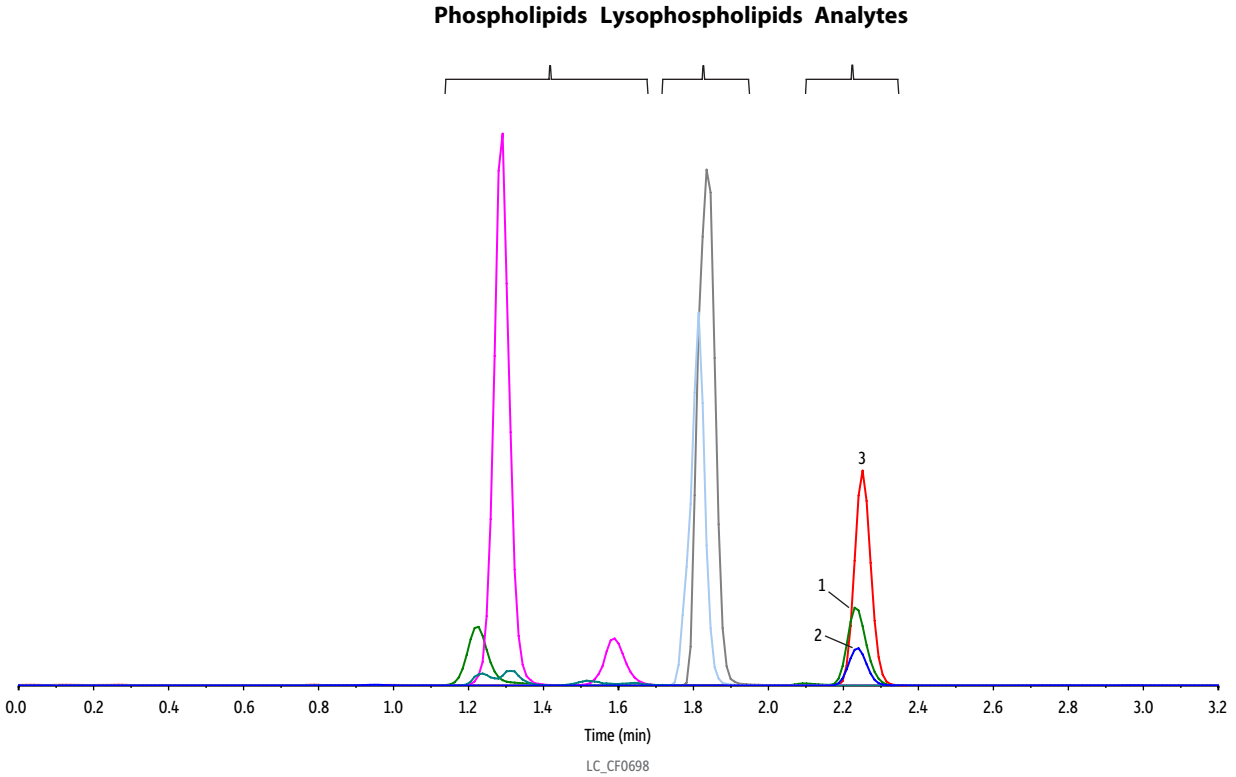
Folate Deficiency Biomarkers in Human Plasma on Raptor HILIC-Si by LC-MS/MS



Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion
1. 5-Formyl tetrahydrofolate	2.23	50	474.2	327.0
2. Folic acid	2.23	25	442.2	295.0
3. 5-Methyltetrahydrofolic acid	2.25	25	460.3	313.0

Column Raptor HILIC-Si (cat.# 9310A5E)
Dimensions: 50 mm x 3.0 mm ID
Particle Size: 2.7 µm
Temp.: 30 °C
Standard/Sample
Diluent: 20 mM Ammonium acetate in acetonitrile:water (80:20) containing 10 mg/mL 2-mercaptoethanol
Inj. Vol.: 5 µL
Mobile Phase
A: 50:50 Water:acetonitrile, 20 mM ammonium acetate
B: 20:80 Water:acetonitrile, 20 mM ammonium acetate

Time (min)	Flow (mL/min)	%B
0.00	0.5	100
3.00	0.5	0
3.20	0.5	0
3.21	0.5	100
5.21	0.5	100

Max Pressure: 344 bar
Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC

Sample Preparation
1. Aliquot 380 µL of human plasma (K2EDTA, 2x charcoal stripped) containing 100 µg/mL 2-mercaptoethanol and add 20 µL fortification solution.
2. Vortex for 2 min and centrifuge for 2 min at 4000 rpm.
3. Condition EVOLUTE EXPRESS WAX 30 mg SPE plate (Biotage 604-0030-PX01) with 1 mL methanol and then equilibrate with 1 mL 2% formic acid in water. Apply vacuum to dry the plate completely.
4. Load 400 µL of sample onto the plate and apply vacuum to initiate the flow.
5. Wash the plate with 1 mL water. Apply vacuum to dry the plate completely.
6. Elute samples with 300 µL 5% ammonium hydroxide in methanol containing 10 mg/mL 2-mercaptoethanol. Apply vacuum for elution.
7. Evaporate extracts to dryness under nitrogen at 30 °C.
8. Reconstitute in 200 µL mobile phase B containing 10 mg/mL 2-mercaptoethanol.

Notes
Note: The whole sample preparation process was performed under dim light.
Endogenous peaks for phospholipids and lysophospholipids are displayed because they are common matrix interferences and are known to suppress ionization efficiency.