

# HPLC Application

ID No.: 25778

## Extraction of 5-Fluorouracil from Human Serum Using Strata-X PRO SPE on a Kinetex 2.6 µm PS C18 (ESI -Ve Mo

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

**Flow Rate:** 0.2 mL/min

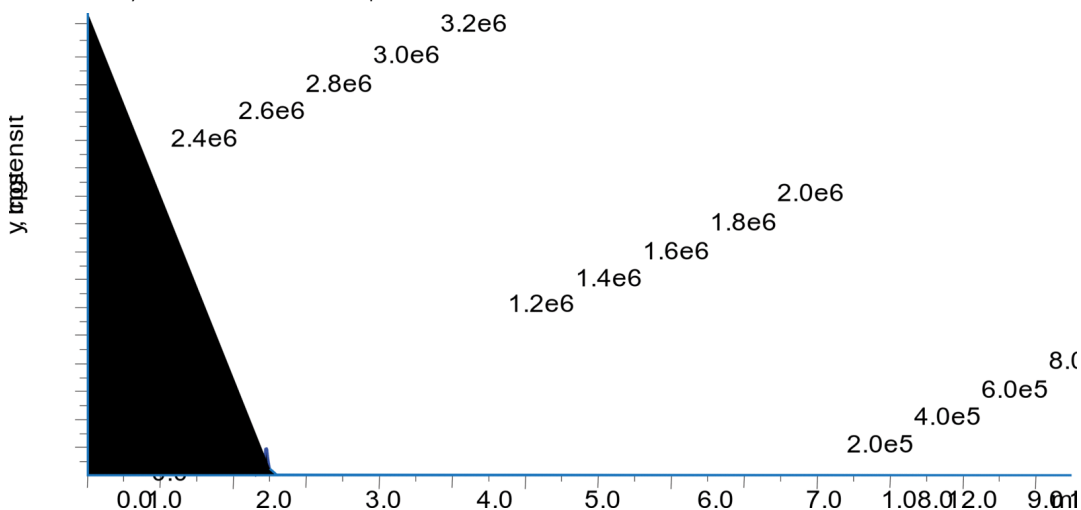
**Col. Temp.:** 25 °C

**Detection:** LC/MS/MS @ 0.000000000 m/z (mass per charge) (25 °C)

**Analyst Note:** Add 100 µL Serum (doubly stripped) to tube. Add 900 µL of chilled (~00C) Acetonitrile 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 5FU

Retention Time: 7.07 min

2 5 Cl Uracil (-Ve IS)

Retention Time: 10.82 min



# Sample Preparation Details

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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:

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#### Load:

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#### Wash:

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#### Dry:

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#### Elute:

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### Final Prep and Analysis:

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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 5FU	100			89	
2 5 Cl Uracil (-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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