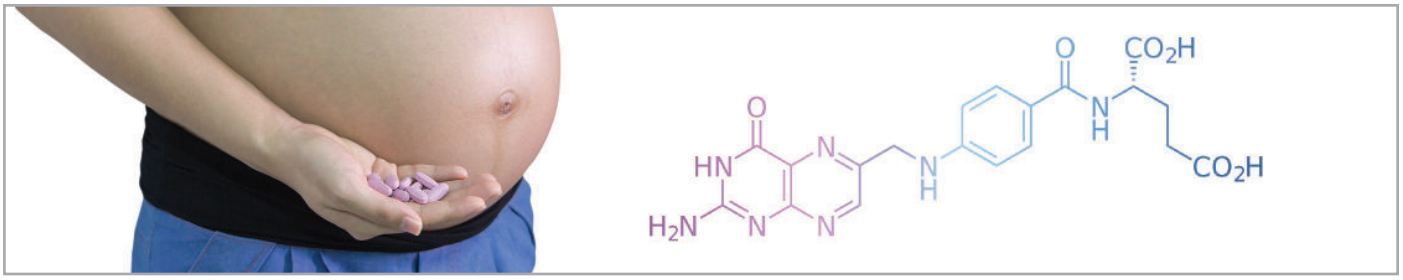


Featured Application: *Folate Deficiency Biomarkers in Plasma on Raptor HILIC-Si*

Fast, Accurate LC-MS/MS Method for Folate Deficiency Biomarkers in Plasma

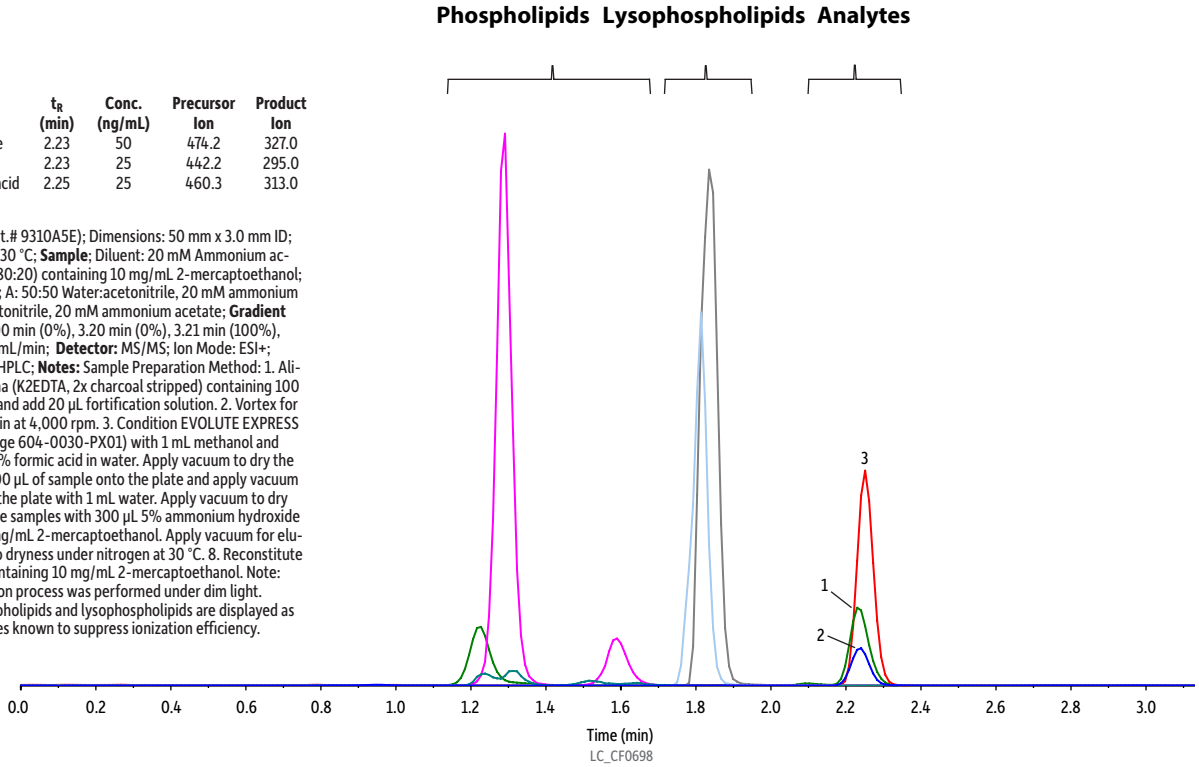
- Strong retention on a Raptor HILIC-Si column prevents matrix interference.
- Complete separation from phospholipids ensures accurate results in a quick, 5-minute analysis.
- Divert matrix to waste to keep your MS source clean and reduce downtime for maintenance.

Folate deficiency is considered a risk factor for a wide range of human health problems, including neural tube defects in newborns, cardiovascular diseases, Alzheimer's disease, and certain forms of cancer. Plasma levels of folic acid and its metabolites (5-formyl tetrahydrofolate and 5-methyltetrahydrofolic acid) are used as biomarkers to diagnose folate deficiency. However, LC-MS/MS methods for folate deficiency biomarkers in plasma can be very challenging because these small, polar compounds are not retained well on traditional reversed-phase LC columns. Retention can be improved using a HILIC method, but in this case, the column must provide strong enough retention to prevent coelution with the phospholipid components in the plasma sample matrix. Although a good sample preparation protocol will help, 100% removal of phospholipids is very difficult, and even low levels of phospholipids can interfere with target analytes, compromise quantitation, and contaminate the MS source.

Using a HILIC approach with a Raptor HILIC-Si column is a much better alternative for LC-MS/MS methods for folate deficiency biomarkers in plasma because you can quickly and completely separate the matrix interferences from the target analytes. The increased retention obtained on a Raptor HILIC-Si column ensures good separation of folate deficiency biomarkers from phospholipids and allows labs to accurately quantitate these important compounds, even at just 25–50 ng/mL, with no ion suppression from matrix interferences. In addition, more resolution between analytes and matrix components lets you divert matrix to waste, which keeps your MS cleaner longer. The LC-MS/MS method for folate deficiency biomarkers in plasma shown here allows folic acid, 5-formyl tetrahydrofolate, and 5-methyltetrahydrofolic acid to be accurately analyzed with no matrix interference in a fast, 5-minute analysis.

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; **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; **Gradient (%B):** 0.00 min (100%), 3.00 min (0%), 3.20 min (0%), 3.21 min (100%), 5.21 min (100%), **Flow:** 0.5 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM**; Instrument:** UHPLC; **Notes:** Sample Preparation Method: 1. Aliquot 380 µL of human plasma (K2EDTA, 2x charcoal stripped) containing 100 µg/mL 2-mercapto ethanol and add 20 µL fortification solution. 2. Vortex for 2 min and centrifuge for 2 min at 4,000 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. Note: The whole sample preparation process was performed under dim light. Endogenous peaks for phospholipids and lysophospholipids are displayed as common matrix interferences known to suppress ionization efficiency.



Raptor HILIC-Si LC Columns (USP L3)

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
2.7 µm Columns			
30 mm	9310A32		
50 mm	9310A52	9310A5E	9310A55
100 mm	9310A12	9310A1E	9310A15
150 mm	9310A62	9310A6E	9310A65

EXP Reusable Fittings for HPLC & UHPLC

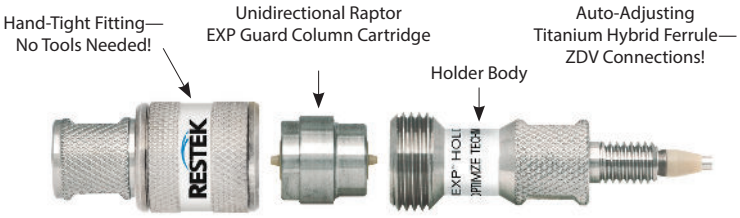
for 10-32 fittings and $^{1}/_{16}$" tubing

Effortlessly achieve 8700+ psi HPLC seals by hand! (Wrench tighten to 20,000+ psi.) Hybrid titanium/PEEK seal can be installed repeatedly without compromising your seal.

Description	qty.	cat.#
EXP Hand-Tight Fitting (Nut w/Ferrule)	ea.	25937
EXP Hand-Tight Fitting (Nut w/Ferrule)	10-pk.	25938
EXP Hand-Tight Nut (w/o Ferrule)	ea.	25939

Hybrid Ferrule U.S. Patent No. 8201854, Optimize Technologies. EXP Holders U.S. Patent No. 8696902, Optimize Technologies. EXP2 wrench U.S. Patent No. D766055, Optimize Technologies. Other U.S. and Foreign Patents Pending. The Opti- prefix is a registered trademark of Optimize Technologies, Inc.

Raptor EXP Guard Cartridges



Protect your investment and extend the life of our already-rugged LC columns and change guard column cartridges by hand without breaking fluid connections—no tools needed!

EXP Direct Connect Holder

Description	qty.	cat.#
EXP Direct Connect Holder for EXP Guard Cartridges (includes hex-head fitting & 2 ferrules)	3-pk.	25808

Maximum holder pressure: 20,000 psi (1400 bar)

Raptor EXP Guard Column Cartridges

Description	Particle Size	qty.	5 x 2.1 mm cat.#	5 x 3.0 mm cat.#	5 x 4.6 mm cat.#
Raptor HILIC-Si EXP Guard Column Cartridge	2.7 µm	3-pk.	9310A0252	9310A0253	9310A0250

Maximum cartridge pressure: 600 bar/8700 psi (2.7 µm) or 400 bar/5800 psi (5 µm).

Raptor SPP LC columns combine the speed of SPP with the resolution of USLC technology.
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