2 Stereoselective pharmacological effects of β<sub>2</sub>-agonists

β<sub>2</sub>-Agonists have been used in treating asthma and obstructive pulmonary disease (COPD) for a long time. Long-acting  $\beta_2$ -agonists are effective in the prevention of COPD and bronchial asthma exacerbation induced by a viral infection, including infection with coronaviruses (HCoV-229E). Recently, it has been found that formoterol inhibited HCoV-229E replication partly by inhibiting receptor expression and/or endosomal function, and it could modulate infection-induced inflammation in the airway [21].

Based on more recent indications,  $\beta_2$ -agonists are also used for alleviating symptoms in amyotrophic lateral sclerosis [22].

They were mostly used in the form of racemates with a 50:50 ratio of (R)- and (S)-isomer. Their therapeutical effects were described in various overviews and publications [3–8,23–34]. Due to the chirality of  $\beta_2$ -agonists, the pharmacological properties of their single stereoisomers were also studied. Some of them are already in clinical practice as pure enantiomers (levalbuterol, arformoterol, indacaterol, olodaterol, and vilanterol). The bronchodilatory activity of most  $\beta_2$ -agonists is higher in the (R)-isomer. Studies made on different animal models confirmed significantly higher bronchodilatory and antidepressant activity of (R)-enantiomer of clenbuterol, albuterol, formoterol, and terbutaline [23]. In some cases in treating asthma and obstructive pulmonary disease, only this stereoisomer is used (levalbuterol – (R)-form of albuterol, arformoterol – (R,R)-form of formoterol) [23–25].

Albuterol also known as salbutamol is largely used as bronchodilators in the management of asthma, both in control of acute symptomatic attacks and chronic. long-term prevention and management. Levalbuterol (levosalbutamol) is the (*R*)-isomer (Figure 5) of albuterol. Its β-adrenoreceptive activities were about 80 times more efficient than activities of (S)-enantiomer [26]. Compared with the racemate, it has a much higher effect on respiratory diseases. Its preferential use has not even been altered by several preclinical and in vitro studies in adults and children with asthma, which confirmed that the (S)-enantiomer is not inert but has a pro-inflammatory effect [27]. The racemate of albuterol is still being widely used in comparison with levalbuterol to treat acute asthma exacerbations. Levalbuterol is recommended for severe asthma diseases, although many works indicate conflicting opinions concerning its use [28-30].

In formoterol that has two stereocenters, effects of the enantiomers of formoterol on inherent and induced tone in isolated human bronchi with that on guinea-pig trachea in vitro were compared. In both human bronchus and guineapig trachea, (S,S)-formoterol was more than 1,000 times less potent than (R,R)-formoterol. Thus, the relaxing effect of formoterol in human airways and guinea-pig trachea was shown to lie with the (R,R)-enantiomer [31].

The (R,R)-isomer of arformoterol (Figure 6), indicating a high tolerance, has recently been introduced into the clinical practice [32].

Salmeterol (Figure 7) belongs to the long-acting  $\beta_2$ -agonists used in clinical practice as a racemate [33]. Clinical studies evaluating the efficacy of this  $\beta_2$ -agonist in the maintenance treatment of asthma demonstrated that salmeterol was more effective than albuterol

Figure 5: Chemical structures of (a) (RS)-(±)-albuterol (salbutamol) and (b) (R)-(-)-albuterol (salbutamol).

Figure 6: Structure of arformoterol.

Figure 7: Structure of salmeterol.

Figure 8: Chemical structure of isoxsuprine.

(short-acting  $\beta_2$ -agonist) in improving pulmonary function and controlling asthma symptoms [34]. For salmeterol,  $\beta_2$ -adrenoceptor pharmacological activity (B2ADR) resides in (R)-salmeterol, while (S)-salmeterol is generally considered to be inert at this target. The difference in B2ADR activity between (R)- and (S)-salmeterol has been reported to be approximately 40-fold [35] less than for other  $\beta_2$ -agonists.

Isoxsuprine has three stereogenic centers (Figure 8), and theoretically, eight stereoisomers can be created. It stimulates  $\beta$ -receptors, and it is known as a vasodilator and a tocolytic with anti-inflammatory and hemorheological properties [36,37]. In the *in vivo* model of stroke, its neuroprotective effect has also been shown [38]. Studies of bindings confirmed that (–)-isoxsuprine has higher activity than (+)-isoxsuprine in the binding on

the  $\alpha$ -receptor, while the (+) isomer has an affinity similar to the  $\beta$ -blocker propranolol [39].

The long-acting bambuterol is used to treat asthma (Figure 9). Structurally, it is a biscarbamate which, as the form of a prodrug, can be hydrolyzed by butyrylcholinesterase and transitioned to an active form of terbutaline. The study found that (R)-bambuterol inhibited butyrylcholinesterase 5-fold faster than the (S)-isomer. The (R)-isomer is more active in response to histamine-induced asthma. Both isomers increase the heart rate [40-42].

Many of the  $\beta_2$ -agonists show anabolic activity, and their use is banned in certain types of sports [43]. This may concern clenbuterol (Figure 10), for which the anabolic activity of individual (R)- and (S)-stereoisomers was monitored. The activity was monitored by measuring the tissue mass and determining the protein content. The results showed that both stereoisomers had the same anabolic activity, but (S)-(+)-clenbuterol exhibited a significant increase in the mass of the heart muscle [44].

Trantinterol (also known as SPFF) and mabuterol (Figure 11) were prepared as potent long-acting bronch-odilators with relatively higher  $\beta_2$ -adrenoceptor selectivity. The affinity of (–)-enantiomer of trantinterol to  $\beta_2$ -adrenoceptor was 6- and 164-fold greater than that of (±)- and (+)-trantinterol, respectively. In addition, the isomeric difference of overall selectivity between (–)-enantiomer and (+)-enantiomer was 10.7-fold for lung versus atria [45].

Many of the  $\beta_2$ -agonists have found use in tocolytic therapy, particularly in the risk of premature birth or in miscarriage, due to the higher selectivity for inhibition of uterus contractions [46]. Ritodrine is a short-acting  $\beta_2$ -agonist with a hydroxyphenol group on a basic nitrogen forming a hydrogen bond. Since ritodrine has a bulky N-substituent, it has high  $\beta_2$  selectivity. It is used as a tocolytic drug [47,48]. In animal experiments, the (–)-ritodrine showed 40 times higher contractions of the uterus than the (+)-enantiomer [49]. In terms of stereochemistry, diastereoisomers of ritodrine in pregnant women were studied, and the duration of

Figure 9: Structures of (a) bambuterol and its active form (b) terbutaline.

Figure 10: Chemical structure of clenbuterol.

Figure 11: Chemical structures of (a) trantinterol and (b) mabuterol.

pregnancy was compared in women expecting one child with women expecting twins. The differences were observed in the serum concentration of (–) and (+)-ritodrine [50].

Indacaterol [51–53] and carmoterol, containing in lipophilic part a carbostyril skeleton, are ultra-long-acting  $\beta_2$ -agonists (Figure 12) that are well tolerated. They have a rapid onset of action, and their long duration of action allow them to be administered once a day. Indacaterol is used in therapeutic practice as pure (R)-enantiomer and carmoterol as the (R,R)-form [54,55].

Abediterol (Figure 13) is a novel long-acting  $\beta_2$ -adrenoceptor agonist currently in development for oncedaily combination maintenance therapy of asthma and chronic obstructive pulmonary disease (COPD). In the preclinical stage, abediterol exhibited (5–20)-fold higher activity when compared with other bronchodilators [56].

In the case of vilanterol, the basic nitrogen contains a bulky chain with substituted phenyl and two ether oxygens, something that differentiates it from salmeterol [57]. This group also includes olodaterol with the dihydrobenzoxazine skeleton in the lipophilic and hydrophilic parts of the molecule with substituted

Figure 13: Chemical structure of abediterol.

hydrogen in 2-methylpropan-2-yl methoxyphenyl [56]. Vilanterol and olodaterol have 1 stereocenter with the (R)-configuration in their structures (Figure 14).

#### 3 Stereoselective pharmacokinetics of β<sub>2</sub>-agonists

Although  $\beta_2$ -agonists have been used in clinical practice as racemic mixtures for a long period, the data on stereoselective pharmacokinetics are incomplete for the  $\beta_2$ -agonists.

 $\beta$ -Agonists are absorbed from the gastrointestinal tract by diffusion in which individual enantiomers behave differently. The plasma concentrations of the enantiomers of anti-asthma drugs may differ as a reflection of stereoselectivity in clearance, the volume of distribution, and the route of administration [58].

Enantioselectivity of salbutamol in plasma was also evident in experiments on horse models, where the values for the (S):(R) ratio were 1.25 and 1.14. Enantioselectivity was not observed in pulmonary lining fluid and the central lungs [59].

In the case of terbutaline, different bioavailability was detected [60]. In human studies, the bioavailability calculated from the plasma values was 7.5% for the (R)-(+)-terbutaline and 14.8% for the (S)-(-)-terbutaline. These differences indicate differences in absorption for both enantiomers of terbutaline and their first-pass metabolism. Bioavailability of the racemate was similar to the (S)-(-)-enantiomer [61–63].

Figure 12: Chemical structure of (a) indacaterol and (b) carmoterol.

Figure 14: Chemical structure of (a) vilanterol and (b) olodaterol.

The distribution of  $\beta_2$ -agonists is closely related to their physical and chemical properties. For hydrophilic compounds, their binding to plasma proteins is negligible. For lipophilic  $\beta_2$ -agonists, such as clenbuterol, the binding on plasma proteins is 97% [64].

Indacaterol is relatively highly bound to plasma proteins regardless of concentration. No stereoselective binding on plasma proteins has been reported with albuterol [65].

Interesting results were found by Boulton [66] in the study of transplacental albuterol distribution after administration to women before the caesarean section. The (R):(S) albuterol ratio in cord plasma was significantly lower than in maternal plasma. The distribution of (S)/(R)-enantiomers of albuterol in plasma and skeletal and cardiac muscle was compared in animal models using more recent analytical methods (LC-MS/MS) [67]. The results confirmed higher distribution of the (R)-enantiomer in individual muscles when compared with the plasma, which is of importance for anti-doping assessments. Jacobson et al. [68] observed an enantioselective distribution of salmeterol in the central part of the lungs. (R)-enantiomer exhibited about 30% higher distribution than (S)-enantiomer.

Metabolism of  $\beta_2$ -agonists induces sulfation and glucuronidation, which differs from the  $\beta_2$ -agonist type, e.g., albuterol and salbutamol are biotransformed by sulfation into an ineffective metabolite (Figure 15), whereas fenoterol induces glucuronidation in addition to sulfation, and the case of formoterol, it is only glucuronidation. Glucuronidation of formoterol occurs at the phenolic position as well as the formation of a benzyl glucuronide (Figure 16) [69–71].

Figure 15: Structure of salbutamol sulfate.

Figure 16: Structure of formoterol glucuronides.

The racemate of salbutamol undergoes stereoselective sulfation by sulfotransferases mainly in the intestine and liver, and it has been found that (*S*)-salbutamol is retained in the body longer and reaches higher plasma levels than (*R*)-salbutamol [71,72]. (*R*)-Salbutamol is metabolized up to 12 times faster than (*S*)-salbutamol. Following oral or inhaled administration of enantiomerically pure salbutamol, a small quantity (6%) is transferred to the second enantiomer, probably through acid-catalyzed racemization in the stomach [73]. Indacaterol also undergoes glucuronidation, where the most common metabolites are 8-*O-a-N*-glucuronides [74].

Some  $\beta_2$ -agonists reported enantioselectivity in renal clearance. Following intravenous administration of albuterol in the form of the racemate and individual enantiomers, clearance exceeded creatine clearance. Renal clearance of (R)-albuterol is 2- to 3-fold higher than (S)-albuterol [75]. Differences in renal clearance were studied also in the case of terbutaline. The statistically significant value for intravenous and oral administration of (+)-terbutaline was slightly higher than for (–)-terbutaline [63].

# 4 Stereoselective analysis of β<sub>2</sub>-agonists

The investigation of stereoselectivity in action and disposition of chiral drugs together with the preparation of pure enantiomer calls for efficient stereoselective analytical methods.

Methods for separation and quantification of the isomers in biological samples are also desirable for

Table 2: Direct chromatographic enantioseparation of  $\beta\text{-agonist}$ 

Methods	CSP	Analyte	Mobile phase	Matrix	Reference
Macrocyclic antibiotic-based CSP	based CSP				
LC-MS/MS (ESI)	Chirobiotic T	Fenoterol	Methanol with 0.2% acetic acid and 0.01% triethylamine	Human plasma	80
LC-MS/MS (ESI)	Chirobiotic T	Bambuterol	Methanol-water (20:80, v/v) with 20 mM ammonium acetate and 0.005% formic acid	Human plasma	92
(1)(1)(1)(1)(1)(1)(1)(1)(1)(1)(1)(1)(1)(	T - (1 - (1 - (1)		(-) (-) (-) (-) (-) (-) (-) (-) (-) (-)	1	6
LC-MS/MS (APCI)	Chirobiotic I	Bambuterol, terbutaline	ZU MIM AMMONIUM ACETATE—METNANOT (10:90, V/V)	каг ріаѕта	33
LC-MS/MS (APCI)	Chirobiotic T	Terbutaline	0.05% ammonium trifluoroacetate in methanol	Human plasma	94
LC-MS/MS (ESI)	Chirobiotic T	Clenbuterol	Methanol-water (95:5, v/v) with 2.5 mM ammonium formate	Rat plasma, urine and bile	95
LC-MS/MS (ESI)	Chirobiotic T	Salbutamol	5 mM ammonium formate in methanol	Porcine urine	96
LC-MS/MS (ESI)	Chirobiotic T	Salbutamol, sulfate metabolite	Methanol–acetic acid–ammonia (994:5:1,v/v/v)	Human plasma and	26
				urine	
LC-MS/MS (ESI)	Chirobiotic T	Salbutamol	Methanol-0.02% formic acid (60:40, v/v) with 0.1%	Dog plasma	86
- C	F 10 1 1 1 1 1 1 0		Mathematical and the final and a second and		Ç.
LC-FLD (Aex = 250, Aem = 310)	Cilliobiotic	Salbutamot	methanor-acetonithe (40:00, v/v) with 0.5% gladat acetic and 0.2% diethylamine	numan ptasma	66
LC-UV ( $\lambda = 227 \text{ nm}$ )	Chirobiotic T, chirobiotic V	Salbutamol	Methanol-20 mM ammonium acetate buffer (pH 4) (98:2, v/v)	Waste water	100
LC-MS/MS (ESI)	Chirobiotic V	Salbutamol	Methanol-water with 20 mM ammonium acetate buffer (pH 4)	Waste water	101
			(90:10, v/v)		
LC-UV ( $\lambda = 254 \text{ nm}$ )	Chirobiotic V	Mabuterol	Methanol with 0.01% acetic acid and 0.01 % triethylamine	Rat plasma	102
LC-UV ( $\lambda = 280 \text{ nm}$ )	Chirobiotic V	Salbutamol, terbutaline	10 mM ammonium nitrate in ethanol, (pH 5.1)	Water	103
LC-MS/MS (ESI)	Chirobiotic V	Trantinterol	Acetonitrile-methanol (60:40. v/v) with 0.05% ammonia and	Rat plasma	104
			0.1% acetic acid		
LC-MS/MS (ESI)	Chirobiotic V	Trantinterol	Acetonitrile-methanol (60:40, v/v) with 0.01% ammonia and	Human plasma	105
Polysaccharide-based CSP	SSP		0.02% acetic acid		
$IC_{-IIV}(\lambda = 254 \text{ nm})$	Chiralnak AS-H	Trantinterol	Hexane-ethanol (98:2 v/v) with 0 1% triethylamine	Water	106
LC-UV ( $\lambda = 254 \text{ nm}$ )	Chiralpak AD, Chiralcel OD	Isoxsuprine	Hexane—propan-2-ol (80:20, v/v) with 0.1% triethylamine	Water	107
	Chiralcel OJ	-			
LC-UV ( $\lambda = 220 \text{ nm}$ ,	Chiralpak AD Chiralcel	Bambuterol salbutamol	Hexane-propan-2-ol (70:30, v/v) with 0.1% diethylamine	Water	108
$\lambda = 225 \text{nm}$	OD-RH				
LC-UV ( $\lambda = 230 \text{ nm}$ )	Chiralpak AD	Fenoterol	Hexane-propan-2-ol (88:12, v/v) with 0.1% triethylamine,	Water	109
LC-UV ( $\lambda = 225 \text{ nm}$ )	Chiralpak AD Chiralpak IA	Bambuterol	Hexane-propan-2-ol (100:30, v/v) with 0.1% diethylamine	Water	110
Protein-based CSP					
LC-UV ( $\lambda = 276 \text{ nm}$ )	Chiral-AGP	Salbutamol	0.01 M phosphate buffer, 0.13%, v/v, propan-2-ol, (pH 7.5)	Spiked plasma	84
LC- ECD (300 and	Chiral-AGP	Formoterol, glucuronide	1.5% propan-2-ol, 50 µM EDTA-Na, (pH 6.8)	Urine	112
630 mV)		conjugate			
LC-ECD (700 mV)	Chiral-CBH	Salmeterol, α-	5% propan-2-ol in 25 mM sodium phosphate buffer (pH 6.0),	Human liver	113
		hydroxysalmeterol	50 mM EDTA-Na	microsomes	
Pirkle CSP					

Hexane-1,2-dichloroethane-ethanol-trifluoroacetic acid (80:10:10 v/v/v) **Mobile phase** Clenbuterol Analyte Chirex 3022 CSP  $-C-UV (\lambda = 254 \text{ nm})$ Fable 2: continued Methods

LC-UV (A = 276 nm)	Chirex 3022	Salbutamol	Hexane-1,2-dichloroethane—methanol with 0.67% trifluoroacetic acid (160:93:17 v/v/v);	Spiked plasma	84
APCI, atmospheric pre	oCI, atmospheric pressure chemical ionization; CSP, ch		iral stationary phase; ESI, electrospray ionization; ECD, electrochemical detection; FLD, fluorescence detection; UV, ultraviole	.D, fluorescence detection;	UV, ultraviole

olet

Reference

Matrix

Spiked plasma, urine

pharmacological studies, pharmacokinetic studies, and therapeutic drug monitoring [76–78]. Recently, the enantioseparation of chiral pharmaceuticals in the environmental matrices becomes more important. Concerning biodegradation and environmental fate, the recognition of enantioselectivity is essential to provide a more realistic risk assessment of chiral compounds [79].

Several different technologies have been reported for the enantioseparation of  $\beta_2$ -agonists enantiomers, including predominantly liquid chromatography (LC, HPLC) using chiral stationary phase (CSP) [80] or derivatizing reagents [81] as well as GC, TLC, and capillary electrophoresis (CE) using cyclodextrins or their derivatives as chiral selectors [63,82].

# 4.1 Stereoselective liquid chromatographic analysis of $\beta_2$ -agonists

#### 4.1.1 Direct chiral chromatographic separation

Generally, chiral separation can be carried out by (a) direct separation of the enantiomers using CSPs or chiral mobile additives or (b) indirect separation by conversion of the enantiomers to diastereoisomers using a chiral reagent followed by chromatography on a chiral column [82,83].

Direct chromatographic enantioseparation on CSP has appeared as the most effective and convenient way of determining the enantiomeric composition of chiral drugs. Different structural types of CSPs including the  $\pi$ -donor/acceptor phases (Pirkle phase) [84], derivatized polysaccharides [85], proteins [86],  $\beta$ -cyclodextrin [87], and macrocyclic antibiotics [88] were utilized for the enantiomeric separation of  $\beta_2$ -agonists (Table 2).

The following molecular interactions with CSP are supposed to play a role in chiral discrimination of enantiomers: H-bonding,  $\pi$ – $\pi$  interaction, hydrophilic, dipole–dipole, and steric interactions [89]. The possible interactions between analytes depend on the type of CSP and mobile phase selection, which determines that enantiomers fit into the three-dimensional structure of sorbent [90].

In earlier applications of HPLC stereoisomers, the analysis was performed using ultraviolet (UV) and fluorescence detection (FLD). Currently, the liquid chromatography and mass spectrometry (LC-MS, LC-MS/MS) applications are utilized. Using tandem mass spectrometry assays allows for increasing the sensitivity

without losing enantioselectivity [91]. Furthermore, HPLC combined with tandem mass spectrometry is particularly attractive for the simultaneous quantification of drug molecules and their metabolites in biological matrices.

Several papers have recently reported direct liquid chromatography enantioseparation methods for β<sub>2</sub>-agonists in biological fluids. The majority of enantioseparation was performed on CSP based on macrocyclic antibiotics (teicoplanin and vancomycin).

The fenoterol enantiomers were determined in plasma using a sensitive chiral LC-MS/MS method [80]. Enantiomers of fenoterol were separated  $(R_s = 1.4)$  on Astec Chirobiotic T analytical column containing teicoplanin as a chiral selector. The mobile phase was methanol containing 0.2% acetic acid and 0.01% triethylamine. The method was validated and applied to study the bioavailability of fenoterol after oral administration of the single enantiomer formulation and after the administration of the racemic formulation. The results showed a potential pre-systematic enantioselective interaction, in which (S,S')-fenoterol reduces the sulfation of the active (R,R')-fenoterol.

The LC-MS/MS method using Astec Chirobiotic T-chiral column was developed for the simultaneous determination of bambuterol (bis-dimethylcarbamate prodrug of terbutaline), its metabolite-monocarbamate bambuterol and terbutaline enantiomers in human plasma. Enantioseparation was performed under isocratic elution with a mobile phase consisting of methanol and water with the addition of 20 mM ammonium acetate and 0.005% (v/v) formic acid. The method was successfully applied to an enantioselective pharmacokinetic study of racemic bambuterol [92].

The similar LC-MS/MS method using a teicoplaninbased column for the analysis of bambuterol, and its active metabolite terbutaline enantiomers in plasma samples was described by Luo et al. and Xia et al. [93,94].

The chiral chromatography assay (LC-MS/MS with Chirobiotic column) was utilized for the study of enantioselectivity in the disposition of clenbuterol following the administration of clenbuterol racemate to rats. The results indicated that there are differences in the distribution and excretion of the clenbuterol enantiomers, and these may be predominantly due to enantioselective protein binding [95].

The enantiomers of salbutamol in porcine urine samples were assayed using the Chirobiotic T column with a mobile phase constituted of 5 mM ammonium formate in methanol. The sequential solid-phase extraction sample clean up method for the determination salbutamol in porcine urine was developed. Enantiomers of salbutamol were detected and quantified by the LC-ESI-MS/MS method [96].

Teicoplanin as a chiral selector served for the determination of the enantiomers of salbutamol and its 4-O-sulphate metabolite in human plasma and urine [97].

Wu et al. [98] presented an automated chiral LC-MS/ MS method for the determination of salbutamol in dog plasma. The method employed on-line sample extraction using turbulent flow chromatography coupled to a Chirobiotic T column with a polar organic mobile phase - methanol, 0.02% formic acid, and 0.1% ammonium formate.

Detection and determination of low levels of salbutamol enantiomers in dog plasma were also achieved on the teicoplanin CSP using HPLC analysis with fluorescence detection [99].

Chiral liquid chromatography methods with teicoplanin and vancomycin CSP were applied for the analysis of enantiomers of salbutamol and other pharmaceuticals in environmental water and wastewater [100,101].

The vancomycin-based CSP (Chirobiotic V column) with a mobile phase containing methanol with 0.01% acetic acid and 0.01% triethylamine, was used for enantiomeric HPLC separation of mabuterol during pharmacokinetics study in rats. Results indicated that enantioselective pharmacokinetics between mabuterol enantiomers occur within the metabolism phase [102].

The effect of chromatographic conditions on the chiral separation of terbutaline and salbutamol on the Chirobiotic V column was investigated. The salt concentration in the mobile phase and pH value was found to be the most important factors affecting separation. In polar mobile phase containing ammonium nitrate in 100% methanol, pH = 5.1 value was found to give the best separation [103].

Enantioseparation of a new  $\beta_2$ -agonist trantinterol was achieved on the Chirobiotic V column with a mobile phase consisting of acetonitrile-methanol (60:40, v/v) containing 0.05% ammonia and 0.1% acetic acid [104]. The LC-MS/MS method based on this separation condition was applied to a study of stereoselective pharmacokinetics of trantinterol in rats and humans [105].

The enantioseparations of different derivatives of the same drug were also investigated on amylose-based CSP – Chiralpak AS-H column – amylose [tris(S)- $\alpha$ methylbenzylcarbamate]. Trantinterol enantiomers were resolved ( $R_s = 2.73$ ) within 14 min using hexane-ethanol (98:2, v/v) with 0.1% triethylamine as the mobile phase. The method was applied for the enantiomeric impurity determination of (–)-trantinterol bulk samples [106].

HPLC enantioseparation of isoxsuprine was studied on three different polysaccharide type CSPs: Chiralcel OJ – cellulose tris(4-methylbenzoate), Chiralcel OD – cellulose tris(3,5-dimethylphenylcarbamate), and Chiralpak AD – amylose tris(3,5-dimethylphenylcarbamate). The chromatographic analyses were performed in the normal phase with hexane–ethanol–triethylamine. Both of cellulose-based CSPs did not result in the acceptable enantioseparation. Baseline enantioseparation of isoxsuprine was obtained on amylose-based CSPs in the presence of triethylamine in the mobile phase [107].

Chiralpak AD and Chiralcel OD columns were also used for the enantioseparation of bambuterol and salbutamol [108] and for the separation of enantiomers of fenoterol [109] –  $\beta_2$ -agonist with two chiral centers.

Gazić et al. [110] attempted the separation of bambuterol enantiomers on a new generation of chiral polysaccharide stationary phase Chiralpak IA, a 3,5-dimethylphenylcarbamate derivative of amylose immobilized onto silica, which is the immobilized version of Chiralpak AD. An advantage of Chiralpak IA over Chiralpak AD is that immobilization allows the use of the most common organic solvents. However, Chiralpak IA showed a much weaker enantioresolution with the mobile phase already determined as optimal for bambuterol on Chiralpak AD (hexane:propan-2-ol, 100:30 with 0.1% diethylamine).

The urea type CSP made of (S)-indoline-2-carboxylic acid and (R)-( $\alpha$ -naphthyl)ethylamine known as Chirex 3022 column were used for the enantioseparation of clenbuterol [111] in the normal phase condition.

The resolution of salbutamol and formoterol enantiomers was achieved also on the  $\alpha_1$ -glycoprotein chiral stationary phase (AGP) under the reverse phase condition [84,112]. Protein-based chiral column with cellobiohydrolase as a chiral selector (CBH) served for the simultaneous determination of salmeterol and its main human metabolite  $\alpha$ -hydroxysalmeterol. Two-pair enantiomers were detected by electrochemical detectors, and the method was applied to a study of human hepatic metabolism of salmeterol *in vitro* [113].

#### 4.1.2 Indirect chiral chromatographic separation

The conversion of enantiomers into diastereoisomers using chiral derivatizing agents allows the separation on a nonchiral column. This mode is sometimes simpler, less expensive than the use of CSP, and often results in

better resolution, because of the more sensitive detector response. On the other hand, this method requires high optical purity derivatizing reagents and is sometimes time-consuming [114]. Various derivatizing reagents were used for the enantioseparation of  $\beta_2$ -agonists.

Enantioseparation of fenoterol was performed by indirect separation on an octadecyl reverse phase column after chiral derivatization by (S)-(+)-1-naphthyl) ethyl isocyanate. However, better sensitivity was achieved by achiral derivatization by 1-naphtyl isocyanate followed by chiral separation on a Chiracel column. Detection was carried out fluorometrically with a detection limit in the low nanograms per milliliter. The method was adapted to the determination of fenoterol enantiomers in hat heart perfusates [82].

(S)-(-)- $\alpha$ -Methylbenzyl isocyanate was applied for the resolution of salbutamol enantiomers in human urine. After solid-phase extraction, the diastereoisomeric derivatives were resolved ( $R_{\rm s}=1.6$ ) on the  $C_{18}$  column using 47% methanol as a mobile phase with fluorescence detection [115]. The same derivatizing reagents were utilized for the determination of terbutaline enantiomers by reversed-phase HPLC method ( $R_{\rm s}=1.4$ ) [81].

One of the later synthesized isocyanate derivatizing agents, 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl isothiocyanate was used for stereoanalysis of salbutamol in human urine. The salbutamol diastereoisomeric derivatives were resolved ( $R_{\rm s}=1.8$ ) on a  $C_{18}$  column using 35% acetonitrile in 0.05 M ammonium acetate buffer as a mobile phase with electrochemical detection [116].

Chiral derivatizing reagents prepared by substitution of the fluorine atoms in 1,5-difluoro-2,4-dinitrobenzene (DFDNB) with three amino acids: L-alanine, L-valine, and (S)-benzyl-L-cysteine cysteine were used for enantioresolution of racemic  $\beta_2$ -agonist isoxsuprine on reversed-phase HPLC and reversed-phase thin-layer chromatography [117].

Enantioseparation of salbutamol was also achieved by both TLC and RPHPLC via an indirect approach. A new chiral reagent, (*S*)-naproxen benzotriazole ester, was used to synthesize diastereomeric derivatives of salbutamol under microwave irradiation. Diastereoisomers were separated by both TLC and RPHPLC methods [118].

# 4.2 Stereoselective gas chromatographic (GC) analysis of $\beta_2$ -agonists

Although liquid chromatography is the most frequently used method for the enantioseparation of  $\beta_2$ -agonists,

other techniques were also occasionally described for that purpose.

Separation of enantiomers of  $\beta_2$ -agonists by GC requires derivatization with various derivatizing agents, *e.g.*, trimethylsilylation associated with acylation (bambuterol, salbutamol, and terbutaline) or formation of cyclic methylboronates (bambuterol, clenbuterol, formoterol, salbutamol, and salmeterol) [119,120].

Phosgene has been used to form cyclic derivatives of chiral diols, α-hydroxy acids, N-methylamino acids, and other di- and trifunctional compounds - terbutaline, salbutamol, and orciprenaline; the enantiomers were then separated by gas chromatography on the chiral polysiloxane phase XE-60 L-valine-(R)-α-phenylethylamide. In this way, phosgene can supplement the isocyanates as a versatile reagent for enantiomer separation. Factor of separation was 1.03 and (-)-enantiomer eluated before (+)-enantiomer [121]. L-α-Chloroisovaleryl chloride was used for other aminoalcohols to prepare diastereoisomers, and the samples were then dissolved in dichloromethane and N-methyl-N-trimethylsilyltrifluoro-acetamide [122]. These methods require high chemical and optical purity of the derivatizing agent and are therefore poorly used [123-125], and GC is currently being replaced by other separation techniques.

## 4.3 Stereoselective thin layer chromatography (TLC) of $\beta_2$ -agonists

TLC is one of the methods by which it is also possible to separate individual enantiomers in the group of  $\beta_2$ -agonists. It is possible to use a direct and indirect enantioseparation method.

The individual methods applied to  $\beta_2$ -agonists were likewise used with  $\beta$ -adrenolytic drugs. TLC served for the resolution of enantiomers of isoxsuprine using thin silica gel layers impregnated with L-glutamic acid. The limit of detection of each enantiomer was 11–13 ng/mL [117].

Enantiomers of salbutamol were resolved by adopting different modes of loading or impregnating the Cu(II) complexes of L-proline, L-phenylalanine, L-histidine, *N*,*N*-dimethyl-L-phenylalanine, and L-tryptophan on commercial precoated normal phase plates. The detection limit was 0.18 mg for each enantiomer [126].

Direct enantiomeric resolution of salbutamol and five racemic  $\beta$ -adrenolytics has been achieved by thinlayer chromatography using bovine serum albumin (BSA) as a chiral additive in the stationary phase. The effect of variation in pH, temperature, amount of BSA as the additive, and composition of the mobile phase on the resolution was systematically studied [127].

## 4.4 Stereoselective CE method for determination of $\beta_2$ -agonists

Compared with HPLC, the CE analytical method is advantageous in which the chiral column is substituted by the chiral selector added to the base electrolyte using a small volume of the sample and buffer solution [128]. Recently, this method has been largely used for the enantioseparation of chiral compounds and  $\beta_2$ -agonists. The most common chiral selectors for  $\beta_2$ -agonists include various types of cyclodextrins (CDs) (Table 3).

CDs and their derivatives as chiral selectors are frequently used for enantioseparation due to their good solubility in aqueous buffers and wide availability [129].

Native  $\beta$ -cyclodextrin ( $\beta$ -CD) and its derivatives, namely, ethyl- $\beta$ -CD, methyl- $\beta$ -CD, hydroxypropyl- $\beta$ -CD, and hydroxy- $\beta$ -CD, as chiral selectors were used for simultaneous CE enantioseparation of trantinterol, mabuterol, clenbuterol, and bambuterol. Hydroxypropyl- $\beta$ -CD was found to be the most effective complexing agent that allows excellent chiral resolution when compared with other CDs [130].

Hydroxy propyl- $\beta$ -cyclodextrin was also used for stereoselective determination of clenbuterol in human urine [131] and for enantioresolution of salbutamol, terbutaline, salmeterol, and bambuterol [132].

Esquisabel et al. [133] developed the CE method using another cyclodextrin derivative – heptakis(2,6-di-O-methyl)- $\beta$ -cyclodextrin (DM- $\beta$ -CD). The method was applied for the study of the release of salbutamol enantiomers from the matrix tablet.

Determination of salbutamol enantiomers in different pharmaceutical preparation – syrups, oral solutions, and tablets was performed by the CE method using carboxymethyl- $\beta$ -cyclodextrin (CM- $\beta$ -CD) in 25 mM acetate buffer (pH 5.0) [134].

The CM- $\beta$ -CD as a chiral selector was utilized for the enantiomeric separation of clenbuterol [135] and the other five  $\beta_2$ -agonists. The optimal separation was obtained using the following buffer: 50 mM phosphonic acid with 10 mM CD at pH 3.5 [136].

Sulfated  $\beta$ -cyclodextrin (HS- $\beta$ -CD) was chosen for the enantioseparation of some  $\beta_2$ -agonists (terbutalin, salbutamol, clenbuterol, dobutamine, and orciprenaline) [137,138] and for the quantitative determination of

Table 3: Enantiomeric separation of β-agonists by CE methods

Chiral selector	Analyte(s)	Buffer (pH)	Application	Reference
HE-β-CD	Clenbuterol	200 mM phosphoric acid (3.3)	Human urine	131
HP-β-CD	Terbutaline	0.1 M sodium phosphate (2.5, 3.5,		132
	Clenbuterol	4.5, 5.5.)		
	Salbutamol			
	Bambuterol			
α-CD, β-CD, HDMS-β-CD	Salbutamol	40 mM Tris-base in water (2.5)	Tablets	133
CM-β-CD	Salbutamol	25 mM acetate (5.0)	Syrups	134
			Oral solution	
			Tablets	
CM-β-CD	Clenbuterol	50 mM monopotassium		135
		phosphate (3.5)		
CM-β-CD	Procaterol	50 mM phosphoric acid (3.5)		136
	Clenbuterol			
	Bambuterol			
	Tulobuterol			
	Salbutamol			
S-β-CD	Terbutaline	40 mM monosodium		137
	Orciprenaline	phosphate (2.5)		
S-β-CD	Terbutaline	50 mM monosodium		138
	Clenbuterol	phosphate (2.5)		
	Salbutamol			
	Dobutamine			
S-β-CD	Clenbuterol	50 mM monosodium		139
	Salbutamol	phosphate (2.4)		
	Tulobuterol			
HDAS-β-CD	Salbutamol	10 mM ammonium formate	Urine	140 and 141
Glu-β-CD	Terbutaline	120 mM phosphate buffer (2.5-4.0)		142
	Clenbuterol			
$\alpha\text{-CD},\beta\text{-CD},\gamma\text{-CD},\text{CM-}\beta\text{-CD},\text{HS-}\beta\text{-CD},\text{SBE-}\beta\text{-CD},$	Isoxsuprine	100 mM triethanolamine		107
SU- $\beta$ -CD, HDAS- $\beta$ -CD, HDMS- $\beta$ -CD, HS- $\beta$ -CD,	·	phosphate (3.0)		
DM-β-CD, TM-β-CD				
Clarithromycin	Clenbuterol	100 mM citric acid, 10 mM sodium	Urine	143
		hydroxide, 240-300 mM boric acid		
Lactobionic acid/p-(+)-xylose-boric acid	Cycloclenbuterol	14.4 mM triethylamine in methanol		144
complex	Clenbuterol			
	Bambuterol			
	Tulobuterol			
	Terbutaline			
	Clorprenaline			

CD, cyclodextrine; CM- $\beta$ -CD, carboxymethyl- $\beta$ -CD; DM- $\beta$ -CD, dimethyl- $\beta$ -CD; Glu- $\beta$ -CD, glutamic acid- $\beta$ -CD; HE- $\beta$ -CD, hydroxyethyl- $\beta$ -CD; HDAS- $\beta$ -CD, heptakis(2,3-di- $\theta$ -acetyl- $\theta$ -O-sulfo)- $\beta$ -CD; HDMS- $\beta$ -CD, heptakis(2,6- $\theta$ -methyl- $\theta$ -CD; HP- $\theta$ -CD, hydroxyethyl- $\theta$ -CD; HS- $\theta$ -CD, highly sulfated- $\theta$ -CD; S- $\theta$ -CD, sulfobutyl- $\theta$ -CD; TM- $\theta$ -CD, trimethyl- $\theta$ -CD.

clenbuterol, salbutamol, and tulobuterol enantiomers by the CE method [139].

Other sulfo derivatives of  $\beta$ -cyclodextrin – heptakis (2,3-di-O-acetyl-6-O-sulfo)- $\beta$ -cyclodextrin (HDAS- $\beta$ -CD) were applied for the enantioselective determination of the low concentration of salbutamol in human urine by nonaqueous capillary electrophoresis (NACE) [140,141].

Satisfactory CE enantioseparation of terbutaline and clenbuterol was achieved also using glutamic acid- $\beta$ -CD,

which was prepared as a novel single-isomer cyclodextrin derivative [142].

Chankvetadze et al. [107] tested different native and derivatized CDs:  $\alpha$ -CD,  $\beta$ -CD,  $\gamma$ -CD, CM- $\beta$ -CD, HS- $\beta$ -CD, SBE- $\beta$ -CD, SU- $\beta$ -CD, HDAS- $\beta$ -CD, HDMS- $\beta$ -CD, HS- $\beta$ -CD, DM- $\beta$ -CD, and TM- $\beta$ -CD for the enantioseparation of isoxsuprine.  $\beta$ -CD exhibited the highest enantioseparation ability from native CDs and  $\gamma$ -CD, the lowest one. Neutral  $\beta$ -CD derivatives such as DM- $\beta$ -CD and TM- $\beta$ -CD

also exhibited significant enantioseparation ability toward the enantiomers of isoxsuprine.

In nonaqueous CE, clarithromycin as a chiral selector was used for the enantioseparation of clenbuterol [143]. Two new chiral selectors, lactobionic acid-boric acid complex and D-(+)-xylose-boric acid complex, were synthesized in situ in nonaqueous background electrolytes containing methanol and triethylamine and were found to be applicable for the enantioseparation of several  $\beta_2$ -agonists [144].

Capillary electrochromatography (CEC) has also become an important mode of CE for the separation of enantiomers. It is a hybrid of CE and HPLC and combines the high efficacy of CE and high selectivity of HPLC. Chiral separation by CEC can be performed in a wallcoated open tubular, packed, and monolithic capillary column. Enantioseparations of salbutamol were performed using a packed capillary column with CSP onbase teicoplanin [145] and vancomycin [146].

#### 5 Stereoselective synthesis of β<sub>2</sub>-agonists

In addition to the enantiomeric separation, the individual enantiomers may also be prepared by stereoselective synthesis. For many  $\beta_2$ -agonists, the  $\alpha$ -chloroacetophenone or α-bromoacetophenone derivative is the base for this synthesis. Their preparation is based on the reaction with chloroacetyl chloride with a substituted aromatic nucleus, followed by stereogenic reduction with various reducing agents (Figure 17).

In the case of salmeterol, the enantioselective reduction was performed with BH3·S(CH3)2 in THF under chiral Borodin catalysis [147] and reduction with NaBH<sub>4</sub> [148].

For the enantioselective synthesis of (*S*)-salmeterol, azido ketones reduced with the aid of Pichia angusta veast were prepared [149].

For the synthesis of (R)-(-)-salmeterol, Goswami et al. [150] used the stereoselective reduction of

Figure 17: Scheme of stereogenic reduction of acetophenone derivatives X = Cl, Br. (a)  $BH_3 \cdot S(CH_3)_2$  in THF or  $NaBH_4$  or Rhodotorula rubra microbial culture; sodium lauryl sulfate.

4-benzyloxy-3-hydroxymethylbromoacetophenone with Rhodotorula rubra (a yeast microbe isolated from local brewery waste) in the presence of sodium lauryl sulfate, an anionic surfactant. Epoxide was regioselectively opened by nucleophilic attack with (R)-6-(4-phenylbutoxyhexyl)-1-amine in reasonable yield (94%). Catalytic hydrogenation cleaved the benzyl protecting group to give (R)-(-)-salmeterol (Figure 18).

Further, other enantioselective synthetic approaches of salmeterol are summarized in the published overviews [151,152].

In the case of bambuterol, to prepare the (R)enantiomer, the (-)-DIP chloride was used for asymmetric reduction. To prepare the (S)-stereoisomer, its dextrorotatory form of (+)-DIP chloride was used. The optical purity was verified by HPLC. Optical properties were confirmed by optical rotation and the absolute configuration by circular dichroism [153].

Asami et al. [154] used whole cells of Williopsis californica JCM 3600 for asymmetric reduction of chloroketone in the synthesis (*R*)-bambuterolol.

In the work of Huang et al. [155], a 6-stage stereoselective synthesis of (R,R)-formoterol was developed starting from 4-hydroxy-3-nitroacetophenone (Figure 19). The key step is the reduction of the ketone group with Rh-complex as a hydrogenation catalyst.

In the stereoselective synthesis of (R)-terbutaline, the starting O-protected cyanohydrins were used that are converted to amides via the Ritter N-tertiary butylation. In the next step, hydrogenation provided the amino alcohol, from which the (R)-terbutaline was obtained by the group deprotection [156].

A similar procedure was also used to prepare (R)salbutamol. In the next step of its preparation, selective hydrogenation of protected cyanohydrin was used. A 2-aminoethanol derivative was prepared by NaBH<sub>4</sub> hydrogenation of tert-butylamine iminoderivative. Desilylation was carried out with LiAlH<sub>4</sub> but the hydrolytic cleavage of the protected acetal was accompanied by racemization [156].

#### 6 Conclusion

In the presented overview, attention is focused on β<sub>2</sub>-agonists from the stereochemical point of view. Most of  $\beta_2$ -agonists is mainly used in the treatment of asthma and obstructive pulmonary disease. In addition to the bronchodilatory activity, they also have a tocolytic and anabolic effect, and, from the more recent indications,

Figure 18: Synthesis of (R)-(–)-salmeterol. Reaction conditions: (a)  $K_2CO_3$ , THF/MeOH; (b) N,O-bis(trimethylsilyl)acetamide, DMSO, 3,4-dimethoxyphenylamine, (c) Pd–C/H. Note: Bn = benzyl.

Figure 19: Synthesis of (R,R)-formoterol. Reaction conditions: (a)  $K_2CO_3$ , THF/MeOH; (b) HCOOH-Ac $_2O$ . (c) (R)-1-(4-Methoxyphenyl)-N-(R)-1-phenylethyl)propan-2-amine, 120°C; (d) Pt/C, H $_2$ , MeOH. Note: Ac = acetyl; Ph = phenyl; Bn = benzyl.

they make it possible to monitor the amelioration of symptoms in amyotrophic lateral sclerosis. Many β<sub>2</sub>agonists have different pharmacological and pharmacokinetic activity due to the different stereochemistry and, likewise, indicate varying degrees of toxicity. The overview focuses on β<sub>2</sub>-agonists with one stereocenter (albuterol, bambuterol, clenbuterol, salmeterol, and trantinterol) and with multiple stereocenters (formoterol, isoxsuprine, and ritodrine). From these  $\beta_2$ -agonists, pure enantiomers of levalbuterol or levosalbutamol, such as the (R)-isomer of albuterol or salbutamol and the arformoterol (R,R)-isomer of formoterol, were introduced into the therapy by the way of the racemic switch process. More recently, long-acting drugs (indacaterol, carmoterol, abediterol, olodaterol, and vilanterol) have already been introduced into clinical practice as pure enantiomers.

Individual phases of pharmacokinetics enabled the observation of absorption, distribution, and metabolism of different  $\beta_2$ -agonists. Nonetheless, many lack complete data on the pharmacokinetics of individual (R)-and (S)-stereoisomers.

To obtain enantioselective separation of racemates to individual enantiomers, chromatographic and electrophoretic methods were used. In the case of HPLC, various chiral phases were used for the direct methods based on CDs, macrocyclic antibiotics (vancomycin, teicoplanin) on substituted carbamates of cellulose and amylose, many of which are the basis of chiral selectors in the direct methods in CE. Besides the preparative chromatographic methods, the overview lists many methods of stereoselective synthesis enabling pure enantiomers of this group of drugs to be obtained.

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