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## Enantioseparation and Enantioselective Analysis of Chiral Herbicides

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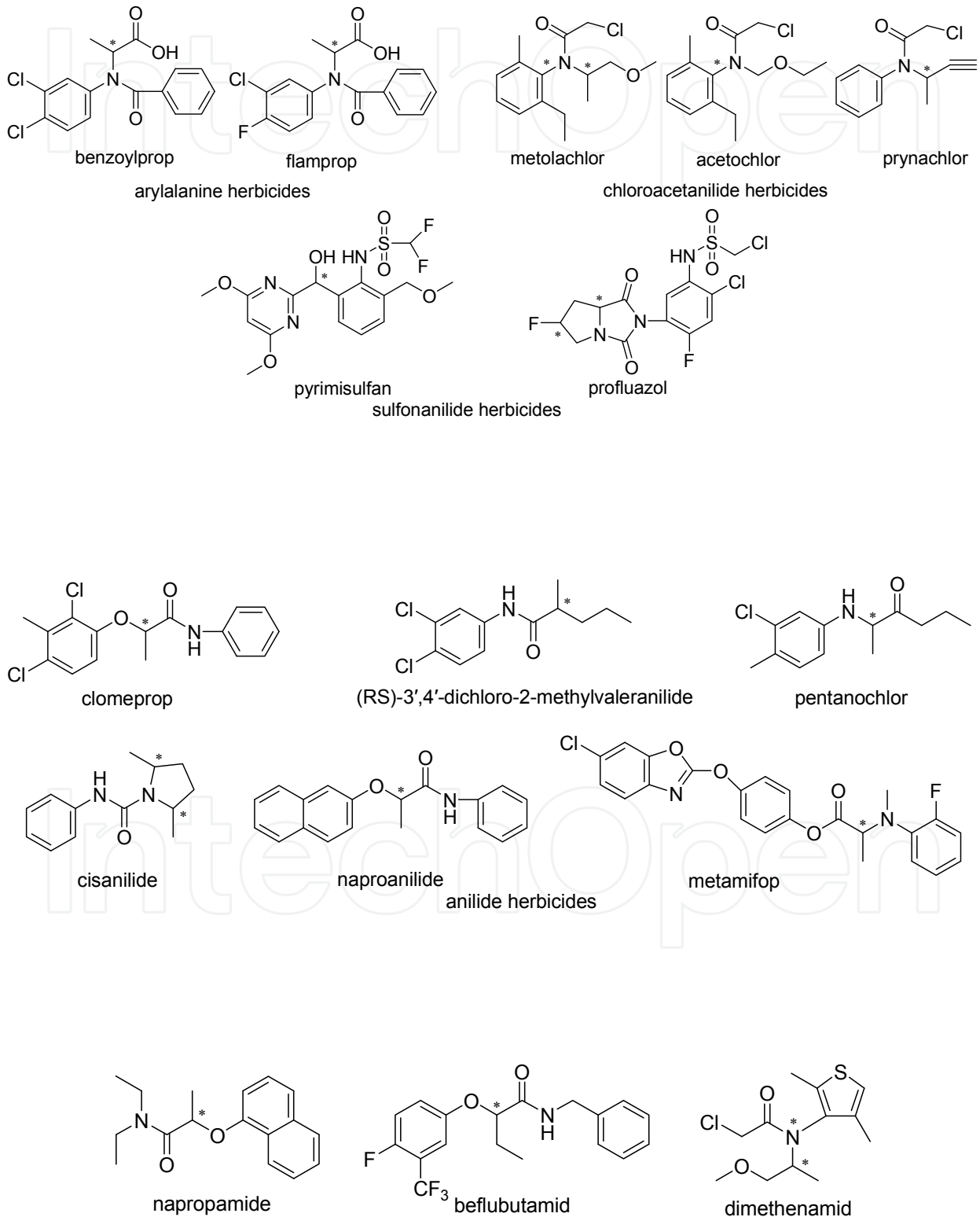
### 1. Introduction

Many commercial agrochemicals in current use contain chiral structures and thus consist of enantiomers. Here chiral herbicide is one of the most important agrochemicals which are widely used. Enantiomers of a chiral compound have identical physical-chemical properties and appear as a single compound in standard analysis. However, the biological effects of enantiomers such as toxicity, mutagenicity, carcinogenicity, and endocrine disruption activity, are generally different, due to the inherent enantioselectivity of biological interactions.

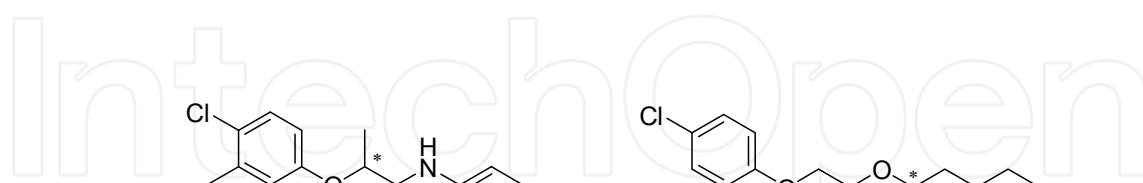
According to the chemical structure, the familiar chiral herbicides have been classified with amide herbicides, phenoxy herbicides, imidazolinone herbicides, organophosphorus herbicides and so on. The analysis and preparation of pure enantiomer herbicides have been summarized with HPLC, GC, CE and SFC methods. Finally, information concerning the stereoselective toxicity and degradation of chiral herbicides in environmental behavior has been offered.

## 2. Classification of chiral herbicides

### 2.1 Amide herbicides

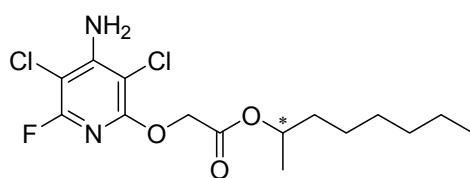


## 2.2 Phenoxy herbicides

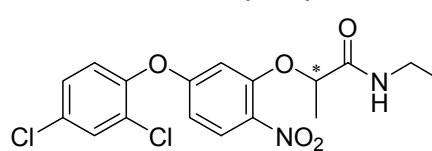


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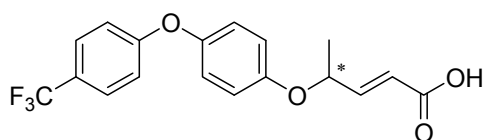
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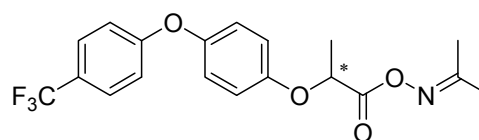
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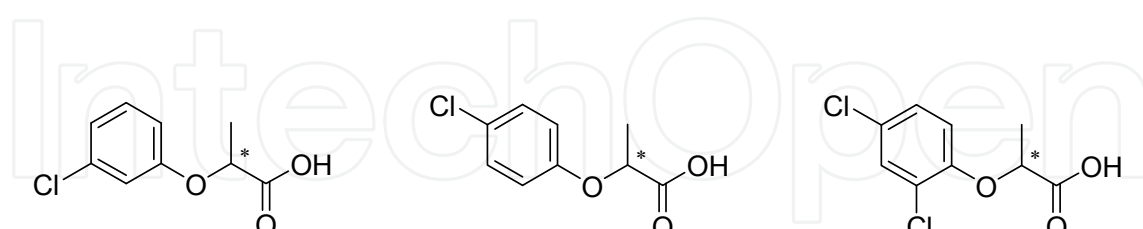
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difenopenten



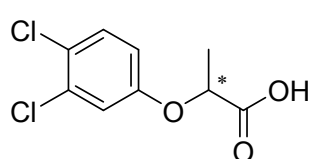
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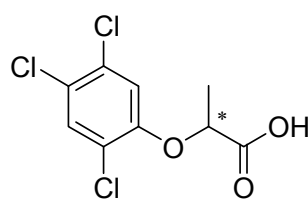
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4-CPP

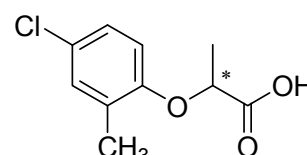
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3,4-DP

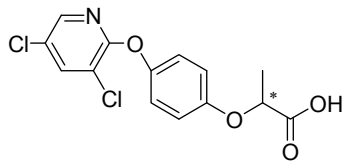


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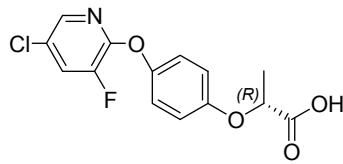


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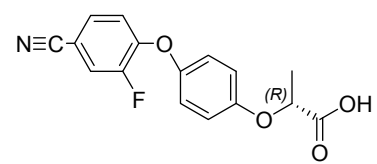
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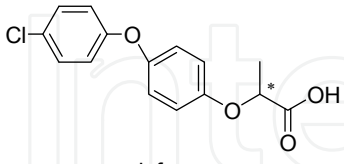
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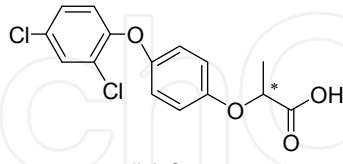
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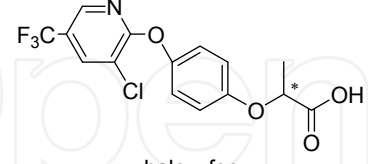
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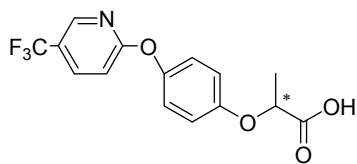
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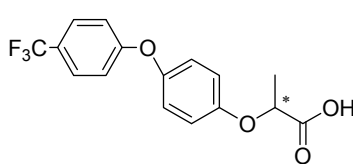
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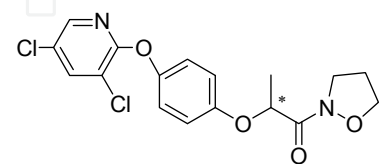
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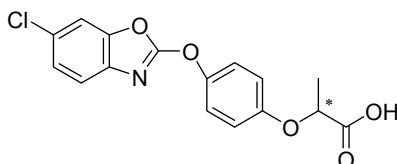
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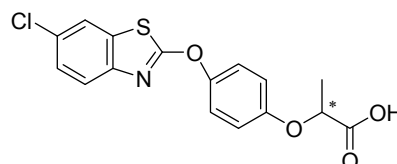
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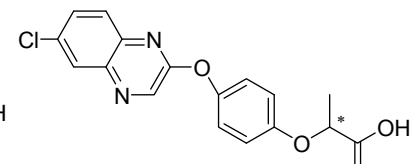
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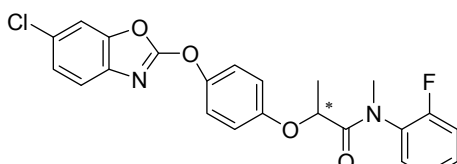
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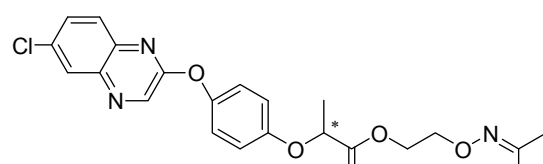
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quizalofop



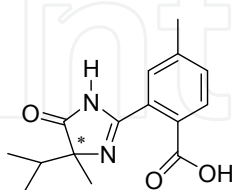
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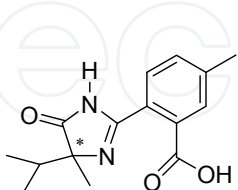
propaquizafop

aryloxyphenoxypropionic herbicides

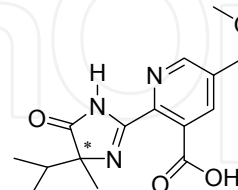
### 2.3 Imidazolinone herbicides



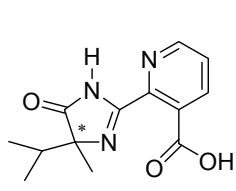
imazamethabenz



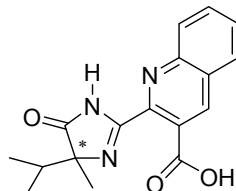
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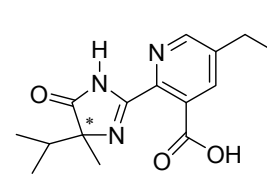
imazapic



imazapyr

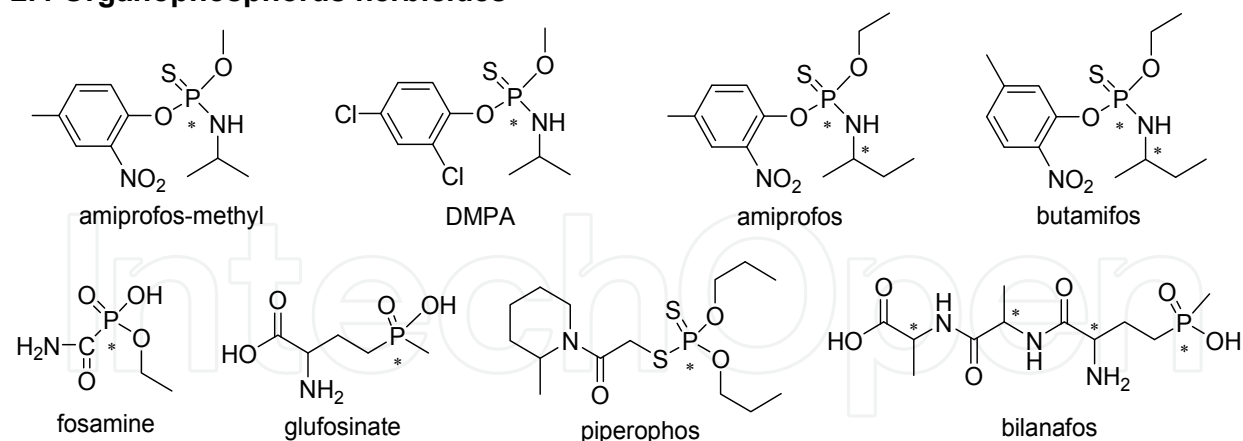


imazaquin

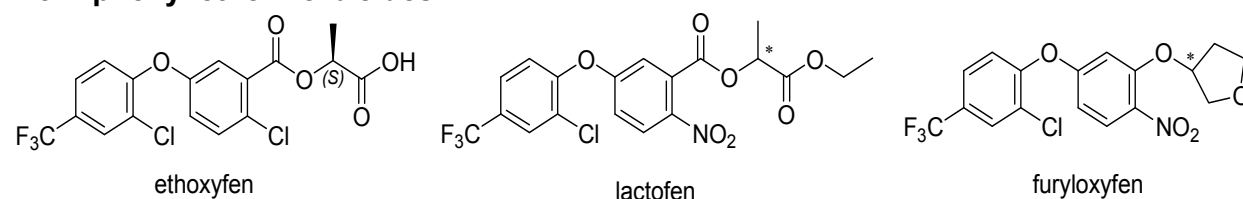


imazethapyr

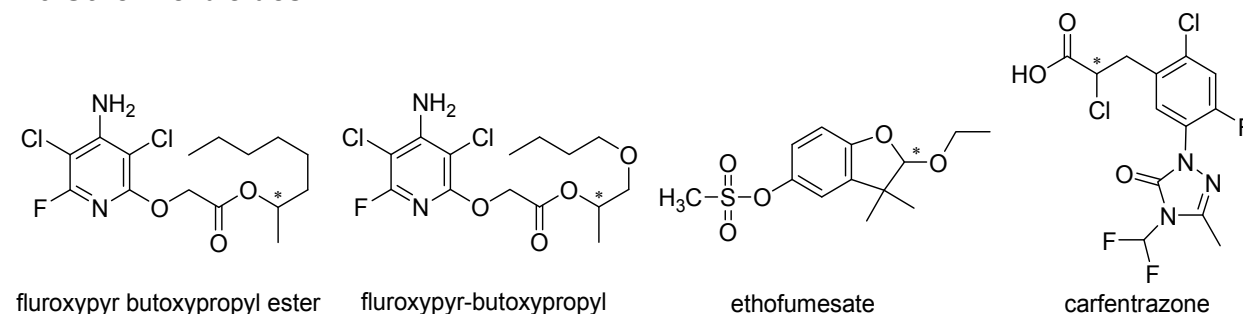
## 2.4 Organophosphorus herbicides



## 2.5 Diphenyl ether herbicides



## 2.6 Other herbicides



## 3. Chromatographic methods for chiral herbicides

### 3.1 Separation of chiral herbicides by HPLC

HPLC combined with kinds of CSPs is one of the most common and easily obtained approaches for enantiomer analysis and preparation. Today, CSPs have been developed at least seven classes, including Pirkle-type CSPs, polysaccharides CSPs, cyclodextrins CSPs, macrocyclic glycopeptide antibiotics CSPs, proteins CSPs, crown ethers CSPs and ligand exchange CSPs, etc. Profiting from the development of CSPs, chiral HPLC methods have held the balance both for determining optical purity of enantiomers and for preparing enantiopure standards.

A group of herbicides, diclofop-methyl, quizalofop-ethyl, lactofen, fluroxypyr-meptyl, acetochlor, ethofumesate, clethodim, napropamide, fenoxaprop-ethyl and carfentrazone-ethyl, were partial or near-baseline separated on self-prepared amylose tris-(S)-1-phenylethylcarbamate CSP by HPLC with  $\eta$ -hexane/isopropanol as mobile phase (Wang *et al.*, 2006).

Chiral pyrazole phenyl ethers (PPE) are highly active herbicides which were resolved by direct HPLC on commercially available CSPs derived from N-3,5-dinitrobenzoyl derivatives of  $\alpha$ -amino acids or amines (Whelk-O 1). Chromatographic resolution obtained was suitable for determination of enantiomeric purities and, in some cases, for preparative resolution of the enantiomers with ee>99% (Hamper *et al.*, 1994). (+)- and (-)-enantiomers of thiobencarb sulfoxide were collected with purities more than 99.0% ee and 99.8% ee on a Chiralcel OB column at 25 °C, 1 mL/min 95/2.5/2.5 hexane/EtOH/MeOH as a mobile phase (Kodama *et al.*, 2002).

### 3.1.1 Enantioseparation of amide herbicides by HPLC

Amide herbicides are a group of important chiral herbicides, and metolachlor, which contains two chiral elements (an asymmetrically substituted carbon and a chiral axis), consists of four stereoisomers stable at ambient temperature with aSS-, aRS-, aSR-, and aRR-configurations. Two of the four metolachlor isomers were isolated from rac-metolachlor in enantio- (ee>98%) and diastereomerically pure forms by a combination of achiral Hypercarb PH and chiral Chiralcel OD-H HPLC with 98/2 n-hexane/IPA. The enantiomer elution sequence is aS prior to aR (retention times, aSS<aRS and aSR<aRR) and 1'S prior to 1'R (retention times, aSS<aSR and aRS<aRR) (Muller *et al.*, 2001). Baseline separation of four metolachlor isomers by HPLC was achieved on Chiralcel OD-H using 91/9 Hex/diethyl ether as the mobile phase by Polcaro *et al.* (Polcaro *et al.*, 2004). Enantiomers and diastereomers of some acetamide pesticides, alachlor, acetochlor, metolachlor, and dimethenamid, were separated using achiral and chiral high-resolution GC/MS (HRGC/MS) and chiral HPLC. Chiral HPLC using modified cellulose and phenylglycine columns also showed some isomer resolution. A novel thermal equilibration procedure allowed distinction among axial-chiral and C-chiral enantiomers (Buser *et al.*, 1995). Additionally, acetochlor enantiomers were partially identified with a cellulose derivative fixed phase CDMPC by HPLC with n-hexane or petroleum ether with different percents alcohol (Peng *et al.*, 2005). And dimethenamid-P was completely resolved on a normal phase Chiralpak AD-H column (Saito *et al.*, 2008).

Another typical amide herbicide, napropamide, was separated both by normal phase HPLC and by reverse phase HPLC by Liu *et al.* (Chen *et al.*, 2006, Zhou *et al.*, 2006). In the former research, a method for the chiral separation and micro-determination of napropamide in water was established on a Chiralcel OJ-H column. The linearity of calibration curve for racemic mixture was 10-100 ng/mL and the correlation coefficient was 0.99 (Chen *et al.*, 2006). In the latter, the enantiomers were resolved using Chiralcel AD-RH and Chiralcel OD-RH with MeCN/H<sub>2</sub>O as mobile phase. The stereoselectivity of Chiralcel AD-RH was better than Chiralcel OD-RH for napropamide (Zhou *et al.*, 2006). In a report by Zhou *et al.* (Tian *et al.*, 2010), napropamide was partially separated ( $R_s$  1.05) under 40/60 MeCN/water reverse phase HPLC on amylose tris(3,5-dimethylphenylcarbamate) CSP (ADMPC).

Flamprop was resolved on 150×4.6 mm I.D. terguride-based CSP (selectivity factor  $\alpha$  1.09) by using 45% 0.02 M potassium acetate buffer (pH 3.5) and 55% MeCN as the elution solvent by HPLC (Padiglioni *et al.*, 1996).

### 3.1.2 Enantioseparation of phenoxy herbicides by HPLC

Phenoxy herbicides are a large group of chiral herbicides with widespread application in agriculture. The most representative herbicides are diclofop, mecoprop (MCPP),



dichlorprop (DCPP) and their derivatives as classified with phenoxypropionic acids herbicides, which are widely applied to control broad-leaf weeds. In Padiglioni's study (Padiglioni *et al.*, 1996), MCP, DCPP, diclofop, fenoxaprop, fenoprop, fluazifop, haloxyfop, quizalofop-ethyl ester and quizalofop were well resolved on 150×4.6 mm I.D. tergride-based CSP by using 0.02 M potassium acetate buffer (pH 3.5)-MeCN as the mobile phase by HPLC. Furthermore, a semipreparative-scale separation of fenoprop enantiomers was carried out on a 250×7.8 mm I.D. column, yielding approximately 1.0 mg of each enantiomer in a single chromatographic run, with a recovery of 88% and optical purity greater than 99%.

Several phenoxypropionic acid herbicides were separated on two CD-derivatived CSPs, Nucleodex  $\alpha$ -PM and Nucleodex  $\beta$ -PM. Phenoxypropionic acids can be divided into three different groups. The first one has one or two small substituents such as methyl, chlorine or hydroxyl at the aromatic ring (e.g. MCP, DCPP). The separation of MCP and DCPP was possibly conducted using NUCLEODEX  $\alpha$ -PM CSP, whereas the methyl ester of these compounds was resolved by both Nucleodex  $\alpha$ -PM and Nucleodex  $\beta$ -PM. A further substitution (e.g. fenoprop R1, R2, R3=C1, R4=H) leads to the second group and results in the failure of the permethylated  $\alpha$ -CD to achieve separation, but fenoprop can be sufficiently resolved by Nucleodex  $\beta$ -PM. The third group contains compounds like fenoxaprop or diclofop with large substituents at the aromatic ring. In this case only the methyl or ethyl esters can be separated by permethylated  $\beta$ -CD. No resolution can be obtained with Nucleodex  $\alpha$ -PM (Riering *et al.*, 1996). Resolution of MCP and DCPP and 2,4-D were proved to be obtained on Nucleodex- $\alpha$ -PM-CD CSP with 70% MeOH and 30% 50 mM NaH<sub>2</sub>PO<sub>4</sub> as eluent by Kohler *et al.* (Zipper *et al.*, 1999) and Bjerg *et al.* (Rugge *et al.*, 2002). MCP and DCPP, and bromacil with a pyrimidinedione ring were better resolved on the native teicoplanin CSPs than the aglycone teicoplanin CSPs with 100% MeOH containing 0.1% TEA and 0.1% acetic acid (v/v) and 20/80 MeOH/water buffered at pH 4.1 by 1% TEAA for bromacil by HPLC (Berthod *et al.*, 2000a). Furthermore, MCPM and DCPPM were better resolved on the native teicoplanin CSPs with 20% MeOH/80% aqueous buffer (pH 4.1 by TEAA, 1%). However, the resolution for bromacil with a pyrimidinedione ring was higher on teicoplanin structurally related A-40926 CSP than on teicoplanin CSP (R<sub>s</sub> 2.8 vs. R<sub>s</sub> 2.5) (Berthod *et al.*, 2000b). Rac-diclofop methyl and rac-diclofop acid were baseline separated on a chiralcel OJ-H column using chiral HPLC coupled with fluorescence detection with a mobile phase of Hex/IPA/HAc (90:10:0.2, v/v) at a flow rate of 0.5 mL/min under 20 °C (Lin *et al.*, 2006) while in a report by Zhou *et al.* (Gu *et al.*, 2010), they were completely resolved on CDMPC CSP with Hex/IPA(98:2) containing 0.1% TFA as mobile phase by HPLC-DAD.

A group of 2-aryloxypropionic acids (TR-1 to 13) and their esters (TR-19 to 20) were used to evaluate four new brush-type CSPs (CSP I-IV) comprising N-3,5,6-trichloro-2,4-dicyanophenyl-L- $\alpha$ -amino acids by HPLC. The best separation of these herbicides was obtained with CSP I, and the (-)-S enantiomer were regularly eluted first. The mechanism of chiral recognition implies a synergistic interaction of carboxylic acid analyte with the chiral selector and achiral free  $\gamma$ -aminopropyl units on silica. (Vinkovic *et al.*, 2001) In a study by Badjah-Hadj-Ahmed (Tazerouti *et al.*, 2002), eleven 2-aryloxypropionic acids and esters herbicides were partially separated on the prepared phenylated  $\beta$ -CD CSP when using heptane and either IPA or chloroform as organic mobile phase modifier.



Enantioseparation of 2,4-DP and MCPP was obtained completely using enantioselective HPLC on a chirobiotic T column with 5:95 MeOH and 1% TEAA as mobile phase (Schneiderheinze *et al.*, 1999).

Fenoxaprop-ethyl could obtain baseline separation on ADMPC CSP by reversed phase HPLC with MeOH/water or MeCN/water at a flow rate of 0.5 mL/min, while the enantiomers of quizalofop-ethyl, fluroxypyr-meptyl and 2,4-D-ethylhexyl got partial separation (Tian *et al.*, 2010).

A group of chlorophenoxypropionic acid herbicides 2,2-CPPA, 2,3-CPPA and 2,4-CPPA were separated in capillary LC, while with 0.1 mM teicoplanin in the mobile phase was sufficient for the baseline enantioresolution of 2,2-CPPA and 2,4-CPPA (Kafkova *et al.*, 2005). Eight commercially available herbicides, dimethenamid-P, dichlorprop-P, fluazifop-P butyl, mecoprop-P, quizalofop-P ethyl, were completely resolved by HPLC combined with a photodiode-array (PDA) detector and a circular dichroism (CD) detector on a normal phase Chiralpak AD-H column (Saito *et al.*, 2008). Optical purity measurement was developed. The enantiomeric excess (ee) of some herbicides investigated was approximately over 95%, while of quizalofop-P ethyl and fluazifop-P butyl was in the range 34.1-94.5%.

### 3.1.3 Enantioseparation of imidazolinone herbicides by HPLC

Imidazolinones are a class of chiral herbicides that are widely used. They inhibit branched-chain amino acid biosynthesis in plants by targeting acetolactate synthase (ALS). Five imidazolinone herbicides imazapyr, imazapic, imazethapyr, imazamox and imazaquin and their methyl derivatives were separated using reversed phase HPLC on Chiralcel OD-R and normal phase HPLC on Chiralcel OJ (Lao *et al.*, 2006a). Enantiomers of imazethapyr, imazaquin, and imazamox were separated on a Chiralcel OD-R column using 50 mM phosphate buffer-MeCN as mobile phase. Enantiomers of imazethapyr, imazaquin, and imazamox were separated on a Chiralcel OD-R column using 50 mM phosphate buffer-MeCN as mobile phase. Enantiomers of imazapyr, imazapic, imazethapyr, imazamox, imazaquin and their five methyl derivatives were resolved on a Chiralcel OJ column using Hex (0.1% TFAA)-alcohol as mobile phase. The described normal phase method was successfully applied for chiral analysis of two imidazolinone herbicides (imazapyr and imazaquin) in spiked soil samples. In a further report (Lao *et al.*, 2006b), temperature affects on enantioseparation of these five imidazolinone herbicides and conformation of CSP were conducted on Chiralcel OJ. The van't Hoff plots of retention factor ( $k'$ ), distribution constant ( $K$ ) and separation factor ( $\alpha$ ) for imazapyr, imazapic, imazethapyr, and imazamox were linear within 15-50 °C. Nonlinear van't Hoff plots of  $\alpha$  were observed for imazaquin with mobile phase of Hex (0.1% TFA)-IPA at 70/30 or 60/40 (v/v). Chiralcel OJ column may yield satisfactory results at 15-50 °C but not at  $\leq 15$  °C.

Recently, Lin *et al.* (Lin *et al.*, 2007) also investigated the enantiomeric separation of imazethapyr, imazapyr, and imazaquin on Chiralpak AS, Chiralpak AD, Chiralcel OD, and Chiralcel OJ columns. Chiralcel OJ column showed the best chiral resolving capacity among the test columns. The optimal chromatographic conditions for complete separation of imidazolinone enantiomers were a mobile phase of Hex/EtOH/HAc (77/23/0.1, v/v/v), flow rate of 0.8 mL/min, and a column temperature in the range of 10-30 °C. It was showed that small amounts of enantiopure imidazolinones may be prepared by using the analytical chiral HPLC approach.

Enantiomers of imazethapyr were separated by HPLC on Chiralcel OJ with a Hex/EtOH/HAc solution (75/25/0.5 by volume) (Zhou *et al.*, 2009, Zhou *et al.*, 2010), and their absolute configurations were confirmed as S-(+)-IM and R-(-)-IM by the octant rule as shown in Fig. 3-1.

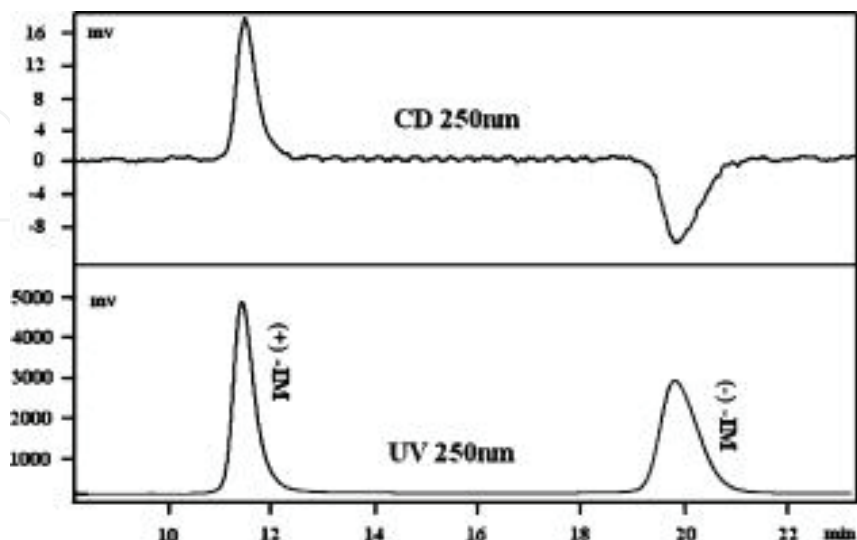


Fig. 3-1 HPLC chromatogram for the enantiomeric separation of imazethapyr on Chiralcel OJ. (Zhou *et al.*, 2009)

### 3.1.4 Enantioseparation of organophosphorus herbicides by HPLC

Five chiral O-aryl O-alkyl N-alkylphosphoramidothioates herbicides were nearly baseline separated on a pirkle-type column OA-4700 (Chirex(S)-LEU & (R)-NEA) by HPLC. The chromatographic elution order is S>R, and the S-enantiomer showed higher herbicidal activity than R-enantiomer and/or racemates (Gao *et al.*, 2000).

In another report by our group (Li *et al.*, 2008), enantioselective separation and biological toxicity of a series of 1-(substituted phenoxyacetoxy)alkylphosphonates organophosphorous compounds (OPs compounds 1-5) were investigated on Chiralpak AD, Chiralpak AS, Chiralcel OD, and Chiralcel OJ. All the analytes investigated obtained baseline resolution ( $R_s > 1.5$ ) on Chiralpak AD, which showed best chiral separation capacity. The acute aquatic toxicity of enantiomers and racemate to *Daphnia magna* (*D. magna*) were assessed. The in vivo assays showed that compound 3 was about 2-148.5 times more toxic than the other four analogues to *D. magna*. The racemates of compounds 3 and 5 showed intermediate toxicity compare to their enantiomers, while those of compounds 1, 2, and 4 showed synergistic or antagonistic effect. These results suggest that the biological toxicity of chiral OPs to nontarget organisms is enantioselective and therefore should be evaluated with their pure enantiomers.

### 3.1.5 Enantioseparation of diphenyl ether herbicides by HPLC

Ethoxyfen-ethyl and lactofen were separated using HPLC on polysaccharide CSPs by Zhou *et al.* (Wang *et al.*, 2006, Tian *et al.*, 2010, Hou *et al.*, 2002, Diao *et al.*, 2009). Enantioseparation of a novel herbicide ethoxyfen-ethyl was conducted on self-prepared CDMPC, and with the content of IPA in hexane in mobile phase decreased to 1%, the resolution factors increased to 3.95 (Hou *et al.*, 2002). The two enantiomers of the herbicide lactofen in soils were baseline

separated and semiprepared on CDMPC using a normal phase HPLC (n-Hex/IPA 95/5). However, the baselined separation was not obtained on a self-prepared tris-(S)-1-phenylethylcarbamate CSP (Wang *et al.*, 2006). And lactofen also could be completely resolved ( $R_s$  2.07) by a reserved phase HPLC using 80/20 MeOH/H<sub>2</sub>O as mobile phase on ADMPC (Tian *et al.*, 2010).

### 3.2 Separation of chiral herbicides by GC

GC is more suitable in analyzing because of its higher sensitivity, higher precision and less injection volume than HPLC system. Besides, contaminants and impurities usually can be separated from the analytes easily by GC.

The most common chiral selectors used for GC are a group of CD and CD-derivatives. Enantiomers and diastereomers of some acetamide pesticides, alachlor, acetochlor, metolachlor, and dimethenamid, were separated using achiral and chiral high-resolution GC/MS (HRGC/MS) and chiral HPLC. Whereas alachlor is achiral, all other compounds are axial- and/or C-chiral and consist of two or four stereoisomers (enantiomers and diastereomers). Chiral HRGC using a  $\beta$ -CD derivative showed varied resolution of diastereomers and/or enantiomers; achiral HRGC showed no resolution of diastereomers. Resolution of C-chiral enantiomers was easier than resolution of axial-chiral enantiomers (atropisomers) (Buser *et al.*, 1995). And all four metolachlor isomers were identified by HRGC (Muller *et al.*, 2001).

Leachate samples from a waste disposal site in Switzerland and groundwater samples downstream of the landfill were analyzed for residues of MCP, DCP, and 2,4-D esterified with 2,3,4,5,6-Pentafluorobenzyl (PFB) by means of enantiomer-specific GC-MS (Zipper *et al.*, 1999, Zipper *et al.*, 1998). The PFB esters of MCP and DCP were nearly baseline separated ( $R_s=0.9$ ) on a 15 m glass column (0.25 mm i.d.) with an OV1701 polysiloxane phase containing 35% heptakis(2,3-dimethyl-6-tert-butyl-dimethylsilyl)- $\beta$ -CD (TBDM- $\beta$ -CD) as the chiral selector.

A capillary column BGB-172 (20% tert-butyl-dimethylsilyl- $\beta$ -CD dissolved in 15% diphenyl-polysiloxane and 85% dimethyl-polysiloxane, GBG Analytik, Adliswil, Switzerland) was used for chiral GC separation of some herbicides by Liu *et al.* (Wen *et al.*, 2004, Ma *et al.*, 2006, Ma *et al.*, 2009). DCP methylated by diazomethane in water was separated and determined with a recovery about 90% (Wen *et al.*, 2004). They also separated rac-metolachlor and S-metolachlor in soil. However, the baseline separation was not achieved because of the presence of two chiral elements (asymmetrically substituted carbon and chiral axis nitrogen) (Ma *et al.*, 2006). Furthermore, the enantiomeric separation of DCPM was investigated by GC on BGB-172 and HPLC on Chiralcel OJ-H by this group. Baseline separation by both GC and HPLC was achieved (Ma *et al.*, 2009).

### 3.3 Separation of chiral herbicides by SFC

As complementary analytical techniques for HPLC, packed-column SFC with sub- and/or supercritical fluid contains kinds of organic polar solvents is becoming a very popular chromatographic technique, for both analysis and small-scale preparation of optically pure chemicals and enantiomers identification, especially as CSPs are becoming easily available and widely applied. Nearly all the conventional HPLC CSPs could be applied in SFC mode except the chiral crown ester CSPs and the protein-based CSPs. Sub- and supercritical carbon dioxide (CO<sub>2</sub>) remains the most commonly used fluid for SFC. Mechanistically, SFC

plays a unique role acting as a bridge between GC and LC. Owing to the good diffusibility and low viscosity of supercritical fluids, column equilibration is accomplished more rapidly and enables faster flow rates in SFC than in HPLC. Besides, the higher diffusivity between mobile phase and CSPs yields greater efficiency (smaller plate heights) in resolving a sample.

Generally, SFC shows notable advantages and superior developmental potential on enantiomer separation. The advantages contain environmental friendly with low organic solvent consumption of mobile phase, simple method development, high efficiency on enantioseparation, low column pressure drop besides ease of coupling with chiral columns or MS. However, the high investment of SFC apparatus restricts its widespread application in enantioseparation. To date, the research about chiral herbicides separation by SFC is very limited. One herbicide example that can be resolved by SFC is the diastereomeric compound metolachlor. The ability to quickly detect and identify metolachlor and its isomeric ratios in low concentration samples is possible, via SFC (Cole *et al.*, 2007).

### 3.4 Separation of chiral herbicides by CE

CE is shown to be a simple, efficient, and inexpensive way with unique versatility to chiral separation because it can be applied to a wide variety of analytes flexibly with various modes. Hitherto, six separation modes of CE has been successfully used in chiral separation, including capillary zone electrophoresis (CZE), capillary electrochromatography (CEC), micellar electrokinetic chromatography (MECC or MEKC), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), capillary isotachopheresis (CITP) (Li *et al.*, 2010), where CZE, CEC and MEKC are the most successful CE modes. For the enantioseparation of chiral herbicides by CE, CD and its derivatives are often added to the electrophoresis buffer as the chiral selectors.

Some chlorophenoxy acid herbicides and their enantiomers, 2,4-dichlorophenoxy-acetic acid (2,4-D), 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP), 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB), 4-chloro-2-methylphenoxyacetic acid (MCPA), were successfully separated within 7 min by adding 4 mM  $\alpha$ -CD and 1 mM  $\beta$ -CD in the buffer in CE (Hsieh *et al.*, 1996). Analyzing the herbicides by CE posed the advantages of a high resolution, high separation efficiency and good reproducibility.

A novel, selective precolumn derivatization reaction was introduced and evaluated in the fluorescence labeling of phenoxy acid herbicides including 2,4-D, (2,4,5-trichlorophenoxy)-acetic acid (2,4,5-T), 2-phenoxypropionic acid (2-PPA), MCPA, 2-(2-chlorophenoxy)propionic acid (2,2-CPPA), 2-(3-chlorophenoxy)propionic acid (2,3-CPPA), 2-(4-chlorophenoxy)propionic acid (2,4-CPPA), DCPA and silvex with 7-aminonaphthalene-1,3-disulfonic acid (ANDSA) by CE (Mechref *et al.*, 1996a). The ANDSA-phenoxy acid herbicide enantiomers exhibited higher chiral resolution than their underivatized counterparts in the presence of CD in the running electrolyte. The best enantioselectivity was achieved when 2,3,6-tri-O-methyl- $\beta$ -CD (TM- $\beta$ -CD) was used as the chiral selector. Mixed CDs based on  $\beta$ -CD and TM- $\beta$ -CD proved to be the most effective as far as the enantiomeric resolution of the chiral analytes is concerned.

A novel chiral nonionic surfactant, namely octyl-*b*-D-maltopyranoside (OM), was evaluated in chiral CE of fluorescently labeled phenoxy acid herbicides (Mechref *et al.*, 1997a). The labeling of the analytes with 7-aminonaphthalene-1,3-disulfonic acid (ANDSA) permitted a concentration detection limit of  $5 \times 10^{-10}$  M using laser-induced fluorescence detection. The tagging of the phenoxy acid herbicides with ANDSA increased the hydrophobicity of the



analytes, thus favoring an enhanced solubilization of the derivatized herbicides in the OM micellar phase. The net results of this effect were a much shorter analysis time and an improved enantiomeric resolution of the derivatives when compared to underivatized phenoxy acid herbicides. Baseline enantiomeric resolution of phenoxy acid herbicides including silvex, DCP, MCP, 2,4-CPPA, 2,3-CPPA, 2,2-CPPA and 2-PPA was attained without 30 min by CE using 200 mM sodium phosphate buffer, pH 6.5, containing 60 mM n-octyl- $\beta$ -D-maltopyranoside (OM) (Mechref *et al.*, 1996b). Silvex, DCP, MCP, 2,4-CPPA, 2,3-CPPA, 2,2-CPPA and 2-PPA were baseline separated except silvex by performing the separation at 10 °C and using 250 mM sodium phosphate buffer, pH 6.5, containing 50 mM n-nonyl- $\beta$ -glucopyranoside (NG) or 70 mM n-octyl- $\beta$ -glucopyranoside (OG) in CE. (Mechref *et al.*, 1997b)

Vancomycin was used as chiral selector for the enantiomeric separation of several free acid herbicides including MCP, fenoprop, DCP, flamprop, haloxyfop, fluazifop, diclofop and fenoxaprop in CE (Desiderio *et al.*, 1997a). The increase of vancomycin concentration caused a general increase of migration time, resolution and selectivity. Baseline resolution was achieved when a 6 mM vancomycin was used. The CE separation of some herbicidal enantiomers was conducted applying 1-allyltergicide as chiral selector (Ingelse *et al.*, 1997). Baseline separation was shown for the enantiomers of fluazifop, halossifop and fenoxaprop, whereas the optical isomers of flamprop could be partially resolved using 100 mM  $\beta$ -alanine-acetate, 50 mM TEA in 100% MeOH supported with 100 mM allyl-TER. Separation times are short compared to similar analyses, applying HPLC and a tergicide CSP.

The enantiomers of a number of 2-aryloxypropionic acids and their ester and amide counterparts are readily separated on the commercially available  $\beta$ -GEM 1 and Whelk-O 1 CSPs. Of the analytes studied, the N,N-diethylamides typically show the greatest enantioselectivity. The enantiomers of several commercial herbicides from this family, including diclofop ethyl, devrinol, and MCP were separated using the Whelk-O 1 CSP.  $\beta$ -Gem1 is a  $\pi$ -acceptor chiral stationary phase and is prepared by covalently bonding N-3,5-dinitrobenzoyl-3-amino-3-phenyl-2-(1,1-dimethylethyl)-propanoate, to 5  $\mu$ m silica through an ester linkage. (Pirkle *et al.*, 1997)

Baseline enantiomeric separation of a mixture of six pairs of phenoxypropionic acid herbicides (PPAHs) including 2,3-CPPA, 2,2-CPPA, 2,4-CPPA, 2(2,4-DCPPA), 2(2,4,5-TCPPA) and 2-PPA was achieved in less than 30 min by CE with heptakis(6-methoxyethylamine-6-deoxy)- $\beta$ -CD [ $\beta$ -CD-OMe (VII)] as chiral selector. The two most substituted herbicides [2(2,4-DCPPA) and 2(2,4,5-TCPPA)] were best resolved. One of the faster migrating antipodes of 2(2,4,5-TCPPA) co-eluted with one slower antipode of 2(2,4-DCPPA) while both baseline separation was obtained run separately (Fig. 3-2) (Haynes *et al.*, 1998).

DCP and imazaquin was analyzed by CE as an anion (Jarman *et al.*, 2005). DCP was separated using 25 mM sodium tetraborate (Na-TB), pH 8.5, with 25 mM trimethyl- $\beta$ -CD as the chiral selector, while imazaquin was analyzed with 15 mM dimethyl- $\beta$ -CD in 50 mM acetate, pH 4.5. Furthermore, sodium hydrogen phosphate (50 mM) at pH 10.1 containing 30 mM hydroxypropyl- $\beta$ -CD (HP- $\beta$ -CD) was found to be the suitable BGE to separate imazaquin enantiomers in field soils (Yi *et al.*, 2007). In another report (Han *et al.*, 2008), the two imazethapyr enantiomers were separated using 6% hydroxypropyl- $\beta$ -CD as chiral selector in buffer at pH 11.0.

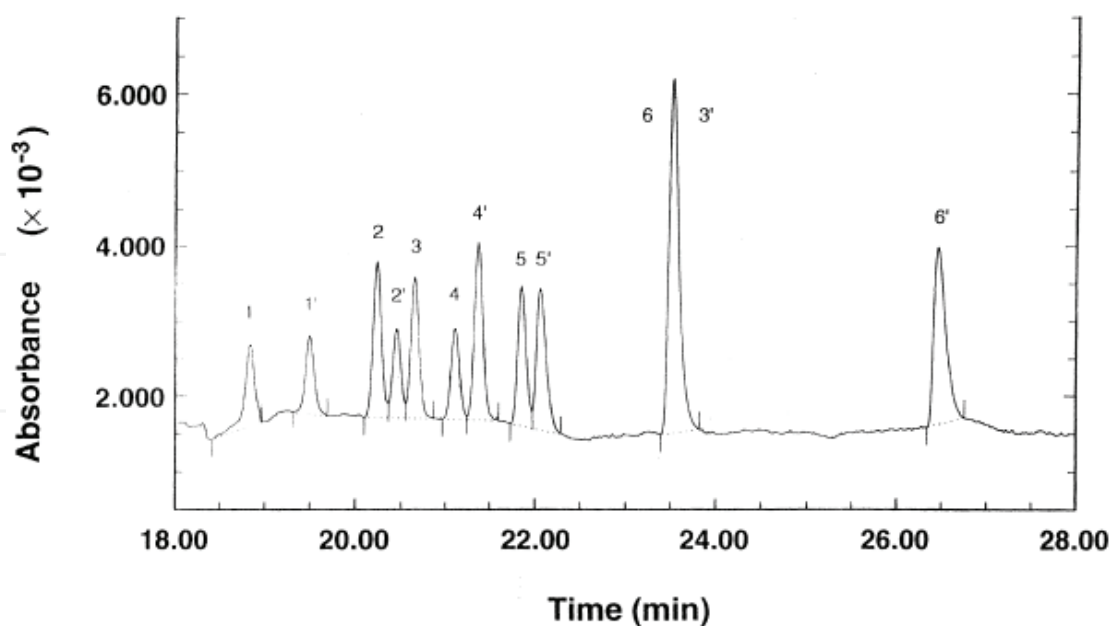


Fig. 3-2. Enantiomeric separation of a standard mixture of 12 ( $\pm$ ) PPAH enantiomers. The BGE contains 50 mM  $\text{NaH}_2\text{PO}_4$  adjusted to pH 6; 3 mM  $\beta$ -CD-OMe (VII); applied voltage was -15 kV, -25  $\mu\text{A}$ ; pressure injection 85 kPa s; sample concentration 0.1 mg/mL in methanol-water (1:1, v/v). 1,1'=2-PPA, 2,2'=2,4-CPPA, 3,3'=2(2,4-DCPPA), 4,4'=2,2-CPPA, 5,5'=2,3-CPPA, 6,6'=2(2,4,5-TCPPA). (Haynes *et al.*, 1998)

### 3.4.1 Separation of chiral herbicides by CZE

The separation mechanism for CZE is based on the differences about the charge/mass ratios. Uncoated fused-silica capillary is filled with some type of electrolyte solution (running buffer or BGE). An electric field is applied to the capillary, and then cations go to the cathode, whereas anions migrate to the anode (Pico *et al.*, 2003).

CD-CZE was applied successfully to the enantiomeric and isomeric separation of chiral herbicides.

Chiral separations of phenoxypropionic acid herbicides were achieved by adding a suitable CD-type chiral selector to the electrophoresis buffer (Nielen, 1993, Otsuka *et al.*, 1998, Zerbinati *et al.*, 2000). DCP, fenoprop and MCP, were baseline separated by the coupling of CE-MS with 20 mM TM- $\beta$ -CD in 50 mM ammonium acetate (pH 4.6) (Otsuka *et al.*, 1998). Separation of the four enantiomers of MCP and DCP was conducted on an ethylcarbonate derivative of  $\beta$ -CD with three substituents per molecule, hydroxypropyl- $\beta$ -CD and native  $\alpha$ -CD as chiral selectors in CZE. Complete resolution of the four optical isomers was obtained with 10 mM ethylcarbonate- $\beta$ -CD in the running buffer of 45 mM  $\text{NaH}_2\text{PO}_4$ , pH 5.6 (Zerbinati *et al.*, 2000).

The separation and detection of 2,4-dichlorophenoxyacetic acid and three optically active phenoxy acid herbicides (DCP, MCP and fenoprop) was investigated in CZE (Garrison *et al.*, 1994). A 50 mM acetate buffer at pH 4.5 gave the best separation. Baseline separation of the two enantiomers of each three optically active herbicides, separately and in mixtures of the three, was accomplished by the addition of 25 mM tri-*O*-methyl- $\beta$ -CD to the acetate separation buffer. Di-*O*-methyl- $\beta$ -CD or  $\alpha$ -CD separated enantiomers of DCP and MCP, but not those of fenoprop;  $\beta$ -CD provided very little separation and  $\gamma$ -CD gave no separation.

Several chiral herbicides, bromacil, chlorbufam, ethofumesate, imazapyr, flamprop-isopropyl, flamprop-free acid, fluazifop-free acid, haloxyfop-free acid, and napropamide, were separated in CZE (Desiderio *et al.*, 1997b). Different  $\beta$ -CD derivatives were investigated for chiral separations and among them the negatively charged sulfobutyl ether  $\beta$ -CD (SBE- $\beta$ -CD) proved to be effective for the stereoselective resolutions of the investigated herbicides. Addition of SBE- $\beta$ -CD (5-50 mg/mL) to the buffer at pH 9 resulted in a general increase of migration times as well as resolution. A CD concentration as low as 5 mg/mL was effective to completely resolve napropamide and ethofumesate enantiomers.

The enantiomeric and isomeric separation of imazaquin, diclofop and imazamethabenz was investigated in CD-CZE (DM- $\beta$ -CD, TM- $\beta$ -CD and HP- $\gamma$ -CD) (Penmetsa *et al.*, 1997). The enantiomers of imazaquin and diclofop, and the isomers of imazamethabenz could be resolved with  $R_s \geq 1.5$  (Fig. 3-3). By employing mixed CDs in the running buffer, the three herbicides were simultaneously separated in a single run (Fig. 3-4).

The separation of DCPD was reported in CZE with  $\alpha$ -,  $\beta$ - and  $\gamma$ -CDs as well as their chemical derivatives C<sub>6</sub>-capped- $\beta$ -CD, ethylcarbonate- $\beta$ -CD, ethylcarbonate- $\gamma$ -CD, methyl- $\beta$ -CD and hydroxypropyl- $\beta$ -CD as chiral selectors. Several of the investigated CDs allowed DCPD enantiomer resolution. In particular, a newly synthesised ethylcarbonate derivative of  $\beta$ -CD showed the best enantiomer resolution properties among the tested compounds. (Zerbinati *et al.*, 1998)

Biological degradation of acetanilide herbicides in soil results in the formation of the ethanesulfonic acid (ESA) and oxanilic acid (OXA) derivatives. These molecules exist in two (alachlor), four (acetochlor), and eight (metolachlor) stereoisomeric forms. Using  $\gamma$ -CD as chiral selector in CZE, complete separation of all four isomers of enantiomerically enriched (5S)-metolachlor OXA was achieved. The enantiomers of acetochlor ESA, acetochlor OXA, and racemic metolachlor OXA were partially separated. (Aga *et al.*, 1999)

CZE was used for the chiral and mutual separation of four phenoxy acid herbicides, fenoprop, dicloprop, MCPD and 2,4-DB, using highly sulphated CD (HSCD) in the buffer. The CE runs were performed with reverse polarity (anode in the outlet vial) using the acidic ammonium formate buffer (20 mmol, pH 3.0). The chiral separation of dicloprop and MCPD were achieved with  $\alpha$ -HSCD but it was not able to resolve fenoprop. With  $\beta$ -HSCD the required base line separation was achieved. The limit of detection (S/N= 3) achieved in present case is 0.15 ppm for fenoprop, 0.14 ppm for dicloprop and MCPD and 0.11 ppm for 2,4-DB. (Malik *et al.*, 2009)

Soil samples taken from a field plot at increasing time intervals after application of Foxtril, a commercial herbicide formulation, were solvent-extracted and analyzed for total DCPD by CZE, using an acetate buffer at pH 4.7. TM- $\beta$ -CD, was then added to the buffer as chiral reagent to effect separation of the (+)- and (-)-enantiomers of DCPD. Baseline resolution allowed calculation of relative concentrations (enantiomer ratios) of the two isomers. The hydrolysis product [methyl 2-nitro-5-(2,4-dichlorophenoxy) benzoic acid] of bifenox methyl ester, another herbicide component of Foxtril, was detected in the soil samples taken at 17 and 31 d. The acetate separation buffer was 50 mM at pH 4.65 and was composed as follows: 0.05 M glacial acetic acid: 0.05 M sodium acetate, 1:1, v:v. The cyclodextrin-containing buffer for enantiomeric separation was prepared by dissolving TM- $\beta$ -CD in the acetate separation buffer to a final concentration of 25 mM cyclodextrin. (Garrison *et al.*, 1996)



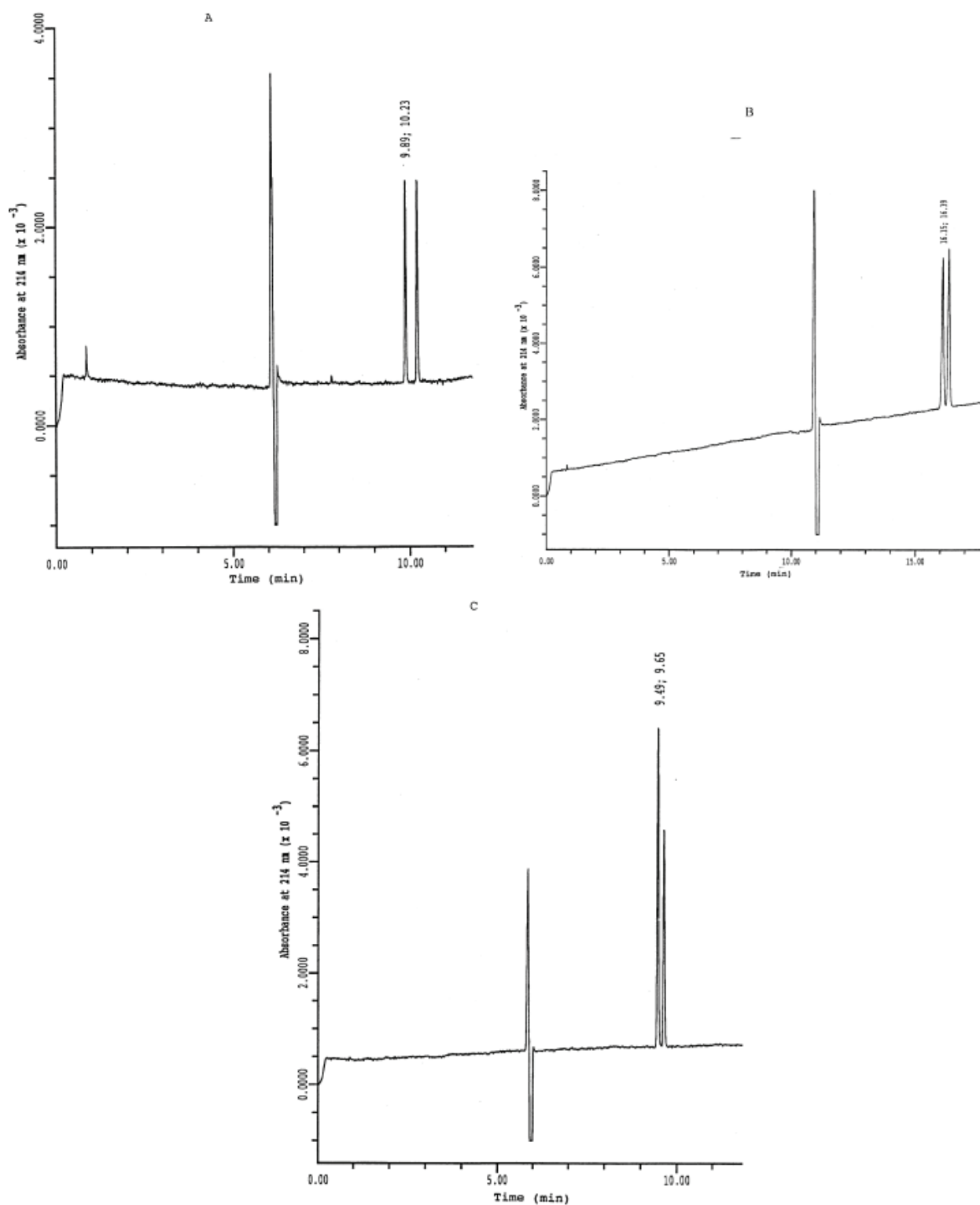


Fig. 3-3. Separation of (A) imazaquin enantiomers, (B) diclofop enantiomers and (C) imazamethabenz isomers (9.49 min, *para* and 9.65 min, *meta* isomers). Analysis conditions: 57 cm (50 cm to detector)  $\times$  50  $\mu$ m I.D. capillary column; pressure injection (2 s=2.4 nl); 25 kV (35  $\mu$ A); 214 nm UV absorbance. Buffer: (A) 50 mM sodium acetate + 10 mM DM- $\beta$ -CD buffer, pH 4.6, (B) 50 mM sodium acetate + 10 mM TM- $\beta$ -CD buffer, pH 3.6 and (C) 50 mM sodium acetate + 10 mM TM- $\beta$ -CD buffer, pH 4.6.

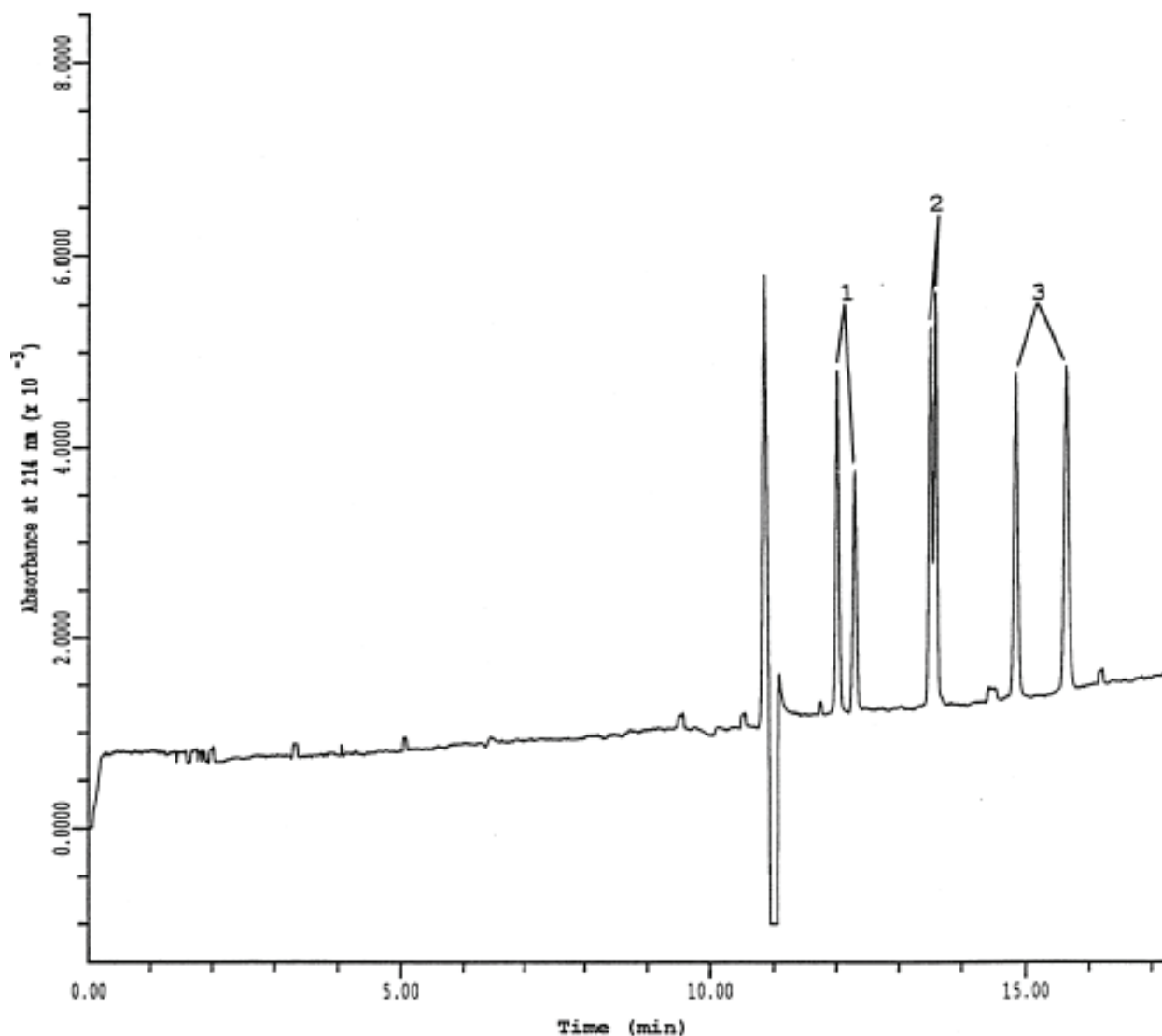


Fig. 3-4. Simultaneous separation of herbicides using mixed cyclodextrins. (1) Imazamethabenz isomers, (2) diclofop enantiomers and (3) imazaquin enantiomers. Analysis conditions: 57 cm (50 cm to detector)  $\times$  50  $\mu$ m I.D. capillary column; pressure injection (2 s=2.4 nl); 50 mM sodium acetate + 10 mM DM- $\beta$ -CD + 10 mM TM- $\beta$ -CD buffer, pH 3.6; 25 kV (35  $\mu$ A); 214 nm UV absorbance. (Penmetsa *et al.*, 1997)

### 3.4.2 Separation of chiral herbicides by CEC

CEC utilises a stationary phase rather than a micellar pseudo-stationary one. CEC is a hybrid technique that couples the selectivity of LC and the separation efficiency of CE. Both charged and uncharged compounds can be separated effectively using CEC.

A series of herbicide molecules (haloxyfop, fluazifop, fenoxaprop, and flamprop free acids, diclofop, MCP, DCP, fenoprop, 2-PPA) were separated using a CSP derived from an L-RNA aptamer by CEC after binding to biotin and grafting upon streptavidin-modified porous glass beads. (Andre *et al.*, 2006)

A porous monolithic chiral column was prepared by in situ copolymerization of glycidyl methacrylate, methyl methacrylate and ethylene glycol dimethacrylate in the presence of formamide and 1-propanol as the porogen solvents to analyze the DCP enantiomers.

Subsequently, the epoxide groups at the surface of the monolith were reacted with (+)-1-(4-aminobutyl)-(5R,8S,10R)-terguride as the chiral selector. Optimum conditions for the herbicide resolution by CEC were found using mobile phases consisting of HAc/TEA mixtures in MeCN:MeOH (9:1 v/v). Under these conditions fully separation of DCPD enantiomers in the presence of clofibrac acid (internal standard) was achieved in about 5 min. (Messina *et al.*, 2007)

A silica based monolithic capillary column derivatized with *O*-9-(tert-butylcarbamoyl)quinidine was prepared for CEC enantiomer separation of chiral 2-aryloxypropionic acid herbicides including *inter alia* DCPD, MCPD and fenoprop. Reasonable baseline separations of enantiomers were accomplished for all analytes after optimization of relevant mobile phase parameters in the anion-exchange CEC system, and the separations were comparable to such obtained on an optimized high density quinidine-carbamate modified organic polymer monolith column. (Buchinger *et al.*, 2009)

### 3.4.3 Separation of chiral herbicides by MEKC

MEKC separation mechanism is based on the differences between interactions of analytes with micelles present in the separation buffer, which can easily separate both charged and neutral solutes with either hydrophobic or hydrophilic properties.

Silvex was separated partially with 50.0 mM *N,N*-bis-(3-*D*-gluconamidopropyl)deoxycholamide as chiral selector, 400.0 mM borate treated fused-silica capillaries at pH 10.0, 15 °C, voltage 20.0 kv in MEKC. (Mechref *et al.*, 1996c)

Enantiomeric ratios of methyl esters of phenoxy acids herbicides and an acetamide herbicide metolachlor were being measured. Each of six CD,  $\alpha$ -CD,  $\beta$ -CD,  $\gamma$ -CD, hydroxypropyl- $\beta$ -CD, dimethyl- $\beta$ -CD and trimethyl- $\beta$ -CD, were then added to the borate-SDS buffer, with and without the organic modifier, to test for separation of the non-chiral compounds and the enantiomers of the chiral racemates by CD-MEKC.  $\gamma$ -CD with MeOH modifier allowed baseline separation of the three phenoxy acid methyl esters and of fenoprop methyl ester, but none of the CDs separated the enantiomers of MCPD and DCPD. Finally, attempts were made to separate the four enantiomers of the herbicide metolachlor; three of the enantiomers were separated by  $\gamma$ -CD with methanol. (Schmitt *et al.*, 1997)

The enantiomeric resolution of chiral phenoxy acid herbicides was performed by MEKC using several neutral and charged CD as chiral pseudophase (CD-MEKC). Among the CDs tested, HP- $\beta$ -CD was found to be the most appropriate for the enantioseparation of phenoxy acids. The use of a 50 mM electrolyte solution in ammonium formate at pH 5 containing 15 mM HP- $\beta$ -CD and a temperature of 40 °C enabled the enantiomeric resolution of four of the six phenoxy acids investigated (2-PPA, 2,3-PPA, 2,4-CPPA, and 2-(2,4-DCPPA)) obtaining migration times ranging from 9 to 15 min. Mixtures of the two phenoxy acids not enantiomerically resolved (2-(4-chlorophenoxy)-2-methylpropionic acid and 2-(2,4,5-trichlorophenoxy)propionic acid) and up to three of the phenoxy acids enantiomerically resolved were separated in about 15 min. (Martin-Biosca *et al.*, 2001)

CD-MEKC was applied to the enantioseparation of thiobencarb sulfoxide, which is produced by *S*-oxygenation of thiobencarb, using  $\gamma$ -CD together with sodium dodecyl sulfate. The optimum running conditions were found to be 20 mM phosphate-5 mM borate buffer (pH 8.5) containing 60 mM hydroxypropyl- $\gamma$ -CD and 100 mM sodium dodecyl sulfate with an effective voltage of +20 kV at 20 °C using direct detection at 220 nm with resolution ( $R_s$ ) approximately 1.7. (Kodama *et al.*, 2002)

### 3.5 Separation of chiral herbicides by other chromatographic methods

A preparative enantiomer separation method of DCPD was developed utilizing a purposefully designed, highly enantioselective chiral stationary phase additive (CSPA) cinchona-derived chiral selector derived from bis-1,4-(dihydroquinidiny)phthalazine in centrifugal partition chromatography (CPC). A solvent system consisting of 10 mM CSPA in methyl tert-butyl ether and 100 mM sodium phosphate buffer (pH 8.0) was identified as a suitable stationary/mobile-phase combination. Complete enantiomer separations of up to 366 mg of racemic DCPD could be achieved, corresponding to a sample load being equivalent to the molar amount of CSPA employed. Comparison of the preparative performance characteristics of the CPC protocol with that of a HPLC separation using a silica-supported bis-1,4-(dihydroquinidiny)phthalazine CSP revealed comparable loading capacities for both techniques but a significantly lower solvent consumption for CPC. Given that further progress in instrumental design and engineering of dedicated, highly enantioselective CSPAs can be achieved, CPC may offer a viable alternative to CSP-based HPLC for preparative-scale enantiomer separation. (Gavioli *et al.*, 2004)

## 4. Enantioselective herbicidal activity and toxicity of herbicide enantiomers

For the amide herbicides, the product enantiomerically enriched with the herbicidally active 1'S-metolachlor (aSS, aRS) has replaced the racemate worldwide after 2004 (Muller *et al.*, 2001). S-metolachlor was more toxic to *C. pyrenoidosa* than rac-metolachlor, and the catalase activity of *C. pyrenoidosa* treated by S-metolachlor was higher than that exposed to rac-metolachlor (Liu *et al.*, 2009). And enantioselective degradation and/or interconversion for metolachlor was determined, S-metolachlor degraded faster in soil than rac-metolachlor (Ma *et al.*, 2006, Kurt-Karakus *et al.*, 2010). After 42-day incubation, 73.4% of rac-metolachlor and 90.0% of S-metolachlor were degraded. However, due to the absence of biological processes the degradation process in sterilized soil showed no enantioselectivity. The results indicated that enantioselective degradations could greatly affect the environmental fate of metolachlor and should be considered when the environmental behavior of these compounds was assessed. Napropamide is a highly active preemergence herbicide whose R-enantiomer has high phytocidal activity to unifacial-leaf and broad-leaf weeds. It was found that R-napropamide was about eight fold more active than S-napropamide, and two more active than rac-napropamide (Chan *et al.*, 1975). The green alga *Scenedesmus acutus* growth was strongly inhibited and fatty acid was desaturated by S-alachlor and S-dimethenamid while the R isomer had no effect (Couderchet *et al.*, 1997). Furthermore, the comparable biological activities of dimethenamid and alachlor indicate that this target is common to both N-phenyl and N-thienyl chloroacetamide herbicides.

Enantioselective herbicidal activity and toxicity of the phenoxy herbicides has been reported profoundly and roundly. The *in vivo* inhibition of R-(+)- and S-(-)-diclofop-methyl affected on root growth was hardly enantioselective (Shimabukuro *et al.*, 1995), while in a report by Liu *et al.* (Ye *et al.*, 2009), the S-diclofop acid was more toxic to leaves and the R-diclofop acid was more toxic to roots of rice Xiushui 63 seedlings. Furthermore, absorption and translocation to the leaf axil of the two-leaf stage plants of diclofop-methyl enantiomers were similar in both susceptible and resistant biotypes, while the rate of metabolism was increased 1.5-fold in this resistant biotype compared to the susceptible (Maneechote *et al.*, 1997). More, the herbicidally inactive S-(-)-enantiomers of both diclofop-methyl and diclofop were similar to or higher than the corresponding R-(+)-forms in toxicity to algae,

depending on specific species. Although no enantiomeric conversion occurred for diclofop-methyl and diclofop, the difference in the enantioselective degradation of these herbicides observed in algae cultures suggested that their application forms were an important factor determining their enantioselective environmental behavior. It was concluded that the enantioselective degradation of diclofop in algae cultures was governed primarily by the facilitated uptake by algae, whereas the enantioselective toxicity was primarily governed by the passive uptake (Cai *et al.*, 2008). And it was proved that the S-diclofop-methyl dissipated faster than R-diclofop-methyl while the generation and degradation rates of S-diclofop were higher than R-enantiomer in the plant by Zhou *et al.* (Gu *et al.*, 2010). However, in a former report of Zhou *et al.* (Diao *et al.*, 2010a), it was found that the degradation of diclofop-methyl in two soils was not enantioselective while the degradation of diclofop was enantioselective under both aerobic and anaerobic conditions, and the S(-)-diclofop was preferentially degraded, resulting in relative enrichment of the R(+)-form. To haloxyfop ethoxyethyl ester, the S-form was degraded faster than R-form (the enantiomeric fraction of R-form was about 72%) (Desiderio *et al.*, 1997a).

Phenoxypropionic acid (PPA) derivatives are widely used in agriculture as selective herbicides. R-enantiomer of PPAs is known for its herbicidal activity while S-isomer is inactive as herbicidal agent (Buser *et al.*, 1997a). A large number of papers have discussed the enantioselectivity of DCP and MCP (Zipper *et al.*, 1999, Rugge *et al.*, 2002, Schneiderheinze *et al.*, 1999, Zipper *et al.*, 1998, Ma *et al.*, 2009, Jarman *et al.*, 2005, Garrison *et al.*, 1996, Messina *et al.*, 2007, Kurt-Karakus *et al.*, 2010, Buser *et al.*, 1997b, Muller *et al.*, 1997, Harrison *et al.*, 2003, Williams *et al.*, 2003, Wen *et al.*, 2009, Wen *et al.*, 2010), thereinto Bidleman *et al.* (Kurt-Karakus *et al.*, 2010) reviewed the concentrations and stereoisomer ratios of DCP, MCP and metolachlor. Mostly, the S-enantiomer of these herbicides degraded faster than the R-enantiomer (Zipper *et al.*, 1999, Zipper *et al.*, 1998, Garrison *et al.*, 1996, Messina *et al.*, 2007, Buser *et al.*, 1997b, Muller *et al.*, 1997). Enantioselective microbial degradation increased the enantiomeric ratio of R- to S-MCP during groundwater passage of the landfill leachate (Zipper *et al.*, 1998). The S-enantiomers of MCP, DCP and 2,4-D were preferentially degraded under aerobic conditions (Zipper *et al.*, 1999). The S(-)-DCP degraded significantly faster ( $t_{1/2} = 4.4$  d) than the R(+)-isomer ( $t_{1/2} = 8.7$  d) in soil (Garrison *et al.*, 1996). No preferential degradation of the R- and S-enantiomers of MCP and of DCP took place in an aerobic field-injection experiment (Rugge *et al.*, 2002, Jarman *et al.*, 2005). However, in the nitrate-reducing microcosm S-MCP did not degrade but R-MCP degraded with zero order kinetics at 0.65 mg/(L·d) to produce a stoichiometric equivalent amount of 4-chloro-2-methylphenol while no biodegradation of MCP was observed in the methanogenic, sulphate-reducing or iron-reducing microcosms. And in aerobic conditions S- and R-MCP degraded with zero order kinetics at rates of 1.90 and 1.32 mg/(L·d), respectively (Harrison *et al.*, 2003, Williams *et al.*, 2003). Chitosan also changed the enantioselective bioavailability of DCP (Wen *et al.*, 2010). The dissipation of S-enantiomer in *Chlorella pyrenoidosa* culture media without chitosan was faster than that of the herbicidally active R-enantiomer, whereas it was inverted to R-enantiomer being faster than S-enantiomer when chitosan was added into the media. In the absence of chitosan, the toxicity of R-enantiomer to *Chlorella pyrenoidosa* was more potent than that of the S-enantiomer. On the contrary, in the presence of chitosan, R-enantiomer was less toxic than S-enantiomer. R-DCP interacted with penicillium expansum alkaline lipase the strongest, followed by Rac-DCP, while S-DCP had the weakest interaction (Wen *et al.*, 2009). R-DCP was preferentially degraded over the S-DCP in different pH solutions (Ma *et al.*, 2009).



Racemic mixtures of 2,4-DP and MCPP were applied to three species of turf grass, four species of broadleaf weeds, and soil. Both herbicides were degraded more quickly and completely by plants than by soil microbes. Preferential degradation of the S(-)-enantiomer of each herbicide was observed in most species of broadleaf weeds and soil, while the degradation in all species of grass occurred without enantioselectivity. The biodegradation in all systems appeared to follow pseudo first-order kinetics with the fastest degradation occurring in broadleaf weeds, followed by the grasses. The slowest degradation was observed in soil. (Schneiderheinze *et al.*, 1999)

Enantioselective herbicidal activity and degradation of imidazolinone herbicides has been reported recently. Imazaquin exhibited nonselective enantiomer loss over its 3 months of incubation time, which could have been due to abiotic or nonselective microbial reactions (Jarman *et al.*, 2005). However, in another report (Yi *et al.*, 2007), the degradation rates of the two imazaquin enantiomers were slightly different, and the pH of the soil, combined with the moisture content in the soil, had a strong influence on the rate of degradation. And the first enantiomer imazethapyr-I, eluted by CE using 6% hydroxypropyl- $\beta$ -CD as chiral selector in buffer at pH 11.0, degraded at a higher rate when compared with imazethapyr-II (Han *et al.*, 2008). The R(+)-enantiomer of all three herbicides, imazapyr, imazethapyr and imazaquin, which has greater herbicidal activity (up to eight times), was found to degrade faster than the less active S(-)-enantiomer (Ramezani *et al.*, 2010). Generally, the R former of imidazolinones was more active than S former. Imazethapyr inhibits elongation of primary roots and shoots, and reduces the number of adventitious roots and the density of root hairs. The maximal root relative inhibition rate reached 80.4%, 67.0%, and 73.5% for R(-)-imazethapyr, S(+)-imazethapyr and (+/-)-imazethapyr at the concentration of 0.5 mg/L, respectively, and the maximal shoot relative inhibition rate reached 77.7%, 26.9%, and 61.7%, respectively (Qian *et al.*, 2009). The inhibition abilities of (+/-)-imazethapyr to the root growth of maize seedlings was between S(+)- and R(-)-imazethapyr (Zhou *et al.*, 2009). Moreover, imazethapyr enantiomers enantioselectively suppressed the *in vitro* and *in vivo* acetolactate synthase (ALS) activity of maize leaves (Zhou *et al.*, 2010). The *in vivo* ALS activity study showed only a 2-fold difference between R(-)-imazethapyr and S(+)-imazethapyr, while the *in vitro* study showed that the difference in inhibition between the enantiomers fell sharply as concentration increased. At the lowest concentration of 40  $\mu$ g/L, R(-)-imazethapyr appeared 25 times more active than S(+)-imazethapyr, but only 7 times at 200  $\mu$ g/L. At the highest concentration of 25 mg/L, *in vitro* ALS activity was almost completely inhibited by S(+)-, R(-)- and (+/-)-imazethapyr, there was only 1.1 times differences between S(+)- and R(-)-imazethapyr.

Thiobencarb was treated with a rat liver microsomal fraction containing cofactors (known as S9mix) (Kodama *et al.*, 2002). The ratio between (+)- and (-)-thiobencarb sulfoxide was found to be 15:85. It was also found that the ratio between (+) and (-)-thiobencarb sulfoxide produced in soil spiked with thiobencarb was 3:7. These results indicated marked enantioselectivities for these metabolisms. The activities of thiobencarb, (+)- and (-)-thiobencarb sulfoxides on 5 $\alpha$ -dihydrotestosterone- and 17  $\beta$ -estradiol-induced transcriptions were also investigated. Thiobencarb and (+)-thiobencarb sulfoxide did not show any activities, (-)-thiobencarb sulfoxide showed significant anti-estrogenic and anti-androgenic activities, suggesting that thiobencarb sulfoxide can act as both an enantioselective anti-estrogen and an enantioselective anti-androgen.

Racemic and the enantiopure S(+)- and R(-)-lactofen were incubated under aerobic and anaerobic conditions. The data from sterilized controls indicated that the dissipation of

lactofen was biological. The dissipation was shown to be enantioselective with S-(+)-enantiomer being degraded faster than the R(-)-enantiomer, resulting in residues enriched with R(-)-lactofen when the racemic compound was incubated. Lactofen was configurationally stable in soil, showing no interconversion of S-(+)- to R(-)-enantiomer and vice versa (Diao *et al.*, 2009). The enantioselective degradation of lactofen enantiomers was proved in a report by Zhou *et al.* (Diao *et al.*, 2010b). In sediments, S-(+)-lactofen or S-(+)-desethyl lactofen was preferentially degraded, resulting in relative enrichment of the R(-)-form. Lactofen and desethyl lactofen were both configurationally stable in sediment, showing no interconversion of S- to R-enantiomers or vice versa. Furthermore, the acute toxicities of lactofen and desethyl lactofen enantiomers to *Daphnia magna* were enantioselective. The calculated LC50 values of S-(+)-, rac-, and R(-)-lactofen were 17.689, 4.308, and 0.378  $\mu\text{g/mL}$ , respectively, and the calculated LC50 values of S-(+)-, rac-, and R(-)-desethyl lactofen were 21.327, 13.684, and 2.568  $\mu\text{g/mL}$ , respectively.

2- $\alpha$ -substituted benzylamino-4-substituted-amino-6-chloro-1,3,5-triazines are herbicidal compounds showing leaf-burning and/or growth inhibition with concomitant greening and stunting. The test compounds inhibited root growth due to interference with a system or systems other than photosynthesis. 4-(R)-sec-butylamino-2-( $\alpha,\alpha$ -dimethylbenzyl)amino-6-chloro-1,3,5-triazine showed the highest inhibitory activity, and 4-methylamino-2-(R)- $\alpha$ -methylbenzylamino-6-chloro-1,3,5-triazine was second. The chiral requirement for a strong inhibition of root growth was the R-configuration, contrasting with the requirement for the S-configuration for an inhibition of photosystem II. (Omokawa *et al.*, 1992)

Differential chiral responses including enantioselectivity and cross intergenus response on root growth between *Oryza* and *Echinochloa* plants against optical active  $\alpha$ -methylbenzyl p-tolylureas were indicated. Rice was more affected by the R-enantiomers and barnyard miller by the S-enantiomers (Omokawa *et al.*, 2001). Plants of the tribe Oryzaceae respond enantioselectively and homogeneously to optically active 1-(C)-methylbenzyl-3-p-tolylurea (MBTU) in root growth inhibition. The root growth of the genus *Oryza* was inhibited more by R-MBTU than by S-MBTU (Omokawa *et al.*, 2004).

## 5. Conclusions

Over the last several decades, the enantioseparation of chiral herbicides has been widely studied and has made a great contribution for studying their stereoselectivity in biological target activity and non-target toxicity. The direct chromatographic separation approaches play a leading role in separation of chiral herbicides. HPLC combined with CSPs shows its superiority for the enantiomer analysis and enantiomer preparation of many common herbicides especially for the group of amide herbicides, phenoxy herbicides and imidazolinone herbicides. GC is powerful in the determination while CE with diversified modes is also useful for its maneuverability. The application of herbicides separation by SFC is relatively limited.

Many herbicides, related to amide herbicides, phenoxy herbicides and imidazolinone herbicides and so on, have shown the enantioselective herbicidal activity and phytotoxicity with their enantiomers. Many chiral herbicides have been commercialized with the pure enantiomer such as S-metolachlor, quizalofop-P-ethyl, haloxyfop-P-methyl, fluazifop-P-butyl, (R)-naproamide *etc.*. Additionally, more work should be conducted on researching enantioselectivity and environmental fate of herbicides.



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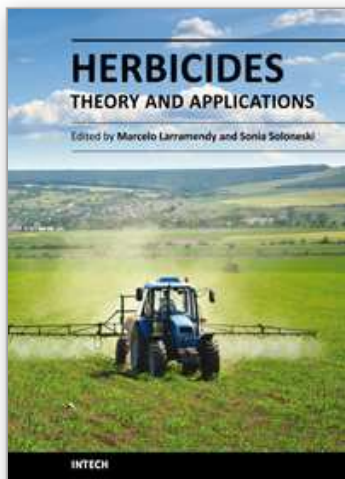
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The content selected in Herbicides, Theory and Applications is intended to provide researchers, producers and consumers of herbicides an overview of the latest scientific achievements. Although we are dealing with many diverse and different topics, we have tried to compile this "raw material" into three major sections in search of clarity and order - Weed Control and Crop Management, Analytical Techniques of Herbicide Detection and Herbicide Toxicity and Further Applications. The editors hope that this book will continue to meet the expectations and needs of all interested in the methodology of use of herbicides, weed control as well as problems related to its use, abuse and misuse.

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