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Nota applicativa

Low Level Enantiomeric Impurity Analysis 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 demonstrates the fast chiral separation of benzyl mandelate and the enantiomeric excess determination at 0.02% impurity level using the Waters ACQUITY UPC^2 System.

Benefits

The high detection sensitivity of the ACQUITY UPC^2 System enables the identification and quantification of enantiomeric impurities in drug substances.

Introduction

According to the September 2005 issue of *Chemical & Engineering News,* 9 out of the top 10 drugs (based on sales figures) have chiral active ingredients, and five of those nine drugs have single enantiomeric active ingredients. The single enantiomeric form of a chiral drug is considered an improved chemical entity, which may offer a higher efficacy, a better pharmacological profile, and a more favorable adverse reaction profile. For the manufacturers of single enantiomeric drugs, the undesired stereoisomers should be considered in the same manner as other organic impurities. Regulatory requirements for the identification, quantification, and control of impurities in drug substances and their formulated products have been explicitly defined by the International Conference of Harmonization (ICH). The threshold for identification and quantification of organic impurities is 0.1% for the majority of compounds, according to the ICH.

Results and Discussion

Benzyl mandelate, shown in Figure 1, is an important synthetic intermediate for pharmaceutical synthesis. A racemic mixture of R- and S-benzyl mandelate (0.20 mg/mL in methanol for each enantiomer) was separated using UltraPerformance Convergenc Chromatography (UPC^2), and the chromatogram is shown in Figure 2. Key experimental parameters are listed in Table 1.

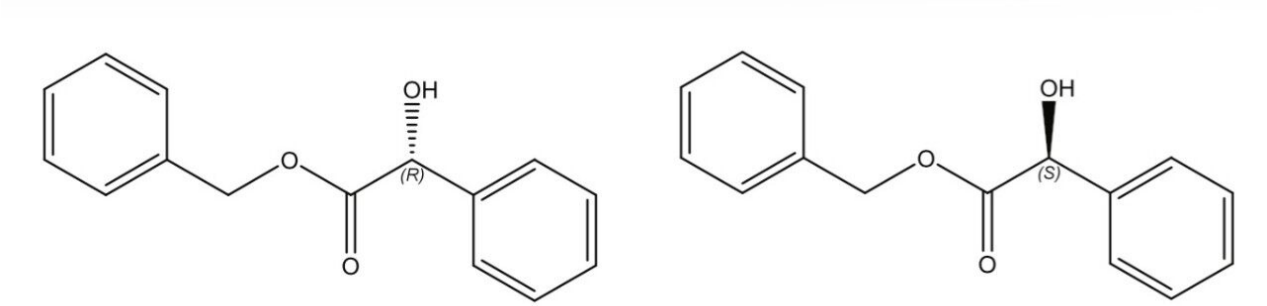

Figure 1. Chemical structures of R- and S-benzyl mandelate.

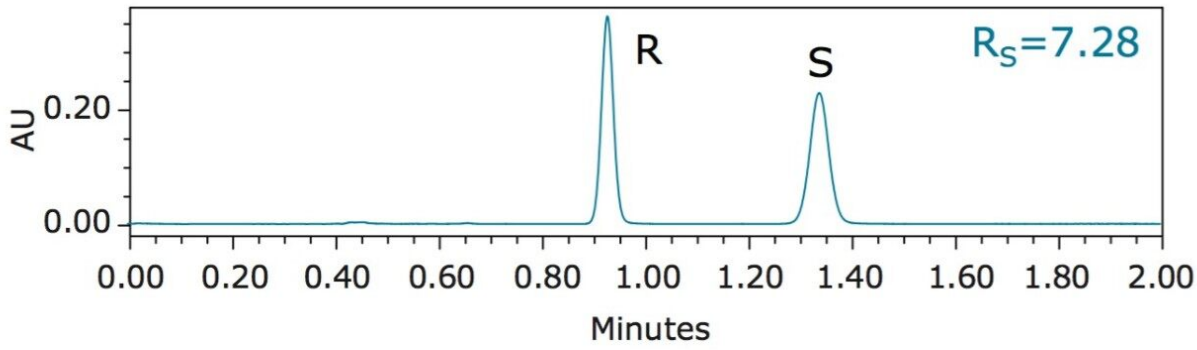

Figure 2. UPC^2 chromatogram of R- and S- benzyl mandelate at 0.20 mg/mL of each enantiomer.

Flow rate	4 mL/min
Mobile phase	CO_2:methanol=70:30
Back pressure	120 bar
Temperature	40 °C
Column	CHIRALPAK AD-H (4.6 x 150 mm, 5 µm)
Injection volume	5 µL

Table 1. Key experimental parameters.

The overall analysis time was less than 1.5 min. Average base peak widths were less than 6 s. Based on the peak area, the ratio of the R- and S-benzyl mandelate was 0.997. Retention time and peak area repeatability measurements were based on five replicate injections, as summarized in Table 2. At 0.20 mg/mL concentration, repeatability for retention time was better than 0.23% RSD and better than 0.5% RSD for

peak area.

Analyte		Benzyl mandelate	
Isomer		R-	S-
Retention time	^{t}R (min)	0.933	1.344
	Std. dev.	0.00179	0.00283
	% RSD	0.23	0.21
Peak area	Peak area (µV*s)	593374	594972
	Std. dev.	2815.4700	2986.2300
	% RSD	0.47	0.50

Table 2. Retention time and peak area repeatability at 0.20 mg/mL of each enantiomer.

Figure 3 shows the UPC^2 chromatogram of R-benzyl mandelate at 2 mg/mL. The minor peak at 1.30 min corresponds to S-benzyl mandelate as confirmed by the UV spectrum (results not shown). This S-benzyl mandelate impurity peak has an S/N of ~3 (LOD) and represents 0.02% of the major peak based on the peak area. This increased detection sensitivity can be attributed to the holistically designed ACQUITY UPC^2 System, which includes an improved pumping system and an optimized detector design. The enantiomeric excess (e.e.) in this case was 99.96%

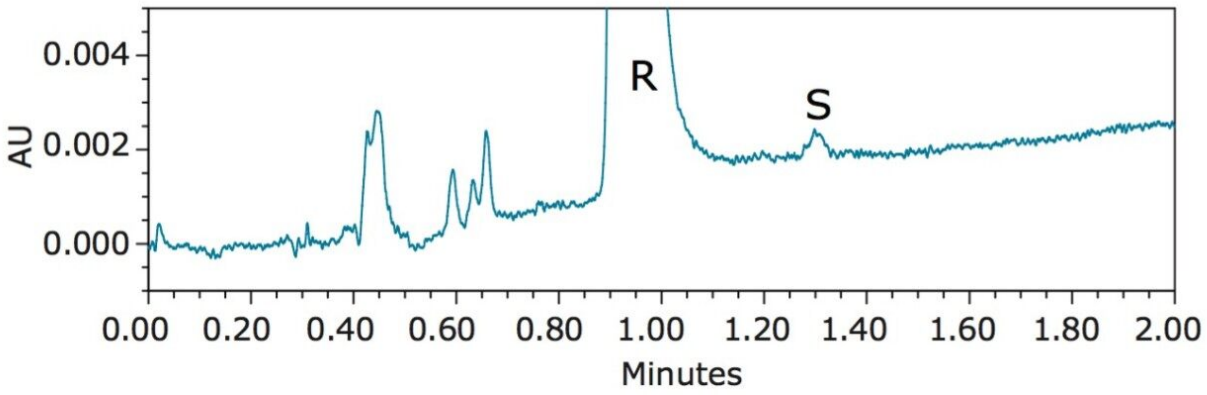

Figure 3. UPC^2 chromatogram of R- and S-benzyl mandelate showing the LOD of the S-benzyl mandelate at 0.02% of the main peak (2 mg/mL)

Conclusion

UPC^2 chiral separations of R- and S-benzyl mandelate in less than 1.5 min were successfully demonstrated using the ACQUITY UPC^2 System. At 0.20 mg/mL concentration of each enantiomer, excellent repeatability (better than 0.23% RSD for retention time and better than 0.5% RSD for peak area) was obtained. Improved detection sensitivity, resulting from a new pumping system and optimized detector design, made detection of a 0.02% enantiomeric impurity and e.e. determination possible. The AQUITY UPC^2 System is suitable for the analysis of low level enantiomeric impurities, enantiomeric excess determinations, and QA/QC analyses.

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ACQUITY UPC2 System <https://www.waters.com/134658367>

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