CHIRAL MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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Abstract

The potential of Micellar Electrokinetic Chromatography to achieve enantiomeric separations is reviewed in this article. The separation principles and the most frequently employed separation strategies to achieve chiral separations by Micellar Electrokinetic Chromatography are described. The use of chiral micellar systems alone or combined with other micellar systems or chiral selectors, as well as of mixtures of achiral micellar systems with chiral selectors is discussed together with the effect of different additives present in the separation medium. Indirect methods based on the derivatization of analytes with chiral derivatizing reagents and the use of achiral micelles are also considered. Preconcentration techniques employed to improve sensitivity and the main approaches developed to facilitate the coupling with Mass Spectrometry are included. The most recent and relevant methodologies developed by chiral Micellar Electrokinetic Chromatography and their applications in different fields are presented.

1. Introduction

Micellar Electrokinetic Chromatography (MEKC) was introduced by Terabe *et al.* in 1984 [1] when they added an anionic surfactant (sodium dodecyl sulfate (SDS)) to the separation buffer in CE. This work supposed an important advance in the field of CE since the use of a surfactant at a concentration above its critical micellar concentration (CMC) enabled the separation of neutral compounds based on their differential partitioning between the aqueous phase and the micellar pseudostationary phase, micellar solubilization operating as the distribution process of solutes [2]. In fact, as the separation in CE is based on the different mobilities of charged analytes based on their charge to mass ratio, under the application of an electric field, the separation of neutral compounds was not possible under these conditions. As a consequence, MEKC is considered as a hybrid technique since electrophoretic and chromatographic phenomena are involved in the separation.

Among the main advantages that have been attributed to MEKC, the following can be highlighted [3]: i) the separation of molecules too small to be separated by gel electrophoresis, ii) the separation of both ionic and neutral compounds in short analysis times, iii) high separation efficiency, iv) minimum consumption of reagents and samples,

v) ability to achieve chiral separations, vi) high sensitivity in small amounts of samples,

vii) the fast separation of complex samples.

Chiral MEKC is considered a powerful tool to achieve the separation of enantiomers of a variety of compounds with different polarities and characteristics. Since the enantiomers of a chiral compound have the same charge to mass ratio, their separation by CE is not possible unless a chiral selector is added to the separation medium. The use of chiral micellar systems alone or combined with other chiral selectors, mixtures of chiral and achiral micelles, or mixtures of achiral micelles with chiral selectors has a big potential for the separation of enantiomers by MEKC. Even though a broad range of surfactants are commercially available, a limited number of them are widely used in chiral MEKC. The requirements that they should meet to be suitable include: (i) their solubility in the buffer solution must be sufficient to form micelles; (ii) the micellar solution must be homogeneous and compatible with the detector system employed; and (iii) the viscosity of the micellar solution must be low [3].

Table 1 lists the most used surfactants in chiral MEKC along with their most relevant properties. In addition to them, the use of other surfactants such as ionic liquid-based surfactants, amino acid-based surfactants, and amphiphilic cyclodextrin derivatives has also been reported to develop different chiral MEKC methodologies as it will be described in next paragraphs.

In spite of the interest that chiral MEKC presents nowadays as an interesting alternative to solve analytical problems raised in many fields of social interest, a comprehensive and specific review devoted to chiral MEKC has not been published in the last two decades. Previous reviews on chiral MEKC were focused on the use of monomeric and polymeric chiral surfactants as pseudostationary phases for chiral separations [9], chiral surfactants in MEKC [10], chiral EKC using dipeptide polymeric surfactants [11], chiral glycosidic surfactants for enantiomeric separation in CE [12], and enantiomeric separations by use of polymeric surfactants EKC [13]. Other articles were aimed to review more concrete aspects of chiral MEKC such as the enantiomeric separation of drugs using chiral surfactants [14]. In addition to the above-mentioned review articles, different reviews were focused on general aspects of MEKC in which a section or paragraph devoted to enantiomeric separations was included [4, 15-22]. Therefore, the present article constitutes the first comprehensive review specifically devoted to chiral MEKC in the last twenty years.

The potential of MEKC to achieve enantiomeric separations is described in this article. With this aim, the separation principles of chiral MEKC together with the main micellar systems employed and the different separation strategies developed to achieve enantiomeric separations will be presented. Preconcentration techniques used to improve method sensitivity and strategies reported to facilitate the coupling with MS will also be summarized. Finally, the most recent and relevant applications of chiral MEKC in different fields will be discussed including those articles published from 2015 to present and grouping the characteristics of these methodologies in tables. In all searches, different combinations of the following search terms were used as keywords: "MEKC", "micellar electrokinetic chromatography", "chiral", "enantio*", "ligand-exchange", “LE-MEKC”, "surfactant", “SDS”, “bile salts”, “*cholate”, “polymeric micelles”, “chiral selector”, “cyclodextrin”, “antibiotic”,

"CD-MEKC", "mass spectrometry", "MEKC-MS", "preconcentration", "extraction", "sweeping", "stacking", "FLEC", "two dimensional", "mechanism", "NMR", "molecular modelling" "counter*", "partial filling". Papers reviewed in this article were searched through the following databases: Web of Science database, Scopus, SciFinder, Google Scholar, Medline and PubMed. The search results were analyzed, compared, summarized, and grouped for exposition throughout the text.

2. Enantioseparation principles in MEKC

The most frequent strategies to achieve a chiral separation in MEKC are: i) using chiral micellar systems or mixtures of chiral and achiral micellar systems, and ii) the combination of chiral selectors with micellar systems. When a cyclodextrin (CD) is employed, the separation mode is called cyclodextrin-modified MEKC (CD-MEKC) [23]. Even though achiral surfactants are usually employed in this strategy, chiral micelles can also be used. In this case, two chiral selectors are simultaneously present in the separation medium so that they can lead to cooperative or opposite effects on the chiral resolution [24].

Figure 1 shows, in a simple way, a scheme representing the enantioseparation principle of a neutral chiral compound in chiral MEKC when using an anionic chiral micelle as pseudostationary phase and a capillary in which a cathodic electroosmotic flow (EOF) exists. As shown in **Figure 1**, the electrophoretic migration of the micelle is towards the anode whereas the EOF migrates towards the cathode. Under neutral or alkaline conditions, the EOF is stronger than the electrophoretic migration of the micelle, so that it transports the bulk solution to the detector making the micelles to move to the cathode at a velocity lower than that of the EOF. The mechanism of enantioseparation relies on the interaction established between the chiral analyte and the chiral micellar phase. In principle, three type of interactions can take place: the adsorption of the solute on the micelle surface by electrostatic or dipole interactions, the solute acts as a co-surfactant by participating in the formation of the micelle, and the solute is incorporated into the core of the micelle [24]. The micelle surface or the nature of the polar groups of the surfactant have a high effect on the separation selectivity of highly polar chiral compounds. On the contrary, if due to its nature, the chiral compound can be incorporated into the

micelle, the hydrophobic part of the surfactant also becomes relevant [25]. Then, the separation of enantiomers in chiral MEKC takes place by a difference in the mobility of the enantiomers as a result of their different distribution between the micelle and the aqueous phase. The migration time of each enantiomer (t_{E1} and t_{E2}) will be comprised between the migration time of the EOF (t_0) and the migration time of the micelle (t_{mc}). The period between t_0 and t_{mc} is frequently referred in the literature as migration time window. To perfectly define this window, it is necessary to know t_0 and t_{mc}. To do that, markers of the bulk solution and the micelle must be injected into the system. Compounds electrically neutral which do not interact with the micelle (for instance, methanol or acetone, among others) are appropriate to measure the time of the bulk solution. These compounds can be easily detected using a UV detector since when they pass through the detection zone, a baseline disturbance is observed by a change in the refractive index [26]. On the contrary, a compound which will be totally incorporated into the micelle is required to know the micelle time [27]. Sudan III or IV are often used as markers but sometimes their low solubility in water makes not possible to observe their peaks in the electropherograms. Timepidium bromide or quinine hydrochloride are good markers for anionic micelles [4]. The chiral recognition in micellar systems can be affected by the fact that micelles do not have a definite configuration, but they are in a dynamic association-dissociation equilibrium with monomeric surfactants in the aqueous phase [24]. In this line, an interesting study was performed by Meier *et al.,* providing insight on how bile surface chemistry gives rise to chiral selection with different bile salts and how discrete steps within processive aggregation affects the interactions between the micelle and the enantiomers of a model chiral compound [28].

CD-MEKC is particularly useful for the chiral analysis of hydrophobic compounds [23]. These compounds are incorporated into the micellar phase which does not allow their separation. In this case, it is very useful to employ a CD in the medium. Here, a differential partitioning of compounds among the micellar system, the CD and the aqueous phase is established. The enantioseparation principle for a neutral compound using an anionic micelle and a neutral CD (which is the most common combination employed in CD-MEKC) is shown in **Figure 2**. Due to its hydrophilic rims,

the neutral CD (which moves at the same velocity as the EOF) is soluble in the aqueous phase and is not incorporated into the micellar phase (although it is possible the inclusion of the surfactant in the CD cavity). Despite this fact is generally accepted, a study carried out by Tsianou and Fajalia, provides direct evidence on the location of CDs (namely γ-CD and HP-β-CD) in the core of SDS [29]. Solutes migrating with the EOF will originate a peak at t_0, solutes totally incorporated into the micelle (without interacting with the CD) will give a peak at t_{mc}, whereas those solutes interacting with the CD will have a differential partitioning between the aqueous phase and the micelle. In fact, the presence of the CD in the separation medium modifies the distribution of the analytes from the micelle to the aqueous phase as a consequence of the interaction between the CD and the analytes [30]. Hydrophobic compounds will be incorporated either in the micelle or in the CD. On the contrary, hydrophilic compounds may be distributed among the micelle, the CD and the aqueous phase. Thus, the solutes partitioned into the micelle or CD will have different mobilities which affects to the selectivity. The enantioselective recognition of the enantiomers in this system involves the formation of enantiomer-CD complexes of different stability for each enantiomer. It should be mentioned that as it has been previously mentioned, it is also possible to combine a chiral micelle with the chiral selector in this MEKC mode. In that case, the enantiomer interacts enantioselectively not only with the CD but also with the chiral micelle.

Selectivity and chiral discrimination modification in CD-MEKC can be done by varying the type of surfactant and the CD as well as varying their concentrations. Also, it is relevant the effect of the presence of buffer additives since they could be included in the CD cavity which may or may not favor the chiral recognition process [24].

3. Separation strategies in chiral MEKC

MEKC offers a variety of possibilities to achieve a chiral separation. The different separation strategies developed in chiral MEKC can be included within the two groups usually employed in chiral separation methods: direct and indirect working modes [31-33].

3.1. Direct strategies

In the direct working mode, a chiral selector is employed in the separation medium which originates transient diastereomeric intermediates with the chiral analytes giving rise to the separation of the enantiomers. In MEKC this chiral selector can be a chiral micellar system or a mixture of a chiral micelle with other chiral selectors but mixtures of achiral micelles with chiral selectors have also been successfully employed. The high variety of chiral selectors commercially available confers a big potential to the direct mode in MEKC when using different chiral and achiral micelles. As the main advantage of the direct working mode, the fact that derivatization of the analytes is not necessary to make possible the chiral discrimination can be cited. Some of the most frequent strategies employed within the direct methods are the following:

3.1.1. A chiral micelle as the sole chiral selector.

The use of a chiral micellar system as the sole chiral selector in the separation medium is the simplest strategy that can be used in chiral MEKC. Natural surfactants such as bile salts have shown to be useful chiral micellar systems and are commercially available [4]. Sodium cholate (SC), sodium deoxycholate (SDC), sodium taurocholate (STC) and sodium deoxytaurocholate (SDTC) have mainly been employed. However, other bile salt micellar systems have also been used. When ten bile salts (cholic, taurocholic, glycocholic, deoxycholic, taurodeoxycholic, glycodeoxycholic, chenodeoxycholic, taurochenodeoxycholic, glycochenodeoxycholic, dehydocholic acids) were compared as chiral selectors for the enantioseparation of tri aza aromatic ligand compounds of iron (II), it was shown that depending on the bile salt employed, significant changes in the enantiomeric resolution were observed [34]. Dihydroxy bile salts were superior in this case to trihydroxy bile salts in terms of resolution. In addition, taurine or glycine conjugated bile salts gave rise to improved results compared with unconjugated bile salts being taurochenodeoxycholic or glycochenodeoxycholic acids the bile salts originating the best results in presence of 10-15% acetone. In addition to organic modifiers such as acetone or methanol which showed to improve enantiomeric resolutions, neutral surfactants such as Brij-35 have been added to the separation medium. In fact,

Brij-35 improved fluorescence intensity of amino acid derivatives [35]. Applications of MEKC using bile salts as micellar systems include the analysis of a variety of analytes such as drugs, amino acids, hormones, compounds of biological interest such as bilirubin, aromatic ligand compounds, alkylphenol etoxylates or model compounds such as binaphthol derivatives, among others.

Amino acid-derived surfactants such as sodium N-dodecanoyl-L-valinate [4] or L-undecyl-leucine [36], a new amphiphilic CD derivative (heptakis(2,3-O-benzyl-6-O-sulfobutyl)cyclomaltoheptaose) [37], a vesicle-forming chiral cationic surfactant ((1R,2S)-(-)-N-dodecyl-N-methyl-ephedrinium bromide) [38] or vesicle-forming single-tailed amino acid derivatized surfactants (sodium N-[4-n-dodecyloxybenzoyl]-L-leucinate and sodiumN-[4-n-dodecyloxybenzoyl]-L-isoleucinate) [39] are other types of chiral surfactants than have been successfully employed. As an example, the potential of the amphiphilic CD derivative (heptakis(2,3-O-benzyl-6-O-sulfobutyl)cyclomaltoheptaose was shown in the enantioseparation of serine (previously derivatized with naphthalene-2,3-dicarboxaldehyde and cyanide) by MEKC-laser induced fluorescence (MEKC-LIF) [37].

Polymerized chiral surfactants constitute other interesting possibility in chiral MEKC. A big variety of polymeric micelles have been employed in chiral MEKC in the last years. Molecular micelles based on amino acids (L-leucine, L-isoleucine, L-valine, L-alanine) [40-52] or carbohydrates (α- and β-glucopyranoside-based polymeric surfactants) [7, 53] have been described and applied to the enantioseparation of different compounds of interest such as binaphthyl derivatives [40-43], ephedrine alkaloids [7, 43-45, 53], β-blockers [7,45-47, 53], warfarin [48, 49], venlafaxine and O-desmehtylvenlafaxine [50], barbiturates [51], benzodiazepines and benzoxazocine [52], and phenylethylamines [45]. Most of the articles based on the use of molecular micelles in chiral MEKC employed MS detection [7, 40-52]. As the coupling of chiral MEKC with MS using conventional surfactants at concentrations above their CMC is challenging [15, 17, 54], the use of micelle polymers has shown to be an interesting alternative to overcome the difficulties associated with MS detection as will be commented in section 6.

3.1.2. A mixture of chiral and achiral micellar systems.

The use of mixed micelles of two or more different surfactants has been considered an effective strategy to improve the separation selectivity in MEKC [15]. Following this approach, mixtures of micellar systems have been employed in chiral MEKC [4, 17]. As one possibility, mixed micelles of SDS and bile salts have been used. Thus, a mixture of SDS and SDC has been reported to be useful in the separation of D-amino acids [55]. Also, mixed molecular micelles of two polymeric surfactants (poly-sodium N-undecenoxy carbonyl-L-leucinate (poly-L-SUCL) and poly-sodium N-undecenoyl-L,L-leucylvalinate (poly-L-SULV)) were successfully employed for on-line coupling of MEKC to atmospheric pressure photoionization (APPI) MS. The simultaneous enantioseparation of four photoinitiators (hydroxybenzoin, benzoin, benzoin methyl ether, benzoin ethyl ether) was carried out under optimized conditions selected using multivariate central composite design [56]. Due to the big variety of achiral and chiral micellar systems, mixed micelles offer a wide range of possibilities to improve enantiomeric separations of different families of compounds of different nature.

3.1.3. A mixture of a micellar system and a chiral selector.

One of the most effective approaches for achieving enantiomeric separations by MEKC is to employ a system based on the combination of chiral or achiral micelles with a chiral selector in the separation medium.

Most articles published in the last twenty years dealing with the use of a mixture of a chiral micelle and a chiral selector based their potential on the cooperative effect of a bile salt with a CD. SC, SDC, STC and STDC have been the bile salts employed in combination mainly with β-CD [57-65], although other CDs such as γ-CD [66, 67], trimethyl-β-CD (TM-β-CD) [68], hydroxypropyl-β-CD (HP-β-CD) [69], sulfated-β-CD (S-β-CD) [70] or even the dual system HP-β-CD/S-β-CD [71] have also been used to obtain the chiral separation of a broad range of compounds of interest such as amino acids, drugs, flavonones, pyrethroids, or polychlorinated biphenyls (PCBs). An interesting work showed a multidimensional separation of amino acid mixtures in a multi-layered three-dimensional hybrid microfluidic/nanofluidic device [72]. Here two consecutive electrophoretic separations were performed, the first being an achiral separation followed by a chiral separation based on the use of β-

CD as chiral selector and STC as the micelle-forming agent. In addition to CDs, bile salts were also combined with other chiral selectors. Thus, the binary system based on the mixture of SC and human serum albumin was employed for the chiral analysis of aspartic acid and glutamic acid fluorescently tagged with 5-(4,6-dichloro-s-triazin-2-ylamino) fluorescein [73] and the combined use of SC micelles with a specific DNA aptamer as chiral selector was useful for the enantiomeric determination of DL-tryptophan [74].

Although the above-mentioned approaches have been the preferred options, other possibilities have also been reported. Thus, it has been demonstrated the potential of the combined use of a polymeric chiral surfactant poly-sodium N-undecanoyl-D-valinate (poly-D-SUV) and hydroxypropyl-γ-CD (HP-γ-CD) to obtain the enantioseparation of different highly hydrophobic PCBs [75] or of different combinations of the D- and L-configurations of poly(sodium N-undecanoyl alaninate), poly(sodium N-undecanoyl leucinate), and poly(sodium N-undecanoyl valinate) with β-CD or γ-CD to carry out the enantioseparation of three binaphthyl derivatives [76]. It is worthy to mention that SDS was added to dual systems formed by a chiral micelle and a chiral selector, with the aim of improving the chiral separation [77-80].

Regarding MEKC methodologies based on the combined use of an achiral micelle and a chiral selector in the separation medium, it should be highlighted that even though some achiral micelles such as Brij 35 [81], tetradecyl trimethyl ammonium bromide (TTAB) [82], or even pyrrolidinium- or imidazolium-based ionic liquids surfactants [83] were employed in combination with CDs, the mixture SDS plus CD was undoubtedly the most employed separation strategy in chiral MEKC. Basically, HP-β-CD or HP-γ-CD have been the preferred CDs to establish the binary system with SDS [84-93], but the use of other CDs, such as β-CD [94-97], carboxymethyl-β-CD (CM-β-CD) [98], TM-β-CD [99], or mono-3-O-phenylcarbamoyl-β-CD [100] as chiral selectors was also reported. Even some works described the combined use of two different CDs as dual chiral selectors in combination with SDS [101-104]. Note that when MEKC methodologies were used to perform the separation of fluorescein 5-isothiocyanate-labeled amino acids enantiomers in microchip devices, the

micellar system chosen was the mixture SDS plus γ-CD [105, 106]. The applicability of all the above mentioned SDS/CDs systems has been demonstrated by the successful chiral separation of a broad range of different compounds including drugs, amino acids, dipeptides, catechins, hydroxyflavanones, fungicides or organophosphorus insecticides, among others.

Although CDs were the chiral selectors most employed to form binary systems with SDS, it is worthy to mention that other chiral selectors were also used in a lesser extent. For instance, the enantioseparation capability of the antibiotic clindamycin phosphate as chiral selector combined with SDS or a new imidazolium-based ionic liquid surfactant 1-butyl-3-methylimidazolium dodecyl sulfate ($[C_4MIm][C_{12}SO_4]$), was demonstrated through the chiral analysis of different model compounds including drugs and amino acids [107, 108]. The comparison of the results obtained using clindamycin phosphate in combination with $[C_4MIm][C_{12}SO_4]$ instead of SDS for the chiral separation of six different model compounds (namely, nefopam, citalopram, propranolol, chlorphenamine, duloxetine and tryptophan) demonstrated that the chiral separation improved, in terms of resolution and peak shape, when employing the ionic liquid-based surfactant [108]. Neutral cyclosophoraoses and highly sulfated cyclosophoraoses were also successfully applied as chiral selectors with SDS for the separation of some chiral flavonoids by MEKC [109].

The use of ligand-exchange complexes as chiral selectors together with micellar systems gave rise to ligand-exchange MEKC (LE-MEKC) which has proven its potential to achieve chiral separations in the last twenty years [110-129]. In fact, the addition of SDS as micellar system in the separation medium using ligand-exchange complexes as chiral selectors could enhance the enantiomeric resolution of analytes. Moreover, it was also shown to have a big influence on the enantiomer migration order which is a subject of high interest in many fields [130]. The combination of the enantioselectivity associated to the ligand-exchange principle with the advantages of MEKC has been seen as a hybrid strategy improving the results obtained by the separate use of ligand-exchange complexes as chiral selectors in CE or the use of MEKC [130].

The most frequent strategy was to use Cu(II) as central ion and an amino acid enantiomer or one of its derivatives as the ligand. Among the amino acid enantiomers most employed as ligands, L-Lysine [110], L-isoleucine [111], L-valine [112], and L-proline [113] can be cited, and L-N-(2-hydroxy-propyl)-phenylalanine [114], 4-hydroxy-L-proline [115] or N-alkyl derivatives of proline and hydroxyproline [116] were used as amino acid derivatives. Ligand-exchange complexes formed by L-Isoleucine or L-Valine with Cu(II) as chiral selectors were combined with SDS to carry out for the first time the enantioselective separation of ofloxacin and four related compounds [111], and the separation of the enantiomers of p-hydroxyphenylglycine (a synthesis intermediate of antibiotics) [112], respectively. Other ligands such as D-penicillamine [117], L- or D-beta-amino alcohols [118] or L-prolinamide [119] were also employed although in a lesser extent, and even a novel amino acid ionic liquid, tetramethylammonium L-hydroxyproline ([TMA][L-OH-Pro]), was applied as chiral ligand to evaluate its enantioselectivity towards several underivatized aromatic amino acids such as tryptophan, phenylalanine, histidine, tyrosine and 3, 4-dihydroxyphenylalanine (DOPA) [120]. Results showed that an increase in the enantiomeric resolution of tryptophan and the partial resolution of phenylalanine and histidine were achieved using a concentration of 10 mM SDS in the background electrolyte (BGE), whereas the enantioseparation of DOPA decreased in presence of SDS, and the enantiomeric separation of tyrosine was not reached in any case.

In addition to Cu(II), other central ions have also been used. It is worthy to comment that borate ion was used as central ion in LE-MEKC with (5S)-pinanediol as chiral selector for the separation of three compounds having a 1,2-diol structure [121]. When (5S)-pinanediol was replaced with (S)-1,2-propanediol, (S)-1,2,4-butanetriol or (S)-3-tert-butylamino-1,2-propanediol, no enantioseparation occurred for any of the analytes showing the importance of the hydrophobic interactions between the chiral selector and the analytes to achieve the chiral separation. Higher concentrations of borate were needed as compared to the use of Cu(II) as central ion since the compounds studied seemed not to form stable complexes with borate and (5S)-pinanediol-borate in aqueous solution.

In the above-mentioned works, SDS was generally been employed as micellar system. However, different studies were developed aimed to investigate the influence of the type of the surfactant on the chiral separation. Thus, a comparative study on the separation selectivity in LE-MEKC using different anionic surfactants such as SDS, sodium alkyl polyoxyethylene sulfate and sodium alpha-sulfonated fatty acid methyl esters showed that SDS provided the best selectivity [122]. Also, sodium n-decyl sulphate, SDS and sodium n-tetradecyl sulphate were employed [123]. In both of these works, CMC values of these anionic surfactants were estimated by the LE-MEKC methods developed. Other micellar system employed was 4'-octadecylneamine derivative that was designed to serve both as micelle-forming surfactant (by its hydrophobic part) and as central ion-complexing ligand (by its hydrophilic part). The enantioseparation properties of the octadecylneamine derivative-Cu(II)-MEKC system were evaluated (and compared with those of the native neamine-Cu(II)-CE system) for the chiral separation of tryptophan showing its potential to carry out the enantioseparation of other analytes such as 1-methyl-tryptophan, 3,5-diiodo-tyrosine and 1-napthyl-alanine [124].

Interesting results were observed on the reversal of the enantiomer migration order in LE-MEKC in some of the articles published. As examples, the migration order of D- and L- phenylalanine was strongly affected by the addition of SDS [115] which also caused the reversal in the migration order of other amino acids [123, 125] being the point of reversal correlated in some cases with the CMC [123].

Although most applications of chiral LE-MEKC described the enantioseparation of underivatized and derivatized protein and non-protein amino acids, the separation of the enantiomers of other compounds was also reported including antibiotics and related substances such as their impurities [110, 111], intermediates for the synthesis of semi-synthetic antibiotics [112], 1,2-diols compounds [121] or hydroxy acids [126]. In those works where a comparative study was achieved between LE-CE and LE-MEKC, a better performance of LE-MEKC was reported indicating the favourable effect of the presence of the micellar system in the separation medium to improve the enantioseparation [120, 125] and the selectivity between analytes [119]. Finally, LE-MEKC has shown to be feasible

in microchips with good enantiomeric resolution for derivatized amino acids and short analysis times [127].

3.2. Indirect strategies

Regarding the indirect methods, chiral analytes are derivatized with a chiral reagent in order to originate the corresponding stable diastereomers to be separated under achiral conditions. The main advantages of these methods are the possibility of reducing the limits of detection (LODs) of the diastereomers using UV, fluorescence or MS detection [31-33]. As the main drawbacks the following can be cited: i) the derivatizing reagent should have a high enantiomeric purity to avoid interferences, which in turn derives in a high cost for some reagents, and ii) the derivatization step increases the analysis time except when in-capillary derivatization is used.

Although different chiral derivatizing reagents can be used to originate the formation of stable diastereomers of a chiral compound to be subsequently separated, (±)-1-(9-fluorenyl)ethyl chloroformate (FLEC) has shown to have very interesting characteristics as described by Moldovan *et al.* in their excellent review [131]. Among the main advantages of FLEC, the following can be cited [131]:

i) (+) and (-) enantiomers of FLEC react fast and quantitatively with primary and secondary amines forming stable and non-racemizing diastereomers that can be easily separated and quantitated,

ii) a simple derivatization procedure in comparison with other derivatizing reagents,

iii) high separation efficiency compared with direct methods,

iv) excellent enantioselectivity using conventional instrumentation avoiding the use of expensive chiral selectors,

v) increase in the sensitivity with UV or fluorescence detection for molecules not possessing chromophore or fluorophore groups,

vi) compatibility with MS detection when using a volatile micellar system for the separation of diastereomers avoiding the problems of the MEKC-MS coupling with non-volatile chiral selectors in the separation medium.

After chiral derivatization with FLEC, the most employed micellar systems for the separation of the formed diastereomers by MEKC have been SDS and ammonium perfluorooctanoate (APFO). SDS is mainly used when UV, direct fluorescence or LIF–detection is employed [132]. Thus, the chiral separation of derivatized amino acids and their subsequent sensitive fluorescence detection (after a broad-band UV-excitation using a Xe-Hg lamp) showed that the sensitivity achieved by the developed methodology, in which SDS was used as surfactant, was better when compared to the chiral analysis of FLEC-amino acids by MEKC-UV (up to a factor of 400) or even by MEKC-MS (up to a factor of 50) [132]. The volatile micellar system APFO is advantageous with MS detection [133, 134] although it has also been employed with other detection systems [135]. In some of the works reported, in-capillary derivatization with FLEC was achieved [133, 136] which enabled the fully automatized chiral analysis of amino acids with application to artificial cerebrospinal fluid samples [133]. In addition to endogenous molecules, the analysis of compounds of pharmaceutical, food, and environmental interest has been reported using FLEC as derivatizing chiral reagent.

4. Mechanistic studies on chiral recognition in MEKC

An interesting and relevant research area in the field of chiral separations is devoted to the study of the mechanistic aspects of selector-selectand interactions. **Table 2** details the experimental conditions in which these studies were performed in the last two decades. As it can be observed in this table, different drugs or amino acids were mainly used as model compounds. For instance, the analysis of palonosetron (a chiral drug with two chiral centers in its structure) using SC as surfactant and chiral selector was employed to investigate its separation mechanism using the bile salt in a wide range of concentrations below and above the CMC [137]. The chiral differentiation was achieved by the interactions between the stereoisomers and the SC micelle. However, this only provided chiral recognition for 3a carbon chiral center whereas the resolution of 2 carbon chiral center was obtained by the difference of mobility in continuous phase. This chiral drug was also used to evaluate the effect of different water-soluble organic solvents on the enantiomeric separation [138], and to establish a

thermodynamic model for the prediction and elucidation of complex changes in the migration order patterns with the experimental conditions [139]. On the one hand, it was found that methanol provided the best separation among ten water soluble organic solvents [138]; on the other hand, following the results obtained in the proposed thermodynamic model it can be concluded that the interactions of the two chiral centers in both neutral molecules and protonated ions with SC are not independent. For this reason, the chiral recognition in two enantiomeric pairs and for the diastereomers could not be estimated from the contribution of each stereogenic center due to their correlation [139]. Wang *et al.* investigated the interactions taking place in the enantioseparation of fenoprofen by a MEKC methodology based on the combined use of TM-β-CD and a cationic ionic liquid type surfactant (N-undecenoxycarbonyl-L-leucinol bromide (L-UCLB)) [140]. Authors hypothesized that the ionic liquid surfactant acted as an inhibitor reducing the interaction between the CDs and the model compound. To demonstrate this idea, they proposed a general model for the ternary interactions among the three compounds. The results obtained suggested that the inhibition observed in the enantioseparation of fenoprofen was due to a competitive inhibition between the ionic liquid and the CD.

Using the combination of chiral MEKC and NMR, Pasquini *et al.* performed a comprehensive investigation not only on the separation mechanism but also on the inclusion complexation of ambrisentan with γ-CD which was used as chiral selector in the micellar medium [141]. To do that, the authors combined the data obtained from MEKC analysis, NMR experiments and molecular modeling. The results obtained in this work showed the high affinity between SDS and the CD cavity, and its influence on the enantiorecognition process. Eckenroad *et al.* also combined MEKC and NMR experiments to shed light on the structural basis for the selective solubilization of binaphthyl derivatives by bile salt micelles such as SC, SDC or chenodeoxycholate [142]. The combination of the data obtained from both analytical techniques demonstrated that the presence of the OH^- group in the carbon 12 of the chiral micelles was of vital importance since it contributed to stabilize the interactions between the S-enantiomer and the bile salt. By using NMR experiments, Hebling *et al.*

studied the chiral separation of (R,S)-1,1′-binaphthyl-2,2′-diylhydrogenphosphate (BNP) in presence of different concentrations (between 0-100 mM) of SC in basic solutions with the aim of providing information about the bile salt micelle formation and selector-selectand interactions [143]. The NMR data obtained indicated that the cmc-like aggregation processes occurred at SC concentrations of 7 ± 1 mM and 14 ± 1 mM, even though MEKC chiral resolution was only obtained at SC concentrations of about 14-15 mM or higher. Data also showed a pseudo-cmc behavior for secondary aggregation at a SC concentration of 50-60 mM and the role of the H5-H7 edge of BNDHP in the selective interactions with primary cholate micelles. Binaphthyl derivatives were also used as model compounds to investigate the potential as chiral selector of an amino acid-based surfactant (L-undecyl-leucine) when a cationic amino acid (L-arginine) was employed as counterion instead of sodium [36]. In addition, NMR experiments were conducted for a better understanding of the interaction between the surfactant and the enantiomers. Results obtained suggested that the pH and the type of counterions play a significant role in the characteristics of the L-undecyl-leucine micelles. Valle *et al*. not only combined the data obtained from MEKC and NMR experiments, but also achieved a study by steady-state fluorescence anisotropy to gain a better understanding of chiral separations involving dipeptide molecular micelles (poly-SULV). This was possible by determining the primary site of interaction between a selected group of neutral or anionic chiral analytes (see **Table 2**) and the micelle [144]. Altering the stereochemistry of the amino acids of the polymeric micelle in a systematic way, it is possible to control its enantiorecognition ability. Data obtained showed that the chiral separation mechanism of the amino acid studied (phenylalanine, tryptophan, leucine and norleucine derivatized with dansyl chloride) relies on the interaction with the valine chiral center of poly-SULV, whereas the enantiomeric separation of 1,1'-binaphthyl-2,2'-diylhydrogenphosphate (BNP) and 1,1'-bi-2-naphthol (BOH) occurs as a consequence of their interaction with the leucine chiral center. Inconclusive results were obtained for 1,1′-binaphthyl-2,2′-diamine (BNA) and Troger's base due to solubility limitations and a suspected impurity, respectively. MEKC, NMR and fluorescence spectroscopy as complementary analytical techniques were also used

by Yarabe *et al*. to study the binding constants between two polymeric chiral surfactants (poly-L-SUV and poly-sodium N-undecenoyl-L-isoleucinate (poly-L-SUI)) and two binaphthyl derivatives (BNP and BOH) [145]. Stoichiometry and stereoselective apparent binding constants of the inclusion complexes of BNP enantiomers and the polymeric micelles were calculated using NMR while steady-state fluorescence enabled the determination of the stoichiometry and stereoselective apparent binding constants of BOH enantiomers with the micelles. This study demonstrated that R-enantiomers have stronger affinity with the polymeric surfactants than S-enantiomers. The results of MEKC corroborated those of the apparent binding constants of binaphthyl derivatives obtained with NMR and fluorescence spectroscopy.

5. Preconcentration techniques in chiral MEKC

Sensitivity is an analytical feature of high relevance in chiral analysis. As examples, the analysis of enantiopure drugs to assess that their enantiomeric impurities are not present in the pharmaceutical formulation at percentages higher than 0.1 % according to the ICH guidelines or the analysis of biological or environmental samples can require highly sensitive methodologies. Bearing in mind the limited concentration sensitivity provided by UV detection, which continues being one of the most employed in chiral analysis, the use of preconcentration techniques is of great interest. Both off-line sample treatment techniques and in-capillary preconcentration techniques based on electrophoretic principles have been employed in chiral MEKC during the last two decades to improve detection sensitivity. Interesting reviews have reported the advantages of the use of these techniques in MEKC [15, 54, 146].

Solid phase extraction (SPE) has been the preferred off-line sample treatment technique employed to carry out the analysis of chiral compounds by MEKC. It has been used mainly to perform the treatment of biological samples in which reaching an adequate sensitivity is imperative [48, 50, 51]. Thus, Wang *et al.* [48] employed SPE with an anionic exchange cartridge (Oasis MAX) for the extraction of warfarin and hydroxywarfarin related compounds from plasma. After elution with

acetonitrile/methanol/formic acid (50/50/5, v/v/v) and evaporation, the samples were reconstituted (5 times more concentrated) in acetonitrile/water (40/60, v/v). LODs between 2 and 5 ng/mL were achieved. Cationic exchange cartridges were also employed for biological sample preparation. For instance, Wang *et al.* [51] used a cationic exchange cartridge (Oasis MCX) for the treatment of human serum samples in which the chiral analysis of methobarbital, pentobarbital and secobarbital was performed by MEKC-MS. The analytes were eluted with acetonitrile and after evaporation, samples were reconstituted in acetonitrile/water (80:20, v/v). In this case, LODs of 7.8 μg/mL were reached [51]. A strong cation exchange cartridge (Strata-X-C polymeric) was used by Liu *et al.* to purify venlafaxine and O-desmethylvenlafaxine in human plasma prior to their chiral analysis by MEKC-MS/MS [50]. Analyte elution was carried out with ammonium hydroxide and methanol (5/95, v/v), and after evaporation, the sample was reconstituted (nine times more concentrated) in methanol/water (10/90, v/v). LODs of 10.5 ng/mL of venlafaxine and 15 ng/mL O-desmethylvenlafaxine were obtained after the chiral analysis by MEKC-MS/MS [50]. Although SPE has been the main off-line treatment to preconcentrate biological samples, other sample treatments have also been employed. For instance, a liquid-liquid extraction was used to concentrate testosterone, epitestosterone, methyltestosterone, nandrolone, gestrinone, dihydrogestrinone and tetrahydrogestrinone in urine samples [147]. Also, immunoaffinity extraction based on the immobilization of anti-epitestosterone monoclonal antibody on a Sepharose 4B stationary phase was used for sample clean-up, extraction and preconcentration of testosterone and epitestosterone in urine samples [148].

Other chiral MEKC methodologies were based on the use of in-capillary preconcentration techniques with the aim to propose highly sensitive methods [57, 85, 114, 149-158]. To carry out the preconcentration of ketoprofen in wastewater samples, Petr *et al.* addressed a setup based on electrokinetic accumulation on the pH boundary followed by the enantioselective mobilization by the electrolyte containing SDS, S-β-CD and TM-β-CD [149]. By using a simple filtration as a clean-up step under the most adequate experimental conditions, the determination of nanomolar concentration levels of ketoprofen enantiomers was achieved. In fact, LODs were 2.5 nM and 3.4 nM, which

represent enhancement factors of 9921 and 8529, respectively. The potential of large-volume sample stacking with an electroosmotic flow pump (LVSEP) as preconcentration strategy was demonstrated by Kitagawa *et al.* [150, 151]. First, they combined a MEKC methodology employing SDS and γ-CD with LVSEP for the enantioseparation of arginine, methionine and leucine previously derivatized with fluorescein isothiocyanate (FITC) [150]. By using this methodology, a 1300-fold sensitivity increase was achieved enabling the detection of 100 pM amino acids without much loss of resolution. More recently, LVSEP in combination with field-amplified sample injection was employed for the chiral analysis of amino acids in a coated capillary. Under optimal conditions, a long electrokinetic injection time of up to 20 min could be applied resulting in a sensitive enhancement factor of 6420 and 4500 for D- and L-leucine enantiomers [151].

On the other hand, sweeping has also been employed as in-line preconcentration technique in chiral MEKC. Concentration factors up to 12-fold were achieved using sweeping as preconcentration approach in the determination of different neutral and hydrophobic compounds (namely galaxolide, tonalide, traseolide and phantolide) in perfumes by MEKC-UV [152]. Also, compared to conventional injection, sweeping injection of triadimenol for its chiral separation by MEKC-UV using HP-γ-CD and SDS enabled around 10-fold increase in sensitivity of the UV detection [153]. Some studies compared the sensitivity improvement obtained when applying two different in-line preconcentration techniques. For instance, Ibrahim *et al.* [154] compared the increase in detection sensitivity obtained when stacking or sweeping were employed as preconcentration techniques in the determination of propiconazole, fenbuconazole and tebuconazole enantiomers in grapes by CD-MEKC. Better LODs (0.4 μg/mL) were reached using sweeping than stacking (LOD, 2.0 μg/mL) [154]. Subsequently, employing an off-line preconcentration based on the use of SPE with a C-18 cartridge (where the analytes were eluted with dichloromethane, evaporated and reconstituted (200 times more concentrated) in the buffer solution), and after optimizing the sweeping-CD-MEKC conditions, LODs of 0.09-0.1 μg/mL were achieved for fungicide enantiomers. Later, the same authors employed both stacking with a reverse migrating micelle (SRMM) and sweeping in the chiral

analysis of three triazole fungicide enantiomers (hexaconazole, penconazole and myclobutanil) by CD-MEKC [155]. With the first strategy, an increase of 9-10 times in the detection sensitivity (LODs between 1.2 and 4.0 mg/L) was achieved whereas using sweeping the sensitivity increased between 62- and 67-times giving rise to LODs between 0.1 and 0.2 mg/L.

More recently, Ghiasvand *et al.* reported an interesting work related to the use of micelle-to-cyclodextrin stacking (MCDS) as preconcentration technique to carry out the enrichment and separation of cationic, neutral and chiral analytes (chlorpheniramine, fenoprop, dichlorprop and mecoprop) by MEKC [156]. In this system, the analyte in the solution with SDS is transported to a "dynamic boundary" formed between the BGE containing SDS and the BGE containing γ-CD. At the stacking boundary, the micelle and the CD form a stable inclusion complex. Then, the CMC of SDS increases and the concentration of free SDS could fall below the CMC causing the micelle collapse what releases the analyte from the micelle. There was reversal (for charged) or nulling (for neutrals) of the analyte´s electrophoretic mobility generating the analyte accumulation at the boundary. **Figure 3** shows the MCDS-chiral MEKC mechanism and the proof-of-concept using fenoprop as model compound. Using this methodology, enrichment factors from 54 to 146 were achieved for the four chiral compounds analyzed. Even though this preconcentration strategy has been successfully applied to metabolic stability studies of small molecules with HepG2 cell line, until now its applicability to the enantiomeric analysis of real samples has not been demonstrated.

Sometimes, the use of a single preconcentration strategy is not enough for the sensitive chiral determination of different compounds. This implies the need of the combined use of different strategies. On the one hand, it is possible to combine off-line sample treatments with in-line preconcentration techniques. Thus, Duan *et al.* developed a methodology of SPE combined with large-volume sample stacking to make possible the enantioselective determination of selenomethionine enantiomers in selenized yeast by LE-MEKC [114], and Choy *et al.* demonstrated for the first time the feasibility of combining salting-out solvent extraction and acetonitrile stacking for sensitive enantiomeric determination of two substituted naphthyl enantiomers using SC as chiral

micelle [157]. On the other hand, two interesting methodologies were reported by Hsieh *et al.* to carry out the preconcentration of different protein amino acids and their enantiomeric determination by chiral MEKC in soymilk and beer samples [57, 85]. These methodologies were based on the use of a discontinuous MEKC system. Using a solution containing poly(ethylene oxide) (PEO), SDS, and HP-β-CD in the buffer vial and a solution containing SDS and HP-β-CD into the capillary, the sensitive determination of aspartic acid enantiomers (derivatized with naphthalene-2,3-dicarboxaldehyde) in beers and soymilk samples was achieved [85]. Under these conditions, two different preconcentration phenomena take place; the difference in viscosity between the sample zone and PEO lead to a preconcentration by stacking, and the interaction between the amino acid enantiomers and SDS gives rise to a preconcentration by sweeping. Thus, 100 and 110-fold enhancement in the sensitivity of D- and L- enantiomers was reached, respectively. Employing the same principle but slightly modifying the components of the buffer vial solution (PEO, STDC, β-CD, and isopropanol) and the capillary solution (STDC, β-CD, and isopropanol), the sensitive chiral analysis of tryptophan, phenylalanine and glutamic acid (derivatized with 9-fluorenylmethoxycarbonyl chloride (FMOC)) in beer samples was also obtained [57]. In this case, an increase in the sensitivity between 100 to 1000 times (which enabled to reach LODs in the nM level) was achieved. Finally, a cation-selective exhaustive injection was also combined with sweeping to achieve LODs of around 80-90 pg/mL methamphetamine enantiomers by CD-MEKC-UV [158].

6. Chiral MEKC-MS

The coupling of chiral MEKC with MS has proven its potential to achieve enantioseparations with increased sensitivity and selectivity compared with other detection systems. The main drawback of this coupling is the interferences caused by the presence of non-volatile surfactants in the separation medium which can deteriorate the efficiency in the ionization [17]. In order to overcome these difficulties, some strategies have been developed such as the use of chiral polymeric micelles in the

separation buffer when the direct separation mode is employed or the use of volatile micelles and a chiral derivatizing reagent when the indirect separation mode is applied.

The most frequent strategy to avoid interferences in the ionization source in MS in the last twenty years has been the use of high molecular weight polymeric surfactants (molecular micelles) using electrospray ionization (ESI) [159, 160]. The use of micelle polymers has shown to be an interesting alternative due to their favourable characteristics such as [159]:

- zero CMC, this allowing to use them at significantly lower concentrations than the CMC values corresponding to surfactant monomers,
- lower surface activity and low volatility, which provide a stable electrospray in MS detection and therefore less analyte signal suppression,
- high electrophoretic mobility,
- high stability in the presence of organic solvents (even at high concentrations) since covalent bonds between monomers are not affected, this allowing to use these modifiers to improve the separation of some compounds,
- improved mass transfer of analytes giving rise to shorter analysis times and better signal to noise ratio values.

Different polymeric micelles have been synthesized, characterized and proposed for chiral MEKC-MS. The most popular ones have been amino acid-based polymeric micelles [40-52, 56] and carbohydrate-based polymeric surfactants [53]. Thus, poly(sodium N-undecanoyl-L-valinate) (poly-L-SUV) demonstrated its feasibility to achieve the separation of the enantiomers of 1,1'-binaphtol and their detection using electrospray ionization mass spectrometry (ESI-MS) by selected ion monitoring (SIM) in the negative mode. Optimization of the ESI-MS parameters and the concentrations of poly-L-SUV and the volatile background electrolyte and pH enabled the successful enantiomeric separation [40]. The potential of poly-L-SUCL to carry out the simultaneous enantioseparation and detection of eight structurally similar β-blockers was investigated by using tandem UV and MS detection. The use of this polymeric micelle was shown to considerably improve

separation efficiency and detection sensitivity for β-blockers as compared with unpolymerized micelle of L-SUCL additionally generating lower separation currents [46]. This molecular micelle enabled to reach LODs of 0.2 μg/mL for atenolol and metoprolol enantiomers by using a 2-acrylamido-2-methyl-1-propane-sulfonic acid (AMPS) coating on the capillary walls which increased the reproducibility of the method and allowed its application to the analysis of tablets [47]. Poly-L-SUCL was also used by Hou *et al.* for the simultaneous chiral analysis of four different ephedrine alkaloids by MEKC-ESI-MS [43, 44]. A central composite design was carried out to optimize the electrospray chamber parameters and the sheath liquid conditions in order to achieve the maximum sensitivity. Using the most appropriate MS parameters it was possible to obtain a significantly higher sensitivity than that obtained by MEKC-UV for the stereoisomers of ephedrine and related compounds [43]. Subsequently, once the MEKC-MS method was validated in terms of linearity, LOD, LOQ, precision and robustness, it was successfully applied to the enantiomeric determination of five ephedrine alkaloids in dietary supplements standard reference materials after a SPE treatment for sample cleaning [44]. The enantiomeric separation of different binaphthyl derivatives was also achieved using poly-L-SUCL in the coupling MEKC-MS. For instance, BNA was enantiomerically separated in less than 20 min by MEKC-ESI-MS in positive ion mode. The optimal separation conditions were selected after studying the influence of different parameter by a multivariate optimization approach [41]. The chiral analysis of BNP and BOH using a dual system with poly-L-SUCL and poly-L-SULV by MEKC-ESI-MS was also reported [42]. Separation parameters such as background buffer composition, voltage, temperature, and nebulizer pressure were optimized using a multivariate central composite design. Both pairs of enantiomers were baseline separated in less than 10 min. Poly-L-SULV showed also its potential as chiral selector for MEKC-MS in the development of analytical methods enabling the base line separation of warfarin [49], and warfarin with its five metabolites and the internal standard [48]. These methods were successfully applied to the analysis of the studied compounds in human plasma samples [48, 49]. Also, the molecular micelle poly-sodium N-undecenoyl-L,L-leucylalaninate (poly-L,L-SULA) was applied to the chiral analysis of

venlafaxine and O-desmethylvenlafaxine in human plasma by MEKC-MS with LODs 10.5-15 ng/mL [50]. Poly-sodium N-undecenoxy carbonyl-L-isoleucinate (poly-L-SUCIL) showed a great potential for the enantiomeric separation of barbiturates (mephobarbital, pentobarbital and secobarbital) by MEKC-MS [51]. After a SPE treatment and a multivariate approach to optimize the experimental conditions, LODs of 7.8 μg/mL were achieved for each analyte. Hou *et al.* evaluated a set of six different polymeric micelles as pseudostationary phases to carry out the chiral separation of two benzodiazepines (oxazepam and lorazepam) and one benzoxazocine (nefopam) [52]. Among the micelles studied, poly-sodium N-undecenoxy carbonyl-L,L-leucyl-valinate (poly-L,L-SUCLV) and poly-sodium N-undecenoyl-L-leucinate (poly-L-SUL) were chosen as chiral selectors for the development of chiral methodologies by MEKC-MS. While Poly-L,L-SUCLV originated enough enantioseparation in the simultaneous analysis of the two benzodiazepines, poly-L-SUL enabled the baseline separation of nefopam [52]. Rizvi *et al.* performed the synthesis and characterization of three amino acid-derived (L-leucinol, L-isoleucinol, L-valinol) sulfated chiral surfactants and carried out a study about their discrimination power to achieve the enantioseparation of phenylethylamines, β-blockers and ephedrine compounds [45]. The results obtained in this work showed that poly-sodium N-undecenoyl-L-isoleucine sulfate (poly-L-SUCILS) was the most appropriate pseudostationary phase to obtain the chiral separation of the studied compounds. In addition, the developed methodology, which enabled to reach a LOD of 325 ng/mL, was successfully applied to the determination of (±)-pseudoephedrine in human urine.

It is worthy to mention that alpha- and beta-glucopyranoside-based polymeric surfactants with two different chain lengths and head groups showed their fully compatibility with electrospray ionization MS and the clear superiority of MEKC-MS over MEKC-UV analysis of different drugs [7, 53]. In fact, a MEKC-MS methodology was successfully developed based on the use of a polymer of α-D-glucopyranoside-based surfactant (namely, poly-α-D-SUGP) as chiral selector for the enantioselective analysis of different ephedrine alkaloids and β-blockers, and LODs of 10-100 ng/mL were reached [7].

Although ESI has been the most widely ionization source applied in MEKC-MS, APPI has also been applied to achieve the enantiomeric separation of benzoin derivatives using two polymeric surfactants (poly-L-SUCL and poly-L-SULV) [56]. APPI is a softer ionization source than ESI, so it could be an attractive research area in the future [15].

The second strategy implemented to avoid problems in the coupling MEKC-MS with chiral micelles is based on the indirect separation mode. As described in section 3, derivatization with a chiral reagent such as FLEC together with the use of a volatile micelle such as APFO has shown to have a big potential in this context. As an example, the development of analytical methodologies for the enantiomeric determination of amino acids by MEKC-ESI-MS with LODs ranging from 0.13-11.92 nM and their application to the analysis of biological samples (cerebrospinal fluids) can be cited [133, 134].

7. Applications of chiral MEKC to the analysis of real samples

Table 3 groups the characteristics and applications of some of the most recent and relevant chiral methodologies developed by MEKC for the analysis of food, biological, pharmaceutical, forensic or environmental samples, covering the period of time from 2015 to present. As it can be observed in **Table 3**, the direct working mode was predominant as chiral separation strategy in MEKC, as expected. Most works were based on the use of mixtures of an achiral surfactant and a single CD. The preferred achiral surfactant to be combined with native or derivatized CDs was the anionic SDS although the positively charged surfactant cetyltrimetrylammonium bromide (CTAB) or the ionic liquid-based surfactant (N-dodecyl-N-methylpyrrolidinium bromide (C_{12}MPYB)) were also employed combined with α-CD and HP-β-CD, respectively. As CDs, the three native CDs (α-CD, β-CD and γ-CD) were employed as well as some of their neutral or anionic derivatives. Even a mixture of two CDs was employed in a few cases (sulfobutylether-β-CD (SBE-β-CD)/TM-β-CD or β-CD/HP-β-CD) in combination with SDS. Other achiral ionic liquid-based surfactant employed in one of the works reported was [C_4MIm][$C_{12}SO_4$] that was combined with a lincosamide antibiotic

(clindamycin phosphate) as chiral selector instead with a CD. The use of a chiral surfactant such as STC combined with γ-CD or a mixture of a chiral micelle (SDC) with an achiral micellar system (SDS) was also reported. Other strategies employed in chiral MEKC within the direct working mode were the use of a chiral micellar system as the sole chiral selector in the separation medium (bile salts, chiral polymeric surfactants, amino acid-based surfactants and amphiphilic CD derivatives), or the use of ligand-exchange complexes such as those formed by L-Lysine, L-Isoleucine, L-Valine or L-hydroxyproline with Cu^{2+} as chiral selectors in the LE-MEKC mode. In the above-mentioned developed direct methods, it was quite frequent to add an organic modifier to the separation medium to improve the results. Thus, the use of isopropanol at percentages of 15% or 30% (v/v), methanol at percentages of 5%, 12% or 20% (v/v), or acetonitrile at 5% (v/v) was reported.

In addition to these strategies, indirect methods were also developed although in a lesser extent. In this case, chiral derivatizing reagents such as FLEC and (S)-(+)-4-(*N,*N-dimehtylaminosulfonyl)-7-(3-aminopyrrolidin-1-yl)-2,13-benzoxadiazole (DBD-Apy) were employed to form the diastereomers of the analytes that were subsequently separated using a volatile micelle in the separation buffer (APFO) or the anionic micelle SDS, respectively.

Regarding detection systems, MS, direct fluorescence, LIF and UV were employed being UV that used in most of the works reported in the period of time considered. MS detection was only employed in four of the works described in **Table 3** which were based on the use of an indirect method (FLEC and APFO) or on the use of chiral polymeric micelles (poly-L,L-SULA–or poly-α-D-SUGP). LIF detection was employed using DBD-Apy as chiral derivatizing reagent.

7.1. Food analysis

As shown in **Table 3**, different MEKC methodologies have demonstrated their potential for the enantiomeric separation of several food components. For instance, a CD-MEKC method based on the use of SDS and HP-β-CD in borate-phosphate buffer (pH 2.5) enabled the simultaneous determination of the content of catechins and methylxanthines in a high variety of tea samples from different geographical origin [161]. (+)-Catechin, (-)-catechin and (-)-epicatechin were

enantioselectively separated due to the presence of HP-β-CD in the medium. The developed methodology along with a chemometric approach for data analysis enabled to discriminate green tea samples according to their geographical origin which is a relevant parameter to determine the quality of commercial tea products. A similar MEKC method, also based on the combination of SDS and a CD (in this case 2,6-di-O-methyl-β-CD (DM-β-CD)) was also applied to the chiral analysis of six major catechins and theanine in different green tea samples [162]. Because of the poor UV absorption of theanine, it was previously derivatized with o-phthaldialdehyde in the presence of N-acetyl-L-cysteine. Results obtained in this work provided useful information about the fermentation process and thermal treatment of green tea samples since the presence of D-theanine is an indicator of enzymatic or microbial processes and the presence of (-)-catechin is related with a thermal degradation. A mixture of SDS and a CD was also successfully applied for the enantiomeric separation of lipids. In this line, Kodama *et al.* developed a method for the separation of both regioisomers and enantiomers of different hydroxyeicosatetraenoic acids using HP-γ-CD and SDS [163]. Subsequently, this methodology was applied to evaluate the stereochemistry of 8-, and 12-hydroxyeicosatetraenoic acids from two marine red algae (*Gracilaria vermiculophylla* and *Gracilaria arcuate*). The potential of MEKC to achieve the chiral analysis of protein and non-protein amino acids was demonstrated by developing two different methodologies in the last five years. On the one hand, the use of SDS as surfactant, a dual system of CDs (β-CD and HP-β-CD) as chiral selector, isopropanol as organic modifier (to improve the β-CD solubility), and D-fructose as additive (which increases the viscosity which slightly reduces the EOF and increases the migrations times) allowed the enantioseparation of six different protein amino acids (previously derivatized with FMOC [164]. This methodology was applied to the determination of glutamic and aspartic acids enantiomers in rice wine (see **Figure 4**). Although some amounts of D-glutamic acid and D-aspartic acid were found in the rice wine samples, they could not be correlated with the rice wine age. On the other hand, a MEKC indirect approach based on the use of FLEC as chiral derivatization reagent and APFO as pseudostationary phase was proposed to carry out the fast (<6 min) enantioselective separation of

DL-selenomethionine [135]. In addition, this method was successfully applied to the quality control of food supplements through the determination of L-selenomethionine. This approach allowed to achieve LODs of 3.1 and 3.7 μM for D- and L-selenomethionine, respectively. Another indirect MEKC method was reported by Yamamoto *et al.* for the separation of DL-aldopentoses and aldohexoses diastereomers using DBD-Apy as chiral derivatization reagent and phenylboronate buffer containing SDS as background electrolyte (BGE) [165]. The applicability of the developed MEKC-LIF method was demonstrated by the chiral analysis of DL-galactose in the hydrolyzate of commercial red seaweed samples.

7.2. Analysis of biological samples

MEKC has also proven its potential to perform the enantiomeric separation of compounds of interest in biological samples. In this research field and as **Table 3** shows, the preferred detection systems have been fluorescence and MS. The simultaneous chiral analysis of protein amino acids by MEKC with fluorescence detection was carried out employing two different micellar systems. The combination of SDS with β-CD (in a borate buffer containing 15% (v/v) isopropanol) was employed by Prior *et al.* for the enantioselective analysis of FMOC-derivatized amino acids in cerebrospinal fluid demonstrating the potential of this methodology for the analysis of low D-amino acids concentrations in the presence of abundant amounts of L-amino acids (**Figure 5**) [166]. Conversely, Evans *et al.* used the combination of SDS and the bile salt SDC as surfactants for the enantiomeric determination by MEKC-LIF of protein amino acids previously derivatized with 2,3-naphthalenedicarboxaldehyde in the islets of Langerhans (an endocrine portion of the pancreas) whose functions are dysregulated under type 2 diabetes mellitus disorder [55]. The LODs obtained by this methodology were lower than 10 nM. Even though the MEKC-MS coupling offers several advantages due to the possibility to provide qualitative and quantitative information about the analytes separated, it is still problematic due to the presence of non-volatile selectors in the MS chamber. To overcome this incompatibly, MEKC-MS approaches based on the use of APFO as a volatile pseudostationary phase have been developed by different authors [133, 134]. In these approaches,

off-line [134] and in-capillary [133] derivatization procedures with FLEC were performed to form stable diastereomers with the enantiomers of a high number of protein amino acids. The applicability of these methodologies to biological samples was tested by analyzing both artificial cerebrospinal fluid samples [133] and human cerebrospinal fluid samples [134]. When off-line derivatization was employed, it was possible to reach lower LODs (0.13-0.63 μM) than when an in-capillary derivatization was used (2.99-11.92 μM). Another way of making MEKC compatible with MS is using polymeric surfactants. For instance, Liu *et al.* employed poly-L,L-SULA as chiral selector to develop an enantioselective MEKC-MS/MS method to carry out pharmacokinetic and pharmacodynamic studies of venlafaxine and O-desmethylvenlafaxine in human plasma [50]. This study demonstrated the potential of MEKC-MS to identify drug-drug interactions involving the enantiomers of the two antidepressants while administering indinivar therapy (which acts as an inhibitor to prevent the breakage of polyproteins in patients with HIV).

7.3. Pharmaceutical analysis

In the field of pharmaceutical analysis, only four MEKC methodologies have been reported in the period of time considered. All of them have been properly validated following the International Conference on Harmonisation (ICH) guidelines. As it can be seen in **Table 3**, three of these methodologies make use of the combination of SDS with a CD [167], a dual system of CDs [168], or a ligand-exchange complex [110] in alkaline media to determine the chiral purity of three chiral drugs marketed as pure enantiomers. Orlandini *et al.* employed 50 mM γ-CD and 100 mM SDS in 100 mM borate buffer (pH 9.0) for the enantiomeric separation of ambrisentan and its separation from three different impurities [167]. For method development, the authors applied a Quality by Design approach which is a relevant procedure in the pharmaceutical industry. Under the optimal experimental conditions ambrisentan enantiomers were separated (resolution of 1.07) and a LOD of 1.5 μg/mL was achieved. The application of the developed method to the analysis of a pharmaceutical formulation allowed the determination of the enantiomeric impurity of ambrisentan at levels of 0.1%. The combination of SDS with a dual system of CDs was used by Flor *et al.* to develop for the first

time a stereoselective MEKC method for the simultaneous determination of montelukast enantiomers and diastereomers and its degradation products in bulk product, pouches and chewable tablets [168]. The use of the two CDs, one of them neutral (TM-β-CD) and the other anionic (SBE-β-CD), was imperative since the neutral one was crucial for the simultaneous separation not only of the montelukast enantiomers (R and S-trans) but also of another pair of enantiomers (R- and S-cis). In this case, the combination of the "one factor at time" approach and a multivariate design was applied to evaluate the influence of different experimental parameters on the stereoselective separation. Under the best conditions, the method allowed determining a percentage of 0.02% of the enantiomeric and diastereoisomeric impurities. On the other hand, Sari *et al.* developed a pressure-assisted MEKC methodology for the enantiomeric purity analysis of ampicillin employing the principle of ligand exchange *via* the combination of SDS with a L-Lysine-Cu(II) complex [110]. The optimization of several parameters, using the "one factor at time" approach, enabled to select the optimal conditions to achieve the separation in a short time (7 min) with a high-sensitivity and low LOD (1.25 μM).

More recently, and as previously mentioned in section 6, Akter and Shamsi [47] have demonstrated the usefulness of the combined used of a covalently bonded AMPS capillary column and the polymeric micelles poly-L-SUCL to the simultaneous separation of three beta-blockers (atenolol, metoprolol and, pindolol (which was used as internal standard)) commercialized as a racemic mixture. By using the optimized conditions (see **Table 3**) the developed methodology was applied to the analysis of commercial atenolol and metoprolol tables (see **Figure 6**).

7.4. Forensic and environmental analysis

Although in a lesser extent (just two works have been published during the last five years), MEKC has also demonstrated its potential in the development of chiral methodologies in other interesting research fields such as forensic and environmental analysis. In the former, Mikuma *et al.* developed a cation-selective exhaustive injection and sweeping CD-MEKC methodology to achieve a high-sensitive analysis of methamphetamine [158]. In this work, sulfated-γ-CD (S-γ-CD) as chiral selector was added to the micellar buffer including SDS without any other change in the buffers employed to

preserve the online concentration mechanism (see **Figure 7A**). By using this procedure, over 10000-fold sensitivity increase was achieved which provided a LOD in the pg/mL level. The suitability of this methodology was demonstrated by analysing methamphetamine (and its main chiral metabolite (amphetamine)) in hair samples as it is illustrated in **Figure 7B**. In the environmental field, Creamer *et al.* developed a chiral methodology based on MEKC with LIF detection for the enantiomeric analysis of different neutral amino acids in water samples from the Mono Lake in USA (which is considered an analogue for astrobiologically relevant targets) [67]. This approach was based on the combined use of γ-CD and STC as chiral system and it was applied to the analysis of neutral amino acids (previously derivatized with 5-carboxyfluorescein succinimidyl ester) and enabled to reach LODs between 5 and 100 nM for the amino acids analyzed.

8. Conclusions

MEKC has shown to have a big potential for the enantiomeric separation and determination of a wide variety of chiral compounds in different pharmaceutical, food, biological, forensic or environmental samples. Different separation strategies can be followed to carry out these enantiomeric separations. Among the different possibilities, the simplest one implies the use of a chiral micelle such as a bile salt, a polymeric micelle or a mixed micellar system, but the fact is that in many cases this option is not enough to obtain an adequate enantioselective separation. For this reason, the most effective strategy, which has been employed in most of the articles devoted to the use of chiral MEKC, is based on the combined use of a chiral or an achiral micelle with a chiral selector in the separation medium. As chiral selectors to be combined with the micellar system in MEKC, CDs have been the preferred ones being the mixture of SDS as micellar system plus a CD the most popular choice without any doubt to carry out a chiral separation by MEKC. Other chiral selectors successfully used have been antibiotics or ligand-exchange complexes.

The most common detection system has been UV absorption followed by LIF and MS. The lowest LODs obtained with these detection systems were at the pg/mL, nM or ng/mL level, respectively,

Thus, the combination of UV detection with cation-selective exhaustive injection and sweeping as preconcentration technique enabled to achieve a LOD in the pg/mL level for the determination of methamphetamine in hair samples. On the other hand, LODs lower than 10 nM were obtained by applying a MEKC-LIF method to the analysis of derivatized protein amino acids in an endocrine portion of the pancreas~~, and~~. Also, LODs in the ng/mL level were reached using a polymeric micelle as chiral selector in a MEKC-MS method, which included a previous step of solid-phase extraction and could be applied to the determination of venlafaxine and O-desmethylvenlafaxine in human plasma.

It is relevant to mention here, that despite the advantages that the MEKC-MS coupling provides (it enables to obtain qualitative and quantitative information about the analytes separated), the presence of non-volatile micelles and chiral selectors makes challenging the use of MS detection. However, interesting MEKC-MS approaches based on the use of polymeric micelles (direct working mode) or volatile pseudostationary phase (such as APFO) (indirect working mode) have demonstrated a great potential for the development of sensitive methodologies to be applied to the analysis of complex matrices. For these reasons, new developments are expected in this field in the near future. Undoubtedly, the use of new surfactants compatible with MS detection and robust preconcentration methods will enhance the sensitivity and selectivity obtained by chiral MEKC. This will increase the applicability of this chiral separation mode to solve analytical problems demanding an adequate solution in different fields of social interest.

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Figure captions.

Figure 1. Scheme of the separation principle in chiral MEKC.

Figure 2. Scheme of the separation principle in CD-MEKC.

Figure 3. (a) Micelle-to-cyclodextrin stacking (MCDS) chiral MEKC mechanism, (i) a CD solution plug remained inside the capillary after MCDS and (ii) the micelles from the BGE transported the analytes into the CD zone. (b) Proof-of-concept for MCDS-chiral-MEKC using fenoprop. Experimental conditions: 50 mM SDS in 50 mM phosphoric acid and 50 mM γ-CD in 100 mM phosphoric acid; sample diluent was BGE (i) and 10 mM SDS in 100 mM phosphoric acid (ii–viii); sample concentration was 50 μg/mL (i, ii) and 5 μg/mL (iii–viii); injection was 5 (i) and 50 s (ii–viii) at 50 mbar; a CD solution plug was injected for 20 (iii), 30 (iv), 40 (v), 50 (vi), 75 (vii), and 100 s (viii) before the sample; voltage: 20 kV; UV detection: 200 nm. Reprinted from [156], copyright (2019) with permission from American Chemical Society.

Figure 4. Electropherograms corresponding to the MEKC analysis of amino acid standard, rice wine sample and spiked rice wine sample. Experimental conditions: 30 mM SDS, 35 mM β-CD, 6 mM HP-β-CD, 25 mM D-fructose and 15% isopropanol in 100 mM boric acid (pH 9.5), injection: 3 s at 50 mbar, voltage: 20 kV, temperature: 25 ºC, UV detection: 254 nm. Peak identification: 5, D-glutamic acid; 5', L-glutamic acid; 6, D-aspartic acid; 6', L-aspartic acid; *FMOC. Reprinted from [164], copyright (2017) with permission from Taylor & Francis.

Figure 5. Electropherograms of A) cerebrospinal fluid spiked with 250 nM of 12 DL-amino acids and 2500 nM of DL-tryptophan, and B) blank cerebrospinal fluid by MEKC-FD. Experimental conditions: 30 mM SDS, 30 mM β-CD and 15% isopropanol in 40 mM borate (pH 9.5), injection: 13 s at 0.5 psi, voltage: 25 kV, temperature: 22 ºC; emission wavelength: 331 nm. Reprinted from [166], copyright (2018) with permission from Springer Nature.

Figure 6. Electropherograms corresponding to the analysis of a mixture prepared by using (A) R,S-atenolol and R,S-metoprolol tablets, and (B) 100:1 S-atenolol and R-atenolol. Experimental conditions: capillary column was covalently bonded with 28 mg/mL AMPS, 25 mM poly-L-SUCL

in 20 mM ammonium acetate and 15 mM TEA (pH 8.8), injection: 50 s at 5 mbar, voltage: 30 kV, temperature: 20 °C. Peak identification: 1, S-(-)-atenolol; 1', R-(+)-atenolol; 2, S-(-)-metoprolol; 2',R-(+)-metoprolol; 3, S-(-)-pindolol; 3', R-(+)-pindolol. Reprinted from [47], copyright (2020) with permission from Elsevier.

Figure 7. A) Predicted mechanism of cation-selective exhaustive injection sweeping cyclodextrin modified MEKC-UV. a) The capillary was filled with medium-conductivity buffer (MCB), then partially filled with high-conductivity buffer (HCB); b) electrokinetic injection of samples (normal-polarity); c) SDS and HS-γ-CD were introduced to the capillary with reversed-polarity mode; and d) in MCB, the hydrophilicity was weakened by methanol (the SDS micelles were mostly destroyed), therefore HS-γ-CD from the inlet vial could catch up with the swept methamphetamine and allowed the enantioseparation. B) Electropherograms of a) the hair of a methamphetamine user, b) a fivefold diluted sample of a), c) standards solution of racemic methamphetamine and amphetamine (at 1 ng/mL each enantiomer), and d) a mixture of b) and c). Experimental conditions: 20 mM SDS, 20 mM HS-γ-CD and 20% methanol in 100 mM phosphate (pH 2.7), injection: 10 min at +18 kV, voltage: -18 kV, temperature: 25 ºC, UV detection: 195 nm. Reprinted from [158], copyright (2016) with permission from John Wiley and Sons.

Table 1. Most employed **s**urfactants in chiral MEKC and their CMC, aggregation number (*N*) and Kraft point (K_p).

	SURFACTANT	CMC[a] (M)	N[b]	K_p[c]
Chiral bile salt	Sodium cholate (SC)	13-15 x 10^{-3}	2-4	-
	Sodium deoxycholate (SDC)	4-6 x 10^{-3}	4-10	-
	Sodium taurocholate (STC)	10-15 x 10^{-3}	5	-
	Sodium taurodeoxycholate (SDTC)	2-6 x 10^{-3}	-	-
Polymeric micelles	Sodium N-undecenoyl leucyl valinate (L-SULV)	7 mM	39	-
	Polysodium N-undecenoyl leucyl valinate (poly-L,L-SULV)	0	18	-
	Sodium N-undecenoyl-L,L-leucyl alaninate (L,L-SULA)	-	42	-
	Poly-sodium N-undecenoyl-L,L-leucyl alaninate (poly-L,L-SULA)	0	18	-
	Sodium N-undecenoxy carbonyl-L-leucynate (L-SUCL)	8/7.8 mM	75	-
	Poly-sodium N-undecenoxy carbonyl-L-leucynate (poly-L-SUCL)	0	37/39	-
	sodium N-undecylenyl-α-D-glucopyranoside 4,6-hydrogen phosphate (α-D-SUGP)	3.8 mM	137	-
	poly(sodium N-undecylenyl-α-D-glucopyranoside 4,6-hydrogen phosphate) (poly-α-D-SUGP)	0	62	-
Achiral surfactants which are used in combination with a chiral selector	Sodium dodecyl sulfate (SDS)	8.1 x 10^{-3}	6.5	16
	Sodium tetradecyl sulfate (STS)	2.1 x 10^{-3} (50ºC)	138*	32
	Sodium decanesulfonate	40 x 10^{-3}	40	-
	Sodium dodecanesulfonate	7.2 x 10^{-3}	54	37.5
	Sodium N-lauroyl-N-methyltaurate (LMT)	8.7 x 10^{-3}	-	<0
	Sodium N-dodecanoyl-L-valinate (SDVal)	5.7 x 10^{-3} (40ºC)	-	-
	Dodecyl trimethyl ammonium chloride (DTAC)	16 x 10^{-3} (30ºC)	-	-
	Dodecyl trimethyl ammonium bromide (DTAB)	15 x 10^{-3}	56	-
	Tetradecyl trimethyl ammonium bromide (TTAB)	3.5 x 10^{-3}	75	-
	Cetyl trimethyl ammonium bromide (CTAB)	0.92 x 10^{-3}	61	-

Data obtained from [4-8]

[a]CMC: Concentration of the surfactant above which it forms a micellar solution. The values showed in table were obtained with pure water as solvent at 25ºC, except * which was obtained using 0.10 M NaCl.

[b]N: number of surfactant molecules taking part in micelle formation.

[c]K_p: temperature above which the solubility of the surfactant increases steeply owing to the formation of micelles.

Table 2. Characteristics of the MEKC methodologies employed to perform mechanistic studies on chiral recognition.

Analyte	MEKC mode - detection	BGE	LOD	Ref
Palonosetron	MEKC-UV	15 mM SC in 30 mM borate (pH 9.2)	-	[137]
		30 mM SC + 12% (v/v) methanol in 30 mM borate (pH 9.2)	0.3 μg/mL	[138]
		30 mM SC in 30 mM borate (pH 8.55)		[139]
Fenorofen	CD-MEKC-UV	4 mM L-UCLB + 2-10 mM TM-β-CD in 5 mM ammonium acetate (pH 5.0)	-	[140]
Ambrisentan	CD-MEKC-UV	100 mM SDS + 10 mM γ-CD in 100 mM borate (pH 9.2)	-	[141]
Binaphthyl derivatives	MEKC-UV	20-60 mM SC, SDC or chenodeoxycholate (pH 12.0)	-	[142]
(R,S)-1,1′-Binaphthyl-2,2′-diylhydrogenphosphate	MEKC-UV	SC (pH 12.0)	-	[143]
Binaphthyl derivatives	MEKC-UV	20 mM L-Undecyl-Leucine + L-Arginine counterion in 5 mM borate (pH 7.0)	-	[36]
4 Dansyl-AAs, binaphthyl derivatives and Troger's base	MEKC-UV	24 mM poly-L-SULV in 50 mM phosphate/25 mM borate (pH 9.0)	-	[144]
Binaphthyl derivatives	MEKC-UV	25 mM poly-L-SUV or poly-L-SUI in 100 mM trisaminomethane (pH 9.5),	-	[145]

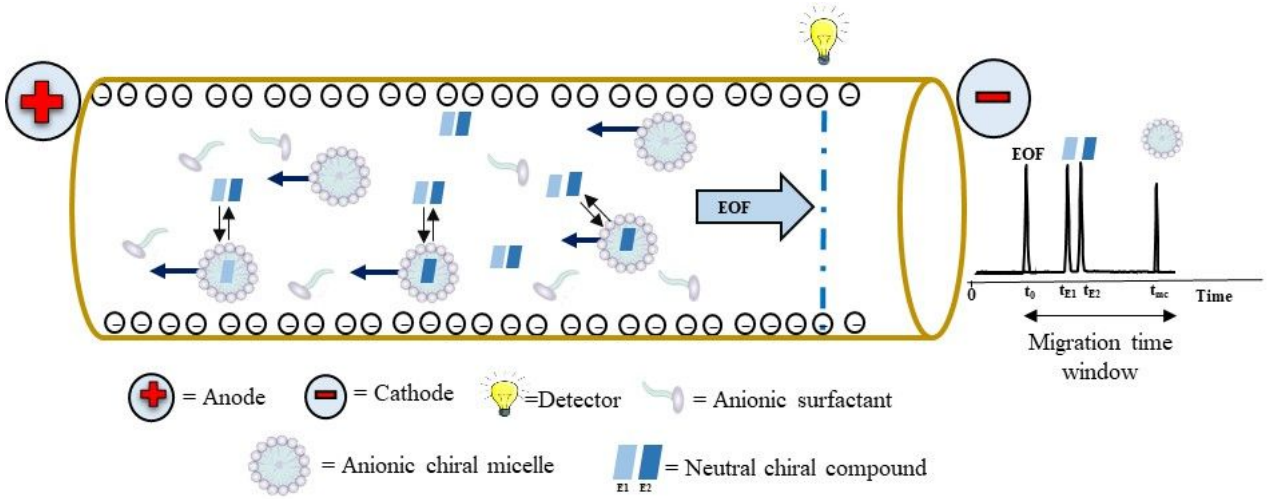
Abbreviations: L-UCLB, N-Undecenoxycarbonyl-Leucinol bromide; poly-L-SUI, poly-sodium N-undecenoyl-L-isoleucinate; poly-L-SULV, poly-sodium N-undecenoyl-L-leucylvalinate; poly-L-SUV, poly-sodium N-undecenoyl-L-valinate; SC: sodium cholate; S-β-CD, sulfated-β-cyclodextrin; SDC, sodium deoxycholate; SDS, sodium dodecyl sulfate; TM-β-CD, heptakis (2, 3, 6-tri-O-methyl)-β-cyclodextrin

Table 3. Characteristics of the most recent chiral methodologies developed by MEKC for the analysis of food, biological, pharmaceutical, forensic or environmental samples (2015-2020).

Analyte	Sample	MEKC mode - detection	BGE	LOD	Ref
Catechins	Green tea	CD-MEKC-UV	90 mM SDS + 25 mM HP-β-CD in 25 mM borate/phosphate (pH 2.5)	-	[161]
Catechins and theanine	Green tea	CD-MEKC-UV	65 mM SDS + 28 mM DM-β-CD in 25 mM borate/phosphate (pH 2.5)	0.1-0.2 μg/mL	[162]
8-, and 12- hydroxyeicosatetraenoic acids	Marine red algae	CD-MEKC-UV	75 mM SDS + 30 mM HP-γ-CD in 30 mM phosphate/15 mM borate (pH 9.0)	0.95-0.99 μg/mL	[163]
Glutamic acid and aspartic acid	Rice wine	CD-MEKC-UV	30 mM SDS + 35 mM β-CD + 6 mM HP-β-CD + 25 mM D-fructose + 15% (v/v) isopropanol in 100 mM borate (pH 9.5)	2.5 μM	[164]
Selenomethionine	Food supplement	(indirect) MEKC-UV	100 mM APFO (pH 8.5)	L-enantiomer: 3.7 μM D-enantiomer: 3.1 μM	[135]
Galactose	Red seaweed	(indirect) MEKC-LIF	50 mM SDS in 50 mM phenylboronate (pH 9.0)	-	[165]
11 protein amino acids	Cerebrospinal fluid	CD-MEKC-FD	30 mM SDS + 30 mM β-CD + 15% (v/v) isopropanol in 40 mM borate (pH 9.5)	10-100 nM D-enantiomers D-trytophan: 536 nM	[166]
13 protein amino acids	Islets of Langerhans	MEKC-LIF	45 mM SDS + 35 mM SDC in 150 mM borate (pH 9.2)	< 10 nM	[55]
14 protein amino acids	Cerebrospinal fluid	(indirect) MEKC-MS	150 mM APFO (pH 9.5)	0.13-0.63 μM	[134]
17 protein amino acids	Artificial cerebrospinal fluid	(indirect) MEKC-MS	150 mM APFO (pH 9.5)	2.99-11.92 μM	[133]
Venlafaxine (VX) and O-desmethylvenlafaxine (O-DVX)	Human plasma	MEKC-MS/MS	15 mM poly-L,L-SULA in 20 mM ammonium acetate/25 mM TEA (pH 8.5)	VX: 10.5 ng/mL O-DVX: 15 ng/mL	[50]
Ambrisentan	Tablets	CD-MEKC-UV	100 mM SDS + 50 mM γ-CD in 100 mM borate (pH 9.2)	1.5 μg/mL	[167]
Montelukast	Bulk drug, pouches and chewable tablets	CD-MEKC-UV	10 mM SDS + 10 mM SBE-β-CD + 10 mM TM-β-CD in 20 mM borate (pH 9.0)	0.30 μg/mL	[168]
Ampicillin	Tablets	LE-MEKC-UV	25 mM SDS + 10 mM L-Lys-Cu^{2+} in 10 mM ammonium acetate (pH 9.0)	1.25 μM	[110]
Metoprolol and atenolol	Tablets	MEKC-MS	25 mM poly-L-SUCL + 10% (v/v) methanol in 20 mM ammonium acetate/15 mM TEA (pH 8.8). Covalently bonded AMPS capillary column.	0.2 μg/mL	[47]
Methamphetamine (MA)	Hair	CD-MEKC-UV	20 mM SDS + 20 mM HS-γ-CD + 20% (v/v) methanol in 100 mM phosphate (pH 2.7)	S-MA: 77.9 pg/mL R-MA: 88.8 pg/mL	[158]
5 neutral amino acids	Lake water	MEKC-LIF	30 mM STC + 30 mM γ-CD + 5% (v/v) acetonitrile in 80 mM tetraborate (pH 9.2)	5-100 nM	[67]

Abbreviations: AMPS, 2-acrylamido-2-methyl-1-propane-sulfonic acid; APFO, ammonium perfluorooctanoate; DM-β-CD, dimehtyl-β-cyclodextrin; FD, fluorescence detector; HP-β-CD, 2-hydroxypropyl-β-cyclodextrin; LIF, laser induced fluorescence; L-Lys-Cu^{2+}, L-Lysine monohydrochloride and copper (II) sulfate pentahydrate; O-DVX, O-desmethylvenlafaxine; poly-L,L-SULA, poly-sodium N-undecenoyl-L,L-leucylalaninate; poly-L-SUCL, poly-sodium N-undecenoxycarbonyl-L-leucinate; SBE-β-CD, sulfobutylether-β-cyclodextrin; SDS, sodium dodecyl sulfate; STC, sodium taurocholate; TEA, triethylamine; TM-β-CD, heptakis (2, 3, 6-tri-O-methyl)-β-cyclodextrin; VX, venlafaxine.

Figure 1.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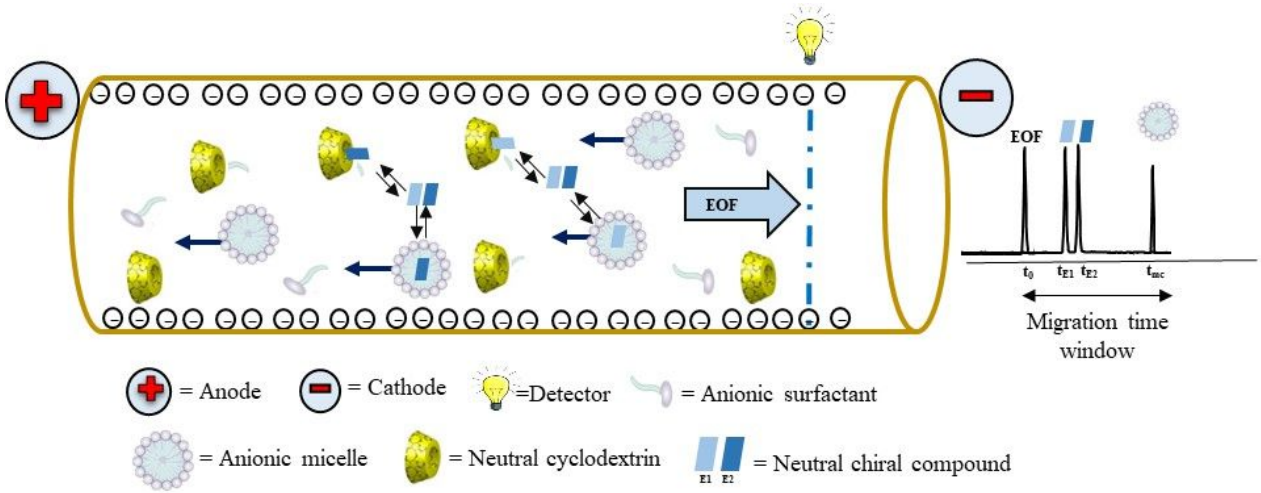
= Anionic chiral micelle
= Neutral chiral compound
E1 E2

Figure 2.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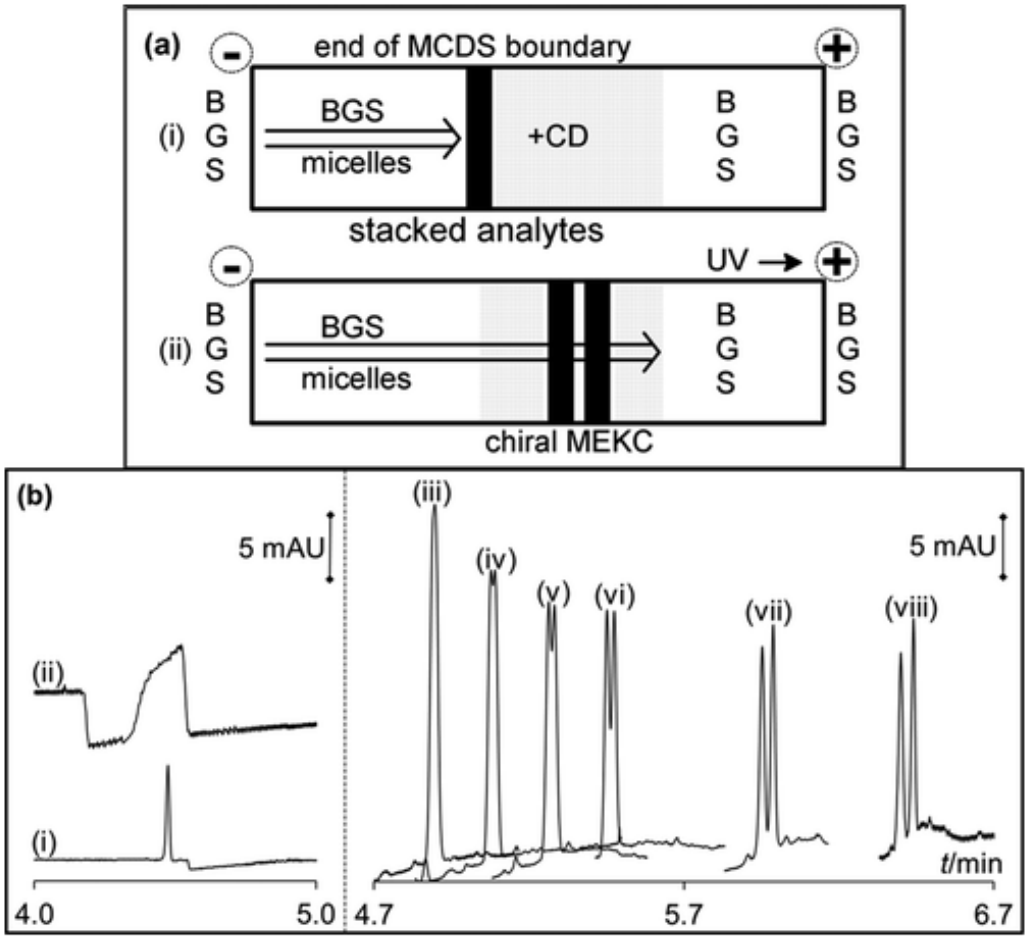
= Neutral chiral compound

Figure 3.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+CD
stacked analytes
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chiral MEKC
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4.0
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(iv)
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(vi)
(vii)
(viii)
4.7
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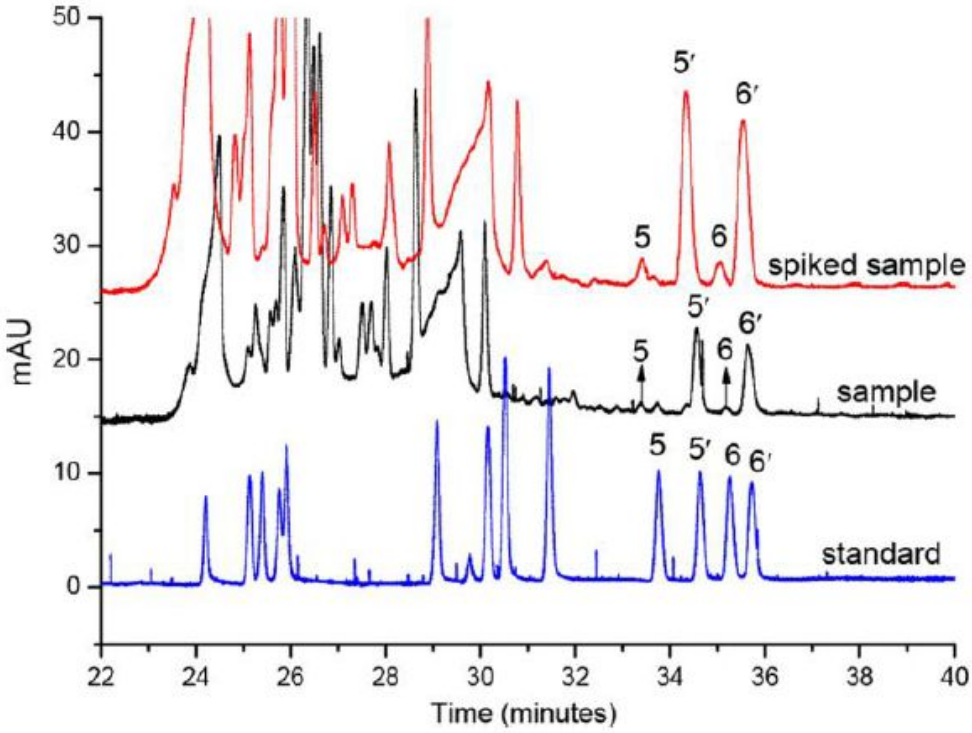
6.7
t/min

Figure 4.



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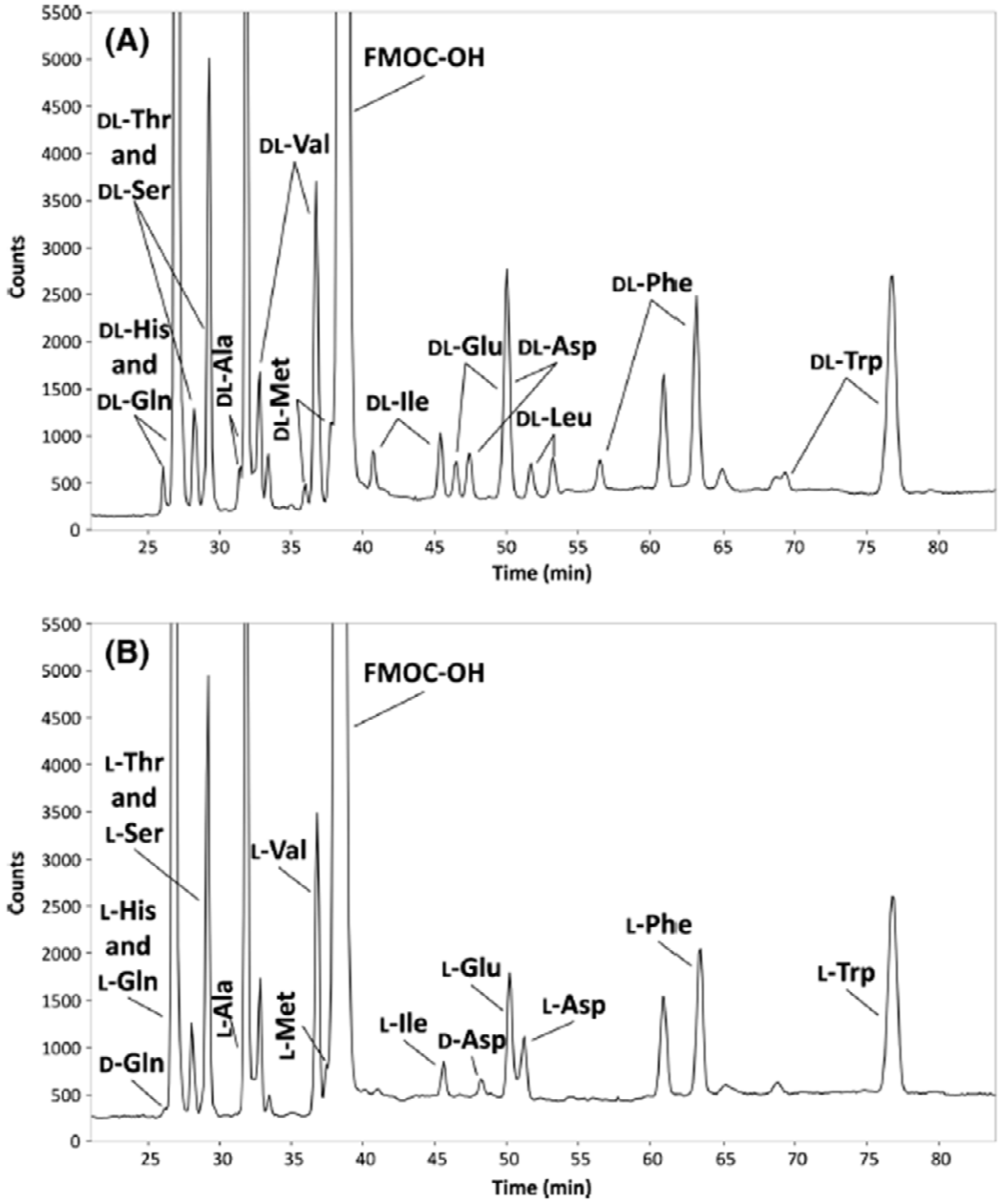
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DL-Val
DL-His and DL-Gln
DL-Ala
DL-Met
DL-Ile
DL-Glu
DL-Asp
DL-Leu
DL-Phe
DL-Trp
Counts
Time (min)
(B)
FMOC-OH
L-Thr and L-Ser
L-Val
L-His and L-Gln
L-Ala
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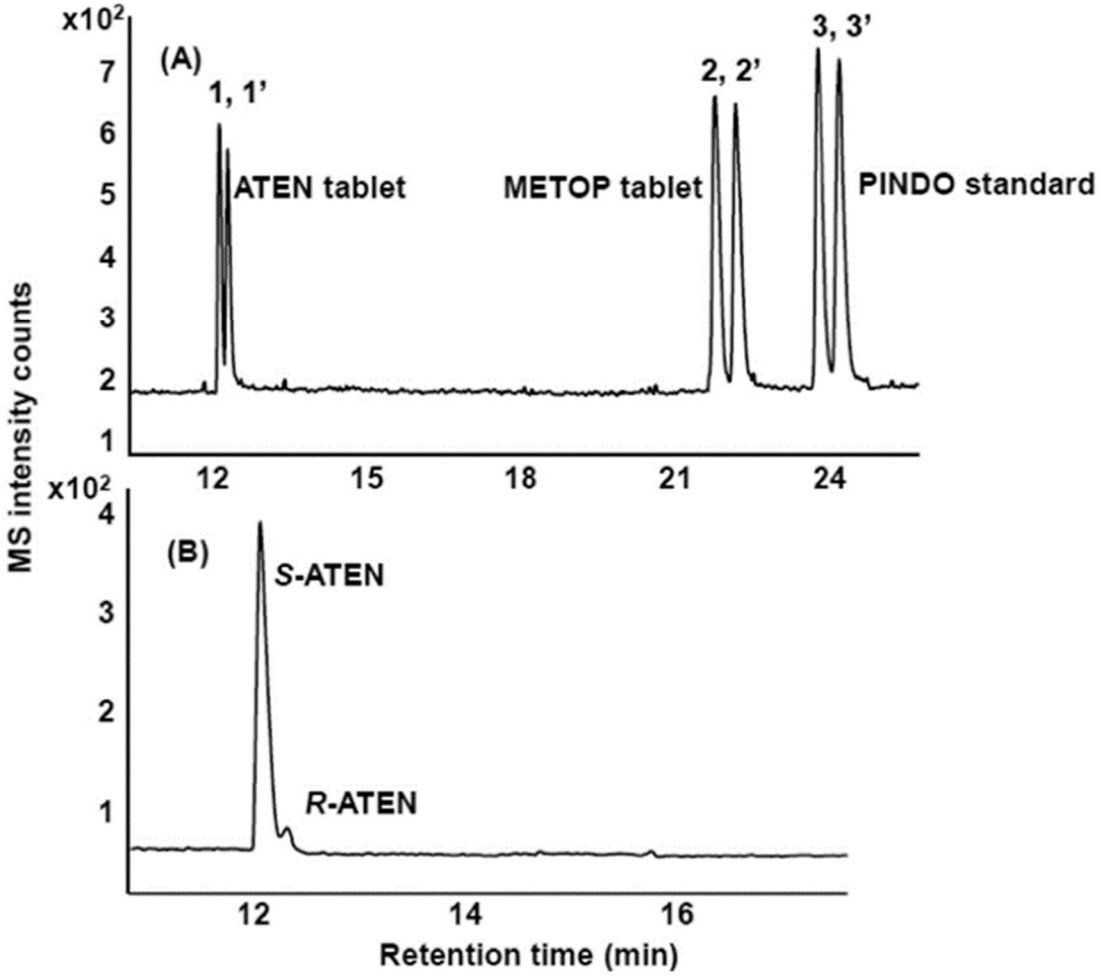
D-Gln
Counts
Time (min)

Figure 6.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METOP tablet
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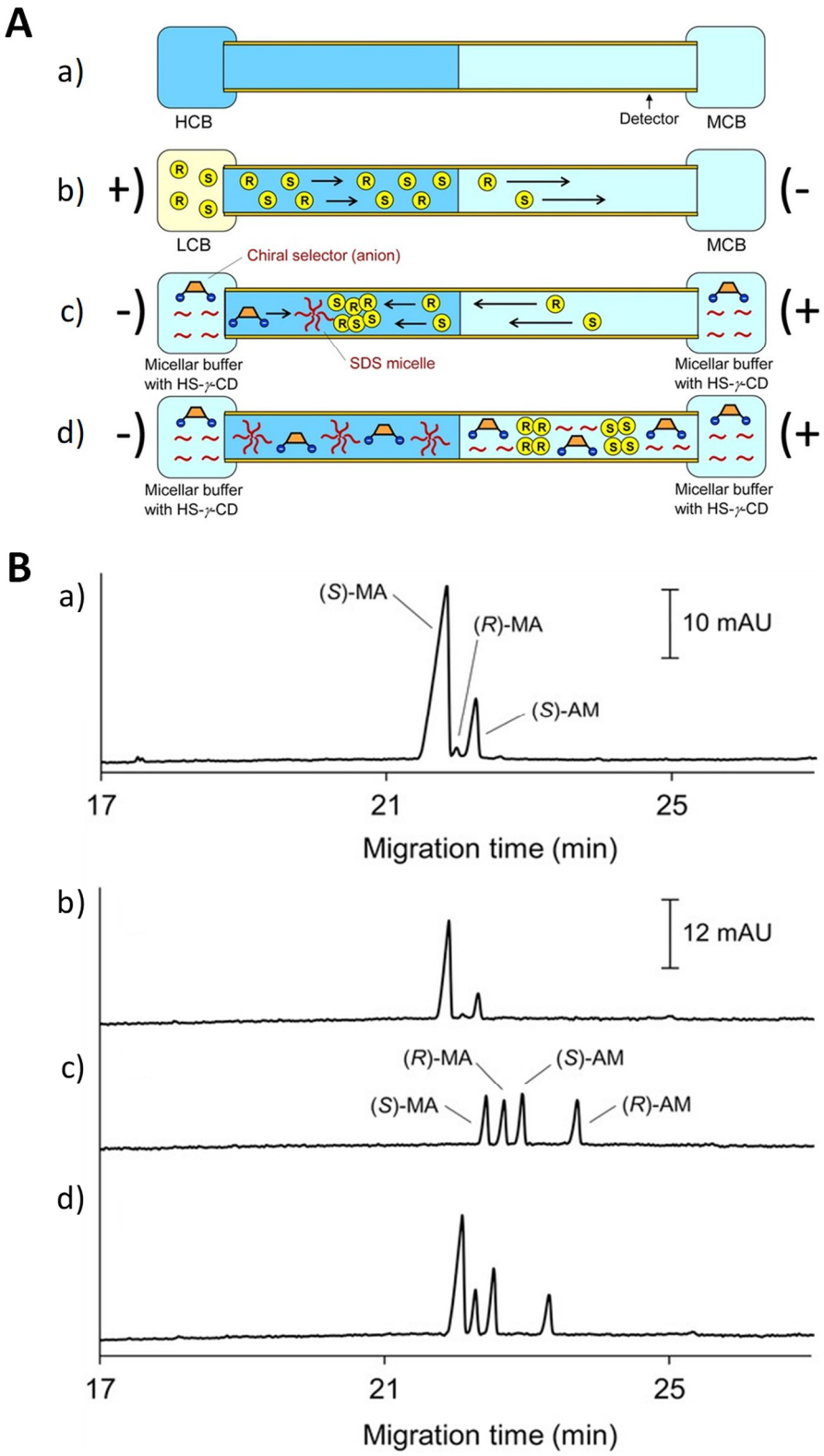
S-ATEN
R-ATEN
Retention time (min)

Figure 7.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HCB
Detector
MCB
b) +)
LCB
MCB
(-
Chiral selector (anion)
c) -)
Micellar buffer with HS-γ-CD
SDS micelle
Micellar buffer with HS-γ-CD
(+
d) -)
Micellar buffer with HS-γ-CD
Micellar buffer with HS-γ-CD
(+
B
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(S)-MA
(R)-MA
(S)-AM
10 mAU
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21
25
Migration time (min)
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d)
17
21
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Migration time (min)