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Enantiomeric determination of drugs in pharmaceutical formulations and biological samples by Electrokinetic Chromatography

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ABSTRACT

Chirality is a relevant issue in the pharmaceutical field due to the different biological activity that enantiomers of a chiral drug can show. In fact, the desired biological or pharmaceutical activity might be present in only one of the enantiomers, while the other enantiomer(s) may have different biological activity, be inactive or even toxic. This has motivated in recent years the development of drugs marketed as pure enantiomers to avoid exposing the organism to the action of enantiomers that may not be active or even harmful to health. Thus, it is of high interest to develop enantioselective analytical methodologies to control the presence of enantiomeric impurities and to understand the enantioselective metabolism of chiral drugs. This review gives an overview about the analytical strategies developed by electrokinetic chromatography (EKC) from 2010 to June 2019 for the enantiomeric determination of drugs in both pharmaceutical formulations and biological samples. The types of chiral selectors used, the migration order of enantiomers, their resolution, the detection technique employed and the sensitivity achieved are revised and compared. Also, applications to assess the enantiomeric purity control of pharmaceutical formulations and to determine chiral drugs in biological samples to study their metabolism are included. Advantages and limitations of the chiral methods developed by EKC are also discussed.

Keywords: Electrokinetic chromatography, Drugs, Pharmaceutical formulations, Biological samples, Chiral selectors, Enantiomers

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Introduction

Nowadays, more than 60% of the drugs marketed are chiral, and among them approximately an 88% are administered in their racemic form [1, 2]. Thus, the determination of enantiomers of chiral drugs is a relevant issue in the pharmaceutical field as they can act in different ways in biological processes [2]. Enantiomers present the same atomic composition, bonds and physicochemical properties. However, they can show different biological, pharmacokinetic and pharmacodynamic activities, since they possess stereospecific recognition to certain receptors and active sites [3]. In this sense, the desired biological or pharmacological activity can be active in one of the enantiomers, while for the other enantiomer(s) different situations may happen:

a) It can be inactive, as in the case of ibuprofen, propranolol or betaxolol [4, 5].
b) It can show the same activity, such as sotalol, which its two enantiomers possess antiarrhythmic properties [6].
c) It can be active, but of less intensity, as is the case of ketamine, for which the *R* enantiomer presents less anesthetic power [7], or colchicine, whose *R* enantiomer has lower antiuremic activity [8].
d) It can have a totally different activity, such as fluoxetine, whose *R* enantiomer is effective against anxiety and sexual dysfunction while its *S* enantiomer is useful against migraines [9], or the case of levomethorphan and dextromethorphan, which are an opioid analgesic and an antitussive, respectively [10].
e) It can be toxic, as is the case of penicillamine, whose *R* enantiomer is neurotoxic [7], bupivacaine, which is an anesthetic administered as racemic and whose *R* enantiomer possesses some cardiac and cerebral toxicity [11] or thalidomide, whose *S* enantiomer is a potent teratogenic agent [7].

For these reasons, pharmacological and toxicological studies need to be performed separately for each enantiomer and compared with the racemic drug, in order to avoid exposing the organism to the

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action of an enantiomer that may not be active or even harmful to health [12]. This has motivated in recent years, the increasing development of drugs marketed as pure enantiomers. Indeed, racemic drugs should only be used in those cases in which the enantiomers possess complementary biological activities. Moreover, even when the characteristics of both enantiomers have been established with certainty, it is still more advantageous to use single enantiomer formulations, as they require lower doses, provide higher safety margin and lower interindividual variability, and cause less drug interactions and side effects [13]. Nevertheless, the increasing use of drug formulations marketed as pure enantiomers requires the development of analytical methodologies using potent and sensitive analytical techniques to control the presence of enantiomeric impurities. In fact, according to the International Conference of Harmonization (ICH) regulation, the enantiomeric impurity must not exceed of 0.1% in the enantiomeric pure drug formulation [14]. For this reason, the ICH, the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) have established different guidelines for the correct validation of analytical methods and the marketing of chiral compounds [2].

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As above mentioned, the stereochemistry of drugs is a relevant topic in biological processes. Therefore, the determination of chiral drugs in biological fluids also acquires great importance to understand the physiological performance of a drug by studying its stereoselective metabolism and the identification of its metabolites. However, biological samples are usually complex matrices where analytes are found at very low concentration levels, what hinders their determination. Thus, generally, a suitable clean-up and preconcentration procedure is required prior to chiral analysis of biological samples. In this sense, different sample preparation techniques can be used, being solid-phase extraction (SPE) and liquid-liquid extraction (LLE) the most common. Nevertheless, the current trend moves toward the use of microextraction techniques which enable less consumption of solvents and samples, such as solid-phase micro-extraction (SPME) or liquid-liquid microextraction (LLME), among others.

The separation techniques most widely employed to perform chiral analysis have been those based on chromatographic and electrophoretic principles, such as Thin-layer Chromatography (TLC), High

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Pressure Liquid Chromatography (HPLC), Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC) and Capillary Electrophoresis (CE). Up to date, HPLC has been by far the most used technique to achieve chiral separations (55% of the publications related to chiral analysis), followed by CE (22%) and GC (15%), while SFC and TLC have been the less employed (5 and 3%, respectively) (**Fig. 1**, data obtained from SciFinder Scholar up to June 2019). Nevertheless, CE presents some advantages over HPLC which make it very suitable for performing enantiomeric separations. For instance, CE is considered a miniaturized and environmentally friendly technique, as it requires very small volumes of sample and solvents [15]. Moreover, HPLC chromatographic chiral columns are quite expensive, while CE does not require any column. Additionally, CE provides high efficiency, versatility, simplicity and feasibility enabling fast method development and high success rate, since the type and the concentration of the chiral selector can be easily optimized, which is impossible in HPLC where, in general, chiral stationary phases (CSP) are usually used for chiral analysis. Also, different chiral selectors can be combined in CE, whereas this is a difficult approach in chromatographic techniques [16, 17]. On the other hand, the main drawback of CE is its complex coupling to mass spectrometry (MS) detection, besides other problems related to repeatability of migration times and peak height/area [16]. Nevertheless, despite these drawbacks, CE is still a powerful technique for chiral analysis and a good alternative to HPLC to perform quick enantiomeric separations with low operating costs.

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There are different CE modes for chiral analysis, such as capillary electrochromatography (CEC), electrokinetic chromatography (EKC) and nonaqueous capillary electrophoresis (NACE). In CEC, the chiral selector is immobilized inside the capillary acting as a stationary phase similar to the HPLC technique, whereas in EKC and NACE a chiral selector is added to the separation buffer [16]. On the other hand, the difference between EKC and NACE is the separation medium. In EKC the chiral separation takes place in an aqueous medium, while in NACE it occurs in an organic solvent [16]. However, most applications of chiral CE are usually performed in aqueous medium. In this context, 82% of the publications related to chiral analysis by CE employed EKC as separation mode, followed by 17%

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which used CEC, while only a 1% were performed by NACE (data obtained from Scifinder Scholar up to June 2019). This is mainly due to the scarce commercial availability of CEC columns as well as their complex homemade fabrication, while EKC is more suitable because of its versatility, since a wide variety of chiral selectors can be added to the buffer solution, such as cyclodextrins (CDs), antibiotics, crown ethers, polysaccharides, proteins or surfactants, among others [16]. Nevertheless, although all of them have been effectively employed, CDs are still by far the most frequently used [17].

Therefore, because EKC is a powerful technique for chiral analysis, this review aims to give an overview about the most relevant analytical strategies developed by EKC in the last ten years for the enantiomeric determination of drugs in both pharmaceutical formulations and biological samples. Owing to the large number of publications, only articles published between 2010 and June 2019, and dealing with stereoselective determination of drugs in these samples, have been considered.

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Enantiomeric determination of drugs in pharmaceutical formulations and biological samples by EKC

Table 1 collects the most relevant works published in the last decade dealing with the chiral analysis of drugs by EKC in pharmaceutical formulations and biological samples. These works have been classified according to the pharmacological activity of the analytes studied. The majority of the methodologies developed in these articles described the individual enantiomeric determination of a specific drug, although there are 20 articles reporting the enantiomeric determination of more than one compound (**Table 1**). Nevertheless, from these articles, the simultaneous enantioseparation of different drugs (belonging to the same family) was carried out in 13 of them under the same experimental conditions [19, 22, 25, 28, 30, 36, 57, 58, 66-69, 72]. In the remaining 7 works, the chiral separation of different compounds was presented in an individual way, using different conditions to achieve the enantioseparation of all the analytes [42, 53, 61, 64, 80, 89, 90]. Moreover, only in 2 of these 7 works the chiral separation of several compounds belonging to different families was achieved [89, 90].

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3 Antihypertensive drugs have been the most studied, followed by antidepressant, psychoactive and
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5 analgesic drugs (**Table 1**).
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9 CDs have been the main chiral selectors used, both in systems with a single selector and in dual
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11 systems. Among all the CDs employed, (2-hydroxypropyl)-β-CD (HP-β-CD) has been the most widely
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13 used, followed by carboxymethyl-β-CD (CM-β-CD), sulfobutylether-β-CD (SBE-β-CD), sulfated β-CD
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15 (S-β-CD), β-CD and (2-hydroxypropyl)-γ-CD (HP-γ-CD) (**Table 1**). In some cases, some additives were
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17 also added to the separation medium which already contained the CD as chiral selector, such as non-
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19 chiral surfactants [36, 37, 43], nanoliposomes [90], natural chiral surfactants [46] o microemulsions [34,
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21 64, 65]. Besides CDs, other chiral selectors have been used. For instance, bovine serum albumin (BSA)
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23 has been employed to separate analgesics [21], while antibiotics as eremomycin (ERM) have been used
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25 to achieve the separation of several drug families [89]. Also, chiral ionic liquids (CILs) have been used
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27 to separate antibiotics [26] and anti-inflammatories [53], and polymeric micelles have been employed
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29 to separate anticoagulants [28], antidepressants [30], sedatives [72] and drugs for Alzheimer's treatment
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31 [74]. Moreover, maltodextrin (MD) [18] and derivatized maltodextrin with several equivalents of
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33 dextrose have been used to perform the enantiomeric separation of different analgesic [19],
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35 antidepressant [29] and antihypertensive [48] drugs.
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39 Concerning the detection mode, the ultraviolet diode array detector (UV-DAD) has been, by far, the
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41 most employed to detect drugs in different samples (**Table 1**). When analytes do not show absorption of
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43 UV radiation it is necessary to carry out a previous derivatization process, such as in the case of
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45 penicillamine which was derivatized with 7-amino-1,3-naphthalenedisulfonic acid (ANDA) [54]. Other
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47 detection techniques employed for the enantiomeric determination of drugs have been conductivity [23],
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49 phosphorescence [35, 75], electrochemistry detection [46] and MS [28, 30, 31, 66, 69, 72]. These
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51 detection systems enabled, in general, to achieve low limits of detection (LODs) in the order of μg/mL
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53 and nM. Despite the high sensitivity achieved with MS, only 6 works have been published in the last
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decade that used EKC coupled to MS, in comparison to the 66 works reviewed using UV-DAD (**Table 1**).

Chiral drugs are usually found at very low concentration levels in high complex matrices, particularly in biological fluid samples, which contain other non-target compounds which may negatively interfere by increasing the analysis time, reducing sensitivity and affecting selectivity and resolution. For this reason, to avoid these matrix interferences a previous sample preparation step is crucial to achieve sample clean-up and preconcentrate the target analytes to enhance their detection and quantification. In this sense, the main sample preparation techniques which have been used in the works reviewed have been SPE [28, 30, 36, 37, 58, 67, 72, 76] and LLE [10, 22, 23, 39, 70, 71]. SPE is one of the most widely used procedure to clean-up and preconcentrate biological samples due to its versatility and low consumption of organic solvents, the great availability of commercial sorbents and its possibility of automation adaptation. On the other hand, despite the conventional LLE technique presents numerous drawbacks, such as the selection of an extraction solvent non-miscible with the sample and the consumption of large volumes of organic solvent, it has been significantly used as sample preparation technique in the enantiomeric determination of drugs. Nevertheless, to overcome these drawbacks other techniques, such as liquid-phase microextraction (LPME) [29], pressurized liquid extraction (PLE) [67], dispersive liquid-liquid microextraction (DLLME) [68] and electromembrane extraction (EME) [33, 40] have been used for sample preparation of biological samples. These techniques have been used for the enantiomeric determination of different drugs in urine, human hair, plasma and several types of paper (packaging, aluminum and plastic). As it is shown in **Table 1**, sample preparation has only been carried out in biological samples, whereas for the analysis of pharmaceutical formulations (tablets, capsules, drugs and injectable) this step has not been necessary in general due to the higher simplicity of the sample matrix, which barely presents matrix interferences.

Analgesic drugs

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Regarding analgesic drugs, 4 articles have been published in the last decade for the separation of tramadol, methadone, meptazinol and tetrahydropalmatine enantiomers by EKC with UV-DAD in positive polarity mode (**Table 1**). Mohammadi et al. developed a chiral methodology by EKC to determine tramadol enantiomers in commercial tablets using MD as chiral selector and borate buffer at pH 10.2 [18]. Migration times were 22.8 and 23.3 min for (+)-tramadol and (-)-tramadol, respectively, with a resolution separation of 2.8, and the LOD for both enantiomers was 1.5 mg/L. More recently, Naghdi and Fakri carried out the simultaneous enantiomeric separation of tramadol and methadone by EKC, using MD with 4-7 equivalents of dextrose (MD-DE 4-7) as chiral selector and phosphate buffer at pH 8.0 [19]. Under these conditions, tramadol enantiomers were separated in 12 min, which is significantly less time than the work previously reported by Mohammadi and co-workers [18]. Nevertheless, the resolution value was lower (1.8) and the LOD higher (2 mg/L). On the other hand, methadone enantiomers were separated in less than 13 min with a resolution value of 1.7, and the LOD was 1.5 mg/L. The chiral methodology developed was applied to the enantiomeric determination of both drugs in tablets, urine and human plasma samples. Regarding meptazinol, a chiral methodology was developed to achieve the enantiomeric separation of this drug and its three intermediate enantiomers by EKC using CM-β-CD as chiral selector [20]. Simultaneous enantiomeric separation of meptazinol and its intermediates III and IV was possible in 16 min using 2.0 mmol/L of CM-β-CD in phosphate buffer at pH 6.0 containing 5% of acetonitrile (ACN). However, the enantioseparation of meptazinol intermediate II was not possible under these conditions, and it was only baseline separated using higher concentration of CM-β-CD (4.0 mmol/L) in phosphate buffer at pH 4.25 and requiring an analysis time of 50 min. On the other hand, the enantioseparation of tetrahydropalmatine was achieved within 9 min using BSA as chiral selector [21]. The analytical performance of the method was successfully evaluated and the sensitivity was significantly improved by using the field-amplified sample injection (FASI) technique, which lower down the LOD to 6 µg/L. Compared with the previous studies described for the enantiomeric analysis of analgesic drugs which also used UV detector, the LODs are one order of

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magnitude higher (mg/L) than with FASI technique (μg/L). This injection mode consists in injecting the sample previously diluted in a solvent of lower conductivity than the buffer electrolyte. This enables high concentration factor of the target analytes inside the capillary, close to the injection end and, thus, higher sensitivity is achieved. Finally, the method was applied to the determination of tetrahydropalmatine enantiomers in a medicinal plant (*Corydalis yanhusuo*). Due to the complexity of the sample, the second migrating enantiomer of tetrahydropalmatine and its coexisting matrix-components could not be completely separated, therefore it was only possible to quantify the first migrating enantiomer of tetrahydropalmatine in the sample (0.13 mg/g). Probably, performing a previous sample preparation step would have improved the sample clean-up enhancing the determination of both tetrahydropalmatine enantiomers.

Anesthetic drugs

In the last decade, the enantiomeric separation of anesthetic drugs by EKC has been reported by using CDs as chiral selectors (**Table 1**). In this sense, Porpiglia and co-workers developed the simultaneous enantiomeric separation of ketamine and its major metabolite norketamine by EKC using highly sulfated-γ-CD (HS-γ-CD) in positive polarity mode and UV-DAD [22]. A complete separation of both drugs was achieved in less than 10 min with good resolution values. Norketamine enantiomers migrated first than ketamine enantiomers, and in both drugs the first migrating enantiomer was the *R*-configuration. The method was developed for its application to the enantioselective determination of both analytes in human hair samples, which were first subjected to LLE prior to analysis. The method developed was validated, allowing the detection of both ketamine and norketamine enantiomers at trace levels in hair samples with a LOD of 0.08 ng/mg, thanks to the preconcentration of the target analytes in the sample preparation step. The method was also tested with hair samples collected from ketamine abusers and it was observed that it could also be suitable for studying the enantioselective metabolism of the drug (**Fig. 2**). On the other hand, the enantioseparation of bupivacaine, another anesthetic drug, was achieved in less than 15 min by EKC in positive polarity mode with SBE-β-CD and using

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conductivity detection [23]. The stereoselective interaction between bupivacaine enantiomers and the CD was studied by calculating the binding energies with a computer-simulation technique. According to the results, the conformational energy formed by *S*-(-)-bupivacaine and SBE-β-CD is smaller than the one formed between the complex *R*-(+)-bupivacaine and SBE-β-CD, leading to a longer migration time of *S*-(-)-bupivacaine. The results also indicated that the complex formed by *R*-(+)-bupivacaine and SBE-β-CD was not complete, forming an entity between NH_4^+ and SBE-β-CD in the peripheral environment which carries more positive charge than that of the entity formed by *S*-(-)-bupivacaine, NH_4^+ and SBE-β-CD. The method developed was validated and applied to the determination of bupivacaine enantiomers in rabbit serum and in a pharmaceutical injection. The serum samples were first subjected to LLE prior to their analysis, while the injectable sample was only diluted in water. The average extraction recoveries of the serum samples ranged between 74-76% and the bupivacaine content of the injectable met the requirements of the labeled content. Thanks to the high sensitivity of the conductivity detection, the LOD of the method was 52 µg/L.

Antiarrhythmic drugs

RS86017 is an antiarrhythmic agent with one enantiomer (SR86017) and two diastereomers (RR86017 and SS86017). Liu et al. [24] developed a chiral methodology by EKC for simultaneously determining all stereo-isomers of RS86017 to assure quality control of this drug according to the international ICH guidelines [14]. Mono-CD (β-CD, HP-β-CD and SBE-β-CD) and dual-CD (combination of HP-β-CD and SBE-β-CD) systems were investigated to achieve the separation of RS86017 stereoisomers. β-CD did not show chiral discrimination for the two pairs of enantiomers, whereas HP-β-CD provided better chiral recognition but not complete separation of the two pairs of enantiomers. Instead, SBE-β-CD allowed the separation of all stereo-isomers, and the dual CD system did not exhibit significant improvement compared with SBE-β-CD. Therefore, SBE-β-CD was chosen as chiral selector. Under the optimal conditions, the analysis time was of 10 min and the migration order

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was as follows: RR86017, SS86017, SR86017 and RS86017. The method was validated and LODs were 0.8 mg/L for all isomers. Additionally, the relative limit of detection (RLOD) corresponding to a relative concentration of the chiral impurities of 0.03% and 0.1% with respect to the active principle was successfully assessed. Finally, the method was applied to investigate the chiral purity of RS86017 in bulk samples, showing that chiral impurities were far below 0.1% in the samples analyzed, complying with the established regulations [14].

Antibiotic drugs

With respect to the chiral separation of antibiotics by EKC, only enantioseparations of ofloxacin and ornidazole have been reported in the last decade (**Table 1**). Al Azzam et al. developed a chiral methodology to achieve the simultaneous enantioseparation of both antibiotics using S-β-CD as chiral selector in positive polarity mode [25]. Under the optimized conditions both compounds were separated in their enantiomers in less than 16 min, with resolutions of 5.5 and 6.3 for ofloxacin and ornidazole, respectively. Ofloxacin enantiomers migrated before ornidazole enantiomers, being the *R*-configuration of ofloxacin the first migrating enantiomer. However, due to a lack of pure standards of ornidazole enantiomers, it was not possible to elucidate their migration order. Nonetheless, authors performed molecular modeling using semi-empirical calculations to further understand the interaction of S-β-CD with the enantiomers of both drugs. The inclusion complexes between S-β-CD and ofloxacin enantiomers showed larger binding energies in comparison to the ornidazole counterparts. It was also noticed, that the complex between S-β-CD and *R*-ornidazole is significantly more favorable than the S-β-CD/*S*-ornidazole complex. Results from these studies revealed that the stronger inclusion complexes lead to shorter migration times. Thus, migration order of ofloxacin was confirmed and it was predicted that *R*-ornidazole migrated before *S*-ornidazole. The analytical characteristics of the method were assessed and it was applied to the determination of the enantiomers of both antibiotics in pharmaceutical formulations. On the other hand, the chiral separation of ofloxacin was also achieved by EKC using a

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3 dual system consisting of HP-β-CD and an ionic liquid (1-ethyl-3-methylimidazolium-L-lactate (EMIM-L-L)) [26]. When using only HP-β-CD as chiral selector, ofloxacin enantiomers were separated with a resolution value of 2.91 and migrated at 17.8 and 18.2 min, which is a worse separation than the one previously described with S-β-CD [25]. Also, it was noticed that *S*-ofloxacin migrated before *R*-ofloxacin, therefore, HP-β-CD provided an inversion of the migration order compared to S-β-CD. It was observed that adding the ionic liquid produced a synergistic effect between HP-β-CD and the cationic part of the ionic liquid by significantly increasing resolution (5.4), but at the expense of a longer analysis time (20.9 and 23.8 min for the *S*- and *R*-enantiomer, respectively). Therefore, it is worth to achieve the enantioseparation of ofloxacin with S-β-CD as sole chiral selector than with the dual system HP-β-CD/EMIM-L-L, as it is quicker and more cost-effective. Nevertheless, the method was validated according to the ICH guidelines and applied to determine the chiral impurity of ofloxacin in bulk samples, showing that *R*-ofloxacin was below the 0.2% in the samples analyzed. Ornidazole was also individually separated in its enantiomers by EKC using sulfated-α-CD (S-α-CD) as sole chiral selector in positive polarity mode [27]. As previously reported, S-β-CD enabled the enantiomeric separation of ornidazole eluting first the *S*-enantiomer [25]. However, with S-α-CD the migration order of the two enantiomer changed, eluting first the chiral impurity (*R*-enantiomer). This is a good order for an impurity control method in which it is desirable for the impurity to elute before the active principle. In addition, peak shape for both enantiomers improved in comparison with the use of S-β-CD and satisfactory separation resolution was achieved. Moreover, the analysis time was less than 7 min, which is faster than the method previously described using S-β-CD, which required 16 min [25]. The proposed method was validated and applied to the enantiomeric purity control of an injectable solution of levornidazole (*S*-ornidazole). No chiral impurity was detected in the sample and the RLOD was assessed at a 0.05%.

Anticoagulant drugs

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Warfarin is the most common anticoagulant which is clinically administered in its racemic form to treat and prevent thromboembolism. Wang et al. developed a sensitive chiral methodology by EKC coupled to tandem MS (MS/MS) to achieve the enantioseparation of warfarin and all its hydroxylated metabolites [28]. For this purpose, they used a polymeric surfactant (polysodium N-undecenoyl-L,L-leucylvalinate) as chiral pseudophase. Under the optimal conditions, baseline separation of warfarin and its five metabolites was achieved in 45 min. The method was evaluated in human serum samples, which were first subjected to an extraction procedure. In this sense, LLE and SPE were compared for the extraction of warfarin and its hydroxylated metabolites from human plasma samples. With LLE recovery values were less than 50%, whereas with SPE were higher than 80%. Therefore, SPE was selected as extraction technique, since it was more effective than LLE, and best results were obtained using mixed-mode anion-exchange cartridges (MAX). The method was validated and thanks to the sample preparation step and MS detection it showed high sensitivity, achieving low LODs for warfarin (0.5 μg/L) and its five metabolites (3.0 μg/L). Finally, the method was successfully applied to the analysis of warfarin and its metabolites in plasma samples of 55 patients undergoing warfarin treatment. Results revealed that the *R*/*S* ratio of warfarin enantiomers in normal patients was above 1, being the *R*-enantiomer more concentrated than the *S*-enantiomer. In contrast, subjects with mutant gene have an *R*/*S* ratio of warfarin enantiomer below 1.

Antidepressant drugs

The chiral separation of different types of antidepressant drugs by EKC has been described in the last 10 years (**Table 1**). In most of these works, the separation of enantiomers was achieved by using CDs as chiral selectors [31-37]. Nevertheless, the separation of citalopram enantiomers was performed by using MD as chiral selector [29], while the simultaneous enantioseparation of venlafaxine and its structurally-similar major metabolite O-desmethylvenlafaxine was achieved by employing poly-sodium-N-undecenoyl-L,L-leucylalaninate (poly-L,L-SULA) as chiral selector after screening other dipeptide polymeric chiral surfactants with the same N-terminal amino acid but different C-terminal amino acid

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[30]. It was observed that migration time significantly increased when the hydrophobicity of the side chain of the C-terminal amino acid increased. On the other hand, an assembling molecular film constructed with poly(diallyldimethylammonium-chloride) and β-CD *via* inclusion complexation was used as chiral selector to achieve the separation of the two pairs of enantiomers of sertraline by Microemulsion Electrokinetic Chromatography (MEEKC) [34]. In general, in all these works, UV detection was used and, despite the high sensitivity of MS, it was only reported for the detection of venlafaxine and duloxetine [30, 31]. In fact, Sanchez-López and coworkers developed two methodologies with UV and MS detection for the chiral separation of duloxetine [31]. MS enhanced the sensitivity, increasing it up to 10 times in comparison to UV detection (LOD 200 ng/mL by CE-UV and 20 ng/mL by CE-MS), enabling to detect 0.02% of duloxetine impurity (**Fig. 3**) and accomplishing the ICH guidelines. Nonetheless, when transferring the method from the CE-UV to the CE-MS system the resolution value decreased, so it was necessary to increase the capillary length and consequently migration times took longer, from 20 to 30 min. In addition, detection based on Laser Induced Phosphorescence (LIP) in direct and sensitized mode was carried out in the enantioseparation of bupropion using biacetyl as acceptor [35]. The phosphorescence intensity greatly improved with the sensitized mode in comparison with the direct phosphorescence mode, achieving a LOD about 40 times lower than the one obtained using direct phosphorescence detection and about 30 times more sensitive than with conventional UV detection. The majority of these chiral methods were applied to the analysis of pharmaceutical formulations (tablets and capsules) [31, 32, 34, 35] and biological samples (urine and human plasma) [29, 30, 33, 35]. Because of the low concentration at which drug enantiomers can be found in biological samples and due to their complex matrix, a sample preparation and preconcentration technique was necessary prior to chiral separation by EKC in the analysis of urine and human plasma [29, 30, 33]. In this sense, venlafaxine and its metabolite were extracted by SPE using Strata-X-C polymeric strong cation cartridges using ammonium acetate/MeOH (5/95, v/v) as elution solvent, achieving recovery values higher than 80% for both analytes [30]. Also, three-phase hollow fiber

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supported liquid-phase microextraction (HF-LPME) was used to extract citalopram enantiomers in urine samples [29]. The analyte was extracted into 1-octanol that was immobilized in the wall pores of a porous hollow fiber and was back extracted into the acceptor phase located in the lumen of the hollow fiber. As compared with SPE, the consumption of organic solvents in HF-LPME is minimum, and it avoids possible carry-over problems since the hollow fiber can be discarded after each extraction. Nevertheless, the recovery values achieved for both enantiomers were lower than 60%. A similar procedure was carried out by Fakhari and coworkers in plasma and urine samples for the extraction and preconcentration of trimipramine enantiomers [33]. In this case, they performed EME, where enantiomers migrated from the sample solution through a thin layer of 2-nitrophenyl octyl ether immobilized in the pores of a hollow fiber and into the acceptor phase located inside the lumen of the fiber (**Fig. 4**). However, as in the previous work [29], recovery values were low, ranging between 48-51 % and 58-60% in plasma and urine samples [33]. The low recoveries achieved from the plasma extraction were due to the strong binding of trimipramine enantiomers to the plasma proteins, while the low recoveries achieved for urine were related to the high ionic strength of these samples. In contrast, the pharmaceutical formulations were only dissolved and diluted before analysis [31, 32, 34, 35].

Antifungal drugs

The chiral separation of tioconazole, isoconazole, fenticonazole and econazole by EKC has been described by different authors using CDs as chiral selectors and UV detection (**Table 1**). Ibrahim et al. developed the simultaneous enantiomeric separation of tioconazole, isoconazole and fenticonazole in less than 15 min using a combination of HP-γ-CD and heptakis (2,6-di-O-methyl)-β-CD (DM-β-CD) since, separately, none of the CDs studied gave complete enantioseparation of the target analytes [36]. On the other hand, Hermawan et al. achieved the enantioseparation of econazole in 9 min using only HP-γ-CD as chiral selector [37]. In both works the chiral method was applied to the determination of these imidazole drugs in cream samples. Due to the complex matrix of creams, SPE was carried out with diol cartridges before chiral analysis, achieving good recovery values for tioconazole (97-10%),

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isoconazole (94-96%) and econazole (93%) [36, 37]. In addition, tioconazole, isoconazole and fenticonazole were also extracted by SPE from urine samples using Bond Elut Plexa cartridges [36]. Recoveries of tioconazole and isoconazole were good (ranging between 95-106%), but lower than 75% for fenticonazole enantiomers due to their higher hydrophobicity. Among these imidazole drugs, fenticonazole was the one with the lowest LOD (2.7 mg/L), while tioconazole and econazole showed similar LOD values (4.2 and 4.3 mg/L, respectively). On the other hand, the LOD of isoconazole was the highest (7.7 mg/L).

Antihypertensive drugs

In the last decade, several authors have described the enantiomeric separation by EKC of different types of antihypertensive drugs, mainly β-blockers, calcium ion antagonist and calcium channel blockers, among others (**Table 1**). It is worth mentioning that from all the articles reviewed for the chiral analysis of antihypertensive drugs, there are no works describing a simultaneous separation of different drugs, instead, each work only described the enantiomeric separation of a single drug. Despite Fang et al. carried out the chiral separation of 10 β-blockers, it was not a simultaneous separation method, since different optimal separation conditions were established for each analyte individually analyzed [42]. In addition, in all these works, CDs are used as chiral selectors to achieve the enantiomeric separation. For instance, chiral separation of β-blockers was performed using CM-β-CD [39, 42], HP-β-CD [40, 41] and octa(6-O-sulfo)-γ-CD (OS-γ-CD) [38], while γ-CD, heptakis (2,3,6-tri-o-methyl)-β-CD (TM-β-CD), β-CD, random methylated-β-CD (RAMEB) and SBE-β-CD were used for the enantiomeric separation of other antihypertensive drugs [43, 45-47, 49]. There is only one exception in which MD-DE 4-7 is used as chiral selector to achieve the enantiomeric separation of the antihypertensive amlodipine drug [48]. In this work, amlodipine enantiomers were separated with MD-DE within 30 min, being *R*-amlodipine the first migrating enantiomer, which is the less active enantiomer. In the following year, Hancu et al. also reported the chiral separation of amlodipine, but in this case using RAMEB as chiral selector [47].

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The migration order was the same as in the work described above [48], however, the analysis time significantly decreased, achieving separation in less than 6 min with higher resolution values (2.5 compared to 1.8) [47]. Nevertheless, the LOD was lower when MD-DE was used as chiral selector instead of RAMEB [47, 48]. In all these works reviewed, UV detection was employed, except the one carried out by Cheng and coworkers in which electrochemical detection is used, enabling to achieve higher sensitivity and, consequently, lower LODs [46]. Also, two chiral methodologies were developed for the separation of perindopril enantiomers by HPLC and EKC in order to compare both techniques [44]. HP-β-CD was used as chiral selector in EKC while a chiral column consisting of β-CD chemically bonded to spherical silica gel particles was used as chiral stationary phase in HPLC. A reverse order of enantiomers was observed among both techniques. HPLC showed advantages over EKC in terms of shorter analysis time, as retention times were 2.2 and 6.0 min for *S*- and *R*-perindopril, respectively, while migration times were 14.7 and 17 min for *R*- and *S*-perindopril, respectively. Nevertheless, *S*-perindopril is the active principle, therefore, the migration order of the EKC method is more suitable to accomplish the ICH guidelines. In addition, it was observed that the time required for the development and optimization of the EKC method was shorter than the one for the chiral HPLC method, since it did not require extensive equilibration times. Another advantage of the HPLC method was it higher sensitivity, as it was five times more sensitive than the EKC method developed. In contrast, the EKC method was advantageous in the higher resolution achieved, its lower consumption of solvent and buffer additives and its use of less expensive chiral selectors compared to HPLC chiral columns. Almost all of these chiral methods were applied to the analysis of pharmaceutical formulations (tablets, capsule and injectable), thus, sample preparation was very simple consisting in dissolution and dilution procedures [38, 41-49]. Only two works applied the chiral method developed for the enantiomeric determination of carvedilol and propranolol in biological samples [39, 40]. Therefore, due to the complexity of the sample matrix, different sample preparation techniques were employed before chiral analysis. In this sense, Hamidi et al. extracted carvedilol enantiomers from human plasma samples using DLLME, achieving

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good recovery values ranging from 91-107% [39]. On the other hand, Tabani and coworkers carried out EME based on a polypropylene hollow fiber impregnated with 2-nitrophenyl-octyl ether to extract propranolol enantiomers from urine and plasma samples [40]. Recovery values were better than the ones previously reported for the antidepressant drugs [29, 33], ranging between 78-95% [40]. In addition, the application of this method for the analysis of a urine sample from a patient under treatment with racemic propranolol 35 min after of its oral administration revealed a lower content of *S*-propranolol than of *R*-propranolol, suggesting the enantioselective metabolism of this drug, where *S*-propranolol is more pharmaceutically active than *R*-propranolol.

Antihistamine drugs

The enantiomeric separation of cetirizine and pheniramine by EKC has been reported by different authors using CDs as chiral selectors and UV detection (**Table 1**). Cetirizine is a second generation selective H1 receptor antagonist with one chiral center, thus it has two enantiomers: levocetirizine (*R*-configuration, the active principle) and dextrocetirizine (*S*-configuration, less active enantiomer). Hancu et al. achieved the enantiomeric separation of cetirizine in less than 8 min using SBE-β-CD in positive polarity mode [50]. Under the optimized conditions, the first migrating enantiomer was the active principle levocetirizine with a resolution value higher than 2. On the other hand, using S-β-CD as chiral selector in positive polarity mode reversed the migration order of cetirizine enantiomers, as reported by Deng et al. [51]. Under the optimized conditions described by Deng et al., the chiral separation of cetirizine was also achieved in less than 8 min, but the enantiomeric impurity (dextrocetirizine) migrated before the active principle (levocetirizine), unlike with SBE-β-CD [50], which is a better migration order to accomplish the requirements of the reporting limit for impurities stated by the ICH guidelines [14]. Both methods were applied to the enantiomeric purity control of cetirizine in tablets. The analysis carried out by Deng and coworkers enabled the detection and quantification of the enantiomeric impurity in commercialized levocetirizine pure tablets, observing a 0.42% of dextrocetirizine as compared to the

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amount of levocetirizine [51]. On the other hand, pheniramine is another highly potent and widely used antihistamine with one pair of enantiomers, being the *S*-configuration the active principle. The chiral separation of pheniramine was described by using HP-β-CD as chiral selector in positive polarity mode within less than 14 min, being the *R*-pheniramine the first migrating enantiomer [52]. The method was applied to the analysis of pheniramine enantiomers in a pharmaceutical eye drop preparation, indicating that the drug was present as racemate. As antihistamine drugs were only analyzed in pharmaceutical formulations, sample preparation was simple and only based in dissolution and dilution steps (**Table 1**).

Anti-inflammatory drugs

The chiral separation of 5 non-steroidal anti-inflammatory drugs (NSAIDs) by EKC was reported by Zhang et al. [53]. However, they did not develop a simultaneous method for this purpose. Instead, they used individual conditions for each analyte. In this sense, they studied the synergistic effect of two different CILs (based on amino acid ester, L-alanine and L-valine tert butyl ester bis (trifluoromethane) sulfonamide) in the enantiomeric separation of these drugs when used in combination with the antibiotic vancomycin as chiral selector. Additionally, these CILs were evaluated as sole chiral selectors, but no enantiomeric discrimination was found for any of the target analytes when each CIL was used alone. The combined use of vacomycin with the CILs produced a synergistic effect by significantly improving the resolution separation compared to vacomycin-alone cases. Nevertheless, the addition of CILs also increased the migration times, leading to longer analysis times than using vacomycin alone (**Fig. 5**). Therefore, both dual systems (vacomycin-CIL1 and vacomycin-CIL2) were applied to test the chiral impurity of a naproxen drug sample. On the other hand, Song et al. developed a chiral methodology to separate penicillamine using neutral β-CD as chiral selector [54]. Penicillamine was derivatized with 7-amino-1,3-naphthalenedisulfonic acid monopotassium salt (ANDA) for the introduction of both chromophore and strong charge groups in order to enhance detection sensitivity and facilitate electromigration of the analyte. Different BGEs were tested, changing the type of buffer (acetate,

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phosphate and boric buffers), the β-CD concentration and the pH. In addition, negative polarity mode was evaluated for acetate buffer, whereas positive polarity mode was evaluated for phosphate and boric buffers. Better resolution was achieved using acetate and phosphate buffer, however, better sensitivity was achieved with boric buffer. Under the optimized conditions for each BGE the method was applied for the enantiomeric determination of penicillamine in tablets (**Table 1**).

Antiparasitic drugs

Praziquantel is an anthelmintic indicated in schistosomiasis, which has one pair of enantiomers, being the *R*-configuration the active principle, while the *S*-enantiomer is the inactive one. Szabó et al. developed a chiral strategy to achieve the quality control of *R*-praziquantel by EKC with CDs and using UV detection [55]. For this purpose, they screened nine anionic CDs in positive polarity mode using tris/ acetate buffer (pH 6.5). Seven of the nine CDs tested showed enantiomeric discrimination, and in 6 cases baseline resolutions were achieved (with sulfated and sulfobutylated CDs). Best results were achieved with sulfated-γ-CD (S-γ-CD), since the separation of enantiomers was achieved in less time, however, the migration order was unfavorable to achieve the ICH requirements as the active principle (*R*-enantiomer) was the first migrating enantiomer. Thus, the migration order was reversed by switching to negative polarity mode. Nevertheless, changing the polarity not only reversed the migration order but it also resulted in longer migration times and extreme resolution values (35). Therefore, the BGE conditions were optimized by changing the buffer type and the CD concentration, suppressing EOF (pH 2) and employing short-end injection to reduce analysis time. After optimization, the enantiomeric separation of praziquantel was achieved with a resolution value higher than 10 in less than 10 min, being *S*-praziquantel the first migrating enantiomer. The method was validated according to the ICH guidelines, proving to be suitable to quantify the enantiomeric impurity at 0.1% with respect to the active principle, and it was applied for the enantiomeric determination of praziquantel in commercial tablets containing racemic mixture of the drug (**Table 1**).

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Antipsychotic drugs

The single chiral separation of sulpiride, an antipsychotic drug which acts as a selective antagonist of central dopamine receptors, was reported by EKC in reverse polarity using a dual selector system based on the combination of two CDs, S-β-CD and methyl-β-CD (M-β-CD) and UV detection [56]. Under the optimized conditions, base-line separation of sulpiride enantiomers was achieved in 12 min, being the enantiomeric impurity (*R*-sulpiride) the first-migrating enantiomer. The analytical characteristics of the method were assessed according to the ICH guidelines and it was applied to determine the enantiomeric purity of *S*-sulpiride in pharmaceutical blisters, which were just diluted in water. On the other hand, Yu et al. developed a quick chiral strategy to simultaneously separated five pairs of D, L-phenothiazines by EKC coupled to UV detection using HP-γ-CD as chiral selector and poly(diallyldimethylammonium chloride) (PDDAC) as additive, which suppressed the adsorption of the analytes on the capillary [57]. Under the optimized conditions, base-line separation of the 10 phenothiazines enantiomers was achieved in less than 4 min, always eluting first the D-configuration of each pair of enantiomers (**Fig. 6**). The method was applied to the enantiomeric determination of the five pairs of D, L-phenothiazines in urine samples without performing any previous sample preparation technique, achieving LODs ranging from 2 to 8 μM (**Table 1**). More recently, the same chiral method was used to achieve the separation of the same five pairs of D,L-phenothiazines but with slight modifications [58]. In this case, the formate buffer concentration, the capillary length and the voltage were increased from 75 to 100 mM, from 22.5 to 40 cm and from -9.5 to -10 KV, respectively. The other separation conditions remained the same (**Table 1**). Consequently, resolution values increased but also the analysis time took longer, approximately 14 min. The migration order remained the same. The new method was also applied for the enantiomeric determination of the five pairs of D, L-phenothiazines in urine samples, but in this case SPE with C18 cartridges was performed before chiral analysis. Good recovery values were achieved, ranging from 89-101%, and thanks to the preconcentration step the

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sensitivity improved, as LODs for the same analytes were in the order of nM which are significantly lower than the ones achieved in the previous work (μM) [57] (**Table 1**).

Antitussive and analgesic drugs

Methorphan drug is a synthetic analogue of codeine, which has two enantiomers with strongly different activity. The L-enantiomer, named levomethorphan, acts as an opioid agonist, consequently is a narcotic analgesic with strong respiratory depression considered an illicit drug, since its use is usually characterized by habituation, tolerance, physical dependence and withdrawal syndrome. Therefore, levomethorphan is classified as a controlled substance worldwide. On the contrary, the D-enantiomer, named, dextromethorphan, is an opioid agonist with antitussive action which shows no narcotic activity, for this reason it is present in the formulation of several anti-cough medications [10, 59]. Krait et al. achieved the separation of methorphan enantiomers by EKC using a dual selector system involving two different CDs, S-β-CD and methyl-α-CD (M-α-CD) [59]. Under the optimized conditions, the separation of both enantiomers was achieved in less than 5 min with a resolution value higher than 2, being dextromethorphan the first migrating enantiomer. In contrast, Bertaso et al. performed the enantiomeric separation of methorphan using HP-β-CD as single chiral selector [10]. The migration order was the same as with the previous dual system [59], but in this case the analysis time took longer, approximately 22 min [10]. The chiral methodology developed by Krait et al. was applied to the analysis of a capsule formulation, therefore sample preparation was simple [59]. On the other hand, Bertaso and coworkers applied their method to the analysis of post-mortem blood samples from subjects who died because a heroin overdose [10]. Since blood samples are biological samples with a complex matrix, a sample preparation step was required before analysis. In this sense, blood samples underwent LLE with a saturated solution of sodium carbonate and of hexane/ethyl acetate (50/50, v/v). Among the positive blood samples for methorphan, dextromethorphan was found in all of them ranging from 214 to 1282 ng/mL and together with its main metabolite dextrorphan. UV detection was used in both methods [10, 59]. However, due to the LLE procedure, which enabled extraction and preconcentration of the analytes,

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the LODs obtained in the blood samples were significantly lower than the ones obtained by Krait et al. (**Table 1**). Additionally, the lower LODs achieved in the blood samples were also due to the sample injection under FASI stacking conditions.

Antiuremic drugs

The chiral separation of colchicine, which is a chiral antiuremic drug commercialized as pure enantiomer (*S*-colchicine), was described by EKC with UV detection using different chiral selectors [60]. After a screening with 13 anionic CDs, only 4 of them provided enantiodiscrimination of colchicine enantiomers. With carboxyethylated-β-CD (CE-β-CD), carboxyethylated-γ-CD (CE-γ-CD) and succinyl-γ-CD (Succ-γ-CD) the first migrating enantiomer was *S*-colchicine, whereas S-γ-CD originated the opposite migration order. Due to the different migration order of S-γ-CD and the higher resolution achieved with Succ-γ-CD, both CDs were selected as chiral selectors for further optimization of the chiral separation of colchicine. Under the optimized conditions for each CD, colchicine enantiomers were separated in 8 min and 12 min with S-γ-CD and Succ-γ-CD, respectively, obtaining higher resolution with Succ-γ-CD. In addition, the stoichiometry for the enantiomer-CD complexes were calculated by nuclear magnetic resonance (NMR), resulting in a 1:1 stoichiometry in both cases. Nevertheless, the apparent and averaged equilibrium constants for the enantiomer-CD complexes could be only determined in the case of Succ-γ-CD, and suggested that the electrophoretic mobility was the predominant factor in the enantiomeric migration order, rather than the stability of the complexes. Moreover, the analytical characteristics of both methods were determined according to the ICH guidelines. However, only Succ-γ-CD enabled to detect up to 0.1% of *R*-colchicine, while with S-γ-CD it was only possible to detect a 0.3%. Both methods were applied to the analysis of pharmaceutical formulations of colchicine.

Bronchodilator drugs

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Different chiral methods have been described for the separation of several β-agonists by EKC using CM-β-CD as chiral selector [42, 61]. Nonetheless, although these works describe the successful enantiomeric separation of multiple β-agonist drugs, they do not report a simultaneous separation of them, instead each compound was analyzed in an individually way (**Table 1**). Comparing the results obtained for the common analytes in both works, it can be observed that the conditions described by Fang et al. [42] are more suitable than the ones reported by Zhou et al. [61], in terms of time and resolution, by using the same CD and type of buffer, but at different experimental conditions, such as the temperature, voltage, capillary, CD concentration and buffer concentration (**Table 1**). It is worth mentioning the high resolution achieved for procaterol enantiomers with CM-β-CD in both works (**Table 1**). Glycopyrrolate is an anticholinergic drug which can act as bronchodilator and also as antispasmodic. This drug possesses two chiral centers, being the *RS*-enantiomer the active principle, whereas the *SR*-, *RR*- and *SS*-enantiomers are the chiral impurities. Zuo et al. studied the chiral separation of glycopyrrolate by EKC in different polarity modes using S-β-CD as chiral selector [62]. They observed that the migration order of the 4 enantiomers was reversed by switching the polarity and changing the pH of the BGE without changing almost the analysis time. Since it is usually desired to have the migration of the minor enantiomer in front of the major enantiomer to minimize quantitative error caused by peak overlapping, the use of phosphate buffer at pH 7.0 in positive polarity mode was selected as the best choice. Under these conditions, the migration order was as follows: *RR*-, *SR*-, *SS*- and *RS*-glycopyrrolate enantiomers in less than 15 min. The method was validated and applied to control the chiral impurities of *RS*-glycopyrrolate enantiomeric pure drug. On the other hand, higenamine is a β-agonist which can be used as bronchodilator and also for obesity treatment. The chiral separation of this drug was studied by EKC using different β-CD derivatives (S-β-CD, CM-β-CD, HP-β-CD and M-β-CD) as chiral selectors [63]. The optimal conditions for each CD were determined to achieve the separation of higenamine enantiomers. In this sense, the best conditions stated by the authors were 15 mg/mL of CM-β-CD in positive polarity mode (24 min and resolution 9.4), 20 mg/mL of S-β-CD in

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negative polarity mode (12 min and resolution 11.7), 30 mg/mL HP-β-CD in positive polarity mode (14 min and resolution 5.8) and 20 mg/mL M-β-CD in positive polarity mode (14 min and resolution 2.3). Among them, authors selected the conditions of CM-β-CD as the most effective, probably because the amount of chiral selector needed is lower than for the other CDs. However, in terms of efficiency they should have select S-β-CD, since it enabled the separation in less time with better resolution, even using a lower concentration of 20 mg/mL. In fact, authors could have used lower concentrations of all the CDs employed since, as stated in their work, using lower concentrations of the CDs enabled to achieve separation in less time with enough resolution values.

Psychoactive drugs

The chiral separation of different psychoactive drugs by EKC has been carried out by several authors (**Table 1**). Most of these works describe the simultaneous enantiomeric separation of different psychoactive drugs [66-69], whereas in other articles only the individual separation of a single drug, such as amphetamine and threo-methylphenidate [65, 70], was achieved. Moreover, Borst and Holzgrabe performed the chiral separation of six phenethylamines which act as adrenergic agonists of the receptors of the central nervous system (ephedrine, N-methylephedrine, norephedrine, pseudoephedrine, adrenaline and diethylnorephedrine) [64]. However, it was not a simultaneous separation method, since they analyzed each analyte in an individual way (**Table 1**). Nevertheless, in all these works CDs were used as chiral selectors, particularly S-β-CD, HS-γ-CD, β-CD and HP-β-CD. HS-γ-CD and β-CD proved to be the most suitable CDs to perform the simultaneous chiral separation of different psychoactive drugs [66-69]. In fact, Merola and coworkers compared the efficiency of both CDs to achieve the enantiomeric separation of 12 cathinone analogs [69]. With β-CD all the compounds analyzed were separated in their enantiomers, except 4-fluoromethacathinone and methedrone, which did not show stereospecific interactions with this chiral selector under the conditions tested. In contrast, these two compounds were enantiomerically separated using HS-γ-CD as chiral selector, but other compounds were not fully

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3 separated by this CD, such as dymethylcathinone, ethcathinone, buphedrone and pentedrone [69]. In addition, in this work the separation of the 12 cathinones analogues was evaluated using both UV and MS detection. β-CD was first used in the EKC method coupled to UV detection, achieving the enantiomeric separation of 10 compounds in less than 20 min. LODs of these compounds were good, ranging from 4.2 to 7.0 μg/L [69]. On the other hand, for the EKC method coupled to MS detection, β-CD was replaced by HS-γ-CD, since the highly sulfated form migrated counter to the electroosmotic flow in the capillary, reducing problems with noise and contamination. The change from a neutral to a charged CD produced a change in the migration order and despite it was necessary to extend the capillary length when coupling the CE system to the MS detector, the analysis time was lower (15 min). Nevertheless, with the EKC-MS method only the enantiomeric separation of 8 compounds out of 12 was achieved [69]. LODs with MS detection ranged from 1.0 to 11 μg/L, providing lower LODs for 9 out of 12 analytes (**Table 1**). The combination of both chiral methods enabled the separation of all the target compounds and which could be correctly identified thanks to MS detection. Cui et al. also used HS-γ-CD and MS detection to achieve the chiral separation and identification of 8 amphetamine-type stimulants within 40 min [66]. One of the advantages of MS detection is the possibility to detect simultaneously several compounds with the same or similar migration time, since this technique discriminates based on the different mass of the target ions. Thus, peak overlapping is not a problem in MS detection, as long as the target compounds have precursor ions with different mass. The other works reported for the chiral analysis of psychoactive drugs were based on the use of UV detection [64, 65, 67, 68, 70]. Many of these works were applied to the analysis of pharmaceutical formulations using simple sample preparation [65, 66, 69]. In contrast, in those works applied to the analysis of biological samples, different sample preparation techniques were employed before chiral analysis [67, 68, 70]. In this sense, Baciu et al. developed a chiral methodology to achieve the simultaneous separation of mephedrone, 4-methylephedrine and methylenedioxypyrovalerone which were first extracted by PLE from human hair samples [67]. Afterwards, the sample extracts were purified by online SPE coupled to the CE system by

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a short length capillary packed with Oasis HLB (hydrophilic-lipophilic balance) sorbent inserted near to the inlet end of the CE capillary. Analytes were eluted from the sorbent using MeOH containing 2% of formic acid as elution solvent. The recovery values obtained were higher than 80% and thanks to the preconcentration steps LODs were low ranging between 0.02 and 0.1 ng/mg, which is a similar magnitude order as the ones obtained using MS detection (**Table 1**). Despite this method proved to be fast, simple, cost-effective and highly environmentally friendly, its main drawback was the large amount of hair sample (100 mg) which was needed to carry out the analysis. On the other hand, Meng et al. used DLLME to extract different illicit drugs from forensic samples [68]. The extraction procedure was based on the formation of small droplets of an organic extractant in the sample solution using a water-immiscible organic solvent (chloroform) dissolved in a water-miscible organic dispersive solvent (isopropanol). The extraction time was 1 min. Recoveries ranged from 86 to 95% and very low LODs were achieved (0.05-0.20 µg/L). Alternatively, Theurillat and Thormann carried out the extraction of threo-methylphenidate enantiomers from oral fluid samples by LLE using water/hexane (24/76, v/v) [70]. However, recovery values were low, ranging from 53.6-53.9%. Additionally, the samples were injected in FASI stacking mode. Therefore, thanks to the preconcentration achieved with the sample treatment and the injection, good sensitivity was achieved, obtaining a LOD value of 1.5 µg/L (**Table 1**).

Sedative drugs

CM-β-CD has been used as chiral selector to achieve the enantiomeric separation of methadone and zoplicone by EKC coupled to UV detection [71, 73]. In the case of methadone, the separation of its enantiomers was achieved in less than 15 min with a resolution value of 2.0 [71]. Nevertheless, the migration order of methadone enantiomers was not provided by the authors. This method was validated and applied to the determination of methadone enantiomers in exhaled breath condensate (EBC), serum and urine samples from patients undergoing methadone maintenance therapy. Due to the complexity of serum and urine samples, a pretreatment step was necessary to extract and concentrate methadone

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enantiomers. Therefore, a miniaturized LLE with chloroform of these samples was carried out before chiral analysis. Recovery values ranged from 86 to 102%. In contrast, EBC samples were directly injected in the CE system by using a cooling trap system. In addition, all samples were injected in FASI mode to enhance sensitivity, enabling very low LODs (0.05 mg/L) (**Table 1**). On the other hand, zoplicone enantiomers and its impurities were separated in 8 min, being *R*-zoplicone (enantiomeric impurity) the first eluting enantiomer [73]. In this case, the method was applied for the enantiomeric determination of zoplicone in tablets. Thus, sample preparation was simple including an evaporation and reconstitution step which enabled preconcentration, achieving low LODs (**Table 1**). Also, a chiral strategy to achieve the simultaneous separation of different barbiturates (mephobarbital, pentobarbital and secobarbital) was developed by EKC using MS detection and a polymeric chiral surfactant as chiral selector [72]. After testing eleven amino acid polymeric surfactants, polysodium N-undecenoxycarbonyl-L-isoleucinate (poly-L-SUCIL) was selected as the best chiral selector. In a single run, the chiral separation of all three barbiturates was achieved in less than 32 min in the following migration order: mephobarbital enantiomers, pentobarbital enantiomers and secobarbital enantiomers. The method was applied to the enantiomeric determination of these drugs in human serum samples. As explained before, due to the complexity of the matrix, SPE of the sample was carried out before chiral analysis using Oasis mixed-mode cation-exchange cartridges (MCX) and ACN as elution solvent. Recovery values were good, ranging from 84 to 101%. Moreover, the method was evaluated using both UV and MS detection. MS was 3-5 fold times more sensitive than UV detection. Nevertheless, despite using MS, the LODs were higher than the ones reported in the previous works described for the other sedative drugs [71, 73].

Alzheimer's treatment

Huperzine A is a naturally occurring alkaloid which can be extracted from the plant *Huperzia serrata*. Due to its action as potent and highly specific inhibitor of acetylcholinesterase it can be used to treat Alzheimer's disease. However, only its (-)-enantiomer, known as selagine, is biologically active.

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In order to control the purity of (-)-huperzine A, Tsioupi et al. developed a chiral methodology by EKC using polymeric surfactants as chiral selectors, obtaining the best results with poly(sodium N-undecanoyl-LL-alanyl-valinate) [74]. Nevertheless, bad peak shape was obtained, so to solve this problem the addition of tert-butanol as modifier was necessary to improve the efficiency of the peaks. Under the optimized conditions, base-line separation of huperzine A enantiomers was achieved within 10 min and, to prove the feasibility of the method, it was successfully applied in the purity control of (-)-huperzine A in a pharmaceutical formulation.

Cancer, rheumatoid arthritis and psoriasis treatment

L-methotrexate is an antagonist of folic acid, as well as an inhibitor of the synthesis of purines and pyrimidines, this means that in the presence of this drug key reactions in cell division are shut down. For this reason, L-methotrexate can be used for the treatment of different diseases such as cancer, rheumatoid arthritis and psoriasis. Its D-enantiomer has similar inhibitory effect, but its antitumor effect is lower, thus it is considered the chiral impurity of methotrexate. The chiral separation of this drug has been reported by EKC using HP-β-CD as chiral selector, achieving separation of methotrexate enantiomers in less than 25 min with a resolution value of 1.5, being the chiral impurity the first migrating enantiomer [75]. Moreover, phosphorescence detection was used based on dynamic quenching of the strong emission of the phosphorophore 1-bromo-4-napthalene sulfonic acid by methotrexate under deoxygenated conditions. With this detection technique very low LODs were achieved, which were an order of magnitude better than with UV detection. The method developed was applied to the enantiomeric determination of methotrexate in a pharmaceutical formulation and in a cell culture extract from human leukemia cells (**Table 1**). On the other hand, Martínez-Girón et al. described the development of a chiral method by EKC to achieve the separation of a pyroglutamic acid derivative which is undergoing drug development for cancer treatment due to its interesting pharmacological activity as immunomodulator [76]. This drug possesses two chiral centers, leading to two pair of enantiomers: RR, SS, SR and RS, being the SR isomer the active principle, while the others are

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3 considered chiral impurities. After screening 16 different CDs (neutral and anionic), the separation of these 4 isomers was achieved using highly sulfated-β-CD (HS-β-sulfate) within 25 min and resolution values higher than 2.0. The migration order was as follows: RR, SS, RS and SR. The analytical characteristics of the method were assessed according to the ICH guidelines and the method was applied to the determination of the purity of the chiral drug, to the determination of its chiral stability in the solid stage and to investigate its possible *in vivo* inversion in metabolism samples (plasma). To achieve good sensitivity, the plasma samples were subject to SPE using Oasis MCX cartridges and 10% NH_4OH in MeOH as elution solvent. Additionally, the FASI mode was also used to enhance sensitivity. This injection technique allowed to improve the sensitivity about 3000-fold compared with the conventional injection mode. Results revealed no racemization of this drug during its storage and manufacture, and neither during *in vivo* metabolism.

Diabetes treatment

The enantiomeric separation of different drugs used for the treatment of Diabetes (alogliptin, repaglinide and vildagliptin) has been described by EKC using CDs as chiral selectors and UV detection (**Table 1**). After screening native CDs and 10 CDs derivatives enantiodiscrimination of alogliptin was achieved with γ-CD, sulfopropylated-γ-CD, sulfopropylated-β-CD, SBE-β-CD and sulfobutylether-γ-CD (SBE-γ-CD) [77]. It was observed a reversed migration order between γ and β-CD derivatives. In this sense, with β-CD derivatives *S*-alogliptin (impurity) reached the detector before *R*-alogliptin (active principle), while with γ-CD derivatives the migration order was the opposite. Therefore, SBS-β-CD was chosen as chiral selector since it provided the best separation in terms of resolution and migration order. Under the optimized conditions, alogliptin enantiomers were separated in less than 11 min with a resolution value of 8.4. In the case of vildagliptin, sulfobutylether-α-CD (SBE-α-CD) was selected as the best chiral selector after screening 13 negatively charged CDs [79]. Under the optimized conditions, vildagliptin enantiomers were separated in approximately 6 min with very good resolution (5.2), being

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the enantiomeric impurity (*R*-vildagliptin) the first migrating enantiomer. On the other hand, the chiral separation of repaglinide was achieved with SBE-β-CD and DM-β-CD [78]. However, DM-β-CD was selected since it demonstrated better separation level than with SBE-β-CD. Under the optimized conditions, separation of repaglinide enantiomers was achieved within 10 min, *S*-repaglinide (active principle) migrating before than the *R*-enantiomer (impurity). All the chiral methods developed for the analysis of these drugs were applied for their enantiomeric determination in pharmaceutical formulations, thus sample preparation was simple without carrying out preconcentration procedures (**Table 1**).

Dyspepsia treatment

Omeprazol and pantoprazole are the most frequently prescribed proton pump inhibitors to treat dyspepsia, pectic ulcer, gastroesophageal reflux disease, laryngopharyngeal reflux and Zollinger-Ellison syndrome. In both chiral drugs their *S*-enantiomer is the active principle, while the *R*-enantiomer presents less pharmacological activity. The chiral separation of omeprazole by EKC coupled to UV detection has been achieved using different CDs as chiral selectors (**Table 1**). In this sense, the chiral discrimination of omeprazole was observed with β-CD, HP-β-CD and RAMEB [80, 81]. Hancu and coworkers selected RAMEB as the best chiral selector to develop the enantiomeric separation of omeprazole, as it provided, under the same conditions, separation of its enantiomers with better resolution than β-CD and HP-β-CD in similar analysis time [80]. Under the optimized conditions, the separation was achieved within 9 min and the first-migrating enantiomer was the chiral impurity *R*-omeprazole. In contrast, Estevez et al. chose HP-β-CD as chiral selector to achieve the separation of omeprazole enantiomers [81]. Nevertheless, despite the migration order was the same as the one obtained with RAMEB, the analysis time took longer, approximately 17 min and, in addition, resolution was lower. Therefore, RAMEB is more effective to achieve the chiral separation of omeprazole. Moreover, Hancu et al. reported that the use of 20 mM of HP-β-CD in 50 mM phosphate buffer (pH 2.5) at 20 ºC and 20 kV, enabled the separation of omeprazole enantiomers in less than 9 min with a resolution value of 2.0 [80], which is a more effective

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3 separation than the one achieved by Estevez et al. using the same amount of HP-β-CD (20 mM) in 100
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5 mM Tris-phosphate buffer (pH 2.5) at 15 ºC and 28 kV as described before [81]. On the other hand,
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8 separation of pantoprazole enantiomers by EKC with UV detection has only been described with SBE-
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10 β-CD as chiral selector [80, 82]. With this CD *R*-pantoprazole was the first-migrating enantiomer.
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12 However, when SBE-β-CD was used in 50 mM phosphate buffer (pH 7.0) at 15 ºC and 20 kV,
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15 pantoprazole enantiomers were separated in less than 7 min [80], while using this CD in 50 mM borax-
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17 150 mM phosphate buffer (6.5) at 16 ºC and 10 kV provided the separation of enantiomers in more than
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19 30 min [82]. All these methods were validated according to the ICH guidelines and were applied to the
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22 analysis of pharmaceutical formulations which did not required complex sample preparation (**Table 1**).
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25 ***Enuresis treatment***
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28 Darifenacin is a potent muscarinic receptor antagonist indicated for enuresis treatment as it acts by
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30 blocking acetylcholine, what helps to relax the involuntary smooth muscle found in the wall of the
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33 urinary bladder. This drug has one pair of enantiomers, being the *S*-configuration the active principle.
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35 Bawazeer et al. developed two chiral methods to achieve the separation of darifenacin enantiomers by
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37 HPLC and EKC, both using UV detection [83]. For the development of the EKC method, different types
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40 of CDs were screened, observing enantiomeric discrimination for darifenacin with HS-γ-CD, HS-α-CD,
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42 SBE-β-CD and HP-β-CD. Best resolution (9) was obtained with HS-γ-CD achieving the separation of
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44 darifenacin enantiomers in approximately 6 min, being the enantiomeric impurity the first migrating
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47 enantiomer. In contrast, with the HPLC method the separation was achieved in less than 3 min using a
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49 Daicel CROWNPAK CR(+) column as chiral stationary phase based on (3,3-diphenyl-1,1-binaphthyl)-
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51 crown-6 coated onto a silica support. As in CE, *R*-darifenacin reached the detector before *S*-darifenacin.
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54 LODs were lower in HPLC (0.7 mg/L) than in EKC (12.3 mg/L). Thus, the HPLC method was more
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56 sensitive and quicker than the EKC method. However, the EKC method was advantageous in terms of
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58 higher resolution and cost-effectiveness, as it used a less expensive chiral selector and lower
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60 consumption of solvents.

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Hyperparathyroidism treatment

Cinacalcet drug is an allosteric regulator of the calcium sensor receptor which acts lowering the release of parathyroid hormone. Thus, is indicated for the treatment of secondary hyperparathyroidism. It is commercialized as its single *R*-enantiomer, as it is 75 times more potent than its *S*-enantiomer. In the last 10 years, two works reported the enantiomeric separation of cinacalcet by EKC with UV detection using the same chiral selector, HP-γ-CD (**Table 1**). Ginterová et al. carried out the experimental optimization of the chiral separation of cinacalcet [84]. Several types of CDs (native and substituted) were tested, observing that the enantiomeric separation of cinacalcet was obtained with substituted γ-CDs, such as HP-γ-CD and S-γ-CD. The migration order of the enantiomers was the same for both selectors, being *S*-cinacalcet the first enantiomer reaching the detector. Despite HP-γ-CD provided lower resolution than S-γ-CD, it was chosen because it afforded better repeatability of migration times and areas, as its non-ionic nature is not affected by Joule heating unlike S-γ-CD. Under the optimized conditions, the separation was achieved within 12 min. More recently, Pasquini et al. performed the same chiral separation but optimizing the different conditions by response surface methodology (RSM) [85]. They observed that also (2-carboxyethyl)-β-CD showed chiral resolving capability towards cinacalcet, but it provided the opposite migration order than HP-γ-CD, being the enantiomeric impurity (*R*-cinacalcet) the first-migrating enantiomer. For this reason, HP-γ-CD was more suitable. The optimal conditions established by the modeled optimization were very similar to the ones previously reported by Ginterová et al. [84], as only slight changes were introduced concerning the chiral selector and MeOH concentration, the temperature, the voltage and the injection (**Table 1**). Nevertheless, despite these optimizations, the analysis time was very similar in both methods, achieving separation in about 11 min with similar resolution. Both methods were validated according to the ICH guidelines and applied to the analysis of cinacalcet tablets.

Migraine and headache treatment

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Khan et al. developed a chiral strategy by EKC using UV detection to achieve the separation of zolmitriptan, which is a serotonin 5-hydroxytryptamine receptor agonist indicated in the treatment of migraine [86]. The *S*-enantiomer is the active principle while the *R*-enantiomer is considered the enantiomeric impurity. A wide variety of CDs (γ-CD, DM-β-CD, TM-β-CD, HP-β-CD and SBE-β-CD) and chiral surfactants (deoxycholic acid sodium salt (SDC) and taurodeoxycholic acid sodium salt (STDC)) were evaluated as chiral selectors to achieve the enantiomeric separation of zolmitriptan. Enantiomeric discrimination of zolmitriptan was only obtained with HP-β-CD and SBE-β-CD, and resolution was found to be better with SBE-β-CD. Thus, this CD was chosen as chiral selector, and under the optimized conditions, the enantiomers were separated in less than 20 min with a resolution value of 3.0 and being *R*-zolmitriptan the first-migrating enantiomer. The method developed was applied to determine the chiral purity in bulk drug samples and tablets without requiring complex sample preparation (**Table 1**).

Obesity treatment

Sibutramine is a serotonin and noradrenaline re-uptake inhibitor used for obesity treatment. It is a chiral drug with two enantiomers which is commercialized in its racemic form. Nevertheless, both enantiomers show enantioselective behavior, as *R*-sibutramine decreases body weight and food uptake while *S*-sibutramine has the opposite effect. For this reason, sibutramine should be marketed as a single pure enantiomer (*R*-sibutramine). The enantiomeric separation of this drug by EKC with UV detection was described by Lee et al. [87]. For this purpose, M-β-CD was used as chiral selector achieving the separation of sibutramine enantiomers in less than 33 min, being *S*-sibutramine the first-migrating enantiomer. Moreover, a structure of the inclusion complex formed between the CD and the enantiomer was proposed through proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) studies. Finally, the chiral method was applied to the quantitative determination of sibutramine enantiomers in commercial drug capsules.

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Parkinson's treatment

Pramipexole is a dopamine receptor agonist used for the treatment of early-stage Parkinson's disease. The biological activity of this chiral drug is mainly attributed to the *S*-enantiomer, while the *R*-enantiomer is considered the chiral impurity. The separation of pramipexole enantiomers by EKC using UV detection was described by Deng et al. [88]. To achieve the chiral separation, they screened seven different neutral CDs and seven negatively charged CDs as chiral selectors. None of the neutral CDs provided enantiomeric discrimination for pramipexole. In contrast, the chiral separation was achieved with three charged CDs: carboxymethyl-α-CD (CM-α-CD) and CM-β-CD in positive polarity mode and S-β-CD in negative polarity mode. Considering the separation efficiency and the influence on the electric current of these three CDs, CM-β-CD was selected as the best chiral selector. Under the optimized conditions, pramipexole enantiomers were separated in less than 6.5 min, being the *S*-enantiomer the first migrating enantiomer. Despite the impurity reached the detector after the active principle, the separation resolution was high enough (3.7) to correctly validate the method achieving the ICH requirements. Finally, the method was applied to the enantiomeric determination of pramipexole tablets which were subjected to simple sample preparation (**Table 1**).

Mix of drug families

In all the articles reviewed in the period comprising the last 10 years, there are only two works which described the chiral separation of several drugs belonging to different families by EKC (**Table 1**). However, these methods do not provide a simultaneous separation of all the compounds analyzed, instead, they establish the best conditions for each analyte, performing individual chiral separations. In this sense, the use of eremomycin adsorbed in the capillary was evaluated as chiral selector for the enantiomeric separation of mandelic acid, warfarin, coumachlor, flurbiprofen, indoprofen, ketoprofen, fenoprofen and ibuprofen [89]. The separation conditions were the same for mandelic acid, warfarin and coumachlor, while the profens compounds shared other different conditions (**Table 1**). Additionally,

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only the migration order of ibuprofen and ketoprofen enantiomers was determined, being the *S*-enantiomers the first-migrating enantiomers. Moreover, these procedures were only applied to the analysis of warfarin and flurbiprofen in tablets. On the other hand, Li et al. evaluated the combined use of SBE-β-CD and nanoliposomes (consisting of phosphatidylcholine and cholesterol) in the chiral separation of 4 model drugs (naproxen, warfarin, ketoprofen and amlodipine) [90]. This system consisting of the combination of SBE-β-CD with the nanoliposome was compared with the single SBE-β-CD system and the combined system of SBE-β-CD with SDS (sodium dodecyl sulfate). With the SBE-β-CD-nanoliposome system all the tested drugs were base-line separated under their own experimental conditions in less than 15 min, while with the other two comparative systems no separation or poor separation was observed. The procedure was applied to test the chiral purity of a naproxen drug, observing that with this method the active principle (*S*-naproxen) migrated before the chiral impurity (*R*-naproxen).

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Conclusions

As shown throughout this review, EKC is a powerful technique for chiral analysis which, due to its advantages, can be used as an interesting alternative to other chromatographic or electromigration techniques. For instance, the great simplicity and versatility of EKC enable to carry out quick enantiomeric separations with low operation costs, since the type and concentration of the chiral selector can be easily optimized in contrast to chromatographic techniques (HPLC, GC and SFC) in which the CSPs required to achieve chiral separations are expensive. For the same reason, EKC is also more suitable than CEC, since the commercial availability of CEC columns is scarce, and their homemade fabrication complex. On the other hand, chiral separations of drugs in CE are usually performed on an aqueous separation medium rather than in an organic solvent. Many drugs and chiral selectors are soluble in water, therefore, EKC is more suitable and common than NACE electrophoretic mode for the chiral separation of drugs. In addition, the none consumption of organic solvents makes EKC an environmentally friendly microseparation technique which, besides, considerably reduces the volume of

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sample and solvents required compared to chromatographic techniques. Nevertheless, in comparison to chromatographic techniques, EKC presents some limitations regarding reproducibility of migration times as well as difficulties in the coupling to MS mainly due to the frequent use of nonvolatile chiral selectors. However, different strategies are available to overcome these limitations (e.g. partial filling and counter migration techniques in the CE-MS coupling) conferring to EKC a great potential to perform quick and cost-effective enantiomeric separations.

Concerning the chiral selectors explored in drug analyses by EKC in pharmaceutical formulations and biological samples, CDs have been by far the most commonly used, both in single and dual selector systems. In these sense, β-CD derivatives have proved to be the most effective to achieve the enantiomeric discrimination of drugs, being HP-β-CD, CM-β-CD, SBE-β-CD and S-β-CD the most widely used. Whereas, other chiral selectors such as maltodextrin, proteins, antibiotics, chiral ionic liquids and polymeric micelles have been employed to a lesser extent.

UV detection has become the first choice to detect drugs in different samples. Nevertheless, other detection techniques have shown to be more sensitive than UV detection, such as conductivity, phosphorescence, fluorescence, electrochemistry and MS. Indeed, MS superiority is not only due to its high sensitivity but also to its structural elucidation capability, enabling unequivocal identification and confirmation of the target analytes. However, it has been proved that the sensitivity of UV detection can be improved by using FASI mode or performing a sample preparation step prior to chiral analysis, as it overcomes problems related to matrix interferences and to the low concentration levels of the target compounds. It is worth mentioning that these sample preparation procedures have only been performed in biological samples and not in pharmaceutical formulations due to the different complexity of the matrices. In this sense, different sample preparation procedures have been evaluated for biological samples, mainly LLE, SPE, DLLME, LPME, PLE and EME. Among these techniques, SPE has been one of the most effective, whereas LLE has shown low recovery values and higher consumption of organic solvents than the other techniques. In addition, the hollow fiber LPME was not effective for the

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extraction of antidepressant drugs, as low recovery values were achieved with this technique (<60%). In contrast, the combination of the hollow fiber with EME enabled to achieve good recovery values for the extraction of antihypertensive drugs. Therefore, this highlights the importance of considering the nature of the target compounds willing to be extracted in order to use the best sorbents and solvents enabling to achieve the highest extraction efficiency.

In general, the majority of the works reviewed only described the enantiomeric separation of a specific drug, whereas there are only a few methods reporting the simultaneous chiral separation of different drugs. It is worth to mention, that despite some of these works described the enantioseparation of several drugs, their analysis was not simultaneous, as different conditions were established for each compound and analyzed in an individual way. Only a few articles reported the simultaneous separation of more than one drug. However, in these cases all the drugs belonged to the same family. Therefore, the development of multicomponent methods to achieve the simultaneous enantiomeric separation of different kinds of drugs is still a challenge.

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Overall, great advances have been made in the stereochemistry pharmaceutical field in the last years. Nevertheless, is still a great challenge which requires more investigation and the development of chiral strategies through the combination of powerful detection techniques and cost-effective sample preparation techniques to carry out quick, sensitive, precise and environmentally friendly methods to successfully achieve the quality control of drugs marketed as pure enantiomers, as well as understanding the stereospecific metabolism of these drugs. In this sense, the challenges for the next years in this field should focus on the development of quick multicomponent methods to achieve the simultaneous enantiomeric separation of drugs belonging to different chemical families, as well as their environmentally friendly extraction from different matrices by using microextraction techniques with low consumption of time, samples and solvents.

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Disclosure statement

The authors declare no conflicts of interest.

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Figure Captions

Fig. 1 Classification of the number of published articles related to the enantiomeric separation of chiral compounds according to the different chromatographic and electrophoretic separation techniques used. Data obtained from SciFinder Scholar up to June 2019.

Fig. 2 Examples of 3 authentic hair samples **(a-c)** collected from ketamine abusers and extracted by LLE, against a standard solution **(d)** containing ketamine, norketamine and lamotrigine (I.S.). Experimental conditions: BGE: 15 mM phosphate buffer/tris containing 0.1% (w/v) HS-γ-CD at pH 2.5, capillary: 50 µm internal diameter x 35 cm effective length, voltage: 20 kV, temperature: 20 °C, injection: 7 kV x 20 s. Readapted and reproduced with permission of [22].

Fig. 3 **(a)** CE–UV electropherogram corresponding to the LOD of *R*-duloxetine (0.2 µg/mL) in the presence of 100 µg/mL of *S*-duloxetine. Experimental conditions: BGE: 0.5% (w/v) of HP-β-CD in 150 mM phosphate buffer (pH 3.0); capillary: 50 µm internal diameter x 56 cm effective length; temperature, 20 °C; voltage, 30 kV; hydrodynamic injection, 50 mbar × 20 s; UV detection at 220, 4 nm (reference wavelength: 375, 100 nm). **(b)** CE–MS^2 extracted ion electropherograms corresponding to the LOD of *R*-duloxetine (0.02 µg/mL) in the presence of 100 µg/mL *S*-duloxetine. Experimental conditions: BGE, 150 mM ammonium formate buffer (pH 3.0); partial filling technique, 0.5% (w/v) of HP-β-CD in BGE applying 1 bar during 1 min; capillary: 50 µm internal diameter x 104 cm; temperature, 15 °C; voltage, 30 kV; hydrodynamic injection, 50 mbar × 5 s; sheath liquid, 3.3 µL/min of 80:20 (v/v) methanol/water with 0.1% (v/v) of formic acid; nebulizer and drying gas, 3 psi N_2 and 5 L/min N_2 at 200 °C; ESI+ at −4.5 kV with an end plate of −500 V; capillary exit, 57 V; EIE, 153.8 *m/z* (MS^2transition from 298.1 *m/z*); fragmentation by collision-induced dissociation with He, 0.5 V for 40 ms to a fragmentation width of 10 *m/z***.** * Unknown impurity. Readapted and reproduced with permission of [31].

Fig. 4 Schematic illustration of the setup for electromembrane extraction. Readapted and reproduced with permission of [33].

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Fig. 5 Typical electropherograms of the chiral separations of five NSAIDs with vancomycin combined with CIL1 or CIL2. Conditions: fused-silica capillary, 50 µm internal diameter x 24.5 cm effective length; 50 mM phosphate buffer (20% (v/v) of methanol included) containing **(a)** 2 mM vancomycin; **(b)** 2 mM vancomycin+15 mM CIL1; **(c)** 2 mM vancomycin+15 mM CIL2; pH 7.0; voltage, 20 kV; temperature, 25 °C. Reproduced with permission of [53].

Fig. 6 High rapid enantioseparation of **(a)** a blank urine sample, **(b)** five pairs of D,L-phenothiazines spiked urine. Experimental conditions: capillary: 50 µm internal diameter x 22.5 cm effective length; BGE: 75 mM formic acid (pH 3.0), 0.9% PDDAC, and 5 mM HP-γ-CD; voltage: −8.5 kV; hydrodynamic injection at 20-cm height for 10 s; and UV detection at 254 nm. Peak identification (concentration): IS (0.5 mM); 1. D-thioridazine (0.25 mM); 2. L-thioridazine (0.25 mM); 3. D-methotrimeprazine (0.25 mM); 4. L-methotrimeprazine (0.25 mM); 5. D-trimeprazine (0.25 mM); 6. L-trimeprazine (0.25 mM) 7. D-ethopropazine (0.25 mM), 8. L-ethopropazine (0.25 mM); 9. D-promethazine (0.25 mM); 10. L-promethazine (0.25 mM). Reproduced with permission of [57].

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Table 1. Chiral drug separations by EKC in pharmaceutical formulations and biological fluids described in the literature from 2010 to June 2019

Analyte (Specific family)	**Application**	**Sample treatment**	**Separation conditions and chiral resolution (Rs)**	**LOD / LOQ Linearity Recovery**	**Ref.**
			Analgesics		
Tramadol (Noradrenaline and serotonin reuptake inhibitor)	Enatiomeric determination in tablets	Grinding, dissolving in water, filtration and dilution with water.	BGE: 50 mM borate buffer, pH 10.2 + MD (10%, w/v) Capillary: 75 µm i.d. x 50 cm e.l.; T: 20 ºC; V: 20 kV; Injection: 30 mbar x 5 s; Detection: UV 214 nm Rs: 2.8	1.5 / 5 mg/L 5 - 100 mg/L 99 - 102%	[18]
(a) Tramadol (Noradrenaline and serotonin reuptake inhibitor) (b) Methadone (Opioid agonist)	Simultaneous enantiomeric determination in tablets, urine and human plasma	Tablets: grinding, dissolving in MeOH, centrifugation, dilution of the supernatant and filtration. Urine: dilution (1/1, v/v) with water. Plasma: dilution (1/3, v/v) with water. Both samples were spiked with the corresponding racemic standards.	BGE: 100 mM phosphate buffer, pH 8.0 + MD-DE 4-7 (20%, w/v) Capillary: 50 µm i.d. x 47 cm e.l.; T: 25 ºC; V: 16 kV; Injection: 65 mbar x 10 s; Detection: UV 214 nm Rs: (a) 1.8; (b) 1.7	(a) 2 / 7 mg/L -- -- (b) 1.5 / 5 mg/L -- --	[19]
Meptazinol and 3 synthesis intermediates (Opioid agonist)	Enatiomeric determination in tablets	Grinding and dissolving in MeOH.	BGE: 20 mM phosphate buffer, pH 6.0 + 2 mM CM-β-CD + ACN (5%, v/v) Capillary: 50 µm i.d. x 40 cm e.l.; T: -; V: 15 kV; Injection: 0.5 psi x 5 s; Detection: UV 237 nm (one intermediate compound); 271 nm (Meptazinol and 2 intermediate compounds) Rs: 2.0	2.5 / 7.5 mg/L 10.0 - 125 mg/L 98 - 102%	[20]
Tetrahydropalmatine (Calcium channel inhibitor)	Enatiomeric determination in medicinal plant *(C. yanhusuo)*	Extraction with EtOH, centrifugation, filtration and extract dilution in water.	BGE: 20 mM phosphate buffer, pH 7.4 + 50 µM BSA Capillary: 50 µm i.d. x 57.6 cm e.l.; T: 25 ºC; V: 15 kV; Injection: 15 kV x 12 s;	6 / 20 µg/L 40 - 5000 µg/L 106%	[21]

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			Detection: UV 200 nm; Rs: 2.0		
		Anesthetics			
(a) Ketamine (b) Norketamine (Antagonists of glutamate receptors)	Simultaneous enantiomeric determination in human hair	Washing with SDS, water and MeOH. Incubation of the spiked sample in HCl at 37 ºC for 12 h. Afterwards, pH adjustament of the sample extract to 10.0 with NaOH followed by LLE with hexane/EtOAc (50/50, v/v). Finally, the organic extract is treated with HCl, evaporated and reconstituted in water/HCl (98/2, v/v).	BGE: 15 mM phosphate buffer/Tris, pH 2.5 + HS-γ-CD (0.1%, w/v) Capillary: 50 µm i.d. x 35 cm e.l.; T: 20 ºC; V: 20 kV; Injection: 7 kV x 20 s; Detection: UV 200 nm Rs: (a) 2.4; (b) 2.0	0.08 / 0.25 ng/mg 0.5 - 8 ng/mg (a) 49 - 89% (b) 64 - 91%	[22]
Bupivacaine (Sodium channel blocker)	Enantiomeric determination in rabbit serum and injectable	Serum: LLE with chloroform/ether (10/1, v/v) of the spiked sample previously treated with NaOH. Evaporation of the organic extract to dryness and reconstitution in MeOH/acetate buffer (1/1, v/v). Injectable: dilution in water.	BGE: 4 mM ammonium acetate buffer, pH 4.0 + 0.48 mM SBE-β-CD Capillary: 25 µm i.d. x 55 cm e.l.; T: -; V: 12 kV; Injection: 10 kV x 3 s; Detection: Conductivity Rs: 5.0	0.052 / 0.17 µg/mL 0.52 - 10.4 µg/mL 97 - 11%	[23]
		Antiarrhythmics			
RS86017 (Calcium channel inhibitor)	Enantiomeric determination in drug[1]	--	BGE: 25 mM phosphate buffer, pH 8.0 + 28 mg/mL SBE-β-CD + ACN (20%, v/v) Capillary: 50 µm i.d. x 40 cm e.l.; T: 20 ºC; V: 22 kV; Injection: 50 mbar x 8 s; Detection: UV 206 nm Rs: 5.8	0.8 / 2.5 mg/L 2.5 - 100 mg/L 97 - 98%	[24]
		Antibiotics			

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(a) Ofloxacin (DNA gyrase enzyme inhibitor) (b) Ornidazole (--)	Simultaneous enatiomeric determination in tablets	Grinding, dissolving in EtOH, dilution with water and filtration.	BGE: 50 mM phosphate buffer/Tris, pH 1.9 + 30 mg/mL S-β-CD Capillary: 50 µm i.d. x 31.5 cm e.l.; T: 25 ºC; V: 18 kV; Injection: 50 mbar x 15 s; Detection: UV 230 nm Rs: (a) 5.5, (b) 6.3	(a) 0.5 / 1.6 mg/L[2] 5 - 100 mg/L 97 - 103% (b) 0.9 / 3.1 mg/L[2] 5 - 100 mg/L 98 - 104%	[25]
Ofloxacin (DNA gyrase enzyme inhibitor)	Enantiomeric determination in drug[1]	--	BGE: 50 mM phosphate buffer, pH 2.75 + 40 mM HP-β-CD + 30 mM EMIM-L-L Capillary: 50 µm i.d. x 41 cm e.l.; T: -; V: 20 kV; Injection: -; Detection: UV - Rs: 5.4	0.5 /1.6 mg/L 1.6 - 8.0 mg/L 97 - 105%	[26]
Ornidazole (--)	Enantiomeric determination in injectable	Filtration.	BGE: 20 mM phosphate buffer/Tris, pH 2.1 + S-α-CD (2 %, w/v) Capillary: 50 µm i.d. x 50 cm e.l.; T: 25 ºC; V: 30 kV; Injection: 0.5 psi x 5 s; Detection: UV 277 nm Rs: > 2.0	0.3 /1.0 mg/L 2.5 - 6000 mg/L 100 - 105%	[27]
			Anticoagulants		
(a) Warfarin (Inhibitor of the liver enzyme that reduces vitamin K) (b) Five hidroxylated metabolites of warfarin	Simultaneous enatiomeric determination in human serum	Dilution with percloric acid (10%, v/v), centrifugation, SPE of the supernantant with MAX cartridges, elution with ACN/MeOH/formic acid (50/50/5, v/v/v), evaporation of the extract and reconstitution in ACN/water (40/60, v/v).	BGE: 25 mM ammonium acetate buffer, pH 5.0 + 25 mM poly-L,L-SULV + MeOH (15%, v/v) Capillary: 50 µm i.d. x 120 cm t.l.; T: -; V: 30 kV; Injection: 5 mbar x 2 s; Detection: MS Rs: (a and b) > 2.0	(a) 0.5 / 2.0 µg/L 2 - 5000 µg/L 90 - 111% (b) 3.0 / 10 µg/L[3] 10 - 1000 µg/L 85 - 113%	[28]
			Antidepressants		
Citalopram (Serotonin reuptake inhibitor)	Enantiomeric determination in urine	Dilution (1/3, v/v) with water, pH adjustment to basic pH and spiking. Hollow-fiber liquid-phase microextraction and elution at acid pH.	BGE: 50 mM phosphate buffer, pH 5.0 + MD-DE 4-7 (15%, w/v) Capillary: 50 µm i.d. x 50 cm e.l.; T: 20 ºC; V: 18 kV; Injection: 60 mbar x 5 s; Detection: UV 214 nm Rs: 4.5	20 / 60 µg/L 60 - 300 µg/L 58 - 59%	[29]

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(a) Venlafaxine (Serotonin and noradrenaline reuptake inhibitor) (b) Metabolite	Simultaneous enatiomeric determination in human plasma	Dilution with phosphoric acid (4%, v/v) and centrifugation. SPE of the supernatant with Strata-X-C cartridges, elution with ammonium acetate/MeOH (5/95, v/v), extract evaporation and reconstitution of the residue in MeOH/water (10/90, v/v).	BGE: 20 mM ammonium acetate buffer/25 mM TEA, pH 8.5 + 25 mM Poly-L,L-SULA Capillary: 50 µm i.d. x 60 cm t.l.; T: - ºC; V: 20 kV; Injection: 5 mbar x 100 s; Detection: MS Rs: (a) 1.1; (b) 1.5	(a) 10.5 / 31 µg/L 150 - 5000 µg/L 82 - 113% (b) 15 / 45 µg/L 150 - 5000 µg/L 99 - 110%	[30]
Duloxetine (Serotonin and noradrenaline reuptake inhibitor)	Enatiomeric determination in capsules	Dissolution in DMSO, centrifugation and dilution of the supernatant in water.	BGE: 150 mM phosphate buffer, pH 3.0 + HP-β-CD (0.5%, w/v) Capillary: 50 µm i.d. x 56 cm e.l.; T: 20 ºC; V: 30kV; Injection: 50 mbar x 20 s; Detection: UV 220 nm and MS Rs: 2.5	UV: 0.2 / 0.7 µg/mL 0.5 -10 µg/mL 5 - 120 µg/mL 104 - 107% MS: 0.02 / 0.07 µg/mL 0.05 - 5 µg/mL 1 - 100 µg/mL --	[31]
Fluoxetine (Serotonin reuptake inhibitor)	Enantiomeric determination in tablets	Grinding and dissolution in the BGE.	BGE: 30 mM phosphate buffer, pH 8.0 + HS-β-CD (0.25%, w/v) Capillary: 50 µm i.d. x 21 cm e.l.; T: 30 ºC; V: 25 kV; Injection: 0.5 psi x 5 s; Detection: UV 200 nm Rs: 2.4	-- 5 - 50 mg/L 92 - 105%	[32]
Trimipramine (Serotonin, norepinephrine and dopamine reuptake inhibitor)	Enantiomeric determination in (a) plasma and (b) urine	Spiked samples extracted with a polypropylene hollow-fiber electromembrane (liquid membrane of 2-nitrophenyloctyl ether).	BGE: 100 mM phosphate buffer, pH 2.0 + 10 mM α-CD Capillary: 50 µm i.d. x 50 cm e.l.; T: 20 ºC; V: 18 kV; Injection: 60 mbar x 5 s; Detection: UV 214 nm Rs: 2.9	(a) 10 / 30 µg/L 30 - 500 µg/L 48 -51% (b) 8 / 25 µg/L 25 - 500 µg/L 58 - 60 %	[33]
Sertraline (Serotonin reuptake inhibitor)	Enantiomeric determination in tablets	Dissolution in EtOH (75%, v/v), filtration and dilution in the tetraborate buffer.	BGE: 20 mM sodium tetraborate buffer, pH 8.84 + β-CD (1%, w/v) + PDDAC (0.1%, v/v) + SDS (1.2%, w/v) + BuOH (21%, v/v) + ACN (18%, v/v) + Hexano (0.8%, v/v) Capillary:50 µm i.d. x 45 cm e.l.; T: 20 ºC; V: -20 kV; Injection: -; Detection: UV 210 nm	0.30 / 1.0 mg/L[2] 10 - 500 mg/L 100 - 105%	[34]

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			Rs: 2.4		
Bupropion (Dopamine and norepinephrine reuptake inhibitor)	Enantiomeric determination in tablets and urine	Tablets: grinding, dissolution in water, filtration and dilution with water. Urine: sample spiked with racemic and diluted 1/10 (v/v) with water.	BGE: 25 mM phosphate buffer, pH 3.0 + 5 mM S-α-CD Capillary: 75 μm i.d. x 50 cm e.l.; T: 20 ºC; V: -20 kV; Injection: 50 mbar x10 s; Detection: LIP ($\lambda_{Excitation}$: 250 nm y $\lambda_{Emision}$: 453 nm) Rs: >3.0	0.2 / 0.7 μM 1- 300 μM --	[35]
			Antifungals		
(a) Tioconazole (b) Isoconazole (c) Fenticonazole (Inhibitors of ergosterol biosynthesis)	Simultaneous chiral separation. Simultaneous enantiomeric determination in urine, and Individual enantiomeric determination in cream	Urine: Dilution (1/10, v/v) in water, filtration, spiking and SPE with Bond Elut Plexa cartridges, elution with MeOH, extract evaporation to dryness and reconstitution of the residue in MeOH. Cream: Dissolution in DCM, filtration and SPE with diol cartridges, elution with MeOH, extract evaporation to dryness and reconstitution of the residue in MeOH.	BGE: 25 mM phosphate buffer, pH 7.0 + 35 mM HP-γ-CD + 10 mM DM-β-CD + 50 mM SDS + ACN (20%, v/v) Capillary: 50 μm i.d. x 56 cm e.l.; T: 30 ºC; V: 25 kV; Injection: 3 kV x 3 s; Detection: UV 200 nm Rs: (a, b and c) > 1.6	(a) 4.2 / 14.0 mg/L[2] 25 - 200 mg/L 103 - 106% (b) 7.7 / 25.7 mg/L[2] 25 - 200 mg/L 95 - 99% (c) 2.7 / 9.0 mg/L 25 - 200 mg/L 72 -73%	[36]
Econazole (Ergosterol biosynthesis inhibitor)	Enantiomeric determination in cream	Dissolution in DCM, filtration, SPE with diol cartridges, elution with MeOH, extract evaporation and dilution in MeOH.	BGE: 20 mM phosphate buffer, pH 8.0 + 40 mM HP-γ-CD + 50 mM SDS Capillary: 50 μm i.d. x 56 cm e.l.; T: 25 ºC; V: 30kV; Injection: 3 kV x 3 s; Detection: UV 200 nm Rs: 2.2	4.3 / 14.3 mg/L[2] 50 - 200 mg/L 93%	[37]
			Antihypertensive		

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Pindolol (β-blocker)	Enantiomeric determination in injectables and capsules	Injectable: dilution in DMSO. Capsules: dissolution in the BGE, filtration and dilution in DMSO/BGE mixture.	BGE: 80 mM MOPS buffer, pH 7.12 + 6 mM OS-γ-CD Capillary: 50 µm i.d. X 40 cm e.l.; T: 25 ºC; V: 25kV; Injection: 20 mbar x 6 s: Detection: UV 214 nm Rs: > 2.0	0.6 / 2.0 mg/L 5 - 55 mg/L 50 - 300 mg/L 95 - 103%	[38]
Carvedilol (β-blocker)	Enantiomeric determination in human plasma	Sample spiked with the racemic standard. Afterwards, protein precipitation with acetone, centrifugation of the supernatant and adition of NaOH (pH 10.0). LLE with cloroform/acetone, evaporation of the organic extract to dryness and reconstitution of the residue in water/ACN.	BGE: 100 mM phosphate buffer/TEA, pH 2.5 + CM-β-CD (0.6%, w/v) + MeOH (30%, v/v) Capillary: 50 µm i.d. x 41 cm e.l.; T: 15 ºC; V: 25 kV; Injection: 15 kV x 30 s; Detection: UV 241 nm Rs: 4.0	4 / 12.5 µg/L 20 - 160 µg/L 91 - 107%	[39]
Propranolol (β-blocker)	Enantiomeric determination in (a) plasma and (b) urine	(a and b) Extraction with an electromembrane based on a polypropylene hollow fiber impregnated with 2-nitrophenyl-octyl ether.	BGE: 80 mM ammonium acetate buffer, pH 2.5 + 8 mM HP-β-CD Capillary: 50 µm i.d. x 50 cm e.l.; T: 20 ºC; V: 18 kV; Injection: 50 mbar x 5 s; Detection: UV 214 nm Rs: 1.6	(a) 10 / 30 µg/L 30 - 300 µg/L 66 - 67% (b) 7 / 20 µg/L 20 - 300 µg/L 54 - 55%	[40]
Carvedilol (β-blocker)	Enantiomeric determination in tablets	Grinding, dissolution in MeOH, centrifugation and supernatant dilution with MeOH.	BGE: 25 mM phosphate buffer, pH 2.5 + 20 mM HP-β-CD Capillary: 50 µm i.d. x 40 cm e.l.; T: 15 ºC; V: 20 kV; Injection: 50 mbar x 1 s; Detection: UV 242 nm Rs: 2.7	1.2 / 3.6 mg/L[2] 2.5 - 50 mg/L --	[41]

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(a) Propranolol (b) Carteolol (c) Metoprolol (d) Atenolol (e) Pindolol (f) Esmolol (g) Bisoprolol (h) Bevantolol (i) Arotinolol (j) Sotalol (β-blockers)	Individual enantiomeric separation. Application to the enantiomeric determination of propranolol in a drug[1]	--	(a and b) BGE: 30 mM phosphate buffer, pH 3.5 + 15 g/L CM-β-CD (c) BGE: 30 mM phosphate buffer, pH 3.5 + 5 g/L CM-β-CD (d, e, g and h) BGE: 30 mM phosphate buffer, pH 4.5 + 8 g/L CM-β-CD (f and i) BGE: 30 mM phosphate buffer, pH 4.5 + 5 g/L CM-β-CD (j) BGE: 30 mM phosphate buffer, pH 4.0 + 5 g/L CM-β-CD Capillary: 50 μm i.d. x 40 cm e.l.; T: 20 ºC; V: 25 kV; Injection: 10 cm x 5 s; Detection: UV 210 nm Rs: In all cases > 2.0	(a) 0.3 / 2.0 mg/L 2.0 - 2000 mg/L 97 - 101%	[42]
Ambrisentan (Endothelin receptor antagonist)	Enantiomeric determination in tablets	Grinding, dissolution in MeOH, centrifugation and supernantant dilution with water.	BGE: 100 mM borate buffer, pH 9.2 + 50 mM γ-CD + 100 mM SDS Capillary: 50 μm i.d. x 64.5 cm t.l.; T: 22 ºC, V: 30 kV; Injection: 50 mbar x 5 s; Detection: UV 200 nm Rs: 1.5	1.5 / 3.0 mg/L 1800 - 3600 mg/L 3.0 - 30 mg/L 98 - 104%	[43]
Perindopril (Acetylcholinesterase inhibitor)	Enantiomeric determination in tablets	Grinding, dissolution in phosphate buffer/MeOH (20%, v/v) and filtration.	BGE: 100 mM phosphate buffer, pH 7.0 + 5 mM HP-β-CD + MeOH (15%, v/v) Capillary: 50 μm i.d. x 56 cm e.l.; T: 20 ºC; V: 15kV; Injection: 50 mbar x 10 s; Detection: UV 210 nm Rs: > 2.0	14.7 / 25.0 mg/L 25 - 800 mg/L 99 - 100%	[44]
Lercanidipine (Calcium channel blocker)	Enantiomeric determination in tablets	Grinding and dissolution in MeOH.	BGE: 200 mM acetate buffer, pH 4.0 + 10 mM TM-β-CD Capillary: 50 μm i.d. x 50 cm e.l.; T: 15 ºC; V: 25kV; Injection: 0.5 psi x 10 s; Detection: UV 237 nm Rs: 1.3	0.8 / 2.4 mg/L[2] 12.5 - 100 mg/L 101%	[45]

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1-phenyl-R, S-tetrahydro-isoquinoline (Muscle M3 receptor blocker)	Enantiomeric determination in drug[1]	Drying at 40 ºC, dissolution in EtOH (75%), filtration and dilution with the buffer.	BGE: 35 mM phosphate buffer, pH 7.9 + 20 mM β-CD + 30 mM SDC + ACN (20%, v/v) Capillary: 25 µm i.d. x 40 cm e.l.; T: -; V: 20 kV; Injection: Electrocinética; Detection: Electrochemistry Rs: 2.7	0.5 / 1.7 µM[2] 10 - 550 µmol/L 97 - 104%	[46]
Amlodipine (Calcium ion antagonist)	Enantiomeric determination in tablets	Grinding, dissolution in MeOH, centrifugation and supernantant dilution with MeOH.	BGE: 50 mM phosphate buffer, pH 3.0 + 20 mM RAMEB Capillary: 50 µm i.d. x 40 cm e.l.; T: 15ºC; V: 25 kV; Injection: 50 mbar x 3 s; Detection: UV 238 nm Rs: 2.5	2.4 / 8.2 mg/L[2] 2 - 50 mg/L 98 - 102%	[47]
Amlodipine (Calcium ion antagonist)	Enantiomeric determination in tablets	Grinding and dissolution in water of HPLC grade.	BGE: 100 mM phosphate buffer, pH 4.0 + MD-DE 4-7 (10%, w/v) Capillary: 50 µm i.d. x 49 cm e.l.; T: 20 ºC; V: 20 kV; Injection: 60 mbar x 6 s; Detection: UV 214 nm Rs: 1.8	0.52 / 1.70 mg/L 2.5 - 250 mg/L 97 - 98%	[48]
Isradipine (Calcium channel blocker)	Enantiomeric determination in capsules. Stability study.	Dissolution in ACN, centrifugation and supernantant dissolution in ACN.	BGE: 15 mM borate buffer, pH 9.3 + SBE-β-CD (2.5%, p/v) Capillary: 50 µm i.d. x 51.5 cm e.l.; T: 25 ºC; V: 30 kV; Injection: 50 mbar x 7.5 s; Detection: UV 239 nm Rs: 5.9	3.7 / 11.6 mg/L[2] 50 - 300 mg/L 98 - 102%	[49]
			Antihistamines		
Cetirizine (Histamine H1 receptor antagonist)	Enantiomeric determination in tablets	Grinding, dissolution in water, centrifugation, filtration and dilution in water.	BGE: 25 mM phosphate buffer, pH 7.0 + 5 mM SBE-β-CD Capillary: 50 µm i.d. x 40 cm e.l.; T: 20 ºC; V: 20 kV; Injection: 50 mbar x 1 s; Detection: UV 230 nm Rs: > 2.0	2.9 / 8.2 mg/L[2] 2.5 - 50 mg/L --	[50]

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Levocetiricine (Histamine H1 receptor antagonist)	Enantiomeric determination in tablets	Grinding, dissolution in MeOH, centrifugation, dilution in water and filtration.	BGE: 50 mM tetraborate buffer, pH 8.2 + S-β-CD (1%, w/v) Capillary: 50 µm i.d. x 30 cm e.l.; T: 25 ºC; V: 10 kV; Injection: 2 psi x 5 s; Detection: UV 195 nm Rs: > 3.0	0.075 / 0.25 mg/L 0.25 - 2.5 mg/L 15 - 100 mg/L 84 - 109 %	[51]
Pheniramine (Histamine H1 receptor antagonist)	Enantiomeric determination in eye drops	Dilution (1/40, v/v) in phosphate buffer.	BGE: 50 mM phosphate buffer, pH 3.0 + 50 mM HP-β-CD Capillary: 75 µm i.d. x 66 cm e.l.; T: 25 ºC; V: 30 kV; Injection: 50 mbar x 5 s; Detection: UV 214 nm Rs: 1.8	0.5 / 5 µM 5 - 500 µM 101 - 106%	[52]
			Anti-inflammatories		
(a) Naproxen (b) Carprofen (c) Ibuprofen (d) Ketoprofen (e) Pranoprofen (Prostaglandin synthetase inhibitors)	Individual chiral separation. Application to the enantiomeric determination of naproxen in drug[1]	Dissolution in MeOH/water (50/50, v/v).	(a, c, d and e) BGE: 50 mM phosphate buffer, pH 7.0 + 2 mM VC + 15 mM CIL1 + MeOH (20%, v/v) (b) BGE: 50 mM phosphate buffer, pH 7.0 + 2 mM VC + 15 mM CIL2 + MeOH (20%, v/v) Capillary: 50 µm i.d. x 24.5 cm e.l.; T: 25 ºC; V: 20 kV; Injection: 50 mbar x 4 s; Detection: UV (a) 235 nm, (b) 230 nm, (c and d) 225 nm and (e) 210 nm Rs: (a) 4.9; (b) 3.1; (c) 2.6; (d) 4.1; (e) 3.5	(a) 9.0 / 30 mg/L 30 - 400 mg/L --	[53]
Penicillamine (Collagen cross-linking inhibitor and immune system modulator)	Enantiomeric determination in tablets	Grinding, dissolution in sodium bicarbonate, centrifugation and supernatant dilution (1/5, v/v) with sodium bicarbonate.	Analyte derivatization with ANDA (a) BGE: 200 mM acetate buffer, pH 4.5 + 19 mM β-CD (b) BGE: 30 mM phosphate buffer, pH 7.4 + 17 mM β-CD Capillary: 50 µm i.d. x 40 cm e.l.; T: 23 ºC; V: -15 kV (+15 kV at pH 7.4); Injection: 0.5 psi x 2 s; Detection: UV 254 nm Rs: (a and b) 7.0	(a) 2.6 / 8.7 mg/L 8.56 - 856 mg/L 93 - 98% (b) 2.6 / 8.7 mg/L 8.56 - 1710 mg/L 98 - 101%	[54]

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			Analyte derivatization with ANDA BGE: 80 mM borate buffer, pH 9.7 + 17 mM β-CD Capillary: 50 µm i.d. x 40 cm e.l.; T: 23 ºC; V: 15 kV; Injection: 0.5 psi x 2 s; Detection: UV 254 nm Rs: 4.0	1.4 / 4.7 mg/L 8.56 - 1710 mg/L 99 - 105%	
			Antiparasitic		
Praziquantel (Anthelmintic, causes damage in the parasitic tissues and tetanic contraction of the musculature)	Enantiomeric determination in tablets	Grinding, dissolution in MeOH/phosphoric acid (99.7/0.3, v/v), filtration and dilution with phosphate buffer.	BGE: 50 mM phosphate buffer, pH 2.0 + 15 mM S-β-CD Capillary: 50 µm i.d. x 40 cm e.l.; T: 25 ºC; V: 15 kV; Injection: 50 mbar x 2 s; Detection: UV 200 nm Rs: 16.8	0.75 / 2.00 mg/L 2 - 200 mg/L 50 - 500 mg/L 96 - 102%	[55]
			Antipsychotics		
Levosulpiride (Dopamine receptor antagonist)	Enantiomeric determination in blisters	Dilution (25/75, v/v) with water.	BGE: 5 mM Britton-Robinson buffer, pH 3.45 + 10 mM S-β-CD + 34 mM M-β-CD Capillary: 50 µm i.d. x 24.5 cm e.l.; T: 16 ºC; V: 14 kV; Injection: 50 mbar x 2 s; Detection: UV 214 nm Rs: > 1.5	1.2 mg/L / 2.0 mg/L 1200 - 2400 mg/L and 2.0 - 20 mg/L 99.5 - 102%	[56]

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(a) Etopropazina (b) Metotrimeprazina (c) Prometazina (d) Tioridazina (e) Trimeprazina (Antagonists of histamine receptors and of muscarinic acetylcholine receptors)	Simultaneous enantiomeric determinatation in urine	Centrifugation and spiking with racemic standards.	BGE: 75 mM formate buffer, pH 3.0 + 5 mM HP-γ-CD + 0.9 % PDDAC Capillary: 50 µm i.d. x 22.5 cm e.l.; T: - ºC; V: -9.5 kV; Injection: 20 cm x 10 s; Detection: UV 254 nm Rs: > 1.8	(a) 4 / 13.3 µM[2] -- 95 - 96% (b) 3 / 10 µM -- 94 - 96% (c) 8 / 26.7 µM[2] -- 94% (d) 3 / 10 µM[2] -- 89 - 92% (e) 3 / 10 µM[2] -- 107 - 109%	[57]
(a) Etopropazina (b) Metotrimeprazina (c) Prometazina (d) Tioridazina (e) Trimeprazina (Antagonists of histamine receptors and of muscarinic acetylcholine receptors)	Simultaneous enantiomeric determinatation in urine	Sample pH adjustment to 7.0 with NaOH, centrifugation and spiking of the supernatant. SPE C_{18} cartridges, elution with MeOH, extract evaporation to dryness and reconstitution of the residue in water/MeOH (50/50, v/v).	BGE: 100 mM formate buffer/Tris, pH 3.0 + 5 mM HP-γ-CD + PDDAC (0.9%, v/v) Capillary: 50 µm i.d. x 40 cm e.l.; T: - ºC; V: -10 kV; Injection: 20 cm x 10 s; Detection: UV 254 nm Rs: > 2.0	(a) 2.9 / 9.7 nM[2] 10 - 800 nM 89 - 101% (b) 2.6 / 8.7 nM[2] 9 - 600 nM 89 - 101% (c) 2.3 / 7.7 nM[2] 6 - 400 nM 89 - 102% (d) 6.3 / 21 nM[2] 20 - 1500 nM 89 - 101% (e) 2.6 / 8.7 nM[2] 9 - 600 nM 89 - 101%	[58]
		Antitussives and analgesics			

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Methorphan (Levomethorphan: Opioid receptor agonist. Dextromethorphan: opioid agonist, glutamate receptor antagonist and decreaser of tachykinin production)	Enantiomeric determination in capsules	Dissolution en EtOH (96%), centrifugation, evaporation of a portion of the supernantant and reconstitution in HCl 10 mM.	BGE: 30 mM phosphate buffer, pH 6.5 + 16 mg/mL S-β-CD + 14 mg/mL M-α-CD Capillary: 50 μm i.d. x 30 cm e.l.; T: 20 ºC; V: 20 kV; Injection: 0.7 psi x 5 s; Detection: UV 200 nm Rs: > 2.0	0.3 mg/L / 1.0 mg/L 1.0 - 15 mg/L 89 - 106%	[59]
Methorphan (Levomethorphan: Opioid receptor agonist. Dextromethorphan: opioid agonist, glutamate receptor antagonist and decreaser of tachykinin production)	Enantiomeric determination in post-mortem blood samples	Spiked sample extracted by LLE with a saturated dissolution of sodium carbonate + hexane/EtOAc (50/50, v/v), centrifugation, evaporation and reconstitution in MeOH/HCl 10 mM (90/10, v/v).	BGE: 150 mM phosphate buffer, pH 4.4 + 5 mM HP-β-CD + MeOH (20%, v/v) Capillary: 50 μm i.d. x 50 cm e.l.; T: 15 ºC; V: 25 kV; Injection: 0.5 psi x 8 s; Detection: UV 200 nm Rs: 2.0	8 μg/L / 25 μg/L 25 - 500 μg/L 86 - 105%	[10]
			Antiuremics		
Colchicine (Inhibitor of microtubule polymerization by binding to its constitutive protein tubulin)	Enantiomeric determinacion in tablets	Grinding, dissolution in water/DMSO (1/1, v/v), centrifugation. Supernantat dilution with water and filtration.	(a) BGE: 50 mM borate buffer, pH 9 + 7 mM Succ-γ-CD Capillary: 50 μm i.d. x 50 cm e.l.; T: 16 ºC; V: 20 kV; Injection: 0.7 psi x 5 s; Detection: UV 243 nm (b) BGE: 25 mM borate buffer, pH 9 + 17 mM S-γ-CD Capillary: 50 μm i.d. x 50 cm e.l.; T: 20 ºC; V: 20 kV; Injection: 0.7 psi x 5 s; Detection: UV 243 nm Rs: (a) 5.6; (b) 3.2	(a) 0.3 / 1 mg/L 1 - 10 mg/L 5 - 360 mg/L 98 - 101% (b) 0.3 / 1 mg/L 1 - 10 mg/L 5 - 120 mg/L 97 - 102%	[60]
			Bronchodilators		
(a) Clenbuterol (b) Procaterol (c) Bambuterol (d) Tranterol (e) Salbutamol (f) Terbutalina (β_2 adrenergic agonists)	Individual chiral separation	--	(a, b, c and f) BGE: 30 mM phosphate buffer, pH 3.5 + 5 g/L CM-β-CD (d and e) BGE: 30 mM phosphate buffer, pH 4.0 + 8 g/L CM-β-CD Capillary: 50 μm i.d. x 40 cm e.l.; T: 20 ºC; V: 25 kV; Injection: 10 cm x 5 s; Detection: UV 210 nm Rs: in all cases > 2.0	--	[42]

(a) Clenbuterol (b) Bambuterol (c) Procaterol (d) Salbutamol (e)Tulobuterol (β_2 adrenergic agonists)	Individual enantiomeric determination in different tablets	Grinding, dissolution in water and filtration.	BGE: 50 mM phosphate buffer, pH 3.5 + 10 mM CM-β-CD Capillary: 75 µm i.d. x 40 cm e.l.; T: 15 ºC; V: 20 kV; Injection: 10 kV x 5 s; Detection: UV 200 nm Rs: (a) 7.7; (b) 8.5; (c) 25.4; (d) 2.2; (e) 2.3	--	[61]
Glycopyrrolate (Antagonist of acetylcholine muscarinic receptors)	Enantiomeric determination in drug[1]	--	BGE: 30 mM phosphate buffer, pH 7.0 + S-β-CD (2.0%, w/v) Capillary: 50 µm i.d. x 40 cm e.l.; T: 25 ºC; V: 20 kV; Injection: 0.5 psi x 5 s; Detection: UV 210 nm Rs: > 2.0	0.3 / 1 mg/L 1.00 – 12.00 mg/L 97.5 – 101%	[62]
Higenamine (β_2 adrenergic agonists)	Enantiomeric determination in *Nelumbo nucifera* seeds	Grinding, dissolution in MeOH, filtration and dilution with water.	BGE: 30 mM phosphate buffer, pH 3.5 + 15 mg/mL CM-β-CD Capillary: 50 µm i.d. x 40 cm e.l.; T: 25 ºC; V: 20 kV; Injection: 0.5 psi x 20 s; Detection: UV 210 nm Rs: 9.4	-- 4.93 - 49.3 mg/L 93 - 101%	[63]
			Psychoactives		
(a) Ephedrine (b) Pseudoephedrine (c) Metilephedrine (d) Norephedrine (e) Diethylnorephedrine (f) Adrenaline (Adrenergic agonist of the CNS receptors)	Individual enantiomeric determination of ephedrine synthesis impurities	--	BGE: 20 mM phosphate buffer, pH 2.5 + S-β-CD (4.0%, w/v) + EtOAc (0.5%, v/v) + SDS (1.0%, w/v) + BuOH (4.0%, v/v) + i-PrOH (3.0%, v/v) Capillary: 50 µm i.d. x 40 cm e.l.; T: 20 ºC; V: -15 kV; Injection: 3.4 kPa x 4 s; Detection: UV 200 nm Rs: in all cases > 2.0	(a) 1.9 / 6.3 mg/L 3.2 - 32 mg/L -- (b) 1.6 / 5.3 mg/L 2.8 - 28.4 mg/L --	[64]

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Amphetamine (Indirect agonist of the presynaptic norepinephrine and dopamine receptors of the CNS)	Enantiomeric determination in a commercial drug	Dissolution in water.	BGE: 50 mM sodium phosphate buffer, pH 3.0 + S-β-CD (5.5%, w/v) + SDS (1.5%, w/v) + EtOAc (0.5%, v/v) + BuOH (3.5%, v/v) + i-PrOH (2.5%, v/v) Capillary: 50 µm i.d. x 42 cm e.l.; T: 20 ºC; V: -14 kV; Injection: 0.5 psi x 4 s; Detection: UV 200 nm Rs: > 2.0	1.5 / 3 mg/L 3 - 150 mg/L -- (Levoamphetamine)	[65]
(a) Ephedrine (Adrenergic agonist of the CNS receptors) (b) Methylpseudoephedrine (c) Amphetamine (d) Methamphetamine (e) Ethylamphetamine (f) (3,4-methylenedioxyamphetamine) (g) (3,4-methylenedioxymethamphetamine) (h) (3,4-methylenedioxy-N-ethylamphetamine) (Indirect agonist of the presynaptic norepinephrine and dopamine receptors of the CNS)	Simultaneous enantiomeric determination in amphetamines.	Dissolution in water an filtrarion.	BGE: 50 mM ammonium formate buffer, pH 2.2 + HS-γ-CD (0.26%, w/v) Capillary: 50 µm i.d. x 90 cm t.l.; T: 25 ºC; V: 20 kV; Injection: 7.25 bar x 10 s; Detection: MS Rs: (a) 2.8; (b) 2.0; (c) 5.3; (d) 2.8; (e) 3.3; (f) 9.8; (g) 3.6; (h) 2.6	(a) 8.6 / 28.7 mg/L^2 -- (b) 1.0 / 3.3 mg/L^2 -- (c) 15.5 / 51.7 mg/L^2 -- (d) 4.8 / 16 mg/L^2 -- (e) 1.8 / 6 mg/L^2 -- (f) 7.4 / 24.7 mg/L^2 -- (g) 3.2 / 10.7 mg/L^2 -- (h) 3.0 / 10 mg/L^2 --	[66]
(a) Mephedrone (b) Methylephedrine (c) Methylenedioxy-pyrovalerone (--)	Simultaneous enantiomeric determination in human hair sample	Spiked sample extracted by PLE with alkalized water at pH 10.0 con ammonium hydroxide (28%, v/v), dilution of the extract with water at pH 10.0 and filtration. Afterwards, on-line SPE with Oasis HLB cartridges and elution with MeOH/formic acid (2% v/v).	BGE: 80 mM phosphate buffer, pH 2.5 + 12 mg/mL β-CD Capillary: 50 µm i.d. x 71.5 cm e.l.; T: 25 ºC; V: 30 kV; Injection: 50 mbar x 5 s; Detection: UV 200 nm Rs: (a) 1.6; (b) 1.6; (c) > 2.0	(a) 0.02 / 0.05 ng/mg 0.05 - 5 ng/mg 85 - 93% (b) 0.02 / 0.05 ng/mg 0.05 - 5 ng/mg 86 - 95% (c) 0.1 / 0.40 ng/mg 0.40 - 10 ng/mg 81 - 91%	[67]

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(a) 3,4-Methylenedioxy-methamphetamine (b) Methamphetamine (Indirect agonist of the presynaptic norepinephrine and dopamine receptors of the CNS) (c) Ketamine (Glutamate receptor antagonist)	Simultaneous enantiomeric determination in differents types of paper (packaging, aluminum and plastic)	Samples in contact with the contaminants during 5 days. Afterwards, extraction with acetic acid and filtration. DLLME with isopropranol/cloroform (92.5/7.5, v/v), centrifugation, evaporation of the organic extract and reconstitution in water.	BGE: 100 mM phosphate buffer, pH 3.2 + 20 mM β-CD Capillary: 50 µm i.d. x 30 cm e.l.; T: 25 ºC; V: 15 kV; Injection: 5 kV x 7 s; Detection: UV 200 nm Rs: in all cases > 1.9	(a) 0.08 / 0.27 µg/L 0.24 - 6000 µg/L 86 - 89% (b) 0.20 / 0.67 µg/L 0.60 - 6000 µg/L 86 - 89% (c) 0.15 / 0.5 µg/L 0.45 - 6500 µg/L 91 - 94%	[68]

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(a) Dimethylcathinone (b) Methcathinone (c) Buphedrone (d) Pentedrone (e) Methedrone (f) Methylone (g) Mephedrone (h) Ethylone (i) 3,4-DMMC (j) Pentylone (k) MDPV (Indirect agonist of the presynaptic norepinephrine and dopamine receptors of the CNS)	Simultaneous enantiomeric determination in drugs.	Dissolution in MeOH and centrifugation.	BGE: 100 mM phosphate buffer, pH 2.5 + 10 mM β-CD Capillary: 50 µm i.d. x 49 cm e.l.; T: 25 ºC; V: 25 kV; Injection: 10 kV x 10 s; Detection: UV 206 nm and MS Rs: (a) 1.6; (b) 1.6; (c) 0.8; (d) > 2.0; (e) NS; (f) 1.8; (g) 1.6; (h) 1.8; (i) > 2.0; (j) > 2.0; (k) > 2.0	(a) 11 / 33 µg/L 12.5 - 500 µg/L -- (b) 1.0 / 3 µg/L 12.5 - 500 µg/L -- (c) 3.5 / 10 µg/L 12.5 - 500 µg/L -- (d) 3.7 / 11 µg/L 12.5 - 500 µg/L -- (e) 5.0 / 15µg/L 12.5 - 500 µg/L -- (f) 9.5 / 31 µg/L 12.5 - 500 µg/L -- (g) 5.0 / 15 µg/L 12.5 - 500 µg/L -- (h) 3.8 / 11 µg/L 12.5 - 500 µg/L -- (i) 5.0 / 15 µg/L 12.5 - 500 µg/L -- (j) 6.7 / 19 µg/L 12.5 - 500 µg/L -- (k) 5.5 / 16 µg/L 12.5 - 500 µg/L --	[69]

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Threo-methylphenidate (Reuptake inhibitor of dopamine and noradrenaline)	Enantiomeric determination in oral fluid samples	LLE with water/hexane (24/76, v/v), centrifugation, dilution of the organic phase with water, evaporation and reconstitution with acetic acid 1M.	BGE: 50 mM phosphate buffer/TEA, pH 3.0 + 20 mg/mL HP-β-CD Capillary: 50 µm i.d. x 30 cm e.l.; T: 25 ºC; V: 20 kV; Injection: 6 kV x 50 s; Detection: UV 200 nm Rs: 2.0	1.5 / 5 µg/L 10 - 200 µg/L --	[70]
			Sedatives		
Methadone (Opioid agonist)	Enantiomeric determination in spiked samples of serum, urine and condensed exhaled air	Serum: Sample treated with NaOH to pH 9.0 and ACN, LLE with cloroform, centrifugation, evaporation of the organic phase and reconstitution in water. Urine: Same procedure as in serum samples, in this case the samples is diluted with water (1/5, v/v). Air: Condensation in cold trap of exhaled air. The three samples were afterwards spiked before analysis.	BGE: 150 mM phosphate buffer/TEA, pH 2.5 + CM-β-CD (0.8%, w/v) + MeOH (30%, v/v) Capillary: 50 µm i.d. x 41.5 cm e.l.; T: 15 ºC; V: 25 kV; Injection: 15 kV x 40 s; Detection: UV 200 nm Rs: 2.0	0.05 / 0.15 mg/L 0.15 - 5.00 mg/L 86 - 102%	[71]
(a) Mephobarbital (b) Pentobarbital (c) Secobarbital (CNS depressants)	Simultaneous enantiomeric determination in human serum	Dissolution with HCl 0.1M, spiking, centrifugation, supernatant collection, SPE with Oasis MCX cartridges, elution with ACN, evaporation and reconstitution in ACN/water (80/20, v/v).	BGE: 40 mM ammonium acetate buffer, pH 7.0 + 50 mM poly-L-SUCIL Capillary: 50 µm i.d. x 60 cm e.l.; T: 20 ºC; V: 25 kV; Injection: 2 mbar x 5 s; Detection: MS Rs: 2.0	(a) 7.8 / 26 mg/L 7.8 - 125 mg/L 84 - 101% (b) 7.8 / 26 mg/L 7.8 - 125 mg/L 84 - 101% (c) 7.8 / 26 mg/L 7.8 - 125 mg/L 84 - 101%	[72]

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Zopiclone (Benzodiazepine receptor agonist)	Enantiomeric determination in tablets	Grinding, dissolution in ACN, Filtration, evaporation and reconstitution in water:ACN (9/1, v/v).	BGE: 80 mM phosphate buffer, pH 2.5 + 5 mM CM-β-CD Capillary: 50 µm i.d. x 42 cm e.l.; T: 25 ºC; V: 27 kV; Injection: 55 mbar x 30 s; Detection: UV 305 nm Rs: > 2.0	-- 400 - 800 mg/L 98 - 99%	[73]
			Alzheimer's treatment		
Huperzine A (Acetylcholinesterase inhibitor and glutamate receptor antagonist)	Enantiomeric determination in tablets	Grinding, dissolution in MeOH, filtration, evaporation and reconstitution in MeOH.	BGE: 50 mM acetate buffer, pH 5.0 + L,L-AV (0.2%, w/v) + t-BuOH (10%, v/v) Capillary: 50 µm i.d. x 55.5 cm e.l.; T: 25 ºC; V: 20 kV; Injection: 30 mbar x 3 s; Detection: UV 230 nm Rs: 1.8	4.17 /13.92 mg/L 50 – 800 mg/L --	[74]
			Cancer, rheumatoid arthritis and psoriasis treatment		
Methotrexate (Folic acid antagonist, and enzyme inhibitor of purine and pyrimidine synthesis)	Enantiomeric determination in human leukemic cell extract	Centrifugation, decanting and solid reconstitution in Hanks saline solution, spiking, centrifugation and supernantant filtration.	BGE: 25 mM phosphate buffer, pH 7.0 + 3 mg/mL HP-β-CD + 1 mM BrNS + MeOH (20%, v/v) Capillary: 75 µm i.d. x 60 cm e.l.; T: 20 ºC; V: 30 kV; Injection: 50 mbar x 5 s; Detection: Phosphorescence with excitation at 294 nm Rs: 1.5	0.3 / 1.1 µM --	[75]
Pyroglutamic acid derivative (--)	Enantiomeric determination in injectable and plasma	Injectable: Dilution with water. Plasma: Rat spiking *in vivo*, collection of blood samples at different times after ingestion. Centrifugation and dilution of the supernantant with water. SPE with Oasis MCX cartridges, elution with 10% NH_4OH in MeOH, evaporation to dryness and reconstitution in water.	BGE: 50 mM phosphate buffer, pH 2.5 + HS-β-CD (2.5%, w/v) Capillary: 50 µm i.d. x 72 cm e.l.; T: 15 ºC; V: -30 kV; Injection: 50 mbar x 15 s; Detection: UV 260 nm Rs: > 2.0	0.15 / 0.45 mg/L 0.25 – 0.75 mg/L 100%	[76]
			Diabetes treatment		

Alogliptin (Dipeptidyl peptidase-4 inhibitor)	Enantiomeric determination in drug[1]	--	BGE: 25 mM acetate buffer, pH 4.75 + 5 mM SBE-β-CD Capillary: 50 µm i.d. x 40 cm e.l.; T: 16 ºC; V: 18 kV; Injection: 20 mbar x 4 s; Detection: UV 200 nm Rs: 8.4	2 / 6 mg/L 6 - 250 mg/L --	[77]
Repaglinide (insulin release stimulator)	Enantiomeric determination in tablets and drug[1]	Tablets: Grinding, dissolution in MeOH, filtration and dilution. Drug: Dissolution in MeOH, dilution in water and filtration.	BGE: 20 mM phosphate buffer, pH 2.5 + DM-β-CD (1.25%, w/v) Capillary: 50 µm i.d. x 36 cm e.l.; T: 20 ºC; V: 20 kV; Injection: Gravity; Detection: UV 243 nm Rs: 2.0	100 / 333 µg/L 12.5 - 400 mg/L 98 - 101%	[78]
Vildagliptin (Dipeptidyl peptidase-4 inhibitor)	Enantiomeric determination in tablets	Grinding, dissolution in water, centrifugation and supernatant filtration.	BGE: 50 mM acetate buffer/Tris, pH 4.75 + 20 mM SBE-α-CD Capillary: 50 µm i.d. x 40 cm e.l.; T: 15 ºC; V: 25 kV; Injection: 50 mbar x 4 s; Detection: UV 200 nm Rs: 5.2	2.5 / 7.5 mg/L 7.5 - 180 mg/L --	[79]
			Dyspepsia treatment		
(a) Omeprazole (b) Pantoprazole (Proton pump inhibitor)	Individual enantiomeric determination in omeprazole or pantoprazole tablets	Grinding, dissolution in MeOH, centrifugation and supernatant filtration.	(a) BGE: 50 mM phosphate buffer, pH 2.5 + 20 mM RAMEB (b) BGE: 50 mM phosphate buffer, pH 7.0 + 5 mM SBE-β-CD Capillary: 50 µm i.d. x 40 cm e.l.; T: 15 ºC; V: 20 kV; Injection: 50 mbar x 1 s; Detection: UV (a) 210 and (b) 300 nm Rs: (a) 3.0; (b) 2.5	(a) 0.9 / 2.8 mg/L[2] 5 - 100 mg/L 95 - 105% (b) 1.1 / 3.4 mg/L[2] 5 - 100 mg/L 95 - 105%	[80]

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Omeprazole (Proton pump inhibitor)	Enantiomeric determination in pills	Dissolution in NaOH 0.1M/borate buffer pH 9.2 (8/92, v/v) and centrifugation.	BGE: 100 mM phosphate buffer/Tris, pH 2.5 + 20 mM HP-β-CD + 1 mM dithionite Capillary: 75 µm i.d. x 50 cm e.l.; T: 15 ºC; V: 28 kV; Injection: 0.5 psi x 5 s; Detection: UV 301 nm Rs: 2.3	0.6 / 2 mg/L 2 - 6 mg/L 98 - 99%	[81]
Pantoprazole (Proton pump inhibitor)	Enantiomeric determination in drug[1]	Dissolution in NaOH 0.1M.	BGE: Borax/phosphate buffer (50/150 mM), pH 6.5 + 20 mg/mL SBE-β-CD Capillary: 50 µm i.d. x 38 cm e.l.; T: 16 ºC; V: 10 kV; Injection: 10 cm x 10 s Detection: UV 290 nm Rs: 3.0	0.9 / 2.5 mg/L 2.5 - 25 mg/L 93 - 105%	[82]
			Enuresis treatment		
Darifenacin (Acetylcholine muscarinic receptor blocker)	Enantiomeric determination in tablets	Grinding, dissolution in phosphate buffer, filtration and dilution in buffer.	BGE: 50 mM phosphate buffer/TEA, pH 2.5 + HS-γ-CD (10%, w/v) Capillary: 50 µm i.d. x 31.2 cm e.l.; T: 25 ºC; V: 25 kV; Injection: 0.6 psi x 3.5 s; Detection: UV 286 nm Rs: 9.0	12.3 / 37.2 mg/L 40 - 300 mg/L 99 - 100 %	[83]
			Hyperparathyroidism treatment		
Cinacalcet (Allosteric regulator of the calcium sensor receptor)	Enantiomeric determination in tablets	Grinding, dissolution in MeOH, dilution wit water and filtration.	BGE: 150 mM phosphate buffer, pH 2.5 + HP-γ-CD (0.5%, w/v) + MeOH (20%, v/v) Capillary: 50 µm i.d. x 40 cm e.l.; T: 20 ºC; V: 25 kV; Injection: 50 kPa x 5 s; Detection: UV 214 nm Rs: 1.8	0.15 / 0.5 mg/L[2] 0.5 - 50 mg/L 99 - 100%	[84]
Cinacalcet (Allosteric regulator of the calcium sensor receptor)	Enantiomeric determination in tablets	Grinding, dissolution in EtOH, centrifugation and supernatant dilution in water.	BGE: 150 mM phosphate buffer, pH 2.7 + 3.1 mM HP-γ-CD + MeOH (2%, v/v) Capillary: 50 µm i.d. x 40 cm e.l.; T: 18 ºC; V: 26 kV; Injection: 50 mbar x 3 s; Detection: UV 220 nm Rs: 2.0	0.8 / 1.0 mg/L 1.0 - 10 mg/L 104%	[85]
			Migraine and headaches treatment		

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Zolmitriptan (Selective agonist of cranial vascular contraction receptors)	Enantiomeric determination in drug[1]	Dissolution with citrate buffer.	BGE: 50 mM citrate buffer, pH 4.6 + 20 mM SBE-β-CD Capillary: 50 µm i.d. x 72 cm t.l.; T: 25 ºC; V: 30 kV; Injection: 50 mbar x 3 s; Detection: UV 225 nm Rs: 3.0	0.3 - 1 mg/L 1 - 31 mg/L 95 - 101%	[86]
Obesity treatment					
Sibutramine (Reuptake inhibitor of serotonin, noradrenaline and dopamine)	Enantiomeric determination in capsules	--	BGE: 50 mM phosphate buffer, pH 3.0 + 10 mg/mL M-β-CD Capillary: 50 µm i.d. x 56 cm e.l.; T: 20 ºC; V: 20 kV; Injection: 50 mbar x 3 s; Detection: UV 225 nm Rs: 2.0	0.5 / 1.7 mg/L 1 - 24 mg/L 95 - 100%	[87]
Parkinson's treatment					
Pramipexole (Dopamine receptor agonist)	Enantiomeric determination in tablets	Grinding, dissolution in water, centrifugation and supernatant filtration.	BGE: 50 mM phosphate buffer, pH 2.8 + 25 mM CM-β-CD Capillary: 75 µm i.d. x 40 cm e.l.; T: 25 ºC; V: 25 kV; Injection: 3.45 kPa x 5 s; Detection: UV 262 nm Rs: 3.7	0.9 / 2.94 mg/L 5 - 75 mg/L 100.5%	[88]
Mix of drug families					

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(a) Mandelic acid (Acne treatment, moisturizing and exfoliating activity) (b) Warfarin (c) Coumachlor (b and c, Anticoagulants, inhibitors of the liver enzyme that reduces vitamin K) (d) Flurbiprofen (e) Indoprofen (f) Ketoprofen (g) Fenoprofen (h) Ibuprofen (d, e, f, g, and h, Antiinflammatories, prostaglandin synthetase inhibitors)	Individual chiral separation. Application to the enantiomeric determination of (b) warfarin in tablets and (d) flurbiprofen in tablets	(b) Grinding, dissolution in MeOH:water (50/50, v/v) and filtration. (d) Grinding, dissolution in MeOH:water (50/50, v/v).	(a, b and c) BGE: 50 mM phosphate buffer/Tris, pH 6.1 + 5 mM ERM Capillary: 75 μm i.d. x 30 cm e.l.; T: 20 ºC; V: -10 kV; Injection: 2.5 kPa x 5 s; Detection: UV 305 nm (d, e, f, g and h) BGE: 50 mM phosphate buffer /Tris, pH 6.1 + 5 mM ERM Capillary: 75 μm i.d. x 30 cm e.l.; T: 20 ºC; V: 1, 2, 3, 4 -10 kV; 5- (-5) kV; Injection: 2.5 kPa x 5 s; Detection: UV 254 nm Rs: (a) 4.6; (b) 2.0; (c) 3.0; (d) 11.4; (e) 10.9; (f) 4.8; (g) 5.0; (h) 9.9	(b) 1 / 3.3 μM[2] 3.2 - 4.9 μM -- (d) 10 / 33.3 μM[2] 40 - 2000 μM --	[89]
(a) Warfarin (Anticoagulant, inhibitor of the liver enzyme that reduces vitamin K) (b) Naproxen (c) Ketoprofen (b and c, Antiinflammatories, prostaglandin synthetase inhibitors) (d) Amlodipine (Antihypertensive, calcium ion antagonist)	Individual chiral separation Application to the enantiomeric determination of naproxen drug[1]	--	(a) BGE: 20 mM phosphate buffer, pH 8.4 + SBE-β-CD (2.0%, w/v) + NLips (1.2%, w/v) (b) BGE: 20 mM phosphate buffer, pH 5.2 + SBE-β-CD (2.0%, w/v) + NLips (1.2%, w/v) (c) BGE: 20 mM phosphate buffer, pH 4.0 + SBE-β-CD (1.5%, w/v) + NLips (1.2 %,w/v) (d) BGE: 20 mM phosphate buffer, pH 2.6 + SBE-β-CD (1.5%, w/v) + NLips (0.96%, w/v) Capillary: 50 μm i.d. x 24.5 cm e.l.; T: 25 ºC; V: (a, b, and c) -6 kV and y (d) -10 kV; Injection: 50 mbar x 5 s; Detection: UV (a) 305 nm, (b) 235 nm, (c) 256 nm and (d) 237 nm Rs: (a) 2.2; (b) 1.6; (c) 1.5; (d) 2.6	(b) 4.5 / 15 mg/L 15 - 450 mg/L --	[90]

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[1]Drug: drug with excipients without being in its commercial form.
[2]Highest LOD of the two enantiomers.
[3]Highest LOD found for the enantiomers corresponding to the different metabolites.
3,4-DMMC: (±)-1-(3,4-dimethylphenyl)-2-(methylamino)propan-1-one; ACN: acetonitrile; ANDA: 7-amino-1,3-naphthalenedisulfonic acid; BGE: background electrolyte; Bond Elut Plexa: polymeric sorbent for retention of neutral and ionic analytes; Britton-Robinson buffer: buffer based on the mixture of 40 mM boric acid + 40 mM phosphoric acid + 40 mM acetic acid; BrNS: 1-bromo-4-naphthalene sulfonic acid; BSA: bovine serum albumin; BuOH: butanol; CD: cyclodextrin; CIL: chiral ionic liquid; CIL1: L-alanine-tert-butylesterbis(trifluoromethane)sulfonimide; CIL2: L-valine-tert-butylesterbis(trifluoromethane)sulfonimide; CM-β-CD: carboxymethyl-β-CD; DCM: dichloromethane; DLLME: dispersive liquid-liquid microextraction; DM-β-CD: heptakis(2,6-di-O-methyl)-β-CD; DMSO: dimethylsulfoxide; e.l.: effective length: EMIM-L-L: 1-ethyl-3-methylimidazolium-L-lactate; ERM: eremomycin; EtOAc: ethyl acetate; EtOH: ethanol; Hank's salt: dissolution rich in bicarbonate ions; HCl: hydrochloric acid; HLB: polymeric sorbent for retention of hydrophilic and hydrophobic compounds; HP-β-CD: (2-hydroxypropyl)-β-CD; HP-γ-CD: (2-hydroxypropyl)-γ-CD; HPLC: high pressure liquid chromatography; HS-β/γ-CD: β/γ-CD with high degree of sulfated substitution; i.d.: internal diameter; i-PrOH: isopropranol; L,L-AV: N-undecanoyl-L,L-alanyl-valinate; LIP: laser induced phosphorescence; LLE: liquid-liquid extraction; LOD: limit of detection; LOQ: limit of quantification; MAX: mixed-mode anion-exchange sorbent; MCX: mixed-mode cation-exchange sorbent; MD: maltodextrin, MD-DE 4-7: maltodextrin with 4-7 equivalent of dextrose; MDPV: (±)-1-(benzo[d][1,3]dioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one; MeOH: methanol; M-α-CD: methyl-α-CD; M-β-CD: methyl-β-CD; MOPS: 3-(N-morpholino)propanesulfonic acid; MS: mass spectrometry; NaOH: sodium hydroxide; NLip: nanoliposome; NS: no separation; OS-γ-CD: octa(6-O-sulfo)-γ-CD; PDDAC: poly(diallyldimethylammonium chloride); PLE: pressurized liquid extraction; Poly-L-SUCIL: poly-sodium-N-undecenoxy carbonyl-L-isoleucinate; Poly-L,L-SULA: poly-sodium-N-undecenoyl-L,L-leucylalaninate; Poly-L,L-SULV: poly-sodium-N-undecenoyl-L,L-leucylvalinate; RAMEB: random methylated β-CD; Rs: resolution; RS86017: (−)7R,13aS-N-p-chlorobenzyl-2,3-methylenedioxyl-9,10-dimethoxyl-5,8,13,13a-tetrahydro-6H-dibenzo[a,g]quinolizinium chloride; S-α-CD: sulfated α-CD; S-β-CD: sulfated β-CD; SBE-β-CD: sulfobutylether-β-CD; SDC: sodium deoxycholate; SDS: sodium dodecyl sulfate; CNS: central nervous system; SPE: solid-phase extraction; Strata-X-C: strong cation-exchange and reversed-phase polymeric sorbent; Succ-γ-CD: succinyl-γ-CD; t-BuOH: tert-Butanol; TEA: triethanolamine; t.l.: total lenght; TM-β-CD: heptakis 2,3,6-tri-o-methyl-β-CD; Tris: tris(hydroxymethyl)aminomethane; UV: ultraviolet; VC: vancomycin.

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Fig. 1

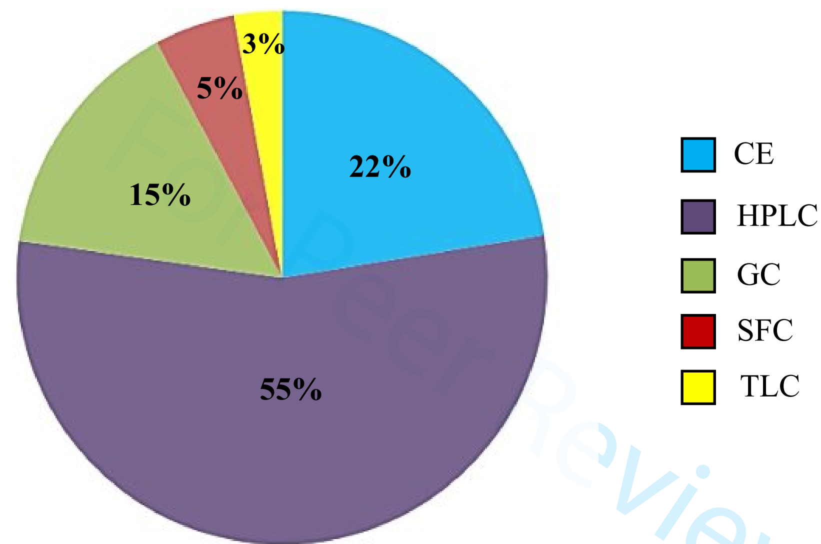

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Fig. 2

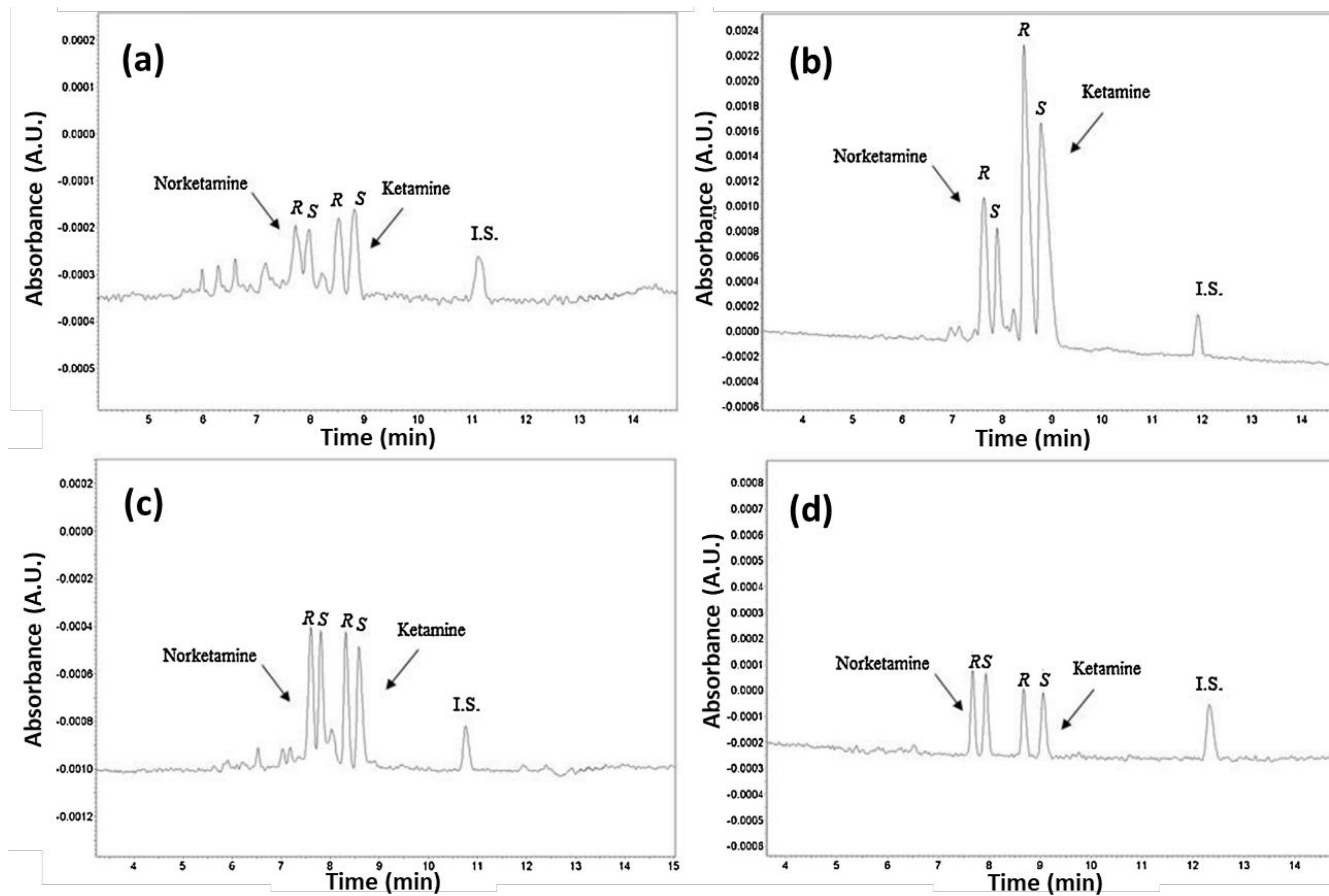

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Fig. 3

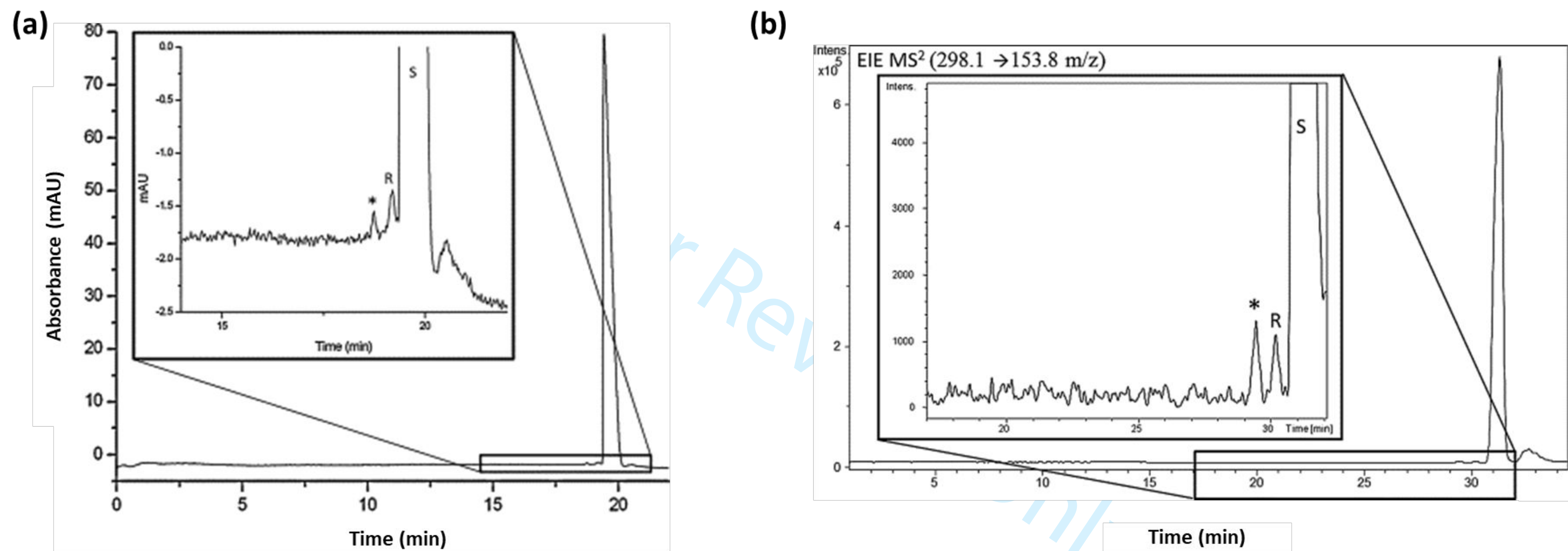

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Fig. 4

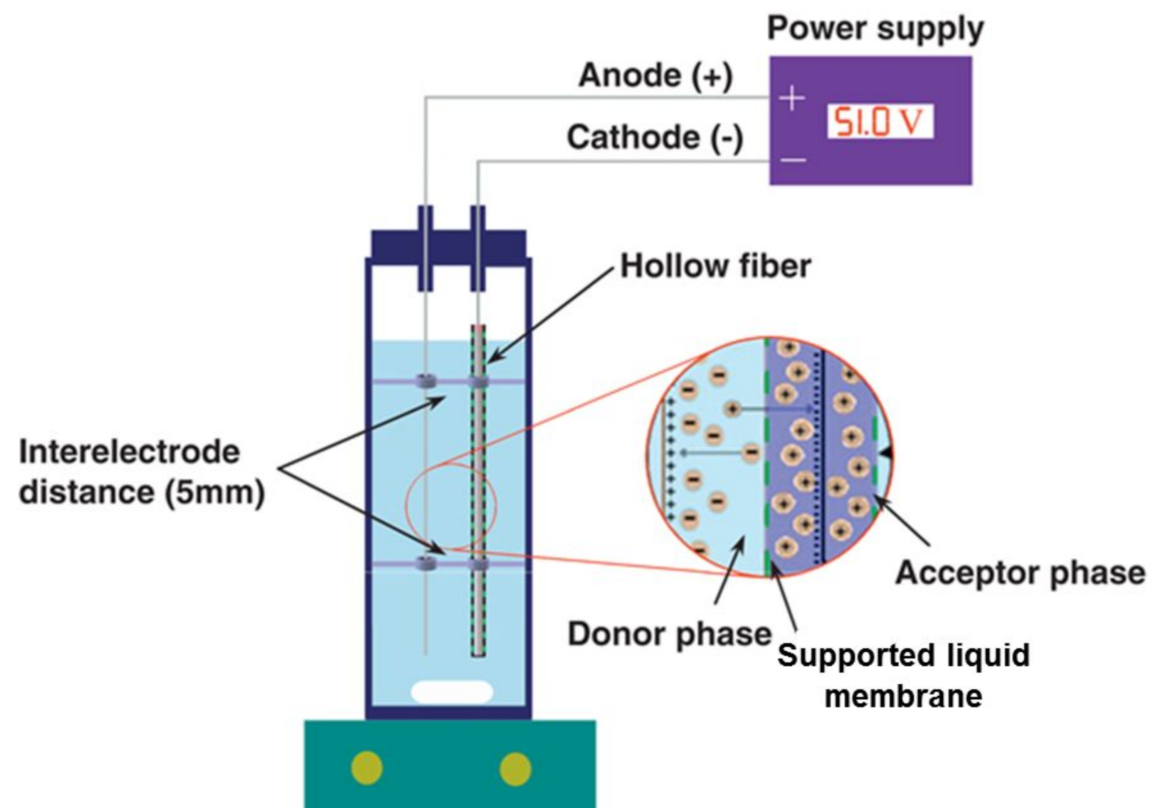

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Fig. 5

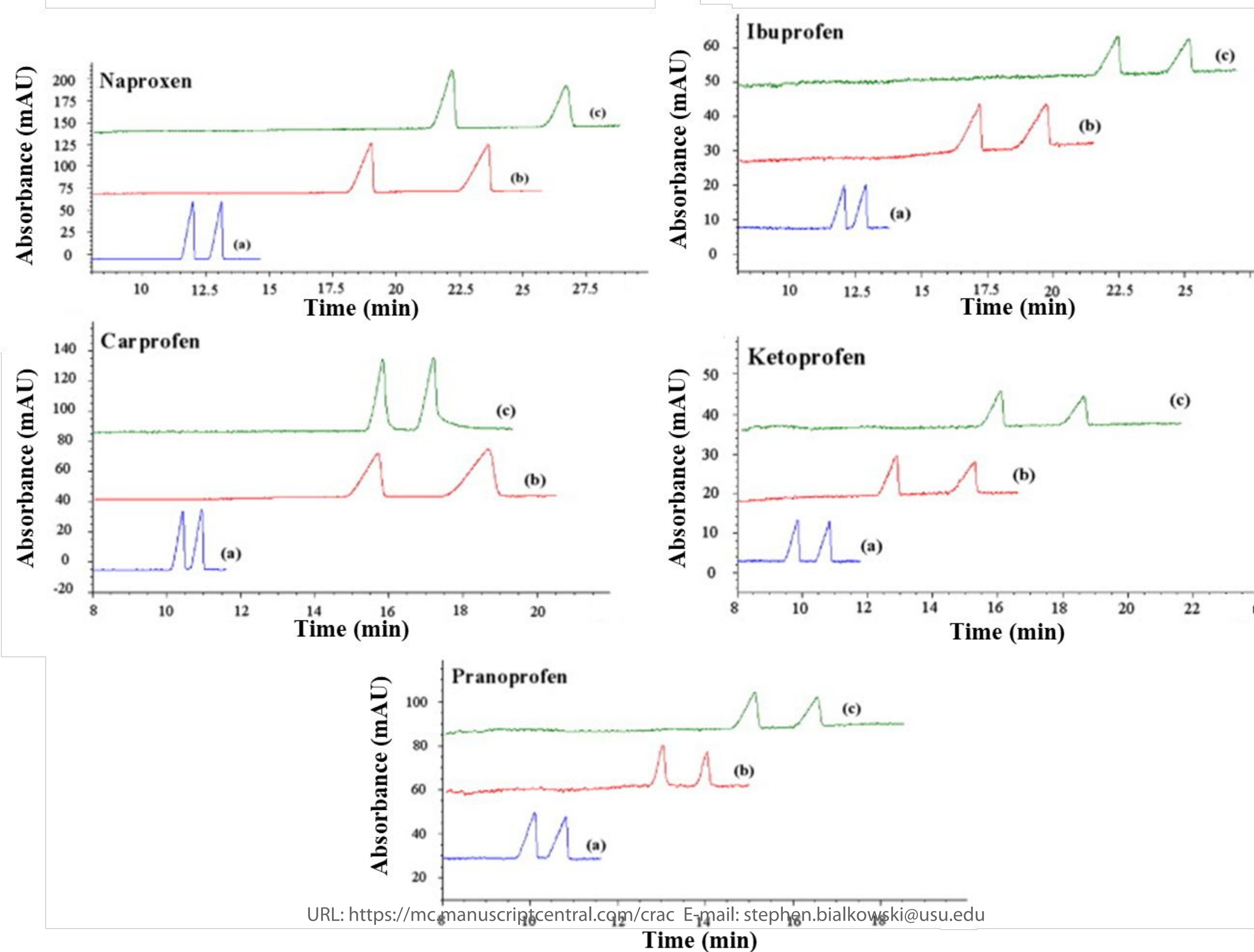

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Fig. 6