

# Separation of an Amino Acid Mixture in HILIC Mode Using a Luna™ NH<sub>2</sub> 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. Some compounds are very polar and are hard to retain by reversed phase HPLC. Due to the polar and hydrophilic nature of amino acids, HILIC chromatography was utilized to separate a 6 amino acid mixture.

In this application note, a Luna 3 μm NH<sub>2</sub> column, in HILIC mode, and a robust LC-MS/MS method was used to successfully separate an amino acid mixture. Amino groups bound to the silica surface of the stationary phase particles serve as a weak anion exchanger and offer greater polar selectivity, thus increased separation of highly polar amino acids.

**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

## LC Conditions

**Column:** Luna 3 μm NH<sub>2</sub>  
**Dimensions:** 150 x 0.3 mm  
**Part No.:** [OOF-4377-AC](#)  
**Mobile Phase:** A: 10 mM Ammonium Formate in Acetonitrile, pH 3.3  
 B: 10 mM Ammonium Formate in Water, pH 3.3

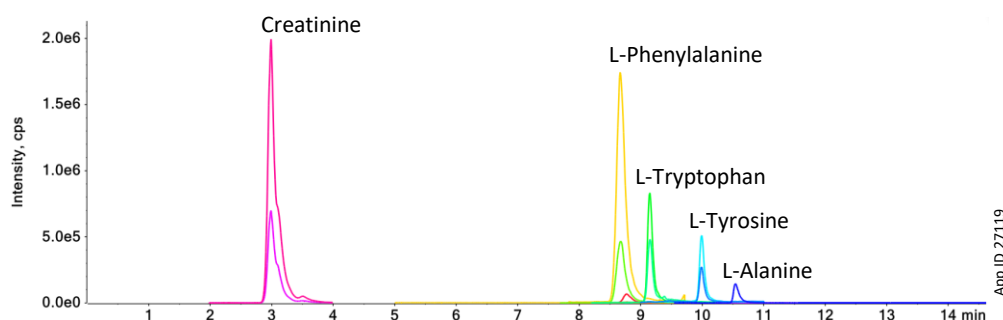
Gradient: Time (min)	%B
0	3
6	3
11.5	50
12.5	98
18.5	98
19.5	3
32	3

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

## 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 Luna 3 μm NH<sub>2</sub> Column Under HILIC Conditions.



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