A Fast and Effective Quantitation Method for Uracil, 5,6-Dihydrouracil, and 5-Fluorouracil from Human Serum by LC-MS/MS

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Overview

Uracil, 5-Fluorouracil (5FU), and 5,6-Dihydrouracil (UH_2) offer unique markers for enzymatic activity. In order to determine the 5FU catabolic rate, uracil and its homologues are tested in correlation to each other to ultimately determine if proper metabolization is occurring. The primary goal of this study was to develop a sample preparation and LC-MS/MS method for quantitative analysis of uracil and its two homologues from human serum. This application focuses on developing a sample preparation method and LC-MS/MS analysis using a Strata®-X PRO solid phase extraction (SPE) and a Kinetex® PS C18 column respectively. The Kinetex PS C18 Column is a positively charged, surface modified C18 phase that caters its unique selectivity to separating the very polar uracil and its homologues in this analysis, while the novel Strata-X PRO greatly reduces the phospholipids in the sample and provides cleaner extracts.

SPE Protocol

Pretreatment: Add 100 µL Human serum (doubly stripped) to a tube. Dispense 900 µL of chilled (~0°C) Acetonitrile to the serum while vortexing. Centrifuge samples at 6000 rpm for 5 minutes.

96-Well Plate:	Strata-X PRO, 30 mg/well
Part No.:	8B-S536-TGA
Condition:	1 mL Acetonitrile
Load:	Pass the supernatant (from pre-treatment) and apply vacuum to collect eluted extract
Dry Down:	Under Nitrogen and heat around 40-45°C
Reconstitute:	100 µL initial mobile phase
Note:	For phospholipid analysis human plasma EDTA was utilized and direct injection (bypass dry down and reconstitution steps) of the eluted sample was made.

LC-MS/MS Conditions

Quantitative Analysis for Uracil Homologues

Column:	Kinetex 2.6 µm PS C18	
Dimensions:	150 x 3.0 mm	
Part No.:	00F-4780-Y0	
Mobile Phase:	A: 0.1% Formic acid in Water B: Methanol	
Isocratic:	**Time (min)**	**% B**
	0	7
	12	7
Flow Rate:	0.2 mL/min	
Injection Volume:	10 µL	
Column Temperature:	25 °C	
Instrument:	Agilent® 1260	
Detection:	MS/MS SCIEX API Triple Quad™ 4500, Dual Polarity (Positive for U and UH_2, Negative for 5 FU)	

Qualitative Analysis for Phospholipids

Column:	Kinetex 2.6 µm C18	
Dimensions:	50 x 2.1 mm	
Part No.:	00B-4462-AN	
Mobile Phase:	A: 0.1% Formic acid in Water B: 0.1% Formic Acid in Methanol	
Gradient:	**Time (min)**	**% B**
	0	40
	0.5	95
	11.5	95
	11.51	40
	13.5	40
Flow Rate:	0.4 mL/min	
Injection Volume:	2 µL	
Column Temperature:	40 °C	
Instrument:	Agilent 1260	
Detection:	MS/MS SCIEX API Triple Quad 4500, ESI Source (Positive)	

Revision: 0
PHEN-RUO-00099

Figure 1. Structure of Uracil and Homologues

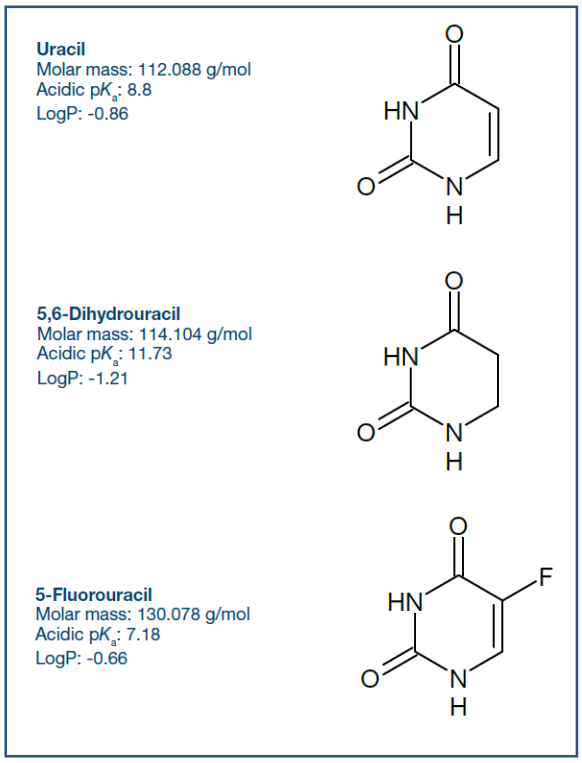

Table 1. Retention Time (RT), MRM Transition and % Recovery for Analytes

Analyte	RT	Q1	Q3	Spike Concentration (ng/mL)	% Recovery	% CV
UH_2	4.31	114.9	55.04	100	90	5.4
Uracil	4.65	112.9	69.8	100	84	3.2
5FU	7.07	128.8	41.9 86.1 58.9	100	89	3.2
Uracil 1,3-$15N_2$ (+Ve IS)	4.65	114.8	96.9	200	N/A	N/A
5 Cl Uracil (-Ve IS)	10.82	145.1	42.1	200	N/A	N/A

Figure 2. Representative Chromatogram of Extracted Human Serum Analyzed by a Kinetex 2.6 µm PS C18 LC Column Under ESI Positive Polarity

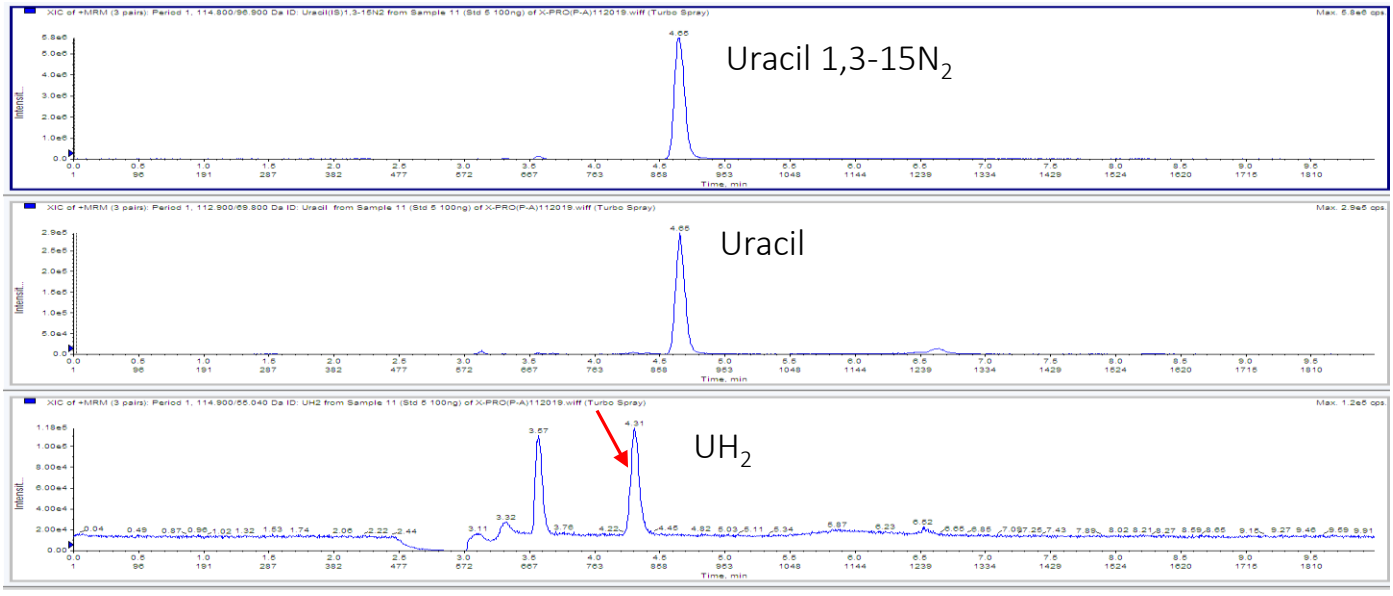

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Figure 3. Representative Chromatogram of Extracted Human Serum Analyzed by a Kinetex® 2.6 µm PS C18 LC column Under ESI Negative Polarity

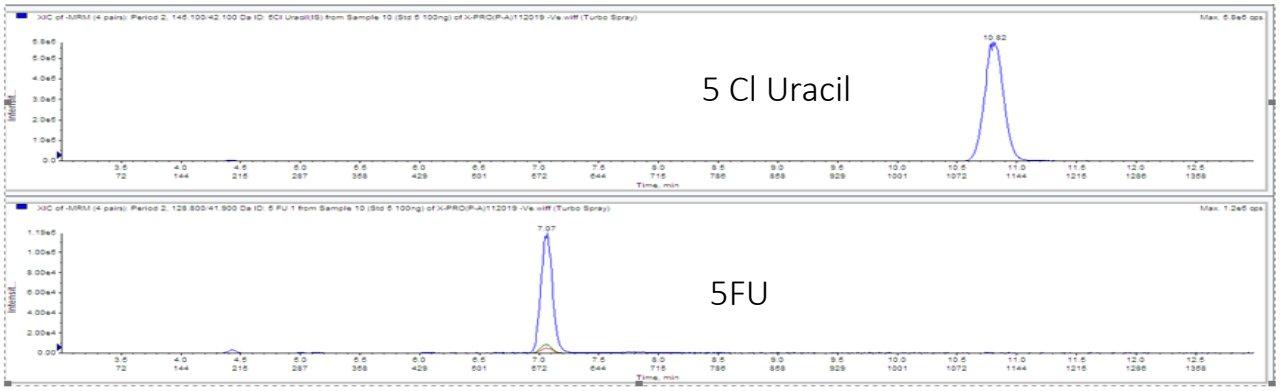

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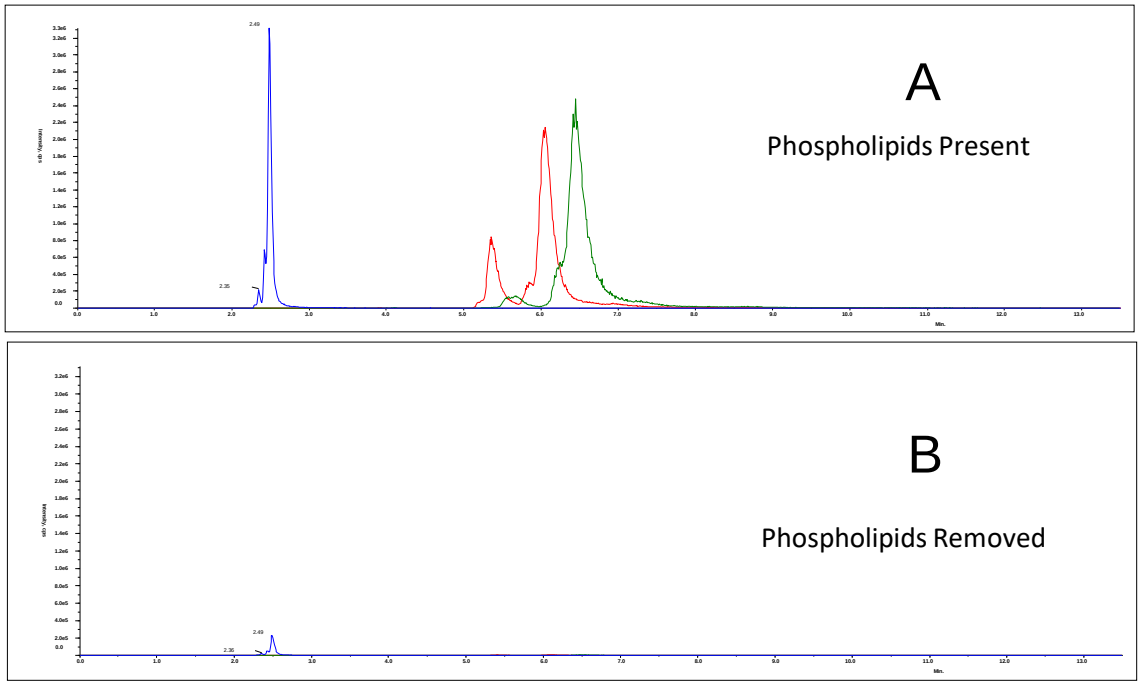
Figure 4. Representative Chromatogram for Qualitative Evaluation of Phospholipid in Extracted Human Plasma Samples, Analyzed by a Kinetex 2.6 µm C18 LC Column (A) Protein Precipitation (B) Strata®-X PRO Extraction



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