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Stereoselective Separation of Triazole Fungicides Using the ACQUITY UPC^2 System and ACQUITY UPC^2 Trefoil Chiral Columns

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Abstract

This application note describes a more rapid chromatographic methodology for enantiomeric and diastereomeric separation and detection by using a combination of ACQUITY UPC^2 and Trefoil chiral columns.

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.

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 (UPC^2) System. Convergence chromatography is a complimentary separation technique to liquid chromatography, that provides orthogonal selectivity, and uses supercritical CO_2 as the primary mobile phase.

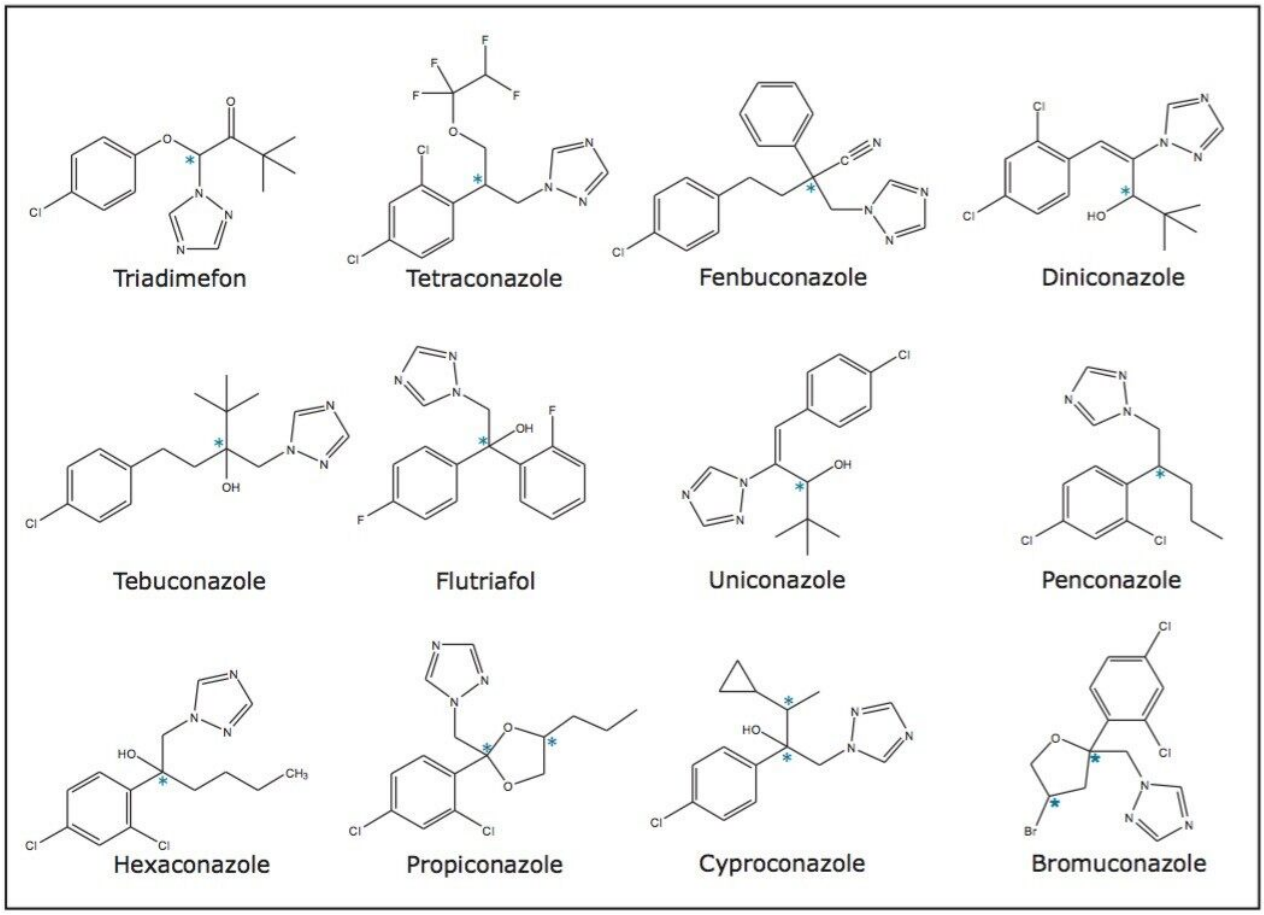

Figure 1. Structures of 12 triazole fungicides.

** denotes the stereogenic center/s.*

Experimental

Instrumentation

All separations were performed using the ACQUITY UPC^2 System. Detection was by ACQUITY UPC^2

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.

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 UPC^2 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.

	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 UPC^2 Trefoil AMY1 and CEL1 column dimensions were 3.0 x 150 mm, 2.5-µm.

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-µm , p/n 186007460), and the optimum co-solvent was methanol.

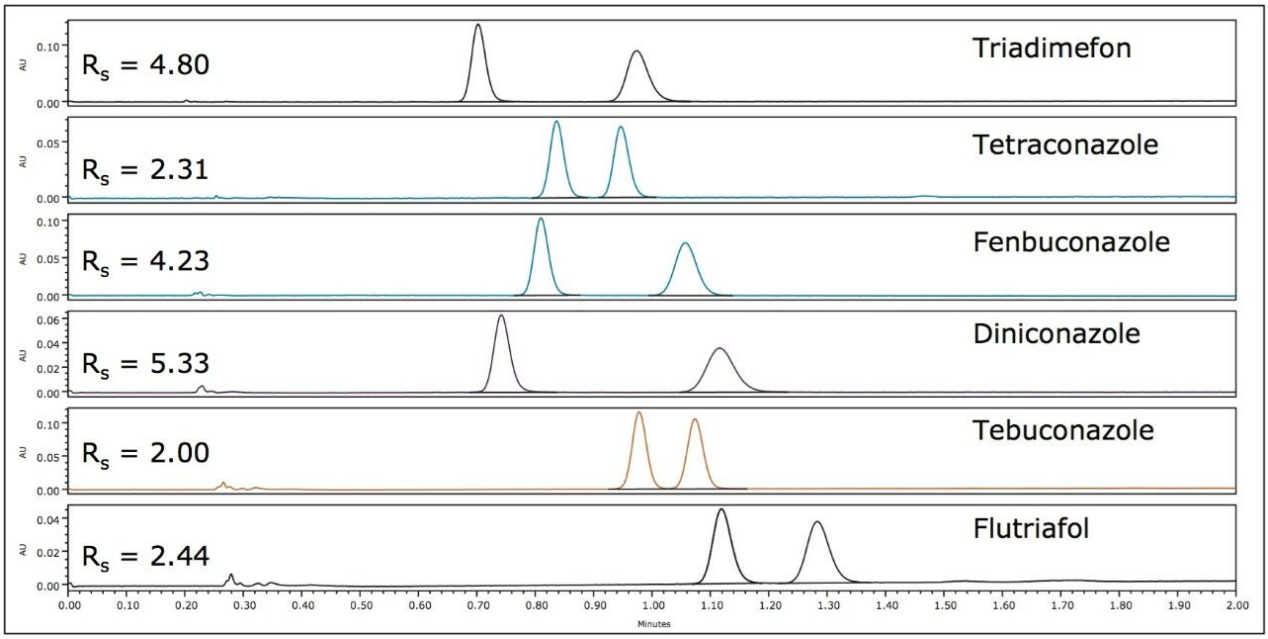

Figure 2. ACQUITY UPC^2 UV chromatograms showing the enantiomeric resolution of the triazole fungicides using an ACQUITY UPC^2 Trefoil AMY1 Column (3.0 x 150 mm, 2.5-µm), with methanol as a co-solvent. The USP resolution (R_s) values obtained are also listed (left).

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-µm, 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.

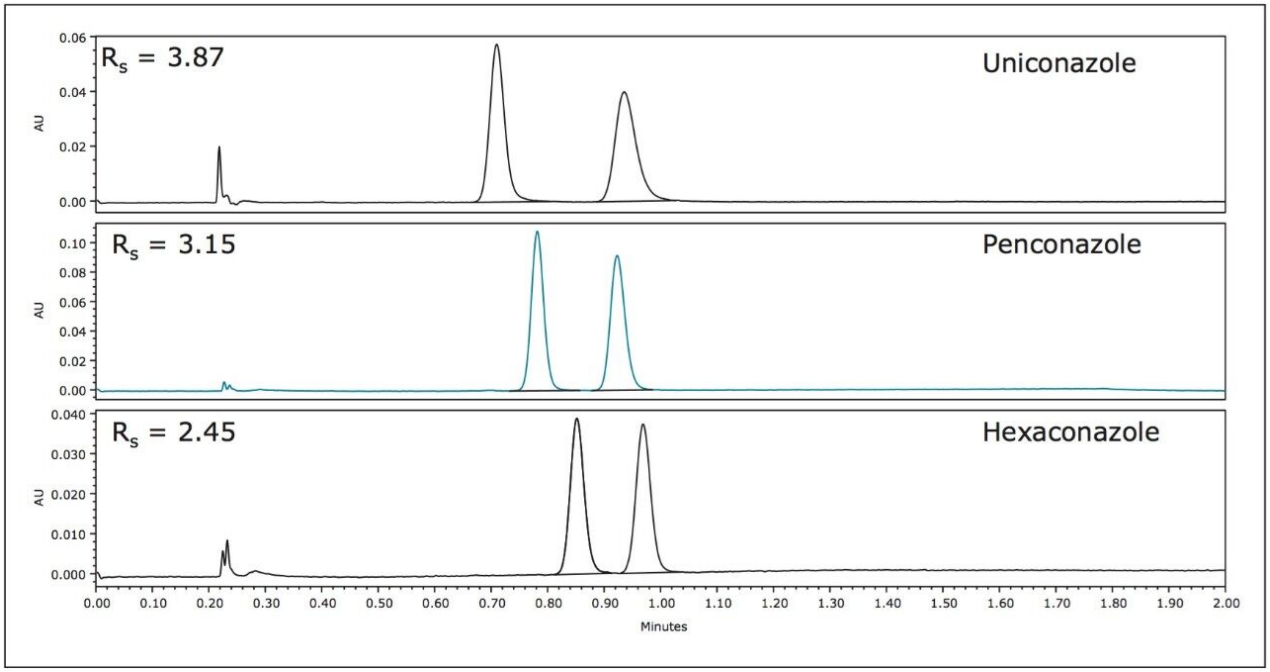

Figure 3. ACQUITY UPC^2 UV chromatograms showing the enantiomeric resolution of the triazole fungicide standards using an ACQUITY UPC^2 Trefoil AMY1 Column (3.0 x 150 mm, 2.5-µm), with 50:50 2-propanol/ethanol as a co solvent. The R_s values achieved are also listed (left).

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 UPC ^{2} Trefoil AMY1 CSP Column (3.0 x 150 mm, 2.5-µm, p/n 186007464).

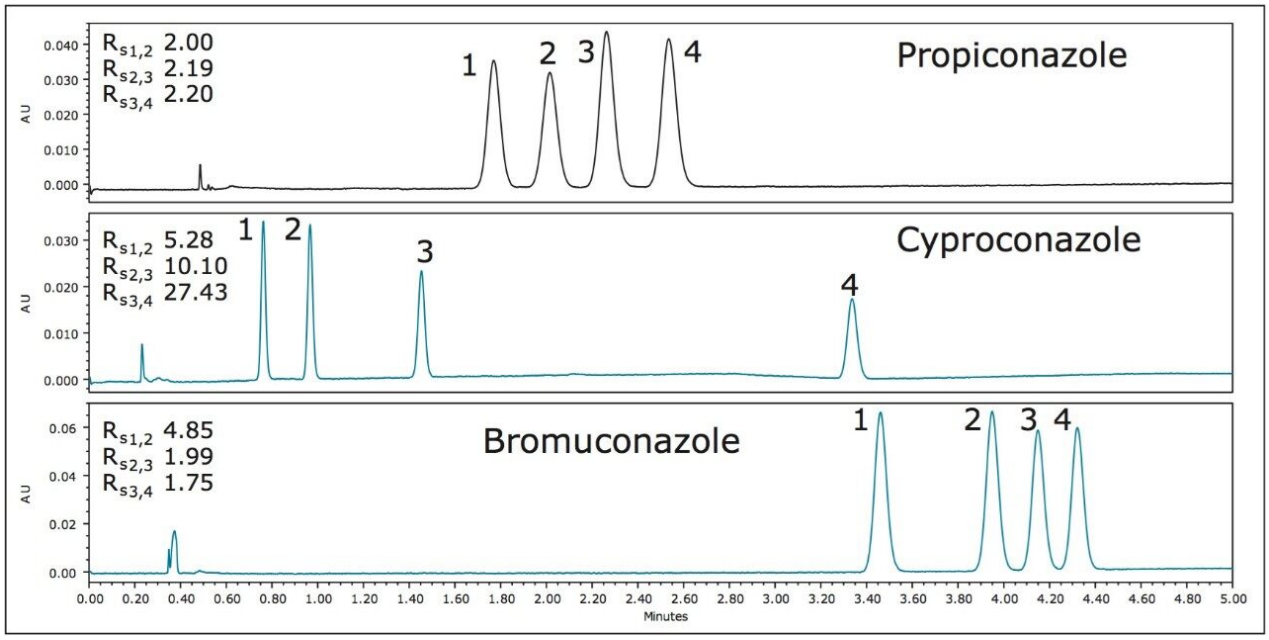

Figure 4. ACQUITY UPC^2 UV chromatograms showing the resolution of the enantiomers and diastereomers present in pesticide standard mixtures using an ACQUITY UPC^2 Trefoil AMY1 Column (3.0 x 150 mm, 2.5-µm) with 50:50 2-propanol/ethanol as a co-solvent for propiconazole, and an ACQUITY UPC^2 Trefoil CEL1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

The optimum column in the case of cyproconazole and bromuconazole was the ACQUITY UPC^2 Trefoil CEL1, 3.0 x 150 mm, 2.5-µm. The resolution of both triazole fungicides is possible in less than 4.5 minutes.

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 UPC^2 can be achieved much faster compared to reverse phase[12-19] (3 to 30X), normal phase[6-11] (8 to 40X), and conventional SFC[4,20] (3 to 10X) separations.

The optimized ACQUITY UPC^2 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.

Conclusion

The study of enantioselectivity is important to the crop protection industry since the knowledge of the efficacy of a more biologically active individualenantiomer 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 UPC^2 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 CO_2 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.

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720005404, May 2015