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Application Note

# Diastereoselective Separation of Permethrin Using the ACQUITY UPC$^2$ System

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

## Abstract

This application brief describes successfull development of a diastereoselective UltraPerformance Convergence Chromatography ($UPC^2$) method for the baseline resolution of all four permethrin isomers using the Waters ACQUITY $UPC^2$ System.


### Benefits

Compared to chiral HPLC methods, $UPC^2$ offers a complete baseline resolution of all isomers with significantly shorter run time; ideal for pesticide manufacturers who routinely perform diastereomer analyses.

## Introduction

Public concern about pesticide use is growing. Twenty-five percent of pesticides currently used are chiral compounds. Chirality plays an important role in the potency, toxicity, metabolic, and environmental fate of these chiral pesticides. As a result, there has been an increasing demand for stereo-selective separation techniques and analytical assays to evaluate the enantiomeric purity of pesticides. Permethrin is a synthetic chemical widely used as an insecticide and an insect repellent.

Permethrin has four stereoisomers (two enantiomeric pairs), arising from the two stereo-centers in the cyclopropane ring as shown in Figure 1. Consequently, separation and quantification of permethrin isomers can be challenging. Great effort has been devoted to developing both normal phase and reversed phase HPLC methodologies to separate permethrin, but with moderate success. As we demonstrated here, a baseline resolution of all four permethrin isomers is achieved in less than six minutes using the ACQUITY UPC$^2$ System.

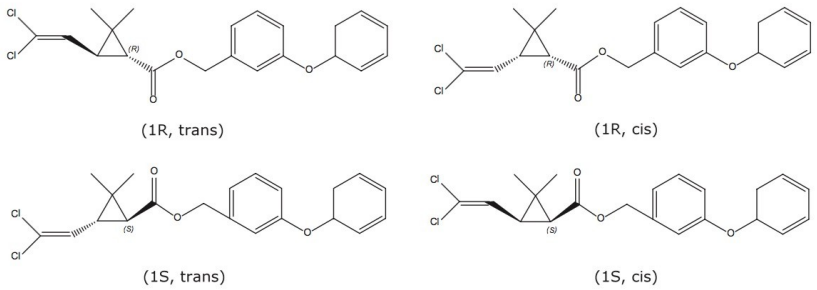


*Figure 1. Chemical structures of permethrin.*

## Results and Discussion

Various chiral stationary phases (CSPs) have been evaluated to separate permethrin using both chiral normal phase and reversed phase HPLC. Lisseter and Hambling reported the use of a Pirkle type CSP for permethrin separation under normal phase HPLC conditions. The total run time was more than 30 min and the mobile phase used was hexane with 0.05% isopropanol (*Journal of Chromatography*, 539 1991; 207-10). However, the enantiomeric cis- and trans- pairs were inadequately resolved. Shishovska and Trajkovska used a chiral β-cyclodextrin CSP for permethrin separation under reversed phase HPLC conditions with methanol and water as the mobile phase ( *Chirality*, 22 2010; 527-33). The total run time was more than 50 min. The resolution of the trans-permethrin enantiomeric pair was <1.5. Alternatively, the CHIRALCEL OJ column under normal phase HPLC conditions was also used for permethrin separation (*Chromatographia*, 60 2004; 523-26). Our experiment, conducted under the same conditions described in Table 1, yielded three separate peaks as shown in Figure 2. This is in agreement with the reported results.

| | Normal phase HPLC | UPC² |
|---|---|---|
| Flow rate (mL/min) | 1 | 4 |
| Mobile phase | Hexane:ethanol=90:10 | $CO_2$:methanol:DEA=95:5:0.2 |
| Back pressure (bar) | n/a | 120 |
| Temp. (°C) | Ambient | 40 |
| Column | CHIRALCEL OJ-H (4.6 x 250 mm, 5 µm) | CHIRALCEL OJ-H (4.6 x 150 mm, 5 µm) |
| Sample conc. | 2 mg/mL | |
| Injection volume (µL) | 10 | |

*Table 1. Key experimental parameters.*

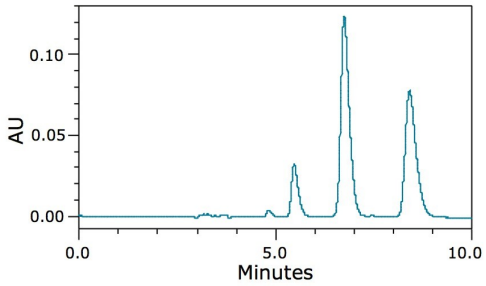


*Figure 2. Chromatogram of permethrin obtained with a CHIRALCEL OJ-H column under normal phase HPLC conditions.*

Figure 3 shows the diastereoselective separation of permethrin using the ACQUITY UPC$^2$ System. Baseline resolution of all four isomers was achieved in less than six minutes with a shorter OJ-H column. Results are summarized in Table 2. Overall, compared to the chiral HPLC methods, the current UPC$^2$ method offers a better resolution and a shorter run time.

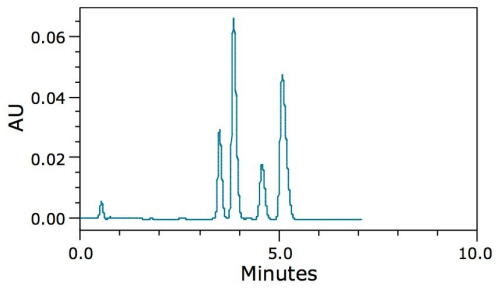


*Figure 3. Chromatogram of permethrin obtained with a CHIRALCEL OJ-H column under UPC$^2$ conditions.*

| Peak | Retention time (min) | K' | α | Resolution | USP tailing factor |
|---|---|---|---|---|---|
| 1 | 3.509 | 5.66 | | | 1.12 |
| 2 | 3.862 | 6.33 | 1.12 | 1.80 | 1.31 |
| 3 | 4.582 | 7.69 | 1.22 | 3.25 | 1.12 |
| 4 | 5.089 | 8.66 | 1.13 | 1.92 | 1.50 |

*Table 2. Retention time, retention factor (K'), selectivity (α), resolution, and USP tailing factor of permethrin obtained under UPC$^2$ conditions with a CHIRALCEL OJ-H column.*

## Conclusion

Successful diastereoselective separation of permethrin was demonstrated using the Waters ACQUITY UPC$^2$ System. Baseline resolution of all four isomers was achieved in less than six minutes. Compared to chiral HPLC methods, the UPC$^2$ method offers a better resolution and a shorter run time. The UPC$^2$ method also eliminated the need for toxic hexane often used in normal phase HPLC methods. The ACQUITY UPC$^2$ System is ideal for pesticide manufacturers who routinely perform diastereomer analyses.

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ACQUITY UPC2 System

720004246, August 2012