

Low Level Enantiomeric Impurity Analysis Using the ACQUITY UPC² System

GOAL

To demonstrate the fast chiral separation of benzyl mandelate and the enantiomeric excess determination at 0.02% impurity level using the Waters® ACQUITY UPC²™ System.

BACKGROUND

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.

The high detection sensitivity of the ACQUITY UPC² System enables the identification and quantification of enantiomeric impurities in drug substances.

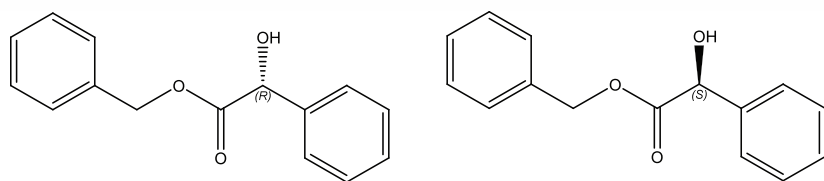


Figure 1. Chemical structures of R- and S-benzyl mandelate.

THE SOLUTION

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 Convergence Chromatography™ (UPC²™), and the chromatogram is shown in Figure 2. Key experimental parameters are listed in Table 1.

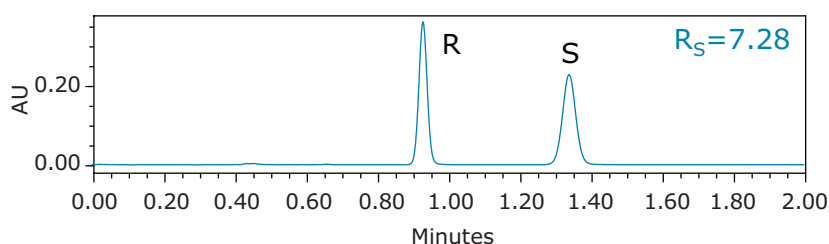


Figure 2. UPC² chromatogram of R- and S- benzyl mandelate at 0.20 mg/mL of each enantiomer.

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	593374	594972
Peak area	Peak area ($\mu V*s$)		
	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 UPC² 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 UPC² System, which includes an improved pumping system and an optimized detector design. The enantiomeric excess (e.e.) in this case was 99.96%.

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 μ m)
Injection volume	5 μ L

Table 1. Key experimental parameters.

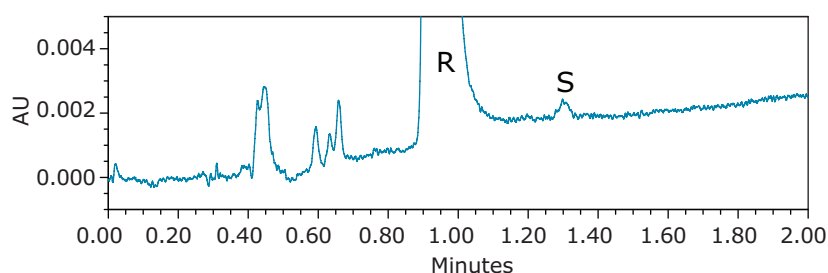


Figure 3. UPC² chromatogram of R- and S-benzyl mandelate showing the LOD of the S-benzyl mandelate at 0.02% of the main peak (2 mg/mL).

SUMMARY

UPC² chiral separations of R- and S-benzyl mandelate in less than 1.5 min were successfully demonstrated using the ACQUITY UPC² 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 ACQUITY UPC² System is suitable for the analysis of low level enantiomeric impurities, enantiomeric excess determinations, and QA/QC analyses.

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