

Separation of an Amino Acid Mixture Using a Kinetex™ XB-C18 Column

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Overview

Amino acids are the building blocks of proteins, making them an extremely well researched compound class. With thousands of naturally occurring amino acids available, separating and quantitating a mixture of amino acids can be challenging. While HPLC is a well characterized method for amino acid analysis, LC-MS/MS analysis of amino acids poses several challenges.

In this application note, a Kinetex 2.6 μm XB-C18 column and a robust LC-MS/MS method were used to separate an amino acid mixture. The protective butyl side chains on the stationary phase allow for superior peak shape and enhanced separation of basic compounds under acidic conditions.

LC Conditions

Column: Kinetex 2.6 μm XB-C18
Dimensions: 150 x 0.3 mm
Part No.: [00F-4496-AC](#)
Mobile Phase: A: 0.1 % Formic Acid in Water
 B: 0.1 % Formic Acid in Methanol
Gradient:

Time (min)	%B
0	2
6	10
12	85
13	2
20	2

Flow Rate: 10 μL/min
Injection: 1 μL
Temperature: 40 °C
Detector: QTRAP® 6500
System: NanoLC™ 425 (SCIEX®)
Detection: MS/MS

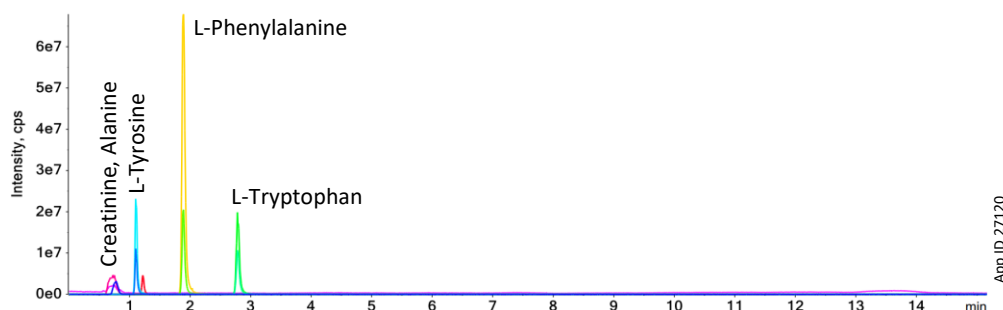
Table 1. Amino Acids Used and Their Respective MRM Transitions.

Metabolite	Q1 (m/z)	Q3 (m/z)
Creatinine (D3)	117	47
L-Phenylalanine (13C6)	172	126
L-Leucine (13C6)	138	91
L-Tryptophan (13C11)	216	155
L-Tyrosine (13C6)	188	142
L-Alanine (13C3/15N)	94	47

MS/MS Conditions

Scan Type: MRM
Polarity: Positive
Source: OptiFlow® Turbo V™
SG1: 20
SG2: 30
CUR: 30
Temperature: 150 °C
Spray Voltage: 5000 V

Figure 1. Extracted Ion Chromatogram of 6 Amino Acids on a Kinetex 2.6 μm XB-C18 Column.



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