



13-Minute, Comprehensive, Direct LC-MS/MS Analysis of Amino Acids in Plasma

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Abstract

A fast, direct method for LC-MS/MS analysis of amino acids in plasma was developed during the course of this study. Using a simple sample preparation procedure without derivatization and a hybrid retention mode Raptor Polar X column, 45 amino acids were simultaneously analyzed in a 13-minute run. Good chromatographic results, including the separation of critical isobaric compounds, were achieved and acceptable accuracy, precision, and linearity results were obtained.

Introduction

Amino acids are critical molecules for biological function as they are the fundamental building blocks of peptides and proteins and also serve as intermediates in various metabolic pathways, such as the urea cycle and the citric acid cycle. Amino acid profiling in human plasma is an important tool for the diagnosis of metabolic disorders, especially for inborn errors of metabolism. A fast and accurate large-panel analysis of amino acids in plasma is critical not only for disorder identification but also for continued monitoring and assessment of the nutritional status of patients and the effectiveness of treatment plans.

Traditionally, amino acids have been analyzed by either post-column or precolumn derivatization methodologies; however, these approaches have significant limitations. The major shortcoming of post-column derivatization methods is that chromatographic cycling times are typically quite long, which limits sample throughput capacity. Precolumn derivatization methods often have poor chromatographic separation, which can reduce the number of amino acids that can be included in comprehensive amino acid panels. Direct LC-MS/MS analysis of amino acids in plasma is an attractive alternative because no costly and time-consuming derivatization step is necessary, but, historically, there has been a relative lack of direct analysis applications due to poor chromatographic retention and insufficient detection sensitivity.

With the recent advancements in hydrophilic-interaction chromatography (HILIC) LC columns and modern MS instrumentation, it is now possible to analyze underivatized amino acids with simplicity and consistency. In this study, a straightforward workflow was developed and implemented for accurate quantification of a large panel of 45 amino acids in human plasma. A fast chromatographic analysis (13 minutes) was achieved using a Raptor Polar X column. This column was selected because the unique hybrid (HILIC and ion exchange) stationary phase has previously been demonstrated to adequately retain and effectively separate a wide range of amino acid chemistries without derivatization [1].

Experimental

Standard and Control Plasma Materials

Two standard mixture solutions (A6282 and A6407) containing a total of 28 amino acids and 17 solid standards were obtained from Sigma-Aldrich. A stock standard solution containing 45 amino acids was prepared in phosphate buffered saline (1x PBS) solution at 250-500 $\mu\text{mol/L}$.

Internal standards were used to compensate for plasma matrix effects. The internal standard (IS) working solution was prepared by mixing a stock solution of 17 isotopically labeled amino acids (MSK-A2-S, Cambridge Isotope Laboratories) with 12 individual deuterium isotopes of α -aminobutyric acid, β -aminoisobutyric acid, γ -aminobutyric acid, β -alanine, N-acetyltyrosine, asparagine, ethanolamine, glutamine, homocysteine, 1-methylhistidine, pipecolic acid, and taurine (CDN Isotopes) in water at 125-250 $\mu\text{mol/L}$.

MassChrom amino acid analysis plasma control level I (0471), II (0472), and III (0473) samples were obtained from Chromsystems.

Calibration Standard Preparation

For this LC-MS/MS analysis of amino acids in plasma, calibration standards were prepared across a range of 1-500 $\mu\text{mol/L}$ in 1x PBS solution. A 25 μL aliquot of calibration standard was mixed with 2.5 μL of 30% sulfosalicylic acid solution, 2 μL of internal standard working solution, and 225 μL of mobile phase B, acetonitrile:water (90:10), 0.5% formic acid and 1 mM ammonium formate, for injection and LC-MS/MS analysis. Exploratory studies demonstrated that the chromatographic retention of three analytes, β -aminoisobutyric acid, γ -aminobutyric acid, and β -alanine, is sensitive to the salt concentration in final injection solution. Therefore, the standards were prepared in PBS in order to match the salt condition in plasma samples and ensure consistent retention times for these three compounds between the standard and sample solutions.

Sample Preparation

Preliminary testing established that overall detection sensitivity was greatly improved by performing a protein precipitation step using 30% sulfosalicylic acid. Therefore, a 50 μL aliquot of control plasma was mixed with 5 μL of 30% sulfosalicylic acid solution. Following centrifugation at 4200 rpm for 10 minutes to pellet the precipitated material, a 27.5 μL aliquot of clear supernatant was mixed with 2 μL of internal standard working solution and 225 μL of mobile phase B prior to LC-MS/MS analysis.

Chromatographic Method

The chromatographic conditions used on a Waters ACQUITY UPLC coupled with a Xevo TQ-S mass spectrometer for this LC-MS/MS analysis of amino acids in plasma are detailed below. The ion transitions and internal standards used for each analyte are provided in Table I.

During method development experiments, several chromatographic parameters were determined to be critical for successful analysis. The column temperature of 35 $^{\circ}\text{C}$ was needed to have a stable, well-formed γ -aminobutyric acid peak. In addition, using a guard column and starting the analysis with 96%B mobile phase provided the most consistent peak shape for ethanolamine. Also, while all analytes elute by eight minutes, adding a one-minute wash with a highly aqueous mobile phase was found to be beneficial in reducing carryover for aspartic acid, argininosuccinic acid, anserine, carnosine, adenosylhomocysteine, homocystine, cystathionine, citrulline, 3-methylhistidine, 1-methylhistidine, and phosphoethanolamine. Finally, sensitivity issues were observed for glycine, and the shallow gradient used here gave better results than the steeper gradients that were also assessed.

Column:	Raptor Polar X (2.7 μm , 100 mm x 2.1 mm ID [cat.# 9311A12])
Guard column:	Raptor Polar X EXP guard column cartridge (2.7 μm , 5 mm x 2.1 mm [cat.# 9311A0252])
Column temp.:	35 $^{\circ}\text{C}$
Injection volume:	5 μL
Mobile phase A:	Water, 0.5% formic acid and 1 mM ammonium formate
Mobile phase B:	Acetonitrile:water (90:10), 0.5% formic acid and 1 mM ammonium formate

Time (min)	%B
0.00	96
2.00	96
10.00	30
10.01	5
11.00	5
11.01	96
13.00	96

Flow rate:	0.3 mL/min
Ion mode:	Positive ESI
Mode:	Scheduled MRM

Table I: Ion Transitions and Internal Standards for LC-MS/MS Analysis of Amino Acids in Plasma.

Peak Identification	Retention Time (min)	Precursor Ion	Product Ion	Internal Standard
Ethanolamine	1.06	62.0	44.1	Ethanolamine-d4
Acetyltyrosine	1.83	224.1	136.1	Acetyltyrosine-d3
γ -Aminobutyric acid	2.25	104.0	69.0	γ -Aminobutyric acid-d4
β -Aminoisobutyric acid	2.27	104.1	30.0	β -Aminoisobutyric acid-d3
β -Alanine	2.52	90.0	30.1	β -Alanine-d4
Tryptophan	3.31	205.1	146.1	β -Alanine-d4
Leucine	3.40	132.1	86.1	Leucine $^{13}\text{C}_6$ ^{15}N
Phenylalanine	3.53	166.1	120.1	Phenylalanine $^{13}\text{C}_9$ ^{15}N
Isoleucine	3.60	132.1	86.1	Isoleucine $^{13}\text{C}_6$ ^{15}N
Alloisoleucine	3.77	132.1	86.1	Methionine $^{13}\text{C}_5$ ^{15}N
Methionine	4.15	150.1	104.1	Methionine $^{13}\text{C}_5$ ^{15}N
Tyrosine	4.27	182.1	136.1	Tyrosine $^{13}\text{C}_9$ ^{15}N
Valine	4.27	118.1	72.1	Valine $^{13}\text{C}_5$ ^{15}N
Arginine	4.52	175.2	70.1	Arginine $^{13}\text{C}_6$ $^{15}\text{N}_4$
α -Aminobutyric acid	4.57	104.0	58.1	α -Aminobutyric acid-d2
Pipecolic acid	4.60	130.0	84.1	Pipecolic acid-d9
1-Methylhistidine	4.62	170.1	124.1	1-Methylhistidine-d3
Histidine	4.65	156.1	110.2	Histidine $^{13}\text{C}_6$ $^{15}\text{N}_3$
Taurine	4.65	126.1	108.1	Taurine-d4
Anserine	4.67	241.2	109.1	Histidine $^{13}\text{C}_6$ $^{15}\text{N}_3$
Carnosine	4.67	227.2	110.1	Histidine $^{13}\text{C}_6$ $^{15}\text{N}_3$
Lysine	4.68	147.1	84.1	Lysine $^{13}\text{C}_6$ $^{15}\text{N}_2$
3-Methylhistidine	4.68	170.0	126.0	Histidine $^{13}\text{C}_6$ $^{15}\text{N}_3$
Ornithine	4.72	133.1	70.0	Lysine $^{13}\text{C}_6$ $^{15}\text{N}_2$
Hydroxylysine	4.84	163.1	128.1	Threonine $^{13}\text{C}_4$ ^{15}N
Alanine	4.86	90.0	44.1	Alanine $^{13}\text{C}_3$ ^{15}N
Proline	4.89	116.1	70.1	Proline $^{13}\text{C}_5$ ^{15}N
Sarcosine	5.05	90.0	44.1	Threonine $^{13}\text{C}_4$ ^{15}N
Threonine	5.15	120.1	74.1	Threonine $^{13}\text{C}_4$ ^{15}N
Glycine	5.19	76.1	30.1	Threonine $^{13}\text{C}_4$ ^{15}N
Hydroxyproline	5.26	132.1	86.1	Threonine $^{13}\text{C}_4$ ^{15}N
Adenosylhomocysteine	5.30	385.0	133.7	Threonine $^{13}\text{C}_4$ ^{15}N
Glutamine	5.43	147.1	84.1	Glutamine-d5
Homocitrulline	5.43	190.1	84.1	Glutamine-d5
Serine	5.46	106.1	60.1	Serine $^{13}\text{C}_3$ ^{15}N
α -Aminoadipic acid	5.53	162.1	98.1	Asparagine-d3
Asparagine	5.54	133.1	74.1	Asparagine-d3
Citrulline	5.61	176.1	159.1	Asparagine-d3
Homocystine	6.20	269.1	136.0	Homocystine-d8
Glutamic acid	6.26	148.1	84.1	Glutamic Acid $^{13}\text{C}_5$ ^{15}N
Cystathionine	6.75	223.1	134.0	Glutamic Acid $^{13}\text{C}_5$ ^{15}N
Cystine	6.99	241.1	152.0	Cystine $^{13}\text{C}_6$ $^{15}\text{N}_2$
Phosphoethanolamine	7.32	142.0	44.1	Cystine $^{13}\text{C}_6$ $^{15}\text{N}_2$
Argininosuccinic acid	7.41	291.1	70.1	Cystine $^{13}\text{C}_6$ $^{15}\text{N}_2$
Aspartic acid	8.00	134.1	74.1	Aspartic Acid $^{13}\text{C}_4$ ^{15}N

Results and Discussion

Chromatographic Performance

A fast, comprehensive method was established for the direct LC-MS/MS analysis of amino acids in plasma. In total, 45 amino acids were simultaneously analyzed on a Raptor Polar X column with a 13-minute total cycle time (Figure 1). As shown in Figure 2, the selectivity of the Raptor Polar X column provided proper chromatographic separation of the isobaric compounds leucine/isoleucine/alloisoleucine and alanine/sarcosine, which is essential for accurate identification and quantification because these compounds cannot be distinguished by MS alone.

Alloisoleucine is of particular concern because it is an important biomarker for diagnosing maple syrup urine disease, so chromatographic separation from its isobars (leucine and isoleucine) is clinically essential. During method development, the balance of ammonium formate and formic acid concentration in the mobile phase proved to be critical for this separation. Using the mobile phases that were established during method development and starting the run with a two-minute isocratic period, as presented here, proved to be essential for the separation of alloisoleucine, isoleucine, and leucine. In addition, it was observed that isomer resolution can vary among LC instruments, so additional gradient changes and/or adjustments for differences in instrumental dead volume may be necessary when transferring the method between instruments.

Linearity

Various calibration ranges were determined individually for each amino acid based on differences in MS detection sensitivity and diagnostic suitability for all 45 analytes (Table II). All compounds showed acceptable linearity with r^2 value >0.990 and deviations $<20\%$ using quadratic regression (1/x weighted) calibration curves. While the highest quantifiable concentration of $500 \mu\text{mol/L}$ is suitable for most of the analytes, it is necessary to dilute the plasma sample for the analysis of glutamine and alanine into this range due to their relatively higher physiological concentration of $>500 \mu\text{mol/L}$. In addition, due to poor ionization, the LOQ of glycine is much higher than the other amino acid compounds, and the detection sensitivity could vary on different MS systems. Nevertheless, as the physiological concentration of glycine is relatively higher, this LC-MS/MS method is still suitable for glycine quantification in plasma.

Accuracy and Precision

Three control plasma samples containing low to high concentrations of all 45 analytes were used to evaluate method accuracy and precision. The acceptance criteria for method accuracy is that the measured concentrations fall within the labeled concentration ranges for each analyte. A total of three batches were analyzed on different days. Table II shows the accuracy and precision results averaged across all three batches ($n=9$). Method accuracy criteria were met, as demonstrated by the concentration values falling within the nominal ranges for all 45 analytes. In addition, %RSD values were $<20\%$, which indicates that method precision was also acceptable.

Figure 1: Control Plasma Level 2

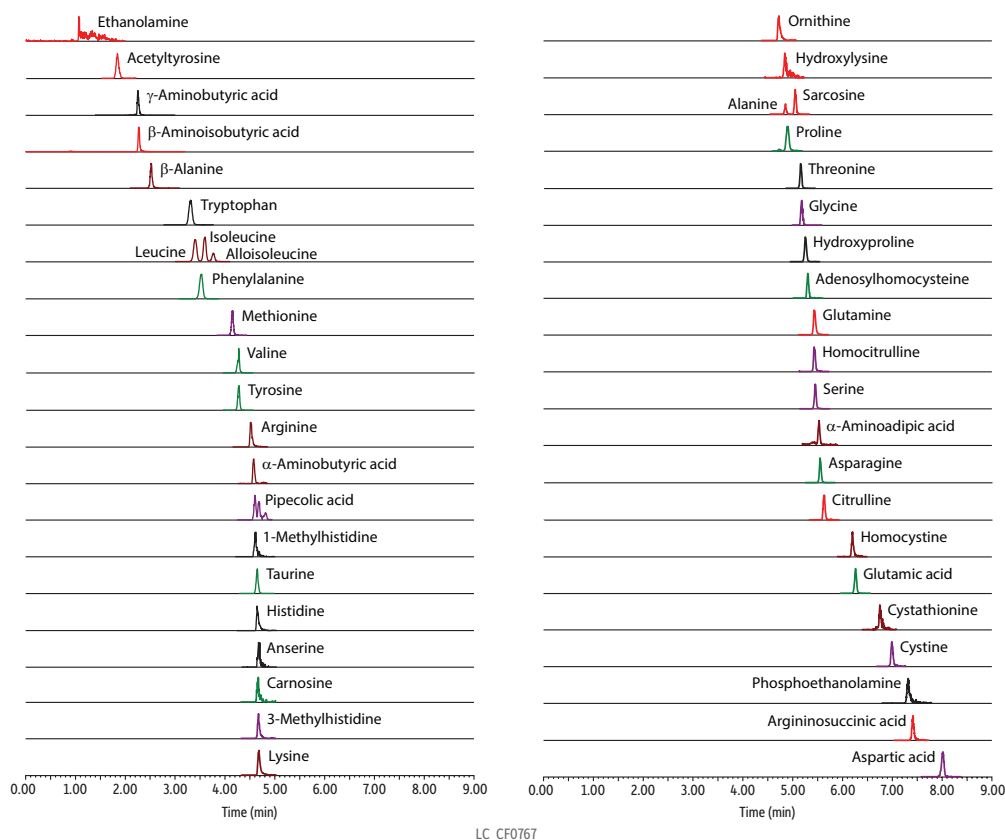


Figure 2: Control Plasma Level 1–Chromatographic Separation of Critical Isobaric Groups (Leucine/Isoleucine/Alloisoleucine and Alanine/Sarcosine).

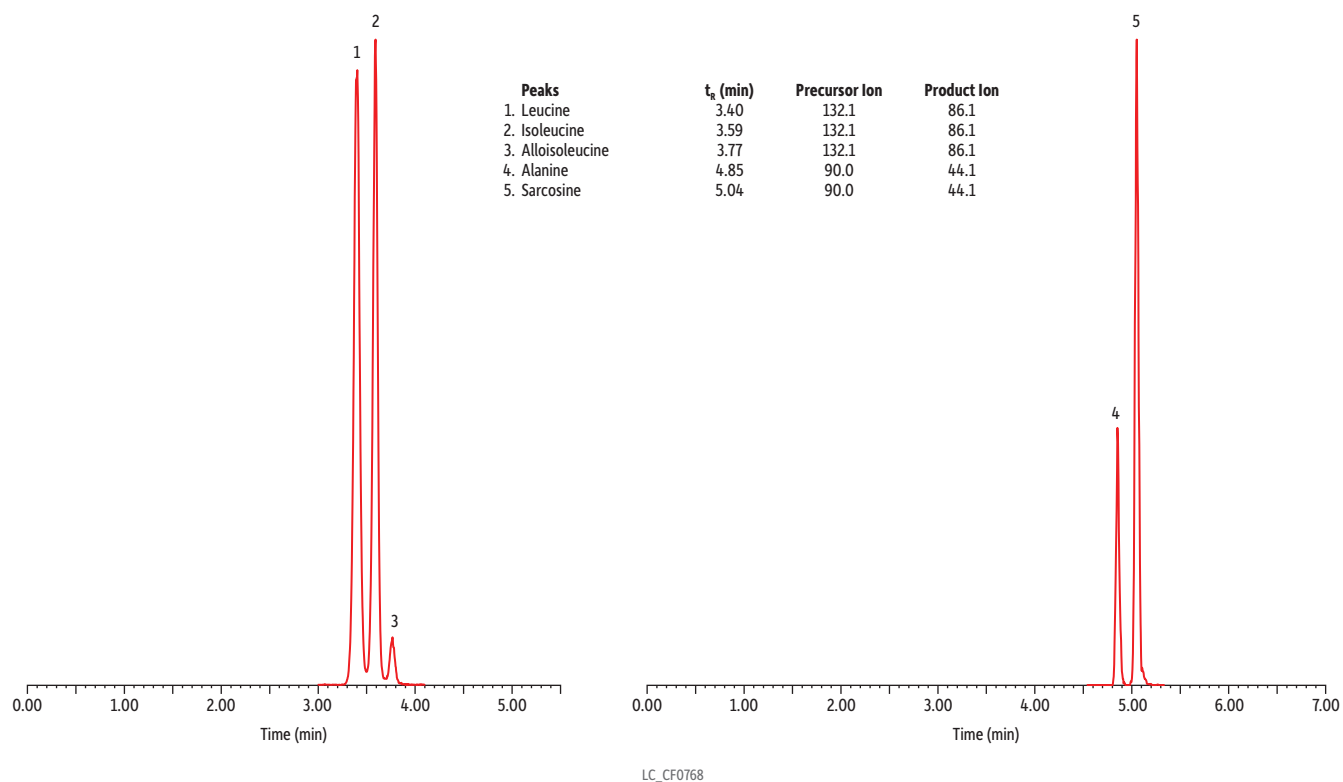


Table II: Accuracy and Precision Results for LC-MS/MS Analysis of Amino Acids in Plasma.

Analyte	Linearity Range (μmol/L)	Control Level 1		Control Level 2		Control Level 3	
		Nominal Conc. Range (μmol/L)	Average Conc. (μmol/L) (%RSD)	Nominal Conc. Range (μmol/L)	Average Conc. (μmol/L) (%RSD)	Nominal Conc. Range (μmol/L)	Average Conc. (μmol/L) (%RSD)
Acetyltyrosine	0.5 - 250	4.10 - 6.15	4.84 (9.76)	32.5 - 48.8	40.5 (9.76)	58.5 - 87.7	73.9 (15.1)
Adenosylhomocysteine	2.5 - 250	2.77 - 4.16	3.16 (15.0)	17.8 - 26.7	20.4 (15.4)	32.5 - 48.8	42.9 (9.98)
Alanine	5 - 500	159 - 239	191 (15.0)	524 - 786	655* (20.0)	890 - 1334	979* (19.4)
β-Alanine	1 - 500	10.4 - 15.5	10.8 (3.13)	42.7 - 64.0	47.9 (3.12)	74.0 - 111	81.1 (3.01)
α-Aminoadipic acid	1 - 500	4.98 - 7.46	5.74 (7.53)	9.92 - 14.9	11.2 (6.75)	14.7 - 22.1	17.9 (11.7)
α-Aminobutyric acid	1 - 500	3.69 - 5.54	4.47 (11.4)	39.5 - 59.2	52.1 (10.9)	73.6 - 110	97.7 (7.43)
β-Aminoisobutyric acid	1 - 500	3.82 - 5.73	4.40 (9.85)	17.0 - 25.5	19.2 (6.10)	29.7 - 44.5	35.3 (7.75)
γ-Aminobutyric acid	1 - 500	3.79 - 5.69	4.75 (5.35)	7.98 - 12.0	9.60 (3.45)	12.0 - 17.9	14.1 (5.60)
Anserine	1 - 500	3.85 - 5.77	4.71 (12.5)	7.65 - 11.5	10.8 (5.82)	11.5 - 17.3	15.9 (7.71)
Arginine	1 - 500	8.63 - 12.9	9.55 (11.5)	124 - 186	133 (3.60)	237 - 356	257 (3.32)
Argininosuccinic acid	0.5 - 250	2.35 - 3.53	2.96 (13.3)	59.7 - 89.6	72.3 (11.1)	115 - 172	141 (7.99)
Asparagine	1 - 500	21.4 - 32.1	31.3 (2.46)	97.0 - 145	143 (1.39)	173 - 259	250 (2.22)
Aspartic acid	1 - 500	18.5 - 27.7	19.2 (1.83)	101 - 152	105 (3.54)	182 - 274	189 (2.89)
Carnosine	1 - 500	7.69 - 11.5	10.3 (9.66)	18.9 - 28.3	23.7 (10.1)	29.2 - 43.8	36.6 (10.2)
Citrulline	5 - 500	12.7 - 19.1	15.6 (13.9)	83.8 - 126	88.8 (4.17)	153 - 230	161 (1.63)
Cystathionine	1 - 500	4.86 - 7.29	5.76 (6.47)	20.1 - 30.1	23.1 (8.07)	35.3 - 52.9	40.7 (9.63)
Cystine	0.5 - 250	8.54 - 12.8	10.3 (9.50)	62.7 - 94.0	78.8 (6.33)	116 - 174	144 (9.05)
Ethanolamine	5 - 250	12.3 - 18.4	15.2 (11.1)	98.5 - 148	125 (11.3)	182 - 273	206 (3.53)
Glutamic acid	1 - 500	45.8 - 68.7	53.7 (6.16)	304 - 456	335 (8.12)	553 - 829	593* (8.49)
Glutamine	1 - 500	286 - 429	371 (3.58)	748 - 1122	975* (5.46)	1209 - 1814	1587* (8.40)
Glycine	25 - 500	154 - 231	177 (10.2)	517 - 775	613* (7.54)	864 - 1295	995* (10.9)
Histidine	1 - 500	35.9 - 53.9	47.6 (5.27)	121 - 182	157 (7.27)	210 - 316	266 (4.35)
Homocitrulline	2.5 - 250	7.88 - 11.8	9.87 (2.05)	22.6 - 33.9	31.3 (3.83)	37.1 - 55.6	53.7 (2.69)
Homocystine	1 - 500	3.29 - 4.93	3.60 (9.17)	10.5 - 15.8	12.8 (5.69)	18.0 - 27.0	22.0 (4.71)
Hydroxylysine	5 - 500	3.97 - 5.96	5.50 (7.60)	14.3 - 21.4	16.58 (11.2)	24.3 - 36.5	28.4 (14.2)
Hydroxyproline	5 - 500	7.94 - 11.9	10.1 (14.2)	72.8 - 109	97.52 (8.40)	136 - 204	198 (5.02)
Allo-isoleucine	0.5 - 250	2.86 - 4.29	3.55 (3.12)	65.5 - 98.2	79.15 (4.56)	128 - 192	150 (5.87)
Isoleucine	5 - 500	18.4 - 27.6	20.1 (3.12)	143 - 215	164.41 (3.01)	260 - 389	282 (2.69)
Leucine	5 - 500	40.2 - 60.3	43.3 (2.57)	239 - 358	288.87 (4.37)	444 - 666	512* (4.67)
Lysine	1 - 500	21.9 - 32.9	28.2 (5.79)	231 - 346	315.89 (4.13)	436 - 655	621* (4.61)
Methionine	1 - 500	6.80 - 10.2	7.46 (5.29)	64.8 - 97.1	72.02 (3.83)	121 - 181	139 (2.32)
1-Methylhistidine	1 - 500	2.06 - 3.08	2.52 (8.18)	6.17 - 9.25	7.12 (9.84)	10.3 - 15.4	12.4 (9.84)
3-Methylhistidine	5 - 500	5.82 - 8.73	8.03 (8.31)	36.2 - 54.3	43.81 (10.2)	66.3 - 99.5	85.1 (4.10)
Ornithine	5 - 500	13.6 - 20.4	15.2 (3.13)	161 - 242	168.76 (4.35)	301 - 452	322 (5.76)
Phenylalanine	1 - 500	32.2 - 48.3	36.5 (3.38)	248 - 373	297.96 (1.80)	461 - 692	547* (4.31)
Phosphoethanolamine	5 - 500	8.53 - 12.8	9.64 (12.2)	57.4 - 86.1	64.32 (9.09)	105 - 158	127 (9.93)
Pipecolic acid	0.5 - 250	1.62 - 2.43	1.85 (9.98)	13.4 - 20.0	17.25 (8.08)	24.6 - 36.9	31.0 (7.45)
Proline	1 - 500	50.7 - 76.1	58.6 (4.05)	268 - 403	322.45 (3.46)	482 - 722	578* (4.99)
Sarcosine	1 - 500	3.29 - 4.94	4.00 (7.35)	13.5 - 20.3	19.59 (6.51)	23.7 - 35.5	34.8 (4.19)
Serine	5 - 500	102 - 153	126 (3.65)	295 - 443	383.86 (6.51)	484 - 726	627* (5.89)
Taurine	5 - 500	14.7 - 22.1	18.2 (6.28)	173 - 260	234.74 (3.51)	325 - 488	433 (6.26)
Threonine	1 - 500	36.7 - 55.1	42.6 (2.76)	203 - 304	246.86 (7.01)	366 - 549	464 (4.16)
Tryptophan	1 - 500	13.5 - 20.2	16.9 (11.5)	88.4 - 133	107.52 (7.72)	158 - 237	188 (10.3)
Tyrosine	1 - 500	22.3 - 33.5	25.4 (5.35)	129 - 194	148.82 (2.58)	232 - 348	264 (3.40)
Valine	1 - 500	74.4 - 112	91.3 (6.73)	256 - 384	318.69 (7.67)	442 - 664	532* (9.56)

*Concentration is outside of the calibration range.

Conclusion

This comprehensive method for the direct LC-MS/MS analysis of amino acids in plasma demonstrated that 45 amino acids could be simultaneously measured using a simple sample preparation procedure and a Raptor Polar X column. Acceptable accuracy, precision, and linearity results were obtained without derivatization in a fast, 13-minute run, making this approach suitable for high-throughput analysis of amino acids.

References

[1] Restek Corporation, Fast, direct analysis of underivatized amino acids in infant formula. 2021, <https://www.restek.com/en/technical-literature-library/articles/fast-direct-analysis-of-underivatized-amino-acids-in-infant-formula/> (accessed July 23, 2021).

This method has been developed for research use only; it is not suitable for use in diagnostic procedures without further evaluation.

Raptor Polar X LC Columns

- 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.

Storage Conditions: When not in use, Raptor Polar X columns must be kept in 100% acetonitrile. If using a buffered mobile phase, first flush thoroughly with 50:50 water:acetonitrile, then fill with acetonitrile for storage.

Analyzing polar compounds using liquid chromatography has historically been a challenge due to poor retention, long equilibration times, low sensitivity, and the need to mitigate these problems with time-consuming sample derivatization or complex ion-pairing approaches. However, with the development of the Raptor Polar X column—a novel column that is specifically designed for the analysis of a broad range of polar compounds—scientists can avoid these problems. These new columns feature a unique phase chemistry that combines both HILIC and ion-exchange retention mechanisms on a single ligand. Because this ligand is bound to superficially porous particles, Raptor Polar X columns both reliably retain and efficiently separate a wide variety of polar analytes with simple mobile phase changes. By analyzing polar compounds on a column that provides purpose-built resolving power, you can avoid complex sample preparation procedures, save time and money, and reduce opportunities for error. Simplify the analysis of polar compounds with the resolving power of Raptor Polar X columns from Restek.



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 #.

Pore Size: 90 Å
Particle: 2.7 μm superficially porous particle (SPP) silica
Surface Area: 130 m^2/g
End-Cap: Proprietary
Carbon Load: Proprietary
USP Phase Code: NA
Phase Category: Proprietary
Ligand Type: Proprietary
Recommended Usage:
pH Range: 2-8
Maximum Temperature: 60 °C
Maximum Pressure: 600 bar/8700 psi

Properties:

- Excellent resolution and separation of a wide variety of polar compounds.
- Combines HILIC and ion-exchange retention mechanisms together in a single ligand.
- Broadly applicable for polar compound analysis spanning different industries and methods.

Switch to a Raptor Polar X when:

- You are analyzing neutral, acidic, basic, or permanently charged polar compounds.
- Performing LC-MS/MS analysis of polar compounds.
- You are struggling to retain or elute polar compounds and considering ion chromatography.

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

Raptor EXP Guard Column Cartridges

- Free-Turn architecture lets you change cartridges by hand without breaking inlet/outlet fluid connections—no tools needed.
- Patented titanium hybrid ferrules can be installed repeatedly without compromising high-pressure seal.
- Auto-adjusting design provides ZDV (zero dead volume) connection to any 10-32 female port.
- Guard column cartridges require EXP direct connect holder (cat.# 25808).
- Pair with EXP hand-tight fitting (cat.# 25937–25938) for tool-free installation.

Great with any Raptor column to get ultimate protection from particulates and matrix contamination, especially when using dilute-and-shoot or other minimal sample preparation techniques.

Raptor SPP LC columns combine the speed of SPP with the resolution of USLC technology. Learn more at www.restek.com/raptor

To help protect your investment and further extend the life of our already-rugged LC columns, Restek offers the patent-pending guard column hardware developed by Optimize Technologies. A Restek LC guard cartridge in an EXP direct connect holder is the ultimate in column protection, especially when using dilute-and-shoot or other minimal sample preparation techniques.

Description	Particle Size	Size	qty.	cat.#
Raptor C18 EXP Guard Column Cartridge	UHPLC	5 x 2.1 mm	3-pk.	9304U0252
	UHPLC	5 x 3.0 mm	3-pk.	9304U0253
	2.7 µm	5 x 2.1 mm	3-pk.	9304A0252
	2.7 µm	5 x 3.0 mm	3-pk.	9304A0253
	2.7 µm	5 x 4.6 mm	3-pk.	9304A0250
	5 µm	5 x 2.1 mm	3-pk.	930450252
	5 µm	5 x 3.0 mm	3-pk.	930450253
	5 µm	5 x 4.6 mm	3-pk.	930450250
Raptor ARC-18 EXP Guard Column Cartridge	UHPLC	5 x 2.1 mm	3-pk.	9314U0252
	UHPLC	5 x 3.0 mm	3-pk.	9314U0253
	2.7 µm	5 x 2.1 mm	3-pk.	9314A0252
	2.7 µm	5 x 3.0 mm	3-pk.	9314A0253
	2.7 µm	5 x 4.6 mm	3-pk.	9314A0250
	5 µm	5 x 2.1 mm	3-pk.	931450252
	5 µm	5 x 3.0 mm	3-pk.	931450253
	5 µm	5 x 4.6 mm	3-pk.	931450250
Raptor Biphenyl EXP Guard Column Cartridge	UHPLC	5 x 2.1 mm	3-pk.	9309U0252
	UHPLC	5 x 3.0 mm	3-pk.	9309U0253
	2.7 µm	5 x 2.1 mm	3-pk.	9309A0252
	2.7 µm	5 x 3.0 mm	3-pk.	9309A0253
	2.7 µm	5 x 4.6 mm	3-pk.	9309A0250
	5 µm	5 x 2.1 mm	3-pk.	930950252
	5 µm	5 x 3.0 mm	3-pk.	930950253
	5 µm	5 x 4.6 mm	3-pk.	930950250
Raptor FluoroPhenyl EXP Guard Column Cartridge	UHPLC	5 x 2.1 mm	3-pk.	9319U0252
	UHPLC	5 x 3.0 mm	3-pk.	9319U0253
Raptor FluoroPhenyl EXP Guard Column Cartridges	2.7 µm	5 x 2.1 mm	3-pk.	9319A0252
	2.7 µm	5 x 3.0 mm	3-pk.	9319A0253
	2.7 µm	5 x 4.6 mm	3-pk.	9319A0250
	5 µm	5 x 2.1 mm	3-pk.	931950252
	5 µm	5 x 3.0 mm	3-pk.	931950253
	5 µm	5 x 4.6 mm	3-pk.	931950250
Raptor HILIC-Si EXP Guard Column Cartridge	2.7 µm	5 x 2.1 mm	3-pk.	9310A0252
	2.7 µm	5 x 3.0 mm	3-pk.	9310A0253
	2.7 µm	5 x 4.6 mm	3-pk.	9310A0250
Raptor Polar X EXP Guard Column Cartridge	2.7 µm	5 x 2.1 mm	3-pk.	9311A0252 NEW!

Maximum cartridge pressure: 1034 bar/15000 psi* (UHPLC), 600 bar/8700 psi (2.7 µm); 400 bar/5800 psi (5 µm)

* For maximum lifetime, recommended maximum pressure for UHPLC particles is 830 bar/12000 psi.

Intellectual Property: optimizetech.com/patents

Raptor
LC Columns



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 #.