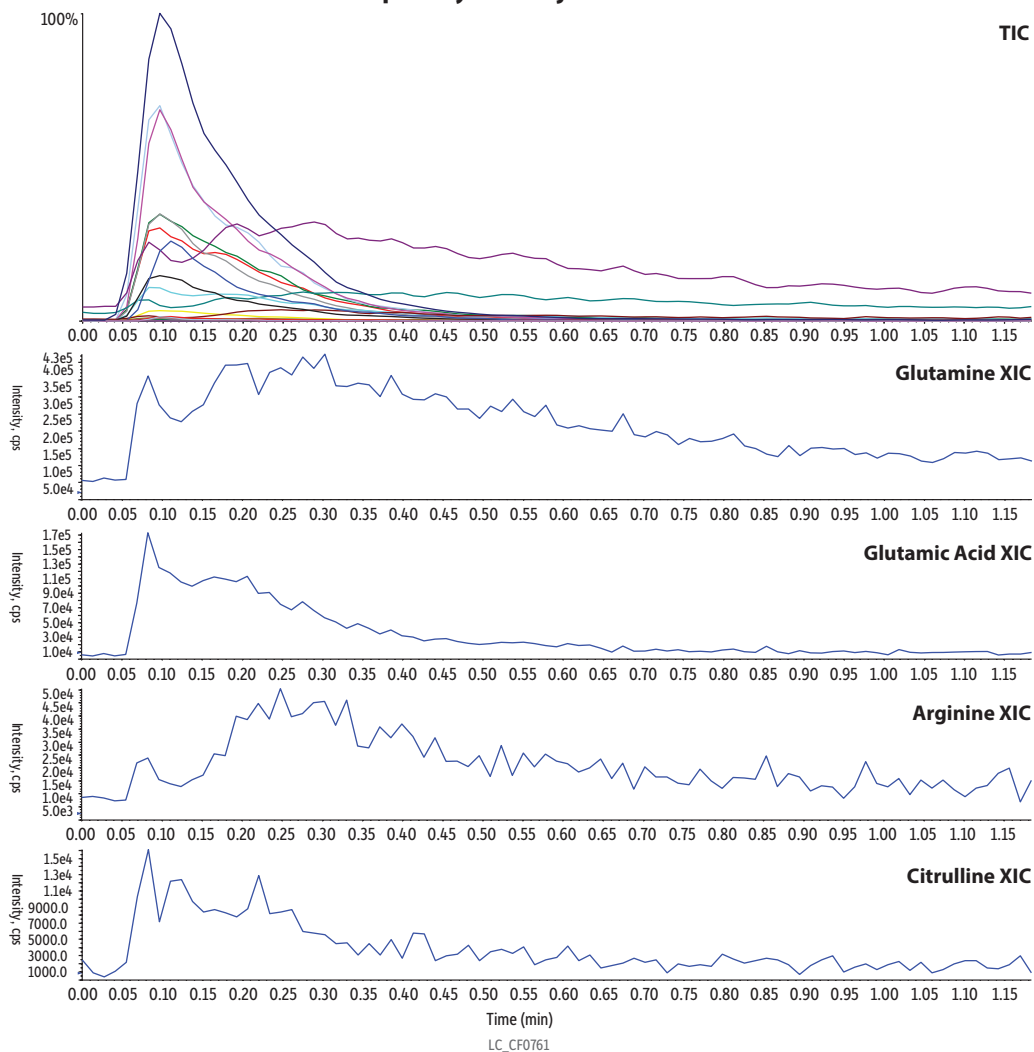


# Acylcarnitines and Amino Acids in Dried Blood Spots by Flow Injection-MS/MS



Peaks	rt (min)	Precursor Ion	Product Ion
1. Phenylalanine	0.1	166.0	120.1
2. Leucine	0.1	132.1	86.0
3. Isoleucine	0.1	132.1	86.1
4. Tyrosine	0.1	182.1	91.0
5. Methionine	0.1	150.1	56.1
6. Valine	0.1	118.1	72.0
7. C20-Eicosanoyl-L-carnitine	0.1	456.4	85.1
8. C18-Stearoyl-L-carnitine	0.1	428.3	85.1
9. C18:1 Oleoyl-L-carnitine	0.1	426.4	85.1
10. C18:2 Linoleoyl-L-carnitine	0.1	424.3	85.1
11. C16-Palmitoyl-L-carnitine	0.1	400.3	85.1
12. C16:1 Palmitoleyl-L-carnitine	0.1	398.3	85.1
13. C14-Myristoyl-L-carnitine	0.1	372.3	85.1
14. C14:1 Tetradecenoyl-L-carnitine	0.1	370.3	85.1
15. C14:2-Tetradecadienoyl-L-carnitine	0.1	368.3	85.1
16. Proline	0.1	116.0	70.1
17. C12-Lauroyl-L-carnitine	0.1	344.3	85.1
18. Alanine	0.1	90.1	44.1
19. C10-Decanoyl-L-carnitine	0.1	316.3	85.1
20. C8-Octanoyl-L-carnitine	0.1	288.3	85.1
21. C7-Heptanoyl-L-carnitine	0.1	274.2	85.1
22. C6-Hexanoyl-L-carnitine	0.1	260.2	85.1
23. Glutamine	0.1	147.1	84.1
24. C5-Valeryl-L-carnitine	0.1	246.2	85.1
25. C5-Isovaleryl-L-carnitine	0.1	246.1	85.1
26. 2-Methylbutyryl-L-carnitine	0.1	246.2	85.1
27. C5:1-Tiglyl-L-carnitine	0.1	244.2	85.1
28. C4-Butyryl-L-carnitine	0.1	232.2	85.1
29. C4-Isobutyryl-L-carnitine	0.1	232.1	85.1
30. Citrulline	0.1	176.1	113.1
31. Glutamic acid	0.1	148.1	83.9
32. C3-Propionyl-L-carnitine	0.1	218.1	85.1
33. C2-Acetyl-L-carnitine	0.1	204.1	85.1
34. Arginine	0.1	175.2	70.1
35. Ornithine	0.1	133.1	70.1

All the analytes were present endogenously at varying concentrations in whole blood. Analytes eluted at 0.1 minute at the solvent front because there is no stationary phase for chromatographic separation in flow injection MS/MS methods. Internal standard peaks not shown.

**Column**  
Temp.: 30 °C  
**Standard/Sample**  
Diluent: 85:15 Acetonitrile:water (v/v)  
Inj. Vol.: 2.0 µL  
**Mobile Phase**  
B: 0.1% Formic acid in methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.3	0	100
1.2	0.3	0	100

**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 flow injection-MS/MS analysis.