

## Enantiomeric Separation of Nebivolol Using UltraPerformance Convergence Chromatography (UPC<sup>2</sup>)

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

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## Abstract

This application brief demonstrates a fast, easy, and cost-effective method for the enantiomeric separation of nebivolol isomers using UltraPerformance Convergence Chromatography (UPC<sup>2</sup>).

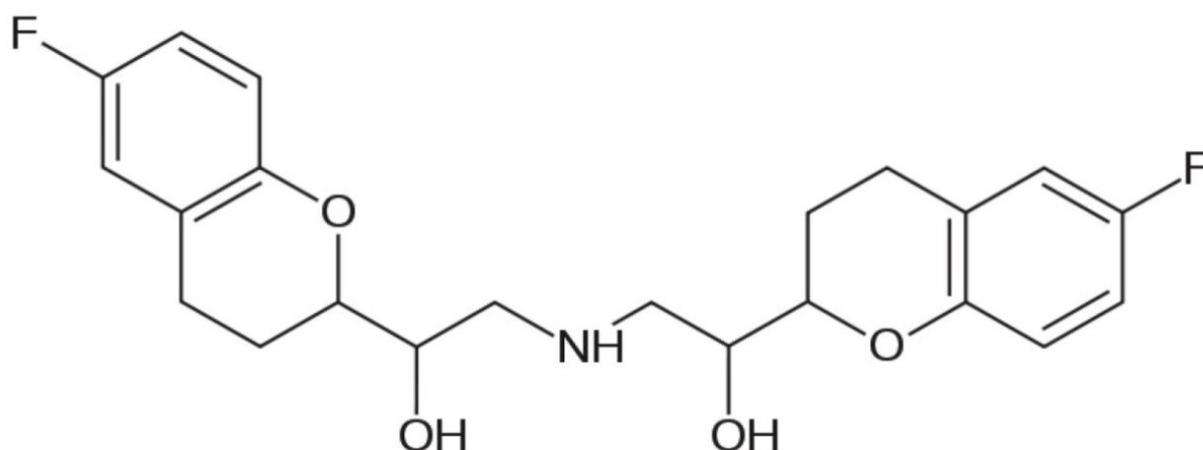
### Benefits

UPC<sup>2</sup> separation of nebivolol allows for up to a 65% reduction in analysis cost and up to 80% improvement in analysis throughput compared to traditional LC methods.

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## Introduction

Nebivolol (Figure 1) is a beta blocker drug with nitric oxide-potentiating vasodilatory effects used in the treatment of hypertension. Beta blockers are one of the most highly recommended and prescribed drugs for the treatment of hypertension and heart diseases throughout the world. Studies show that the D-isomer of nebivolol is primarily responsible for beta selectivity, whereas the L-isomer potentially provides the anti-hypertensive effect of the D-isomer. Enantiomeric profiling is often needed to monitor the metabolic pathways for both isomers.



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Figure 1. Chemical structure of nebivolol.

Chiral method development for these types of compounds is often time-consuming, since screening of multiple chiral stationary phases is required. The analysis and column regeneration times for chiral stationary phases using normal-phase solvents is often very high. The solvents used for traditional normal-phase chromatography such as n-hexane, halogenated solvents, etc., are toxic and costly in terms of both use and disposal.

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## Results and Discussion

Chiral analysis is typically performed in the early stage of drug discovery. Due to its superior resolving power and high speed, convergence chromatography proves to be a powerful analytical technique in chiral analysis. Use of convergence chromatography also eliminates the use of toxic solvents typically associated with normal-phase LC chiral analysis.

The Waters ACQUITY UPC<sup>2</sup> System uses supercritical CO<sub>2</sub> as the primary mobile phase component. UPC<sup>2</sup> technology applies the performance advantages of UPLC to supercritical fluid chromatography (SFC). UPC<sup>2</sup> allows for screening and method development for the enantiomeric separation of pharmaceutical compounds within a very short time, as well as for reduced solvent consumption.

This study demonstrates a fast and effective method for the enantiomeric separation of the isomers of nebivolol using UPC<sup>2</sup>.

For the method demonstrated here, a nebivolol standard was dissolved in 100% methanol at a concentration of 500 ppm. The ACQUITY UPC<sup>2</sup> System was used with a 4.6 x 150 mm, 3- $\mu$ m chiral column, and an ACQUITY UPC<sup>2</sup> PDA Detector at 220-nm wavelength.

Using the method described above, the isomers of nebivolol have a USP resolution of 2.1.

The results obtained are highly reproducible over several consecutive injections. The %RSD value for retention time and area for 20 replicate injections were 0.1 and 1.0, respectively.

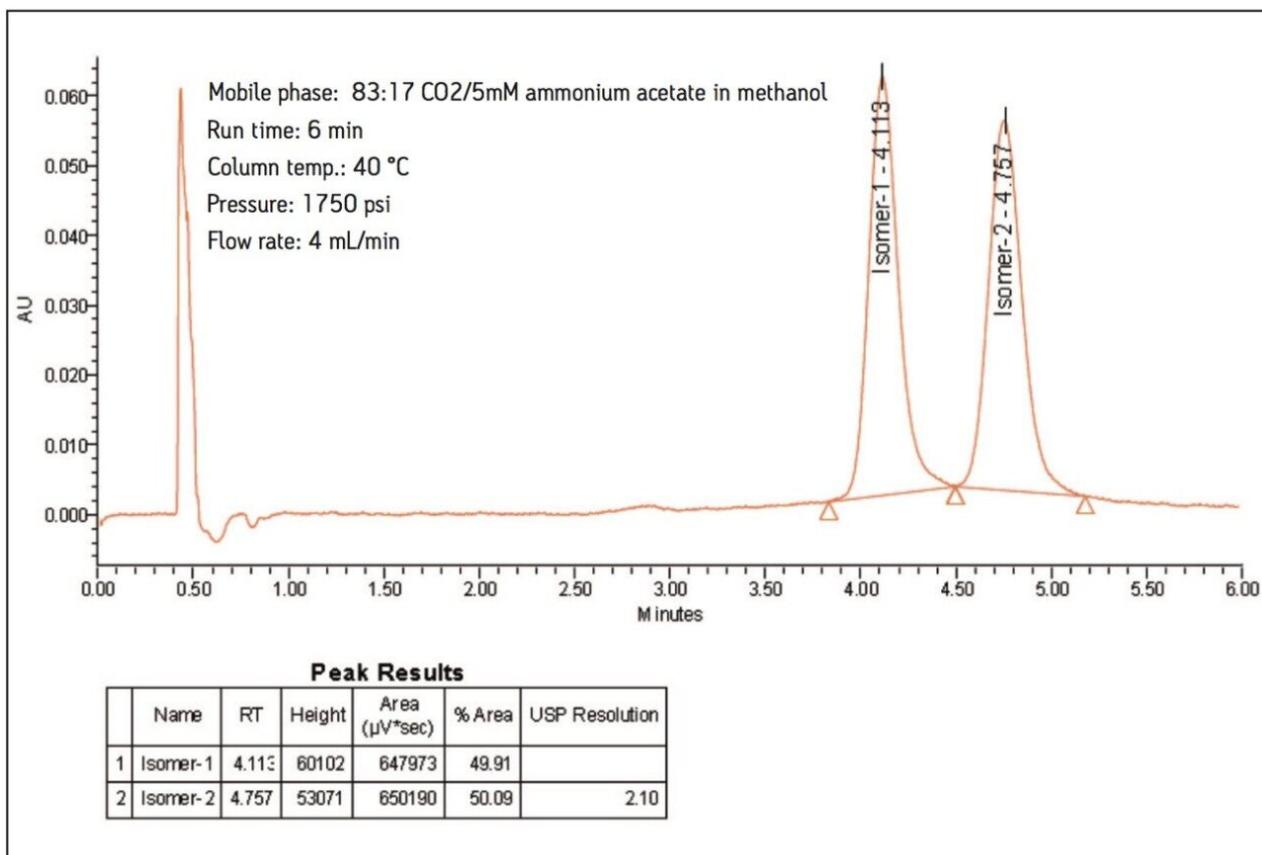


Figure 2. UPC<sup>2</sup> chromatogram of nebivolol.

## Conclusion

The ACQUITY UPC<sup>2</sup> System with a 3-μm column was used to demonstrate an easy and effective method for the enantiomeric separation of nebivolol. The isomers of nebivolol have been separated to base line within 6 minutes. Using the method demonstrated here, up to a 65% reduction in analysis cost and up to 80% improvement in analysis throughput is possible compared to traditional LC methods. At the same time, where some of the current HPLC normal-phase method for nebivolol has recommended column conditioning time of 12 to 15 hours before starting the analysis, 5 to 10 minutes column conditioning is sufficient in UPC<sup>2</sup> when using the method described here. The method is highly reproducible over several injections. The mobile phase used is compatible with a mass spectrometer, thus extending the applicability of UPC<sup>2</sup> to bioanalytical studies.

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

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