[TECHNOLOGY BRIEF]

Facilitating Chiral Method Development Using UPC²/MS

GOAL

To demonstrate the use of an MS detector to facilitate chiral method development using the Waters® ACQUITY UPC²™ System.

BACKGROUND

Chirality plays a critical role in drug profiles because enantiomers can have vastly different biological/pharmacological/toxicological properties. Chiral analysis is typically addressed in the early stage of drug discovery. Due to its superior resolving power and high speed, SFC has gained a strong foothold in the chiral analysis arena. Furthermore, SFC eliminates the use of toxic solvents typically associated with normal phase LC chiral analysis, such as hexane and chloroform.

Analysts often encounter a large number of structurally diverse stereoisomers with varying purity levels, ranging from 70% to 90%. As a result, shortening the cycle time for chiral method development has become a key initiative in improving productivity. To this end, UPC²™/MS has been proposed to expedite chiral method development by leveraging the specificity of MS detection. Although MS detection does not offer selectivity between enantiomers, it can provide complete resolution of different enantiomers and their impurities.

The Waters ACQUITY UPC²/MS System is ideal for laboratories pursuing high throughput enantioselective analyses, enantiomeric excess determination in a complex mixture or matrix, and impurity profiling.

THE SOLUTION

A mixture of two chiral sulfoxides, oxfendazole and pantoprazole (0.2 mg/mL for each compound in methanol), was analyzed. Key experimental parameters are described in Table 1.

Chromatography		3100 SQD MS	
Flow	3 mL/min	Source	APCI +
Co-solvent	methanol	Corona current	5 uA
Back pressure	120 bar	Cone voltage	40 V
Temperature	40 °C	Source temp.	150 °C
Column	CHIRALPAK IC (4.6 x 150 mm, 5 µm)	Probe temp.	450 °C
Gradient	5% to 45% in 4 min, 45% for 1 min, 45% to 5% in 0.5 min, 5% for 0.5 min	Cone gas	70 L/hr
		Desolvation gas	450 L/hr

Table 1. Key experimental parameters.

Typically, chiral SFC method development involves screening a sample with many individual columns and mobile phases using a generic gradient similar to the one described in Table 1. The throughput can be improved by using a parallel approach, where a sample is screened by multiple columns simultaneously (*LCGC Europe, Application Book*, Dec. 2009, 24-25). Alternatively, multiple samples can be screened simultaneously using SFC/MS, the enantiomers are then differentiated by examining the resulting extracted ion chromatograms (XICs) (*Journal of Chromatography A*, 1003 (2003), 157-166), as demonstrated in this brief.

Figure 1 corresponds to the structures of oxfendazole and pantoprazole. UPC^2 chromatograms of the chiral pairs for each compound are shown in Figure 2. Baseline resolution was achieved for both enantiomeric pairs, indicating that an IC column and methanol are a suitable stationary phase and co-solvent, respectively. With *a priori* knowledge of the molecular mass, the compound identity can easily be determined from the XIC. The retention times of the two compounds can subsequently be used for further method optimization. The throughput was improved two-fold in this case, compared to a traditional SFC-UV approach where only a single compound is screened for one set of chromatographic conditions.

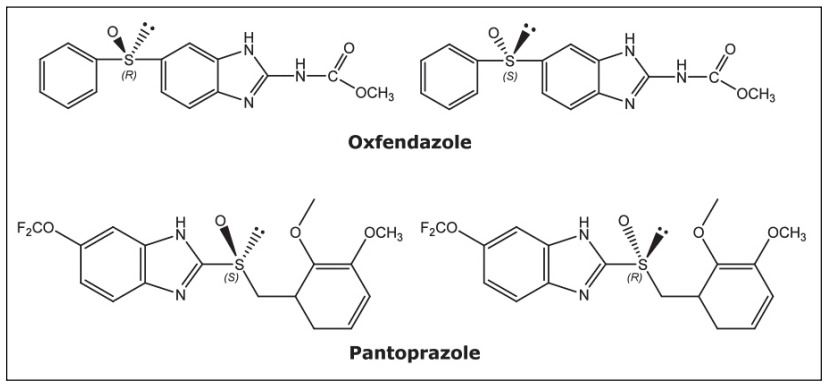

Figure 1. Chemical structures of oxfendazole and pantoprazole.

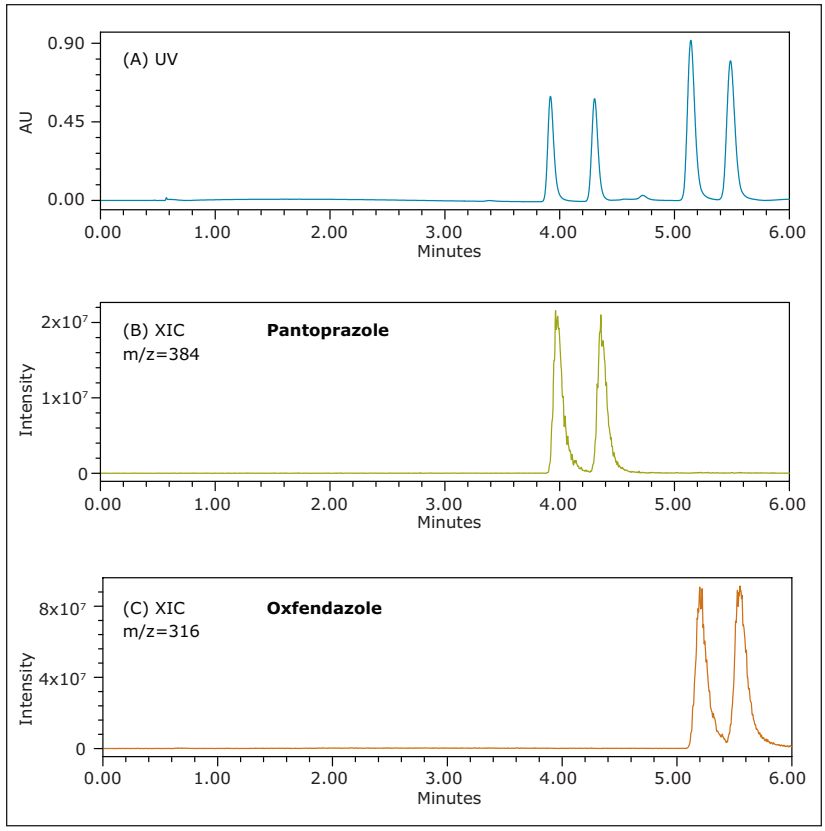

Figure 2. UPC^2 chromatograms of the oxfendazole and pantoprazole mixture.

SUMMARY

The use of MS detection was demonstrated to facilitate chiral UltraPerformance Convergence Chromatography™ (UPC^2) method development. Taking advantage of the specificity of MS detection, a mixture of two enantiomers with different molecular masses was screened simultaneously. By examining the XICs combined with *a priori* knowledge of the compound mass, the compound identity was easily determined. The throughput for chiral method development was, therefore, improved two-fold, compared to a traditional SFC-UV approach. The ACQUITY UPC^2/MS System is ideal for laboratories pursuing high throughput enantioselective analyses, enantiomeric excess determination in a complex mixture or matrix, and impurity profiling.

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