THE ANALYSIS OF PHARMACOLOGICALLY ACTIVE COMPOUNDS AND BIOMOLECULES IN REAL SAMPLES

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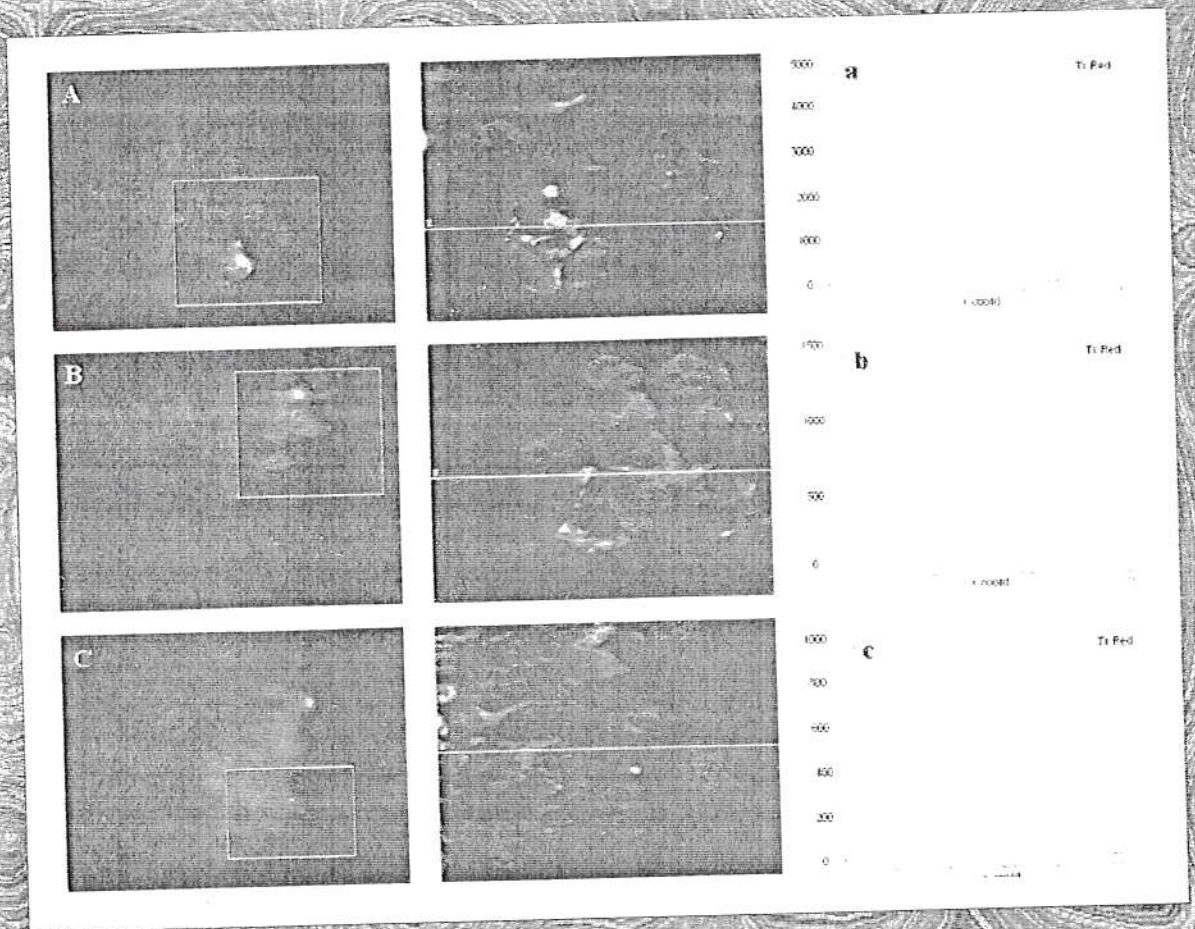

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The Analysis of Pharmacologically Active Compounds and Biomolecules in Real Samples

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3. Recent advances in the applications of selected analytical technics for enantioseparations of ephedrine-type compounds

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Abstract. Ephedrine-type compounds exhibit potent adrenergic activity by agonising the different α (α_1, α_2) and β (β_1, β_2, β_3) receptor subtypes, or releasing the catecholamines from adrenergic neurons vesicles, but some ephedrines show the both modes of action. The chirality of interactions between receptors as chiral entities and related enantiomer of ephedrine-type compound and also as with other drugs is the key factor for pharmacological activity. It is for this reason the ephedrine-type compounds, also as other drugs have to be administrated in their enantiomeric pure form. The enantiomeric purity control requires the development of adequate enantiomeric separation techniques.

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Electromigration techniques (capillary electrophoresis (CE), capillary electrochromatography (CEC)), and high performance liquid chromatography (HPLC) are found to be the choice methods for chiral analysis of ephedrine-type compounds. The mechanisms and modes of these techniques are briefly discussed in this paper. In the cited technics, the crucial factor for chiral separations is the choice of the proper chiral selector, according to the structure of the ephedrines. A series of chiral selectors were presented to provide good resolution in CE, CEC and HPLC.

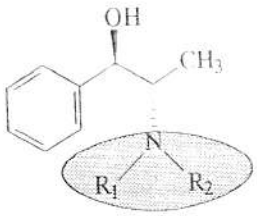
Some enantioseparations of ephedrine-type compounds in raw material, pharmaceuticals, dietary supplements, herbs, herb extracts and biological samples (urine, cerebrospinal fluid and hair) are presented.

Introduction

Drug action is the result of large number of pharmacological and pharmacokinetic processes that take place in the living systems, and the majority of these processes have high degree of stereoselectivity. Most pharmaceutical compounds have chiral centres and effects are due to interactions with chiral biological active sites (receptors or enzymes). Different enantiomers of drugs usually have different pharmacological properties in terms of activity, toxicity, transport mechanism and metabolic route. For these reasons, drugs have to be administrated in their enantiomeric pure form. According to the guidelines issued by the United States Food and Drug Administration (FDA), the presence of the unintended enantiomer in a stereochemically pure drug should be treated in the same way as any achiral impurities [1].

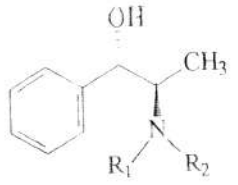
Ephedra sinica, also called *Ma-Huang*, has been used in traditional Chinese medicine for more than 5000 years. The pharmacological effects of *E. sinica* and other *Ephedra* species are principally due to several phenylpropanolamine-type alkaloid components: (1*R*,2*S*)-(-)-ephedrine ((-)-E) (**Figure 1A**), (1*R*,2*S*)-(-)-norephedrine ((-)-NE) (**Figure 1A**), (1*R*,2*S*)-(-)-methylephedrine ((-)-ME) (**Figure 1A**), (1*S*,2*S*)-(+)-pseudoephedrine ((+)-PE) (**Figure 1C**), (1*S*,2*S*)-(+)-norpseudoephedrine ((+)-NPE) (**Figure 1C**), and (1*S*,2*S*)-(+)-methylpseudoephedrine ((+)-MPE) (**Figure 1C**). Their enantiomers: (1*S*,2*R*)-(+)-ephedrine ((+)-E) (**Figure 1B**), (1*S*,2*R*)-(+)-norephedrine ((+)-NE) (**Figure 1B**), (1*S*,2*R*)-(+)-methylephedrine ((+)-ME) (**Figure 1B**), (1*R*,2*R*)-(-)-pseudoephedrine ((-)-PE) (**Figure 1D**), (1*R*,2*R*)-(-)-norpseudoephedrine ((-)-NPE) (**Figure 1D**), and (1*R*,2*R*)-(-)-methylpseudoephedrine ((-)-MPE) (**Figure 1D**) were not found in natural resources [2].

These facts request the determination of the origin of ephedrine-type compounds (natural or synthetic), presented in commercial dietary supplements called "Natural *Ephedra*-containing" products, after enantioseparation. Nowadays, *Ephedra*-containing products are illicit as energy-boosters, weight-loss aids and athletic performance enhancers. Serious side effects, as well as,



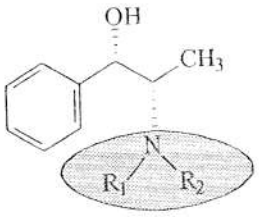
R_1	R_2	Compound
H	H	(−)-NE
CH_3	H	(−)-E
CH_3	CH_3	(−)-ME

A



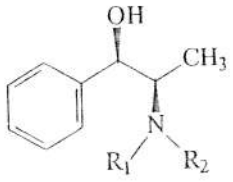
R_1	R_2	Compound
H	H	(+)-NE
CH_3	H	(+)-E
CH_3	CH_3	(+)-ME

B



R_1	R_2	Compound
H	H	(+)-NPE
CH_3	H	(+)-PE
CH_3	CH_3	(+)-MPE

C



R_1	R_2	Compound
H	H	(−)-NPE
CH_3	H	(−)-PE
CH_3	CH_3	(−)-MPE

D

Figure 1. Chemical structure of ephedrine-type compounds. *(A)* Ephedrine derivatives isolated from *Ephedra* sp: (1*R*,2*S*)-(-)-ephedrine((-)-E), (1*R*,2*S*)-(-)-norephedrine((-)-NE) and (1*R*,2*S*)-(-)-methylephedrine((-)-ME); *(B)* Synthesized enantiomers of natural ephedrine derivatives: (1*S*,2*R*)-(+)-ephedrine((+)-E), (1*S*,2*R*)-(+)-norephedrine((+)-NE) and (1*S*,2*R*)-(+)-methylephedrine((+)-ME); *(C)* Pseudoephedrine derivatives isolated from *Ephedra* sp: (1*S*,2*S*)-(+)-pseudoephedrine((+)-PE), (1*S*,2*S*)-(+)-norpseudoephedrine((+)-NPE) and (1*S*,2*S*)-(+)-methylpseudoephedrine((+)-MPE); *(D)* Synthesized enantiomers of natural pseudoephedrine derivatives: (1*R*,2*R*)-(-)-pseudoephedrine((-)-PE), (1*R*,2*R*)-(-)-norpseudoephedrine((-)-NPE) and (1*R*,2*R*)-(-)-methylpseudoephedrine((-)-MPE).

adverse incidents (hypertension, palpitation to stroke), and numerous fatalities were recently reported [3].

Enantiomer (-)-E has strong direct agonistic effect on different α (α_1, α_2) and β (β_1, β_2, β_3) receptor subtypes and some indirect activity, while (+)-E exhibits primarily indirect activity. (+)-PE has virtually no direct adrenergic receptor activity, and it acts indirectly, by releasing cathecolamines from adrenergic neurons vesicles [4-5].

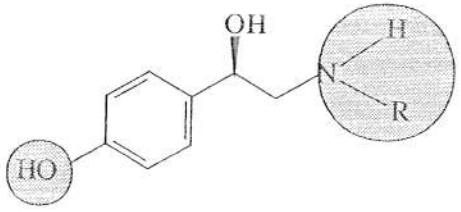
Ephedrine is primarily used in the treatment of asthma or bronchitis, as well as in alleviation of symptoms of cold and influenza, including nasal congestion, cough, fever and chills [6-8].

Nowadays, only the synthetic forms of ephedrine-type compounds are used in commercially available pharmaceuticals.

(1*R*)-(-)-Synephrine ((-)-Syn) (**Figure 2A**) is the main adrenergic amine isolated with (1*R*)-(-)-octopamine ((-)-Oct) (**Figure 2A**), tyramine and N-methyltyramine from unripe peel and edible fruit parts of Bitter Orange (*Citrus aurantium* L. var. *amara*). The synthesized, racemic synephrine ((±)-Syn), because of its low price is widely present on the markets, as a raw material in the production of dietary supplements [9,10].

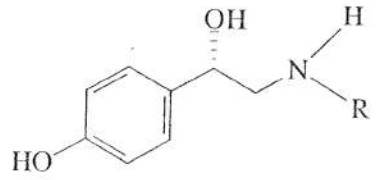
Enantiomer (-)-Syn stimulates predominantly β_3 receptors in the adipose tissue and liver, with a moderate effect on the other subtypes of α- and β-receptors present in the body. This leads to an increased metabolic rate, with minor effect on heart rate and blood pressure [11]. For this reason, (-)-Syn is extensively used in diet pills and weight loss formula. (-)-Syn undergoes chiral inversion *in vitro* and *in vivo*, so the enantioseparation of this compound is crucial in evaluation of activity of herbal extracts and dietary supplements, and in monitoring of (-)-Syn and (+)-Syn levels in urine, after oral ingestions of *Citrus* fruits or dietary-supplements [12].

Chromatographic methods and electromigration techniques have long been the methods of choice in this field and suitable for enantioseparation analysis of ephedrine-type compounds. HPLC can be used either indirectly with chiral derivatization reagents or directly with chiral stationary phases



R	Compound
H	(-)-Oct
CH_3	(-)-Syn

A



R	Compound
H	(+)-Oct
CH_3	(+)-Syn

B

Figure 2. Chemical structure of synephrine-type compounds. ***(A)*** Synephrine derivatives isolated from *Citrus* sp: (1*R*)-(-)-octopamine((-)-Oct) and (1*R*)-(-)-synephrine((-)-Syn); ***(B)*** Synthesized enantiomers of natural synephrine derivatives: (1*S*)-(+)-octopamine((+)-Oct) and (1*S*)-(+)-synephrine((+)-Syn).

(CSPs) or chiral mobile phase's additives (CMPA). In the last decades, capillary electrophoresis (CE), and capillary electrochromatography (CEC) have been developed for routine analysis and extensively applied for the enantioseparation of ephedrine-type compounds, and the main reason being their high efficiency and low solvent and selector consumption.

This paper present an overview of CE, CEC and HPLC techniques for enantoseparation of ephedrine-type compounds.

1. Capillary electrophoresis (CE)

1.1. Capillary zone electrophoresis (CZE)

CZE with its high resolving power has attracted great interest in analysing of different classes of compounds including enantiomers. In CZE chiral selectivity can be obtained either by adding the chiral selector to the running buffer (background electrolyte), or by immobilizing of the chiral selector in the capillary.

Cyclodextrins (CDs) are the most frequently used chiral selectors in CZE. CDs are cyclic oligosaccharide molecules built up of D-(+)-glucopyranose units via α-(1, 4)-linkages. They consist of six (α-CD), seven (β-CD) or eight (γ-CD) glucopyranose units. CDs have a hydrophilic surface and a truncated cone with a hydrophobic cavity. As a chiral selector, CD acts as a host, by incorporating the guest molecule in its hydrophobic cavity to form a 1:1 inclusion complex. The depth of the cavity and the solubility of CDs can be modified by derivatization process. The hydroxyl groups in positions 2, 3 and 6 are available for derivatization [13].

The enantiomers of rac-terbutaline, rac-terbutaline monosulphate, rac-bambuteril, rac-propranolol, rac-ephedrine ((±)-E) and rac-brompheniramine were used as model substances to study the effect of cyclodextrin (type, concentration and degree of substitution), pH and applied running voltage on separation parameters. The CDs used in the experiments were α-CD, β-CD and dimethyl-β-CD (DM-β-CD). The most effective parameters for optimizing resolution were pH, CD type, and concentration level. The running voltage was the most effective in tuning the separation time [14].

The use of charged CDs for enantioseparation of ephedrines often contributes to higher chiral resolving capabilities at lower concentrations than the neutral CDs. The high effectiveness of the charged CDs is the result of an electrostatic interaction between the charged CD and oppositely charged enantiomers as well as the opposite electromobility of the chiral selector and analytes. The self-mobility of sulfated β-CD (HS-β-CD), being opposite to

the electrophoretic mobility of ephedrines, enhances the separation factor provided by the chiral selector [15,16].

Aumatell *et al.* [17] described in detail the enantiomer separation of β-agonists, β-antagonists, phenylethylamines and alcohol stimulants. A total of 42 compounds were enantioselectively resolved using hydroxypropyl-β-CD (HP-β-CD) and 20 with sulfobutyl ether-β-CD (SBE-β-CD). The degree of enantiomeric separation for examined substances is dependent on the modified CDs concentration. The separation efficiency reaches a maximum with a particular CDs concentration, after which the increase of CD concentration causes a progressive decrease in chiral differentiation.

The degree of substitution of carboxymethyl-β-CD (CM-β-CD) and the ionic strength have significant effects on the resolution of some basic racemic drugs (oxprenolol, ephedrine, and aminoethyl-benzodioxane derivative). The ionic β-CD derivatives altered the ionic strength/conductivity of separation media, so the buffer capacity of the applied buffers has to be taken into consideration when the working CM-β-CD concentration is determined [18].

The capillary temperature also plays a significant role in the efficiency of chiral separations with CD-CZE. An improved resolution of enantiomers at subambient temperatures was reported by several authors [19-22]. The separation of enantiomer pairs of β-hydroxyphenethylamine, norephedrine, octopamine, norepinephrine, epinephrine and isoproterenol by CZE was investigated with DM-β-CD as the chiral selector at temperatures ranging from -20° to 40°C. The effect of temperature on enantioselectivity was found to depend on the number of phenolic hydroxyl groups in the molecule. Upon lowering the temperature from 40° to -20°C, the chiral selectivity of the system, as measured by the relative mobility difference, increased tenfold for the amines with two vicinal phenolic groups, whereas the increase was insignificant for those having no phenolic group [23].

The possibility for obtaining reversal ephedrine enantiomer migration order was presented in paper by Schmitt *et al.* [24]. On principle, three different approaches can be used. Firstly, electroosmotic flow (EOF) can be reversed using different additives in the running buffer. Secondly, selecting different CDs as chiral additives can also reverse the migration order due to different separation and complexation mechanisms. Especially derivatized CDs have a great potential for this application and additionally contribute to the number of chiral selectors available in CE. In the third case, varying the pH value charged CDs offer possibility for a reversal migration order [24].

Some CDs have been widely used as chiral selectors in different running buffers for the enantiomeric separations of β-hydroxyphenylethylamines, β-agonists, β-antagonists and other basic compounds: β-CD, DM-β-CD [25,26], CM-β-CD [27].

Liu *et al.* [28] described a simple, systematic method for rapidly screening potential CE separation conditions for small, amine containing enantiomers. During method development, 39 pairs of enantiomers were investigated, and a partial or complete separation was achieved in each and every case. The screening strategy uses a bare fused silica capillary and a pH 2.5 amine-modified phosphate running buffer containing one of the selected CDs: DM-β-CD, HP-β-CD, hydroxypropyl-α-CD (HP-α-CD), hydroxypropyl-γ-CD (HP-γ-CD), and HS-β-CD.

Hellriegel *et al.* [29] compared the ability of different CDs for enantioseparation of (±)-E, and (±)-PE (β-CD, heptakis(2,3-*O*-diacetyl)-β-CD (DA-β-CD) and heptakis(2,6-diacethyl-6-sulfato)-β-CD (DAS-β-CD)), under the same running conditions: uncoated fused silica (60 cm x 50 cm x 50 μm), 25°C, +20 kV, pressure injection (5 s), 194 nm, with 50 mM phosphate running buffer (pH 3.0). The concentrations of CDs were 12 mM for (β-CD, DA-β-CD) and 3 mM (DAS-β-CD). The usage of β-CD, as the neutral CD showed no baseline separation of all enantiomers and diastereoisomers, but the separation was sufficient for determination of the migration order: (+)-PE, (+)-E, (-)-E, (-)-PE. The neutral and modified CD, DA-β-CD, have completely changed the migration order, causing a faster migration of (-)-enantiomers, than in case of the corresponding (+)-enantiomers. The migration time of all isomers was much shorter than with β-CD. With DAS-β-CD, again the (-)-enantiomers were migrating faster than (+)-enantiomers, but the reversal diastereoisomers migration order was obtained instead. A very high resolution power of DAS-β-CD has resulted in the baseline separation of all isomers.

For detection UV cell in CE is easily made by burning off a short section of the polyamide coating on the fused-silica capillary. However, this tiny flow cell limits the detection sensitivity of even UV-absorbing analytes. More importantly, UV is not a specific detector that can provide structural information suitable for characterization of organic compounds [30].

Sheppard *et al.* [30] used ion spray mass spectrometry (IS-MS) in the positive ion mode in providing the structural characterization after CZE enantiomeric separations of terbutaline and ephedrine, while Iio *et al.* [31,32] used same method for identification and quantification of enantiomers of ephedrines and amphetamines after CZE enantiomeric separations with DAS-β-CD, as a chiral selector.

A Contactless conductivity detector is successfully demonstrated for the enantiomeric separation of basic drugs and amino acids in CE. The derivatization of compounds or the addition of a visualization reagent as in indirect optical detection schemes was not required. Non-charged chiral selectors (HP-β-CD) and acidic buffer solutions prepared with lactic or citric acid were employed in case of basic drugs, including ephedrines [33].

Table 1. Application of CE and CEC for enantioseparation of ephedrine-type compounds in raw-materials, pharmaceuticals and dietary supplements.

Compound	Application	Running buffers	Capillaries and running conditions*	Ref.
Ephedrines	NIST SRMs (SRMs 3240 - 3244)	15 mM CH_3COONH_4+35 mM poly-L-SUCL buffer, containing acetonitrile, 30% (v/v),(pH 6.0)	Uncoated fused silica (120 cm x 50 μm); 20°C; +30 kV; pressure injection (5 mbar/2s); 254 nm (60 cm); MS	2
Ephedrine	Raw material	25 mM TEAM-PO_4 buffer (pH 2.5), with 7.5% HS-β-CD	Uncoated fused silica (60 cm x 50 cm x 50 μm); 20°C; -25 kV; pressure injection (21 mbar/5s); 200 nm	34
Amphetamines, Ephedrines	Raw materials, tablets	50 mM TBA-PO_4 buffer (pH 2.5), with 20 mM DM-β-CD	Uncoated fused silica (65 cm x 64.5 cm x 50 μm); 25°C; +30 kV; pressure injection (50 mbar/4s); 200 nm	35
Ephedrines	Tablets, solutions, herbs and herbal extracts	75 mM Tris-phosphate buffer (pH 2.5), with 40 mM DM-β-CD	Uncoated fused silica (57 cm x 50 cm x 75 μm); 25°C; +25 kV; pressure injection (34.5 mbar/5s); 195 nm	36
Ephedrines	Nutritional supplements	Solution of 70 mM HP-β-CD, 30 mM TMA-Cl and 10 mM SDS (pH 2.0, H_3PO_4)	Uncoated fused silica (90 cm x 65 cm x 50 μm); 31°C; +28 kV; vacuum injection (250 mbar/s); 210 nm	37
Ephedrines, synephrine	Dietary supplements	55 mM NaH_2PO_4+10 mM, $Na_2B_4O_7$, buffer (pH 8.6), containing methanol, 30% (v/v), with 20 mM HP-β-CD and 7 mM β-CD	Uncoated fused silica (110 cm x 101.5 cm x 50 μm); 37°C; +30 kV; pressure injection (50 mbar/5s); 210 nm	38
Ephedrines	NIST SRMs (SRMs 3240 – 3244)	25 mM NaH_2PO_4 buffer (pH 2.5), with 2.8% HS-β-CD and 1.2% DM-β-CD	Uncoated fused silica (80.5 cm x 72 cm x 75 μm); 25°C; -15 kV; pressure injection (25 mbar/5s); 210 nm	39
Ephedrines	NIST SRMs (SRMs 3240 – 3244)	25 mM NaH_2PO_4 buffer (pH 2.5), with 4.0% DM-β-CD	Uncoated fused silica (80.5 cm x 72 cm x 75 μm); 25°C; +30 kV; pressure injection (25 mbar/5s); 210 nm	39
Ephedrines	NIST SRMs (SRMs 3240 – 3244)	25 mM NaH_2PO_4 buffer (pH 2.5), with 4.0% HP-β-CD	Uncoated fused silica (80.5 cm x 72 cm x 75 μm); 25°C; +25 kV; pressure injection (25 mbar/5s); 210 nm	39
Ephedrines	Unregulated drugs	50 mM KH_2PO_4 buffer (pH 2.6), with 20 mM DM-β-CD	Uncoated fused silica (64.5 cm x 56 cm x 50 μm); 20°C; +30 kV; pressure injection (50 mbar/3s); 195 nm	40

Table 1. Continued

Ephedrines	*Ephedra* plant extracts	20 mM phosphate buffer (pH 9.0), containing 2-propanol, 20% (v/v), with 10 mM L-leucine.	Uncoated fused silica (43 cm x 38.2 cm x 50 μm); 25°C; +15 kV;pressure injection (1379 mbar/s); 190 nm	44
Ephedrine	Raw material	100 mM (-)-DIKGA and 40 mM KOH in methanol/ethanol mixture (40:60, v/v)	Polyacrylamide coated fused silica (32 cm x 23.5 cm x 50 μm); 25°C; +30 kV; pressure injection (35 mbar/5s); 200 nm	58
Ephedrine	Raw material	acetonitrile-methanol mixture (80:20, v/v), containing 50 mM HCOOH and 25 mM 2-amino-1-butanol	(*S*)-penicillamine sulfonic acid-derived coated fused silica (33.5 cm x 25cm x 100 μm); 20°C; +15 kV; external pressure 8000 mbar; electrokinetic injection (+5 kV/5s); 200 nm	59

* The pressure units in the cited papers are converted into mbar.

CZE is widely used for the enantiomer separations of ephedrine-type compounds in raw material, drug formulations, herbal material and dry extracts [34-40,44] (**Table 1.**).

Commercial HS-β-CDs are usually sulfated either at position 6 or at positions 2 and 6, but not at position 3. The average sulfate content usually falls within the range of 6 to 8 per CD molecule. MS and nuclear magnetic resonance spectroscopy (NMR) were employed to characterize these HS-β-CDs. Enantiomeric separations by CZE using HS-β-CDs as chiral selectors and the running buffer containing triethylammonium phosphate (TEAM-PO_4), showed that these CDs exhibited similar chiral selectivity and resolution of ephedrine enantiomers. One derivative of HS-β-CD was tested for the enantiomeric purity evaluation of (-)-ephedrine contained in the raw material. The quantification was achieved by comparing the corrected peak areas of the minor enantiomer and (-)-ephedrine [34] (**Table 1.**).

The CZE method using DM-β-CD as a chiral selector, and tetrabutylammonium phosphate (TBAM-PO_4) as a cation has been successfully developed and optimized for the simultaneous chiral separation of the amphetamine-type stimulants and their ephedrine enantiomer precursors. The sensitivity and precision of the method were found to be sufficiently good for application in the chiral analysis of seized amphetamines in both crystalline and tablet forms [35] (**Table 1.**).

Liu *et al.* [36] (**Table 1.**) reported the method for enantioseparation of ephedrines, using DM-β-CD, with hydroxymethyl aminomethane (Tris)-phosphate buffer, in a series of drugs such as antitussive, antirheumatic

drugs, the drug for rhinitis and the Chinese herbal preparations made from the plants in the genus *Ephedra* (*Ma-Huang* in Chinese). The detection limits (S/N = 3) ranged from 65-161 ng/mL, and the linear range was 0.15 to 101.00 μg/mL, for pressure injection. In real samples, the recoveries of ephedrine and related compounds ranged from 97.6 to 103.5%.

Using the running buffer containing HP-β-CD, tetrabutylammonium chloride (TBAM-Cl) and sodium dodecyl sulfate (SDS) at pH 2.0, the separation, identification and quantification of ten stereoisomers in the ephedrine family was described. Calibration plots of the ephedrines were linear over the range 4-100 μg/mL. The method was used in the analysis of nutritional supplements in the form of capsules containing *Ma-Huang* [37] (**Table 1.**).

From National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs) enantiomers of ephedrines and synephrine were separated, after extraction using different electrophoretic conditions [38,39] (**Table 1.**).

Unregulated drugs, referred to as dietary supplements in U.S.A., have been legally used as tonic agents, but illicit substances such as ephedrines were often detected. CZE was applied to the enantiomeric separation of chiral ephedrine derivatives in these materials, using DM-β-CD [40] (**Table 1.**).

The application of CM-β-CD as a chiral selector, and a borate running buffer in the CZE separation of different neurotransmitters (serotonin, phenylalanine, dopamine, adrenaline and DOPA) including ephedrine and propranolol in cerebrospinal fluid and pharmaceuticals, using single run analysis was presented by Maruszak *et al.* [41] (**Table 2.**).

Applications of CZE/MS [31,32], and CZE/UV [42] (**Table 2.**) for the separation and detection of enantiomers of ephedrines and amphetamines in urine samples, from methamphetamine and dimethylamphetamine addicts and patients under selegiline pharmacotherapy, without further extraction process (with simple filtration), were reported.

The chiral separation of phenethylamines in a CD-CE-based forensic analysis, and its preliminary application to the analysis of human urine and hair, using β-CD, after liquid-liquid extraction procedure was published in 1997 [43] (**Table 2.**).

The separation and determination of ephedrine enantiomers by CE was performed using L-leucine as a chiral selector. This method is suitable for the analysis of ephedrine and related compounds in *Ephedra* plant extracts [44] (**Table 1.**).

Crown ethers are macrocyclic polyethers known to form host-guest complexes with alkali- and earth-metal ions as well as primary ammonium cations. If a chiral crown ether is used, the inclusion of primary amines is stereoselective. The formation of hydrogen bonds between 3 hydrogens attached

Table 2. Application of CE CEC for enantioseparation of ephedrine-type compounds in biological samples.

Compound	Application	Running buffers	Capillaries and running conditions*	Ref.
Amphetamines, ephedrines	Urine	1 M HCOOH (pH 1.7), with 0.85 mM DAS-β-CD	Uncoated fused silica (100 cm x 50 μm); 20°C; +30 kV; pressure injection (50 mbar/12s); MS	31
Amphetamines, ephedrines	Urine	1 M HCOOH/1 M $HCOONH_4$ (pH 2.0) (10:0.2 v/v), with 1.5 mM DAS-β-CD	Uncoated fused silica (100 cm x 50 μm); 20°C; +30 kV; pressure injection (50 mbar/12s); MS	32
Neurotransmiters, ephedrine	Cerebrospinal fluid, pharmaceuticals	20 mM $Na_2B_4O_7$ buffer (pH 7.5), with 20 mM CM-β-CD	Uncoated fused silica (75 cm x 65 cm x 50 μm), room temp.; +20 kV; hydrostatic injection (5s); 214 nm	41
Ephedrines	Urine and nutritional supplements	260 mM Tris-phosphate buffer (pH 3.5), with 13.3 mM DM-β-CD	Uncoated fused silica (48.5 cm x 40 cm x 75 μm), 25°C, +30 kV; pressure injection (25 mbar/20s); 195 nm	42
Amphetamines, ephedrine	Urine, hair	150 mM phosphate buffer (pH 2.5), with 15 mM β-CD	Uncoated fused silica (47 cm x 40 cm x 50 μm); 17.5°C, +10 kV; pressure injection (34.5 mbar/5s); 200 nm	43
Ephedrine	Urine	25 mM Na_2HPO_4+ 25 mM $Na_2B_4O_7$ buffer, (pH 8.8), with 50 mM (*S*)-DDCV	AccuSep capillary (60 cm x 52.5 cm x 50 μm); room temperature; +12 kV; hydrostatic injection (2s); 214 nm	62

*The pressure units in the cited papers are converted into mbar.

to the nitrogen of the analyte and dipoles of the macrocyclic ether oxygen are assumed to be the main interactions. Additionally, crown ether substituents are perpendicular to the plane of the macrocyclic ring, forming a chiral barrier, which divides the space available for the substituents at the chiral centre of the analyte into two domains. Thus, two different diastereomeric inclusion complexes are formed [45].

The only one chiral crown ether used up to now in CE is 18-crown-6-tetracarboxylic acid (18C6H4), and that had found application in the chiral separation of sympathomimetics and aminoalcohols [46,47].

Dual selector systems containing either two chiral selectors or one chiral selector and a separation-supporting agent have been found to improve or even enable amino alcohols enantioseparation in several cases, by forming the "three body" complexes between the amine group, CD and 18C6H4. The enantiomers of adrenaline, noradrenaline, ephedrine and pseudoephedrine were separated by CE on a micromachined device (microchip-CE). The detection was carried out with a new two-electrode amperometric detector, eliminating the need for individual counter and reference electrodes. The separation of investigated isomers was achieved by employing CM-β-CD, as a chiral selector in the buffer, partly with the additional inclusion with 18C6H4 [48].

Macrocyclic antibiotics, rifamycins, have a highly substituted aliphatic bridge, but they differ in the type and location of the substituent in their

naphthohydroquinone ring. Rifamycin B, as the most exploited rifamycin in separations of enantiomers, has nine stereogenic centres, and in addition to its aromatic ring systems, it has four hydroxyl groups, one carboxylic acid moiety, one carboxymethyl group, and one amide bond. The semirigid basket-shaped aglycan, having hydrophobic properties, enables the formation of host-guest inclusion complexes and due to pendant polar arms, which can form hydrogen bonds. Ionic, dipole-dipole, π-π, hydrophobic interactions and steric repulsion are assumed to have effects in complex formation [49].

Armstrong *et al.* [50] showed that negatively charged rifamycin B could associate with several chiral amino alcohols (including (±)-Syn, (±)-Oct, (±)-E and (±)-PE) and enantioselectively resolved them. In resolving overcome detection problems arising from the strong UV-absorption of the selector, a counter-current process has been applied. The migration times, electrophoretic mobilities and enantioselectivity were controlled and optimized by adjusting the concentration of the chiral selector (25 mM) in the running buffer, as well as the pH (7.0), organic modifier type (2-propanol, 30% w/w), buffer type and capacity (0.1 M phosphate buffer).

1.2. Chiral ligand exchange capillary electrophoresis (CLE-CE)

CLE-CE has been showed to be a simple and rapid approach for the chiral resolution of chelate complex-formatting compounds. The basic separation principle was found to be applicable to the capillary electrophoresis, by simply using the chiral selector-metal complex, as an additive to the electrolyte.

An *N*-(2-hydroxyoctyl)-L-4-hydroxyproline (OH-L-Hypro)-copper(II) complex in molar ratio of 1:2 is found to be the most efficient for enantiomeric separation of amino alcohols (symmphatomimetics and β-blockers).

Optimal pH value for the complexation of these amino alcohols was found to be 12 in the running buffer containing ammonia [51]. For *R*-(-) enantiomers were found to elute before the *S*-(+)-enantiomers. Schmid *et al.* [52] found and commented that the selector is negatively charged at high pH, and migrates in opposite direction than the EOF. This results in lower analytes migration velocity than the EOF [52]. The conclusion that the more strongly retained *S*-(+) isomer must show higher affinity to the selector does not seem to be logical. The moment when the selector forms a ternary copper complex with the analyte, the total charge of the new species becomes neutral or positive, depending on the dissociation of the amino alcohol hydroxyl group. Therefore, the stronger bound enantiomer should be expected to move faster, as a component of a neutral ternary complex, not as a free anion. In any case, one must consider relative charges and possible electrophoretic

mobilities of the analyte-containing species only, not those of the selector itself. It is noteworthy that drugs having one (e.g. octopamine) or two (e.g. orciprenaline) weak acidic phenolic groups migrate substantially slower [53].

The enantiomeric resolution of ephedrine and pseudoephedrine, using L-ornitine (L-Orn) and copper(II) in constant molar ratio of 2:1, with acetate buffer at pH 8.0 was reported [54].

The crucial parameter for an optimal complexation of L-tartaric acid and L-threonine (as chiral selectors) with copper(II) for enantioselective CLE-CE of drugs containing amino alcohol structure was the pH value of 12. In the case of tartaric acid, a selector/copper(II) molar ratio of 2:1 was found to be optimal, but in the case of threonine the optimal molar ratio was about 4:3 [55].

1.3. Non-aqueous CE (NACE)

Non-aqueous solvents show several advantages regarding solubility of chiral selectors or samples and reduce undesirable interactions with the capillary wall. Often an increase in selectivity can be observed in non-aqueous solvents. Different forms of chemical equilibria in aqueous and non-aqueous systems can lead to different selectivities. Weak interactions disrupted by water can become effective in non-aqueous systems. A lower Joule heating is produced and so a higher voltage can be applied, with resultant shorter retention times [56].

Vincent and Vigh demonstrated the advantages of use of a single isomer DAS-β-CD in pure methanol for the chiral separation of basic drugs, including the ephedrines [57].

Hedeland *et al* [58] (**Table 1.**) presented the validated method for determination of enantiomeric purity of the (-)-ephedrine raw material using 2*R*,3*S*,4*R*,5*S*-(-)-2,3:4,6-di-*O*-isopropylidene-2-keto-L-gulonic acid [(-)-diisoproylideneketogulonic acid; (-)-DIKGA] as a chiral ion-pair selector in NACE. Using 1*S*,4*R*-(+)-ketopinic acid, as a chiral ion-pair selector in NACE was not efficient for enantioresolution of ephedrine.

2. Capillary electrochromatography (CEC)

CEC can be regarded as a hybrid method between CE and HPLC combining the efficiency between the CE and selectivity of stationary phases. While in HPLC a conical flow profile is produced by the hydrodynamic flow, in CEC a rather plug-like flow profile is generated by electroosmotic flow, resulting in higher efficiency. A chiral selector can be covalently attached or coated on the inner surface of a capillary (open tubular CEC), or an achiral stationary phase is combined with a chiral mobile-phase mode (CMP) or a chiral stationary phase mode (CSP), (packed CEC) [13].

Bicker *et al.* [59] (**Table 1.**) reported the validated nonaqueous CEC method for the enantioselective impurity profiling of D-ephedrine, using a low-molecular-weight strong chiral cation exchanger, based on penicillamine sulfonic acid, immobilized on thiol-modified silica particles (3.5 μm). Under the optimized conditions, the ephedrine enantiomers were separated on this CSP, with an enantioselectivity of 1.11, resolution value of 4.77, and satisfactory average efficiency. For L-ephedrine raw material the strong cation-exchange (SCX)-type CSP with opposite configuration was utilized, so that the enantiomeric impurity eluted before the main component peak and yielding similar results in terms of separation and validation [59].

Hebenstreit *et al.* [60] synthesized and evaluated SCX CSPs based on β-amino sulfonic acid-terminated dipeptide derivatives as chiral selectors, immobilized on thiol-modified silica particles (3.5 μm), for enantiomer separations of chiral bases by CEC. These studies included a variation of the acid-terminal amino sulfonic acid residue, a variation of the configurations, *i.e.*, a comparison of the diastereomeric (*S*,*S*)- and (*R*,*S*)-configurations of the sulfopeptides, and finally a comparison of sulfodipeptide selectors with corresponding β-amino sulfonic acid analogues.

Boer *et al.* [61] used spherical molecularly imprinted polymer particles for full baseline separation of racemic ephedrine within 10 min. Analysis were performed via a partial filling technique using (+)-ephedrine-imprinted microspheres (100-200nm), which were polymerized from methacrylic acid and 1,1,1-Tris(hydroxymethyl)propanetrimethacrylate, using acetonitrile as the solvent.

2.1. Micellar electrokinetic capillary chromatography (MEKC)

MEKC is the mode of CE which has the ability to separate charged and uncharged compounds simultaneously. In addition, the MEKC approach facilitated the separation of several equally charged analytes. Under conditions of MEKC, partition of all components in the system occurs between the bulk solution and the "pseudo-stationary phase", micelles formed by the surfactant [53]. Analytes in the mobile EOF are separated by differential interactions with the micelles and their electrophoretic mobilities.

Bile salts do not allow reversal enantiomer migration order, and because of their low aggregation number, they have only found the application for hydrophobic analytes [62].

Aumatell *et al.* [63] described attempts to resolve enantiomers of β-agonists, β-antagonists, phenylethylamines and diclofensine using HP-β-CD with sodium taurodeoxycholate and sodium SBE-β-cyclodextrin with SDS.

The resolution factor values of many phenylethylamine and amino alcohols (ephedrines) with concentrations of 60 and 120 mM HP-β-CD (average MSs 0.6 and 0.8) in 50 mM sodium taurodeoxycholate solution (anion surfactant), 5% (v/v) 1-propan-ol and 50 mM borate buffer at 9.5 were 0.98. This result pointed to a weak interaction present between the drug and negatively charged sodium taurodeoxycholate at pH 9.5 and positively charged molecules of drugs (pK_a values between 9.2 and 11.0).

Synthetic chiral surfactants, with a chiral head group: (*S*)- and (*R*)-*N*-dodeoxycarbonylvaline (DDCV) were the most used for achieving enantiomeric separations of basic pharmaceutical compounds by MEKC. For ionisable compounds like amines, enantioselectivity was optimized by changing the composition and pH values of the running buffer. The partitioning was optimized through the surfactant concentration, organic additives and pH value [64].

Mazzeo *et al.* [62] described the separation of ephedrine enantiomers in urine, using 50 mM (*S*)-DDCV and 25 mM Na_2HPO_4+25mM $Na_2B_4O_7$ buffer, pH 8.8. The only step in a sample preparation was a simple filtration procedure (**Table 2.**).

It has been shown that by using the two enantiomers of DDCV individually, a reversal enantiomer migration time can be achieved. For the quantification of enantiopurity of raw material or pharmaceutical formulations, it is desirable that the trace enantiomeric impurity be eluted before a tailed parent enantiomer peak. Conversely, in the case of fronted peaks, it would be desirable for the trace enantiomer to be eluted last. The reversal enantiomer migration order was found to be beneficiary for the identification of enantiomeric compounds in a complex mixture [65].

Three monovalent counterions (Li^+, Na^+ and K^+) influence on the peak shape, efficiency, selectivity and retention in amino alcohol enantiomers separation with DDCV micelles. A much better (more symmetrical) peak shape was observed when Li^+ was employed, due to a better, but still imperfect match of analyte and counterion mobilities; average asymmetry factors in LiDDCV, NaDDCV, KDDCV buffers were 1.9, 3.7, and 4.2, respectively [66].

Pascoe and *et al.* [67] studied the application of formed mixed micelles with DDCV and the second anionic surfactant, SDS, as well as vesicles containing DDCV and cationic surfactant cetyltrimethylammonium bromide (CTAB). Enantioselectivity, as well as other chromatographic and electrophoretic parameters were compared between the mixed micelles, vesicles and DDCV micelles. The hydrophobocity of the DDCV system was also evaluated as a predictor of n-octanol-water partition coefficients for 15 investigated β-amino alcohols.

Complete extraction procedures, enantioselective separations, with MS and UV detection and quantification of ten enantiomers of ephedrine and related compounds from NIST SRMs, using MEKC-MS and polysodium N-undecenoxycarbonyl-L-leucinate (poly-L-SUCL) were described in detail [2] (**Table 1.**).

Some applications of CE and CEC for enantioseparation of ephedrines in raw materials, pharmaceuticals and dietary supplements are presented in **Table 1.**, while the applications of CE and CEC for enantioseparation of ephedrine-type compounds in biological samples are given in **Table 2.**

3. High performance liquid chromatography (HPLC)

HPLC for the enantiomeric separation of ephedrines can be used either directly (with chiral stationary phases or chiral mobile phase selector) or indirectly (with chiral derivatization reagents).

3.1. Chiral ligand-exchange chromatography (CLEC)

CLEC, invented by Davankov, in the early seventies, was the first liquid chromatographic technique successfully applied in a complete and reliable separation of optical isomers of amino acids, hydroxy acids, amino alcohols and other solutes capable to form coordination compounds with transition metal ions (copper(II) is most frequently used). The chiral selector may be present in the mobile phase (CMP), coated on the stationary phase (chiral coated phase mode, CCP), or chemically bonded on the stationary phase (CSP). In CLEC, the interaction between the chiral selector and enantiomers does not occur in the direct contact. The interaction is mediated by a metal ion which coordinates simultaneously with a chiral selector and the enantiomers to be separated with the formation of ternary mixed-ligand complexes [68, 69].

Davankov *et al.* [70] proposed notably simple method of converting commercially available reversed-phase HPLC columns into highly efficient chiral ligand exchangers by a dynamic coating of complexing chiral selector *N*-alkyl-L-hydroxyproline (*N*-alkyl-Hyp). In the CCP chiral selector loaded permanently on the surface of packing due to strong hydrophobic interactions between the *N*-alkyl substituent of the selector and silica-bonded alkyl groups, whereas the hydrophilic part of the selector remained exposed at the interphase layer to the aqueous eluent and solutes molecules. Yamazaki *et al.* [71,72] demonstrated the usefulness of a *N*-*n*-dodecyl-L-hydroxyproline (C_{12}-Hyp) modified reversed-phase octadecylsilanized silica (RP C_{18}) column by resolving successfully racemic norephedrine and its analogues, with

aqueous solution containing copper(II) as the mobile phase, with pH value of 6.0. The enantiomeric separation of underivatized aromatic and aliphatic amino alcohols with a primary and secondary alcohol moiety on a column packed with C_{12}-Hyp-coated RP C_{18} was improved by addition of barbital (BB) to the mobile phase containing copper(II) [73].

Oi *et al.* [74] introduced new chiral ligand, Schiff base of amino alcohol (*N*-salicylidene-(*R*)-amino-1,1-bis(2-butoxy-5-tert-butylphenyl)-3-phenyl-1-propanol) for an efficient resolution of racemic amino acids, hydroxyl acids, amines and important amino alcohols. The same research group [75] employed *N*,*S*-dioctyl-D-penicillamine and *N*,*S*-dioctyl-*N*-methyl-D-penicillamine as chiral coatings on a RP C_{18} column to obtain chiral columns capable of resolving different classes of substances (including amino alcohols).

Determinations of synephrine enantiomers were achieved using commercial columns with CCP: Sumichiral OA-5000 (based on D-penicilamine) [76], or Sumichiral OA-6000 (based on L-tartaric acid-1-(*R*)-1-(α-naphthyl)-ethylamine) [77], on HPLC systems with electrochemical detection. The method described in [77] (**Table 3.**), with mobile phase containing copper(II) and ammonium acetate buffer (pH 6.4), was applied for synephrine enantiomer separation in *Citrus unshiu* fruit, marmalade and samples of human urine after intake of *C. unshiu* pulp.

CMP includes the system of a conventional achiral RP C_{18} column with an eluent containing the chiral selector. CMP differs from the CCP only in that the selector predominantly loaded in the stationary phase in the latter systems, whereas it predominantly moves with the mobile phase in CMP systems. The important consequence of these partitions is that the enantiomer more strongly bounded to the selector will be retained longer in the CCP column, whereas it will elute first with the CMP. There is also a whole palette of intermediate systems in which chiral selector partitions between the mobile and stationary phases and acting in two opposite directions. CMP mode of CLEC is not convenient for enantiomeric separation of amino alcohols, because the pH optimum for the complexation of amino alcohols, as mentioned, is about 12, but pH values of mobile phase higher than 7.0 leads in destroying of the conventional RP C_{18} column. In contrast to CLEC, in CLE-CE the usage of high pH enables optimum complexation equilibria of amino alcohols and improved separations [51,52,55]. Despite this fact, the enantioseparation of amino alcohols, using RP C_{18} column and a chiral mobile phase containing copper(II), L-proline and barbital was achieved. Although barbital is achiral, addition of BB to the mobile phase was critical for the separation [78,79]. Tanaka *et al* [79] supposed that barbiturates participate in the formation of the mixed-ligand ternary complexes responsible

for the chiral recognition and in such a way affect on the selectivity of separation.

Gübitz *et al.* [80], using Chiral ProCu column suggested a general approach of CSP for resolving amino alcohols after derivatization of their amino function with bromoacetic acid, which converts the latter into glycine. The achiral glycine coordinates to copper (II), whereas the asymmetric atom in the N-substituent provides for the enantiodifferentiation. A whole range of important adrenergic drugs, and β-blocking agents having β-amino alcohol structure with secondary amino groups were resolved in this manner with α-values up to 2.6 (synephrine). The phosphate buffer (pH 4.5) containing 0.1 mM copper(II) and 20% methanol was found to be the most suitable mobile phase for these separations. Operating the column at 50°C has improved its efficiency.

Bazylak [81] reported the HPLC method for enantioseparation of primary and secondary amino alcohols, including ephedrine analogues with acetonitrile-water as the mobile phase containing a synthesized neutral, square-planar, helically distorted nickel(II) chelate. The influence of changes in the mobile phase (concentration of chelate and acetonitrile) and the flow rate on the observed enantioseparation was discussed.

3.2. CDs as a CSPs and a chiral selectors

CDs can be immobilised on column, giving a chiral stationary phase, or they can be added as a chiral selector to the mobile phase [82]. The enantioseparation of ephedrines, synephrine and some adrenergic agonists in β-CD columns was reported [83].

Pumera *et al.* [84] 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 enantioselectively separated by β-CD modified column and twenty β-adrenergics, β-blocking agents and similar amino alcohol types were analysed using α-CD column. Chiral discriminations occured by steric hindrance to form different interactions between the CDs and the drugs [85]. The separation of the enantiomers and diastereoisomers of ephedrines for impurity profiling in the methamphetamine synthesis pathway and raw material, with phenyl-β-CD type column, and mobile phase containing 20 mM KH_2PO_4 (pH 4.6) and acetonitrile was developed by Makino *et al.* [86] (**Table 3.**).

CM-β-CD and carboxyethyl-β-CD (CE-β-CD) have proved to be effective chiral mobile phase additives for the resolution of enantiomers of ephedrine, hexobarbital and an aminomethylbenzodioxane derivative.

The type and concentration of the CDs, pH and type of base used to adjust the pH value were varied in order to optimize the method. For the enantioseparation of ephedrine using of Nucleosil 300-5 C_4 cartridge column, with mobile phase containing 5 mg/mL aqueous CE-β-CD solution-ethanol (90:10 v/v) and pH 5.0, adjusted with triethylamine (TEA) gave the good results [87].

Pseudoephedrine was selected as a model compound for evaluation of different strategies for the quantification of partially coeluting optical isomers. For HPLC, LiChrospher 100 RP 18 column, mobile-phase consisting of methanol and 2.0 g/L solution of CM-β-CD (pH 5.2 adjusted with triethylamine) and UV detector set at 215 nm were used. The results of study demonstrated that the best methodology for the quantification of partially overlapping UV peaks of enantiomers and for obtaining the enantiomeric excess is the usage of principal component regression (PCR) model with peak heights, perpendicular drop peak areas and deconvoluted peak areas as the original variables [88].

Herráez-Hernández *et al.* [89] investigated two possibilities for enantioseparations of ephedrines: application of a CSP consisting of immobilized β-CD, and use of β-CD, methyl-β-CD (M-β-CD), CE-β-CD, CM-β-CD and HP-β-CD as different mobile phase selectors. The results of the study demonstrated that the applying of immobilized β-CD provided better enantioseparation in shorter times of analysis.

3.3. Miscellaneous CSPs

(*R*)-1-naphthylglycine and 3,5-dinitrobenzoic acid as a Brush-type (Pirkle-type) of CSP was used for baseline enantiomeric separation of ephedrines with n-hexane, 1,2-dichloroethane, methanol and acetic acid containing mobile phase [90]. Newly synthesized polysaccharide-based [91,92], cellulose/amylose based [93], and cation-exchange [94,95] CSPs have enabled an enantioseparation of many drugs, including ephedrines with reversed-phase HPLC, but Matthij *et. al.* [91] and Caccamese *et al.* [92] demonstrated that polysaccharide-based CSPs could be used in normal-phase HPLC. The adsorption of (+)- or (-)-camphorsulfonic acid on Chiralcel OD column (silica gel column, coated with cellulose tris (3,5-dimethylphenylcarbamate)) has significantly impoved the enantioseparation of ephedrines [96].

The separation of synephrine enantiomers from *Citrus* fruit and dietary supplements, using Chiral CBH column (protein based CSP, with cellobiohydrolase as the chiral selector), with 2-propanol, phosphate buffer and disodium EDTA containing mobile phase was reported by Pellati *et al.* [97] (**Table 3.**).

3.4. Indirect enantiomeric separation

Indirect methods have been traditionally preferred for the analysis of ephedrines at low concentration levels, for example, in biofluids. The reason being that, owing to the low UV absorbances and a very little natural fluorescence, a derivatization reaction is often needed to overcome the lack of sensitivity. Therefore, derivatization can also facilitate enantioresolution of ephedrines. In this respect, derivatization of the amino groups with a chiral reagent is the most commonly used approach because the stereoisomers originated can be resolved in conventional (achiral) stationary phases [98]. These reagents are capable of reacting under mild conditions only with primary and secondary amino groups, and no applications to the enantioseparation of ME and MPE have been reported. Successful results have been obtained with the chiral reagents: 2,3,4,6-tetra-*O*-acetyl-*β*-glucopyranosyl isothiocyanate [99], (1*S*,2*R*)-1-acetoxy-1-phenyl-2-propyl isothiocyanate [100], *β*-(+)-1-(fluorenyl)ethyl chloroformate [101], 9-fluorenylmethyl chloroformate-L-proline [102], (-)-1-(9-fluorenyl)ethyl chloroformate [103] and 4-(4,5-diphenyl-1*H*-imidazole-2-yl)-benzoyl chloride (Dansyl chloride) [104,105].

Some applications of HPLC for enantioseparation of ephedrines in real samples are presented in **Table 3.**

Table 3. Application of HPLC for enantioseparation of ephedrine-type compounds in real samples.

Compound	Application	Mobile phase	Columns and running conditions	Ref.
Synephrine	Food, urine	20 mM CH_3COONH_4 buffer, (pH 6.4) with 1 mM copper(II) acetate	Sumichiral OA-6000 (150 mm x 4.6 mm x 5μm); Temp. not specified; 5 μL; 1.5 mL/min; electrochemical detection (1.0 V vs Ag/AgCl)	77
Ephedrines, amphetamines	Raw material	20 mM KH_2PO_4 (pH 4.6)–acetonitrile (4:1 v/v)	Chiral Drug (150 mm x 1.5 mm), 20°C; 1 μL; 0.1 mL/min; 210 nm	86
Synephrine	*Citrus* fruits, dietary supplements	10 mM sodium phosphate buffer (pH 6.0), with 2-propanol (5% w/w) and 50 μM disodium EDTA	Chiral-CBH (150 mm x 4.0 mm x 5μm), 20°C; 20 μL; 0.8 mL/min; 225 nm	97

Summary

Electromigration techniques (CE and CEC) and HPLC represent the most efficient methods for a chiral pharmaceutical and biomedical analysis of ephedrine-type compounds.

In the cited techniques, the crucial factor for chiral separations is the choice of the proper chiral selector, according to the structure of the ephedrines and other amino alcohols. A series of chiral selectors providing a sufficient resolution in CE, CEC and HPLC techniques were presented.

Apart from the general advantages of CE and CEC (high separation efficiency and short analysis times) in CE and CEC enantioseparations the amounts of samples and running buffer are much less than those used in HPLC, the chiral selectors are dissolved in the running electrolyte, and thus the expensive chiral columns are not required. On the other hand the disadvantages of CE and CEC enantioseparations, in comparison to HPLC are lower reproducibility, lower sensitivity and fewer possibilities of the preparative applications.

The process of derivatization, in an indirect HPLC mode of enantioseparation of ephedrines requires the usage of high enantiomeric purity derivatization reagents, and this reaction represents an additional step, which can involve undesirable side reactions (formation of decomposition products and racemisations).

The direct mode of HPLC, with commercial columns, containing CSPs is more convenient and applicable for preparative purposes also, but high column prices limit their using in a routine enantioseparation analysis of ephedrines. The CMP approach represents a simple and flexible alternative, which is, however, not always applicable in ephedrines enantioseparation. Because of large amounts of CMP selectors in mobile phases necessary for the enantioseparation, as well as the impossibility of reusing the CMP selectors, this mode is not applicable in a routine analysis.

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