



# Enantioseparation of four amide herbicide stereoisomers using high-performance liquid chromatography



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## ABSTRACT

The chirality of herbicides has been the focus of research. However, there is little information on the enantioseparation of amide herbicides with different chiral elements. In this study, the need for different chiral stationary phases (CSPs), mobile phases, temperatures and flow rates for the separation of napropamide, acetochlor and propisochlor was discussed in detail and compared to metolachlor. Resolution of C-chiral enantiomers was easier than that of axial-chiral enantiomers. Metolachlor and acetochlor could achieve baseline separation only on AY-H and AS-H columns, respectively. Propisochlor had satisfactory separations on OD-H and AS-H columns. Napropamide was separated on OJ-H, AY-H and AS-H columns. Both the structures of the compounds and CSPs and the interactions between them played significant roles in the enantioseparations. Molecule dockings were also used to elucidate the separation mechanisms. C-chiral enantiomers had perfect symmetry in their optical properties, whereas the axial-chiral enantiomers did not. The elution order for napropamide, acetochlor and propisochlor, with a single chiral location, was R- prior to S-. These results were the first that compare the enantioseparations of four amide herbicides with different chirality, and they provided the absolute configurations for the herbicides. The paper also illustrated certain mechanisms for enantioseparations.

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## 1. Introduction

Amide herbicides are routinely used as pre-emergence selective herbicides to increase soybean, corn, peanut, cotton, potato, rapeseed, sugarcane, garlic and other crop yields. The amount of amide herbicides used is considerable, and together with the amino acid herbicides (glufosinate, glyphosate) and sulfonylurea herbicides, they compose the majority of herbicides used in China. The water solubility of amide herbicides is moderate, and they can be rapidly absorbed into plants. [1]

Amide herbicides are commonly chiral molecules. The hindered rotation about the phenyl-nitrogen bond and the asymmetrically substituted C-atom in the alkyl moiety contribute to the chirality of these compounds [2].

Metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide], napropamide [N,N-diethyl-2-(1-naphthalenyloxy) propanamide], acetochlor

[2-chloro-N-ethoxymethyl-6'-ethylacet-o-toluamide] and propisochlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(isopropoxymethyl) acetamide] are popular amide herbicides. These four herbicides include compounds with different elements of chirality. Napropamide is C-chiral because of the presence of an asymmetrically substituted C-atom in the carboxy alkyl moiety. Acetochlor and propisochlor are axial-chiral because of the asymmetric phenyl substitution. Metolachlor has both axial- and C-axial chirality. They are marketed as racemic products, although their transformation reactions in biological systems and the environment may show enantioselectivity. To obtain the enantiomers of the four herbicides, gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and other techniques for chiral separation have been developed, and HPLC is the most frequently used technique. GC-CSP [2],  $\gamma$ -CD-MEK [3] and HPLC [4,5] have been used for metolachlor separation. Using only a mixture of fresh diethyl ether (DEE) and *n*-hexane as the mobile phase on an OD-H column, baseline-separated peaks were eventually obtained [5]. Napropamide separation has been accomplished using an amylase-3-[(S)-1 phenyl ethyl carbamate] column with *n*-

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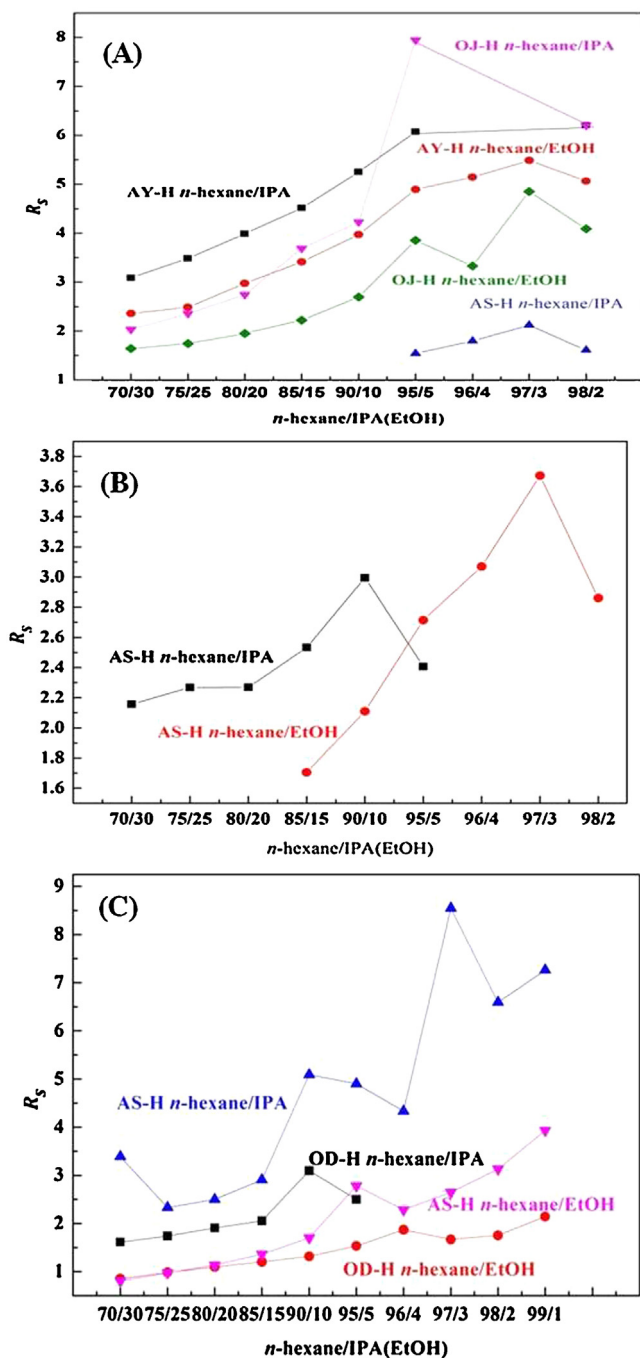


Fig. 1. Resolution obtained for (A) napropamide, (B) acetochlor and (C) propisochlor using different organic modifiers.

hexane/isopropanol (90/10), and the resolution was 1.14 [6]. Peak resolution tended to be progressively better when an OJ-H column was used for napropamide [7]. For acetochlor, partial resolution has been obtained on a CDMPC with *n*-hexane/isopropanol (98/2) [6], and a satisfactory separation was achieved on a Chiralpak AS-H column with *n*-hexane/isopropanol (97/3) [8]. For propisochlor, using *n*-hexane/IPA (97/3) on an AS-H chiral column achieved a baseline separation [8]. However, little effort has been put forth over the last decades to achieve the enantioseparation of these acetamide herbicides. Only one or two columns have been utilized to separate these amide herbicides, and the resolution has not always been satisfactory. Additionally, no comparisons of

the different efficiencies for resolution have been made for the different columns.

By studying the optimized HPLC separation conditions for metolachlor, napropamide, acetochlor and propisochlor, we hope to achieve a deeper understanding of the rules for resolution of amide herbicides with different chiral elements. Amide herbicides with only a C-chirality are often easier to resolve. CSPs and mobile phases may play significant roles in the resolution. Lower flow rates may increase resolution. Resolution are enthalpy-driven. The separation mechanisms may relate to the structures of both the herbicides and CSPs and the interactions between them. The results from the present study may offer additional information for better resolution of the four amide herbicides with different chiral elements and permit a deeper analysis of the separation mechanisms.

## 2. Materials and methods

### 2.1. Chemicals and materials

Napropamide was supplied by the Jiangsu Rudong pesticide factory (Rudong, China). Acetochlor, propisochlor and metolachlor were from Shandong Qiaochang Chemical (Binzhou, China). The stereo configurations of the selected amide pesticides are given in Fig. S1. These compounds were dissolved in *n*-hexane or isopropanol (IPA) at a concentration of 1000 mg L<sup>-1</sup> and stored in the dark at 4 °C prior to analysis and separation by HPLC. Other chemicals and solvents used in this study were HPLC grade.

### 2.2. Preparation and absolute confirmation of the enantiomers

Chiral separations of napropamide, acetochlor, propisochlor and metolachlor were resolved by a Jasco HPLC system consisting of a pump, autosampler, column oven, photo diode array detector, and circular dichroism detector (Jasco models PU-4180, AS-4050, Co-4061, MD-4010 and CD-4095, respectively). In the preliminary experiments, chiral columns with different chiral stationary phases (CSPs), including Chiralcel OD-H [cellulose tris-(3, 5-dimethylphenylcarbamate)], Chiralcel OJ-H [cellulose tris-(4-methylbenzoate)], Chiralcel AS-H [amylose tris ((S)-1-phenylcarbamate)] and Chiralcel AY-H [amylose tris-(5-chloro-2-methylphenylcarbamate)] were tested for their resolution. The structures of the CSPs are shown in Fig. S2. For the analysis process, all the columns were 250 × 4.6 mm i.d. with a 5 μm particle size. The UV signals were recorded at 220 nm, and the CD detection wavelength was set at 235 nm for all analyses. A volume of 10 μl of the herbicide was injected for the enantioseparation, and the analytical runs were performed using a normal-phase eluent. The resolved fractions were individually collected at the outlet of the UV detector, and the purities were found to be >99%. The capacity factor ( $k'$ ), separation factor ( $\alpha$ ), and resolution factor ( $R_s$ ) for the enantiomers, which were usually used as chromatographic parameters for the enantioselectivity evaluation of the CSP, were calculated.

Electronic circular dichroism (ECD) spectra for the stereoisomers were recorded on a Jasco J-1500 CD spectrometer. A quartz cuvette with a 1 mm path length was used. Throughout the ECD measurements, the following parameters were set: bandwidth, 1.00 nm; temperature set point, 25 °C; wavelength start, 400 nm; wavelength end, 185 nm; speed, 200 nm min<sup>-1</sup>. Vibrational circular dichroism (VCD) was acquired in a Jasco FVS-6000 VCD spectrometer from 2000 to 850 cm<sup>-1</sup>. A cell with a 0.05 mm path length and BaF<sub>2</sub> windows was used. The raw spectra of the solvents were subtracted from all presented spectra.

The ECD and VCD intensities for the enantiomers were calculated utilizing Gaussian 09 [9]. Time-dependent density functional

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