

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

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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 demonstrates gradient separation of nucleic acid bases.

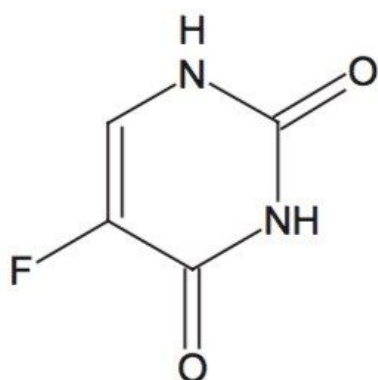
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## Introduction

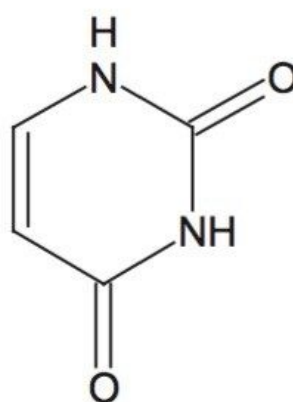
Compounds that are used in this study includes:

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

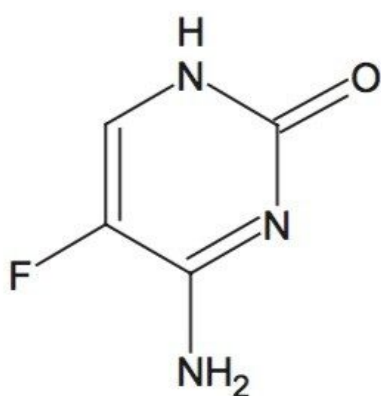
5-Fluorouracil



Uracil



5-Fluorocytosine



Cytosine

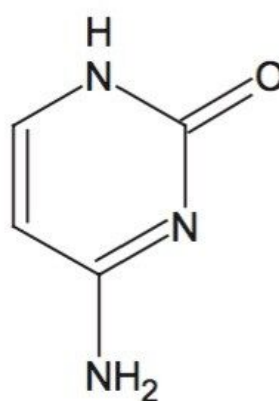


Figure 1: Structure of the compounds analysed.

## 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 $\mu$ 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

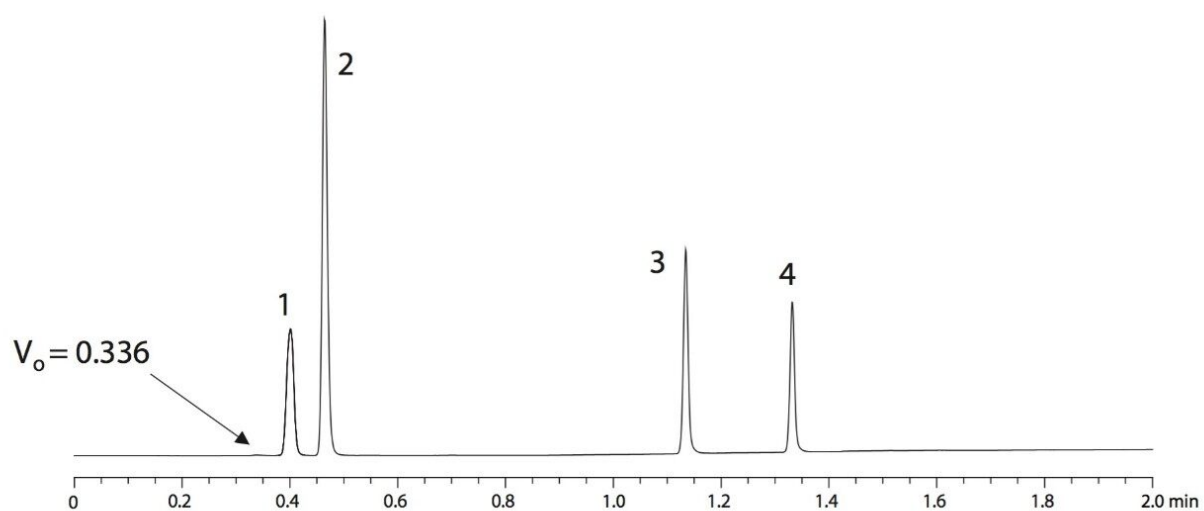
## Gradient

Time(min)	Profile
	%A
0.0	0.1

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

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## Results and Discussion



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

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## Featured Products

ACQUITY UPLC System <<https://www.waters.com/514207>>

ACQUITY UPLC Tunable UV Detector <<https://www.waters.com/514228>>

WA60138, August 2009

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