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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

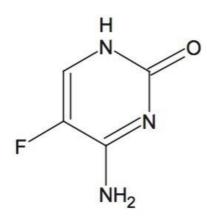
Compounds that are used in this study includes:

- 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 μ m
Part Number:	186003461
Mobile Phase A:	20 mM CH ₃ COONH ₄ , 0.05% CH ₃ COOH in 50:40:10 ACN:MeOH:H ₂ O
Mobile Phase B:	4 mM CH ₃ COONH ₄ , 0.01% CH ₃ COOH in 95:3:2 ACN:MeOH:H ₂ 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

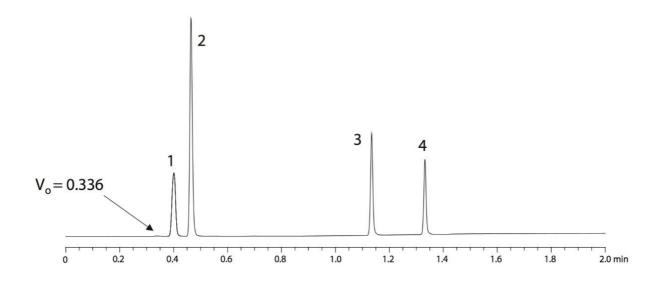


Figure 2: Chromatogram of 1. 5-Fluorouracil 2. Uracil 3. 5- Fluorocytosine 4. Cytosine

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

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