Supelco®
Analytical Products

Application Note

LC-MS/MS Analysis of 20 Underivatized Amino Acids on Supel™ Carbon LC column

Introduction

Amino acids are the building blocks of proteins and peptides in biological systems. Amino acids have also been used as supplements to support health and as indicators for certain diseases. The challenge with analyzing amino acids is due to the wide-ranging polarities spanning across all 20 amino acids (**Figure 1**). Due to this complexity, methods in the past have relied on derivatization of the amino acids; however, derivatization can lead to further complexity due to the presence of derivatized/underivatized amino acids and interferences from the derivatizing reagent itself. This application outlines an LC-MS/MS method for analyzing all 20 amino acids, without derivatization, utilizing the Supel™ Carbon LC column. All 20 amino acids are retained on the column, with good peak shape.

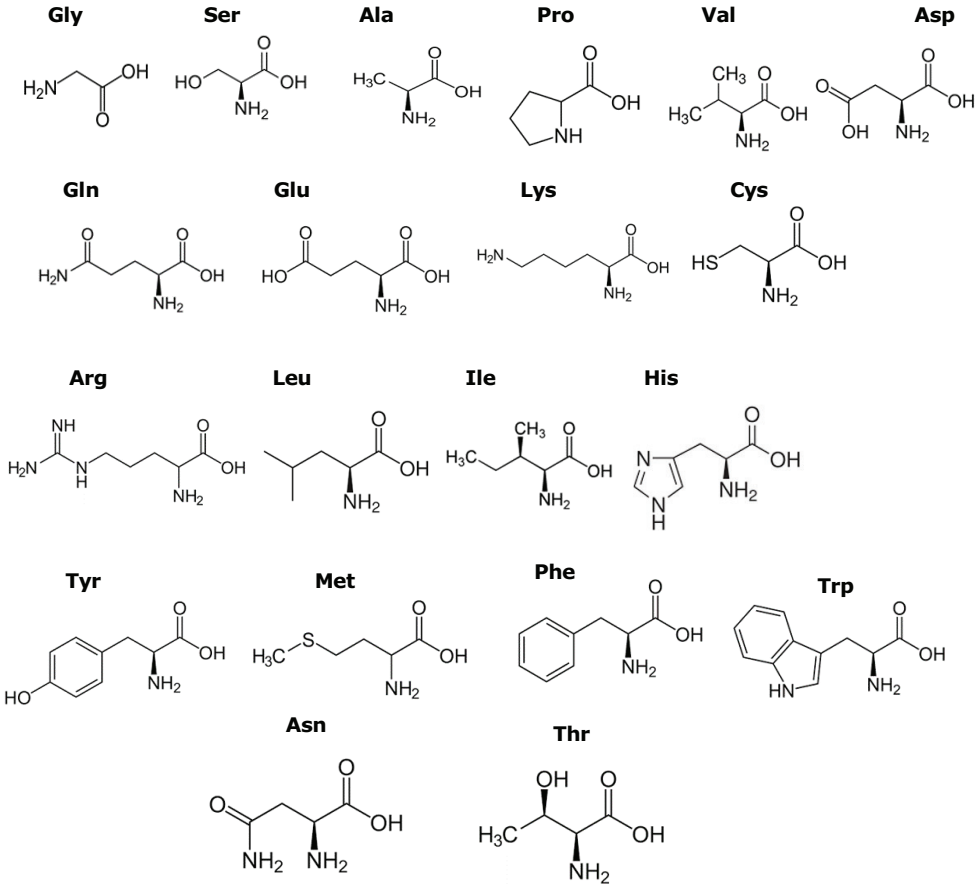

Figure 1: Structures and three letter codes of amino acids.

Results/Conclusion

Figure 2 displays the MS spectral results for the analysis of 20 underivatized amino acids while **Table 1** displays the optimized MS conditions for the separation and **Table 2** displays the fragment ions and fragmentation parameters for the amino acids.

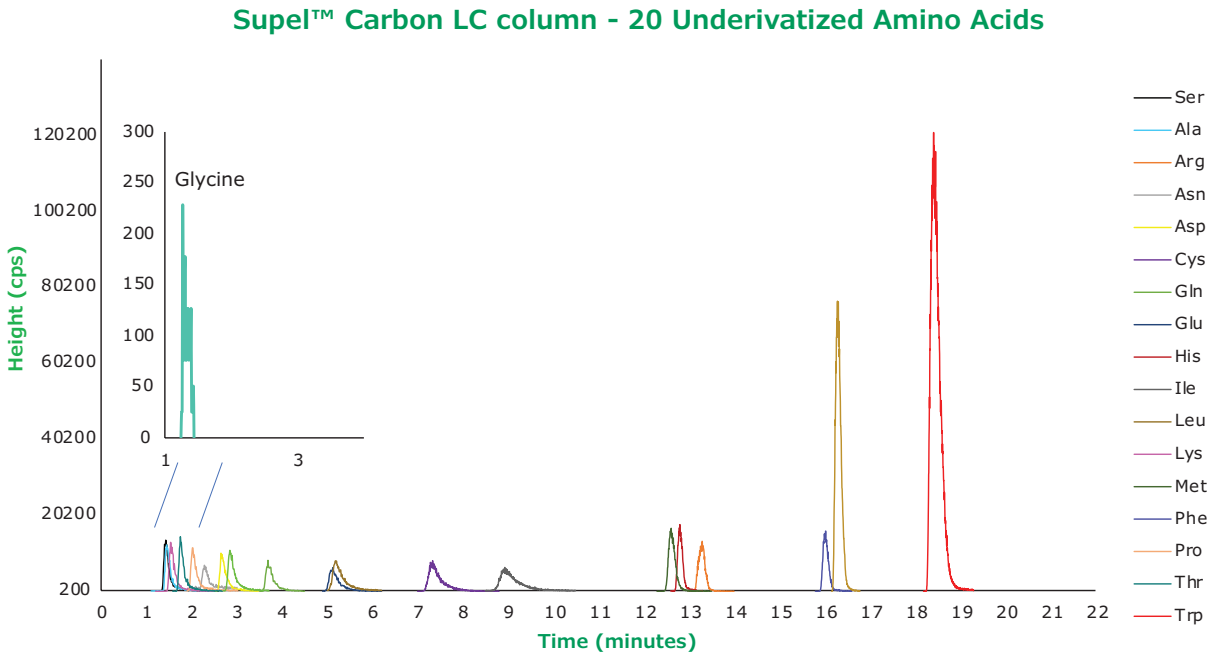

Figure 2: Separation of 20 underivatized amino acids by LC-MS/MS. Conditions: Column: Supel™ Carbon LC, 10 cm x 2.1 mm I.D., 2.7 µm; Mobile Phase: [A] Water (0.1% (v/v) DFA); [B] Acetonitrile (0.1% (v/v) DFA); Gradient: Hold at 0% B for 7 min; 0% B to 5% B in 5 min; 5% B to 100% B in 10 min; Flow Rate: 0.2 mL/min; Column temp.: 12 °C; Detector: MSD; Injection: 1.0 µL; Sample: Amino Acid Mix, varied concentration, water (0.1% (v/v) DFA)

Elution Order	Compound	Retention Time (min)
1	Glycine	1.27
2	Serine	1.43
3	Alanine	1.45
4	Lysine	1.54
5	Threonine	1.75
6	Proline	2.03
7	Asparagine	2.29
8	Aspartic Acid	2.65
9	Valine	2.85
10	Glutamine	3.69

Elution Order	Compound	Retention Time (min)
11	Glutamic Acid	5.13
12	Leucine	5.18
13	Cystine	7.34
14	Isoleucine	8.93
15	Methionine	12.61
16	Histidine	12.81
17	Arginine	13.30
18	Phenylalanine	16.03
19	Tyrosine	16.29
20	Tryptophan	18.42

Table 1: MS Conditions for the Separation of 20 Amino Acids

Ion Source Type	Turbo Spray
Curtain Gas	25
Ion Spray Voltage	4000 V
Temperature	300 °C
Ion Source Gas 1	20
Ion Source Gas 2	30
Interface Heater	On

Table 2: MRM Fragmentation Parameters and Ions for the 20 Amino Acids

Name	Q1	Q3	DP	EP	CEP	CE	CXP
Lysine	147.1	84.0	21.7	7.0	5.3	21.0	3.0
Proline	116.1	70.1	22.2	7.9	6.2	19.1	4.1
Aspartic Acid	134.1	74.0	17.0	8.3	12.8	17.0	3.7
Serine	106.1	60.0	13.0	4.0	8.8	15.0	3.1
Glycine	76.1	30.0	7.0	7.0	4.9	17.0	5.5
Alanine	90.1	44.0	16.0	6.0	4.9	16.9	5.6
Threonine	120.1	56.0	19.0	5.3	7.0	22.0	3.3
Asparagine	133.1	87.0	17.0	3.3	8.2	14.0	2.6
Valine	118.1	72.0	14.5	6.0	7.5	14.0	3.2
Glutamine	147.1	84.0	12.0	6.8	6.1	23.0	3.5
Leucine	132.1	86.0	16.0	7.4	10.2	14.0	3.2
Glutamic Acid	148.1	84.0	24.0	6.1	9.0	20.0	3.0
Isoleucine	132.1	86.0	16.0	7.4	10.2	14.0	3.2
Methionine	150.2	104.0	16.0	5.5	11.8	13.6	3.9
Histidine	156.1	110.0	24.0	3.5	11.0	19.0	3.7
Arginine	175.2	70.0	25.2	6.7	10.0	37.7	3.0
Phenylalanine	166.2	120.2	18.0	7.0	9.1	16.8	3.2
Tryptophan	205.2	146.2	19.4	4.8	17.7	23.0	4.0
Tyrosine	182.2	136.2	19.2	7.7	6.4	18.04	4.0
Cystine	241.2	120.0	26.0	7.8	9.8	26.0	4.1

This application demonstrated the effectiveness of the Supel™ Carbon LC column in resolving 20 amino acids without the need for a derivatization reagent. Analysis of amino acids is typically done with speciality, silica-based columns, ion exchange columns, or normal phase columns. However, by utilizing tandem MS/MS detection, analyses of amino acids can be accomplished on Supel™ Carbon LC column with fast run times compared to most commercial approaches. Due to porous graphitic carbon's unique ability to discriminate three-dimensional differences between compounds, Leucine and Isoleucine can be resolved with exceptional resolution.

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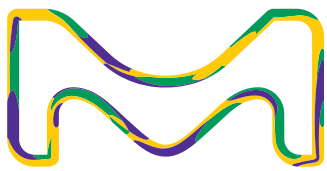

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