

Application Note

## Gradient Separation of Nucleic Acid Bases on ACQUITY UPLC BEH HILIC

Waters Corporation



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

---

## Abstract

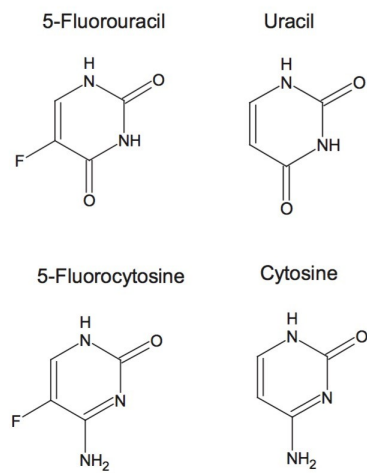
This application brief demonstrates gradient separation of nucleic acid bases.

---

# Introduction

Compounds that are used in this study includes:

1. 5-Fluorouracil
2. Uracil
3. 5-Fluorocytosine
4. Cytosine



---

*Figure 1: Structure of the compounds analysed.*

---

# Experimental

## Test Conditions

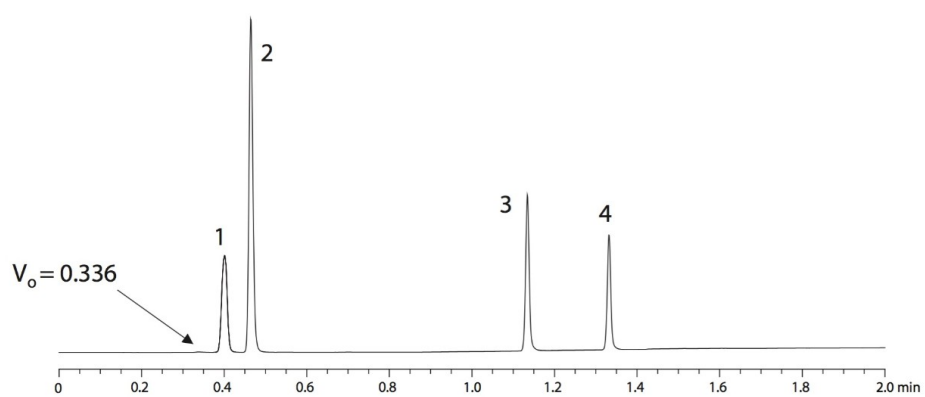
Column:	ACQUITY UPLC BEH HILIC, 2.1 x 100 mm, 1.7 $\mu$ m
Part Number:	186003461
Mobile Phase A:	20 mM $\text{CH}_3\text{COONH}_4$ , 0.05% $\text{CH}_3$ COOH in 50:40:10 ACN:MeOH:H <sub>2</sub> O
Mobile Phase B:	4 mM $\text{CH}_3\text{COONH}_4$ , 0.01% $\text{CH}_3$ COOH in 95:3:2 ACN:MeOH:H <sub>2</sub> O
Flow Rate:	0.790 mL/min
Injection Volume:	0.8 $\mu$ L
Sample Concentration:	25 $\mu$ g/mL
Sample Diluent:	75:25 ACN:MeOH with 0.2% HCOOH
Temperature:	30 $^\circ$ C
Detection:	UV @ 254 nm
Sampling Rate:	20 pts/sec
Time Constant:	0.1
Instrument:	Waters ACQUITY UPLC with ACQUITY TUV

## Gradient

Time(min)	Profile	
	%A	%B
0.0	0.1	99.9
0.37	0.1	99.9
1.71	99.9	0.1
1.74	0.1	99.9
1.98	0.1	99.9

---

## Results and Discussion



---

Figure 2: Chromatogram of 1. 5-Fluorouracil 2. Uracil 3. 5- Fluorocytosine 4. Cytosine

---

## Featured Products

[ACQUITY UPLC System](#)

[ACQUITY UPLC Tunable UV Detector](#)

WA60138, August 2009

©2019 Waters Corporation. All Rights Reserved.