Chiral analysis of pollutants and their metabolites by capillary electromigration methods

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Abbreviations:

allyl-TER: 1-allylterguride; **ANDSA:** 7-aminonaphthalene-1,3-disulfonic acid; **ANSA:** 5-aminonaphthalene-1-sulfonic acid; **ANTS:** 8-aminonaphthalene-1,3,6-trisulfonic acid; **BGE:** background electrolyte; **CM- γ -CD:** carboxymethylated- γ -cyclodextrin; **MCPA:** (4-chloro-2-methylphenoxy)-acetic acid; **MCPB:** (4-chloro-2-methylphenoxy)butyric acid; **2,2-CPPA:** 2-(2-chlorophenoxy)-propionic acid; **2,3-CPPA:** 2-(3-chlorophenoxy)propionic acid; **2,4-CPPA:** 2-(4-chlorophenoxy)-propionic acid; **CMBA:** 2-(4-chlorophenyl)-3-methylbutanoic acid; **CA:** chrysanthemic acid; **MEGA:** decanoyl-*N*-methylglucamide; **DCA:** dichlorochrysanthemic acid; **2,4-D:** (2,4-dichlorophenoxy)acetic acid; **2,4-DB:** 4-(2,4-dichlorophenoxy)butiric acid; **2,4-DCPPA:** 2-(2,4-dichlorophenoxy)propionic acid; **DM- β -CD:** 2,6-di-*O*-methyl- β -cyclodextrin; **β -CD-(NH₃)₂²⁺:** 6,6'-dideoxy-6,6'-L-diamino- β -cyclodextrin; **ECD:** electron capture detector; **HS- β -CD:** hepta-6-sulfato- β -cyclodextrin; **β -CD-EA:** heptakis(6-hydroxyethylamino-6-deoxy- β -cyclodextrin; **γ -HCH:** γ -hexachlorocyclohexane; **2,3-DM- α -CD:** hexakis(2,3-di-*O*-methyl)- α -cyclodextrin; **HP- β -CD:** (2-hydroxypropyl)- β -cyclodextrin; **HP- γ -CD:** (2-hydroxypropyl)- γ -cyclodextrin; **β -CD-NH₂:** 6-monodeoxy-6-monoamino- β -cyclodextrin; **OG:** n-octyl- β -D-glucoside; **OM:** n-octyl- β -D-maltopyranoside; **γ -PCCH:** γ -pentachlorocyclohexene; **PM- β -CD:** permethylated- β -cyclodextrin; **PMMA- β -CD:** permethyl monoamino- β -cyclodextrin; **2-PPA:** 2-phenoxypropionic acid; **2-PBA:** 2-phenoxybutanoic acid; **PCBs:** polychlorinated biphenyls; **PAHs:** polycyclic aromatic hydrocarbons; **Poly-D-SUV:** polysodium *N*-undecanoyl-D-valinate; **SDC:** sodium deoxycholate; **SC:** sodium cholate; **STC:** sodium taurocholate; **STDC:** sodium taurodeoxycholate; **S- β -CD:** sulphated- β -cyclodextrin; **Succ- β -CD:** succinylated- β -cyclodextrin; **Succ- γ -CD:** succinylated- γ -cyclodextrin; **SBE- β -CD:** sulfobutyl ether- β -cyclodextrin; **SPE- α -CD:**

sulfopropylether- α -cyclodextrin; **2,4,5-T**: 2,4,5-trichlorophenoxyacetic acid; **3,4,5-TP**:
2-(3,4,5-trichlorophenoxy)propanoic acid; **TM- β -CD**: 2,3,6-trimethyl- β -cyclodextrin

Abstract

Chiral separation of enantiomers is one of the most challenging tasks for any analytical technique including capillary electrophoresis (CE). Since the first report in 1985 showing the great possibilities of CE for the separation of chiral compounds, the amount of publications concerning this topic has quickly increased. Although chiral electromigration methods have mainly been used for enantioseparation of drugs and pharmaceuticals, they have also been applied to analyze chiral pollutants. This article intends to provide an updated overview, including works published till January 2005, on the principal applications of CE to the chiral analysis of pollutants and their metabolites, with especial emphasis on articles published in the last ten years. The main advantages and drawbacks regarding the use of CE for chiral separation of pollutants are addressed including some discussion on the foreseen trends of electromigration procedures applied to chiral analysis of contaminants.

1. Introduction.

Separation of chiral compounds is an interesting and challenging topic of research in many analytical chemistry areas, especially in the biomedical, pharmaceutical and environmental fields where pure enantiomeric forms are widely required. It is already well-known that enantiomers, in spite of their very similar structure, when exposed to an identical biological environment can show very different biological activity. Furthermore, as a general rule, when the toxicological effect of an active substance is directly related to its desired biological activity, the most active isomer is usually the most toxic one [1]. Among environmental contaminants, chemical pollutants (pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phenols, metals, etc.), are considered the most toxic. Some pollutants have shown chirality and, as a result, their different enantiomers may have different toxicity, an important fact to be considered when analyzing these environmental samples. Furthermore, some nonchiral pollutants can give rise to different degradation products that can be chiral in nature showing, therefore, different toxicity.

Traditionally, the techniques most frequently used for chiral separations have been GC, HPLC or SFC. In the last decade, however, capillary electrophoresis (CE) has shown to be a very powerful and effective analytical tool for this task [2]. As compared to HPLC, chiral separations by CE have the advantages of higher separation efficiency, speed of analysis and flexibility allowing the incorporation of various chiral selectors at different concentrations (in HPLC chiral selectors are usually fixed onto the stationary phase and hence the concentrations of the chiral selectors cannot be varied). Since Gassman et al. [3] first reported the great possibilities of CE for the separation of chiral compounds, the

amount of publications concerning this topic has quickly increased mostly in the last decade. In this sense, nowadays, more than half of all papers dedicated to chiral analysis involve CE being the majority related to drug analysis [4,5]. Various CE modes such as capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC) and capillary electrochromatography (CEC) have been used together with a suitable chiral selector for this purpose, being CZE the CE mode most commonly used.

Enantiomers have identical charge to mass ratio and, therefore, they can not be separated by CE without the presence of a suitable chiral selector as, for instance, cyclodextrins (CDs), chiral surfactants, chiral crown ethers, ligand-exchange complexes or linear polysaccharides [6] capable of discriminating between enantiomers. In this sense, separations of enantiomers by CE are carried out with these chiral selectors e.g., bonded to the capillary wall, included into a gel or directly added into the separation electrolyte. The most convenient and easy way to perform chiral separations by CE is the addition of the chiral selector to the supporting electrolyte. The number of chiral selectors available for CE is relatively large being CDs the most frequently used. CDs are cyclic oligomers of α -D-glucose linked through the 1,4 position. The most common CDs (neutral CDs) are α -, β - and γ -CD that consist of 6, 7 and 8 glucopyranose units, respectively. They have a truncated cone shape, with a relatively hydrophobic open cavity and a hydrophilic outside (due to hydroxyl groups in positions 2, 3 and 6 of the glucopyranose ring) that can be entered by appropriate molecules. Chiral discrimination by CDs can be modified by substituting the hydroxyl groups on the ring, changing their asymmetry and, as a result, increasing enantioselectivity. This modification can provide neutral (methyl, hydroxyethyl, hydroxypropyl, acetyl groups), anionic (carboxymethyl, carboxyethyl, sulphobutylether, phosphated, sulphated, succinyl) or cationic CDs

(quaternary ammonium substituted CDs). Besides CDs, other compounds as chiral surfactants have also been used for enantioseparation by CE. These naturally occurring detergents belong to three families: bile salts, digitonins and saponins, being the bile salts the most common. They consist of four saturated fused rings with side chains containing hydroxyl groups and a carboxyl bonded to either taurine or glycine. Among them, particularly deoxycholic derivatives have proven to be highly effective in enantioseparations by CE.

Although chiral separations by CE have been recently reviewed in food analysis [7], drugs [4,5], peptides [8], in non-aqueous media [9,10] etc., few articles have reviewed the chiral separation of pollutants [11-13]. The most recent of these articles [13] reports the chiral resolution of some environmental pollutants, mainly pesticides, without compiling the published articles concerning this topic (in that paper, special emphasis was put on the influence of different parameters as type of chiral selector, pH of BGE, applied voltage, temperature, etc. on the chiral resolution). In view of the importance of the separation of chiral contaminants, the goal of this work is, therefore, to review the chiral separation of pollutants and metabolites by capillary electromigration methods, with especial emphasis on those articles published in the last ten years.

2. Chiral analysis of pesticides.

Nowadays, pesticides constitute a class of important pollutants that are widespread over the environment. Fast and feasible analytical procedures are required to monitor their presence in different types of matrixes (waters, soils, fruits, biological fluids, etc.). In this sense, capillary electrophoresis is increasingly gaining importance in pesticide

determination, as can be seen in recent (non-chiral) review articles [14,15]. In this sense, about 25% of agrochemicals that exist contain chiral centers and are produced and used as racemic mixtures [16]. In some cases, only one of the isomers is active as pesticide, while the other may have less activity or even toxic effects against non-target organisms; surprisingly, this fact is often not taken into account into state regulations [1]. As an example, phenoxy acid herbicides (*R*) isomers show much higher herbicide activity and different metabolism than their (*S*) isomers [17]; in fact, only (+)-isomers of dichlorprop, mecoprop and diclofop-methyl are active as herbicides [18]. Also, several pyrethroid insecticides have 2-8 stereoisomers (e.g., cypermethrin [1]) but only one of them shows biological activity. A clear example can also be found in the imidazolinones, whose activity as herbicide resides in the (*R*)-enantiomer being about eight times more active than the (*S*)-enantiomer [19]. Apart of the different activity linked to the different enantiomers, some nonchiral pesticides can degrade in the environment into toxic and/or chiral products. A first example is γ -hexachlorocyclohexane (γ -HCH), an achiral pollutant that degrades into γ -pentachlorocyclohexene (γ -PCCH) enantiomers, which are toxic. A second example is atrazine, which is transformed into its chiral metabolites 2-chloro-4-ethylamino-6-(1-hydroxy-2-methylethyl-2-amino)-1,3,4,5-triazines.

Another fact that has to be taken into account is that when racemic mixtures are used as pesticides, the enantiomers are often degraded at different rates [17,20]. Therefore, it can be concluded that the chiral separation of pesticides is a very important challenge that will allow optimizing enantioselective production processes, assessing the enantiopurity of formulations and monitoring their presence in the environment.

Table 1 shows examples of the application of CE to the enantioseparation of pesticides in the last ten years. As it can be seen, phenoxy acid herbicides constitute the main group of pesticides studied (see in Figure 1 some structures of these phenoxy acids). One important detail that should be realized from Table 1 is that only few of these works deal with CE enantioseparation of pollutants in real matrixes (e.g., wine [52], water [24,33,43] or soil [22,38]), being most of the studies carried out with pure standards. A possible explanation can be the relatively low sensitivity of CE; this limitation makes the direct application of CE to real life samples difficult since pesticides are usually in very low concentrations there.

Recently, Kodama et al. [52] have analyzed vinclozolin in wine, a dicarboximide fungicide widely used in Europe to protect fruits, vegetables, ornamentals and turf grasses. In order to achieve the chiral separation of vinclozolin enantiomers by MEKC-UV, several CDs (DM- β -CD, TM- β -CD, HP- β -CD, HP- γ -CD and γ -CD) were tested in the separation buffer that contained SDS, phosphate and borate. The best separation was achieved in 20 mM phosphate-5 mM borate buffer at pH 8.5 containing 50 mM γ -CD and 100 mM SDS. Vinclozolin was extracted from wine samples using PS-2 SPE cartridges before HPLC purification and detected in wine at sub-ppb level. The peak area ratio of (+) and (-) vinclozolins (2:3) detected in the samples suggested that the degradation rates during the wine-making process were different.

Wu et al. [21] have proposed an interesting approach for the chiral separation of triazole-type fungicides. Most triazole fungicides have at least one chiral center that is expected to play an important role in the bioactivity of these fungicides. In their work, 12 of the 14 triazoles could be separated using a phosphate buffer with 2% of sulphated-

β -CD (w/v) as charged chiral selector at pH 3.0 (Figure 2 shows an electropherogram of this separation). The other two triazoles could be enantio-separated by adding urea to the previous separation buffer. Another work of the same group [22] reported the chiral separation of triadimefon and triadimenol enantiomers (triadimenol has four stereoisomers, with the 1*S*, 2*R* isomer giving the highest antifungal activity) using sulphated- β -CD added into the phosphate separation buffer. Although they tested several CDs, only sulphated- β -CD allowed the enantiomeric separation. The optimum BGE consisted of 2% sulphated- β -CD in a phosphate buffer at pH 3.0. Once the electrophoretic separation was optimized, they studied the stereoselectivity of the biotransformation or bioactivation of triadimefon into triadimenol by the joined action of microorganisms in the soil. It has been reported [53], that the antifungal activity of triadimefon is large, due to its transformation in the environment (i.e. soil, water, plant bodies) into triadimenol by the creation of an additional chiral center. In that work [22], a soil sample spiked with both pesticides was incubated up to 20 days. During that time, an exponential decrease in the concentration of both enantiomers of triadimefon and also an exponential increase in triadimenol concentration were observed (exponential curves are typical of these types of transformation processes). Interestingly, the diastereomeric pair of triadimenol, which contains the most effective antifungal component (1*S*, 2*R*), was the predominant transformation product. In their work it was also pointed out, that the incubation of other types of soils could provide different diastereoselectivity.

Capillary electrochromatography (CEC), a hybrid technique combining the high separation efficiency of CE with the separation mechanism of HPLC, has also been used for pesticide enantioseparation [30,48]. Zhang and El Rassi have used CEC-UV

for the chiral separation of organochlorine pesticides [48] and phenoxy acid herbicides [30]. In the first of these works [48], they used a diol-silica stationary phase dynamically coated with the chiral selector HP- β -CD. With this procedure, the achiral diol-silica stationary phase is transformed into a chiral one. The group of chiral organochlorine pesticides studied included *o,p*-DDT, *p,p'*-DDT, diclofop methyl and silvex isooctyl ester. Several parameters as ionic strength of the mobile phase, HP- β -CD concentration, pH and the influence of different organic modifiers were evaluated. The enantioresolution of all pesticides was successfully achieved using a mobile phase consisting of 1 mM sodium phosphate, 2.5 mM sodium borate and 2 mM HP- β -CD, and composed of 30% v/v methanol, 50% v/v acetonitrile and 20% v/v aqueous buffer at pH 5.0. In the second work [30], seven enantiomeric phenoxy acids (i.e. silvex, dichlorprop, mecoprop, 2,4-CPPA, 2,3-CPPA, 2,2-CPPA, 2-PPA) were enantioseparated using a HP- β -CD-sulfonated silica stationary phase. This stationary phase consisted of silica with a covalently attached hydrophilic sulfonated sublayer with a chiral top layer of HP- β -CD. The sulfonated sublayer provides a strong EOF, essential for the CEC separation of these compounds, while the HP- β -CD layer ensures the enantiomeric separation. In this case, after studying the use of organic modifiers, ionic strength and pH, the seven racemic phenoxy acids (except 2-PPA) were successfully enantioseparated using a mobile phase consisting of 2.0 mM sodium phosphate, containing 60% v/v acetonitrile and 40% v/v aqueous phosphate buffer at pH 6.0 (see Figure 3).

Polcaro et al. [33], separated mecoprop, fenoprop, fluazifop and haloxyfop enantiomers using vancomycin and γ -CD as chiral selectors. These pesticides were determined in extracts from ground and river water obtained by solid phase extraction (SPE) on styrene-divinylbenzene cartridges (SBD-1). The partial filling technique was used in

order to leave the detector path free of chiral agent, which strongly absorbs at the detection wavelength used (205 nm). The method allowed the determination of these pesticides enantiomers in the range 0.1-0.13 ppb in ground water and 0.4-0.54 ppb in river water.

Although laser induced fluorescence (LIF) was already used together with CE in 1985 [3], it was applied to pesticide analysis for the first time in 1995 by Jung and Brumley [54]. Pesticides and their metabolites do not frequently show fluorescence and, therefore, a pre-column or post-column derivatization step with a suitable probe is necessary, introducing an additional step in the analytical procedure that has to be carefully optimized. That is the main reason why LIF detection has not been as widely used as UV, although some CE-LIF works have demonstrated the good possibilities of this procedure for the chiral separation of pesticides [29,42]. Thus, Karcher and El Rassi [29] evaluated the use of ANSA, ANDSA and ANTS derivatizing agents in the precolumn derivatization of chiral phenoxy acids (dichlorprop, 2,4-CPPA, 2-PPA) and chiral transformation products from pyrethroid insecticides (chrysanthemic acid, CA, dichlorochrysanthemic acid, DCA, 2-(4-chlorophenyl)-3-methylbutanoic acid, CMBA). Enantiomeric CE separation was carried out using two alkylglycoside chiral surfactants in the separation buffer, namely, n-octyl- β -D-glucoside (OG) and n-octyl- β -D-maltopyranoside (OM). Among the different derivatizing agents, ANDSA-derivatives were the best enantioresolved. A change in the enantioseparation was observed when either the ionic strength of the running buffer (sodium phosphate) and the concentration and type of chiral surfactant were changed. Previous works of the same group have also used chiral alkylglycoside surfactants for the separation of derivatized pesticides or their degradation products [55,56] and underivatized [39,57]. Another interesting work

related to CE-LIF of pesticides enantiomers was developed by the same group [42]. In that work [42], nine phenoxy acid herbicides (seven of them were chiral) fluorescently labeled with ANDSA were successfully separated by CE-LIF and MEKC-LIF.

Chiral analysis by CE-MS has also been proposed using CDs as chiral selectors [58,59], however, the use of CDs as well as other non-volatile additives can contaminate the ion source, reducing considerably the MS signal intensity and, as a result, decreasing the sensitivity. Some strategies have been proposed to avoid these problems, being the partial filling technique the most frequently applied. Probably due to the mentioned limitations, CE-MS analysis of chiral pesticides has hardly been studied [35]. Otsuka et al. [35] proposed a CE-ESI-MS method for the separation of dichlorprop, fenoprop and mecoprop enantiomers using a buffer consisting of 20 mM TM- β -CD in 50 mM ammonium acetate at pH 4.6. The sheath-liquid was composed of ethanol water 50:50 (v/v) with 1% (v/v) of formic acid. Authors suggested that further studies on the use of non-aqueous buffers containing CDs or other chiral selectors should be done to suppress or decrease the background noise, to reduce the contamination of the ion source, and to increase in that way the detection sensitivity.

The use of stacking techniques has made possible to improve, in some cases considerably, the sensitivity in CE [60,61]. These stacking techniques have also been used in the chiral separation of triadimenol [23] and phenoxy acid herbicides [34]. Otsuka et al. [23] have separated triadimenol enantiomers using two methods: a combination of sweeping-MEKC-UV and also stacking with a reverse migrating pseudostationary phase (SRMP)-CZE-UV procedure. For the MEKC separation, the buffer consisted of 30 mM HP- γ -CD, 50 mM SDS in 50 mM phosphate buffer at pH 2.2

while for CZE it was 20 mM HS- β -CD in 20 mM phosphate buffer at pH 2.2. In both preconcentration strategies, around 10-fold increase in detection sensitivity was achieved. Quirino et al. [34] have also applied stacking and sweeping techniques to the CZE-UV separation of steroids, phenols and phenoxy acid herbicides (fenoprop, mecoprop and dichlorprop). The three pesticides could be enantioseparated using a buffer consisting of 20 mM HS- β -CD in 15 mM phosphoric acid at pH 1.9. The use of SRMP provided the highest sensitivity improvements, between 90 and 158 fold.

3. Chiral analysis of polychlorinated biphenyls (PCBs).

Polychlorinated biphenyls are considered as priority pollutants by the EPA (USA Environmental Protection Agency). They were widely used during 1930s and 1940s, and produced till 1970s as complex mixtures of congeners (about 60-90) [62]. Their chemical inertness, thermal stability and high solubility in oils and fats make them highly environmentally persistent and bio-accumulable. Besides, they are carcinogenic, but also capable of causing other non-cancer short-term illnesses. In these compounds, 1 to 10 chlorine atoms are binded to a biphenyl molecule. They have 209 congeners, and among them 78 have axial chirality but only 19 of them are stable towards racemization at room temperature (those that have three or four chlorine atoms in the ortho position). These 19 compounds, due to the existence of axial chirality, are called atropisomers (they are given in Table 2). Most of these compounds are introduced in the environment as racemates, but atropisomers have shown different toxicities and metabolic pathways [64]. Although, GC (with ECD or MS detection) has been the most frequently used technique to analyze PCBs congeners and enantiomers, CE has also proven to be effective in this field. Recent applications of CE to the enantioseparation of PCBs can

be seen in Table 3. Chiral separation of atropisomeric PCBs by MEKC was first reported by Marina et al. [72,73] although the technique had previously been used to the separation of PCBs congeners (mixtures of trichlorobiphenyl isomers) by Terabe et al. [74]. Marina et al. separated the enantiomers of twelve PCBs (45, 84, 88, 91, 95, 132, 136, 139, 149, 171, 183 and 196) using γ -cyclodextrin [73] and mixtures of β - and γ -cyclodextrins [72] in a buffer containing urea, 2-(N-cyclohexylamino)ethanesulphonic acid (CHES) and sodium dodecyl sulphate (SDS). The use of CHES increased the solubility of these lipophilic compounds in the aqueous media, while urea increased the solubility of both PCBs and CDs.

Lin et al. [70] investigated the enantiomeric separation of a mixture of seven atropisomeric PCBs (45, 84, 88, 91, 95, 132 and 136) using four CD derivatives ((2-hydroxypropyl)- γ -CD, HP- γ -CD, (2-hydroxypropyl)- β -CD, HP- β -CD, 2,6-di-*O*-methyl- β -CD, DM- β -CD, and 2,3,6-trimethyl- β -CD, TM- β -CD) in a buffer consisting of 4 M urea, 100 mM sodium tetraborate, 50 mM sodium phosphate at pH 8.00 and 80 mM SDS. Also three organic modifiers (methanol, acetonitrile and 2-propanol) were added to the separation buffer. All atropisomers could be resolved with HP- γ -CD (60 mM) and 20% (v/v) of 2-propanol in the separation electrolyte. The use of organic solvents in MEKC has provided good results for chiral separations, since these solvents may change the binding constants between solutes and CDs.

García-Ruiz et al. [68] have studied the individual separation of the 19 PCBs atropisomers by CZE using running buffers containing MES, urea, CM- γ -CD as pseudostationary phase mixed with β -CD or PM- β -CD. The use of anionic cyclodextrins (CM- β -CD or CM- γ -CD) in the MES-urea buffer did not allow the

separation of any of the pesticides. However, when CM- γ -CD was used together with β -CD up to 12 chiral PCBs were separated (45, 88, 91, 95, 131, 132, 136, 139, 144, 149, 171 and 176) while with PM- β -CD only eight could be enantioresolved (45, 88, 91, 131, 136, 144 and 197). The optimum buffer composition was 20 mM CM- γ -CD, 10 mM β -CD or 20 mM PM- β -CD, 2 M urea, in 50 mM MES at pH 6.5. In that work [68], PCB 197 was for the first time separated into its two enantiomers by CE. The CE separation of mixtures of the enantioresolved PCBs was also tested using the same buffers. In this sense, only mixtures a) 45, 88, 91, 95 and 176, b) 45, 95, 131 and 136, c) 132, 139, 149 and 171 could be separated.

In a recent work by García-Ruiz et al. [67], the biodegradation of several chiral PCBs (45, 88, 91, 95, 136, 144, 149 and 176) by *Jonibacter sp.* a naturally occurring bacterium isolated from a contaminated soil, was studied. For this purpose, CZE was used together with one of the dual cyclodextrin buffers optimized in a previous work [68] that consists of 50 mM MES at pH 6.5 containing 2 M urea, 20 mM CM- γ -CD and 10 mM β -CD. The study of the biodegradation process was carried out with mixtures of two congeners at a time (149-95, 88-136, 176-144 and 91-45). A high degree of degradation (61 to 94 %), of all selected PCBs after 262 hours of incubation was observed. Among the selected congeners, PCBs 45 and 88, the least chlorinated ones, showed the highest degradation rate.

García-Ruiz et al. [65] have also compared the use of different CDs for the chiral separation of the 19 atropisomeric PCBs. Anionic CDs, β -CD-phosphated, β -CD-sulphated, succinylated- γ -CD (Succ- γ -CD) and succinylated- β -CD (Succ- β -CD), and cationic CD, 6-monodeoxy-6-monoamino- β -CD (β -CD-NH₂) were tested. The best

results were obtained with the cationic CD at a concentration of 30 mM in a 10 mM phosphate buffer at pH 2.0 containing 2M urea, but only individual chiral recognition of 11 PCBs could be achieved. The presence of urea was found crucial to achieve the chiral resolution. Also, the addition of acetonitrile as organic modifier was studied, which improved the resolution of some of the previous 11 PCBs.

Chiral surfactants, more precisely, bile salts, have also been used as chiral selectors, for the separation of PCBs enantiomers. Crego et al. [69] have studied the use of the bile salts: sodium cholate, SC, sodium deoxycholate, SDC, sodium taurocholate, STC, and sodium taurodeoxycholate, STDC, alone or mixed with SDS for the chiral separation of these compounds. By using STDC as surfactant in a buffer consisting of 2 M urea in 0.05 M 2-(N-cyclohexylamino)-ethanesulphonic acid (CHES) at pH 9.0, only three chiral PCBs (84, 95 and 176) could be separated; however, the simultaneous separation of these three PCBs was not possible in a single run. The addition of SDS to the CHES-urea buffer containing any of the four bile salts tested, allowed the separation of the same three PCBs. The use of these bile salts in this CHES-urea buffer with SDS and γ -CD, was also studied. In this case, the combined use of these two chiral selectors (γ -CD, as chiral additive, and SC, as chiral surfactant) together with SDS, at appropriate concentrations, allowed the separation of a higher number of PCBs enantiomers (up to 15). In this sense, the separation of enantiomers 131, 135, 144 and 175 was achieved for the first time by CE. This buffer allowed the separation of a group of 8 PCBs in a single run (Figure 4). In a previous work [71], a buffer containing sodium cholate, 2 M urea and CHES at pH 10 allowed the individual separation of three chiral PCBs (i.e. 84, 95 and 176), showing for the first time the chiral separation of the PCB 176 by CE.

4. Chiral analysis of other pollutants.

Despite their beneficial therapeutic effects, some drugs may have negative effects on the natural environment. In this sense, several studies have shown the widespread occurrence of pharmaceutical compounds in the environment [75], establishing these compounds as a new class of priority pollutants. As it has been previously indicated, nowadays, most of the papers dedicated to chiral CE separations are related to drug analysis [4,5]. Although the discussion on chiral analysis of drugs by CE is out of the scope of this review, it is necessary to point out that the chiral determination of drugs in environmental samples has not been fully explored. Thus, pharmaceutical and personal care products have received increasing attention as environmental pollutants in the last years. Fragrances, for example, are mixtures of terpenes, esters and musks [76]. These last ones are ubiquitous, persistent, bioaccumulative pollutants and some of them can generate toxicologically active compounds. Among them, polycyclic musks (PCMs) are of special relevance. The most frequent of these compounds are HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran) and AHTN (1-[5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl]-ethanone). Since they are consumed in high volumes, show low degradability and constitute a potential hazard to human health, they constitute an important class of organic residues in the aquatic environment, especially in urbanized areas. These widespread compounds [77], delivered in wastewaters and sewage, can have one (AHTN, AHDI: 1-[2,3-dihydro-1,1,2,3,3,6-hexamethyl-1H-inden-5-yl]-ethanone, DPMI: 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4H-inden-4-one) or two (HHCB, HHCB-lactone: 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran-1-one; ATII: 1-[2,3-dihydro-1,1,2,6-tetramethyl-3-(1-methyl-ethyl)-1H-inden-5-yl]ethanone) chiral centers. In fact,

they are used as racemic mixtures in commercial products [78]. To our knowledge, concerning chiral and not chiral separations, this group of compounds has not been separated by CE till now. Furthermore, they have not been fully identified nor their environmental fate studied, in fact, unlike PAHs, PCBs or pesticides, musks lack of any kind of regulation.

Another class of persistent and widespread pollutants are polybrominated biphenyls (PBBs) that have extensively been applied as flame-retardants in textiles, electronic equipment and plastics. Likewise their chlorinated analogues (PCBs) they exist in a theoretical variety of 209 congeners. [79] and they can show atropisomerism too. However, due to their more bulky bromine substituents, it can be assumed that more than 19 atropisomeric forms (like the ones for PCBs) exist at ambient temperature. Up to now, few atropisomers have been identified [79,80] and separated by GC or HPLC. To our knowledge, no method has been developed till now for the chiral separation of PBBs atropisomers by CE, constituting other research line to be explored.

5. Concluding remarks and future trends.

CE is increasingly being used in separation science due to its inherent advantages over GC and HPLC. In this sense, CE provides a high resolving power, rapid method development, easy sample preparation and low operation expense compared with chromatographic techniques. However, these chromatographic techniques also have their own advantages, for instance, it is well known that GC is a robust technique widely employed for the analysis of volatile compounds. Although CE has not been fully exploited in many fields of research, and it is far from being considered a routine

technique in many analytical laboratories, concerning chiral analysis, CE is an impressive tool that is increasingly being used specially in pharmaceutical and environmental fields.

Although much work has been carried out in the separation of chiral pollutants by CE, stills more effort needs to be done in order to be able to analyze these compounds and their metabolites in real samples. For this purpose, the use of on-line and off-line preconcentration strategies (sample stacking, sweeping, SPME, SBSE, SPE...) can be a good strategy [81,82], allowing the determination of these compounds at trace levels in environmental samples.

Another aspect to be addressed is the development of new chiral selectors able to extend the application of chiral CE analysis [83,84]. In this sense, the attainment of new chiral selectors compatible with MS detection can open the application of chiral CE-MS analysis, since this technique has hardly been used for the chiral analysis of pollutants due to the inhibition of the MS signal provided by the actual non-volatile chiral selectors (mainly CDs).

The continue need for sensitive and portable tools for field analysis could be a good opportunity for CE and more probably for microchip-CE. The great potential of microchip separations for pesticide analysis has also been shown for the separation of paraquat and diquat [85]. The application of this powerful tool for the analysis of chiral pollutants is also a very attractive topic that has to be developed in the non distant future.

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Figure captions

Figure 1. Structures of the most frequently separated chiral phenoxy acid herbicides.

Figure 2. Chiral separation of twelve triazole fungicides by CE with S- β -CD. Running electrolyte: phosphate buffer with 2% S- β -CD, pH 3.0. High voltage, -18 kV. Capillary 60 cm (53 cm effective length) x 50 μ m i.d. UV detection, 220 nm. Peak identification: 1, cyproconazole; 2, penconazole; 3, hexaconazole; 4, tetraconazole; 5, triadimenol; 6, diniconazole (9:1 *R:S* mixture); 7, paclobutrazol (dominated by 2*RS*,3*RS* isomers); 8, tebuconazole; 9, flutriafol; 10, propiconazole; 11, triadimefon and 12, myclobutanil. Reprinted from [21], with permission.

Figure 3. Electrochromatogram of the enantiomers of phenoxy acid herbicides. Capillary column, 20.5 cm/27 cm x 100 μ m i.d., whole packed with 5 μ m CDSS stationary phase; mobile phase, hydro-organic eluent containing 2 mM sodium phosphate and composed of 60% v/v acetonitrile and 40% v/v aqueous phosphate buffer at pH 6.0; running voltage, 15 kV; electrokinetic injection at 1 kV for 1sec. Solutes: 1) silvex; 2) dichlorprop; 3) mecoprop; 4) 2,4-CPPA; 5) 2,3-CPPA; 6) 2,2-CPPA; 7) 2-PPA. Reprinted from [30], with permission.

Figure 4. Electropherogram corresponding to the enantiomeric separation of (a) PCBs 88, 45, 144, 131, 176, 171 and 175; and (b) PCBs 88, 45, 139, 136, 84, 95, 183 and 196. Separation buffer: 0.05 M SDS, 0.05 M SC, 0.05 M γ -CD, 0.1 M CHES at pH 9 and 2 M urea. Reprinted from [69], with permission.

Silvex/fenoprop

Mecoprop

Dichlorprop

2-PPA

2,4-CPPA

2,2-CPPA

2,3-CPPA

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Table 1. Chiral CE analysis of different pesticides.

<i>Pesticide</i>	<i>Chiral Selector</i>	<i>Matrix</i>	<i>CE mode</i>	<i>Buffer</i>	<i>Comments</i>	<i>Reference</i>
Triazoles						
Bitertanol, difenoconazole, cyproconazole, penconazole, hexaconazole, tetraconazole, triadimenol, diniconazole, paclobutrazol, tebuconazole, flutriafol, propiconazole, triadimefon, myclobutanil	Sulfated- β -CD	Spiked buffer	CZE or MEKC-UV (220 nm)	1 M phosphate, 2 M urea (pH 3), 2% CD	-	[21]
Triadimenol, triadimefon	Sulfated- β -CD	Soil	CZE-UV (220 nm)	Phosphate buffer with 2% sulphated- β -CD, pH 3.0	Study of the biotransformation by soil microorganisms	[22]
Triadimenol	HP- γ -CD (MECK) HS- β -CD (CZE)	-	MEKC-UV and CZE-UV (200 nm)	MEKC: 30 mM HP- γ -CD-50 mM SDS in 50 mM phosphate buffer (pH 2.2) CZE: 20 mM HS- β -CD in 20 mM phosphate buffer (pH 2.2)	Sweeping (MECK), stacking (CZE)	[23]
Propiconazole, bitertanol, triadimenol	Several CDs, SC	Water	MEKC-UV (214 nm)	Several buffers containing NaH ₂ PO ₄ , SDS or SC, CDs	SPE preconcentration (Oasis HLB)	[24]
Cinnamic acid Dimethomorph,	Several CDs, SC	Water	MEKC-UV (214 nm)	Several buffers containing NaH ₂ PO ₄ , SDS or SC, CDs	SPE preconcentration (Oasis HLB)	[24]
Phenoxy acids						
2-PPA, 2,3-CPPA, 2,4-CPPA, dichlorprop, mecoprop, silvex	HP- β -CD	-	CZE-UV (220 nm)	50 mM ammonium formate pH 5, 15 mM HP- γ -CD	Apparent binding constants calculated (20 and 40 °C)	[25]
MCPB, fenoprop, mecoprop, dichlorprop, MCPA, 2,4-D,	PMMA- β -CD	-	CZE-UV (202 nm)	40 mM boric, acetic and phosphoric acid buffers in a ratio of 1:2:2 pH	MCPB, MCPA, 2,4-D achiral.	[26]

Fenoprop, dichlorprop, mecoprop, fluazifop, fenoxaprop, flamprop, 2-PPA, 2-PBA, 2,2-CPPA, 2,3-CPPA, 2,4-CPPA, 3,4,5-TP	(β -CD-(NH ₃) ₂) ²⁺	-	CZE-UV (200 nm)	6.5, 10 mM PMMA- β -CD 1mM β -CD-(NH ₃) ₂ ²⁺ (AB)*, 100mM acetate buffer pH 5.0 (coated Neural [®] capillary)	Flamprop not resolved	[27]
Mecoprop, dichlorprop	Ethylcarbonate- β -CD	-	CZE-UV (214 nm)	45 mM Na ₂ HPO ₄ pH 5 (citric acid), 8.5-9.2 mM CD	Experimental design used to optimize	[28]
Dichlorprop, 4-CPPA, 2-PPA	OG, OM	-	CE-LIF (He-Cd: 325 nm; Filter: 380, 420 nm)	Several buffer containing sodium phosphate, OM or OG	Derivatization with ANSA, ANDSA, ANTS	[29]
Silvex, dichlorprop, mecoprop, 2,4-CPPA, 2,3-CPPA, 2,2-CPPA, 2-PPA	HP- β -CD (bounded to the stationary phase)	-	CEC-UV (254 nm)	2.0 mM sodium phosphate, 60% v/v ACN, 40% v/v aqueous phosphate buffer, pH 6.0	Sulfonated silica stationary phase having surface bound HP- β -CD. 2-PPA not resolved. Chiral dansyl amino acids also separated.	[30]
Mecoprop, dichlorprop, 2,4-D, 2,4-CPPA, Silvex, 2,4,5-T, 2,3-CPPA, 2,2-CPPA, 2-PPA	2,3-DM- α -CD, SPE- α -CD	-	CZE-UV (230 nm)	0.1 M borate-0.05 M phosphate (pH 9.0), 5 mM 2,3-DM- α -CD, 2.5 mM SPE- α -CD	2,4-D, 2,4,5-T, not chiral.	[31]
Mecoprop, dichlorprop, 2,4-D, 2,4-CPPA, Silvex, 2,4,5-T, 2,3-CPPA, 2,2-CPPA, 2-PPA	2,3-DM- α -CD, α -CD	-	CZE-UV (230 nm)	0.1 M borate-0.05 M phosphate (pH 9.0), 5 mM 2,3-DM- α -CD, 2.5 mM α -CD	2,4-D, 2,4,5-T, not chiral	[32]
Mecoprop, fenoprop, fluazifop, haloxyfop	Vancomycin, γ -CD	Ground and river waters	CZE-UV (205 nm)	75 mM Britton-Robinson buffer (pH 5), Partial filling: with 8 mM vancomycin in 10 mM γ -CD containing running buffer	SPE preconcentration (SBD-1 cartridges).	[33]
Fenoprop, mecoprop, dichlorprop	HS- β -CD	-	CZE-UV (225 nm)	20 mM HS- β -CD in 15 mM	Evaluation of stacking	[34]

			nm)		phosphoric acid (pH 1.9)	procedures.	
Dichlorprop, fenoprop, mecoprop	TM- β -CD	-	CZE-ESI-MS		20 mM TM- β -CD, 50 mM NH ₄ OAc (pH 4.6)	-	[35]
Haloxifop, fluazifop, diclofop, fenoxaprop, flamprop, mecoprop, fenoprop, dichlorprop	Hepta-tyr glycopeptide antibiotic*	-	CZE-UV (205 nm)		50 mM Britton-Robinson buffer pH 5: ACN (80:20, v/v), 1mg/mL Hepta-tyr	Chiral selector evaluation. Partial filling.	[36]
Fluazifop, haloxifop, fenoxaprop, flamprop	allyl-TER	-	CZE-UV (230 nm)		100 mM β -alanine-acetate, 50% MeOH (pH 5.3) supported with 25 mM allyl-TER	Flamprop partially resolved	[37]
Mecoprop, fenoprop, dichlorprop flamprop, haloxifop, fluazifop, diclofop, fenoxaprop	Vancomycin	Soil (haloxifop)	CZE-UV (205 nm)		75 mM Britton-Robinson buffer pH 5, 6 mM vancomycin	Partial-filling	[38]
Silvex, dichlorprop, mecoprop, 2,4-CPPA, 2,3-CPPA, 2,2-CPPA, 2-PPA	OG, NG	-	MEKC-UV (230 nm)		250 mM sodium phosphate, pH 6.5, 50 mM NG or 70 mM OG	Silvex not enantioresolved	[39]
2,2-CPPA, 2,3-CPPA, 2,4-CPPA, 2,4-DCPPA, 2,4,5-TP	β -CD-EA	-	CZE-UV (214 nm)		50 mM Na ₂ HPO ₄ pH 6, 20 mM β -CD-EA	Chiral selector evaluation	[40]
2,2-CPPA, 2,3-CPPA, 2-PPA	Vancomycin, ristocetin A, teicoplanin	-	CZE-UV (254 nm)		Several buffers containing borate and chiral selector	Chiral selector evaluation	[41]
2,4-D, 2,4,5-T, 2-PPA, mecoprop, 2,2-CPPA, 2,3-CPPA, 2,4-CPPA, dichlorprop, silvex	Several CDs	-	CZE and MEKC LIF (He-Cd 325 nm; emission: 420 nm)		Several buffers containing different CDs, sodium phosphate, borate, MEGA (MEKC)	Derivatization with ANDSA. 2,4-D, 2,4,5-T achiral	[42]
2,4-DB, MCPB, 2,4-DP, 2,4-D, MCPA, 2,4,5-T, fenoprop	α -CD, β -CD	Water	CZE-UV (200 nm)		Phosphate buffer pH 5.6, 4 mM α -CD, 1 mM β -CD	SPE (C ₁₈) preconcentration. fenoprop and 2,4-DP chiral	[43]
2,2-CPPA, 2,3-CPPA, 2,4-CPPA, 2-PPA	Ristocetin A	-	CZE-UV (254 nm)		0.1 M phosphate buffer, pH 6.0, 2 mM ristocetin	Chiral selector evaluation	[44]

2,4-D, dichlorprop, fenoprop, mecoprop.	TM- β -CD	-	CZE-UV (230 nm)	50 mM acetate pH 4.5, 25 mM TM- β -CD	2,4-D achiral	[45]
Diclofop	TM- β -CD HP- β -CD	-	CZE-UV (214 nm)	50 mM sodium acetate, 10 mM TM- β -CD, 10 mM HP- β -CD, pH 3.6	-	[46]
Flamprop-isopropyl, flamprop, fluazifop, haloxyfop	SBE- β -CD	-	CZE-UV (205 nm)	Several buffers containing borate, SBE- β -CD	-	[47]
Diclofop methyl, silvex isooctyl ester	HP- β -CD (dynamic coating)	-	CEC-UV (254 nm)	1 mM sodium phosphate, 2.5 mM sodium borate, 2 mM HP- β -CD, composed of 30% v/v MeOH, 50% v/v ACN and 20% v/v aqueous buffer (pH 5.0)	Diol-silica stationary phase. Chiral dansyl amino acids also separated	[48]
Fenoprop methyl ester, mecoprop methyl ester, dichlorprop methyl ester	Several CDs	-	MEKC-UV (200 nm)	Several buffers containing borate, CDs and SDS	-	[49]
Pyrethroids						
Cypermethrin, alphamethrin, permethrin, fenpropathrin	γ -CD	-	MEKC-UV (214 nm)	50mM sodium phosphate, pH 2.5, 150 mM SDS, 150 mg/ml γ -CD	Stereoisomers (including isomers)	[50]
CA (Phenothrin)*, DCA (cypermethrin), CMBA (sanmarton)	OG, OM	-	CE-LIF (He-Cd: 325 nm; Filter: 380, 420 nm)	Several buffer containing sodium phosphate, OM or OG	Derivatization with ANSA, ANDSA, ANTS	[29]
Bioallethrin, fenpropathrin, phenothrin	Several CDs, SC	Water	MEKC-UV (214 nm)	Several buffers containing NaH ₂ PO ₄ , SDS or SC, CDs	SPE preconcentration (Oasis HLB)	[24]
Organochlorine						
<i>O,p</i> -DDT, <i>p,p'</i> -DDT	HP- β -CD (dynamic coating)	-	CEC-UV (254 nm)	1 mM sodium phosphate, 2.5 mM sodium borate, 2 mM HP- β -CD, composed of 30% v/v MeOH, 50% v/v ACN and 20% v/v aqueous buffer (pH 5.0)	Diol-silica stationary phase. Chiral dansyl amino acids also separated	[48]

<i>o,p</i> -DDT, <i>o,p</i> -DDD, <i>p,p'</i> -DDT, <i>o,p'</i> -DDE, <i>p,p'</i> -DDD, <i>p,p'</i> -DDE	Several CDs	-	MEKC-UV (200 nm)	Several buffers containing borate, CDs and SDS	<i>p,p'</i> -DDT, <i>o,p'</i> -DDE, <i>p,p'</i> -DDD, <i>p,p'</i> -DDE not chiral	[49]
Organophosphorous						
Fenamifos, isofenfos, ruelene, dialifos, malathion	Several CDs	-	MEKC-UV (200 nm)	Several buffers containing borate, CDs and SDS	-	[49]
Acetamide						
Metolachlor	Several CDs	-	MEKC-UV (200 nm)	Several buffers containing borate, CDs and SDS	-	[49]
Napropamide	SBE- β -CD	-	CZE-UV (205 nm)	Several buffers containing borate, SBE- β -CD	-	[47]
Imidazolinones						
Imazaquin, imazamethabenz	TM- β -CD HP- β -CD	-	CZE-UV (214 nm)	50 mM sodium acetate, 10 mM TM- β -CD, 10 mM HP- β -CD, pH 3.6	Imazamethabenz (<i>m</i> -, <i>p</i> -isomers)	[46]
Imazapyr	SBE- β -CD	-	CZE-UV (205 nm)	Several buffers containing borate, SBE- β -CD	-	[47]
Imidazole						
Fenticonazole	TM- β -CD	-	CZE-UV (200 nm)	20 mM TM- β -CD, phosphate buffer pH 3	HPLC comparison. Other CDs used for impurity separation.	[51]
Benzofuran						
Ethofumesate	SBE- β -CD	-	CZE-UV (205 nm)	Several buffers containing borate, SBE- β -CD	-	[47]
Uracil						
Bromacil	SBE- β -CD	-	CZE-UV (205 nm)	Several buffers containing borate, SBE- β -CD	-	[47]
Carbanilate						
Chlorbufam	SBE- β -CD	-	CZE-UV (205 nm)	Several buffers containing borate, SBE- β -CD	-	[47]

			nm)	SBE- β -CD				
Dicarboximide								
Vinclozolin	γ -CD	Wine	MEKC-UV (203 nm)	20mM phosphate-5mM borate (pH 8.5), 50 mM γ -CD, 100 mM SDS	Previous extraction and separation	SPE-HPLC	[52]	

*Metabolite and pesticide (between parentheses).

Table 2. List of 19 atropisomeric polychlorinated biphenyls (PCBs). Basic structure, IUPAC numbers and systematic names [63].

IUPAC Number	Chiral PCB
45	2,2',3,6-tetrachlorobiphenyl
84	2,2',3,3',6-pentachlorobiphenyl
88	2,2',3,4,6-pentachlorobiphenyl
91	2,2',3,4',6-pentachlorobiphenyl
95	2,2',3,5',6-pentachlorobiphenyl
131	2,2',3,3',4,6-hexachlorobiphenyl
132	2,2',3,3',4',6-hexachlorobiphenyl
135	2,2',3,3',5',6-hexachlorobiphenyl
136	2,2',3,3',6,6'-hexachlorobiphenyl
139	2,2',3,4,4',6-hexachlorobiphenyl

144	2,2',3,4,5',6-hexachlorobiphenyl
149	2,2',3,4',5',6-hexachlorobiphenyl
171	2,2',3,3',4,4',6-heptachlorobiphenyl
174	2,2',3,3',4,5,6'-heptachlorobiphenyl
175	2,2',3,3',4,5',6-heptachlorobiphenyl
176	2,2',3,3',4,6,6'-heptachlorobiphenyl
183	2,2',3,4,4',5',6-heptachlorobiphenyl
196	2,2',3,3',4,4',5',6-octachlorobiphenyl
197	2,2',3,3',4,4',6,6'-octachlorobiphenyl

Table 3. Chiral CE analysis of PCBs.

<i>PCB</i>	<i>Chiral Selector</i>	<i>CE mode</i>	<i>Buffer</i>	<i>Comments</i>	<i>Reference</i>
45, 84, 88, 91, 95, 131, 136, 144, 149, 176 and 197	CM- γ -CD, β -CD, β -CD-NH ₂	CZE-UV (230 nm)	30 mM β -CD-NH ₂ , 10 mM phosphate (pH 2.0), 2M urea	Individual separation of 11 chiral PCBS in their two enantiomers	[65]
45, 88, 135, 149, 171	Poly-D-SUV HP- γ -CD	CZE-UV	5 mM CHES (pH 10.0), 1.5 % (w/v) poly-D-SUV, 16 mM HP- γ -CD, 1M urea, 20% (v/v) MeOH	Separation of a mixture of 5 chiral PCBs	[66]
45, 88, 91, 95, 136, 144, 149 and 176	CM- γ -CD β -CD	CZE-UV (230 nm)	20 mM CM- γ -CD, 10 mM β -CD, 2 M urea in 50 mM MES (pH 6.5)	Monitoring stereoselectivity biodegradation by a naturally occurring soil bacterium	[67]
45, 88, 91, 95, 131, 132, 136, 139, 144, 149, 171, 176, 197	CM- γ -CD β -CD PM- β -CD	CZE-UV (230 nm)	20 mM CM- γ -CD, 10 mM β -CD or 20 mM PM- β -CD, 2 M urea, in 50 mM MES (pH 6.5)	Individual separation of 13 chiral PCBs in their two enantiomers Chiral separation of mixtures a) 45, 88, 91, 95 and 176; b) 45, 95, 131 and 136; c) 132, 139, 149 and 171	[68]
45, 84, 88, 91, 95, 131, 135, 136, 139, 144, 171, 175, 176, 183, 196	γ -CD, SC, SDC, STC, STDC	MEKC-UV (235 nm)	0.05 M SDS, 0.05 M SC, 0.05 M γ -CD, 0.1 M CHES (pH 9), 2 M urea	Individual separation of 15 chiral PCBs in their two enantiomers Chiral separation of mixtures: a) 88, 45, 144, 131, 176, 171, 175; b) 88, 45, 139, 136, 84, 95, 183, 196	[69]
45, 84, 88, 91, 95, 132, 136	HP- γ -CD	MEKC-UV (230 nm)	80 mM SDS in 100mM borate-50mM phosphate (pH 8.0), 60 mM HP- γ -CD, 20% 2-propanol, 4 M urea	Separation of a mixture of 7 chiral PCBs	[70]
84, 95, 176	SC	MEKC-UV (235 nm)	50 mM CHES (pH 10.0), 150 mM SC (100 mM for PCB 176), 2M urea	Individual separation	[71]
45, 84, 88, 91, 95, 132, 136, 139, 149, 171, 183, 196	β -CD, γ -CD	MEKC-UV (235 nm)	0.090 M CHES (pH 10.0), 0.11 M SDS, 2 M urea, 0.079 M β -CD, 0.024 M γ -CD	Individual separation and separation of a mixture of 8 chiral PCBs	[72]
45, 84, 88, 91, 95, 132, 136, 139, 149, 171, 183, 196	γ -CD	MEKC-UV (235 nm)	0.10 M CHES (pH 10.0), 0.11 M SDS, 2 M urea, 0.05 M γ -CD	Individual separation and separation of a mixture of 9 chiral PCBs	[73]

*Selective modified β -CD-(NH₃)₂²⁺. AB regioisomer.