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Chiral Stationary Phase Comparison for the Enantioseparations of 20 Agrochemical Compounds

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Introduction

Approximately 30% of organic chemical pesticides are chiral molecules, and, since 2007, ~44% of new pesticidal molecules contain at least one asymmetric center (Jeschke, 2018). This is significant because, in some cases, only one stereoisomer is responsible for the targeted effects while the others are less active or entirely inactive. Differences in efficacy, toxicity, environmental effects, and degradation rates suggest the potential need for robust characterization and determination of enantiomeric ratios of pesticide products. Chiral chromatography provides both a way to determine these ratios and, if desired, a means to prepare enriched product with better specificity and/or lower toxicity. In this study, high-performance liquid chromatography (HPLC) chiral separations of 20 agrochemical compounds were performed by screening compounds on each of seven chiral stationary phases (CSPs) with each of two different mobile phases. "Hit" rates, determined by resolution values (R_S) ≥ 1, are reported for each column/mobile phase combination. The instances of highest resolution along with the number of unique hits are also reported for each column.

Experimental

HPLC was performed using either a Shimadzu Nexera (Kyoto, Japan) or Agilent 1100 Series HPLC (Palo Alto, CA). All agrochemical standards were acquired from Chem Service Inc. (West Chester, PA), and the molecular structures of these compounds are shown in Figure 1. Samples were prepared in mobile phase and screened on each of seven 5 µm, 25 cm x 4.6 mm chiral stationary phase (CSP) columns from Regis Technologies, Inc. (Morton Grove, IL) under isocratic conditions with each of two different mobile phases. The structures of the chiral selectors are shown in Figure 2, and chromatographic screening conditions are summarized in Table 1.

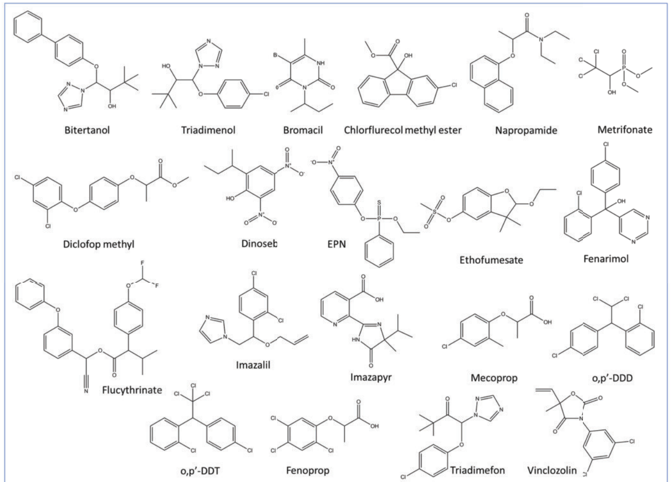

Figure 1: Structures of agrochemical compounds

Column:	1. Reflect C-Amylose A 2. Reflect C-Cellulose B 3. Reflect I-Amylose A 4. Reflect I-Cellulose B 5. Reflect I-Cellulose C 6. Reflect I-Cellulose J 7. Whelk-O1
Mobile Phase:	1. 80:20 n-Hex:EtOH*‡ 2. 80:20 n-Hex:IPA*‡
Flow:	1.5 mL/min
Oven Temp:	30° C
Detection	210 nm

* 0.1% TFA added when screening fenoprop,mecoprop, and imazapyr
‡ 0.1% DEA added when screening dinoseb

Table 1: Chromatographic screening conditions used in this study

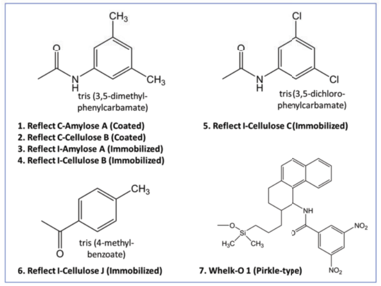

Figure 2: Column names and structures of the chiral selectors used in this study

Results and Discussion

Column hit rates, determined by resolution values (R_S) ≥ 1, are reported for each column/mobile phase combination. Peak resolution (R_S) was determined using the equation:

$$R_S = 2 \times \frac{t_2 - t_1}{w_1 + w_2}$$

where t_1 and t_2 are the retention times, and w_1 and w_2 are the peak widths. When only partial resolution was observed, w and R_S were calculated by the USP tangent method. If no valley was observable, resolution was estimated as R_S = 0. Figures 3 (bromacil), 4 (metrifonate), and 5 (EPN) illustrate the kind of data that were collected for each of the 20 agrochemical compounds. Hits are designated by red outlines.

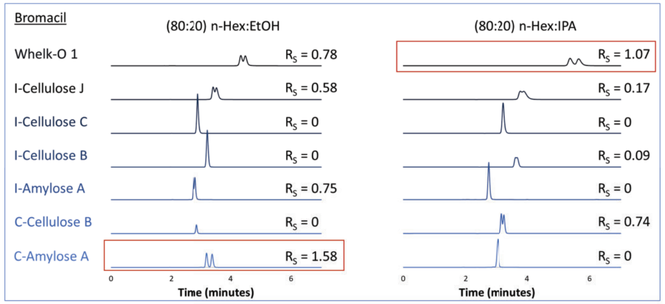

Figure 3: Chromatograms for bromacil on each of the seven columns and two mobile phases

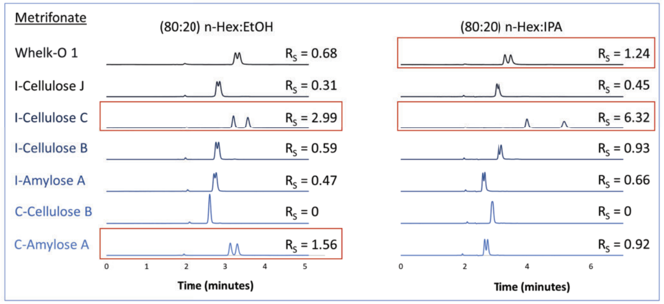

Figure 4: Chromatograms for metrifonate on each of the seven columns and two mobile phases

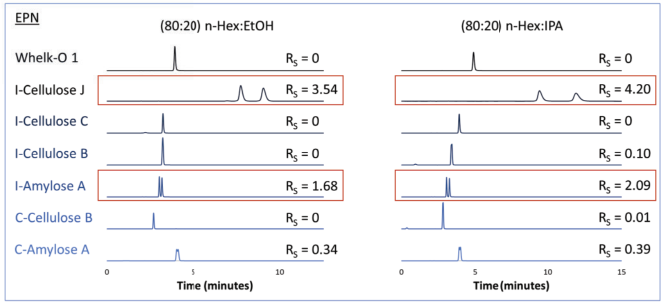

Figure 5: Chromatograms for EPN on each of the seven columns and two mobile phases

Figure 6 summarizes the number of compound hits that were observed for each column. The effect of the mobile phase on selectivity is also noted for cases when one mobile phase modifier (i.e. EtOH or IPA) resulted in a R_S value ≥ 1.5 times higher than the R_S value observed when using the other modifier.

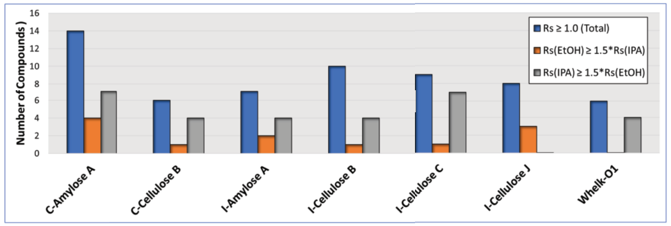

Figure 6: Number of hits on each CSP along with instances when EtOH or IPA as mobile phase modifier resulted in 50% higher resolution over the other modifier.

Figure 7 shows the number of compounds for which each CSP provided the highest resolution. It also highlights the number of compounds for which each CSP provided a unique hit. For example, Reflect C-Amylose A provided a total of 14 compound hits, and seven of those hits were the best that were observed for any of the columns used in this study. One hit was unique, meaning that C-Amylose A was the only column to provide a R_S ≥ 1.0 for that particular agricultural compound.

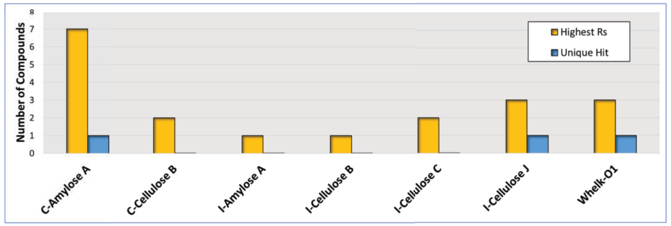

Figure 7: Number of compounds for which each CSP provided the highest resolution along with the number of compounds for which each CSP provided a unique hit.

From Figure 7, it can be determined that 19 of the 20 compounds are represented as having been resolved best by a single CSP. Dinoseb was the only compound to have exhibited no enantiomeric resolution (i.e. R_S = 0) with any of the column/mobile phase combinations. Upon close examination of the peak shapes of the 14 chromatograms collected for dinoseb, it was determined that Reflect C-Amylose A gave the best chance of resolving the enantiomers. As shown in Figure 8, by weakening the mobile phase to 95:5:0.1 n-Hex:EtOH:DEA, a resolution of 1.51 was achieved.

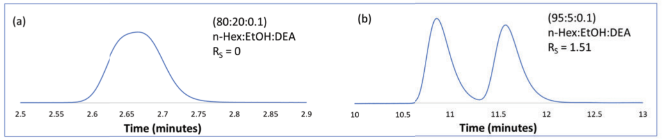

Figure 8: Separation of dinoseb on C-Amylose A with different mobile phase compositions

Compounds containing multiple chiral centers can present special difficulties. In the present study, bitertanol and flucythrinate, each had four stereo-isomers. Each compound hit uniquely on single CSPs such that $R_{S,1}$, $R_{S,2}$, and $R_{S,3}$ were each ≥ 1.0. Weakening the mobile phases allowed for baseline resolution of all four isomers for both compounds. Figure 9 shows the separation of bitertanol with 95:5 n-Hex:EtOH on I-Cellulose J.

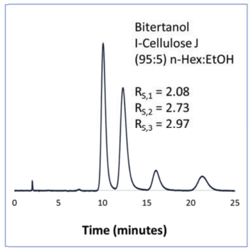

Figure 9: Separation of bitertanol on I-Cellulose J

Summary and Conclusions

- All 20 agrochemicals were ultimately resolvable on at least one of the CSPs
- Each column provided the highest R_S for at least one of the compounds
- Of the seven columns screened, C-Amylose A provided highest hit rate (14/20)
- All compounds could be resolved by using just three columns (C-Amylose A, I-Cellulose J, and Whelk-O1). This information may be useful when prioritizing orthogonal column sets for screening of additional compounds.

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