

Article

Sensitive Analysis of Chiral Phenoxy Acid Herbicides Using Anion-Selective Exhaustive Injection-Sweeping Micellar Electrokinetic Chromatography

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Abstract

Chiral pesticides, comprising enantiomers with identical physical and chemical properties but different biological effects, call for extra efforts in comprehensively evaluating their risk assessment. However, the defect regarding sensitive analysis of chiral pesticides limits the progress of research. Therefore, an efficient and highly sensitive analytical method for the trace-level analysis of chiral pesticides is imperative. Herein, normal micellar electrokinetic chromatography (MEKC), sweeping-MEKC, and anion-selective exhaustive injection-sweeping-MEKC (ASEI-sweeping-MEKC) were optimized to separate and enrich three chiral phenoxy acid herbicides (cloprop, dichlorprop, and fenoprop). Normal MEKC with 30 mM γ -cyclodextrin, 50 mM SDS, pH 2.5, and 50 mM phosphate buffer background electrolyte (BGE) achieved ideal chiral separation of target herbicides. Then, sweeping-MEKC was modified to enhance the detection sensitivity by 4690 to 10,630-folds with the conditions of pH 2.5, 100 mM phosphate buffer high-conductivity buffer (HCB), 60 s injection time + 30 mM γ -CD, 50 mM SDS, pH 2.5, 50 mM phosphate buffer BGE + 120 s sample injection time. ASEI-sweeping-MEKC achieved further improvement of detection sensitivity by 241,000- to 673,000-fold. And the optimal ASEI-sweeping-MEKC conditions for the target chiral phenoxy acid herbicides were 20% ACN water plug, 30 s injection time + pH 2.5, 100 mM phosphate buffer HCB, 60 s injection time + 30 mM γ -CD, 50 mM SDS, pH 2.5, 50 mM phosphate buffer BGE + 12 min sample injection time. All in all, these findings have validated ASEI-sweeping-MEKC as an efficient and powerful analytical method for the trace-level analysis of the enantiomers of three chiral phenoxy acid herbicides. This approach enables a comprehensive risk assessment and provides guidance for the sustainable application of chiral pesticides.

Keywords: anion-selective exhaustive injection; sweeping; micellar electrokinetic chromatography; sensitive analysis; chiral phenoxy acid herbicides



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1. Introduction

The myriad benefits of pesticides have led to the mass production and application of these agricultural chemicals [1]. As a special part, chiral pesticides are well-known for the chiral centers that result in the asymmetrical enantiomers [2]. The enantiomers usually possess identical physical and chemical properties but have different biological effects due to their enantioselective interactions with enzymes or other chiral macromolecules [3]. Therefore, some enantiomers are active against target organisms while the other enantiomers may be inactive or have different effects or even adverse effects on non-target species [4,5]. For example, the bioactivity of (1*S*, 2*R*)-triadimenol is more than 1000 times higher than the other stereoisomers [6]. Dramatic differences between enantiomers of chiral pesticides also have been observed in their effects on non-target species [7,8]. Chiral pesticides account for about 40% of total pesticides currently in use [1]. However, most of the chiral pesticides are commercialized as racemates due to economic reasons but enantioselective enrichment and racemization of these pesticides even make it more complex to understand their ecotoxicological and health risks [4,9]. Hence, it is necessary to further investigate chiral pesticides in an enantiomeric manner. However, pesticides in complex environmental samples usually exist at ultra-trace levels, and analysis of chiral pesticides inevitably involves efficient analytical methods. To meet this demand, developing high-sensitivity analytical techniques for chiral pesticides is of considerable significance, which will help to make comprehensive risk assessments and guide the scientific use of pesticides.

Phenoxy acid herbicides are a kind of herbicide widely employed in the agricultural industry to control annual and perennial broad-leaf weeds [10]. Due to their high polarity and water solubility, phenoxy acid herbicides can subsequently disperse into surface and ground waters when applied to farmlands [11], and growing evidence showed that they are frequently detected in various water ecosystems existing in ng/L levels [12,13]. Additionally, some phenoxy acid herbicides are chiral pesticides, such as cloprop (CP), dichlorprop (DCP), and fenoprop (FP), which all contain chiral centers. Generally, CP, DCP, and FP are commercialized in the form of racemates, but enantioselective differences in these chiral herbicides in biological effects have been reported. For example, the *R*-dichlorprop exhibited herbicidal activity while the *S*-enantiomer did not [14]. Therefore, the efficient analysis of the enantiomers of these phenoxy acid herbicides in environmental samples is quite challenging.

Currently, the most commonly employed analytical techniques for chiral separation of pesticides include gas chromatography (GC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE) [15–20]. For GC-based chiral separations, the direct approach necessitates the use of a chiral stationary phase as a selector. However, this method exhibits significant limitations when dealing with analytes that possess low volatility, high polarity, or thermal instability [9]. HPLC, while highly efficient in chiral separation, typically requires cumbersome sample cleanup procedures to prevent column contamination due to the complexity of environmental matrices. Additionally, detectors with high selectivity, such as tandem mass spectrometry (MS/MS), are often essential for accurate HPLC analysis [21], and the analysis process is also characterized by the lengthy run time and the consumption of hazardous and costly organic solvents, which increase operational expenses and time investment [11]. In comparison, CE offers several advantages, including superior separation efficiency, short analysis time, and minimal sample volume requirements. Traditionally, CE is utilized for the separation of pharmaceutical compounds. However, it can also be adapted for effectively analyzing chiral pesticides by employing chiral selectors (e.g., cyclodextrins) that bind to different enantiomers, enabling efficient chiral separation [22]. Another notable strength of CE is its excellent flexibility, as various chiral selectors can be simply incorporated into the background electrolyte (BGE) solution at different concentrations, whereas in HPLC, such selectors are typically

immobilized on the stationary phase [23,24]. Despite these advantages, the application of CE in chiral pesticide analysis is significantly hindered by its inherently high detection limits. This limitation primarily arises from CE's low concentration sensitivity, which could be attributed to the limited sample loading capacity of the capillary and the restricted path length available for on-capillary optical detection [25]. Therefore, improving these shortcomings can greatly facilitate the application of CE.

To address this problem, several online concentration methods have been developed. Among them, sample stacking is the most widely used capillary-based enrichment technique in capillary zone electrophoresis (CZE). Briefly, sample ions cross the boundary separating low-conductivity and high-conductivity solutions, where their migration significantly decelerates [26]. The injection of a sample zone in stacking can be achieved by either hydrodynamic or electrokinetic strategies, with the latter commonly known as field-amplified sample injection (FASI). Another frequently employed online enrichment method is sweeping, which is particularly applied in micellar electrokinetic chromatography (MEKC) [27]. In this approach, the sample is introduced into a medium with the same resistance as the BGE but without the pseudostationary phase and the concentration efficiency depends on the affinity between the analytes and the pseudostationary phase [28–30]. Cation-selective exhaustive injection-sweeping (CSEI-sweeping), which enables the achievement of a notable enhancement in sensitivity, is the combination of two distinct on-capillary concentration methods, field-amplified sample injection (FASI) and sweeping [31,32]. After modification, a similar approach has been adapted for anion concentration, now referred to as anion-selective exhaustive injection-sweeping (ASEI-sweeping) [33], which is an ideal on-capillary concentration method for acidic compounds.

The aim of this research is to develop a highly sensitive CE method for the analysis of chiral pesticides with three chiral phenoxy acid herbicides (cloprop, dichlorprop, and fenoprop) chosen as representative chemicals to verify the resolution efficiency and sensitivity of the method. Generally, different conditions of the sweeping and anion-selective exhaustive injection-sweeping-MEKC (ASEI-sweeping-MEKC), including chiral selector, injection time, injection length and composition of the water plug, length and conductivity of the buffer, were optimized to achieve the highest detection response and sensitivity. All in all, the optimized ASEI-sweeping-MEKC can achieve highly efficient and sensitive analysis of chiral phenoxy acid herbicides, which could enhance the risk assessment and scientific regulation of these chiral pesticides.

2. Material and Methods

2.1. Reagents

2-(3-chlorophenoxy)-propionic acid (cloprop, CP), 2-(2,4-dichlorophenoxy)-propionic acid (dichlorprop, DCP), and 2-(2,4,5-trichlorophenoxy)-propionic acid (fenoprop, FP) were purchased from Sigma (St. Louis, MO, USA). Detailed information regarding the target herbicides was listed in Table S1. The three herbicide standards were individually prepared in acetonitrile at a stock concentration of 5.00 mg/mL. Then a secondary stock solution was prepared by mixing equal volumes of these stock solutions with acetonitrile, in which the concentration of each herbicide was diluted to 0.10 mg/mL (with each enantiomer being 0.05 mg/mL). The secondary stock solution was further diluted using ultrapure water at desired concentrations for sequential research studies. All solutions were filtered through a 0.45 µm filter prior to CE analysis.

Cyclodextrins (CDs), including β-cyclodextrins (β-CD), hydroxypropyl-β-cyclodextrins (HP-β-CD), and γ-cyclodextrins (γ-CD), as well as a stock solution of 200 mM sodium dodecyl sulfate (SDS) were purchased from Shanghai Chemical Reagent (Shanghai, China). Phosphate buffer was purchased from Sinopharm Chemical Reagent (Shanghai, China).

and prepared at 200 mM as a stock solution. The background electrolyte (BGE) solution was prepared freshly each day with CDs, SDS, and phosphate buffer in ultrapure water.

2.2. Optimization of Normal MEKC Procedure

The optimization of normal MEKC conditions was performed using a Beckman P/ACETM MDQ CE system (USA) with a Ruifeng 60 cm × 75 µm bare-fused silica capillary (Hebei, China). The precondition for the separation capillary was set as follows: first flushed with ultrapure water for 2 min, followed by 1 M NaOH for 5 min, ultrapure water for 2 min, 1 M HCl for 5 min, ultrapure water for 2 min, and finally the BGE solution (CDs, SDS, and phosphate buffer) for 10 min. Between runs, the capillary was flushed with running buffer for 6 min. The analytes were injected hydrodynamically via a pressure of 0.3 psi for varying durations. After injection, the buffer vials at both ends of the capillary were replaced with the BGE solution. The type of CDs (β-CD, HP-β-CD, γ-CD), the concentrations of CD (10, 20, 30, 40 mM), SDS (50, 75, 100 mM), phosphate buffer (50, 100, 150 mM), and the pH (2.0, 2.5, 3.0, 3.5, 4.0) of the BGE solution, as well as the injection time (3, 5 s) were assessed to achieve the optimal normal MEKC condition for the chiral separation of target phenoxy acid herbicides. All running solutions were filtered through a 0.45 µm filter prior to use. Electrophoresis was performed at −20 kV with UV detection set at 214 nm. The collected data were analyzed using Beckman 32 Karat Software version 7.0. The rate of separation (*Rs*) is calculated as follows:

$$Rs = \frac{t_2 - t_1}{(w_2 - w_1)/2}$$

t_1 , t_2 , w_1 , and w_2 are defined as follows: retention time for enantiomer 1 and enantiomer 2, baseline peak width for enantiomer 1 and enantiomer 2, respectively.

2.3. Optimization of the Sweeping-MEKC Procedure

The optimization of sweeping-MEKC conditions was performed based on normal-MEKC analysis using the Beckman P/ACETM MDQ CE system. The precondition for the separation capillary was set as follows: first flushed with ultrapure water for 2 min, followed by 1 M NaOH for 5 min, ultrapure water for 2 min, 1 M HCl for 5 min, ultrapure water for 2 min, and finally the BGE solution (30 mM γ-CD, 50 mM phosphate buffer, 50 mM SDS, pH 2.5) for 10 min. Between runs, the capillary was flushed with running buffer for 6 min. Subsequently, a nonmicellar high-conductivity buffer (HCB) plug (30 mM γ-CD, phosphate buffer, pH 2.5) was injected (1 psi). The analytes were injected hydrodynamically via a pressure of 1 psi for varying durations. After injection, the buffer vials at both ends of the capillary were replaced with the BGE solution. The concentration of phosphate buffer (50, 100, 150 mM) and the injection time (20, 40, 60, 80 s) of the BGE solution, as well as the injection time (30, 60, 90, 120, 150 s) of the sample, were assessed to achieve the optimal sweeping-MEKC condition for the chiral separation of target phenoxy acid herbicides. All running solutions were filtered through a 0.45 µm filter prior to use. Electrophoresis was performed at −20 kV with UV detection set at 214 nm. The collected data were analyzed using 32 Karat Software version 7.0 (Beckman, USA). The sensitivity enhancement factor (*Ef*) is calculated as follows:

$$Ef = \frac{c_0}{c_e} \times \frac{\omega_e}{\omega_0}$$

c_0 , c_e , ω_0 , and ω_e are defined as follows: sample concentration in normal MEKC (mg/mL), sample concentration in sweeping-MEKC (mg/mL), peak height in normal MEKC (AU), and peak height in sweeping-MEKC (AU), respectively.

2.4. Optimization of the ASEI-Sweeping-MEKC Procedure

The optimization of ASEI-sweeping-MEKC conditions was performed based on sweeping-MEKC analysis with slight modification. The precondition for the separation capillary was the same as that in sweeping-MEKC assays. Between runs, the capillary was flushed with running buffer for 6 min. Subsequently, a nonmicellar HCB plug (30 mM γ -CD, phosphate buffer, pH 2.5) was injected (1 psi), and a water plug (containing ACN) was hydrodynamically injected (1 psi). The analytes were injected hydrodynamically via a pressure of 1 psi for varying durations. After injection, sample vials were refreshed to maintain repeatability. The concentration of phosphate buffer (50, 100, 150 mM) and the injection time (20, 40, 60, 80 s) of the BGE solution, the ACN proportion (0%, 10%, 20%, 30%) and the injection time (10, 20, 30, 40 s) of the water plug, as well as the injection time (3, 6, 9, 12, 15 min) of the sample were assessed to achieve the optimal sweeping-MEKC condition for the chiral separation of target phenoxy acid herbicides. All running solutions were filtered through a 0.45 μ m filter prior to use. Separation was performed at -20 kV with the BGE solution at both ends of the capillary. The sensitivity enhancement factor (Ef) is calculated as follows:

$$Ef = \frac{c_0}{c_e} \times \frac{\omega_e}{\omega_0}$$

c_0 , c_e , ω_0 , and ω_e are defined as follows: sample concentration in normal MEKC (mg/mL), sample concentration in ASEI-sweeping-MEKC (mg/mL), peak height in normal MEKC (AU), and peak height in ASEI-sweeping-MEKC (AU), respectively.

2.5. Data Processing

Statistical analyses were conducted using Origin 8.0 software (USA). The results are presented as the mean $\pm$ standard deviation (SD) from three duplications.

3. Results and Discussion

Herbicide residues raise concerns about food safety, environmental contamination, and biodiversity, contributing to unsustainable ecological and environmental management practices. Monitoring the herbicide residue levels in the environment is essential, as it offers valuable insights into the current extent of environmental contamination and potential ecological risks attributed to their application [34]. However, the enantiomer diversity of chiral herbicides exacerbates the difficulty of this process. Therefore, an efficient and highly sensitive analytical method for the trace-level analysis of chiral herbicides is impending. CE has been intensively applied to chiral separation for the convenience offered by chiral selectors added into BGE. To meet the demand for environmental monitoring of trace levels of chiral pesticides existing in environmental matrices, the combination of online concentration methods with chiral separation of typical phenoxy acid herbicides was investigated in detail below.

3.1. Optimization of Normal MEKC Conditions

The different biological effects of chiral pesticide enantiomers press for an efficient method for the trace-level analysis of all enantiomers, but the identical physical and chemical properties of the enantiomers make it quite tricky [3]. Therefore, thorough chiral separation of the enantiomers is the precondition for the optimization of the MEKC conditions. In order to obtain chiral baseline separation of the enantiomers of three kinds of phenoxy acid herbicides in normal MEKC, it was necessary to determine the type of chiral selector added into the buffer to allow chiral separation. The selection of a chiral selector primarily considers factors such as the charge properties, molecular size, and physicochemical nature of the analyte. An ideal chiral selector should be highly water-

soluble, have no UV absorption, exhibit high separation selectivity, and be generally applicable. CDs and their derivatives are most commonly used for the separation of chiral pesticides [35–38]. For instance, β -CD achieved the resolution of the enantiomers of terbutaline, and HP- β -CD was reported in the literature for the chiral separation of seven chiral phenoxy acids [39,40]. γ -CD was also selected as the chiral selector to separate the enantiomers of mecoprop, fenoprop, fluazifop, and haloxyfop [39]. When the analyte is an acidic substance, CDs are typically chosen as the chiral selector for chiral separation in capillary electrophoresis. Under acidic conditions, the electroosmotic flow (EOF) is minimized, causing the analyte to carry a positive charge and migrate toward the negative electrode. Neutral CDs migrate at the same speed as EOF, and enantiomers, due to their differing configurations, form inclusion complexes with varying stabilities with CDs, thereby achieving separation. Phenoxy acid herbicides are acidic in nature; therefore, β -CD, HP- β -CD, and γ -CD were selected as the chiral selectors to evaluate their effects on the chiral separation. As shown in Figure 1A,B, β -CD and HP- β -CD could only separate the CP enantiomers with the R_s of 0.92–10.49 and 0.35–2.82, respectively. However, γ -CD exhibited the most appropriate enantiomer-resolving ability in terms of the resolution of the enantiomers of CP, DCP, and FP, and the optimal concentration was 30 mM (Figure 1C).

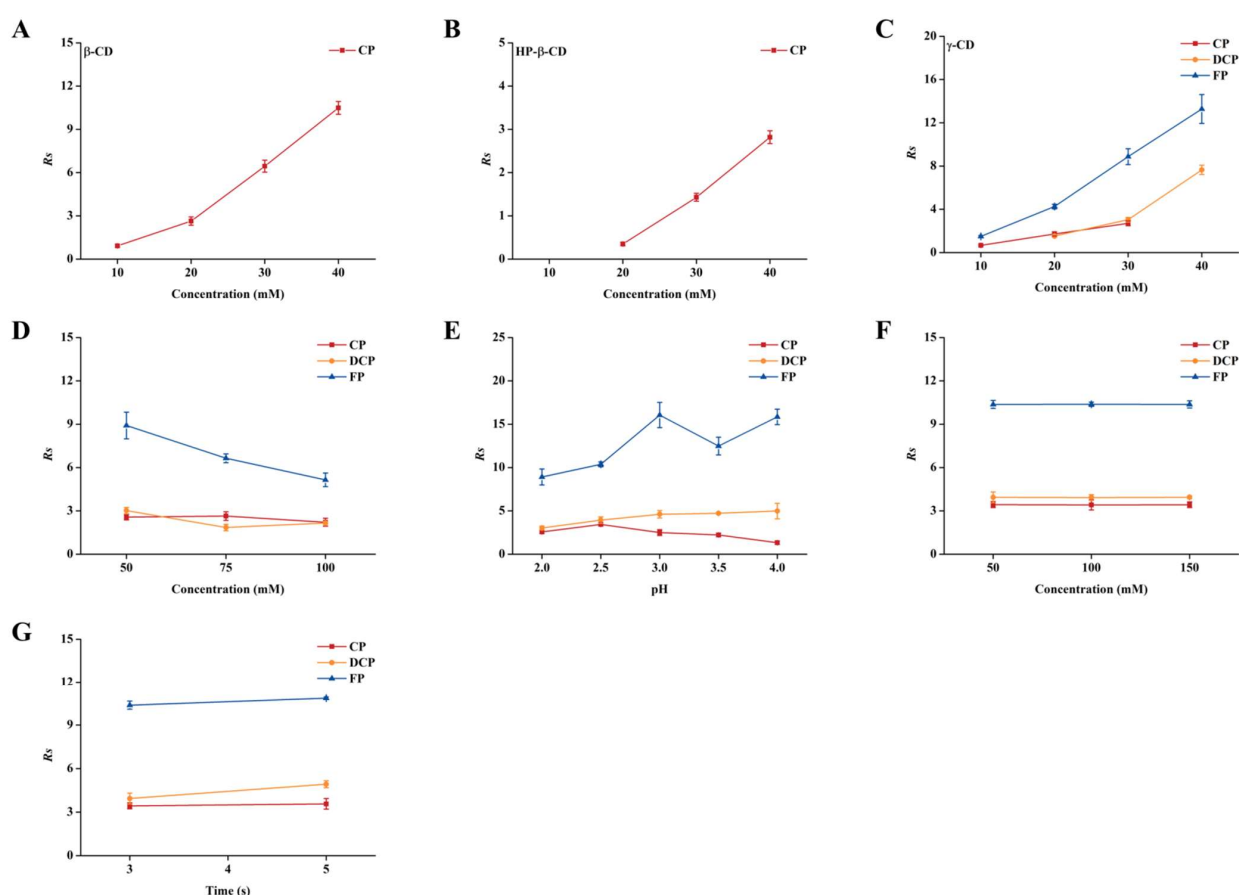


Figure 1. Chiral resolution of three phenoxy acid herbicides by normal MEKC. (A): Influence of the β -CD concentration on R_s . (B): Influence of the HP- β -CD concentration on R_s . (C): Influence of the γ -CD concentration on R_s . (D): Influence of the SDS concentration of BGE on R_s . (E): Influence of the pH of BGE on R_s . (F): Influence of the phosphate buffer concentration of BGE on R_s . (G): Influence of the sample injection time on R_s .

With the aim to further achieve ideal chiral separation of these enantiomers, optimization of those influence factors in normal MEKC was needed as a preliminary to starting an online concentration study, namely the concentrations of SDS, the pH and concentration of phosphate

buffer, as well as injection time. For the concentrations of SDS, CP, DCP, and FP, the best R_s were achieved at the concentration of 50 mM, with the R_s being 2.57, 3.02, and 8.91, respectively (Figure 1D). CP, DCP, and FP also acquired the most suitable resolution at the pH of 2.5 for phosphate buffer (Figure 1E), but the concentration of phosphate buffer did little to improve the resolution of CP, DCP, and FP (Figure 1F). In terms of the sample injection time, the herbicides get better separation with a longer injection time (Figure 1G). In normal MEKC, the sample injection time cannot be extended indefinitely and should generally be kept within 10 s because a longer injection time leads to broader analyte peaks, potentially causing poor separation of the target substances and significantly impacting the enrichment efficiency [41].

Enantiomeric separation is a critical task in chiral analysis, and separation techniques play a pivotal role in achieving this goal. Among the various methods available, HPLC, GC, supercritical fluid chromatography (SFC), CE, and nano-liquid chromatography (nano-LC) stand out as particularly effective tools for resolving enantiomers [42]. Each of these techniques offers unique advantages depending on the specific requirements of the analysis. For example, Zhang et al. achieved the chiral separation of seven phenoxy acid herbicides, including CP, DCP, and FP, using the nano-LC method [42]. Generally, the condition of 30 mM γ -CD, 50 mM SDS, pH 2.5, 50 mM phosphate buffer and BGE + 5 s sample injection time achieved the optical resolution for CP, DCP, and FP in normal MEKC (Figure 2). Detailed information regarding the limit of detection (LoD), the limit of quantitation (LoQ), the coefficient of determination (R^2), and the relative standard deviation (RSD) of all enantiomers for normal MEKC was listed in Table S4. CE, as a widely used capillary separation technique, offers the advantage of low sample and reagent consumption, making it environmentally friendly, although the addition of chiral selectors in CE may lead to incompatibility with mass spectrometry detection [43,44]. Herein, normal MEKC achieved chiral baseline separation of CP, DCP, and FP, but the enrichment efficiency was not satisfactory. Then, sweeping enrichment was applied to enhance the online detection sensitivity of CE for the three phenoxy acid herbicides based on the above-mentioned normal MEKC condition.

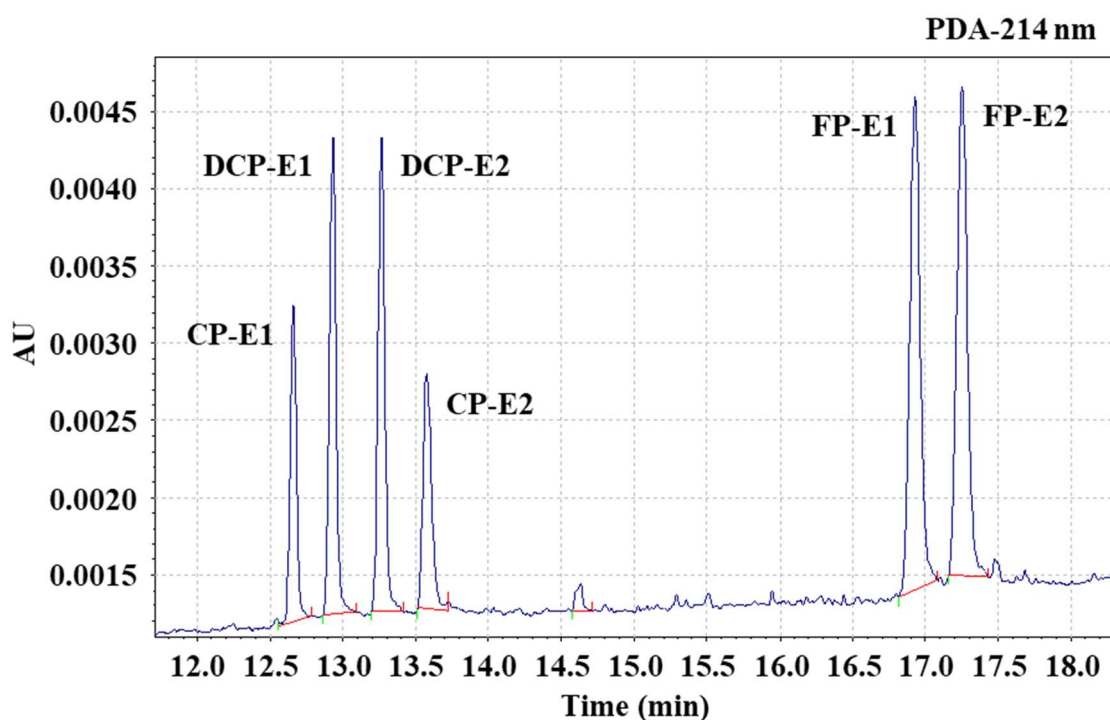


Figure 2. Electropherograms of chiral separation obtained in normal MEKC for the enantiomers of three phenoxy acid herbicides. BGE: 30 mM γ -CD, 50 mM SDS, 50 mM phosphate buffer; 5 s sample injection time.

3.2. Optimization of Sweeping-MEKC Conditions

Under the above-optimized normal MEKC conditions, the enantiomers of phenoxy acid herbicides could be baseline resolved but the sensitivity could not meet the requirement for environmental analysis, in which these enantiomers usually exist in extremely low concentrations. Sweeping enrichment, as an emerging enrichment technique, has been reported to be applied in the detection of pesticide residues. However, due to the limitations of detection sensitivity, there is still limited research on the online enrichment of chiral pesticide residues [45]. Thus, sweeping coupled with MEKC was applied to enhance the online detection sensitivity of CE for the three phenoxy acid herbicides. This enrichment strategy could be briefly described as the focusing of analyte zones by a pseudostationary phase. The high conductivity buffer (HCB) and the BGE used here had a low pH, which could neutralize those anionic analytes and immobilize them in the sample zone [46]. This HCB should not contain any micelles (e.g., SDS) as micelles would interfere with the stacked sample zone and cause peak broadening [47]. Therefore, the injection time and phosphate buffer concentration of HCB, as well as the sample injection time were optimized to achieve higher sensitivity.

Sweeping enrichment is mainly based on the phase distribution process of the sample solution in bands without micelles, and its enrichment effect depends on the distribution capability of the sample in the micellar phase. Thus, the length of HCB, determined by injection time, strongly affects the enrichment effect. Herein, the enantiomers achieved the highest sensitivity enhancement factors (0.76×10^3 – 2.54×10^3) after a 60 s injection (Figure 3A). However, if the injection time of the bands without micelles is too long, it can lead to more complex components in the capillary, slower distribution effects, and even result in the disorder of peak shape or delay of peak elution. Electroosmotic flow (EOF) is not only the driving force for capillary separation but also one of the key parameters for controlling separation time, optimizing separation efficiency, and improving separation selectivity [48]. Generally, great efforts are made to create a minimal EOF to keep it smaller than the mobility of the analytes. The critical factors influencing EOF are the electrostatic equilibrium within the capillary, pH and the electrolyte phosphate. Herein, the pH of HCB remained consistent with the BGE; therefore, the concentration of phosphate buffer was adjusted to optimize the conditions of sweeping-MEKC. As shown in Figure 3B, the three phenoxy acid herbicides achieved the optimal resolution at the concentration of 100 mM phosphate buffer in HCB. The narrowest accumulated analyte zones were found when the sweeping came to an end [47]. Thus, sample injection time has a significant effect on the enrichment efficiency of sweeping-MEKC. In normal MEKC, the sample injection time is usually restricted to 10 s in order to maintain the broadness of the sample peaks. However, the injection time could be longer in sweeping-MEKC to improve the length of the sample plug zones and increase enrichment efficiency. As can be seen in Figure 3C, the sensitivity enhancement factor relative to sweeping-MEKC increased with injection time from 30 s to 120 s. However, when the injection time was extended beyond 120 s, the peaks began to broaden without further improvement in sensitivity enhancement and the resolution also decreased. This is probably because the analytes that were injected overloaded the capillary [49]. Thus, to maintain good sensitivity and resolution, 120 s was selected as the optimum injection time.

Generally, pH 2.5, 100 mM phosphate buffer HCB, 60 s injection time + 30 mM γ -CD, 50 mM SDS, pH 2.5, 50 mM phosphate buffer BGE + 120 s sample injection time achieved the optical resolution for three phenoxy acid herbicides in sweeping-MEKC (Figure 4). Detailed information regarding the limit of detection (LoD), limit of quantitation (LoQ), coefficient of determination (R^2), and relative standard deviation (RSD) of all enantiomers for sweeping-MEKC is listed in Table S4. Sweeping-MEKC is frequently used as an effective

online concentration method in the analysis of pesticides. For example, the sensitivity enhancement factor obtained by sweeping-MEKC ranged between 50- and 181-fold for the studied thirteen benzimidazoles [50]. In terms of enantioselective analysis, 134- and 159-fold enrichment factors were obtained for alprenolol enantiomers by sweeping-MEKC, respectively [51]. Herein, the sensitivity enhancement factor was calculated and summarized in Table S2. Compared with normal MEKC, the sensitivity was improved by 3 orders of magnitude.

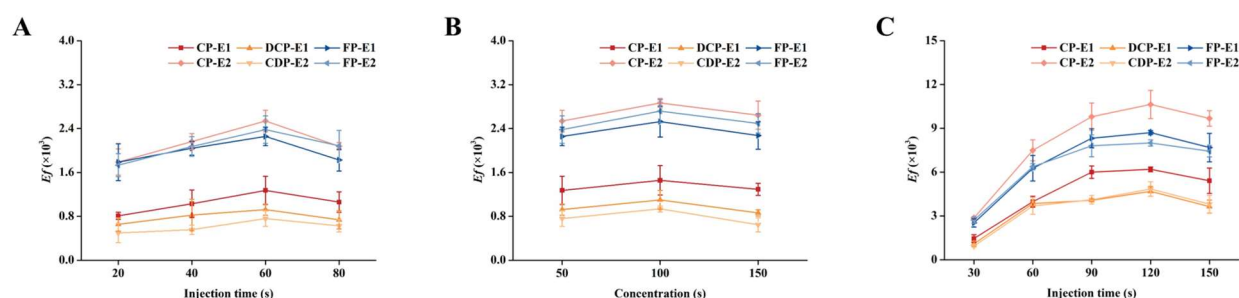


Figure 3. Enrichment efficiency of the enantiomers of three phenoxy acid herbicides by sweeping-MEKC. (A): Influence of the HCB injection time on E_f . (B): Influence of the phosphate buffer concentration of HCB on E_f . (C): Influence of the sample injection time on E_f .

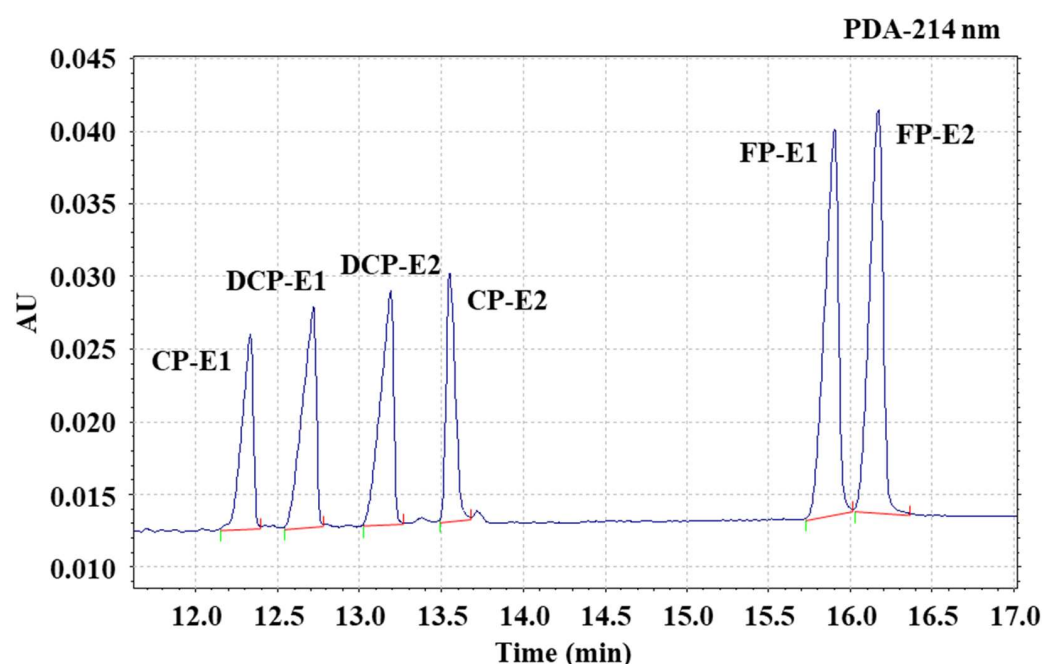


Figure 4. Electropherograms of chiral separation obtained in sweeping-MEKC for the enantiomers of three phenoxy acid herbicides. BGE: 30 mM γ -CD, 50 mM SDS, 50 mM phosphate buffer; HCB: pH 2.5, 100 mM phosphate buffer, 60 s injection time; 120 s sample injection time.

3.3. Optimization of ASEI-Sweeping-MEKC Conditions

Although sweeping-MEKC significantly improved enrichment efficiency, the current detection sensitivity is insufficient to meet the requirements of the trace-level analysis involved in environmental chiral pesticides determination. For a further improvement of sensitivity, ASEI-sweeping-MEKC was chosen to develop a highly sensitive analysis method. To perform ASEI-sweeping-MEKC, the capillary was filled with an acid micellar BGE to suppress EOF, and then an HCB plug was hydrodynamically introduced, followed by a water plug injected also by hydrodynamic injection. After that, electrokinetic injection was employed to introduce the anionic sample into the capillary. After the injection was

completed, the inlet end of the capillary was transferred to the vial containing BGE and a negative voltage (−20 kV) was applied. Consequently, sweeping and subsequent separation were achieved under MEKC mode. In an attempt to obtain the largest improvement in detection sensitivity, parameters including the injection time and phosphate buffer concentration of the HCB, the injection time and composition of the water plug, as well as the sample injection time were investigated in detail.

The HCB is used to reduce the anions' velocity and result in stacking in a narrow zone [52]. Therefore, the stacking efficiency is significantly influenced by the length, pH, and concentration of HCB. Herein, the injection of HCB was performed for different times to test the effect of the length of HCB on stacking efficiency. As the injection time of HCB increased from 20 s to 80 s, the sensitivity enhancement factor rose until reaching its maximum at 60 s. However, when the injection time was extended to 80 s, it resulted in prolonged migration times and compromised peak shapes (Figure 5A). This phenomenon may be attributed to the EOF becoming too low. Then, different concentrations (50 mM, 100 mM, 150 mM) of phosphate buffer in HCB were considered, since the pH remained consistent with that in BGE. Within the 50–150 mM concentration range, the sensitivity enhancement factor reached its maximum at 100 mM but decreased as the phosphate buffer concentration increased to 150 mM (Figure 5B), likely due to the increased ionic strength of the buffer [49].

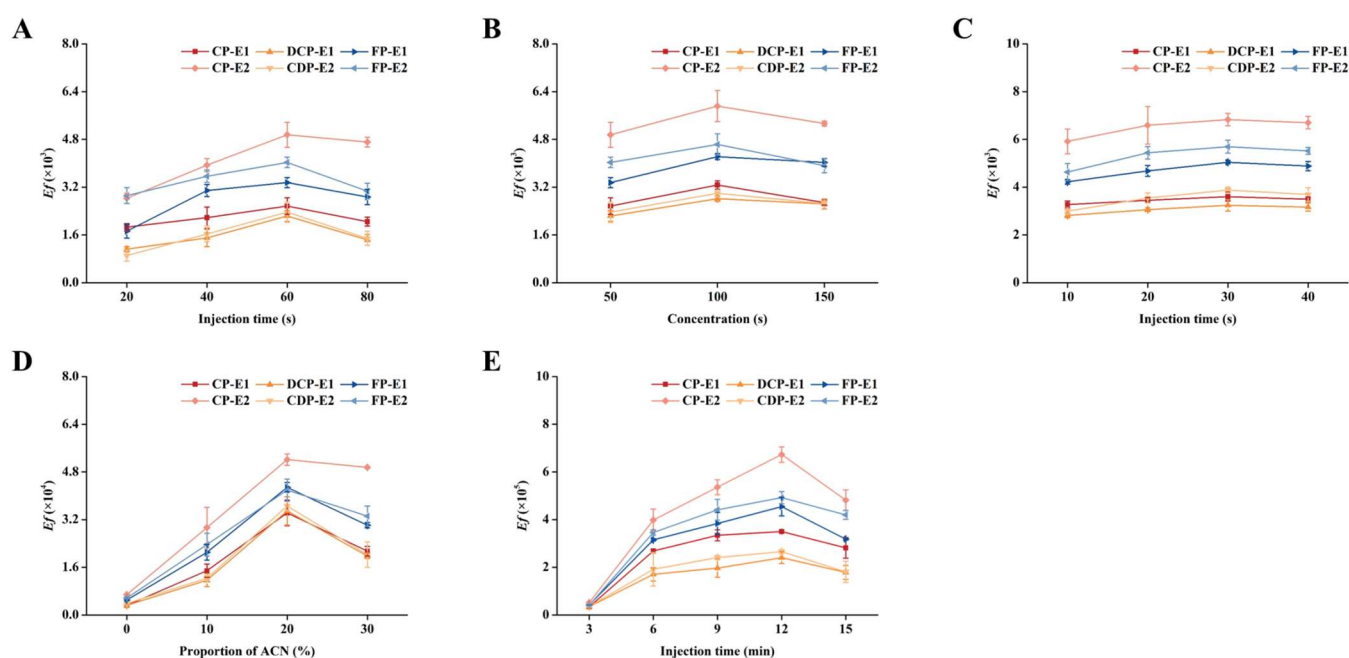


Figure 5. Enrichment efficiency of the enantiomers of three phenoxy acid herbicides by ASEI-sweeping-MEKC. (A): Influence of the HCB injection time on E_f . (B): Influence of the phosphate buffer concentration of HCB on E_f . (C): Influence of the water plug injection time on E_f . (D): Influence of the ACN proportion of the water plug on E_f . (E): Influence of the sample injection time on E_f .

Another influential factor of the stacking efficiency is the water plug injected before the sample, which accelerates the sample into the capillary by creating a high electric field at the tip of the capillary. The function of the water plug is to provide a high electric field at the injection point and act as a medium to hold the sample, which otherwise might be pumped out of the capillary and cause poor repeatability [53]. With the application of negative voltage, the sample ions moved rapidly towards the boundary between the water plug and the acidic HCB and were neutralized and stacked once they entered the acidic HCB. The length of the water plug is dependent on the injection time used to introduce the

water plug. The sensitivity enhancement factor increased as the water plug injection time extended from 10 s to 30 s. Beyond this point, no significant improvement in sensitivity was observed and the baseline noise increased as well (Figure 5C). This is because the water plug was slowly moving out of the column under the effect of the negative potential and thus the water plug should be long enough to hold the ions but should not be too long, which otherwise would take a longer time to pump the water plug out of the column [49]. In addition, the composition of the water plug also has an important effect on the stacking efficiency [25]. Acetonitrile, as an organic modifier, can reduce the conductivity, thereby reducing the EOF and improving the separation efficiency [54]. As shown in Figure 5D, the best sensitivity enhancement factor was achieved at 20% ACN. However, at 30%, the peaks broadened and the analysis time increased. The length of the sample zone depended on the injection time. In principle, the longer the injection time, the longer the sample zone and the better the concentration effect. However, the injection time should not be too long as it may cause overloading and decreased system stability [55]. The sensitivity enhancement factor increased as injection time increased from 3 to 12 min. However, when injection time exceeded 12 min, the concentration effect decreased, and broader peaks were observed (Figure 5E).

Under the optimized conditions mentioned above (20% ACN water plug, 30 s injection time + pH 2.5, 100 mM phosphate buffer HCB, 60 s injection time + 30 mM γ -CD, 50 mM SDS, pH 2.5, 50 mM phosphate buffer BGE + 12 min sample injection time), the electropherogram of samples at a concentration of 2 ng/mL by ASEI-sweeping-MEKC is illustrated in Figure 6 and high-resolution feature was not compromised. Detailed information regarding the limit of detection (LoD), the limit of quantitation (LoQ), the coefficient of determination (R^2), and the relative standard deviation (RSD) of all enantiomers for ASEI-sweeping-MEKC is listed in Table S4. The sensitivity enhancement factor was calculated and is summarized in Table S3. Compared with normal MEKC, the sensitivity was improved by 5 orders of magnitude (241,000–673,000). ASEI-sweeping-MEKC combines two different concentration strategies to generate a higher enhancement of sensitivity. The sensitivity could be even improved by almost 100,000-fold using this combination [25]. Kim et al. demonstrated that the concentration of anions could be accomplished by ASEI-sweeping-MEKC, and about a 1000- to 6000-fold increase in sensitivity was achieved for dansyl amino acids and two naphthalenedisulfonic acid (NDSA) isomers [56]. As to the racemic phenoxy acid herbicides, ASEI-sweeping-MEKC had approached over a 100,000-fold increase in detection sensitivity [25].

Phenoxy acid herbicides are a class of commercial herbicides widely used all over the world; however, their endocrine-disrupting effects have resulted in strict residue monitoring [57]. So far, various methods have been established for detecting these herbicides in different matrices. GC and GC-MS are widely used in the analysis of phenoxy acid herbicides with the limits of detection (LODs) ranging from 0.05 $\mu\text{g/L}$ to 0.19 ng/L [58,59]. On the other hand, the application of commercialized racemates of phenoxy acid herbicides has left the hidden trouble behind due to the distinctive biological effects of the enantiomers [60]. Therefore, optimization of the CE method for phenoxy acid herbicides should meet both chiral separation and detection sensitivity. Herein, the enrichment factors could sufficiently meet the requirement of trace-amount determination of the residues of these pesticide enantiomers in the environment and food samples. After a suitable offline sample cleanup step to remove matrix interferences, this method could be applied to real sample analysis and provide vital information required for the evaluation of health risks connected with individual enantiomers.

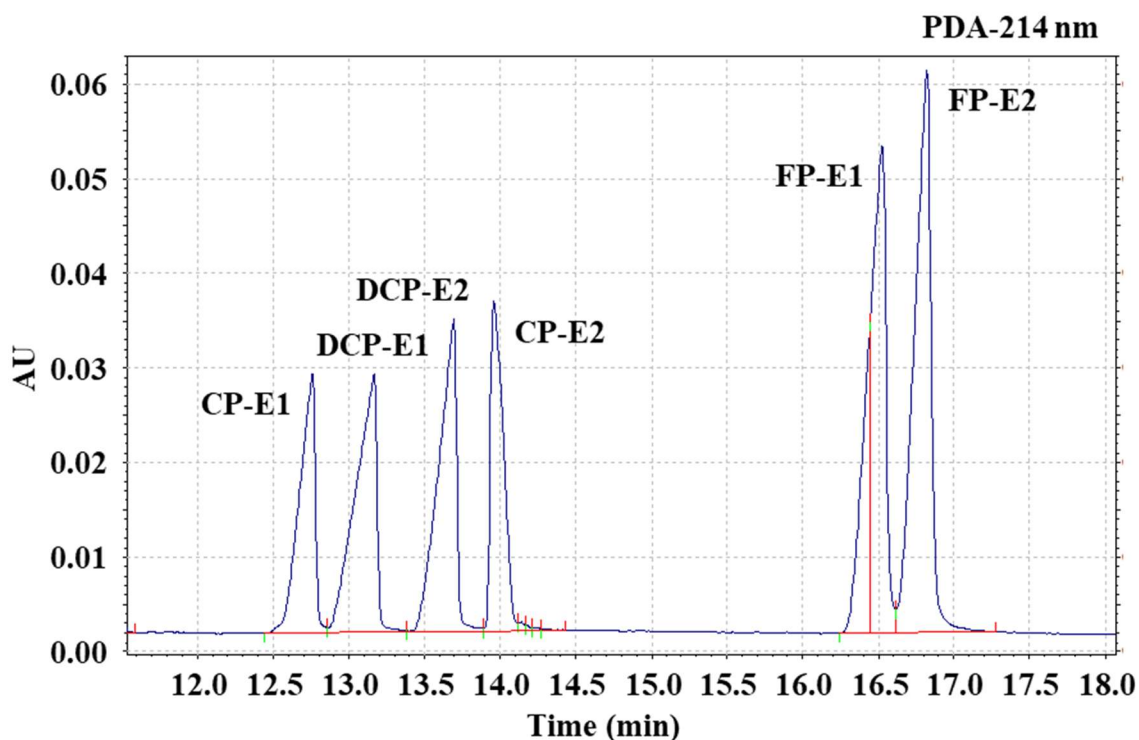


Figure 6. Electropherograms of chiral separation obtained in ASEI-sweeping-MEKC for the enantiomers of three phenoxy acid herbicides. Water plug: 20% ACN, 30 s injection time; HCB: pH 2.5, 100 mM phosphate buffer, 60 s injection time; BGE: 30 mM γ -CD, 50 mM SDS, 50 mM phosphate buffer; 12 min sample injection time.

4. Conclusions

To sum up, ASEI-sweeping-MEKC, an efficient technique for trace-level analysis, could be used for online concentration, chiral separation, and determination of the enantiomers of three phenoxy acid herbicides. Under optimized conditions, the sensitivity enhancement factors for the six enantiomers were 241,000 to 673,000-fold, respectively, and did not cause any loss in resolution when compared with normal MEKC. With regard to the better enhancement of sensitivity, ASEI-sweeping-MEKC is recommended for the ultra-trace chiral separation and determination of chiral pesticides necessarily involved in the analysis of environmental samples, which will in turn help us make a comprehensive risk assessment and guide further use of pesticides.

Several limitations should be acknowledged. Effective pretreatment methods for different environmental samples and analysis of these herbicides in real environmental samples are also indispensable to verify the availability of the presented method.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy16141313/s1>, Table S1. Detailed information regarding three chiral phenoxy acid herbicides; Table S2. The sensitivity enhancement factor of three phenoxy acid enantiomers by sweeping-MEKC; Table S3. The sensitivity enhancement factor of three phenoxy acid enantiomers by ASEI-Sweeping-MEKC; Table S4. The key performance parameters of all enantiomers for different analytical methods.

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