

Stereoselective Separation of Triazole Fungicides Using the ACQUITY UPC² System and ACQUITY UPC² Trefoil Chiral Columns

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APPLICATION BENEFITS

- Improved enantiomeric and diastereomeric resolution.
- Shorter analysis times resulting in higher sample throughput and reduced solvent consumption compared with normal phase separations.
- Reliable and reproducible measurement of the enantiomeric and/or diastereomeric ratios.

WATERS SOLUTIONS

[ACQUITY UPC²® System](#)

ACQUITY UPC² Photodiode Array
(PDA) Detector

[Empower® 3 Software](#)

[ACQUITY UPC² Trefoil™ AMY 1 Column](#)

[ACQUITY UPC² Trefoil CEL1 Column](#)

KEY WORDS

Chiral pesticides, UPC² enantiomer, diastereomer, chiral separation, chiral resolution, triazole fungicide, SFC, supercritical fluid chromatography, chiral columns

INTRODUCTION

The development of analytical methods for the separation of chiral compounds is important in many areas of research, as it is well known that different enantiomers are selectively biologically active.¹ Biochemical reactions can be diastereo or enantioselective. While one isomer may deliver the desired effect to the target species, the other enantiomer may be less effective to the target, completely ineffective, or cause undesirable effects. Additionally, it is known that different isomers can have very different environmental fates. It is estimated that 20 to 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 crop protection 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 the stereoisomeric compositions of these substances, analytical methods that provide reliable and reproducible separations in a rapid time frame are necessary. Supercritical fluid chromatography (SFC) is known as an effective chiral separations technique that has many advantages over conventional high performance liquid chromatography (HPLC).^{3,4} The properties of the supercritical fluid, such as low viscosity and high diffusivity, allow for the achievement of very high efficiency separations with shorter analysis times.⁵

In this application note we present the enantiomeric and/or diastereomeric resolutions of 12 triazole fungicides (Figure 1) using Waters® Trefoil Column Technology. Trefoil Columns use a modified polysaccharide chiral stationary phase (CSP) with a 2.5 µm particle designed for broad-spectrum chiral selectivity. Resolutions were performed using an UltraPerformance Convergence Chromatography™ (UPC²®) System. Convergence chromatography is a complimentary separation technique to liquid chromatography, that provides orthogonal selectivity, and uses supercritical CO₂ as the primary mobile phase.

EXPERIMENTAL

Instrumentation

All separations were performed using the ACQUITY UPC² System. Detection was by ACQUITY UPC² Photodiode Array (PDA) Detector. Empower 3 Software was used for chromatographic data acquisition and processing.

Sample preparation

Racemic pesticide standards were purchased from AccuStandard (New Haven, CT) The pesticide standards were prepared in methanol at a concentration of 1 mg/mL with the exception of cyproconazole and uniconazole which were purchased as 100 µg/mL stocks in methanol and acetonitrile respectively.

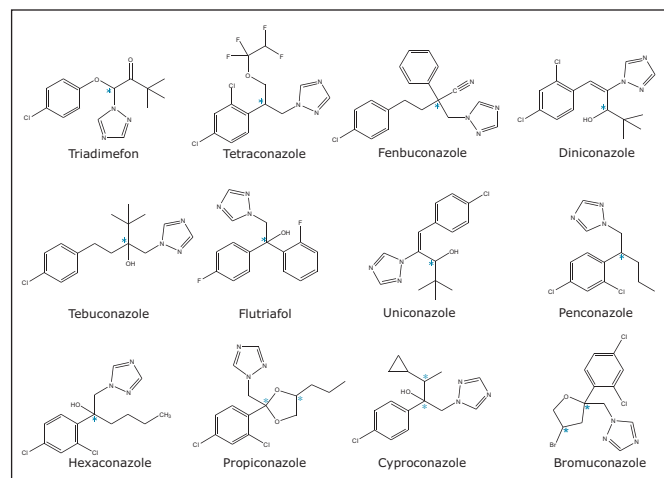


Figure 1. Structures of 12 triazole fungicides. * denotes the stereogenic centers.

Summary of method conditions

	Column	Co-solvent (B)	ABPR psi/bar	Flow rate mL/min	Column temp. °C
Triadimefon	ACQUITY UPC ² Trefoil AMY1	Methanol	1600 psi/110 bar	3.5	35
Tetraconazole	ACQUITY UPC ² Trefoil AMY1	Methanol	1990 psi/137 bar	3.0	15
Fenbuconazole	ACQUITY UPC ² Trefoil AMY1	Methanol	2300 psi/159 bar	3.0	35
Diniconazole	ACQUITY UPC ² Trefoil AMY1	Methanol	1990 psi/137 bar	3.0	10
Tebuconazole	ACQUITY UPC ² Trefoil AMY1	Methanol	2500 psi/172 bar	2.5	45
Flutriafol	ACQUITY UPC ² Trefoil AMY1	Methanol	1990 psi/137 bar	2.5	10
Uniconazole	ACQUITY UPC ² Trefoil AMY1	50:50 2-propanol/ethanol	2200 psi/152 bar	3.0	35
Penconazole	ACQUITY UPC ² Trefoil AMY1	50:50 2-propanol/ethanol	1990 psi/137 bar	3.0	35
Hexaconazole	ACQUITY UPC ² Trefoil AMY1	50:50 2-propanol/ethanol	2500 psi/172 bar	3.0	35
Propiconazole	ACQUITY UPC ² Trefoil AMY1	50:50 2-propanol/ethanol	3000 psi/207 bar	1.5	20
Cyproconazole	ACQUITY UPC ² Trefoil CEL1	Methanol	2200 psi/152 bar	3.0	35
Bromuconazole	ACQUITY UPC ² Trefoil CEL1	Methanol	1500 psi/103 bar	2.0	45

Table 1. Summary of selected analysis conditions used in the study. The ACQUITY UPC² Trefoil AMY1 and CEL1 column dimensions were 3.0 x 150 mm, 2.5-µm.

RESULTS AND DISCUSSION

Method development for the stereoselective resolution of the technical grade fungicides began by using a generic screening gradient with a number of chiral columns and co-solvents, for example methanol, ethanol, 2-propanol, or mixtures of each. The ACQUITY UPC² System has multi-column switching capabilities and a choice of four co-solvents. The screening step can be completed rapidly, due to the shorter analysis times that are possible using this technique. The combination of the co-solvent and column that produced the most promising separation for each compound was then selected for further optimization. The selectivity in a chiral separation can change markedly by varying the temperature, pressure, and flow rates.⁵

Separations in chiral chromatography typically result from multiple interactions between analytes and stationary phases. These interactions can be influenced differently by changing the experimental parameters to produce desired changes in the chromatography. Consequently, each parameter including temperature, pressure, and flow rate should be systematically evaluated to investigate the individual effects each change can have on the compound resolution. A summary of the selected analysis conditions is shown in Table 1.

The chromatograms resulting from the optimized gradient separations of the racemic mixtures of triadimefon, tetraconazole, fenbuconazole, diniconazole, tebuconazole, and flutriafol are shown in Figure 2. In each case, the optimum column was a Trefoil AMY1 (3.0 x 150 mm, 2.5- μ m, [p/n 186007460](#)), and the optimum co-solvent was methanol.

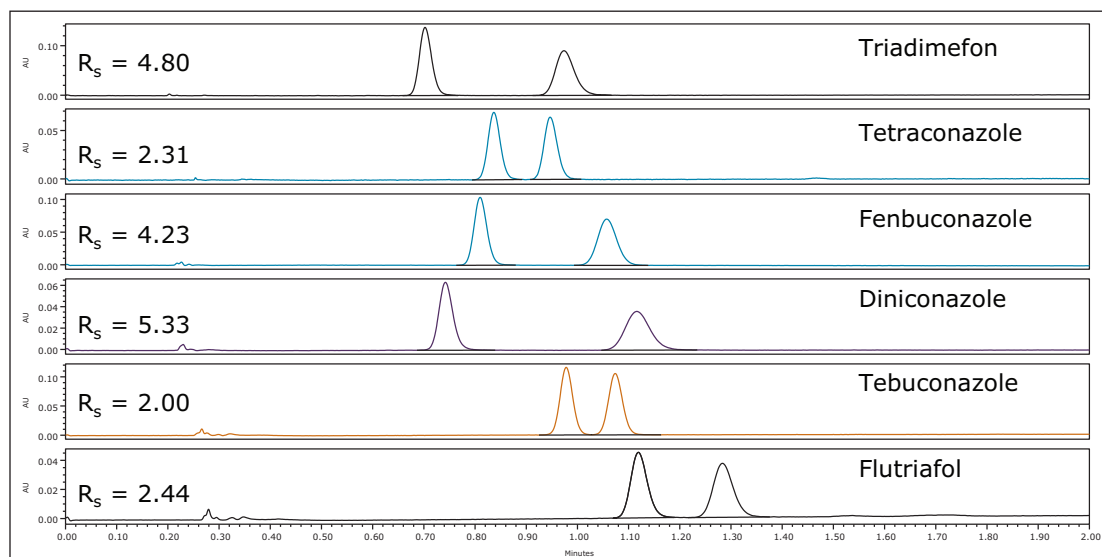


Figure 2. ACQUITY UPC² UV chromatograms showing the enantiomeric resolution of the triazole fungicides using an ACQUITY UPC² Trefoil AMY1 Column (3.0 x 150 mm, 2.5- μ m), with methanol as a co-solvent. The USP resolution (R_s) values obtained are also listed (left).

Baseline R_s was achieved for all pesticides in less than 1.5 minutes. Optimized resolutions for the racemic mixtures of uniconazole, penconazole and hexaconazole are shown in Figure 3. The optimum column in these cases was also a Trefoil AMY1, 3.0 x 150 mm, 2.5- μ m, and the optimum co-solvent was 50:50 2-propanol/ethanol. Baseline resolution was achieved rapidly (less than 1.2 min) for the enantiomers of each triazole fungicide.

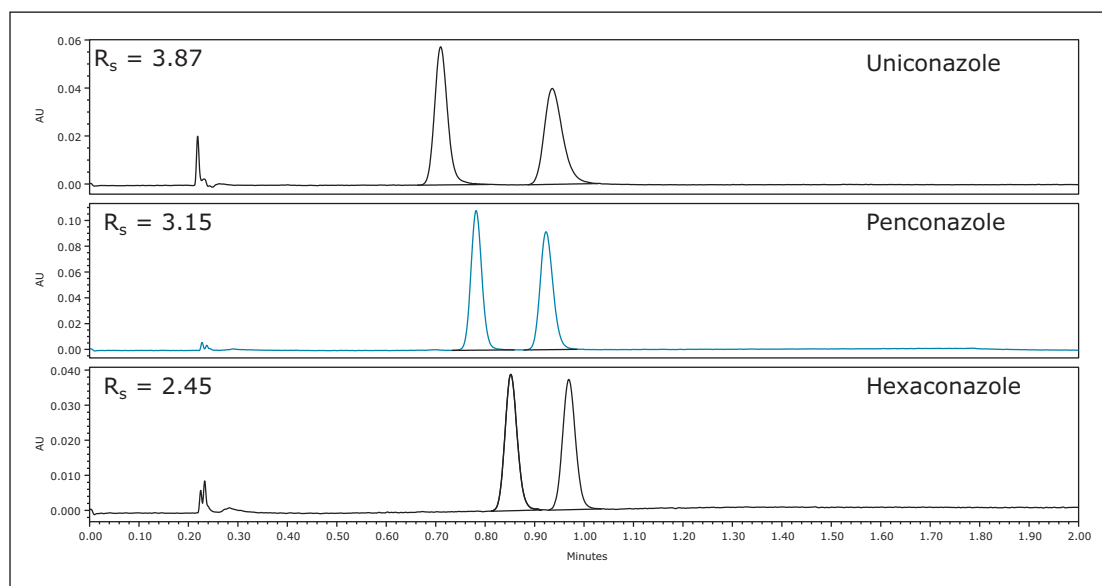


Figure 3. ACQUITY UPC² UV chromatograms showing the enantiomeric resolution of the triazole fungicide standards using an ACQUITY UPC² Trefoil AMY1 Column (3.0 x 150 mm, 2.5- μ m), with 50:50 2-propanol/ethanol as a co-solvent. The R_s values achieved are also listed (left).

The chiral resolutions of propiconazole, cyproconazole, and bromuconazole, each with two chiral centers in their chemical structures, are shown in Figure 4. Despite the increase in the stereochemical complexity in the structures of these compounds, R_s values of >1.75 were achieved for all of the stereoisomers of each pesticide. The chiral separation of propiconazole is possible in less than 2.8 minutes using the ACQUITY UPC² Trefoil AMY1 CSP Column (3.0 x 150 mm, 2.5- μ m, [p/n 186007464](https://www.waters.com/waters/p/n/186007464)).

The optimum column in the case of cyproconazole and bromuconazole was the ACQUITY UPC² Trefoil CEL1, 3.0 x 150 mm, 2.5- μ m. The resolution of both triazole fungicides is possible in less than 4.5 minutes.

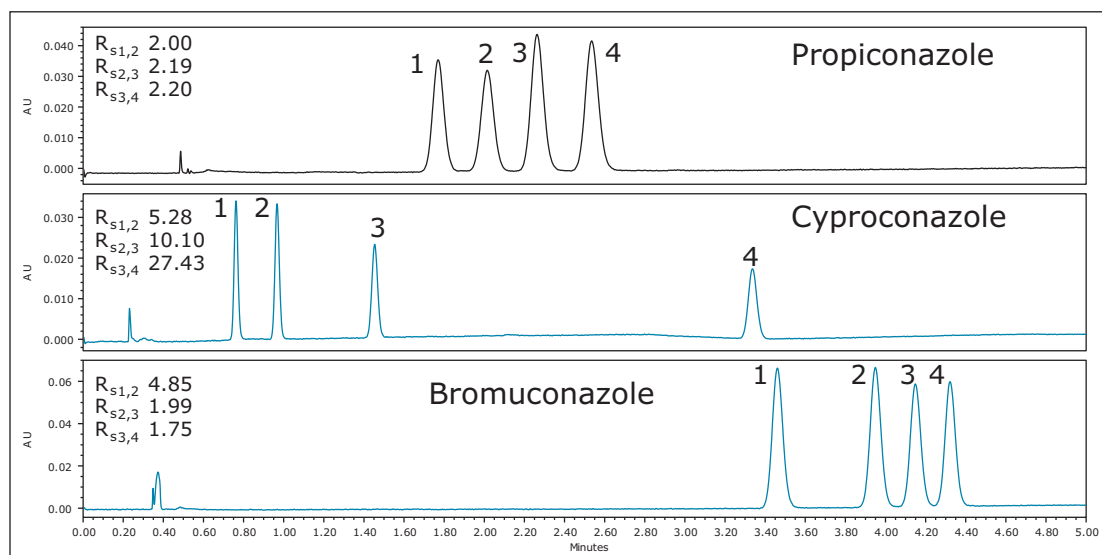


Figure 4. ACQUITY UPC² UV chromatograms showing the resolution of the enantiomers and diastereomers present in pesticide standard mixtures using an ACQUITY UPC² Trefoil AMY1 Column (3.0 x 150 mm, 2.5- μ m) with 50:50 2-propanol/ethanol as a co-solvent for propiconazole, and an ACQUITY UPC² Trefoil CEL1 Column (3.0 x 150 mm, 2.5- μ m) for cyproconazole and bromuconazole, with methanol as a co-solvent. The R_s obtained between the stereoisomers are also listed (left).

A review of the literature indicates that when using normal phase high performance liquid chromatography (HPLC), the chiral resolution of propiconazole is possible in 34 min, and the enantiomeric resolution of tebuconazole ranged from 17 to 45 min.⁶⁻¹¹ Similar resolutions were achieved for propiconazole and tebuconazole using traditional SFC, but the analysis times were reduced to 10 minutes and 10.5 minutes, respectively.⁴

The literature search also revealed two reviews^{6,8} showing that the chiral resolutions of the test compounds using UPC² can be achieved much faster compared to reverse phase¹²⁻¹⁹ (3 to 30X), normal phase⁶⁻¹¹ (8 to 40X), and conventional SFC^{4,20} (3 to 10X) separations.

The optimized ACQUITY UPC² methods developed in this work allow increased sample throughput and improved enantiomeric resolutions, especially when compounds with multiple chiral centers are analyzed.

Reproducibility data (n=8) for retention time, area, area%, height, and USP resolution for bromuconazole are shown in Table 2. The %RSD's were less than or equal to 0.60% for all of the stereoisomers.

	Bromconazole %RSD (n=8)				
	tR	Area	%Area	Height	R _s
Peak 1	0.10	0.57	0.36	0.56	
Peak 2	0.07	0.47	0.15	0.38	0.27
Peak 3	0.07	0.51	0.16	0.42	0.28
Peak 4	0.07	0.48	0.28	0.50	0.60

Table 2. %RSD for eight replicate injections of bromuconazole.

CONCLUSIONS

The study of enantioselectivity is important to the crop protection industry since the knowledge of the efficacy of a more biologically active individual enantiomer could facilitate a significant reduction in the total amount of pesticide applied and result in a more marketable product. The rapid enantioseparation of chiral pesticides has previously been challenging due to the difficulty in chromatographically resolving them in short analysis times. This application note highlights a more rapid chromatographic methodology for enantiomeric and diastereomeric separation and detection by using a combination of ACQUITY UPC² and Trefoil chiral columns. The result was a highly efficient stereoselective separation of 12 triazole fungicides using two CSP's. Further, the methodology shown in this work improves the sample throughput compared with LC-based chiral separations.⁵⁻¹⁹ The %RSD's (n=8) for retention time, area, area%, height, and USP resolution for bromuconazole were less than or equal to 0.60% for all of the stereoisomers. These methods use supercritical CO₂ as the primary mobile phase and alcohol modifiers as the co-solvents. The need to use large volumes of potentially hazardous solvents that are routinely used in normal phase chiral separations is reduced, as well as the cost associated with solvent waste disposal.

References

1. Sekhon BS. Chiral pesticides. *J. Pestic Sci.* 34, 1:1–12, 2009.
2. Liu WP. Pesticide Environmental Chemistry. *Chemical Industry Press*, Beijing, China. 341–343, 2006.
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.* 49: 739-743, October 2011.
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.* 1046: 249–253, 2004.
5. McCauley JP, Subbarao L, Chen R. Enantiomeric and Diastereomeric Separations of Pyrethroids Using UPC². Waters Application note No. [720004530en](#). December, 2012.
6. Perez-Fernandez V, Garcia MA, Marina ML. Chiral separation of agricultural fungicides. *J Chrom A.* 1218: 6561–6582, 2011.
7. Zhou Y, Li L, Lin K, Zhu X, Liu W. Enantiomer separation of triazole fungicides by high performance liquid chromatography. *Chirality.* 21: 421–427, 2009.
8. Ye J, Wu J, Liu W. (2009) Enantioselective separation and analysis of chiral pesticides by high-performance liquid chromatography. *Trends in Analytical Chemistry.* 28, 10: 1148–1163, 2009.
9. Wang P, Jiang S, Liu D, Wang P, Zhou Z. Direct enantiomeric resolutions of chiral triazole pesticides by high-performance liquid chromatography. *J Biochem Biophys Methods.* 62: 219–230, 2005.
10. Wang P, Jiang S, Liu D, Wang P, Zhou Z. Direct enantiomeric resolutions of chiral triazole pesticides by high-performance liquid chromatography. *J Biochem Biophys Methods.* 62: 219–230, 2005.
11. Wang P, Liu D, Jiang S, Xu Y, Zhou Z. The chiral separation of triazole pesticide enantiomers by amylose-tris(3,5-dimethylphenylcarbamate) chiral stationary phase. *J Chrom Sci.* 46: 787–792, 2008.
12. Zhang H, Quian M, Wang X, Wang X, Xu H, Wang Q, Wang M. HPLC-MS/MS enantioseparation of triazole fungicides using polysaccharide based stationary phases. *J Sep Sci.* 35: 773–781, 2012.
13. Zhang Q, Hua X, Shi H, Liu J, Tian M, Wang M. Enantioselective bioactivity, acute toxicity and dissipation in vegetables of the chiral triazole flutriafol. *J Hazard Mat.* 284: 65–72, 2015.
14. Qiu J, Dai S, Zheng C, Yang S, Chai T, Bie M. Enantiomeric separation of triazole fungicides with 3- μ m and 5- μ m particle chiral columns by reverse-phase high performance liquid chromatography. *Chirality.* 23: 479–486, 2011.
15. Chai T, Jia Q, Yang S, Qiu J. Simultaneous stereoselective detection of chiral fungicides in soil by LC-MS/MS with fast sample preparation. *J Sep Sci.* 37: 595–601, 2014.
16. Ye X, Peng A, Qiu J, Chai T, Zhao H, Ge X. Enantioselective degradation of tebuconazole in wheat and soil under open field conditions. *Advanced Materials Research.* 726–731: 348-356, 2013.
17. Wang X, Wang X, Zhang H, Wu C, Wang X, Xu H, Wang X, Li Z. Enantioselective degradation of tebuconazole in cabbage cucumber and soils. *Chirality.* 24: 104–111, 2012.
18. Wang H, Chen J, Guo B, Li J. Enantioselective bioaccumulation and metabolization of diniconazole in earthworms (*Eiseniafetida*) in an artificial soil. *Ecotoxicol Environ Safety.* 99: 98–104, 2014.
19. Li Y, Dong F, Liu X, Xu J, Li J, Kong Z, Chen X. Simultaneous enantioselective determination of triazole fungicides in soil and water by chiral liquid chromatography/tandem mass spectrometry. *J Chrom A.* 1224: 51–60, 2012.
20. del Nozal MJ, Toribio L, Bernal JL, Castano N. Separation of triadimefon and triadimenol enantiomers and diastereoisomers by supercritical fluid chromatography. *J Chrom A.* 986: 135–141, 2003.

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