Optimising the Chiral Separation of the Pesticide Diniconazole

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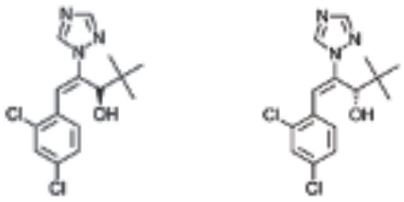
A variety of diseases can attack agricultural crops for many reasons including changes in climatic conditions. These diseases include many fungal diseases derived from dirt and rust. Fungal diseases can sufficiently effect crop yields and agricultural output unless they are controlled or eliminated. The application of chemical pesticides can effectively eliminate plant pest such as fungal diseases. However, with increasing doses and accumulated application chemical pesticides can potentially impact human health [1]. Additionally, about 30% of the commonly used chemical pesticides are chiral, however most of these are sold and used as racemic mixtures [2]. A racemic mixture, or racemate, is one that has equal amounts of left- and right-handed enantiomers of a chiral molecule. Enantiomers of the same chemical have identical physicochemical properties, but may exhibit differences in biological activity, pharmacokinetics, pharmacodynamics and toxicity [3]. One widely used pesticide used to control plant fungal diseases is diniconazole which is sold and used as a racemic mixture of two enantiomers, structures shown in Figure 1. Diniconazole is a broad spectrum triazole germicide/fungicide used to prevent diseases in fruit, vegetable, wheat and tea plants. Diniconazole suppresses the 14 α-demethylation in the biosynthesis of ergosterol causing a deficiency of ergosterol which prevents the formation of disease derived from rust and dirt [4]. Diniconazole is a chiral chemical with one chiral center and commercial Diniconazole sold as an enantiomer mixture of R, S Diniconazole enantiomers. It has reported that R (-) Diniconazole has higher bactericidal activity, while S (+) Diniconazole shows higher plant growth regulator activity [5]. In many cases it is important to know if environmental factors have altered the ratio of R and S Diniconazole residues when applied on agricultural crops [5].



R Diniconazole S Diniconazole

Figure 1. Chemical structures of R and S Diniconazole.

Supercritical fluid chromatography (SFC) is a powerful chromatographic technique for the separation, isolation and analysis of complex mixtures from many different samples. Many chemicals can potentially be used as a supercritical mobile phase for SFC. However virtually all current practitioners of SFC use carbon dioxide (CO_2) which offers several advantages when compared to liquid chromatography [6]. The use of carbon dioxide (CO_2) as the primary component of the mobile phase is one of the key features that benefit the preparative SFC chromatographer and since the CO_2 used for SFC is recovered from the atmosphere it is considered a 'Green' solvent. It is miscible with a wide range of organic solvents, nonflammable, has low UV absorbance at low wavelengths [7-9]. Other advantages of SFC as a technique are the diffusion coefficients of solutes in the SFC mobile phases have been shown to be 3-10 times higher than in normal liquids potentially allowing for very rapid separations; and the viscosity of SFC mobile phases are significantly lower than LC mobile phases producing much lower pressure drop across the column thus allowing the use of much higher mobile phase flow rates [10].

Scope

In a recent manuscript SFC Chiral chromatography coupled with quadrupole-time-flight mass spectrometry has been used as an analytical tool to determine the enantiomeric residue of diniconazole in tea, grape and apples [11]. The authors of this manuscript developed methodology to determine if environmental and processing factors have altered the ratio of R and S Diniconazole residues on the selected agricultural products. The alteration of residual diniconazole on black tea during processing was of particular interest to them. Black tea is a forced oxidised product designed to encourage the increase in activity of the enzyme polyphenol oxidase which leads to browning and ultimately the production of black tea from green tea leaves. In this recent manuscript the authors found changes in the enantiomeric ratios of diniconazole as a result of black tea processing. A significant part of the methodology utilised the SFC separation of enantiomeric diniconazole residues using a chiral column. The chiral column they selected was a ChromegaChiral™ CCA (tris-(3,5-di-methylphenyl) carbamoyl amylose produced by ES Industries. A chromatogram of for the enantiomeric separation of R,S diniconazole on ChromegaChiral CCA is shown in Figure 2. This chromatogram shows near baseline resolution of the two enantiomers in about 10.5 minutes with some peak tailing using 5% isopropanol (IPA) in CO_2. The ChromegaChiral CCA chiral column enabled the authors to complete their study, however, we wanted to investigate if other chiral columns could be utilised to improve the resolution of R,S diniconazole with a significantly quicker analysis time. It is the goal of this current study to investigate other chiral columns to improve the separation and analysis time for determination of R and S diniconazole.

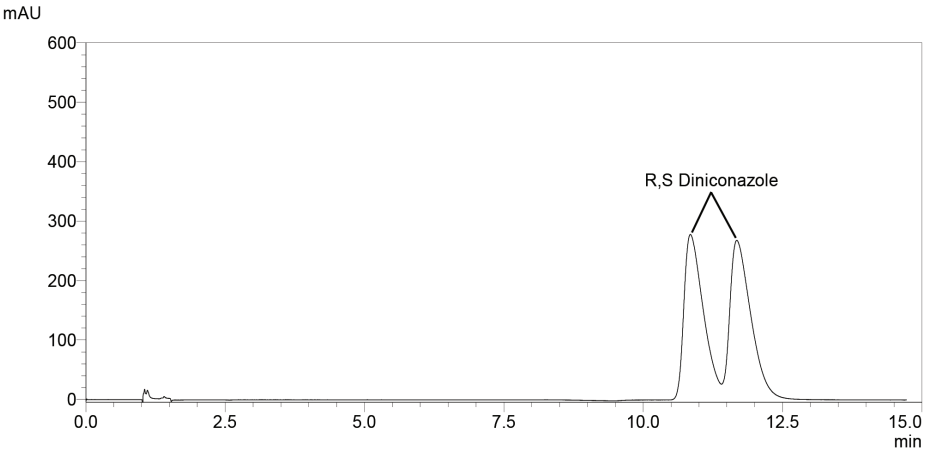


Figure 2. Enantiomeric separation of diniconazole on ChromegaChiral CCA 4.6 mm ID x 150 mm length 5µm particle. Conditions: 5% IPA in CO_2, Flow 2 mL/min, Column oven 25 C, BPR 140 bar, detection UV@254 nm.

Table 1. The 10 different ChromegaChiral stationary phases selected for evaluation.

Stationary Phase Typ	Chiral Selector	Resolution	RT
ChromegaChiral CCA	Tris-(3,5-dimethylphenyl) carbamoyl amylose	1.23	10.85
ChromegaChiral CCO	Tris-(3,5-dimethylphenyl) carbamoyl cellulose	No resolution	10.54
ChromegaChiral CCO-F4	Tris-(4-fluoro 3-methylphenyl) carbamoyl cellulose	No resolution	12.18
ChromegaChiral CC4	Tris-(4-chloro 3-methylphenyl) carbamoyl cellulose	No resolution	14.94
ChromegaChiral CCS	Tris-((S)-αmethylbenzyl) carbamoyl amylose	9.44	8.86
ChromegaChiral CC3	Tris-(5-chloro 2-methylphenyl) carbamoyl amylose	No resolution	7.35
ChromegaChiral CCJ	Tris-(4-methylbenzoate)		
Cellulose	No resolution	9.56	
ChromegaChiral CCU	Bis-(3-chloro 4-methylphenyl) & (methylbenzyl) carbamoyl amylose	2.94	19.33
ChromegaChiral CCA-F4	Tris-(4-fluoro 3-methylphenyl) carbamoyl amylose	1.00	8.22
ChromegaChiral CCC	Bis-(3,5-dichlorophenyl) & (3-chloro 4 methylphenyl) carbamoyl cellulose	0.68	21.42

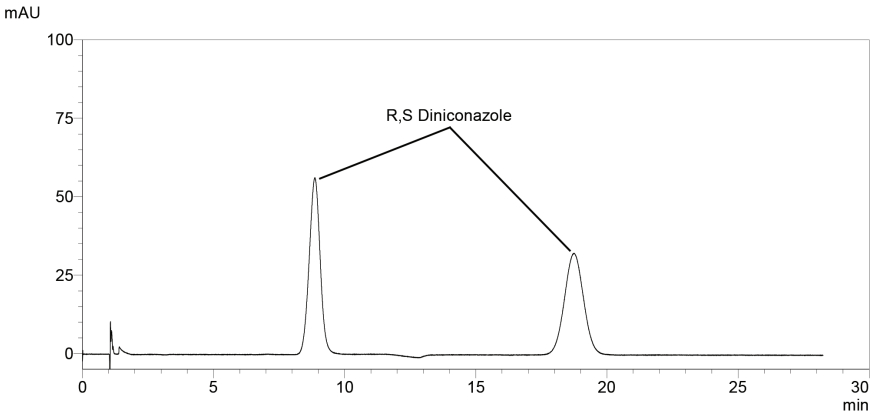


Figure 3. Enantiomeric separation of diniconazole on ChromegaChiral CCS 4.6 mm ID x 150 mm length 5µm particle. Conditions: 5% IPA in CO_2, Flow 2 mL/min, Column oven 25°C, BPR 140 bar, detection UV@254 nm.

Experimental - Chiral Column Selection

There are 14 different ChromegaChiral stationary phases, from ES Industries, available for evaluation for the enantiomeric separation of diniconazole. For this study 10 different ChromegaChiral stationary phases were selected for evaluation (Table 1). These nine stationary phases have been found to have high rates of applicability to many chiral separations and have been successfully applied to wide variety of chiral applications. Initially all tested stationary phases were evaluated at 140 bar back pressure, flow of 2 mL/min, 25°C column oven and 5% IPA co-solvent in CO_2. All columns were 5µm particles 4.6 mm ID and 25 cm length except for ChromegaChiral CCA and ChromegaChiral CCS. These two columns were 5µm particles 4.6 mm ID and 15 cm length. Table 1 includes the stationary phase evaluated, chiral selector, USP calculated resolution for the two enantiomers and retention time (RT) for the first eluting enantiomer.

There are several points that are notable in Table 1:

1. ChromegaChiral CCS provided the highest resolution for diniconazole enantiomers and this chromatogram is shown in Figure 3.
2. Most of the amylose based stationary phases resolved the diniconazole enantiomers with the exception of ChromegaChiral CC3.
3. None of the cellulose based phases did not adequately separate the diniconazole enantiomers.

The resolution of the diniconazole enantiomers on ChromegaChiral CCS is clearly superior to any of the stationary phases evaluated using a co-solvent of 5% IPA and based on this information we decided to raise the percentage of IPA co-solvent in order to try to reduce the analysis time. At first the concentration of IPA co-solvent was increased to 10% chromatogram is shown in Figure 4. At 10% IPA the resolution (6.98) of the diniconazole enantiomers on ChromegaChiral CCS is more than adequate. The next level of IPA used is 15% chromatogram is shown in Figure 5. At 15% IPA the resolution (5.44) of the diniconazole enantiomers on ChromegaChiral CCS is excellent and the separation completed in less than 4.5 minutes. The resolution and rapid analysis at 15% IPA on ChromegaChiral CCS are striking when compared to the resolution (1.23) on ChromegaChiral CCA which has an analysis time of 12.5 minutes.

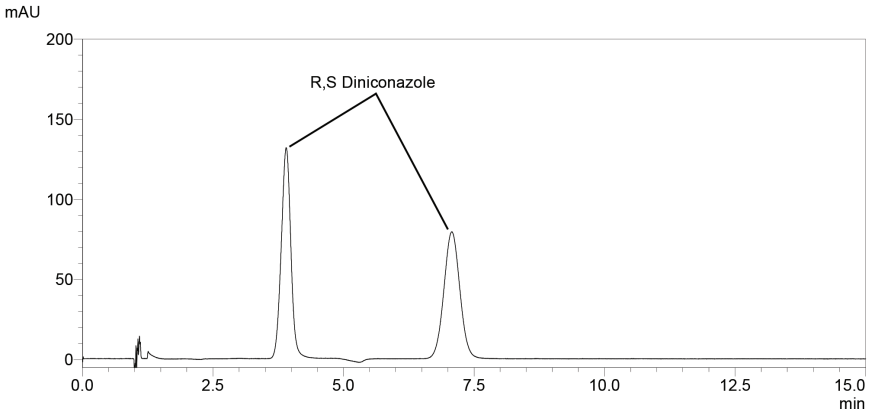


Figure 4. Enantiomeric separation of diniconazole on ChromegaChiral CCS 4.6 mmID x 150 mm length 5µm particle.

Conditions: 10% IPA in CO_2, Flow 2 mL/min, Column oven 25 C, BPR 140 bar, detection UV@254 nm.

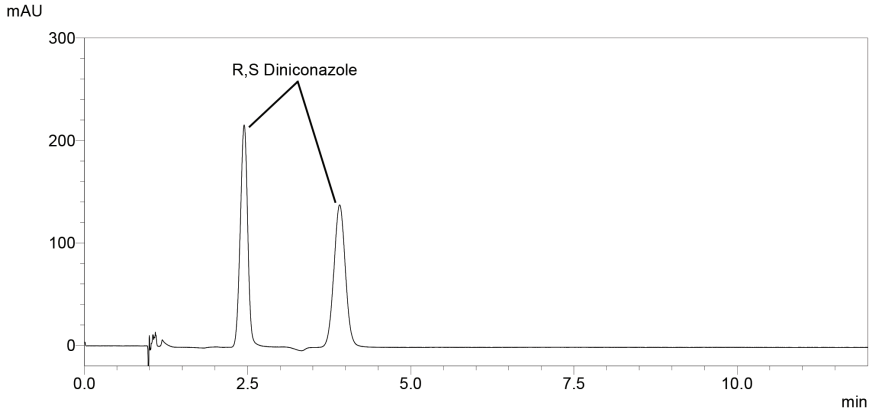


Figure 5. Enantiomeric separation of diniconazole on ChromegaChiral CCS 4.6 mm ID x 150 mm length 5µm particle.

Conditions: 15% IPA in CO_2, Flow 2 mL/min, Column oven 25 C, BPR 140 bar, detection UV@ 254 nm.

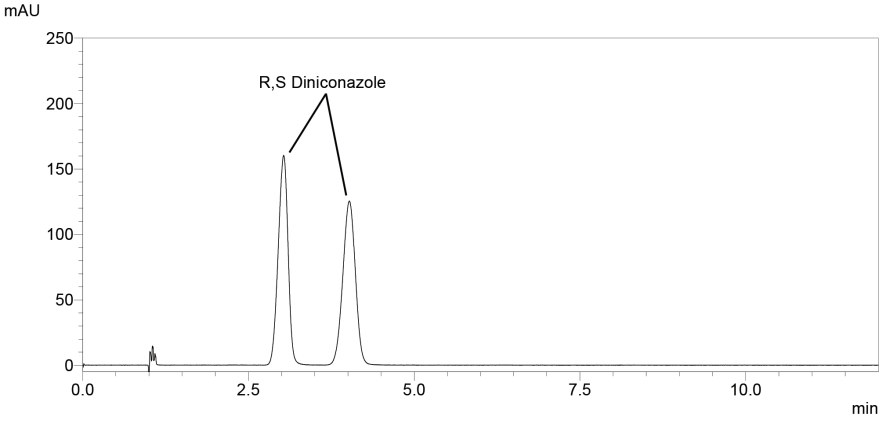


Figure 6. Enantiomeric separation of diniconazole on ChromegaChiral CCS 4.6 mm ID x 150 mm length 5µm particle. Conditions: 5% Methanol in CO_2, Flow 2 mL/min, Column oven 25°C, BPR 140 bar, detection UV@254 nm.

Co-Solvent Selection

The selection of the optimal co-solvent is critical for many SFC chiral separations and the initial selection of IPA as a co-solvent was based on previous work [11]. In order to further optimise the enantiomeric separation of diniconazole we tested other commonly used co-solvents for SFC chiefly ethanol and methanol. In the case of ethanol vs. IPA co-solvent we observed no substantial differences. However, for methanol vs. IPA we observed several differences. We could obtain an enantiomeric separation of diniconazole with an analysis time of less than 4.5 minutes and a resolution of 2.84 with only 5% methanol on ChromegaChiral CCS chromatogram shown in Figure 6. However, on ChromegaChiral CCA the enantiomeric separation of diniconazole was a complete co-elution with 5% methanol. Alternatively, an enantiomeric separation of diniconazole was achieved using 5% methanol on both ChromegaChiral CCO-F4 (Figure 7) and ChromegaChiral CC4 (Figure 8) which are both based on cellulose. It is also noted that the separation is completed quicker on ChromegaChiral CCO-F4, the fluorinated version of ChromegaChiral CC4 (chlorinated). Generally, we have observed that the fluorinated ChromegaChiral stationary phases require less co-solvent than the similar chlorinated phase to obtain the same separation.

Conclusion

Ten different chiral stationary phases were investigated in order to optimise the enantiomeric separation of diniconazole by SFC. We observed that ChromegaChiral CCS provided the highest resolution separation and with adjustments to co-solvent strength we were able to obtain a high-resolution separation in less than 4.5 minutes, which is a substantial improvement over the previously reported enantiomeric separation of diniconazole on ChromegaChiral CCA [11]. In addition, the use of methanol co-solvent improved enantiomeric separation of diniconazole on several chiral stationary phases tested. When developing chiral separations for either analytical or preparative applications it is important to test several different chiral stationary phases to obtain the optimal separation conditions for a given enantiomeric separation. Additionally, for SFC based separations both chiral and achiral co-solvent selection should not be ignored.

References

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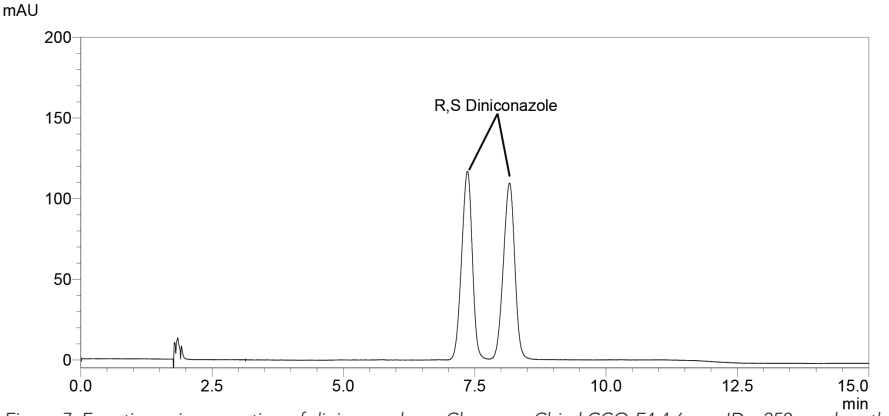

Figure 7. Enantiomeric separation of diniconazole on ChromegaChiral CCO-F4 4.6 mm ID x 250 mm length 5µm particle. Conditions: 5% Methanol in CO_2, Flow 2 mL/min, Column oven 25°C, BPR 140 bar, detection UV@254 nm.

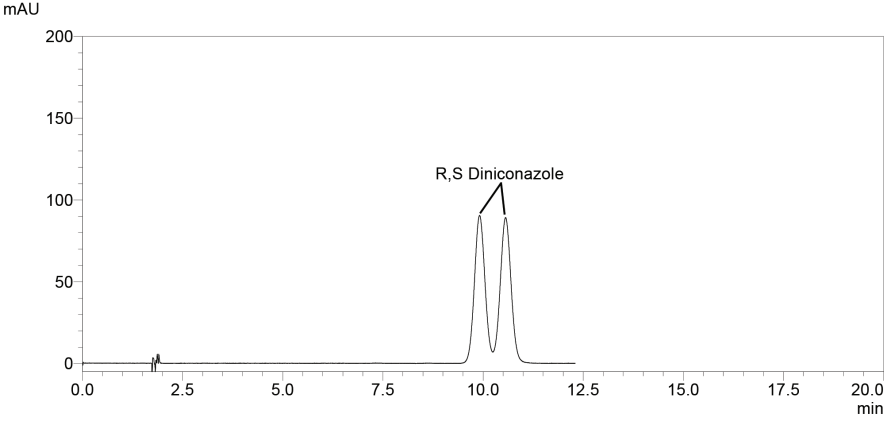

Figure 8. Enantiomeric separation of diniconazole on ChromegaChiral CC4 4.6 mm ID x 250 mm length 5µm particle. Conditions: 5% Methanol in CO_2, Flow 2 mL/min, Column oven 25°C, BPR 140 bar, detection UV@254 nm.

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