

Enantiomeric and Diastereomeric Resolutions of Chiral Pesticides by ACQUITY UPC² with UV Detection

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APPLICATION BENEFITS

- Improved enantiomeric and diastereomeric resolution and shorter analysis times resulting in higher sample throughput and reduced solvent consumption when compared with normal phase separations.
- Reliable and reproducible measurement of the enantiomer and/or diastereomer ratios to accurately determine the correct application rates.

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ACQUITY® UltraPerformance
Convergence Chromatography System™
(UPC²®)

ACQUITY UPLC® Photodiode Array
(PDA) Detector

Empower® 3 Software

KEY WORDS

Chiral pesticides, UPC², enantiomer, diastereomer, chiral separation, chiral resolution, metolachlor, metalaxyl, difenoconazole.

INTRODUCTION

The development of analytical methods for the separation of chiral compounds is important in many areas of research because it is well known that enantiomers can react differently in a chiral environment. Biochemical reactions can be stereo or enantioselective – and while one enantiomer may deliver the desired effect (referred to as the eutomer) to the target species, the other enantiomer may be less effective to the target or completely ineffective. It is estimated that 30% of pesticides on the market today have optical isomers and there are reports that 40% of the pesticides used in China are chiral.^{1,2} The study of enantioselectivity is important to the agricultural chemicals manufacturing industry since the knowledge of the efficacy of each individual enantiomer could facilitate a significant reduction in the total amount of pesticide applied. In order to improve our knowledge of enantioselectivity, analytical methods that provide reliable and reproducible separations in a rapid time frame are needed. Supercritical fluid chromatography (SFC) has become known as an effective chiral separations technique possessing many advantages over conventional high performance liquid chromatography (HPLC).^{3,4} The properties of a supercritical fluid allow high efficiency separations with shorter analysis times to be achieved. The structural complexity of new pesticides is increasing, which means that there is a greater likelihood that multiple chiral centers may be present in a molecule^{5,6,7} and high efficiency techniques are needed to perform successful separations.

In this application note, we present the enantiomeric and/or diastereomeric separations of three pesticides: metalaxyl-M (a phenylamide fungicide), S-metolachlor (acetanilide class of herbicides), and difenoconazole (a triazole fungicide). Metalaxyl has one chiral center, while metolachlor and difenoconazole have two chiral centers. The structures are shown in Figures 1 to 3. Separations were performed using Waters® ACQUITY UltraPerformance Convergence Chromatography System (UPC²). Convergence chromatography is a complimentary separation technique to liquid chromatography, providing orthogonal selectivity and using supercritical CO₂ as the primary mobile phase.

EXPERIMENTAL

Instrumentation

Separations were done using the ACQUITY UPC² System. Detection was by photodiode array (PDA) in combination with single wavelength detection. Empower 3 Software was used for chromatographic data processing.

Sample Preparation: The pesticide standards, supplied by Syngenta, were prepared in 2-propanol.

Method conditions

Metalaxyl-M

Separation mode: Gradient
 Column: Chiralpak IA-3
 4.6 x 150 mm, 3- μ m
 Co-solvent: 2-propanol
 ABPR: 2000 psi/138 bar
 Flow rate: 4.0 mL/min
 UV detection: 215 nm
 Column temp.: 55 °C.
 Injection volume: 1 μ L

S-metolachlor

Separation mode: Gradient
 Column: Chiralpak IA-3
 4.6 x 150 mm, 3 μ m
 Co-solvent: 2-propanol
 ABPR: 2000 psi/138 bar
 Flow rate: 2.5 mL/min
 UV detection: 220 nm
 Column temp.: 35 °C
 Injection volume: 2 μ L

Difenoconazole

Separation mode: Isocratic
 Column: Chiralcel OD-3
 4.6 x 150 mm, 3 μ m
 Co-solvent: 2-propanol/Butan-1-ol
 (70/30)
 ABPR: 2000 psi/138 bar
 Flow rate: 2.0 mL/min
 UV detection: 235 nm
 Column temp.: 35 °C
 Injection volume: 2 μ L

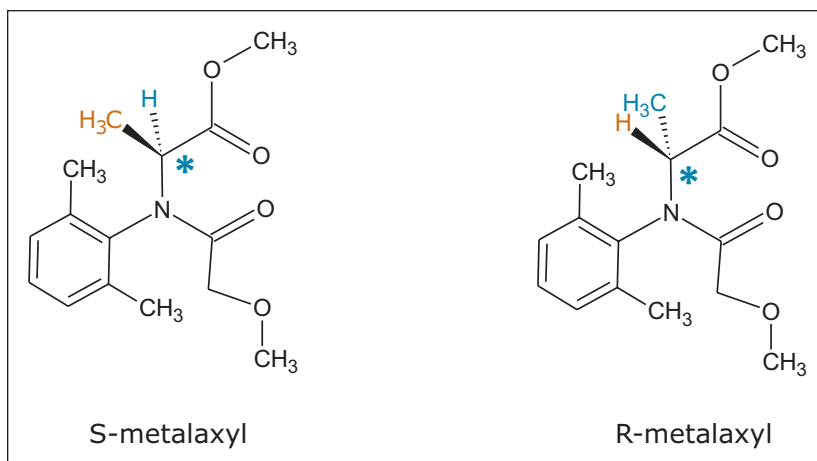


Figure 1. Structures of the two metalaxyl enantiomers. The asterisk indicates the stereogenic center.

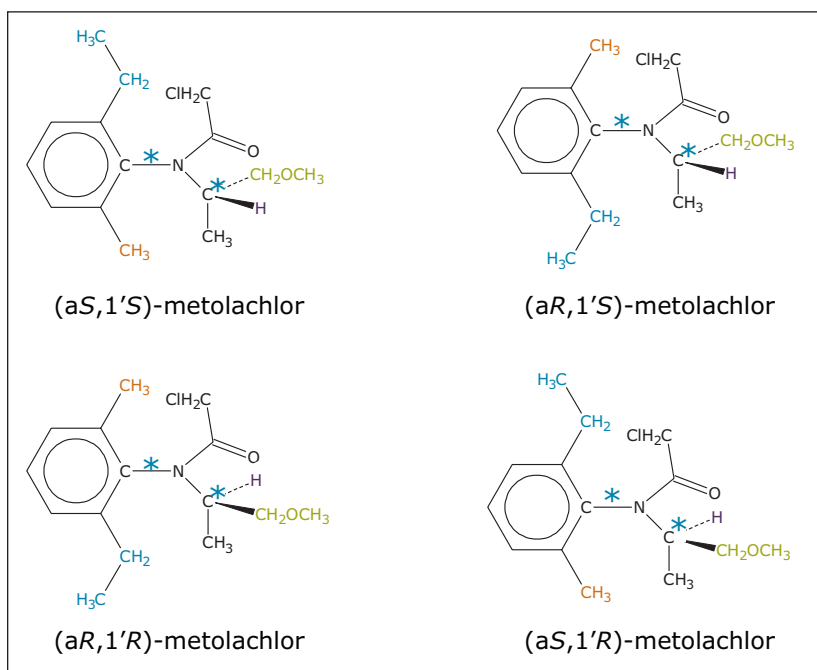


Figure 2. Structures of the four stereoisomers of metolachlor. The asterisks indicate the stereogenic centers.

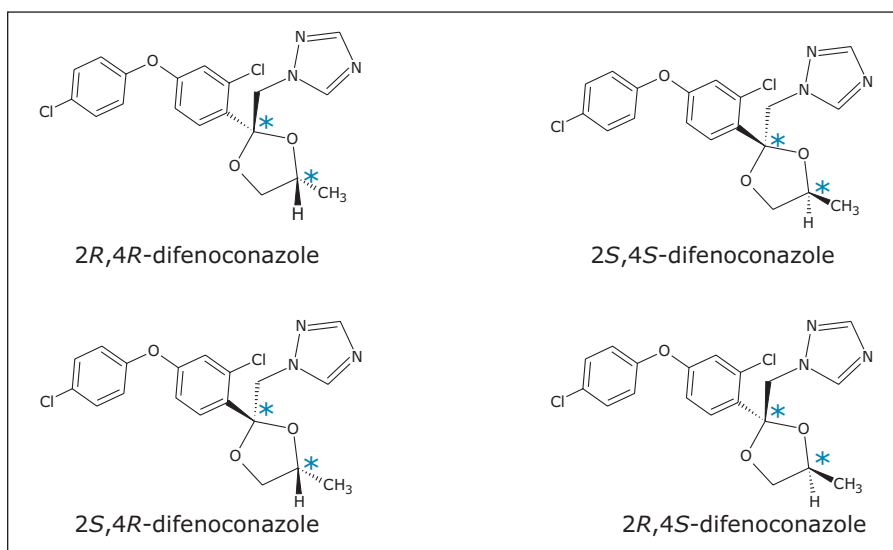


Figure 3. Structures of the four stereoisomers of difenoconazole. The asterisks indicate the stereogenic centers.

RESULTS AND DISCUSSION

The method development for the standard pesticides began by using a generic screening gradient with a number of chiral columns and co-solvents. This screening step can be completed rapidly due to the shorter analysis times that are possible with this technique. The combination of co-solvent and column that produced the most promising separation for each compound was then optimized. The selectivity in a chiral separation can change markedly by varying the temperature, pressure, and flow rate.⁸ Gradient and isocratic separations were evaluated; both resulted in successful separation of the stereoisomers for each compound. The separation that showed optimum resolution (R_s) is reported here. The optimized separations for the standard racemic mixture of metalaxyl and the biologically active R-enantiomer,⁹ metalaxyl-M are shown in Figure 4.

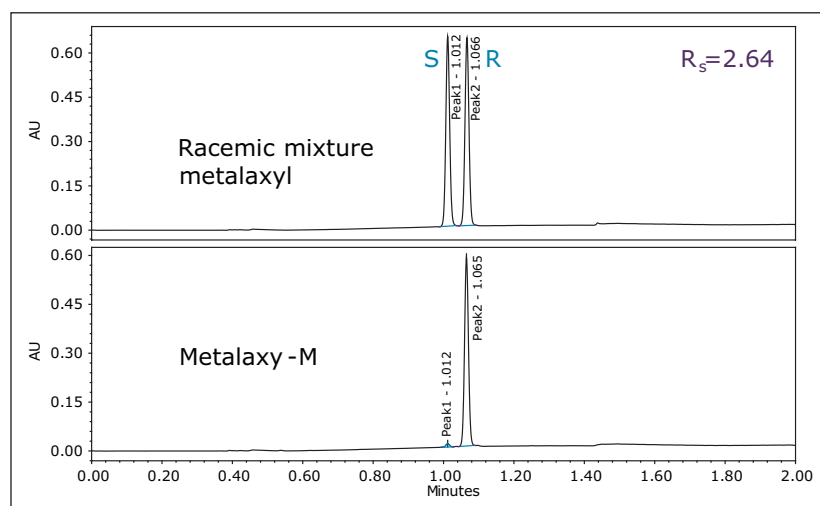


Figure 4. ACQUITY UPC² UV chromatogram at 215 nm, S, and R enantiomers of a racemic mixture of metalaxyl and metalaxyl-M standards.

The R_s between the S and R enantiomers was 2.64 (Table 1). A comprehensive survey of enantioselective separations of chiral pesticides in the literature, published in 2009, reported a normal phase isocratic method using hexane/ethanol (60/40) for the resolution of metalaxyl enantiomers. The R_s was reported to be 1.94 with both enantiomers eluting in just under 15 minutes.^{10,11}

	$R_{S1,2}$	$R_{S2,3}$	$R_{S3,4}$
Metalaxyl-M	2.64	—	—
S-metolachlor	2.36	1.74	1.62
Difenoconazole	3.50	1.50	2.05

Table 1. Resolution for the stereoisomers of metalaxyl-M, S-metolachlor, and difenoconazole.

In the case of metolachlor, 95% of the herbicidal activity is reported to come from the S pair of enantiomers.^{12,13} The separation of all four stereoisomers of metolachlor is shown in Figure 5 with the minimum R_s being 1.62 between peaks 3 and 4, (Table 1). The baseline resolution of the stereoisomers of metolachlor using a normal-phase separation with hexane/diethyl ether (91/9) was also reported in the 2009 article. A figure from this publication showed all four stereoisomers eluting between 20 to 30 min.^{10,14} The ACQUITY UPC² methods reported here have significantly shorter run times for both metalaxyl and metolachlor. In addition, potentially hazardous solvents are not required.

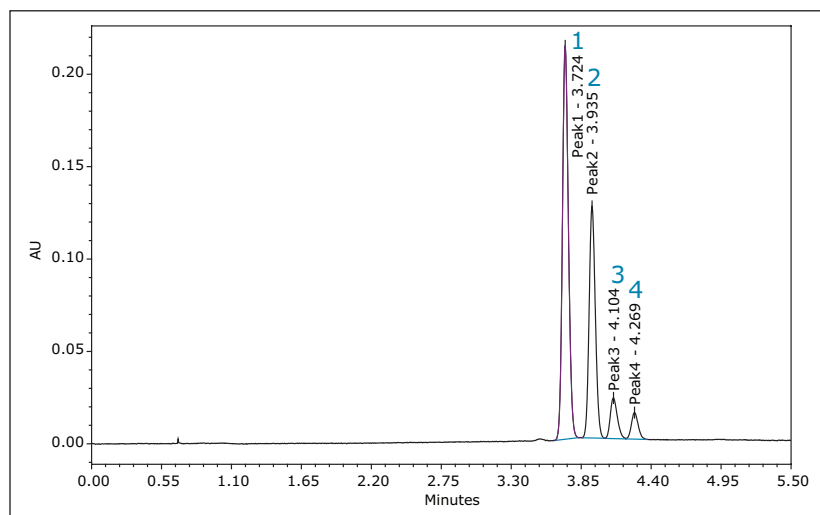


Figure 5. ACQUITY UPC² UV chromatogram at 220 nm using gradient elution of the S and R enantiomeric pairs of standard S-metolachlor.

The separation of the stereoisomers in a racemic mixture of difenoconazole is shown in Figure 6. The minimum R_s of 1.5 was between peaks 2 and 3. A normal-phase separation of the stereoisomers of difenoconazole using hexane/ethanol (90/10) was reported in the previously mentioned paper, resolution of the four stereoisomers took place between 30 and 55 minutes. Baseline separation ($R_s > 1.5$) was not achieved between peaks 1,2 or 2,3 in the reported method.^{10,15}

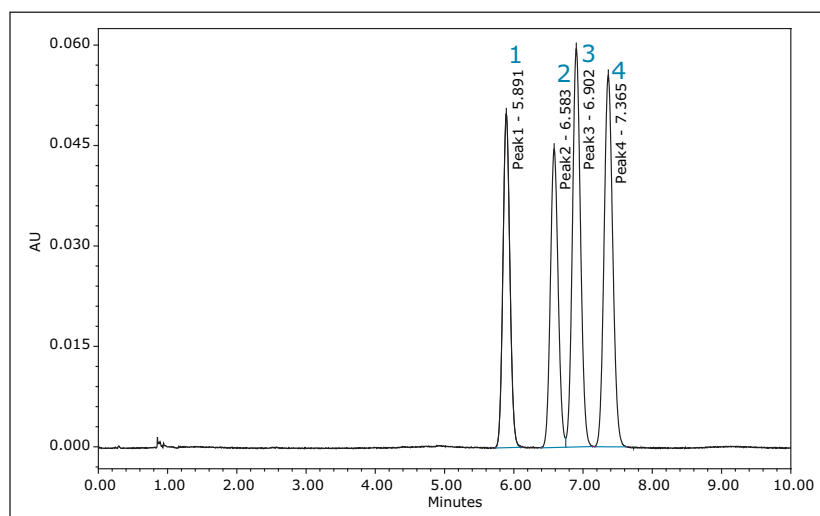


Figure 6. ACQUITY UPC² UV chromatogram at 235 nm using isocratic elution of the stereoisomers of difenoconazole.

The optimized ACQUITY UPC² methods allowed increased sample throughput and improved enantiomeric and diastereomeric resolution. In the case of metalaxyl-M (Figure 4), both enantiomers eluted in one minute; for S-metolachlor all four stereoisomers had eluted in 4.5 minutes (Figure 5). The resolution of the four difenoconazole stereoisomers used isocratic elution in under eight minutes (Figure 6), six times faster than some normal-phase methods reported in the literature. Reproducibility data (n=6) for retention time, area, area %, and height gave %RSD's less than or equal to 1.22 for all the stereoisomers of each compound, (Tables 2 to 4).

Metalaxyl-M %RSD (n=6)				
	Rt	Area	%Area	Height
S	0.04	1.18	1.10	0.77
R	0.03	0.61	0.03	0.66

Table 2. %RSD for six replicate injections of metalaxyl-M.

S-metolachlor %RSD (n=6)				
	Rt	Area	%Area	Height
Peak 1	0.11	0.22	0.09	0.77
Peak 2	0.11	0.19	0.09	0.22
Peak 3	0.07	0.33	0.27	0.29
Peak 4	0.08	1.20	1.22	0.36

Table 3. %RSD for six replicate injections of S-metolachlor.

Difenoconazole %RSD (n=6)				
	Rt	Area	%Area	Height
Peak 1	0.06	0.35	0.13	0.34
Peak 2	0.37	0.50	0.24	0.47
Peak 3	0.44	0.26	0.08	0.45
Peak 4	0.41	0.23	0.12	0.44

Table 4. %RSD for six replicate injections of difenoconazole.

CONCLUSIONS

In this application note we have shown the analysis of chiral pesticides using ACQUITY UPC². ACQUITY UPC² allows high efficiency separations that can significantly increase the sample throughput when compared with traditional normal-phase separations.¹⁰ The time taken to develop a method from the column and co-solvent screening step to the final optimized method is decreased. The methods described here use supercritical CO₂ as the primary mobile phase and predominantly 2-propanol as the organic modifier. The need to use large volumes of potentially hazardous solvents is reduced as is the cost associated with solvent waste disposal. The %RSD's obtained were comparable to those obtained by UPLC[®]/UV methods.

The study of enantioselective toxicity and environmental fate has previously been a challenge due to the difficulty in resolving chiral compounds. The benefit of having faster analytical methods to resolve chiral compounds means that critical information pertaining to their stereoselective behavior can be obtained more rapidly.

References

1. Sekhon BS. Chiral pesticides. *J. Pestic. Sci.* 2009; 34(1):1-12.
2. Liu WP. Pesticide Environmental Chemistry. *Chemical Industry Press*, Beijing, China. 2006; 341-343.
3. Jin L, Gao W, Yang H, Lin C, Liu W. Enantiomeric resolution of five chiral pesticides on a chiralpak IB-H column by SFC. *J Chrom. Sci.* 2011 October; 49:739-743.
4. Toribo L, del Noza MJ, Bernal JL, Jimenez JJ, Alonso C. Chiral separation of some triazole pesticides by supercritical fluid chromatography. *J Chrom A.* 2004; 1046:249-253.
5. Cantrell CL, Dayan FE, O'Duke S. Natural Products As Sources for New Pesticides. *J. Nat. Prod.* 2012; 75(6):1231-1242.
6. Mann RS, Kaufman PE. Natural product pesticides: Their development, delivery and use against insect vectors. *Mini-reviews in Organic Chemistry*, 2012; 9:185-202.
7. Ulrich EM, Morrison CN, Goldsmith MR, Foreman WT. Chiral Pesticides: Identification, Description and Environmental Implications. *Reviews of Environmental Contamination and Toxicology*. Springer U.S. 2012; 217: 1-74.
8. McCauley JP, Subbarao L, and Chen R. [Enantiomeric and Diastereomeric Separations of Pyrethroids Using UPC²](#). Waters Application Note APNT134717217. 2012, December.
9. Zadra C, Marucchini C, Zazzeini A. Behavior of metalaxyl and its pure R-enantiomer in sunflower. *J. Agric. Food Chem.* 2002; 50:5373-5377.
10. Ye J, Wu J, Weiping L. Enantioselective separation and analysis of chiral pesticides by high-performance liquid chromatography. *Trends in Analytical Chemistry.* 2009; 28(10):1148-1163.
11. Saito K, Yato M, Ito T, Iwasaki Y, Ito R, Matsuki Y, Nakazawa H. Verification of the need for optical purity measurement of chiral pesticide standards as agricultural reference materials Accred. *Qual. Assur.* 2008; 13:373-379.
12. Poiger T, Muller MD, Buser HR. Verifying the chiral switch of the pesticide metolachlor on the basis of the enantiomer composition of the environmental residues. *Chimia.* 2002; 56:300-303.
13. O'Connell PJ, Harms CT, Allen JRF. Metolachlor, S-metolachlor and their role within sustainable weed-management. *Crop Protection.* 1998; 17(3): 207-212.
14. Polcaro CM, Berti A, Mannina L, Marra C, Sinibaldi M, Viel S. Chiral HPLC resolution of neutral pesticides. *J. Liq. Chromatogr. Relat. Technol.* 2004; 27:49-61.
15. Zhou Y, Li L, Lin K, Zhu X, Liu W. Enantiomer separation of triazole fungicides by high-performance liquid chromatography. *Chirality.* 2009; 21:421-427.

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