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Review

Recent developments of enantioseparation techniques for adrenergic drugs using liquid chromatography and capillary electrophoresis: A review

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Abstract

This review provides the achievements of enantioseparation of adrenergic drugs and application of these methods in clinical and pharmaceutical analysis. The adrenergic agonist and antagonist drugs are analyzed in the direct and indirect modes by liquid chromatography (LC) and capillary electrophoresis (CE). Other chromatographic enantioseparation methods including super- and sub-critical fluid chromatography (SFC), and capillary electrochromatography (CEC) are presented likewise to analyse the cited compounds. The different separation processes for these drugs are briefly discussed and some applications are presented.

Keywords: Enantioseparation; Adrenergic drugs; Agonists; Antagonists; Liquid chromatography; Capillary electrophoresis

Contents

Abbreviations: ACE, affinity capillary electrophoresis; AEKC, affinity electrokinetic chromatography; AGP, α_1-acid glycoprotein; BGE, background electrolyte; BSA, bovine serum albumin; CBH I, cellobiohydrolase I; CD, cyclodextrin; CDR, chiral derivatization reagent; CD-MEKC, CD-mediated micellar electrokinetic chromatography; CE, capillary electrophoresis; CE-β-CD, carboxyethyl-β-cyclodextrin; CEC, capillary electrochromatography; CGE, capillary gel electrophoresis; CM-β-CD, carboxymethyl-β-cyclodextrin; CMPA, chiral mobile phase additive; CSP, chiral stationary phase; CTA-I, microcrystalline cellulose triacetate; CSA, (+)-10-camphor sulfonic acid; CTPC, cellulose trisphenylcarbamate-based CSP; CZE, capillary zone electrophoresis; EOF, electroosmotic flow; HP-β-CD, hydroxypropyl-β-cyclodextrin; HPLC, high-performance liquid chromatography; HSA, human serum albumin; ITP, isotachophoresis; LC, liquid chromatography; M-β-CD, methyl-β-cyclodextrin; MIP, molecular imprinted polymer; NA, nonaqueous; OT-CEC, open-tubular capillary electrochromatography; P-CEC, packed capillary electrochromatography; SDS, sodium dodecyl sulfate; SFC, super- and sub-critical fluid chromatography; $18C_6H_4$, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid; ZGP, *N*-benzoxycarbonyl-glycyl-L-proline.

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1. Introduction

Adrenergic drugs are currently classified into adrenergic agonists and antagonists (blockers). The classification is based on their stimulation or blocking of the adrenergic receptors. The agonist drugs are mainly developed for the treatment of chronic obstructive pulmonary diseases and used as bronchodilators in the treatment of asthma. They were also studied as banned substances in sport testing and as growth promoters in animals [1]. The structures are shown in Table 1. Adrenergic antagonist drugs are effectively used for the treatment of hypertension, prevention of anginal attacks, suppression of cardiac arrhythmia, prevention of myocardial infarction and possibly, amelioration of congestive heart failure [2]. The structures are shown in Table 2. Most of these drugs in use are chiral. Each isomer possesses a different pharmacological activity, potency, and mode of action. The pharmacological effect is restricted in many of the cases to one of the enantiomers. Commonly, the pharmacologically inactive enantiomer shows unwanted effects, antagonistic function and even toxic effects [3]. Obviously, the pharmacologically inactive enantiomer goes down to waste which is an economic loss. Hence obtaining the pure active enantiomer is very important. There are two ways to obtain the pure active enantiomers, one is enantioselective synthesis. If the asymmetric synthesis approach is used, most or all of the inactive enantiomer would be converted into the desired enantiomer, thereby the yield of desired isomer would be maximized. Another way would be the application of enantioseparation techniques, which have been investigated and applied for the separation of racemic mixtures of intermediate or final products. These techniques have become increasingly relevant on a preparative scale.

Chromatography methods and electromigration techniques have long been the methods of choice in this field and suited for enantioseparation analysis. Chromatography techniques such as high-performance liquid chromatography (HPLC), super- and sub-critical fluid chromatography (SFC) have been developed during the past decades, and they are effectively used to separate chiral adrenergic drugs. HPLC can be used either indirectly with chiral derivatization reagents (CDR) or directly with chiral stationary phases (CSPs) or chiral mobile phase additives (CMPA). In the last decades, capillary electrophoresis (CE), microchip techniques and capillary electrochromatography (CEC) have been popularly developed and increasingly applied for the enantioseparation of these drugs, the main reason being their high efficiency and low solvent and selector consumption [4]. These techniques are popularly used in the pharmaceutical and clinical fields for the enantiomeric resolution of adrenergic compounds.

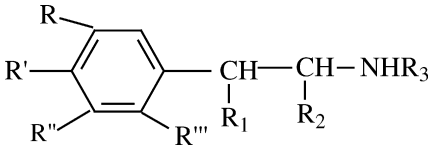
This review will present an overview of LC and CE techniques for adrenergic drugs enantioseparation. Several reviews [5–9] devoted to the enantioseparation have appeared, but specific reviews on the chiral resolution for the adrenergic drugs are rather scarce: reviews of LC enantioseparation of adrenergic β-blocking agents were reported by Egginger et al. in 1993 [10]. Jacek's group [2,11] reported reviews for the enantioseparation of cardiovascular drugs using LC techniques in 1999 and provided an update in 2002. Hence it appeared advisable to summarize the existing methods and techniques for the analytical enantioseparation of adrenergic drugs. The present review provides and updates the information contained therein and reports on the latest achievements in enantioseparation of different classes of adrenergic active compounds using LC, CE and other techniques.

2. Enantioseparation by liquid chromatography

HPLC methods have been developed since a long period for the enantioseparation of adrenergic drugs employing different detectors. The direct approach includes CSP and CMPA, and the indirect approach is used for drug derivatization with CDR [12].

Table 1

Structure of some adrenergic agonists



Drug	R	R′	R″	R‴	R_1	R_2	R_3
Albuterol	CH_2OH	H	OH	H	OH	H	$C(CH_3)_3$
Cimaterol	CN	NH_2	H	H	OH	H	$C(CH_3)_3$
Clenbuterol	Cl	NH_2	Cl	H	OH	H	$C(CH_3)_3$
Clorprenaline	H	H	H	Cl	OH	H	$CH(CH_3)_2$
Dopamine	H	OH	OH	H	H	H	H
Ephedrine	H	H	H	H	OH	CH_3	CH_3
Epinephrine	H	OH	OH	H	OH	H	CH_3
Isoprenalin	H	OH	OH	H	OH	H	$CH(CH_3)_2$
Mabuterol	CF_3	NH_2	Cl	H	OH	H	$C(CH_3)_3$
Methoxamine	OCH_3	H	H	OCH_3	OH	CH_3	H
Norephedrine	H	H	H	H	OH	CH_3	H
Norepinephrine	H	OH	OH	H	OH	H	H
Pseudoephedrine	H	H	H	H	OH	CH_3	CH_3
Salbutamol	H	OH	CH_2OH	H	OH	H	$C(CH_3)_3$
Terbutaline	OH	H	OH	H	OH	H	$C(CH_3)_3$

Adrenergic drug enantiomers are effectively separated using both methods.

2.1. Direct separation

2.1.1. Brush-type CSP

Brush-type CSPs which are also called ‘Pirkle CSPs’ were invented by Pirkle’s group since the early 1980s [13,14]. The chemical structural characteristics of these CSPs are based on the presence of a π-donor, a π-acceptor group or of groups that form multiple hydrogen bonds; they give a good fit to the “three point principle” theory. Some adrenergic drugs were successfully enantioseparated by this type of phases because of their structure: hydroxyl groups and aromatic rings connected with the chiral carbon, the imino (or amino) group often linked to a methylene group which connects with the chiral carbon atom [15]. Compared to the agonist drugs, the difference in the structure of the antagonist drugs is that the phenyl ring part does not directly link to the chiral carbon but to an –OCH_2– group which is connected with the chiral carbon. The interactions of antagonists with the CSP are shown in Fig. 1; both enantiomers of the drugs produce different interactions with the CSP because of the different conformation [16,17].

The applications of enantioseparation of adrenergic drugs have been reported by a series of brush-type CSPs, the corresponding reports being presented in Table 3. The chiral π-acceptor and π-donor phases are used commonly and many commercial columns have been developed. The advantages of these brush-type phases are their facile use, high enantioselectivity and high capacity; on the other hand they are only available to enantioseparate the compounds containing aromaticity, or molecules derivatived with aromatic rings. In addition to these CSPs, other special phases for the chiral separation of β-blockers were reported: *N*-3,5-dinitrobenzoyl-α-amino phosphonate [14], the Whelk-O 1 CSP and ULMO CSP were prepared for the direct separation of the enantiomers of underivatized β-blockers, they are a mixture of CSPs containing a π-donor and a π-acceptor [12].

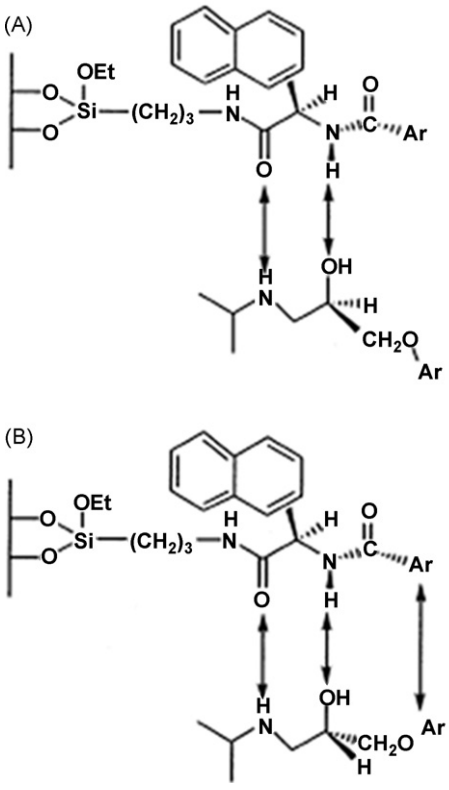
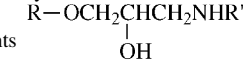
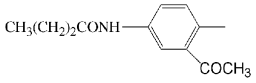
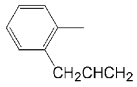
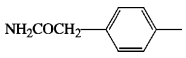
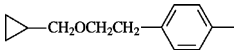
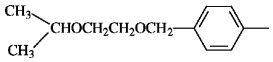
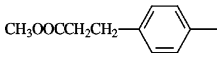
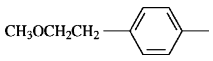
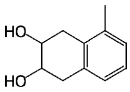
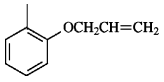
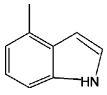
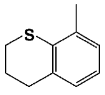
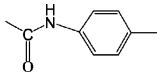
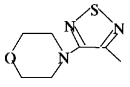


Fig. 1. (A) Interaction between the *R*-enantiomer of antagonists and CSP. (B) Interaction between the *S*-enantiomer of antagonists and CSP. Reprinted from Ref. [17] with permission from Elsevier.

Table 2
Summary of names and structures of oxymethylene β-adrenergic blocking agents



Drug	R	R′
Acebutolol		—CH(CH$_3$)$_2$
Alprenolol		—CH(CH$_3$)$_2$
Atenolol		—CH(CH$_3$)$_2$
Betaxolol		—CH(CH$_3$)$_2$
Bisoprolol		—CH(CH$_3$)$_2$
Esmolol		—CH(CH$_3$)$_2$
Metoprolol		—CH(CH$_3$)$_2$
Nadolol		—C(CH$_3$)$_3$
Oxprenolol		—C(CH$_3$)$_3$
Pindolol		—CH(CH$_3$)$_2$
Propranolol		—CH(CH$_3$)$_2$
Tertatolol		—C(CH$_3$)$_3$
Practolol		—C(CH$_3$)$_3$
Timolol		—C(CH$_3$)$_3$

2.1.2. Cyclodextrin CSP

CDs have been widely used in HPLC; they mainly consist of α-CD, β-CD and γ-CD. β-CD is most easily available because the cavity of β-CD is well suited to a wide variety of drugs including adrenergic drugs [29]. CDs are cyclic oligosaccharides and consist of glucopyranose units. The principle of chiral recognition of the adrenergic drugs is based on inclusion of the hydrophobic group of the drugs into the hydrophobic cavity of the CD and lateral interactions of the hydroxyl groups at the C-2, C-3 and C-6 of the CD with the drugs, the driving force being considered to originate from hydrogen bonds and dipole–dipole interactions.

The first CSPs containing CDs chemically bonded to silica gel were developed by Armstrong and DeMond in 1984 [30]. Recently, not only the native cyclodextrin but also the modified CDs showing improved enantioseparation properties have been used in many cases.

2.1.2.1. Enantioseparation of adrenergic drugs on native CDs. The enantioseparation of ephedrines, synephrine and some adrenergic agonists in β-CD columns has been reported [31,32]. Pumera et al. [33] enantioselectively separated ephedrine, pseudoephedrine and ibuprofen using nano-HPLC by linking the β-CD modifier to the acrylic monolithic phase. Some adrenergic drugs in plasma and urine were enantiomerically separated by β-CD and twenty β-adrenergics and β-blocking agents and similar amino alcohol types were analyzed using α-CD [34]. Chiral discrimination occurs by steric hindrance to form different interactions between the CDs and the drugs.

2.1.2.2. Enantioseparation of adrenergic drugs on derivatized CDs. Enantiomeric adrenergic drugs are not always resolved satisfactorily using native CD columns. Therefore, CD CSPs are modified with hydrophilic or hydrophobic groups to enhance chiral recognition. The most commonly used derivatives are methylated, acetylated, carboxymethylated, apart from naphthylethyl carbamate, 3,5-dimethylphenyl carbamate and hydroxypropylated CDs [3]. In addition to inclusion complexation and hydrogen-bonding interactions similar to the native CD, π–π interactions, hydrophobic interactions and steric repulsion are also assumed to be responsible for chiral recognition.

The applications of enantioseparation of adrenergic drugs by derivatized CDs have been widely reported. Mono-2^A-azido-2^A-deoxyperphenyl carbamoylated β-CD, norborn-2-ene-derivatized β-CDs and mono-6^A-azido-6^A-deoxyperacetylated β-CD, mono-6^A-azido-6^A-deoxy-perphenylcarbamoylated β-CD as CSPs to separate these drugs was reported [35-39]. Lai and Ng [40,41] prepared CSPs by immobilizing mono-6^A-N-allylamino-6^A-deoxy-perphenylcarbamoylated β-CD onto the surface of silica gel via hydrosilylation to separate a variety of racemic β-blockers. Chen et al. [42] made a novel CSP by immobilization of heptakis-(6-azido-6-deoxy-2,3-di-*O*-phenylcarbamoylated)-β-CD. Tazerouti et al. [43] prepared a chemically bonded β-CD CSP for HPLC in a "one pot" process by the reaction of a phenylated β-CD with silica gel; the adrenergic β-blockers were tested on the obtained material. Narimatsu et al. [44] used an Ultron ES-Ph CD column to determine the sulfation of 4-hydroxypropranolol enantiomers from the racemate.

CDs and derivatized CDs have been applied extensively, because of their stable properties, the relative low price and the high-column capacity. In CD-CSPs, temperature, pH, flow rate and ionic strength influence the separation and retention times. Hydrogen bonds are associated with pH, the rate of mobile phase flow influences the interaction between the analytes and the cavity of the CDs. Hence optimization of the chromatographic conditions is necessary for enantioseparation.

Table 3
Some enantiomeric separations of adrenergic drugs on Pirkle-type CSP with HPLC

Adrenergic drug	Type of Pirkle column	Sample resource	Mobile phase conditions	Reference
Ephedrine, norepherine, pseudoephedrine	(*R*)-1-Naphthylglycine 3,5-dinitrobenzoic acid as CSP	Dissolved in methanol	For ephedrine and norephedrine: *n*-hexane:1,2-dichloroethane:methanol (66:24:10, v/v/v) at 25 °C, 1.0 mL/min; for pseudoephedrine: *n*-hexane:1,2-dichloroethane:methanol:acetic acid (63.87:23.95:11.98:0.002) at 25 °C, 1.0 mL/min	[16]
Metoprolol, bisoprolol	(*R*)-1-naphthylglycine 3,5-dinitrobenzoic acid as CSP	Dissolved in methanol	For metoprolol: hexane:1,2-dicholoethane:methanol (65:25:10, v/v/v); for bisoprolol: hexane:1,2-dichloroethane:methanol (65:30:10, v/v/v) at 15 °C, 0.6 mL/min	[17]
Clenbuterol	Chirex 3005	Human serum	*n*-Hexane:1,2-dichloroethane:methanol (54:38:8, v/v/v) at 17 °C, 1.0 mL/min	[18]
Salbutamol	Chirex 3022	Human plasma and urine	Hexane:1,2-dichloromethane:methanol:trifluoroacetic acid (243:140:17:1, v/v/v/v) ambient temperature, 1.0 mL/min	[19]
Salbutamol	Chirex 3022	Human urine	Hexane:dichloromethane:methanol:trifluoroacetic acid (250:218:31:1, v/v/v/v) ambient temperature, 1.0 mL/min	[20]
Clenbuterol	Chirex 3022	Human plasma and urine	Hexane:1,2-dichloroethane:ethanol/trifluoroacetic acid (80:10:10, v/v/v) at 23 °C	[21]
Salbutamol	Sumichiral-OA4700	Human urine		[22]
Albuterol	Sumichiral-OA4700	Human serum		
Metoprolol and its metabolites	Sumichiral-OA4900	Human urine and plasma		[23]
Terbutaline	Sumichiral-OA4900	Human urine	*n*-Hexane:ethyl acetate:dichloroethane:methanol:trifluoroacetic acid (240:220:160:35:1, v/v/v/v/v) 1.0 mL/min	[24]
Terbutaline	Sumichiral-OA4900	Dissolved in mobile phase	*n*-Hexane:ethyl acetate:methanol:trifluoroacetic acid (240:250:25:1, v/v/v/v) 1.0 mL/min	[25]
Propranolol, metoprolol, bisoprolol	(*R*,*R*)-Tartramide derivative as a chiral moiety	Dissolved in chloroform or mobile phase	Hexane:CH_2Cl_2:Et_2NH (84:5:1, v/v/v) at 25 °C, 0.2 mL/min	[26]
Labetalol	Chirex 3022	Plasma and urine	Hexane:dichloroethane:ethanol:trifluoroacetic acid (55.75:35:9:0.25) ambient temperature, 0.6 mL/min	[27]
Propranolol, metoprolol, bufuralol	α-Burke 1 CSP, α-Burke 2 CSP	10 mg/mL in ethanol	Ethanol:dichloromethane (10:90, v/v) with 10 mM ammonium acetate, 2.0 mL/min	[28]

2.1.3. Polysaccharide-based CSP

2.1.3.1. Classification and chiral separation mechanism of CSPs. The polysaccharide-based CSPs consist of cellulose-based and amylose-based CSPs and their derivatives-based CSPs, which show a very broad applicability to different chiral analytes. Various specialized research and reviews reported the applications and development of these CSPs. The first practically useful CSP, i.e., microcrystalline cellulose triacetate (CTA-I) derived from polysaccharides, has the tertiary structure upon swelling and forms chiral cavities which are able to stereoselectively include compounds [45]. Then, CTA-II [46] was produced by coating macroporous aminopropylsilanized silica gel with cellulose triacetate and cellulose trisphenylcarbamate-based CSP (CTPC).

In recent years, a broad range of commercially available polysaccharide phases has been produced by Daicel (Japan), based on cellulose or amylose esters and carbamates. The cellulose-based columns Chiralcel OD and the amylose-based columns Chiralcel AD are frequently used for the enantioseparation of adrenergic drug enantiomers.

2.1.3.2. Application of enantioseparation to adrenergic drugs. Dopamine agonist drugs and antagonist drug enantiomers [47] were separated on a Chiralpak AD column. Many adrenergic blocker enantiomers such as atenolol, alprenolol, acebutolol, metoprolol, bisoprolol and tolamolol were separated using the Chiralcel OD column [48,49]. The interactions are assumed to be the hydrogen bonding and π–π interaction between CSP and drugs. Many β-blocker enantiomers were enantioseparated by this type CSP by different LC methods such as normal- and reversed-phase LC [50–52].

Cellulose-based, amylose-based CSPs and their derivatives-based CSPs have different resolution selectivites, because of the different configuration in the glucose units. The advantages of this type CSPs include their stability properties, facile column preparation, no need for bonding other groups; they are suitable for enantioseparation of drugs containing aromatic rings, such as the adrenergic compounds.

2.1.4. Protein-based CSPs

Proteins consist of amino acids; glycoproteins additionally contain sugar moieties, both of which are chiral. Proteins

can form a three-dimensional structure; hydrophobic, electrostatic interaction and hydrogen bonds are assumed to be the interactions responsible for chiral recognition. So far, many protein-based CSPs have been developed, some studies [53–56] were published on the mechanisms and applications of enantioseparation of chiral drugs including adrenergic compounds.

α_1-Acid glycoprotein (AGP) has been used to separate the enantiomers of several adrenergic drugs, and the column was also used in a switching system designed for the separation of cardiovascular drugs [57]. AGP owns a very acidic character with an isoelectric point of 2.7, hence it can easily bind with the cationic part of the adrenergic drugs. The folding of the secondary structure of AGP could form an apolar cavity, which is able to bind the aromatic groups of the drugs. Besides, hydrogen-bond interactions are also involved in the chiral discrimination mechanism.

Cellobiohydrolase I (CBH I) was found an excellent chiral selector towards a set of β-blocking agents, because of the higher enantioselectively obtained compared with other protein-CSPs. CBH I has a catalytically active three-dimensional structure site, which is composed of an antiparallel β-sandwich and of loops connecting the β-strand, the bonding site being a long tunnel into which cellulose chains can be threaded and cleaved, as shown in Fig. 2(a) [58]. Based on the active site and the carboxylate functions, both enantiomers of adrenergic drugs were modeled in the active site of CBH I [59]. It is shown in Fig. 2(b) that the difference in bonding between both enantiomers gives rise to chiral separation. Based on the same principle, several adrenergic drugs and other cardiovascular drugs have been chirally recognized using these CBH I CSPs [60,61]. Recently, cellobiohydrolase 58 (CBH 58) was reported and its catalytic site was similar to that of CBH I. A series of adrenergic drugs, oxprenolol, metoprolol, atenolol, metanephrine and norephedrine were successfully resolved on this CSP as well.

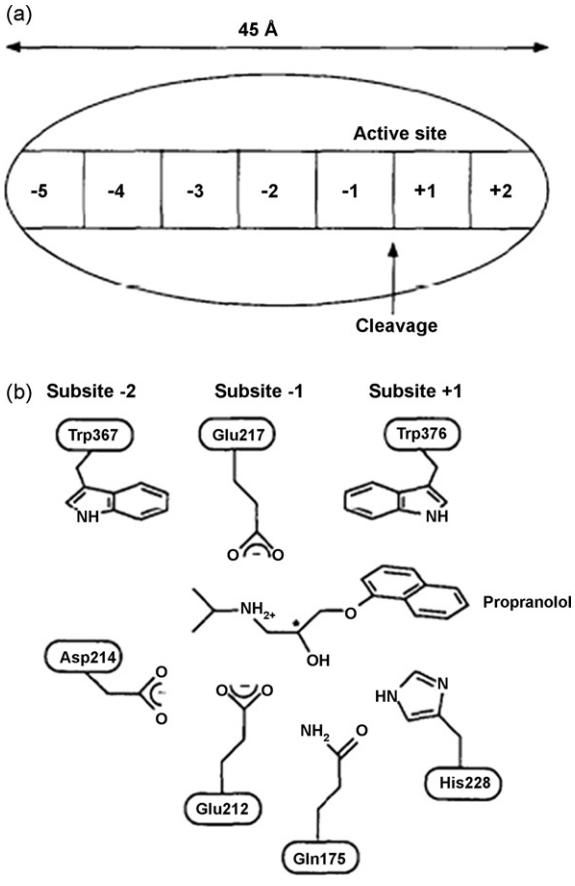


Fig. 2. (a) Schematic representation of the cellulose-binding tunnel in CBH I. (b) Schematic model for the binding of R/S-propranolol in the active site of CBH I. Reprinted from Ref. [58] with permission from Elsevier.

Protein-based columns have provided versatility and broad applicability. However, they feature low-column capacity and shorter lifetimes than other CSPs. Because of the inherent properties of proteins, the columns may be damaged by higher temperatures, organic solvents, inappropriate pH values and salt concentrates.

2.1.5. Macrocyclic antibiotics CSPs

Macrocyclic antibiotics used as chiral selectors were introduced by Armstrong's group [62]. Two main groups of macrocylic antibiotics are the glycopeptides such as ristocein A, avoparcin, vancomycin and teicoplanin, and the ansamycins such as rifamycin B or rifamycin SV. The glycopeptides consist of an aglycon portion of fused macrocyclic rings that form a hydrophobic "basket shape", and this "basket" can include the hydrophobic parts of the adrenergic drugs, the pendent polar arms of the "basket" can form hydrogen bonds and dipole–dipole interactions with polar groups of the drugs; moreover, ionic interactions, steric repulsions and π–π interactions may support the separation. The ansamysin rifamycin B was superior for basic compounds; rifamycin SV was suitable for acidic analytes. However, both of them are more frequently used as chiral additive in CE.

Chirobiotic columns were named by this type of patent chiral selectors. Chirobiotic V (vancomycin-based chiral stationary phase), Chirobiotic T (teicoplanin-based chiral stationary phase), and Chirobiotic TAG (teicoplanin aglycon-based stationary phase) have been used to separate the enantiomers of adrenergic drugs. Aboul-Enein and Ali [63] used the three columns to separate the enantiomers of the adrenergic β-agonists clenbuterol, cimaterol and mabuterol.

Chirobiotic T columns were used for enantioseparation of some adrenergic enantiomers in human and canine plasma analysis [64–66]. Wang and Shen [67] developed a simple and sensitive chiral liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI–MS/MS) method for the separation of *S*-(−)- and *R*-(+)-pindolol in human plasma using the same column. Kafková et al. [68] reported on the enantioseparation of β-adrenergic antagonist drugs using a Chirobiotic T column in capillary liquid chromatography (CLC).

The method on the Chirobiotic V column was estimated as most convenient for the separation of some adrenergic drug enantiomers, some reports presented baseline separation within a few minutes [69].

Hence, these antibiotics form the basket shape for the enantiomers to stereogenically fit in different fashions which results in the chiral discrimination of the enantiomers of the drugs.

2.1.6. Other CSPs

Chiral imprinted polymers, chiral synthetic polymers and crown ethers have also been used as CSP for the enantioseparation of adrenergic drugs.

Fairhurst's group [70] reported enantioseparation of β-adrenergic blockers using a CSP prepared by molecular imprinted polymers (MIPs). The MIPs-approach is based on polymerizing a monomer with a cross-linking agent in the presence of a chiral template molecule. After removing the template molecule, a chiral imprinted cavity remains, which shows high stereoselectivity to the template molecule or closely related molecules. Detailed information was referred to for the specialized analysis of the adrenergic drugs.

Chiral synthetic polymers were also used as CSPs in HPLC. A comprehensive review on the synthesis and application of chiral synthetic polymers was reported by Nakano [71]. The development of polymeric monolithic phases was introduced for chiral separation in the 1980s [72]. Monolithic phases are obtained by *in situ* copolymerization of a monomer, a crosslinker and a selector. Currently, these columns are widely used because of the numerous advantages they offer when compared to the particulate or packed columns. They possess tunable pore sizes, offer fast analysis times, superior flow rates with low-back pressure and high-surface areas for high efficiencies to be reached. The unique feature of monolithic columns is their high permeability, which is nearly twice as high as that of packed columns [73–76]. A series of β-blockers could be enantioseparated using this technique. Moreover, some approaches for synthesizing monolithic polymers and the chiral separation of adrenergic drugs have been presented [77]. A sol–gel process for the preparation of monolithic silica rod columns with a bimodal pore structure, and the preparation of monolithic materials within the confines of fused silica capillaries both demonstrated that the monolithic-type HPLC column could be operated at high-flow rates maintaining high-separational efficiencies.

Crown ethers are macrocyclic polyethers that can form host–guest complexes with alkali and earth-metal ions as well as with primary ammonium cations. The main interactions of chiral separation between the crown ether CSP and the adrenergic drugs are assumed to be the hydrogen bonds between the three hydrogen atoms attached to the nitrogen atom of the drugs and the dipoles of the oxygen atoms of the macrocyclic ether. These phases are only applied for chiral separation of the drugs with primary amino groups. Hyun et al. [78] applied a CSP based on diphenyl-substituted 1,1′-binaphthyl crown ether in resolving a series of racemic amino alcohols.

2.1.7. Chiral mobile phase additives

The effect of mobile phase additives may be attributed to various mechanisms; these chiral additives may activate or block interaction between the solute and the stationary phase. The additives can interact with the solute in the mobile phase to form a mixture of diastereomers, which may have a different selectivity in interacting with the stationary phase; the additives may as well dynamically generate a new stationary phase to separate the enantiomers. This means that the additives may not only change the retention of the enantiomers but also their separation. The frequently used additives are ion-pairing reagents, ligand-exchanging reagents, protein-affinity reagents and CD inclusion reagents. The advantages of this technique include simplicity and flexibility, moreover there is no need for chiral derivatization. However, it is not always applicable in HPLC because of the large amounts of consuming reagents and the long equilibration times.

2.1.7.1. Ion-pairing reagents. The commonly used ion-pairing reagents are quinine, quinidine, (+)-10-camphor sulfonic acid (CSA) and *N*-benzoxycarbonyl-glycyl-L-proline (ZGP) [12]. The principle of enantioseparation is based on the formation of diastereomeric ionpairs between a chiral counter-ion and the enantiomers which can be separated by the adsorption-based columns. Earlier, Pettersson's group [79] used CSA as CMPA for the enantioseparation of β-blockers. Then, ZGP and CSA were found to present higher enantioselectivity for chiral separation of β-blockers [78,80].

2.1.7.2. Ligand-exchanging reagents. Ligand-exchanging reagents are the complexes of amino acids or their derivatives with metal ions (Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}). The chiral metal complexes as additives to the mobile phase are often applied for adsorption onto an achiral stationary phase such as C8 and C18 columns, forming dynamic CSPs. Enantiomers of the adrenergic compounds are separated under these conditions. Yamazaki et al. [81] separated various aromatic β-amino alcohols into their enantiomers using octadecylsilanized silica coated with *N*-*n*-dodecyl-L-hydroxyproline as the stationary phase and acetate buffer containing copper(II) as the mobile phase. A review [82] presented some chiral chromatographic seaparations using the ligand-exchanging reagents.

2.1.7.3. Protein-affinity reagents. Protein-affinity reagents and cholates as CMPA in HPLC have been used for enantioseparation. Proteins such as BSA, AGP and CBH I as chiral additives interact with the enantiomers to form diastereomers, and the latter were separated by C18 or diol-silica-based stationary phases. Hedeland et al. [83] used CBH I as a chiral complexing agent in the mobile phase in HPLC and diol-silica-based stationary phase for the chiral separation of propranolol, oxprenolol and alprenolol, whereas cholates as chiral additives can coat onto the C18 silica-based columns and form dynamic stationary phases for chiral resolution of the enantiomers.

2.1.7.4. CD inclusion reagents. The principle of CDs and their derivatives used as CMPA for chiral separation is inclusion of the hydrophobic group of the enantiomers into the cavity of the CD, then separating the enantiomers on a C8 or C18 column. Native β-CD, methyl-β-CD (M-β-CD), carboxyethyl-β-CD (CE-β-CD), carboxymethyl-β-CD (CM-β-CD) and hydroxypropyl-β-CD (HP-β-CD) were the most frequently used selectors added to the mobile phase to separate the adrenergic enantiomers. Some reports have been presented for this purpose [31,84,85].

Table 4
Enantiomeric separation of adrenergic drugs using derivatization reagents

Derivatization reagent	Adrenergic blocker	References
(1*S*,2*S*)-*N*-[(2-Isothiocyanato)-cyclohexyl]-pivalinoyl amide (PDITC)	Propranolol, mexiletine carvedilol, metoprolol, normetoprolol	[86]
O,*O*′-(*R*,*R*)-Diacylated tartaric acid anhydides	Atenolol	[87]
Dansyl chloride (Dns-Cl); 4-(4,5-diphenyl-1*H*-imidazole-2-yl)-benzoyl chloride (DIB-Cl)	Ephedrine, norephedrine, phentermine, norfenfluramine	[88,89]
2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC)	Propranolol, terbutaline 4-hydroxyl-propranolol, salbutamol, sotalol, atenolol	[90–92]
6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC)	Amphetamine, methamphetamine	[93]
(*R*)-(−)-4-(3-Isothiocyanatopyrrolidin-1-yl)-7-(*N*,*N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazole (DBD-PyNCS)	Propranolol, atenolol, bupranolol, oxprenolol pindolol, alprenolol, . . .	[94]
[1-(9-Fluorenyl)-ethyl]-chloroformate (FLEC)	Propranolol, atenolol, practolol	[95]
(*R*)- or (*S*)-Naphthylethylisocyanate (NEIC)	Oxprenolol, nadolol acebutolol, sotalol, celiprolol, acebutolol	[96,97]
(*S*)-(−)-Methyl chloroformate (MCF)	Sotalol, moprolol, metoprolol	[98]
(*S*)-(−)-α-Methylbenzyl isocyanate reagents	Terbutaline, pindolol	[99,100]
1-(6-Methoxy-2-naphthyl)-ethyl isothiocyanate (NAP-IT); 2-(6-Methoxy-2-naphthyl)-1-propylchloroformate (NAP-C)	Metoprolol, alprenolol, acebutolol, atenolol, diacetolol, propranolol	[101]
N-[2-Isothiocyanato-cyclohexyl]-(3,5)-dinitrobenzoylamide (DDITC)	16 β-blockers	[102]
(1*R*,2*R*)-1,3-Diacetoxy-1-(4-nitrophenyl)-2-propyl isothiocyanate (DANI)	12 β-blockers	[103]

2.2. Indirect separation

The indirect method involving a derivatization step with a suitable chiral tagging reagent is an efficient technique for the separation of many enantiomers. The mechanism of indirect separation is based on the use of chiral derivatization reagents to form a mixture of diastereomers, the diastereomers differing in their physicochemical properties can hence generally be separated by conventional CSPs or by the CSPs mentioned above. The indirect derivatization method is suitable for trace analysis of enantiomers in biological samples because of the option of coupling with highly sensitive reagents that possess high-molar absorptivities or high-fluorescence quantum yields.

Some specialized articles are reported on the use of chiral derivatization reagents for the enantioseparation of several adrenergic drugs. The most frequently used derivatization reagents and their application for the enantioseparation of these drugs in HPLC are listed in Table 4.

2.3. SFC techniques

Although HPLC is the most extensively used technique for enantioseparation of adrenergic drugs, other methods such as SFC have also been reported for this purpose. SFC can be applied to investigate these drugs; carbon dioxide is often being used as mobile phase which can be modified to a certain extent with organic additives; separation can be carried out either in packed or in open columns. Compared to HPLC, SFC has been used less frequently for chiral separation, however some research was performed on adrenergic drugs [104]. Recently, Gyllenhaal and Karisson [105] reported enantiomeric separations of β-blockers by packed-column SFC on hypercarb with L-(+)-tartaric acid as chiral selector. Liu et al. [106] compared three macrocyclic glycopeptide chiral selectors, i.e., Chirobiotic T, its aglycone Chirobiotic TAG and Chirobiotic R, and evaluated them with SFC mobile phases to separate a series of β-blockers. Macrocyclic antibiotic CSPs were evaluated in packed-column SFC for the separation of different types of racemic compounds including β-agonists. Polysaccharide CSPs were used in SFC for the enantioseparation of β-agonists by Medvedovici et al. [107].

3. Enantioseparation by capillary electrophoresis

CE can be considered as complementary to other analytical techniques such as LC for enantioseparation, it presents a number of advantages: (1) the amounts of samples and separation buffer are much less than those used in HPLC; (2) usually the chiral selectors are dissolved in the background electrolyte (BGE) and thus the expensive chiral columns are not required; (3) higher efficiencies and shorter analysis times are obtained than in HPLC. On the other hand the disadvantages of CE in comparison to HPLC are the lower reproducibility, the poorer sensitivity, the smaller possibility of preparative applications, and moreover transfer of CE is not always straightforward in view of high uncertainty in quantitatives analysis [29,108]. CE techniques can also be classified in two ways, either indirect CE using chiral derivazation agents or direct CE using chiral selectors as additives to the electrolyte. The latter method is frequently used in the enantioseparation of adrenergic drugs, and it achieves efficient resolution.

3.1. CDs as chiral selectors

CDs are the most frequently used chiral selectors in CE. The earlier applications of CDs for chiral separations were in ITP, CGE and CZE, and the enantioseparation of adrenergic drugs has also been reported [109–111]. The solubility and the selectivity of the CDs can be improved by derivatization.

3.1.1. Neutral CDs

The neutral CD-derivatives such as heptakis-*O*-methyl-CD, heptakis-(2,6-di-*O*-methyl)-CD (DM-CD) and HP-CD allow the separation of charged analytes, but cannot be used to resolve neutral analytes as neutral CDs migrate with the same velocity as the electroosmotic flow (EOF).

The applications of CDs in CE have been reported in many reviews [112,113], a series of adrenergic drugs were effectively separated. Agonist enantiomers were baseline-separated by DM-CDs and HP-β-CDs in aqueous solution and in plasma samples [114,115]. Our group [116,117] reported on dispersed nanoparticles modified with single layer β-CD and HP-β-CD as chiral selectors for the enhancement of the enantioseparation of clenbuterol and propranolol enantiomers.

Several parameters, such as the concentration of the chiral selector, pH, the nature and ionic strength of the background electrolyte and the addition of organic modifiers were found to have an important influence upon both separational efficency and resolution.

3.1.2. Charged CDs

The use of charged CDs in chiral separations by CE can expand the resolution area in comparison with neutral CDs. Charged CDs offer much higher flexibility in optimizing separation conditions [118]. In the charged mode, they offer additional electrostatic interactions via ion pairing with analytes of opposite charges. This has a large influence upon selectivity and resolution. The negatively charged CDs show a counter-current mobility against EOF, as a result of which they were used to separate neutral and basic chiral compounds. Almost all of the adrenergic drugs are alkaline [119], which can be separated by these negatively charged CDs. The enantiomers of a series of adrenergic drugs were separated using heptakis-(2,3-dimethyl-6-sulfato)-β-CD, CM-β-CD, methylated glucuronyl glucosyl β-CD (Me GUG β-CD), sulfated-β-CD and octakis-(2,3-diacetyl-6-sulfato)-γ-CD at different pH backgrounds by CE and CZE [120-124]; acetonitrile and methanol were used as organic modifiers to improve the resolution. There was a significant increase in migration time, which could be attributed to the decreased EOF, and optimal separating conditions of the enantiomers were achieved.

3.1.3. Dual CD systems

Dual CD systems include a combination of a neutral and a charged CD, or of two oppositely charged CDs. The system may offer a solution when a single CD cannot baseline-separate the enantiomers. For instance, β-CD, DM-β-CD and HP-β-CD are frequently applied as a supplementary selector for enantioseparations.

Recently, a dual mixture of neutral CD, HP-β-CD and DM-β-CD was investigated to enantioseparate the salbutamol enantiomers, and the separation conditions were optimized [125]. Chinaka et al. [109] applied a mixture of β-CD and DM-β-CD to produce simultaneous chiral analysis of adrenergic agonists. Matthijs et al. [126] presented dual CD systems consisting of one highly sulfated (α-, β-, and γ-HSCD) and one neutral CD for the chiral resolution of the enantiomers of atenolol, nadolol, pindolol and propranolol. The same dual CD system was used for separating and quantifying ephedrine and pseudoephedrine stereoisomers [127].

3.1.4. CD-mediated micellar electrokinetic chromatography (CD-MEKC)

CDs have often been used in combination with achiral surfactants such as sodium dodecyl sulfate (SDS). Uncharged CDs migrate with an identical velocity to that of the bulk solution while negatively charged micelles migrate in the direction opposite to the EOF. Partition of a hydrophobic analyte takes place between the bulk solution, the CD and the negatively charged SDS micelle, thus retaining the analyte. This principle enabled the enantioseparation of a broad spectrum of drugs including some adrenergic blockers.

3.2. Linear polysaccharides as chiral selectors

Linear carbohydrates were added to the running buffer solution. The pH of the buffer and the concentration of the polysaccharide are two important factors affecting the enantioselectivity, other than the species of the chiral selector [127].

The chiral separation mechanism is assumed to rely on binding of the aromatic group of the adrenergic drug, lateral-binding forces such as hydrogen bonds and dipole–dipole interactions [128]. The neutral polysaccharides contain dextrin, dextran and amylose, and the charged ones contain heparin, chondroitin, dextran sulfate and λ-carrageenan; the negatively charged polysaccharides have been used for the chiral separation of adrenergic drugs.

Nishi's group produced reviews [128,129] on enantioselectivity in CE with polysaccharides. Phinney et al. [130] applied citrus pectins – a family of heterogeneous polysaccharides found in the cell walls of higher plants contributing to the firmness and structure of plant tissue – to enantiomerically separated salbutamol. Beck and Neau [131] used λ-carrageenan for the enantioseparation of pindolol and propanolol. Gotti et al. [132] designed experiments for the enantioresolution of salbutamol using the mucopolysaccharide dermatan sulfate, and optimized the experimental design; the presence and percentage of the organic modifier (methanol) was tested in order to improve the resolution.

3.3. Proteins as chiral selectors

Up to now, addition of proteins to the running buffer is the most common method employed in protein-based chiral CE. This method which is called affinity electrokinetic chromatography (AEKC) or affinity capillary electrophoresis (ACE) can be applied for the separation of chiral drugs provided that both enantiomers bind the protein to a different extent [56]. The interaction of the drugs with the protein results in a change of the net mobility of the analyte, which means that the solute and solute–protein complexes have different mobilities.

Gagyi et al. [133] studied the enantioseparation of some β-blockers by CZE using human serum transferrin. Xu and Yu [134] separated propranolol with HSA and porcine serum albu-

min by CE in combination with the partial filling technique, and Martínez-Pla et al. [135] described a simple and fast method for enantiomeric resolution of the same drug by ACE using HSA as chiral selector, the effect of several experimental variables such as HSA concentration, temperature, chiral selector plug length and addition of organic modifiers (1-propranol), on the separation was evaluated. Martínez-Gómez's group [136,137] dealt with four adrenergic enantiomers by AEKC-partial filling technique using HSA; protein concentration, running pH and protein solution plug length were optimized. Besides, enzymes were also found to be useful chiral selectors, such as fungal cellulose and CBH I, which were used for the chiral recognition of β-blockers [83,109]. Ovomucoid (OVM) was reported for the enantioseparation of some basic drugs including adrenergic compounds [138].

The use of proteins as selectors for the enantioseparation of drugs presents some advantages and disadvantages: proteins may bear positive or negative charges depending on the pH of the background electrolyte, their charges provide them electrophoretic mobility for separation. However, the absorption of proteins onto the CE column would cause low reproducibilities, zone broadening and low-efficiency effects [12].

3.4. Macrocyclic antibiotics as chiral selectors

Macrocyclic antibiotics possess several asymmetric centers and some groups that can form host–guest inclusion complexes and hydrogen bonds. Ionic, dipole–dipole, π–π, hydrophobic interactions and steric repulsions are also assumed to produce effects. Rifamycin B and rifamycin SV are more frequently used as chiral selectors in CE.

Rifamycin B is negatively charged and is suitable for resolving adrenergic agonists and antagonists, while rifamycin SV seems particularly suited for separating molecular structures containing at least two rings. Ward et al. [139] successfully separated chiral adrenergic drugs which bear two rings in their structures using rifamycin SV in the phosphate buffer with the organic modifier 2-propanol. Erythromycin A (EA) belonging to the group of macrolide antibiotics, was investigated for its potential as chiral selector to enantioseparate adrenergic drugs [140].

Macrocyclic antibiotics have been extensively used in the past decades but are scarcely employed in recent years; the main reasons are their strong UV absorbance, low stability and ready adsorption on the capillary wall, which limits the detection of substrates when these selectors are used. To overcome these problems, adapted methods and counter-current processes were applied [141], a counter current process was created by using coating capillary to suppress EOF, whereby the chiral selectors migrate away from the detection zone, thus improving sensitivity.

3.5. Crown ethers as chiral selectors

Up to date, only (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid ($18C_6H_4$) has been proved effective as a chiral selector for CE. Its selective ability to recognize analytes bearing a primary amine function makes it highly suitable for resolving drugs possessing this group, and the interactions between analytes and $18C_6H_4$ are assumed to be based on inclusion complexes, ionic-, dipole–dipole interactions or hydrogen bonds. Nishi and Mori's groups successfully used $18C_6H_4$ for the enantioseparation of these drugs [142,143]. Chiou and Shih [144] used 18-crown-6 and 15-crown-5 as modifiers for resolving these drug enantiomers, and compared both selectors; the effects of the steric hindrance of drugs, pH value, organic solvent and cations in the buffer were also investigated. A series of applications of the chiral crown ethers to various compounds are given in specialized reviews [145].

Crown ethers as chiral selectors are not extensively used for adrenergic drugs because of their selective ability to recognize analytes bearing a primary amine function. Only a few of these drugs bear this group, hence there is a restriction.

3.6. Chiral metal complexes as chiral selectors

Ligand-exchange capillary electrophoresis (LE-CE) is based on the interchange of the drugs and the ligand around the sphere of a central ion. In this way, Cu(II)-L-prolinamide, Cu(II)-L-ornithine, Cu(II)-3-amino-β-CD and a series of ligands were used for the chiral separation of amino acids and some drugs. It has been reported that the addition of organic modifiers to the background electrolyte improves peak shape avoiding tailing due to a reduction in ligand–receptor interaction, thus improving the efficiency of the separation [135]. Schmid et al. [146] have applied Cu(II)-*N*-(2-hydroxyoctyl)-L-4-hydroxyproline as selector for β-blocker enantioseparation, they added to the enantiomers triethylamine (TEA) to adjust the pH of the buffer.

LE-CE is a most efficiency technique for enantioseparation, however the drug enantiomers must bear the functions that may form metal complexes.

3.7. Chiral selectors in nonaqueous solvents

Nonaqueous capillary electrophoresis (NACE) features several advantages regarding solubility of chiral selectors or samples, reduces unwanted interactions with the capillary wall, reduces the influence between the aqueous solution and the analytes, and increases the selectivity. It has an extensive application foreground in CE. A review on chiral separations by NACE was reported recently [147]. Salbutamol enantiomers were completely resolved using capillary zone electrophoresis (CZE) in nonaqueous solvents, the buffer conditions were optimized with 10 mM ammonium formate and 15 mM derivatized CD in methanol acidified with 0.75 M formic acid [123]; an on-line coupling of NACE with electrospray ionization mass spectrometry was also reported under the same conditions [148]. Some adrenergic drugs were successfully enantioseparated by diisoproylideneketogulonic acid as selector, NH_4Ac in methanol with 2-propanol as buffer using a non-aqueous capillary electrophoretic system with UV and mass spectrometric detection, and the ratio between the chiral selector and buffer was optimized [149]. Ketopinic acid and diisoproylideneketogulonic

acid were used as chiral ion-pair selectors in NACE enantiomeric impurity analysis of *S*-timolol and 1*R*,2*S*-ephedrine, conditions were isotachophoresis (ITP) using a BGE containing chiral selectors and KOH in methanol and ethanol (2:3, v/v) [150]. Marini and et al. [151,152] performed a robustness testing of a chiral NACE method using the BGE consisting of a methanolic formic acid with potassium camphorsulfonic acid for the enantioseparation of timolol maleate sample using derivatized CD as chiral selector.

3.8. Chiral surfactants as selectors

Chiral surfactants which contain natural and synthetic compounds are commonly used as chiral selectors in MEKC: the former group contains bile salts, saponines and digitonin, the latter *N*-alkanoyl-L-amino acids, *N*-dodecoxycarbonyl amino acids, tartaric acid-based surfactants and steroidal glucoside-based surfactants [153]. These surfactants are amphiphilic molecules composed of a polar head group and a hydrophobic tail and they bear some chiral centers for enantioseparation. The mechanism of enantioseparation is based on the ability of the adrenergic drugs to form aggregates with the micelles and on the partition coefficients of the drugs between the chiral micelle phase and the electrolyte bulk phase.

Detailed descriptions of the applications of adrenergic drugs enantioseparations are presented: the use of CD-MEKC with bile salts micelles and *N*-dodecoxycarbonyl amino acids has been successful for enantiomeric separations [154]. Eight structurally similar β-blockers were simultaneously enantioseparated by using a new and more versatile alkenoxy amino acid molecular micelle poly(sodium *N*-undecenoxy carbonyl-L-leucinate) as chiral selector, and the parameters of separation conditions were optimized including the temperature, flow rate, pH and the organic modifier [155].

3.9. Indirect separation

Indirect separations are based on diastereomer formation through precolumn derivatization using a chiral derivatizing reagent followed by separating the diastereomers with an achiral running buffer [156]. Several chiral derivatization reagents used in HPLC have also been applied in CE. Recently, enantioseparations of the adrenergic drugs using this technique in CE were reported: 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosylisothiocyanate (GITC) [157] was used to separate the enantiomers of amphetamine, methamphetamine, ephedrine, pseudoephedrine, norephedrine and norpseudoephedrine; *o*-phthaldialdehyde (OPA) [158] was used for dopamine analysis; (+)-1-(9-fluorenyl)-ethyl chloroformate (FLEC) [159] was applied for indirect resolution of β-blocker agents such as propranolol, oxprenolol, pindolol, metoprolol and atenolol.

In CE, since this indirect approach is more complicated and requires an additional reaction step, direct separation is usually preferred. In the indirect mode, the derivatization reagent has to bear high purity (approximately 100%) and corresponding functional groups need to be present in the analyte.

4. Enantioseparation by microchip techniques

In recent years, microchip techniques attracted increasing interest in chiral separation by CE. Comparing the results of chiral separation between microchips and conventional CE, it can be stated that microchip-CE provides overall better analytical performance and higher efficiency, the analysis times being only a few minutes down to seconds [160]. The enantiomers of adrenaline, noradrenaline, ephedrine and pseudoephedrine were separated by CE on a micromachined device by employing CM-β-CD as chiral selector, partly with the additional inclusion of the crown ether 18-crown-6 [161]. Male and Luong [162] designed a CE system equipped with an array of microfabricated interdigitated platinum electrodes and applied the system to chirally recognize epinephrine, norephedrine and isoproterenol by using heptakis-(2,6-di-*O*-methyl)-β-CD as chiral selector.

Microfabrication technology offers the possibility of producing micrometer-scale columns to separate chiral adrenergic drugs, and offers the advantages of high speed and high resolution. Without any doubt, microchip-CE will become a potentially useful tool for high-throughput chiral analysis in the pharmaceutical industry.

5. Enantioseparation by capillary electrochromatography

CEC can be performed in open-tubular capillaries (OT-CEC) and packed capillaries (P-CEC). In the former method, the chiral selector is covalently attached or coated on the inner surface of a capillary; in the latter one, a CSP or an achiral stationary phase in combination with a chiral mobile phase can be used for chiral separations. During the past years, some reviews were reported on this technique [3,112].

Open tubular capillaries are often prepared by physical adsorption of proteins to the capillary wall and covalently linking different types of CDs onto the capillary wall for the enantioseparation.

In the packed capillary technique, CSPs are more frequently used than the achiral stationary phase with a chiral mobile phase. The CSPs contain CD-CSP, brush-type CSP, macrocyclic antibiotics-CSP, polysaccharide-CSP, protein-CSP and others, which are similar to the CSPs in HPLC. The chiral recognition mechanisms are also based on interactions such as hydrogen bonding, π–π interactions and some forces as those occurring in HPLC. Chirobiotic V and Chirobiotic T chiral columns in pressurized CEC were investigated for enantioseparating some adrenergic drugs [163]. Strong cation exchange-type CSP based on β-amino sulfonic acid-terminated dipeptide derivatives and penicillamine sulfonic acid as chiral selectors, immobilized on thiol-modified silica particles (3.5 μm) were synthesized and applied to enantiomerically separate β-blockers by CEC [164,165]. Krause's group [166] reported enantioseparations of β-blockers in fused-silica capillaries packed with silica gel which was modified by covalent attachment of poly-*N*-acryloyl-L-phenylalanineethylester (Chiraspher) or by coating with cellulose tris(3,5-dimethylphenylcarbamate). These modified silica columns could overcome some shortfalls of the

silica-based columns: the pH stability of silicas is restricted to a small range; peak tailing and peak asymmetry can arise from the adsorption of basic analytes on the slightly acidic columns, therefore, the free silica columns are undesirable for the separation of basic samples [167,168]. CEC with and without pressure-assistance was performed in these capillaries using essentially the same experimental setup. Fanali et al. [169] used vancomycin silica stationary phase in packed CEC for the enantioseparation of some adrenergic drugs.

MIPs are used in CEC, either coated as a thin film to the capillary wall, or in the form of a packed capillary, or as monolithic phases. MIPs are prepared by polymerization of a mixture of monomers, a cross-linking agent, an initiator and a chiral template. After polymerization, the chiral template is removed leaving an imprint that can enantioselectively recognize the original template molecule. Schweitz et al. [170] demonstrated the possibility of rapid enantioseparation of propranolol using MIP monoliths prepared by a photoinitiated polymerization reaction and studied the influence of surfactants as electrolyte additives upon the selectivity and chromatographic efficiency of super-porous MIP monoliths to separate metoprolol, atenolol and propranolol in CEC. They also designed a multiple target approach using MIP nanoparticles to chirally separate propranolol [171]. De Boer et al. [172] described a similar method using MIPs with (+)-ephedrines to recognize ephedrine and salbutamol enantiomers.

CEC is considered to be a hybrid method between CE and LC combining the characteristically high-peak efficiency of electrically driven separation methods with the high selectivity of chromatographic stationary phases [9]. As CEC is a relatively young analytical separation technique, more researchers will undoubtedly explore this domain to achieve better separation parameters.

6. Conclusions

The enantioseparation of adrenergic drugs demonstrates that chromatographic and electromigration techniques comprise the most effective methods for chiral pharmaceutical and biomedical analysis. LC can provide outstanding achievements in the resolution of the enantiomers of adrenergic drugs, because in LC there are a variety of CSPs and CMPAs that fit for the enantioseparation of these compounds. CE towards its competitive technique, LC, features mainly low sensitivity and uncertainty in quantitative analysis, but on the other hand high-resolution capacity and versatility. CEC has reached increasing interest and shows extensive applications in recent years because of its high efficiency. In CE and CEC techniques chiral resolution of the adrenergic drugs was successfully achieved because of the multiform chiral selectors.

In the cited techniques, the crucial factor for chiral separations is the choice of the proper chiral selector, which always needs to be properly carried out according to the structure of the adrenergic drugs to be analyzed. A series of chiral selectors were presented that provide good separation resolution in LC and CE. Hence, the research for and the development of novel chiral selectors are most important to enantioseparation analysis in the future. It is expected that the development and improvement of the separation techniques will solve an increasing number of difficult chiral separation problems and extend the applicational scope of chiral analysis.

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