

# Chiral Capillary Electrophoresis of Agrochemicals in Real Samples

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Many agrochemicals have one or more chiral centers and are used as racemic mixtures. Monitoring the stereoselective degradation and/or transformation of agrochemical enantiomers is an important target in environmental chemistry. For chiral analysis, capillary electrophoresis has advantages over other chromatographic methods, in resolution, cost performance and simplicity. This review summarizes chiral separation techniques of real samples of agrochemicals using capillary electrophoresis.

**Key words** — capillary electrophoresis, enantioseparation, agrochemicals, soil, food

## INTRODUCTION

Worldwide use of agrochemicals (mainly fertilizers and pesticides), which is in the millions of tons per year, has raised concerns about pollution of the environment. Many compounds used in the agrochemical industries contain one or more chiral centers and are used as racemic mixtures, in which each chiral center gives rise to two mirror image isomers called enantiomers. In many situations, one enantiomer of the chemicals elicits undesirable responses or has a more toxic effect on nontarget species or has no effect at all on the target species. Agrochemicals can be transformed by biotic and abiotic processes. Usually, abiotic processes, which may include photolysis, hydrolysis, redox, sorption, or other chemical reactions, are not enantioselective, but biotic processes such as microbial transformation are enantioselective. If the effective enantiomer was transformed at a different rate than its counterpart, the application and exposure information should be revised to reflect this aspect of agrochemicals use.

The total amounts of agrochemicals released into the environment can be greatly reduced by using enantiomerically pure products. The development of more pathways for generating chiral compounds will encourage the development of single isomer compounds to capitalize on their advantages. Unfortunately, limited availability of chiral raw materials and economic synthesis pathways are key reasons why single isomers are less common than they might be.

Capillary electrophoresis (CE) with its high resolution and separation efficiency has already shown promise in the area of agrochemicals analysis. However, most of articles in recent reviews<sup>1-4)</sup> have reported enantioseparation of agrochemicals in standard solution, which does not contain matrices, only several publications that deal with the enantioseparation of agrochemicals in real samples by using CE technique that contain complex and various matrices has been reported until now. Analytical separation methods are needed to monitor the stereoselective synthetic process used in agrochemical production, assess the enantiopurity of commercial agrochemical production, detect the various enantiomeric agrochemicals in environmental matrices and foods, and understand the discrimination of enantiomers in environmental compartments.

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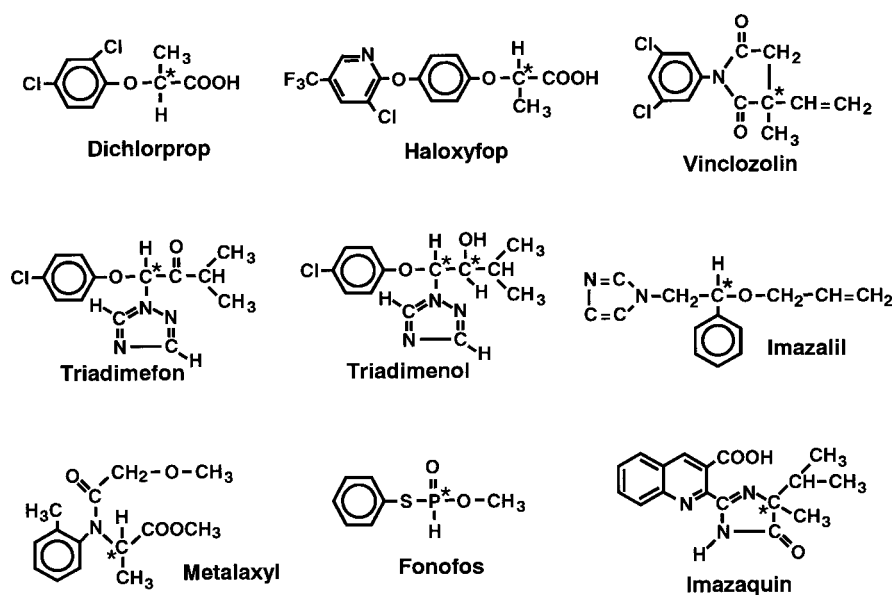


Fig. 1. Chemical Structures of Agrochemicals Described in this Review

Thus, much effort has been focused on the development of methodologies suitable for the separation of chiral agrochemicals. This review describes chiral CE methods for agrochemicals in real samples (Fig. 1).

### Enantioseparation with Capillary Electrophoresis

Electromigration of charged entities (compounds, stable or transient complexes, associates, and aggregates) in an electric field depends on their size, shape, and/or net charge. The main advantages of chiral separation with CE are the high efficiency, the availability of a broad variety of chiral selectors which can simply be added to the background electrolyte without any need of a chiral stationary phase, the possibility to use new and even expensive selectors as usually only small amounts of them are required for CE separations.<sup>5)</sup> CE with its various modes of operation including capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), and capillary isotachopheresis has proved very useful in the analysis of agrochemicals.

The enantioseparation mechanism in CE is based on chromatographic principles, whereas the migration mechanism is electrophoretic.<sup>6)</sup> Enantioseparation with CE usually involves the formation of either non-covalent transient diastereomer or inclusion complex in a dynamic equilibrium process. Small differences in the behavior of enantiomers in

a chiral medium results a small thermodynamic enantioselectivity in their non-covalent interaction with chiral selectors. The low thermodynamic selectivity of recognition combined with high separation efficiency may turn the separations that are impossible to observe by chromatographic techniques well observable in CE. This is a major advantage of CE compared with other instrumental techniques.<sup>7)</sup>

### Principle of Enantioseparation in Capillary Electrophoresis

The structure of the analyte determines the choice of the chiral selectors and separation conditions. However, among structurally related compounds, slight differences in structure may prevent enantiomeric separation, making it very difficult to predict enantioseparation conditions. Therefore, optimization of the chiral separation conditions usually involves the use of the classical trial and error methods.<sup>8)</sup> Chiral separation with CE can be easily accomplished by adding a chiral selector to the background electrolyte. The availability of many chiral selectors make CE an important tool for chiral analysis.<sup>1-5,8,9)</sup> For example, cyclodextrins, linear polysaccharides, crown ethers, chiral surfactants, proteins, macrocyclic antibiotics, and chiral ion-pairing reagents are used as selectors. Of these selectors, cyclodextrins (CDs) and their derivatives have been most widely applied in CE for the separation of enantiomers of many compounds including agrochemicals.

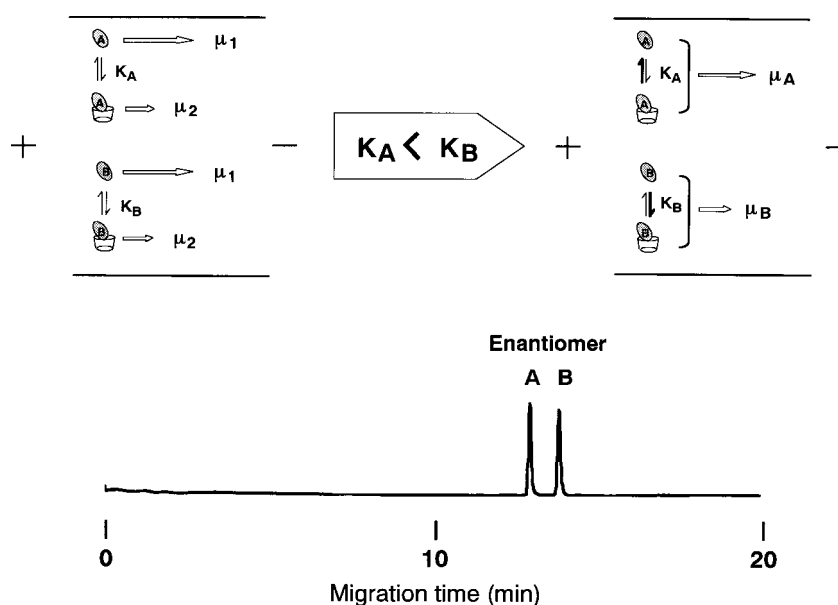


Fig. 2. Enantioseparation Model in Chiral CZE (Upper) and Electropherogram Pattern (Lower) Redrawn from [12]

In a process called inclusion complexation, the cavity of the native or derivatized CDs hydrophobically interacts with the hydrophobic part of the compounds, such as an aromatic ring. This process plays an important role in the stereoselective interaction. The migration times of analytes in CE were well characterized by Guttman *et al.*<sup>10)</sup> Wren and Rowe<sup>11)</sup> developed a theoretical model relating the mobility to the concentration of a CD selector as follows;



where  $m_1$  is the electrophoretic mobility of the analyte in free solution,  $m_2$  is the electrophoretic mobility of the analyte-chiral selector complex, and  $K_A$  and  $K_B$  are equilibrium constant. A and B are a pair of enantiomers which have the same electrophoretic mobility in free solution. They interact with a chiral selector C dissolved in the buffer to form the inclusion complexes AC and BC, which are assumed to have the same electrophoretic mobility (Fig. 2). The concentration of inclusion complex [AC] is expressed by using the equilibrium constant  $K_A$  and the concentration of enantiomer [A] and selector [C] as

$$[AC] = K_A[A][C] \quad (1)$$

If the two enantiomers have different affinities for the chiral selector, that is,  $K_A$  and  $K_B$  are differ-

ent, and the electrophoretic mobilities of the free and complexed enantiomers are different, then chiral resolution is possible. Electrophoretic mobility of enantiomer A ( $\mu_A$ ) is defined as

$$\begin{aligned}
 \mu_A = & \{ [A] / ([A] + [AC]) \} \mu_1 \\
 & + \{ [AC] / ([A] + [AC]) \} \mu_2
 \end{aligned} \quad (2)$$

Substitution of eqn. (1) to eqn. (2) and rearrangement yield

$$\mu_A = (\mu_1 + \mu_2 K_A [C]) / (1 + K_A [C]) \quad (3)$$

The difference in the apparent electrophoretic mobilities of A and B is

$$\Delta\mu = \{ [C](\mu_1 - \mu_2)(K_B - K_A) \} / \{ 1 + [C](K_A + K_B) + K_A K_B [C]^2 \} \quad (4)$$

As shown in Fig. 2, imazalil was enantioseparated by a CZE method with a background electrolyte containing 4 mM 2-hydroxypropyl- $\beta$ -cyclodextrin, 5 mM ammonium dihydrogenphosphate-50 mM phosphate buffer (pH 3.0).<sup>12)</sup> Enantiomer A [(-)-imazalil] migrates faster than enantiomer B [(+)-imazalil], suggesting that (+)-imazalil formed stronger diastereomer complexes with a selector than (-)-imazalil.

From eqn. (4), it is clear that the electrophoretic mobility difference is zero if  $K_A = K_B$ ,  $\mu_1 = \mu_2$ ,  $[C] = 0$ , or  $[C]$  is very large. The optimum concentration of chiral selector,  $C_{opt}$ , can be found from eqn. 5 by the use of differential calculus as

$$C_{\text{opt}} = (K_A K_B)^{-1/2} \quad (5)$$

In fact, the resolution ( $R_s$ ) of imazalil showed a maximum at 4 mM 2-hydroxypropyl- $\beta$ -CD (Fig. 3).<sup>12)</sup>

Vancomycin, which is a macrocyclic antibiotic, is another important selector for enantioseparation of agrochemicals. Since vancomycin shows high UV absorbance, the partial filling method has been used in order to increase the sensitivity of CE methods. That is, coated capillaries were used to eliminate or minimize electroosmotic flow and vancomycin was not present at the detector path during the detection due to its positive charge.

### ANALYSIS OF AGROCHEMICALS IN REAL SAMPLES

The enantioseparation of agrochemicals in real samples with CE was first reported by Garrison *et al.*<sup>13)</sup> In their CZE method, 2,3,6-tri-O-methyl- $\beta$ -CD was used as a chiral selector for the enantioseparation of dichlorprop, a phenoxy acid herbicide. Dichlorprop degrades in moist soil to 2,4-dichloranisole and only the (*R*)-(+)-dichlorprop is herbicidally active. Garrison *et al.* sprayed a 100-ha field in an experimental farm with Foxtril, a commercial herbicide formulation. Soil samples taken from a field plot at different times were solvent-extracted and analyzed for dichlorprop enantiomers by CZE. They found that (*S*)-isomer degraded significantly faster ( $t_{1/2} = 4.4$  d) than the (*R*)-isomer ( $t_{1/2} = 8.7$  d).

Desiderio *et al.*<sup>14)</sup> reported the enantioseparation of 8 acidic herbicides including haloxyfop by using CZE with vancomycin as a chiral selector. Haloxyfop is usually utilized as an ester and is rapidly hydrolyzed in soil to the corresponding acid that is the biologically active. Racemic haloxyfop ethoxyethyl ester was spiked to a soil sample, and after 72 hr at room temperature haloxyfop free acid metabolite was extracted and analyzed by the CZE method. The metabolite was found to be a mixture of (*R*)- and (*S*)-enantiomers where the (*R*)-enantiomers was about 72%.

Triadimefon and triadimenol are triazole fungicides. The antifungal activity of triadimefon is largely due to the transformation of triadimefon to triadimenol under environmental and biological conditions. Triadimefon and triadimenol have one and two asymmetrical carbon atoms, respectively, so triadimenol is composed of 4 isomers and the

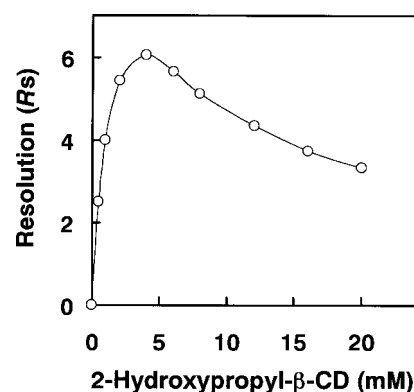
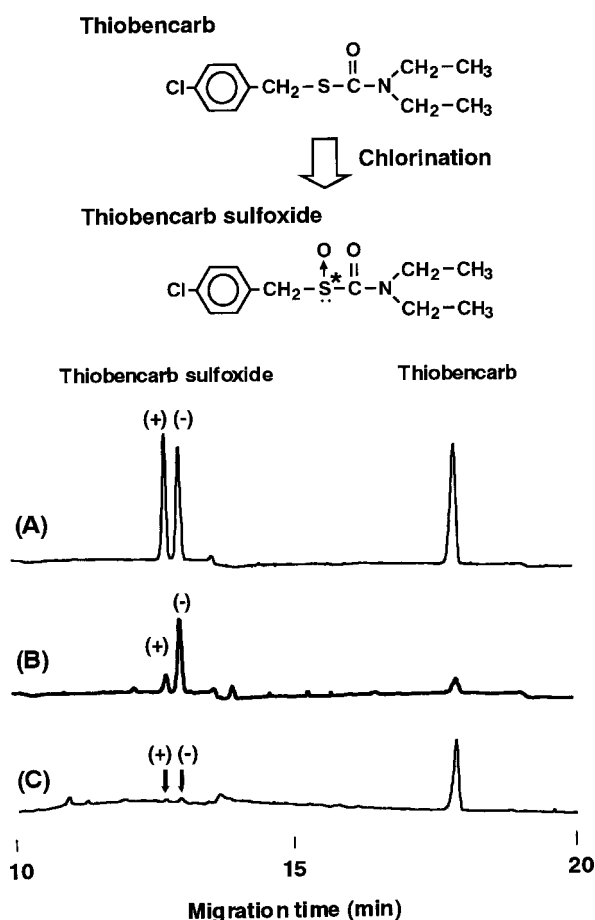


Fig. 3. Effect of the Concentration of a Selector, 2-hydroxypropyl- $\beta$ -CD, on the Resolution ( $R_s$ ) of Racemic Imazalil  
Redrawn from [12].

(*1S,2R*)-isomer shows the highest antifungal activity. Wu *et al.*<sup>15)</sup> reported that triadimefon and triadimenol enantiomers were enantioseparated by CZE using a background electrolyte with sulfated- $\beta$ -CD as a chiral selector. Racemic triadimefon was added to a soil sample and incubated at 35°C for 20 days. The biotransformation process was marked by persistent declines in the concentrations of the two enantiomers of triadimefon and steady increases in the concentrations of the four isomers of triadimenol. They found that the (*1S,2R*)-triadimenol was the predominant biotransformation product.

Thiobencarb is a carbamate herbicide that has been widely used for weed control in rice crops. Thiobencarb can be easily oxidized to thiobencarb sulfoxide under environmental and biological conditions.<sup>16,17)</sup> Thiobencarb is achiral, but its sulfoxide has an asymmetric sulfur. Kodama *et al.*<sup>18)</sup> reported that thiobencarb sulfoxide was enantioseparated by hydroxypropyl- $\gamma$ -CD modified MEKC (Fig. 4). They also found that the ratio between (+)- and (-)-thiobencarb sulfoxides produced in soil that had been spiked with thiobencarb was 3 : 7. And thiobencarb was treated with a rat liver microsomal fraction containing cofactors (known as S9mix). The ratio between (+)- and (-)-thiobencarb sulfoxides was found to be 15 : 85. These results indicated marked enantioselectivities for these metabolisms. (-)-Thiobencarb sulfoxide, but not (+)-thiobencarb sulfoxide, showed significant anti-estrogenic and anti-androgenic activities (through inhibition of 5 $\alpha$ -dihydrotestosterone- and 17 $\beta$ -estradiol-induced transcriptions). This suggests that thiobencarb sulfoxide can act as both an enantioselective anti-estrogen and an enantioselective anti-androgen.



**Fig. 4.** Electropherograms of Thiobencarb Sulfoxide by CE/UV (A): standard solution, (B): thiobencarb sulfoxide produced by S9 catalysis of thiobencarb and (C): thiobencarb sulfoxide produced in thiobencarb-spiked soil. (+) and (-) represent (+)- and (-)-thiobencarb sulfoxides, respectively. Redrawn from [18].

Vinclozolin is a dicarboximide fungicide that has been widely used in Europe to protect fruits, vegetables, ornamental plants and turf grasses. CD-modified MEKC was applied to the enantioseparation of vinclozolin in wine using  $\gamma$ -CD together with sodium dodecyl sulfate.<sup>19)</sup> The peak area ratio of (+)- and (-)-vinclozolin was found to be 2 : 3, suggesting that the degradation rate of (+)-vinclozolin was greater than that of (-)-vinclozolin. The anti-androgenic activity of (+)-vinclozolin tended to be stronger than that of (-)-vinclozolin, suggesting the possibility that vinclozolin can act as an enantioselective anti-androgen.

Imazalil, which is widely used as a post-harvest fungicide, can control a wide range of fungal diseases on fruits, vegetables and ornamentals by inhibiting ergosterol biosynthesis. In Japan, the maximum permitted imazalil concentration is 5 ppm for citrus fruits and 2 ppm for bananas. Imazalil was

enantioseparated by CE using 2-hydroxypropyl- $\beta$ -CD as a chiral selector.<sup>12)</sup> Imazalil was detected in seven out of eight oranges. In four of these seven oranges, the level of (-)-imazalil was the same as that of (+)-imazalil, but in the other three oranges, the level of (-)-imazalil was found to be lower than that of (+)-imazalil, suggesting that (-)-imazalil was degraded more quickly than (+)-imazalil in oranges.

Recently, Jarman *et al.*<sup>20)</sup> reported the transformation of three chiral agrochemicals that were spiked to aqueous slurries of soil samples. Metalaxyl was enantioseparated by CZE with  $\gamma$ -CD as a chiral selector, and fonofos and imazaquin were enantioseparated by MEKC with  $\gamma$ -CD and dimethyl- $\beta$ -CD, respectively, as chiral selectors. The half-life of the target active (*R*)-metalaxyl was shorter than that of (*S*)-metalaxyl, suggesting that metalaxyl was enantioselectively transformed, but imazaquin and fonofos exhibited nonselective transformation.

## CONCLUDING REMARKS

CE has a high potential for the enantioseparation of agrochemicals in real samples. Chiral separation with CE can be easily accomplished by adding a chiral selector to the background electrolyte. Various selectors such as cyclodextrins, macrocyclic antibiotics and chiral surfactants are available. However, sample preparation is critical when analyzing real samples with CE. Further development and application of preconcentration and on-line concentration techniques should help to achieve high sensitivity. We expect that CE will be used more widely for the chiral analysis of agrochemicals in real samples.

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