Chiral Analyses of Pollutants by Capillary Electrophoresis

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Abstract: The determination of enantiomeric composition of chiral xenobiotics and pollutants is a very difficult job due to their low amounts and poor detection by commonly used UV detector. But this sort of analysis is an important issue from the health point of view. The chiral analyses of these notorious pollutants by capillary electrophoresis have been discussed in this article. This review discusses the new trends and advancements, which have been achieved in the analyses of chiral pollutants using capillary electrophoresis and the state-of-art of their enaniomeric resolution. This article focuses on sample treatment, applications, optimization, detection, mechanisms of chiral resolution and future perspectives of CE in chiral resolution of xenobiotics. Besides, efforts have also been made to suggest the improvement in CE machine to make it ideal for the analyses of chiral xenobiotics at trace levels.

Keywords: Capillary electrophoresis, environmental pollutants and xenobiotics, nanoscale analyses, optimization, future perspectives.

1. INTRODUCTION

The presence of chiral pollutants and xenobiotics in our environment is a serious issue due to the different toxicities of their enantiomers [1]. Moreover, these molecules degradate stereo-specifically, leading to more toxic products. Besides, bio-transformation of the chiral pollutants may be stereo-specific making different uptake, metabolism and excretion of enantiomers. Normally, metabolites of the chiral xenobiotics are chiral and the enantiomeric composition of the chiral pollutants may be changed in these processes. The simple analyses of these compounds do not give any idea about their toxicities but rather creating confusion among scientist and society. It has been estimated that about twenty five percent agrochemicals are chiral and are sold as their mixtures. Therefore, the chial analysis of these sorts of xenobiotics is essential and required to understand their toxicities, degradation and metabolism. Therefore, environmentalists, clinicians, nutritional experts, agricultural scientists and regulatory authorities of the world are interesting in chiral analyses of these kinds of pollutants. In view of these facts, various workers developed enantiomeric analytical methods for monitoring chiral xenobiotics in our environment.

Among various analytical techniques, chromatographic and capillary electrophoretic methods have achieved a good reputation in the area of chiral analyses [2,3]. Of course chromatographic modalities are the ideal methods but the low amounts of chiral xenobiotics in the environment compel scientists to work with capillary electrophoretic methods. Capillary electrophoresis (CE) is a suitable technique for such type of analyses due to its requirement of low amount of samples and low detection limits. Besides, its versatility, high speed and sensitivity are the additional advantages. Due to these facts, some people used CE for the analyses of chiral xenobiotics and few reviews have already appeared in the literature on this issue [4-7]. The present article discusses the latest development in this area and a state-of art of chiral analyses of xenobiotics by CE in the environment.

2. DETERMINATION OF CHIRAL XENOBIOTICS BY CAPILLARY ELECTROPHORESIS

The determination of chiral pollutants involves two steps i.e. sample preparation and analyses by CE. Both steps are integral parts of the complete work and sample preparation is carried out by liquid-liquid extraction and spild phase extraction (SPE). But nowadays, sample preparation unit is hyphenated with mail analytical unit, which provide the more sensitivity, reproducibility and time saving. The complete protocol of the determination of chiral pollutants in the environment by CE is given in Fig. (**1**). The complete analyses of chiral xenobiotics are described below.

2.1. Sample Treatment

The enantiomeric analysis of xenobiotics is very sensitive and the presence of any impurity effect drastically. We know that thousands of constituents are present in the environmental. The complete sample preparation requires filtration, extraction, concentration, purification etc. A thorough search of literature was carried out and it was observed that almost all analyses of chiral xenobiotics by CE was carried out in laboratory based synthesized samples.

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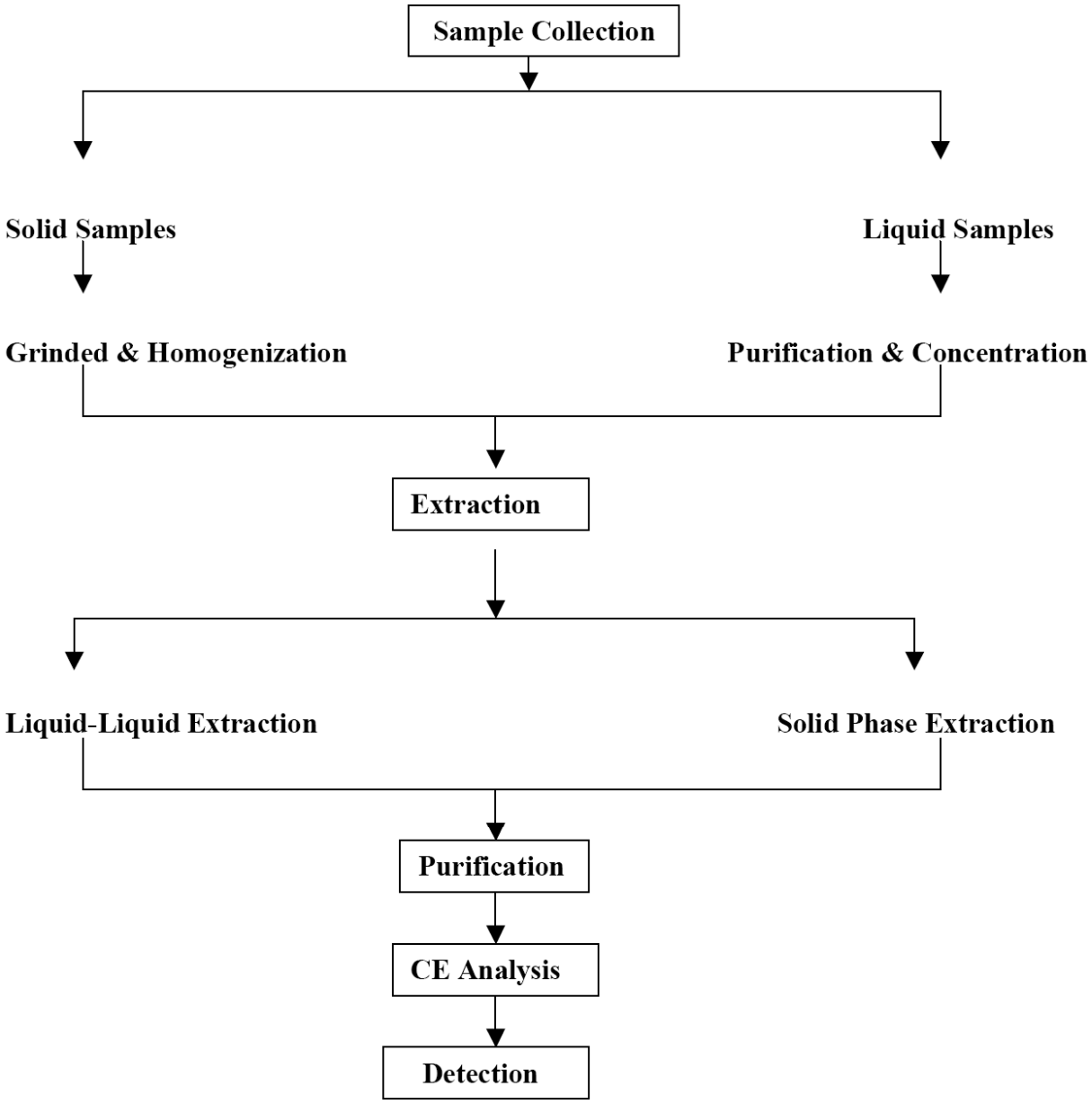

Fig. (1). The protocol for the analyses of chiral xenobiotics by CE. *Note*: This is the brief outline of the procedure to follow in chiral separation by CE. However, other variations may be carried out.

However, few reports are available for achiral analyses of xenobiotics by CE. And, of course, these approaches may be utilized for chiral analyses too. It is intresting to note that among few sorts of sample preparation SPE has achieved a good reputation in analytical science due to its ease of operation, selective, time and costly chemical saving nature [8]. Some reviews have been published on the pre-treatment and sample preparation methodologies for the achiral analysis of pollutants [9-13]. Dabek-Zlotorzynska *et al*. [10] reviewed the sample pretreatment methodologies for environmental analysis prior to CE. Whang and Pawliszyn [14] developed an interface, which enables the solid-phase micro-extraction (SPME) fiber hyphenation to CE. The authors prepared a semi-custom made polyacrylate fiber to reach the SPME-CE interface. They used the developed interface to analyze phenols in water, which may be used for the chiral resolution of the pollutants.

2.2. Analyses by Capillary Electrophoresis

In spite of good advancement in CE still chiral resolution is achieved by adding chiral selectors in back ground electrolyte (BGE). However, few chiral capillaries are available in market but, unfortunately, only few publications available that deal with the chiral resolution on a capillary coated with the chiral selector in CE [15]. The most commonly used chiral BGE additives are polysaccharids, cyclodextrins, macrocyclic glycopeptide antibiotics, proteins, crown ethers, ligand exchangers, and alkaloids [16,17].

2.2.1. Applications

As per the literature search the various chiral organic pollutants analyzed by CE include pesticides, polynuclear-aromatic hydrocarbons, amines, carbonyl compounds,

surfactants, dyes, and other toxic compounds. Some chiral resolutions of xenobiotics by CE are discussed in this section. Some reviews appeared in the literature describing the chiral resolution of environmental pollutants by CE [18-23]. Weseloh *et al*. [24] described CE method for the resolution of biphenyls with phosphate buffer as BGE containing cyclodextrin as the chiral additive. Otsuka *et al*. [23] reported the coupling of capillary electrophoresis with mass spectrometry and used for the chiral analysis of phenoxy acid herbicides. The electro spray ionization (ESI) method for the CE-MS interface has been described. Zerbinati *et al*. [25] separated four enantiomers of the herbicides mecoprop and dichlorprop using an ethylcarbonate derivative of β-CD with three substituents per molecules of hydroxyl propyl-β-CD and native β-CD. The performances of these chiral selectors have been quantified by means of two-level full factorial designs and the inclusion constants. Miura and co-workers [26] described CE for the chiral analyses of seven phenoxy acid herbicides using methylated cyclodextrins as the BGE additives. Sarac *et al*. [27] resolved the enantiomers of 2- hydrazino-2-methy1- 3-(3,4-dihydroxypheny1) propionic acid using cyclodextrin as the BGE additive. The cyclodextrins used were native, neutral, and ionic in nature with phosphate buffer as BGE. Similarly, Tsunoi *et al*. [28] resolved MCPP, DCPP, 2,4-D, 2,4-CPPA, 2,4,5-T, 2,3-CPPA, 2,2-CPPA, 2-PPA, and silvex pesticides using cyclodextrins, with negatively charged sulfonyl groups, as the chiral BGE additives.

Zerbinati and co-workers [24] used heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM- β-CD) as chiral selector for the resolution of three phenoxy acid herbicides. The authors used negative-ionization mode, along with a methanol-water-formic acid solution as a sheath liquid and nitrogen as a sheath gas for stereoselective resolution and detection of these herbicides. The BGE used was 50 mM ammonium acetate buffer (pH 4.6) containing 20 mM TM-β-CD. Gomez-Gomar *et al*. [29] reported the analyses of (±)-cizolirtine and its impurities, (±)-N-desmethylcizolirtine, (±)-cizolirtine-N-oxide, and (±)-5-(hydroxybenzyl)-l-methylpyr-azole, by capillary electrophoresis. Jarman *et al*. [30] resolved enantiomers of metalaxyl, imazaquin, fonofos (dyfonate), ruelene (cruformate) and dichlorprop in CE with CD chiral selectors. Metalaxyl underwent enantioselective transformation; in one soil with 17 and 69 days as half life for R-(+)- and S-enantiomers. Contrarily, imazaquin and fonofos exhibited non-selective enantiomer loss over their three months of incubation time. Furthermore, ruelene and dichlorprop were transformed selectively in a variety of soils. According to authors, CE is a simple, efficient and inexpensive technique to study the transformation of chiral pesticides. Klein *et al*. [31]. analyzed stereoisomers of metolachlor and its two polar metabolites [ethane sulfonic acid (ESA) and oxanilic acid (OXA)] by CE by using γ-cyclodextrin. Garrison *et al*. [20] reported chiral resolution of pesticides in soils and sediment samples by using cyclodextrins as the chiral selectors. For neutral pesticides sodium dodecyl sulfate was added as micelleing agent. The chiral analyses of the environmental pollutants by CE are summarized in Table **1**. To show the nature of the electropherograms, the chiral separation of phenoxy acid herbicides using 175 mM sodium phosphate, pH 6.5 containing 10 mM, 60 mM and 100 mM n-octyl-β-D-maltopyranoside (OM) respectively are shown in Fig. (**1**).

2.2.2. Optimization

As mentioned earlier, the chiral analyses of pollutants by CE is very sensitive and, hence, is controlled by a number of experimental parameters. The optimization parameters may be divided into two categories i.e., the independent and dependent parameters. The independent parameters are under the direct control of the analyst. These parameters include the choice of the buffer, pH of the buffer, ionic strength of the buffer, type of chiral selectors, voltage applied, temperature of the capillary, dimension of the capillary, BGE additives, and various other parameters. On the other hand, the dependent parameters are those directly affected by the independent parameters and are not under the direct control of the operator. These types of parameters are field strength (V/m), EOF, Joule heating, BGE viscosity, sample diffusion, sample mobility, sample charge, sample size and shape, sample interaction with capillary and BGE, molar absorptivity, etc. Therefore, the optimization of the chiral resolution can be controlled by varying all of the parameters mentioned above. For detailed information on the optimization of chiral analysis, one should consult our earlier book [3].

Mechref and El Rassi [32] carried out a remarkable work on the optimization of chiral separations of phenoxy acid herbicides. The authors optimized phosphate buffer concentrations, pH, ionic strength, temperature, BGE additives and concentrations of n-octyl-β-D-maltopyranoside (OM) surfactant to achieve the best resolution. The effects of these parameters on chiral resolution are shown in Figs. (**3-7**), respectively. Tsunoi *et al*. [28] used a mixture of 0.1 M borate and 0.05 M phosphate buffers (pH 9.0) as BGE for the chiral resolution of phenoxy acid herbicides. Weseloh *et al*. [22] reported the chiral resolution of biphenyls using phosphate buffer of different concentration and 2.4 pH. Sarac *et al*. [27] described 2.5 as the best pH of phosphate buffer for the chiral resolution of propionic acid derivatives. Yi *et al*. [33] reported a fast simple and sensitive CE method to study the separation and degradation of imazaquin enantiomers in field soils. used 30 mM phosphate buffer of pH 2.4 as the BGE for the chiral resolution of biphenyl pesticides. Nelson *et al*. [34] reported no heating of the capillary up to 30 kV as the applied voltage when borate buffer was used while heating of the capillary was observed even at 10 and 12 kV using CAPS and phosphate buffers respectively.

2.2.2. Detection

Of course, a sensitive and of low limit method of detection is required as chiral xenobiotics are found at low concentrations in the environment. Most commonly used detectors in the chiral CE are UV, electrochemical, fluorescence, and mass spectrometry. Normally, the detection of the chiral drugs and pharmaceuticals in CE has been achieved by a UV mode [26, 32] but the detection of the chiral pollutants is difficult due to the transparent nature of mostly chiral xenobiotics. Besides, few chiral selectors, such as proteins and macrocyclic glycopeptide antibiotibiotics are UV absorbing in nature. Therefore, the

Table 1. Chiral Analysis of Some Environmental Pollutants by Capillary Electrophoresis

Chiral Pollutants	Electrolytes	Detection	Refs.
2-(2-Methyl-4-chlorophenoxy) propionic acid	0.05M lithium acetate containing α-CD	UV 200 nm	[44]
2-(2-Methyl-4-6-dichlorophenoxy) propionic acid	0.05M lithium acetate containing β-CD	UV 200 nm	[44]
2-(2-4-Dichlorophenoxy) propionic acid	0.05M lithium acetate containing Heptakis-(2,6-di-O-methyl)- β-CD	UV 200 nm	[44]
2-(2-Methyl chlorophenoxy) propionic acid and 2-(2-4-Dichlorophenoxy) propionic acid	0.05M lithium acetate containing Heptakis-(2,6-di-O-methyl)- β-CD	UV 200 nm	[45]
2-(2-Methyl chlorophenoxy) propionic acid Phenoxy acid	0.05 M NaOAc, pH 4.5 with α-CD 0.1 M phosphate buffer, pH 6with	UV230 nm	[46]
Imazamethabenz isomers	50mM sodium acetate, 10mM trimethyl-β-CD, pH 4.6	-	[47]
	Vancomycine	-	[38]
	Ristocetin	-	[38,48]
	Teicoplanin	-	[38,49]
	0.1 M phosphate and acetate buffer	-	[32]
	Containing OM		
	OG		[50]
Phenoxy acid derivatives	β-CD and TM β-CD	-	[38,51]
Silvex	0.4 M borate, pH 10 containing N,N-bis-(D-gluconamidopropyl)-cholamide (Big CHAP) and –deoxycholamide (Deoxy big CHAP)	-	[51]
2-Phenoxypropionic and, dichloroprop, Fenoxaprop, fluaziprop, haloxyfop, and Diclofop enantiomers	75mM Britton-Robinson buffer with 6mM Vancomycine	-	[52]
Imazqaquin isomer	50 mM sodium acetate, 10 mM dimethyl- β-CD, pH 4.6	-	[47]
Phenoxy acid herbicides	200 mM sodium phosphate, pH 6.5 with various Concentration of OG	-	[47]
Diclofop	50 mM sodium acetate, 10mM trimethyl-β-CD, pH 3.6	-	[47]
Fenoprop, mecoprop, and dichlororprop	20mM tributyl-β-CD in 50 mM ammonium acetate, pH 4.6	MS	[23]
Dichloroprop and 2-(2,4-dichlorophenoxy) propionic acid	100mM acetic acid- sodium acetate buffer (pH 5.0) containing α-, β-, and γ-CDs	UV 206 nm	[25]
Metalaxyl, imazaquin, fonofos (dyfonate), ruelene (cruformate), and dichlorprop, and 40 mM γ -CD as the chiral selector.	30 mM Na-TB, pH 8.5, buffer with 100 mM SDS as the micelle, 15% can	-	[30]
Imazaquin enantiomers	Sodium hydrogen phosphate (50 mM) at pH 10.1 containing 30 mM hydroxypropyl-β-CD (HP- β–CD)	-	[33]
Glufosinate (D,L-GLUF), a phosphorus- containing amino acid-type herbicide	Phosphate buffer with γ-CD	Fluorescence	[53]
Imazaquin enantiomers in field soils 30 mM hydroxypropyl-β-CD (HP-beta-CD)	Phosphate (50 mM) at pH 10.1 containing	UV	[33]
Polycyclic musks	CHES buffer (pH 9.0) with CDs	UV	[54]

detection of enantiomers becomes poor. Hence, other detection methods are required, which include electrochemical and mass spectrometric detectors. Only few papers are available in the literature dealing with the limits of the detection for the chiral resolution of environmental pollutants by CE, indicating mg/L to μg/L as the limits of the detection. Mechref and EI Rassi [32] described good detection limits for herbicides in the derivatized mode, in comparison to the underivatized mode. The limit of the detection was enhanced by almost 1 order of magnitude from 1×10^{-4} M (10 pmol) to 3×10^{-5} M (0.36 pmol). In the same study, the authors reported 2.5×10^{-6} M and 1×10^{-9} M as the detection limits of the herbicides by fluorescence and laser induced fluorescence detectors respectively. Tsunoi *et al*. [28] carried out an extensive study on the determination of the limits of the detection for the chiral resolution of herbicides. The authors used a 230 nm wavelength for the detection and the minimum limit of the detection reported was 4.7×10^{-3} M for 2,4-dichlorophenoxy acetic acid. Asami and Imura [35] reported trace enantiomeric compositions of glufosinate (D,L-GLUF); a phosphorus-containing amino acid-type herbicide; in a river. The chiral separation and detection (10^{-9} M) were achieved by solid phase extraction and γ-cyclodextrin based CE. Our experience on the CE and the properties of chiral xenobiotics dictates us that mass spectrometric detection is the best choice for this purpose.

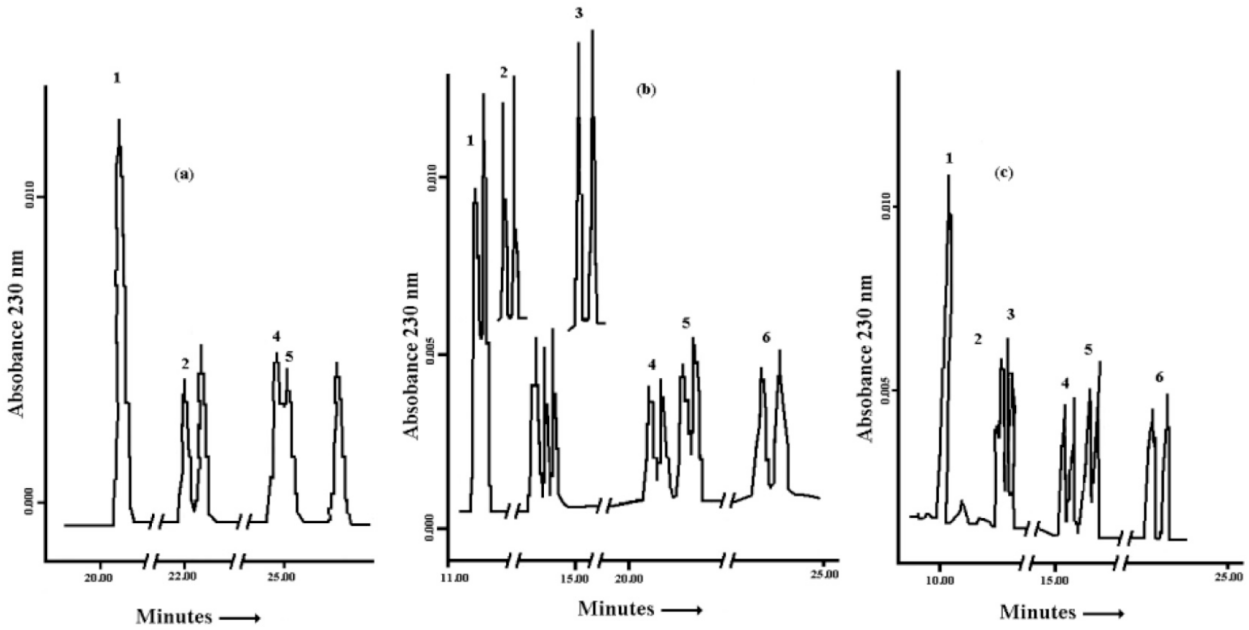

Fig. (2). Electropherograms of phenoxy acid herbicides using 175 mM sodium phosphate, pH 6.5 containing (**a**): 10 mM (**b**): 60 mM and (**c**): 100 mM n-octyl-β-D-maltopyranoside (OM) respectively. Fused silica capillary (57 cm x 50 μm I.D) with 25 kV as applied potential. 1 = silvex, 2 = dichlorprop, 3 = mecoprop, 4 = 2,4-CPPA, 5 = 2,3-CPPA, 6 = 2,2-CPPA and 7 = 2-PPA [32].

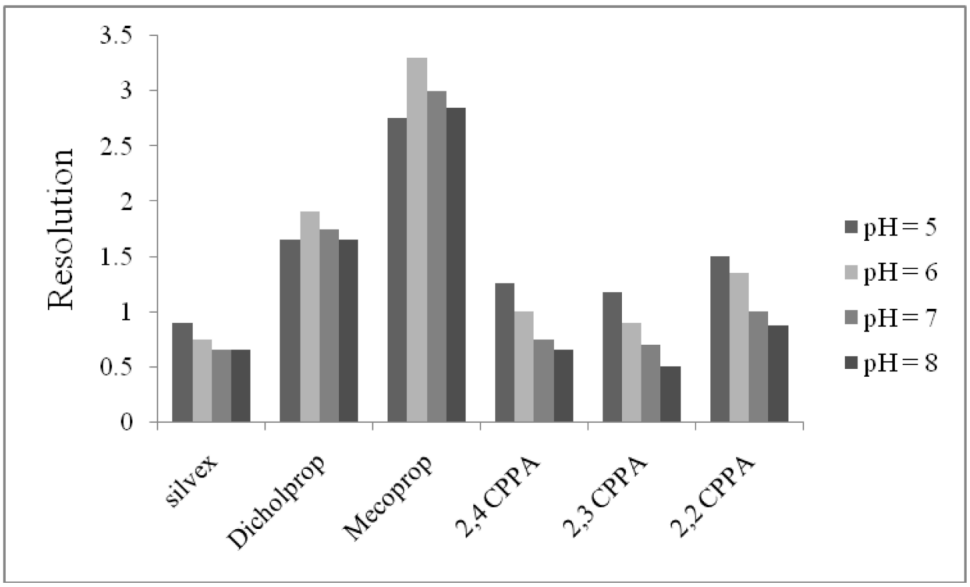

Fig. (3). The effect of pH on enantiomeric separation of phenoxy acid herbicides using 100 mM sodium phosphate sodium acetate buffers containing 60 mM OM as BGE. Other conditions as in Fig. (**2**) [32].

2.2.3. Mechanisms of Chiral Resolution

As discussed above chiral selectors are used to resolve mixtures of racemic xenobiotics in CE. Various chiral selectors have different structures and capabilities to bind enantiomers stereochemically which resulted into the resolution of antipodes. The chiral recognition mechanism of enantiomeric resolution of xenobiotics by CE has not been described in detail. However, this mechanism is available for drugs and pharmaceutical. We believe that the same mechanism may be applicable in case of chiral resolution of xenobiotics. Therefore, the following descriptions are related to the general chiral recognition in CE, which may be applicable in case of xenobiotics. The chiral selectors have special type of structures such as grooves, baskets, cavities etc. in which enantiomers get trapped stereospecifically. The trapping of enantiomers is stabilized by various forces. Most important forces taking place in chiral separations are hydrogen bonding, coordination bonding, electrostatic force of attraction, π- π interactions, van der Waal forces, steric affects, dipole induced dipole attraction, dispersive forces etc. The combination of all these forces is entirely different in each chiral selector. The chiral recognition mechanism depends on the type of the chiral selector used and it varies from chiral selector to selector. As in case of liquid chromatography the chiral environment is essential for the enantiomeric resolution in capillary electrophoresis too. In CE chiral situation is provided by the chiral compound used

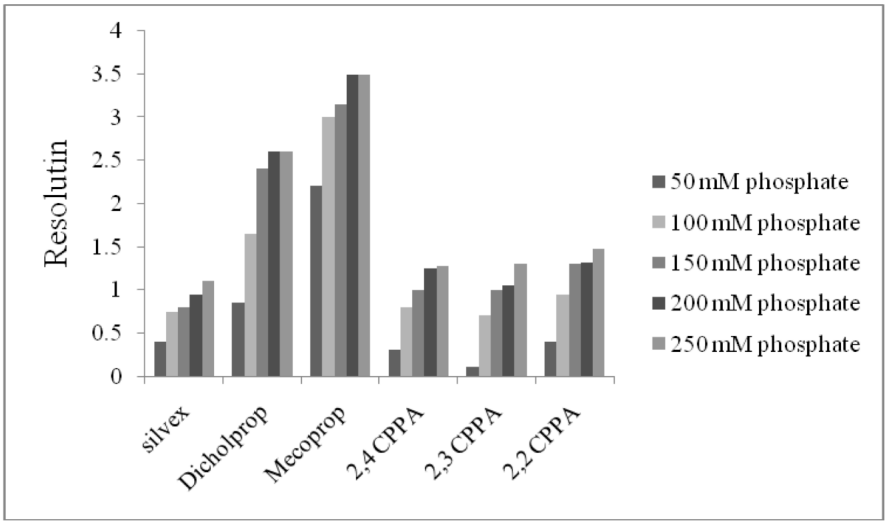

Fig. (4). The effect of ionic strength on on enantiomeric separation of phenoxy acid herbicides using sodium phosphate buffer containing 60 mM OM as BGE. Other conditions as in Fig. (**2**) [32].

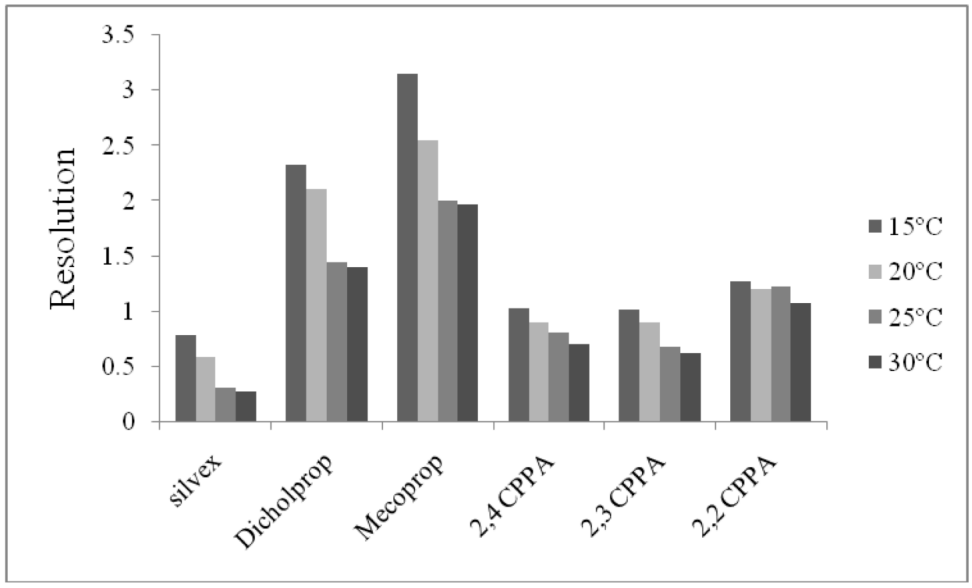

Fig. (5). The effect of the temperature on on enantiomeric separation of phenoxy acid herbicides using 150 mM sodium phosphate buffer (pH 6.5) containing 60 mM OM as BGE. Other conditions as in Fig. (**2**) [32].

in background electrolyte (BGE). Basically, chiral recognition mechanisms in CE are similar to those in liquid chromatography using chiral mobile phase additive mode except that the resolution occurs through different migration velocities of the diastereoisomeric complexes in CE. The chiral resolution in CE occurred through diastereomeric complex formation between the enantiomers of the pollutants and the chiral selector.

Gübitz and Schmidt [19] reviewed the chiral recognition mechanisms in CE. Indirect resolution is achieved by diastereoisomeric complex formation followed by their resolution by CE. Contrarily, direct resolution is achieved by using chiral selectors in BGE. The theoretical consideration of chiral recognition mechanisms in CE was reviewed by Vespalec and Bocek [36]. In case of cyclodextrins, the inclusion diastereomeric complexes are formed which are controlled by a number of interactions such as π-π complexation, hydrogen bonding, dipole-dipole interactions, ionic bindings and steric affects. It is also considered that electrostatic interactions contribute substantially to the chiral recognition processes. Therefore, the chiral selector-enantiomers with a net charge of the same sign must be unfavorable for the chiral separations. Zerbinati *et al*. [24] used ethylcarbonate-β-CD, hydroxypropyl-β-CD and native α-CD for the chiral resolution of mecoprop and dichlorprop. The authors calculated the performances of these chiral selectors by means of two-level full factorial design and calculating inclusion constants from CE migration time data. Furthermore, they have proposed the possible structures of inclusion complexes on the basis of molecular mechanics simulations. Chankvetadze *et al*. [37] explained the chiral recognition mechanisms in cyclodextrin using UV, nuclear magnetic resonance spectroscopy (NMR) and electrospray ionization mass spectrometric methods. Furthermore, the authors determined the structures of the diastereomeric complexes by X-ray crystallographic method. Mostly macrocyclic antibiotics containing ionizable groups, chiral

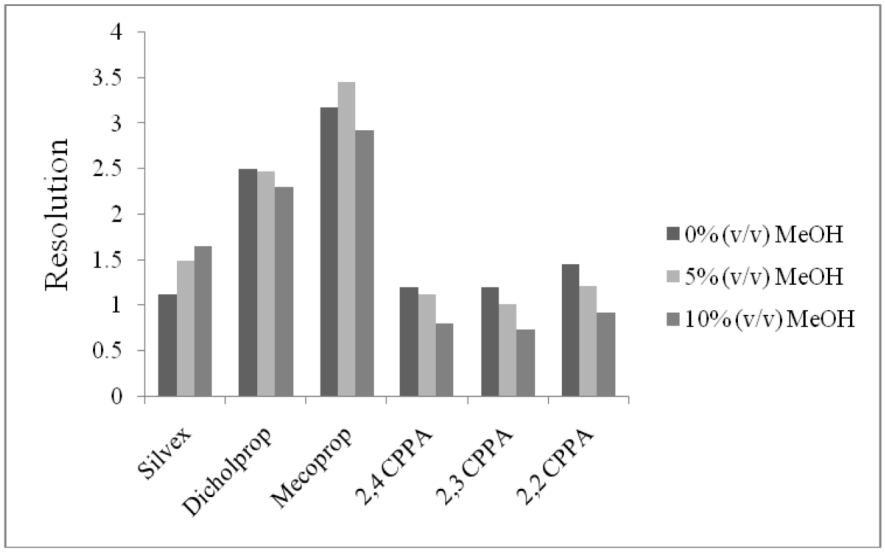

Fig. (6). The effect of methanol concentration on on enantiomeric separation of phenoxy acid herbicides using 200 mM sodium phosphate-sodium acetate buffers (pH 6.5) containing 60 mM OM as BGE. Other conditions as in Fig. (**2**) [32].

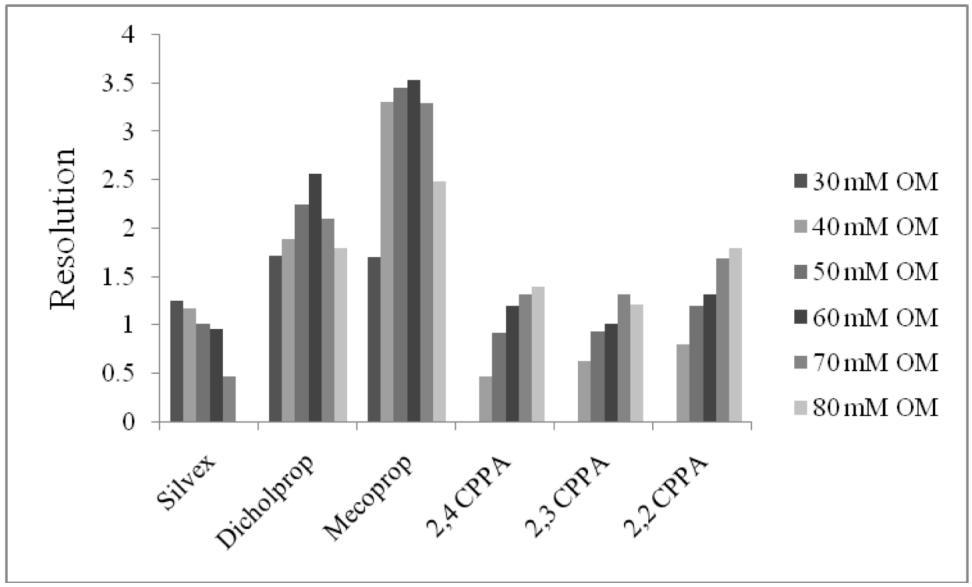

Fig. (7). The effect of the concentration of OM on on enantiomeric separation of phenoxy acid herbicides using 175 mM sodium phosphate buffer (pH 6.5) as BGE. Other conditions as in Fig. (**2**) [32].

baskets, fused rings and sugar moieties change in their charge and possibly three dimensional conformation with different pH and the concentrations of BGE. This allows for an excellent potential to resolve a greater variety of racemates. The possible interactions involved in the formation of diastereomeric complexes are π-π complexation, hydrogen bonding, inclusion complexation, dipole interactions, steric interactions and anionic and cationic binding [38] Accordingly, the diastereomeric complexes possessing different physical and chemical properties separated on the capillary path (achiral phase). The different migration times of the formed diastereomeric complexes depend on their sizes, charges and interaction with the capillary wall and as a result these complexes eluted at different time intervals.

Kuhn *et al*. [39] used chiral crown ether $18C_6H_4$ in CE and explain the chiral recognition mechanisms and as per authors host-guest complexes of enantiomers are formed which are stabilized by hydrogen bonding, electrostatic interactions and steric affects. The carboxylic groups of crown ether; perpendicular to the plane of the ring; form a chiral barrier which divides the space available for the substituents at the chiral centre of the enantiomers into two domains. In this way, two different diastereoisomeric complexes are formed which separated onto CE [40]. Kuhn *et al*. [41] proposed the chiral recognition mechanism based on space availability for the substutents of both the chiral carbon atoms; adjacent to amine functional groups; into two cavities. Koide and Ueno [42] proposed a model and theoretical equations to investigate the enantiomeric recognition of primary amino acids using achiral crown ether with cyclodextrins by capillary electrophoresis and NMR studies. The association constants were calculated by using CE and NMR techniques. The authors reported the formation of diastereoisomeric complexes of amino acid enantiomers-CD-crown ether, which resulted into their

resolution by CE. Zinbinati *et al*. [24] used ethylcarbonate-β-CD and native α-CD for the chiral resolution of mecoprop and dichloprop. The authors calculated the performance of these chiral selectors by means of a two-level full factorial design and calculated inclusion constants from CE migration time data. Furthermore, they have proposed the possible structure of inclusion complexes on the basis of molecular mechanics simulations. Chankvetadze *et al*. [43] explained the chiral recognition mechanisms in cyclodextrin-based CE using UV, NMR, and electrospray ionization mass spectrometric methods Furthermore, the authors determined the structures of the diastereomeric complexes by an X-ray crystallographic method.

2.2.4. Future Perspectives of CE in Chiral Resolution of Xenobiotics

Of course, HPLC is the best technique of chiral resolution but CE is also gaining momentum; especially in the environmental samples where the amount of xenobiotics is very low. CE is a versatile technique with high speed, good sensitivity and selectivity. It also needs low amount samples and provides low detection limits. Moreover, the chiral resolution in CE is achieved using the chiral selectors in the BGE. But, unfortunately, it suffers from reproducibility point of view. However, new advances made in CE machine are successful to over the problem of reproducibility. Therefore, the environmentalists are looking towards CE for the analyses of chiral xenobiotics. And some papers have started to appear into the literature. It is intresting to note that high efficiency of CE is due to the flat profile created and to a homogeneous partition of the chiral selector in the electrolyte which, in turn, minimizes the mass transfer. Generally, the theoretical plate number in CE is much higher in comparison to chromatography and thus a good resolution is achieved in CE. In addition, more than one chiral selector can be used simultaneously for optimizing the chiral analysis.

In spite of the above cited advantages of CE it suffers some other serious draw backs. The other limitations of CE include the waste of the chiral selector as it is used in the BGE. In addition, chiroptical detectors, such as polarimetric and circular dichroism, cannot be used as detection devices because of the presence of the chiral selector in the BGE. Moreover, some of the well-known chiral selectors may not be soluble in the BGE and, thus, a stationary bed of a chiral selector may allow the transfer of the advantages of a stationary bed inherent in HPLC to electrically driven technique, i.e. CE. This will allow CE to be hyphenated with the mass spectrometer, polarimeter, circular dichroism, and UV detectors without any problem. Briefly, at present, CE is getting reputation in chiral resolution of xenobiotics but not fully developed. To the best of our experience and observation a cooling device of capillary, hyphenation of sample preparation unit and MS detector will make this technique ideal for analyses of xenobiotics at nano or low amount levels.

3. CONCLUSIONS

Analysis at trace level is a very important and challenging issue especially in the environmental matrices and CE can accept this challenge by the above cited hyphenation. To make the CE applications more reproducible, the background electrolyte should be developed in such a way ensuring its physical and chemical properties remain unchanged during the experimental run. Besides, other aspects should also be developed so that CE can be used as a routine method in this field. The most important points related to this include the development of new and better chiral selectors, detector devices. In addition, chiral capillaries should be developed and CE device should be hyphenated with sample preparation units, mass spectrometer, polarimetric, and circular dichroism detectors, which may result into good reproducibility and improved limits of detection. All the capabilities and possibilities of CE as analytical technique have not been explored till today and are underway. However, CE will be realized as a widely recognized method of choice in analytical science. Briefly, there are a lot of to be developed for the advancement of CE and, definitely, it will prove itself as one of the best analytical technique within the coming few years.

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ABBREVIATIONS

BGE	=	Background electrolyte
CD	=	Cyclodextrin
CE	=	Capillary electrophoresis
2,2-CPPA	=	2-(2-Chlorophenoxy) propionic acid
2,3-CPPA	=	2-(3-Chlorophenoxy) propionic acid
2,4-CPPA	=	2-(4-Chlorophenoxy) propionic acid
2,4-D	=	(2,4-Dichlorophenoxy) acetic acid
DCPP	=	2-(2,4-Dichlorophenoxy) propionic acid
EOF	=	Electro-osmotic flow
ESI	=	Electron spray ionization
GC	=	Gas chromatograph
HPLC	=	High performance liquid chromatograph
MCPP	=	2-(4-Chlorophenoxy) propionic acid
MS	=	Mass spectrometer
NMR	=	Nuclear magnetic resonance
OG	=	n-Octyl-β-D-glucopyranoside
OM	=	n-Octyl-β-D-maltopyranoside
SPME	=	Solid phase micro extraction
SPME-CE	=	Solid phase micro extraction-Capillary electrophoresis
2,4,5-T	=	(2,4,5-Trichlorophenoxy) acetic acid
TM-β-CD	=	2,3,6-Tri-O-methyl-β-cyclodextrin
UV	=	Ultraviolet

REFERENCES

[1] Ariens, E.J.; Van Rensen, J.J.S.; Welling, W., Eds. *Stereoselectivity of Pesticides, Biological and Chemical Problems: Chemicals in Agriculture;* Elsevier: Amsterdam, **1988**.

[2] Aboul-Enein, H.Y.; Ali, I. *Chiral Separations by Liquid Chromatography and Related Technologies*; Marcel Dekker Inc.: New York, USA, **2003**.

[3] Ali, I.; Aboul-Enein, H. Y. *Chiral Pollutants: Distribution, Toxicity and Analysis by Chromatography and Capillary Electrophoresis*, John Wiley & Sons, Chichester, UK, **2004**.

[4] Ali, I.; Gupta, V.K; Aboul-Enein, H.Y. Chiral resolution of environmental pollutants by capillary electrophoresis. *Electrophoresis*, **2003**, *24,* 1360-1374.

[5] Wong, C.S. Environmental fate processes and biochemical transformation of chiral emerging pollutants. *Anal. Bioanal. Chem.,* **2006**, *386,* 544-558.

[6] Chu, B.; Guo, B.; Wang, Z.; Lin, J.M. Recent advances in enantiomeric separation of chiral environmental pollutants by capillary electrophoresis. *Sepu,* **2007**, *25,* 657-663.

[7] Hernandez-Borges, J.; Rodriguez-Delgado, M.A.; Garcia-Montelongo, F.J.; Cifuentes, A. A chiral analysis of pollutants and their metabolites by capillary electro-migration methods. *Electrophoresis,* **2005**, *26,* 3799-3813.

[8] Ali, I.; Gupta, V.K.; Aboul-Enein, H. Y.; Hussain, A. Hyphenation in sample preparation: Advancement from micro to nano world. *J. Sepn. Sci.,* **2008**, *31,* 2040.

[9] Martinez, D.; Cugat, M.J.; Borrull, F.; Calull, M. S. Solid phase extraction coupled with emphasis on environmental analysis. *J. Chromatogr. A,* **2000**, *902,* 65-89.

[10] Dabek-Zlotorzynska, E.; Aranda-Rodriguez, Reppel-Jones, K. Recent advances in capillary electrophoresis and capilaary electro-chromatography of pollutants. *Electrophoresis,* **2001**, *22,* 4262.

[11] Haddad, P.R.; Doble, P.; Macka, M. Developments in sample preparation and separation techniques for the determination of inorganic ions by ion chromatography and capillary electrophoresis. *J. Chromatogr. A,* **1999**, *856,* 145-177.

[12] Fritz, J.S.; Macka, M. Solid phase trapping of solutes for further chromatographic or electrophoretic analysis. *J. Chromatogr. A,* **2000**, *902*, 137-166.

[13] Pedersen-Bjegaard, S.; Rasmussen, K.E.; Halvorsen, T.G. Liquid-liquid extraction procedure for sample enrichment in capillary zone electrophoresis. *J. Chromatogr. A,* **2000**, *902,* 91-105.

[14] Whang, C.; Pawliszyn, Solid phase microextraction coupled tocapillary electrophoresis. *J. Anal. Commun.,* **1998**, *35,* 353-356.

[15] Jung, M.; Mayer, S.; Schurig, V. Enantiomer separation by GC, SFC and CE on immobilizes siloxane cyclodextrins. *LC-GC,* **1994**, *7,* 340-347.

[16] Blaschke, G.; Chankvetadze, B. Enantiomer separation of drugs by capillary electro-migration and techniques. *J. Chromatogr. A,* **2000**, *875,* 3-25.

[17] Zaugg, S.; Thormann, W. Enantio-selective determination of drug in body fluids by capillary electrophoresis. *J. Chromatogr. A,* **2000**, *875,* 27-41.

[18] Crego, A.L.; Marina, M.L. Capillary electrophoresis: a good alternative for the separation of chiral compounds of environmental interest. *J. Liq. Chrom. & Rel. Technol.,* **1997**, *20,* 1337-1365.

[19] Gubitz, G.; Schmid, M.G. Chiral separation principles in capillary electrophoresis. *J. Chromatogr. A,* **1997**, *792,* 179-225.

[20] Garrison, A.W.; Schmitt-Kopplin, P.; Avants, J.K. analysis of the enatiomers of chiral pesticides and other pollutants in environmental samples by capillary electrophoresis. *Methods Mol Biol.,* **2008**, *384,* 157-170.

[21] Eljarrat, E.; Guerra, P.; Barcelo, D. Enantiomeric determination of chiral persistent organic pollutants and their metabolites. *Trends Anal. Chem.*, **2008**, *27,* 847-861.

[22] Welseloh, G.; Wolf, C.; Konig, W.A. New technique for the determination of inters conversion processes based on capillary zone electrophoresis: studies with axially chiral biphenyls. *Chirality,* **1996**, *8*, 441-445.

[23] Otsuka, K; Smith, J.S.; Grainger, J.; Barr, J.R.; Patterson, D.G., Jr.; Tanaka, N.; Terabe, S. stereoselctive separation and detection of phenoxy acid herbicide enetiomer by cyclodextrin-modified capillary zone electrophoresis-electrospray ionization mass spectrometry. *J. Chromatogr. A,* **1998**, *817,* 75-81.

[24] Zerbinati, O.; Trotta, F.; Giovannoli, C. Optimization of cyclodextrin assisted capillary electrophoresis separation separation of the enantiomers of phenoxy acid herbicides. *J. Chromatogr. A,* **2000**, *875,* 423-430.

[25] Zerbinati, O.; Trotta, F.; Giovannoli, C.; Baggiani, C.; Giraudi, G.; Vanni, A. New derivatives of cyclodextrins as chiral selectors for the capillary electrophoretic separation of dichlorprop enantiomers. *J. Chromatogr. A,* **1998**, *810,* 193.

[26] Miura, M.; Terashita, Y.; Funazo, K.; Tanaka, M. Separation of phenoxy acid herbicides and their enantiomer in the presence of selectively methylated cyclodextrin derivatives by capillary zone electrophoresis. *J. Chromatogr. A,* **1999**, *846,* 359-367.

[27] Sarac, S.; Chankvetadze, B.; Blaschke, G. Enantioseparation of 3,4-dihydoxyphenylalanine and 2- hydrozino-2 methyle-3-(3,4 dihydoxyphenyl) propanoic acid by capillary zone electrophoresis. *J. Chromatogr. A,* **2000**, *875,* 379-387.

[28] Tsunoi, S.; Harino, R.; Miura, M.; Eguchi, M.; Tanaka, M. Separation of phenoxy acid herbicide by capillary electrophoresis using the mixture of hexakis (2,4 di-*O*-methyl) and sulfopropylether-α-cyclodextrins. *Anal. Sci*., **2000**, *16,* 991-993.

[29] Gomez-Gomar, A.; Ortega, E.; Calvet, C.; Merce, R.; Frigola, J. Simultaneous separation of the enantiomers of cizolirtine and its degradation products by capillary electrophoresis. *J. Chromatogr. A,* **2002**, *950,* 257-270.

[30] Jarman, J.L.; Jones, W.J.; Howell, L.A.; Garrison, A.W. Application of capillary electrophoresis to study the enantioselective transformation of five chiral pesticides in aerobic soil slurrirs. *J. Agric. Food Chem.,* **2005**, *53,* 6175-6182.

[31] Klein, C.; Schneider, R.J.; Meyer, M.T.; Aga, D.S. Enantiomeric separation of metolachlor and its metabolites using LC–MS and CZE. *Chemosphere,* **2006**, *62,* 1591-1599.

[32] Mechref, Y.; El Rassi, Z. Capillary electrophoresis of herbicides: Evaluation of octylmamaltopyranoside chiral surfactant in the enantiomeric separation of phenoxy acid herbicides. *Chirality,* **1996**, *8,* 518-524.

[33] Yi, F.; Guo, B.; Peng, Z.; Li, H.; Marriott, P.; Lin, J.M. Study of enantio-separation of imazaquine and enantioselective degradation in field soil by CZE. *Electrophoresis,* **2007**, *28,* 2710-2716.

[34] Nelson, R.J.; Paulus, A.; Cohen, A.S.; Guttmann, A.; Karger, B.L. Use of peltier thermoelectric devices to control column temperature in high-performance capillary electrophoresis. *J. Chromatogr*., **1989**, *480,* 111-127.

[35] Asami, T.; Imura, H. Absolute determination method for trace quantities of enantiomer of glufosinate by gamma-cyclodextrin modified capillary zone electrophoresis combined with solid-phase extraction and on-capillary concentration. *Anal. Sci.,* **2006**, *22,* 1489-1493.

[36] Vespalec, R.; Bocek, P. Chiral separations in capillary electrophoresis. *Chem. Rev*., **2000**, *100,* 3715-3754.

[37] Chankvetadze, B.; Burjanadze, N.; Pintore, G.; Bergenthal, D.; Bergander, K.; Mühlenbrock, C.; Breitkreuz, J.; Blaschke, G. Separation of brompheniramine enantiomers by capillary electrophoresis and study of chiral recognition mechanisms of cyclodextrins using NMR-spectroscopy, UV spectrometry, electrospray ionization mass spectrometry and X-ray crystallography. *J. Chromatogr. A,* **2000**, *875,* 471-484.

[38] Gasper, M.P.; Berthod, A.; Nair, U.B.; Armstrong, D.W. Comparison and modeling of vancomycin, ristocetin A and tecoplanin for CE enantio-separation. *Anal. Chem*., **1996**, *68,* 2501.

[39] Kuhn, R.; Stoeklin, F.; Erni, F. Chiral separations by host-guest complexation with cyclodextrin and crown ether in capillary zone electrophoresis. *Chromatographia*, **1992**, *33*, 32-36.

[40] Kuhn, R.; Steinmetz, C.; Bereuter, T.; Haas, P.; Erni, F. Enantiomeric separations in capillary zone electrophoresis using a chiral crown ether. *J. Chromatogr. A,* **1994**, *666,* 367-373.

[41] Kuhn, R.; Hoffstetter-Kuhn, S. Chiral separation by capillary electrophoresis. *Chromatographia*, **1992**, *34*, 505-512.

[42] Koide, T.; Ueno, K. Mechanistic study of enantiomeric recognition of primary amino compounds using an achiral crown ether with cyclodextrin by capillary electrophoresis and nuclear magnetic resonance. *J. Chromatogr. A*, **2001**, *923,* 229-239.

[43] Chankvetadze, B.; Burjanadze, N.; Pintore, G.; Bergenthal, D.; Bergander, K; Mtihlenbrock, C.; Breitkreuz, J.; Blaschke, G. Separation of brompheniramine enantiomers by capillary electrophoresis and study of chiral recognition mechanisms of cyclodextrins using NMR-spectroscopy, UV spectrometry, electrospray ionization mass spectrometry and X-ray crystallography. *J. Chromatogr. A,* **2000**, *875,* 471-484.

[44] Nielen, M.W.F. Enantiseparation of phenoxy herbicides using capillary zone electrophoresis. *J. Chromatogr. A,* **1993**, *637,* 81-90.

[45] Nielen, M.W.F. LIMS: A report on the 7th International LIMS Conference held at Egham, UK, 8-11 June, 1993. *Trends Anal. Chem.,* **1993**, *12,* 345.

[46] Garrison, A.W.; Schmitt, P.; Kettrup, A. Separation of phenoxy herbicides and their enantiomers by high performance capillary electrophoresis. *J. Chromatogr. A,* **1994**, *688,* 317-327.

[47] Penmetsa, K.V.; Leidy, R.B.; Shea, D. Enantiomeric and isomeric separation of herbicides using cyclodextrin-modified capillary zone electrophoresis. *J. Chromatogr. A,* **1997**, *790,* 225-234.

[48] Armstrong, D.W.; Gasper, M.P.; Rundlet, K.L. Highly enantio-selective capillary electrophoretic separations with dilute solutions of the macrocyclic antibiotic ristocetin A. *J. Chromatogr. A,* **1995**, *689,* 285.

[49] Rundlet, K.L.; Gasper, M.P.; Zhou, E.Y.; Armstrong, D.W. Capillary electrophoretic enantimeric separations using the glycopeptide antibiotics, tecoplanin. *Chirality,* **1996**, *8,* 88-107.

[50] Mechref, Y.; EI Rassj, Z.J. Capillary electrophoresis of herbicides II. Evaluation of alkylglucoside chiral surfactants in the enantiomeric separation of phenoxy acid herbicides. *Chromatogr. A,* **1997**, *757,* 263-273.

[51] Mechref, Y.; EI Rassi, Z. Micellar electrokinetic capillary chromatography with in-situ charged micelles VI. Evaluation of novel chiral micelles consisting of steroidal-glycoside surfactant-borate complexes. *J. Chromatogr. A,* **1996**, *724,* 285-296.

[52] Desiderio, C.; Polcaro, C.M.; Padiglioni, P.; Fanali, S. Enantiomeric separation of acidic herbicides by capillary electrophoresis using vancomycin as chiral selector. *J. Chromatogr. A,* **1997**, *781,* 503-513.

[53] Asami, T.; Imura, H. Absolute determination method for trace quantities of enantiomer of glufosinate by gamma-cyclodextrin modified capillary zone electrophoresis combined with solid-phase extraction and on-capillary concentration. *Anal. Sci.*, **2006**, *12,* 1489-1493.

[54] Martinez-Girona, A. B.; Cregoa, A.L.; Gonzálezb, M. J.; Marina, M.L. Enantiomeric separation of chiral polycyclic musks by capillary electrophoresis: Application to the analysis of cosmetic samples. *J. Chromatogr. A,* **2010**, *1217,*1157.

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