

HPLC Application

ID No.: 25777

Extraction of Uracil and Dihydrouracil from Human Serum Using Strata-X PRO SPE Using Kinetex 2.6 µm PS C18 (

Column: Kinetex® 2.6 µm PS C18 100 Å, LC Column 150 x 3 mm, Ea

Dimensions: 150 x 3 mm ID

Order No: 00F-4780-Y0

Elution Type: Gradient

Eluent A: 0.1% Formic acid in Water

Eluent B: Methanol

Gradient	Step No.	Time (min)	Pct A	Pct B
Profile:	1	0	93	7
	2	12	93	7

Flow Rate: 0.2 mL/min

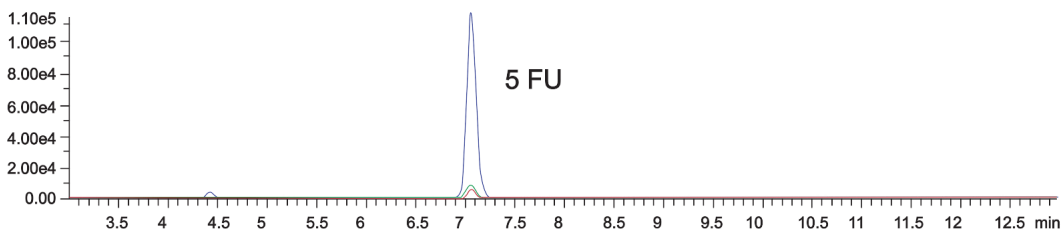
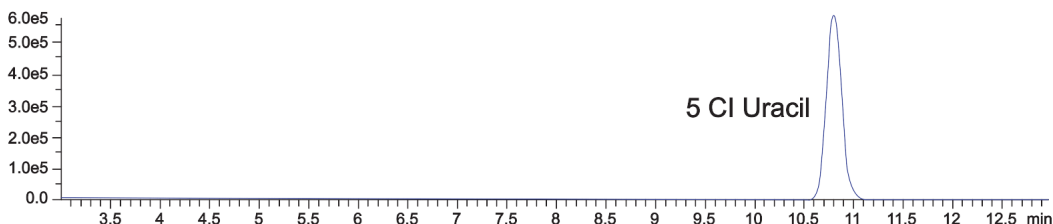
Col. Temp.: 25 °C

Detection: LC/MS/MS @ 0.0000000000 m/z (mass per charge) (25 °C)

Analyst Note: Add 100 µL Serum (doubly stripped) to tube. Add 900 µL of chilled (~00C) ACN to the serum while vortexing. Centrifuge samples at 6000 rpm for 5 minutes and transfer supernatant.
Analyte diluted with initial mobile phase



Products used in this application:



ANALYTES:

- 1 5,6 Dihydrouracil (UH2)
Retention Time: 4.3 min
- 2 Uracil
Retention Time: 4.65 min
- 3 Uracil 1,3-15N2 (+Ve IS)
Retention Time: 4.65 min



Sample Preparation Details

for HPLC Application ID No.: 25777

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PRODUCT DESCRIPTION:

Strata X Pro, 96-Well Plate, 30mg/well

Order No.: 8E-S536-TGA

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Wash:

Dry:

Elute:

Final Prep and Analysis:

Sample Pre-treatment:

- Add 100 µL Serum to tube

Inject: 10 µL on HPLC LC/MS/MS @ 0.000000000 m/z (mass per charge) (25°C)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 5,6 Dihydrouracil (UH2)	100			90	
2 Uracil	100			84	
3 Uracil 1,3-15N2 (+Ve IS)	200				

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals.
Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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