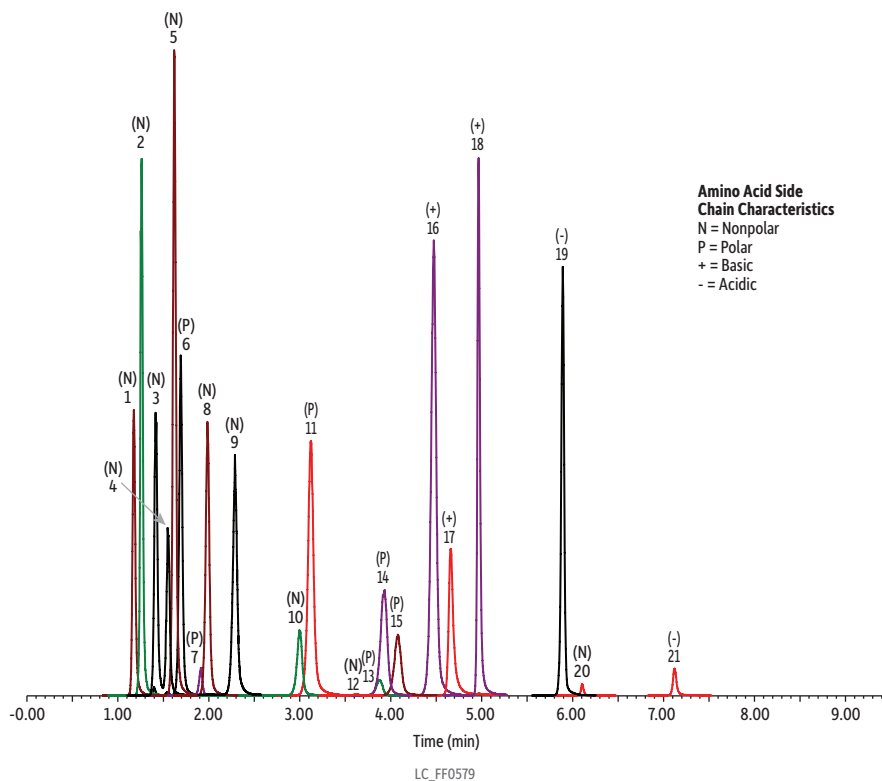


# Underivatized Amino Acids Analysis in Baby Formula on Raptor Polar X



Peaks	tr (min)	Precursor Ion	Product Ion
1. Tryptophan	1.17	205.07	146.08
2. Phenylalanine	1.26	166.13	120.10
3. Leucine	1.41	132.13	86.10
4. Isoleucine	1.55	132.13	86.10
5. Methionine	1.62	150.07	104.10
6. Tyrosine	1.69	182.10	136.08
7. Taurine	1.91	126.07	108.07
8. Valine	1.98	118.13	72.11
9. Proline	2.29	116.13	70.09
10. Alanine	3.00	90.03	44.10
11. Threonine	3.12	120.13	74.08
12. Glycine	3.62	76.10	30.11
13. Glutamine	3.87	147.13	84.07
14. Serine	3.93	106.07	60.09
15. Asparagine	4.08	133.13	74.07
16. Arginine	4.47	175.17	70.09
17. Histidine	4.66	156.07	110.16
18. Lysine	4.97	147.13	84.13
19. Glutamic acid	5.89	148.10	84.10
20. Cystine	6.10	241.13	152.00
21. Aspartic acid	7.12	134.07	74.06

**Column** Raptor Polar X (cat.# 9311A12)  
 Dimensions: 100 mm x 2.1 mm ID  
 Particle Size: 2.7 µm  
 Temp.: 30 °C

**Standard/Sample**  
 Diluent: 20:80 Water:acetonitrile, 0.01 N HCl  
 Conc.: Endogenous amino acids  
 Inj. Vol.: 5 µL

**Mobile Phase**  
 A: Water, 0.5% formic acid  
 B: Mobile Phase B: 9:1 Acetonitrile:20 mM ammonium formate in water (pH 3.0)  
 (The ammonium formate concentration is 20 mM relative to the total volume of mobile phase B. See preparation notes for instructions on diluting a 200 mM aqueous starting solution.)

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	12	88
3.50	0.5	12	88
8.00	0.5	70	30
8.01	0.5	12	88
10.0	0.5	12	88

**Detector** MS/MS  
 Ion Mode: ESI+  
 Mode: MRM  
**Instrument** UHPLC

**Sample Preparation**

A 200 µL aliquot of protein hydrolysate formula (Similac ALIMENTUM) was mixed with 800 µL of acetonitrile and 10 µL of 1 N HCl. After centrifugation at 4000 rpm for 5 minutes, the supernatant was diluted 20-fold with 20:80 water:acetonitrile (0.01 N HCl) and injected for analysis.

**Notes**

**Mobile Phase B Preparation:** To make 500 mL of mobile phase B, measure ~45 mL of water into a small beaker and add 1 mL of 10 M ammonium formate solution. Adjust pH to 3.0 by adding formic acid and then bring the volume to 50 mL with water. Combine this 50 mL ammonium formate solution (pH 3.0) with 450 mL of acetonitrile to complete the preparation.