

## Application Note

# ACQUITY UPLC HILIC Gradient Separation of Ascorbic Acid and Isoascorbic Acids

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Waters Corporation

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

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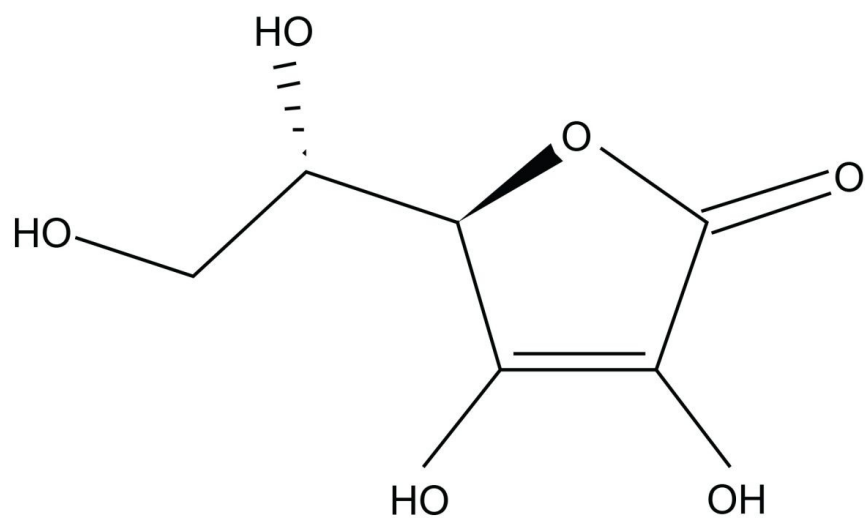
## Abstract

This application brief highlights the gradient separation of ascorbic acid and isoascorbic acids using ACQUITY UPLC BEH Amide Columns.

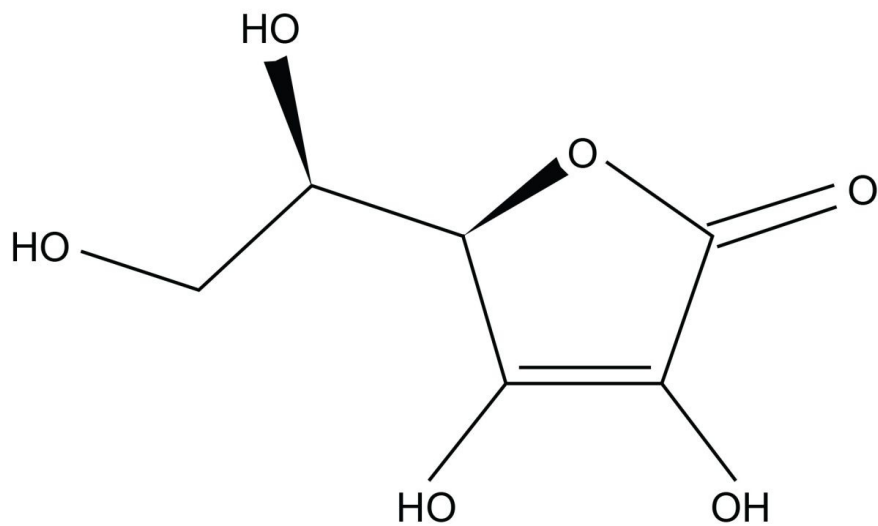
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## Introduction

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**Ascorbic Acid**



**Isoascorbic Acid**

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## Experimental

### Test Conditions

Columns:	ACQUITY UPLC BEH Amide, 2.1 x 100 mm, 1.7 $\mu$ m
Part Number:	186004801
Mobile Phase A:	50/50 MeCN/H <sub>2</sub> O with 10 mM CH <sub>3</sub> COONH <sub>4</sub> and 0.02% CH <sub>3</sub> COOH, pH 5.0
Mobile Phase B:	90/10 MeCN/H <sub>2</sub> O with 10 mM CH <sub>3</sub> COONH <sub>4</sub> and 0.02% CH <sub>3</sub> COOH, pH 5.0
Flow Rate:	0.2 mL/min
Injection Volume:	5.0 $\mu$ L (PLNO)
Sample Concentration:	30 $\mu$ g/mL each
Sample Diluent:	75/25 MeCN/MeOH with 0.2% HCOOH
Column Temperature:	25 $^{\circ}$ C
Weak Needle Wash:	95/5 MeCN/H <sub>2</sub> O
Detection:	UV @ 260nm
Sampling Rate:	20 points/sec
Filter Time Constant:	0.2

Instrument:

Waters ACQUITY UPLC with ACQUITY UPLC PDA  
Detector

## Gradient

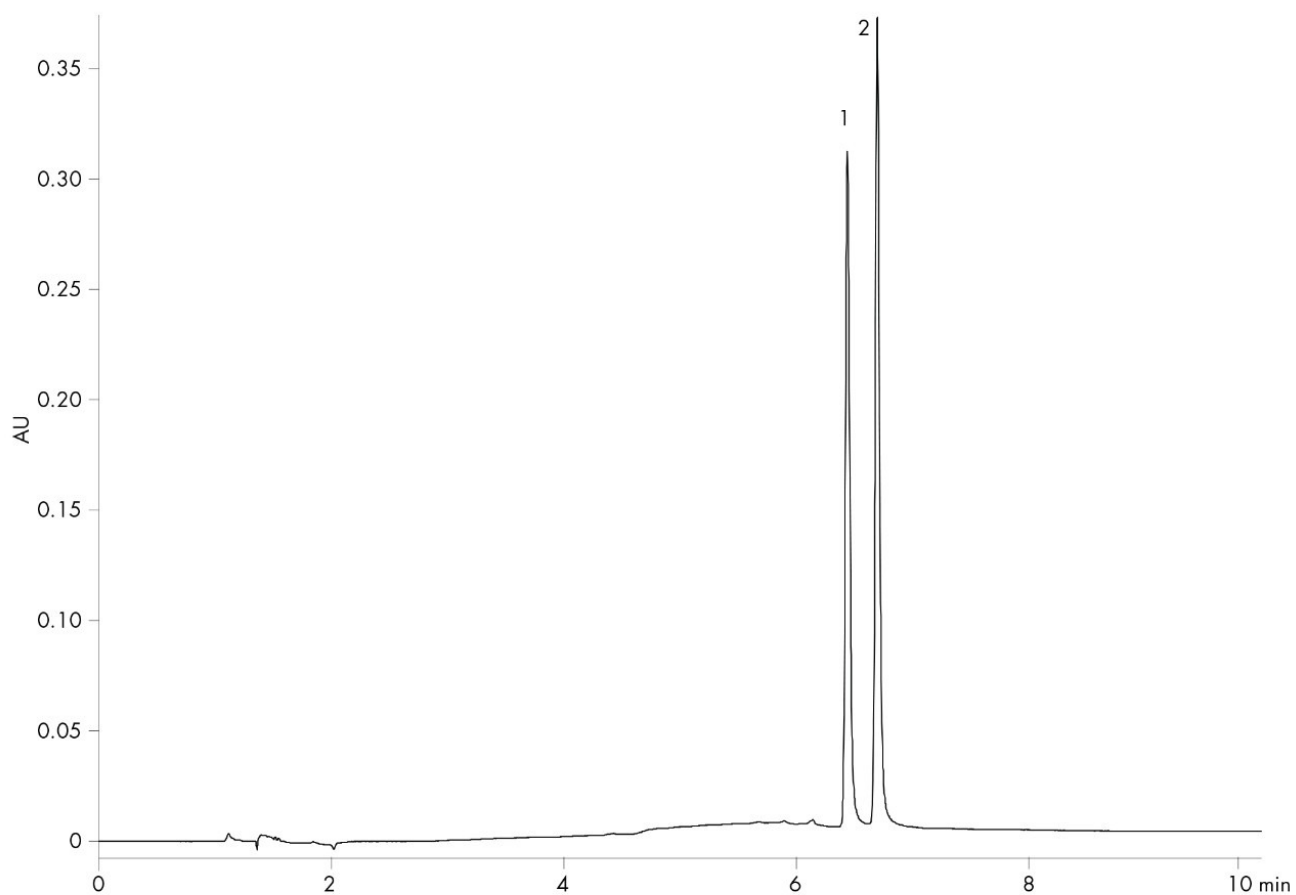
Time (min)	Profile	
	%A	%B
Initial	0.1	99.9
10.00	99.9	0.1
10.01	0.1	99.9
15.00	0.1	99.9

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## Results and Discussion

The compounds used in this study are:

1. Isoascorbic acid
2. Ascorbic acid



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## Featured Products

ACQUITY UPLC PDA Detector <<https://www.waters.com/514225>>

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