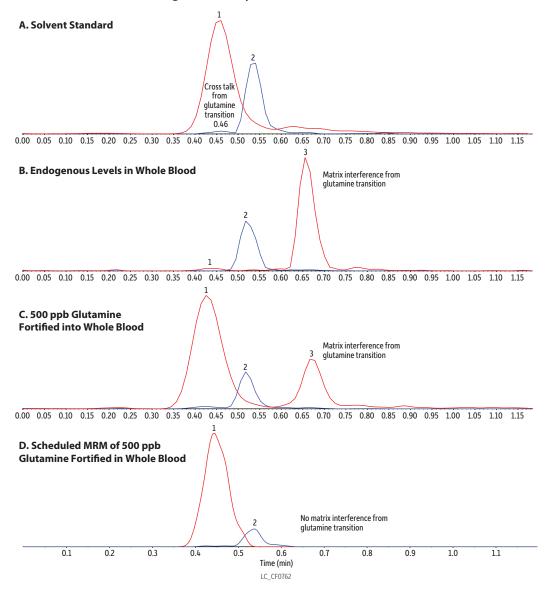
Specificity and Separation from Matrix Interference for Structural Isomer Amino Acids: Glutamine and Glutamic Acid in Dried Blood Spot on Raptor HILIC-Si EXP Guard Cartridge Column by LC-MS/MS

 Raptor HILIC-Si guard cartridge column can separate structural isomers glutamine and glutamic acid from each other and from matrix interferences, preventing false positives.



Peaks	tr (min)	Precursor Ion	Product Ion
1. Glutamine	0.45	147.1	84.1
2. Glutamic acid	0.55	148.1	83.9
3. Matrix interference	0.66	147.1	84.1

All analytes were present endogenously at varying concentrations in whole blood. Only XICs for glutamine and glutamic acid transitions are shown.

Column Dimensions: Particle Size: Temp.: Standard/Sample Diluent:	Raptor HILIC-Si EXP guard cartridge column (cat.# 9310A0252) 5 mm x 2.1 mm ID 2.7 µm 45 °C 85:15 Acetonitrile:water (v/v)			Sample Preparation	
Inj. Vol.: Mobile Phase A: B:	2.0 µL 30 mM Ammonium formate in water Acetonitrile				
	Time (min) 0.00 0.4 0.41 1.2	Flow (mL/min) 0.5 0.5 0.5 0.5	% A 15 70 15 15	%B 85 30 85 85	
Detector Ion Source: Ion Mode:	MS/MS Electrospray ESI+				

 $50~\mu L$ of whole blood was spotted on to Whatman 903 neonatal protein saver cards, which were then dried for 1 hour at room temperature. A 3.0 mm disk (~3.0 μL whole blood) was punched out of the dried spot and into a 2.0 mL Eppendorf tube. 200 μL of 85:15 acetonitrile:water (v/v) that was fortified with known concentrations of stable isotope-labeled internal standards was added, and then the sample was vortexed and incubated for 20 minutes at room temperature on a microplate shaker at a speed of 400 rpm. The sample was then centrifuged for 10 minutes at 4000 rpm, and 150 μL of the supernatant was filtered using a Thomson SINGLE StEP Nano filter vial (cat.# 25882) prior to LC-MS/MS analysis.

Figure Descriptions

A. LC-MS/MS analysis of a 100 ng/mL glutamine and glutamic acid standard prepared in 85:15 acetonitrile:water using a Raptor HILIC-Si guard cartridge column without scheduled MRM.

- B. LC-MS/MS analysis of a blank dried blood spot sample containing endogenous levels of glutamine and glutamic acid using a Raptor HILIC-Si guard cartridge column without scheduled MRM.
- C. LC-MS/MS analysis of a blank dried blood spot sample fortified with 500 ppb glutamine only (glutamine and glutamic acid are present endogenously) using a Raptor HILIC-Si guard cartridge column without scheduled MRM.
- D. LC-MS/MS analysis of a blank dried blood spot sample fortified with 500 ppb glutamine only (glutamine and glutamic acid present endogenously) using a Raptor HILIC-Si guard cartridge column with scheduled MRM.



Instrument

UHPLC