Featured Application: *Underivatized Amino Acids on Raptor Polar X*

Fast, Direct Analysis of Underivatized Amino Acids in Infant Formula

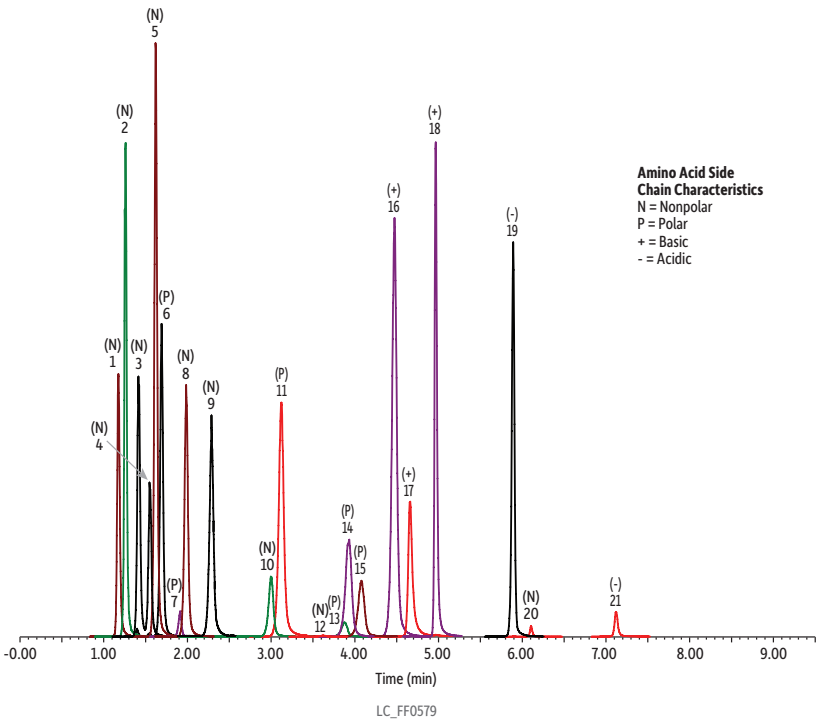
- Simple, one-step sample preparation for direct analysis of underivatized amino acids.
- Simultaneous analysis of nonpolar, polar, positively charged, and negatively charged amino acids in a short 10-minute run.
- Well-designed gradient separates contaminants from target analytes.

To ensure that nutritional requirements are met, reliable analytical methods are needed for the accurate determination of free amino acids in infant formulas. Precolumn derivatization followed by reversed-phase LC analysis is a typical approach, with derivatization being necessary due to the lack of chromatographic retention and poor sensitivity for free amino acids. However, these methods are often time-consuming and labor intensive. The use of perfluorinated acids as ion-pairing reagents to improve the retention of underivatized amino acids on a C18 column is another common technique, but this practice can negatively impact the chromatographic system and mass spectrometer.

In the simpler approach shown here, underivatized amino acids can be measured directly—following a single-step sample preparation procedure—using a Raptor Polar X column paired with an MS/MS detector. Raptor Polar X columns feature a hybrid phase chemistry (HILIC and ion exchange) that provides the balanced retention needed for simultaneous analysis of a wide range of analyte chemistries. As demonstrated, underivatized amino acids with nonpolar, polar, positively charged, and negatively charged side chains were adequately retained and then quickly eluted using a 10-minute gradient. This gradient also separated amino acid system contaminants from target analytes, adding ruggedness to the method. By following this method, time- and labor-intensive sample preparation procedures, which can include expensive derivatization kits, are replaced by a simple protein precipitation and direct analysis of the resulting extract. Direct analysis of underivatized amino acids on a Raptor Polar X column provides excellent results in a fast, easy workflow, making this a beneficial alternative to traditional methods.

Peaks	t_R (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; **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: 9:1 Acetonitrile:water, 20 mM ammonium formate, pH 3.0; **Gradient (%B):** 0.00 min (88%), 3.50 min (88%), 8.00 min (30%), 8.01 min (88%), 10.00 min (88%), **Flow:** 0.5 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument:** UHPLC; **Notes:** *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. *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.



Featured Products

Sample Handling	Analytical Column	Maintenance & Accessories
		
2.0 mL, 9 mm clear vials cat.# 21141 **2.0 mL, 9 mm amber vials** cat.# 21143 **Caps** cat.# 24497	**Raptor Polar X column** **100 mm x 2.1 mm, 2.7 µm** cat.# 9311A12	**Bluestem glass solvent filter** cat.# 26431

Related Products

Waste overflow indicators
4L bottles, cat. # 26543
or
GL-45 bottles, cat.# 26550

Survival kit for HPLC (PEEK)
cat. # 25322

ordering notes

Certificates of analysis for new Restek LC columns are now provided electronically. To view and download, visit www.restek.com/documentation then enter your cat.# and serial #.

Raptor Polar X LC Columns

NEW!

- Reliably analyze a wide variety of polar analytes (acidic, basic, and neutral) without time-consuming derivatization or complex ion pairing.
- Switch between HILIC and ion-exchange retention modes with simple mobile phase changes and short equilibration times.
- 2.7 µm Raptor core-shell particles provide UHPLC-like speed and efficiency on all makes and models of LC systems.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.

ID	Length	qty.	cat.#
2.7 µm Particles			
2.1 mm	30 mm	ea.	9311A32
	50 mm	ea.	9311A52
	100 mm	ea.	9311A12

2.0 mL, 9 mm Short-Cap, Screw-Thread Vials (vial only)

Fit all 2.0 mL, 12 x 32 mm, crimp-top vial-based autosamplers.

Description	Modification	Type	Volume	Color	Size	qty.	cat.#
Short-Cap Vial w/White Graduated Marking Spot	w/White Graduated Marking Spot	9-425 Screw-Thread	2.0 mL	Clear	12 x 32 mm	100-pk.	21140
	w/White Graduated Marking Spot	9-425 Screw-Thread	2.0 mL	Clear	12 x 32 mm	1000-pk.	21141
	w/White Graduated Marking Spot	9-425 Screw-Thread	2.0 mL	Amber	12 x 32 mm	100-pk.	21142
	w/White Graduated Marking Spot	9-425 Screw-Thread	2.0 mL	Amber	12 x 32 mm	1000-pk.	21143
Short-Cap Vial without Graduated Marking Spot	without Graduated Marking Spot	9-425 Screw-Thread	2.0 mL	Clear	12 x 32 mm	100-pk.	21154
	without Graduated Marking Spot	9-425 Screw-Thread	2.0 mL	Clear	12 x 32 mm	1000-pk.	21155

21140

Ideal for Agilent 7673, 7683, 7693 & other autosamplers that process 12 x 32 mm screw-thread vials.

2.0 mL, 9 mm Short-Cap, Screw-Vial Closures (Polypropylene, preassembled)

Description	Type	Cap Size	Color	Septa Material	qty.	cat.#
Short Screw Caps	Screw-Thread	9-425	Blue	PTFE/Silicone/PTFE	100-pk.	24497
	Screw-Thread	9-425	Blue	PTFE/Silicone/PTFE	1000-pk.	24498
	Screw-Thread	9-425	Black	PTFE/Silicone/PTFE	100-pk.	24495
	Screw-Thread	9-425	Black	PTFE/Silicone/PTFE	1000-pk.	24496
	Screw-Thread	9-425	Mixed	PTFE/Silicone/PTFE	500-pk.	24670

Choose preslit caps (available for some vials) to reduce the risk of needle bending, release vacuum from high-volume injections, and improve injection reproducibility when greater than 20% of vial volume is withdrawn.

Visit **www.restek.com/vials** for our full line of vials and caps.

24497

Bluestem Glass Solvent Filter

- Restek Bluestem glass solvent filter provides clean mobile phase to extend the life of columns and pump seals.
- 15 µm borosilicate glass frit sits lower than conventional glass filters to draw more mobile phase from each bottle.
- Connects to standard 1/8" OD (3.2 mm) PTFE tubing using your existing frit adaptor (also sold separately as cat.# 26392).

Description	qty.	cat.#
Frit Adaptor, PTFE	4-pk.	26392
Glass Solvent Filter, 15 µm frit	ea.	26431

26431

25322

Survival Kit for HPLC, PEEK

For start-up and maintenance in all HPLC systems.

The PEEK Survival Kit is an invaluable parts kit that contains tubing, fittings, and tools essential for setting up and maintaining your HPLC system: PEEK tubing, connectors, and elbows; PTFE tubing; a tubing cutter and extra blades; a ValvTool wrench; open-end wrenches; and more.

Description	qty.	cat.#
Survival Kit for HPLC	kit	25322

*Kit contains 1 wrench, replacement (cat.# 20110) is a 2-pk.

26543

Waste Overflow Indicator for LC Systems

- Avoid messy pooling around mobile phase waste containers.
- Audible alarm instantly alerts user, preventing overflow.
- Compact, battery-operated unit.

Description	Certification/Compliance	qty.	cat.#
Waste Overflow Indicator for LC Systems, 4 Liter	CE	ea.	26543
Waste Overflow Indicator for LC Systems, GL-45	CE	ea.	26550

Bottle not included.

RESTEK
Pure Chromatography

Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

www.restek.com

Lit. Cat.# FFFA3436A-UNV