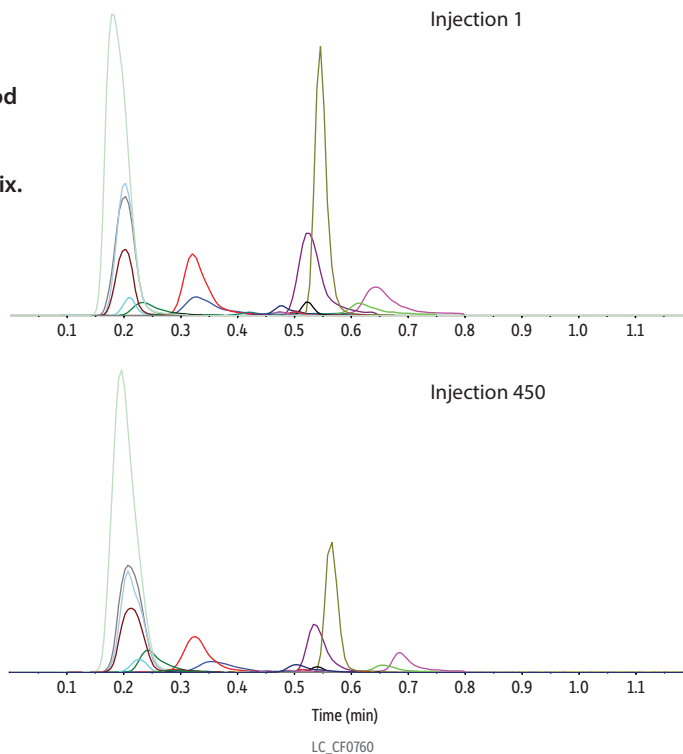


Stable Performance over 450 Injections of Dried Blood Spots on Raptor HILIC-Si EXP Guard Cartridge Column by LC-MS/MS

- Optimized method provides good chromatographic performance that is stable over 450 injections of dried blood spot sample matrix.



| Peaks | tr (min) | Precursor Ion | Product Ion |
|--|----------|---------------|-------------|
| 1. Phenylalanine | 0.20 | 166.0 | 120.1 |
| 2. Leucine | 0.21 | 132.1 | 86.0 |
| 3. Isoleucine | 0.21 | 132.1 | 86.1 |
| 4. Tyrosine | 0.22 | 182.1 | 91.0 |
| 5. Methionine | 0.24 | 150.1 | 56.1 |
| 6. Valine | 0.27 | 118.1 | 72.0 |
| 7. C20-Eicosanoyl-L-carnitine | 0.27 | 456.4 | 85.1 |
| 8. C18-Stearoyl-L-carnitine | 0.28 | 428.3 | 85.1 |
| 9. C18:1 Oleoyl-L-carnitine | 0.29 | 426.4 | 85.1 |
| 10. C18:2 Linoleoyl-L-carnitine | 0.30 | 424.3 | 85.1 |
| 11. C16-Palmitoyl-L-carnitine | 0.30 | 400.3 | 85.1 |
| 12. C16:1 Palmitoleyl-L-carnitine | 0.31 | 398.3 | 85.1 |
| 13. C14-Myristoyl-L-carnitine | 0.32 | 372.3 | 85.1 |
| 14. C14:1 Tetradecenoyl-L-carnitine | 0.33 | 370.3 | 85.1 |
| 15. C14:2-Tetradecadienoyl-L-carnitine | 0.33 | 368.3 | 85.1 |
| 16. Proline | 0.33 | 116.0 | 70.1 |
| 17. C12-Lauroyl-L-carnitine | 0.35 | 344.3 | 85.1 |
| 18. Alanine | 0.36 | 90.1 | 44.1 |
| 19. C10-Decanoyl-L-carnitine | 0.38 | 316.3 | 85.1 |
| 20. C8-Octanoyl-L-carnitine | 0.43 | 288.3 | 85.1 |
| 21. C7-Heptanoyl-L-carnitine | 0.45 | 274.2 | 85.1 |
| 22. C6-Hexanoyl-L-carnitine | 0.47 | 260.2 | 85.1 |
| 23. Glutamine | 0.45 | 147.1 | 84.1 |
| 24. C5-Valeryl-L-carnitine | 0.48 | 246.2 | 85.1 |
| 25. C5-Isovaleryl-L-carnitine | 0.49 | 246.1 | 85.1 |
| 26. 2-Methylbutyryl-L-carnitine | 0.49 | 246.2 | 85.1 |
| 27. C5:1-Tiglyl-L-carnitine | 0.50 | 244.2 | 85.1 |
| 28. C4-Butyryl-L-carnitine | 0.51 | 232.2 | 85.1 |
| 29. C4-Isobutyryl-L-carnitine | 0.51 | 232.1 | 85.1 |
| 30. Citrulline | 0.51 | 176.1 | 113.1 |
| 31. Glutamic acid | 0.55 | 148.1 | 83.9 |
| 32. C3-Propionyl-L-carnitine | 0.54 | 218.1 | 85.1 |
| 33. C2-Acetyl-L-carnitine | 0.56 | 204.1 | 85.1 |
| 34. Arginine | 0.66 | 175.2 | 70.1 |
| 35. Ornithine | 0.69 | 133.1 | 70.1 |

Column Raptor HILIC-Si EXP guard cartridge column (cat.# 9310A0252)
Dimensions: 5 mm x 2.1 mm ID
Particle Size: 2.7 µm
Temp.: 45 °C
Standard/Sample Diluent: 85:15 Acetonitrile:water (v/v)
Inj. Vol.: 2.0 µL
Mobile Phase
 A: 30 mM Ammonium formate in water
 B: Acetonitrile

| Time (min) | Flow (mL/min) | %A | %B |
|------------|---------------|----|----|
| 0.00 | 0.5 | 15 | 85 |
| 0.4 | 0.5 | 70 | 30 |
| 0.41 | 0.5 | 15 | 85 |
| 1.2 | 0.5 | 15 | 85 |

Detector MS/MS
Ion Source: Electrospray
Ion Mode: ESI+
Instrument UHPLC
Sample Preparation 50 µL 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.

All analytes were present endogenously at varying concentrations in whole blood. Internal standard peaks not shown.