[TECHNOLOGY BRIEF]

Enantiomeric Separation of BINOL Using the ACQUITY UPC^2 System

GOAL

To compare the enantiomeric separation of BINOL by normal phase HPLC and UPC^{2}™ using the ACQUITY UPC^{2}™ System.

UltraPerformance Convergence Chromatography™ (UPC^2) demonstrated a faster separation of BINOL enantiomers (by 9 times) compared to normal phase HPLC as well as appreciable cost savings per analysis.

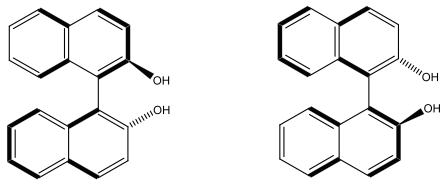
BACKGROUND

Living organisms are composed of chiral biomolecules such as proteins, nucleic acids, and polysaccharides; consequently, they display different biological responses to one of a pair of enantiomers in drugs, food, pesticides, and waste compounds. It is, therefore, important to separate chiral compounds, especially those of pharmaceutical importance. This is manifested by an increasing number of approved chiral drugs in the form of single enantiomers. To comply with the stringent FDA mandate for developing stereoisomeric drugs, the pharmaceutical industry has escalated its emphasis on the generation of enantiomerically pure compounds before undertaking pharmacokinetic, metabolic, physiological, and toxicological evaluations.

In the past decade, SFC has demonstrated great promise as the choice of chromatography for separating stereoisomers, including enantiomers and diastereomers. Compared to traditional chiral high pressure liquid chromatography (HPLC), which is predominantly normal phase HPLC, SFC is on average 3 to 10 times faster. Using inexpensive CO_2 and a polar modifier such as methanol as the mobile phase, SFC is more cost-effective and environmentally friendly by reducing the consumption and disposal of organic solvents.

THE SOLUTION

BINOL is an organic compound with axial chirality as shown in Figure 1. A sample of BINOL was separated using both normal phase HPLC and the ACQUITY UPC^2 System. The key parameters for both methods are described in Table 1.



R-BINOL S-BINOL

Figure 1. Chemical structures of BINOL showing the axial chirality.

	Normal Phase HPLC	UPC^2
Flow rate (mL/min)	2	4
Mobile phase	Hexane:isopropanol=98:2	CO_2:methanol=75:25
Back pressure (bar)	n/a	120
Temp. (°C)	Ambient	40
Column	ChiralPak AS-H (4.6 x 150 mm, 5 µm)	
Sample conc.	2 mg/mL	
Injection volume (µL)	5	

Table 1. Experimental parameters for both normal phase HPLC and UPC.[2]

Figure 2 shows the chiral separations of BINOL by normal phase HPLC (A) and UPC^2 (B). The elution time of the second peak in UPC^2 was 2 min, compared to 18 min in normal phase HPLC, representing a 9 times increase in speed by UPC.^{2} The resolutions (USP) were 1.73 for normal phase HPLC and 2.61 for UPC.^{2} This case also exemplified considerable cost savings per analysis by UPC.^{2} The UPC^2 method used 2 mL of methanol to elute the compound, whereas, the normal phase HPLC method used 35.28 mL hexane and 0.72 mL isopropanol. Based on the organic solvent usage alone, this translated to an estimated $2.85 per analysis for normal phase HPLC and $0.08 per analysis for UPC.^{2}

The peaks in the UPC^2 chromatogram were more symmetrical than those from normal phase HPLC. The tailing factors (USP) for the normal phase HPLC peaks were 1.33, 2.18, respectively; and 1.03, 1.03 for UPC.^{2} The peaks in the UPC^2 chromatogram were also taller and narrower than those in normal phase HPLC, indicating improved sensitivity and peak capacity. Since supercritical CO_2 is used as the main mobile phase in UPC,^{2} the inherent high diffusivity and low viscosity of supercritical CO_2 have a profound impact on the separation. The high diffusivity reduces the peak dispersion resulting from mass transfer between the mobile phase and the stationary phase. The low viscosity enables a high optimal flow rate without generating a formidable pressure drop. Furthermore, a significantly reduced system volume in the ACQUITY UPC^2 System minimizes extra-column band broadening.

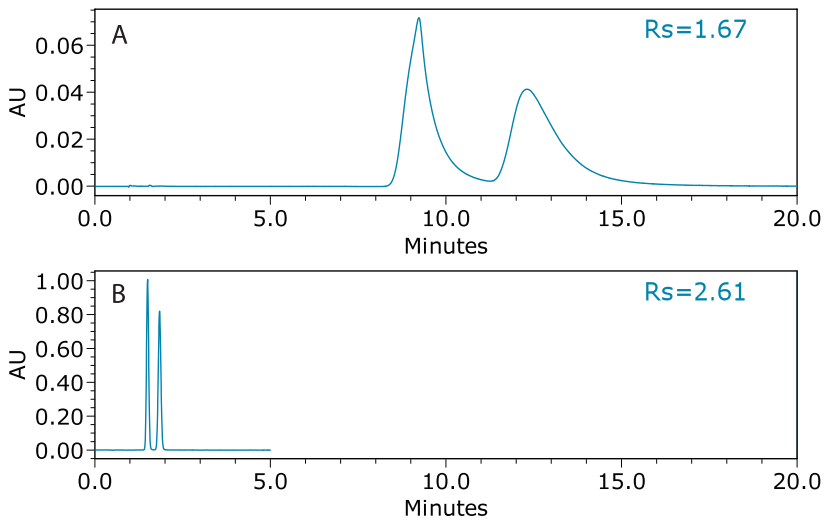


Figure 2. Normal phase HPLC chromatogram (A) and UPC^2 chromatogram (B) of BINOL.

SUMMARY

The ACQUITY UPC^2 System demonstrated a successful UPC^2 separation of BINOL enantiomers in less than 2 min. Compared to normal phase HPLC, UPC^2 was 9 times faster and generated taller and more symmetrical peaks. A significantly reduced system volume in the ACQUITY UPC^2 System minimized extra-column band broadening. The improvement in speed combined with the replacement of hexane with relatively inexpensive methanol led to appreciable cost savings per analysis by UPC^2 ($2.85 per analysis for normal phase HPLC vs. $0.08 per analysis for UPC^2). Waters ACQUITY UPC^2 System is ideal for laboratories routinely performing enantiomer separations.

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