INTRODUCTION

Analytical methods development 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 diastereor or enantioselective and 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 also have very different environmental fates. It is estimated that 20-30% of pesticides on the market today have optical isomers.3,4 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.

To improve our knowledge of these substances stereoisomeric compositions, 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 possessing many advantages over conventional high performance liquid chromatography (HPLC).5 The properties of the supercritical fluid, i.e. low viscosity and high diffusivity, allow for the achievement of very high efficiency separations with shorter analysis times.6

In this poster we present the enantiomeric and/or diastereomeric resolutions of twelve triazole fungicides (Figure 1) using Trefoil column technology. The 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 Ultra Performance Convergence Chromatography System (UPC²).7

RESULTS AND DISCUSSION

Entantoseparation of triazole fungicides with one chiral center

Chromatograms resulting from the optimized gradient separations of the racemic mixtures of triadimenol, tetcraconazole, fenbuconazole, hexaconazole and tebuconazole are shown in Figure 2. The optimum column was a Trefoil AMY1, 3.0 x 150 mm, 2.5-µm and the optimum co-solvent was methanol. Baseline RsR was achieved for all pesticides in <1.5 minutes.

The optimized resolutions for the racemic mixtures of uniconazole, penconazole and hexaconazole are shown in Figure 2. 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 (< 1.2 min) for the enantiomers of each triazole fungicide.

Figure 1. Structures of twelve triazole fungicides. The asterisk(\*) indicates the stereogenic center(s).

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

Entantoseparation of triazole fungicides with two chiral centers

The method development for stereoselective resolution of the 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 combination of co-solvent and column that produced the most promising separation for each compound was selected for further optimisation.

The selectivity in a chiral separation can change markedly by varying the temperature, pressure, and flow rate.8 A summary of selected analysis conditions used in the study is shown in Table 1.

Despite the increase in the stereochromical complexity in the structures of these compounds, RsR values of >1.75 were achieved for all the stereoisomers of each pesticide.

Empower 3 software was used for chromatographic data acquisition and processing.

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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Column</th>
<th>Co-solvent</th>
<th>Flow Rate</th>
<th>Mobile Phase</th>
<th>Temp.</th>
<th>Pressure</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triadimenol</td>
<td>ACQUITY UPC² Trefoil AMY1</td>
<td>Methanol</td>
<td>1.0</td>
<td>50:50 propanol/ethanol</td>
<td>45</td>
<td>1000</td>
<td>10.0</td>
</tr>
<tr>
<td>Tetcraconazole</td>
<td>ACQUITY UPC² Trefoil AMY1</td>
<td>Methanol</td>
<td>1.0</td>
<td>50:50 propanol/ethanol</td>
<td>45</td>
<td>1000</td>
<td>10.0</td>
</tr>
<tr>
<td>Fenbuconazole</td>
<td>ACQUITY UPC² Trefoil AMY1</td>
<td>Methanol</td>
<td>1.0</td>
<td>50:50 propanol/ethanol</td>
<td>45</td>
<td>1000</td>
<td>10.0</td>
</tr>
<tr>
<td>Hexaconazole</td>
<td>ACQUITY UPC² Trefoil AMY1</td>
<td>Methanol</td>
<td>1.0</td>
<td>50:50 propanol/ethanol</td>
<td>45</td>
<td>1000</td>
<td>10.0</td>
</tr>
<tr>
<td>Uniconazole</td>
<td>ACQUITY UPC² Trefoil AMY1</td>
<td>Methanol</td>
<td>1.0</td>
<td>50:50 propanol/ethanol</td>
<td>45</td>
<td>1000</td>
<td>10.0</td>
</tr>
<tr>
<td>Penconazole</td>
<td>ACQUITY UPC² Trefoil AMY1</td>
<td>Methanol</td>
<td>1.0</td>
<td>50:50 propanol/ethanol</td>
<td>45</td>
<td>1000</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Figure 3. ACQUITY UPC² UV chromatograms showing the enantiomeric resolution of the triazole fungicides standards using an ACQUITY UPC² Trefoil AMY1, 3.0 x 150 mm, 2.5-µm column with 50:50 2-propanol/ethanol as a co-solvent.

CONCLUSION

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.

The need for enantioseparation of chiral pesticides has previously been challenging due to the difficulty in chromatographically resolving them in short analysis times.

This application 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 %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. (data not shown)

REFERENCES