# Waters™

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

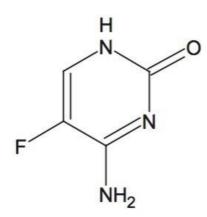
Compounds that are used in this study includes:

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

### 5-Fluorouracil

#### Uracil

# 5-Fluorocytosine



# 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 CH <sub>3</sub> COONH <sub>4</sub> , 0.05% CH <sub>3</sub> COOH in 50:40:10 ACN:MeOH:H <sub>2</sub> O
Mobile Phase B:	4 mM CH <sub>3</sub> COONH <sub>4</sub> , 0.01% CH <sub>3</sub> COOH in 95:3:2 ACN:MeOH:H <sub>2</sub> O
Flow Rate:	0.790 mL/min
Injection Volume:	0.8 µL
Sample Concentration:	25 μg/mL
Sample Diluent:	75:25 ACN:MeOH with 0.2% HCOOH
Temperature:	30 °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
0.0	0.1

Time(min)	Profile
0.37	0.1
1.71	99.9
1.74	0.1
1.98	0.1

#### Results and Discussion

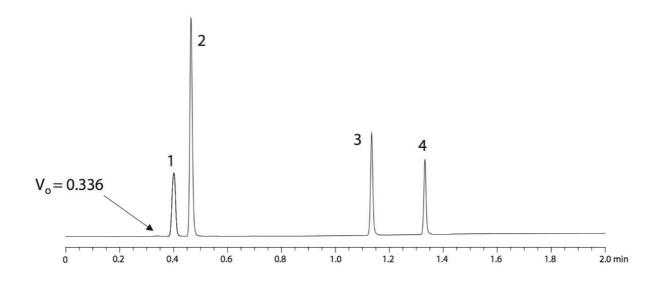


Figure 2: Chromatogram of 1. 5-Fluorouracil 2. Uracil 3. 5- Fluorocytosine 4. Cytosine

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ACQUITY UPLC System <a href="https://www.waters.com/514207">https://www.waters.com/514207</a>

# ACQUITY UPLC Tunable UV Detector <a href="https://www.waters.com/514228">https://www.waters.com/514228</a>

WA60138, August 2009

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