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Robust and Reproducible LC–MS/MS Amino Acids Analysis with an Amide-Based HILIC Column

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Introduction

The accurate and reproducible analysis of amino acids is essential across a wide range of applications including biopharmaceutical development, food and nutritional sciences, and metabolomics. Due to their high polarity and zwitterionic nature, amino acids present well-known challenges for reversed phase liquid chromatography, often requiring derivatization or specialized detection strategies. As analytical demands continue to increase for sensitivity, robustness, and workflow efficiency, alternative chromatographic approaches have gained significant attention.

Amide-based HPLC columns operated under hydrophilic interaction liquid chromatography (HILIC) conditions provide an effective solution for the direct separation of underivatized amino acids. These stationary phases offer strong retention of polar analytes through a combination of hydrophilic partitioning and hydrogen-bonding interactions, enabling improved selectivity and resolution across structurally similar amino acids. When paired with high-organic mobile phases, amide columns also deliver enhanced compatibility with mass spectrometric detection, supporting sensitive and reproducible quantitative analysis. This application note describes the applicability of an amide-based stationary phase for the separation of 22 amino acids (**Table 1**) highlighting the key method considerations such as mobile phase composition, buffer selection and pH control.

UHPLC-MS Conditions

Column: Kinetex Amide
Dimensions: 100 x 2.1mm
Part No.: 00D-4812-AN
Mobile Phase: A: H_2O +0,1 % FA
B: ACN +0,1 % FA

Gradient:

Time (min)	% B
0	95
0,5	95
6	30
8	30
8,1	95
10	95

Flow Rate: 0.40mL/min
Injection Volume: 2µL
Temperature: 40°C
Detection: Shimadzu 8060 NX (MRM)

MS Conditions

Ion Source:	ESI (+)
Heating Gas Flow (L/min)	13
Nebulizing Gas Flow (L/min)	2
Drying Gas Flow (L/min)	10
Capillary spray voltage (kV)	3,0
DL temperature (°C)	250
Heat Block temperature (°C)	400

Table 1. 22 Amino Acids dissolved in H_2O:ACN (20:80) +0.1% Formic Acid

Analyte	Q1 $[M+H]^+$	Q3	Dwell Time (ms)	CE
Alanine	90,1	44,0	21	16
Arginine	175,1	70,1	5	27
		60,1		19
		116,0		14
Aspartic Acid	134,0	74,0	5	19
		88,0		15
		116,0		11
Glutamic Acid	148,0	102,0	9	15
		84,0		25
Glycine	76,1	76,1	9	2
		30,0		19
Histidine	156,1	110,0	9	19
		83,0		34
Isoleucine	132,1	86,0	5	15
		56,0		51
		69,0		23
Leucine	132,1	86,0	5	15
		44,0		31
		43,0		35
Lysine	147,1	130,0	5	13
		84,0		30
Methionine	150,0	133,0	3	13
		104,0		15
		56,0		22
Phenylalanine	166,0	120,0	5	19
		103,0		35
		77,0		51
Proline	116,1	70,0	5	23
		43,0		41
Serine	106,0	60,0	5	15
		42,0		30
Threonine	120,0	103,2	9	25
Tyrosine	182,1	165,0	3	12
		136,0		17
		91,0		38
Valine	118,1	72,0	5	16
		57,0		38
		55,0		27
Cystine	241,1	152,1	5	19
		74,0		20
Tryptophan	205,1	188,0	3	13
		146,0		23
		118,0		36
Serotonin	177,1	132,0	3	30
		115,0		40
Cysteine	122,0	77,0	5	31
		76,0		19
Glutamine	147,1	130,0	9	21
		84,0		24
Asparagine	133,0	74,0	5	21
		87,0		15
		116,0		13

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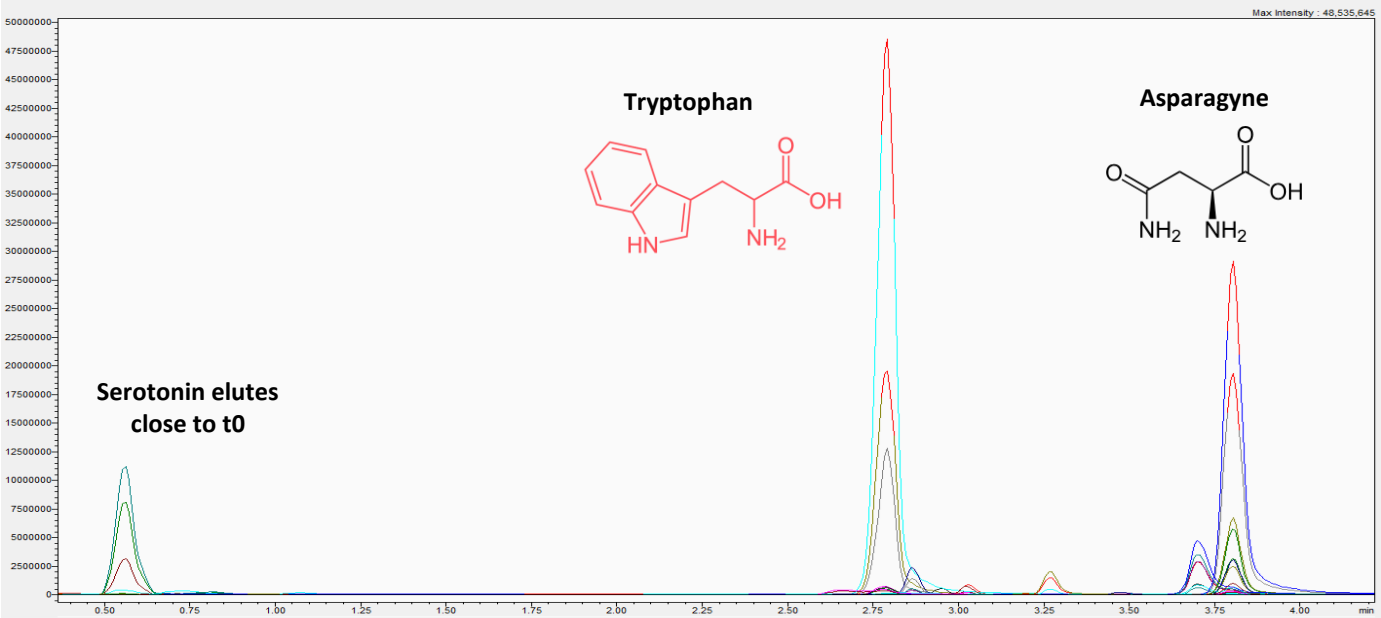
Figure 1. Elution profile of the selected Amino Acids under HILIC conditions.



The Kinetex Amide HPLC column shows effective retention and separation of amino acids under HILIC modality. All tested amino acids display clean and symmetrical peaks, indicating good efficiency and stable retention behavior (**Figure 1**). The amino acid mixture was prepared by diluting appropriate amounts of each stock solution in a water–acetonitrile (20:80, v/v) solution containing 0.1% formic acid. All solutions were freshly prepared prior to analysis and thoroughly mixed to ensure homogeneity.

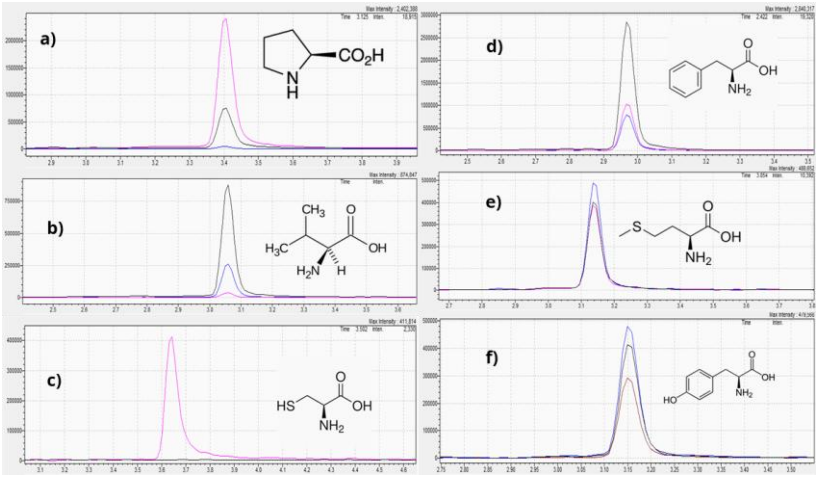
As illustrated in **Figure 2**, depicting representative elution profiles for neutral, aromatic, acidic, and basic amino acid, the Kinetex Amide column delivers consistent selectivity and performance across diverse amino acid classes.

Despite their different chemical properties, all analytes follow the characteristic HILIC elution pattern and exhibit good peak shape, confirming the Kinetex Amide as a well-performing column for non-derivatized amino acid analysis.

Conclusion

The column's performance was evaluated in terms of retention, resolution, and reproducibility, confirming its suitability for both routine and advanced amino acid analyses. The results demonstrate that amide-based HILIC columns can streamline amino acid workflows while delivering robust, reliable, and high-quality chromatographic performance.

Figure 2. Chromatographic profile of Proline (a), Valine (b), Cysteine (c), Phenylalanine (d), Methionine (e), and Threonine (f)



Ordering Information

2.6 µm Kinetex Amide Columns (mm)				Cartridges‡
Phases	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Amide Polyol	00B-4812-AN	00D-4812-AN	00F-4812-AN	AJ0-9800
				for ID: 2.1mm

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

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