

Application News

LCMS-8060RX High Performance Liquid Chromatograph Mass Spectrometer

Amino Acid Analysis by the Triple Quadrupole LCMS-8060RX

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User Benefits

- ◆ Amino acid confirmation and quantitation
- ◆ Calibration spanning 4 to 6 orders of magnitude from as low as 5 nM to as high as 150 µM with $R^2 > 0.99$ and accuracy $\pm 20\%$
- ◆ New RX source utilizes CoreSpray to improve performance and reproducibility and IonFocus to increase robustness by drastically reducing the presence of unwanted molecules that contribute to contamination and matrix effects

Background

Amino acids are the building blocks of proteins. As such, accurate quantitation of amino acids is essential across many areas in biology, chemistry, and medicine. One of the most powerful tools for amino acid analysis in a variety of matrices is LC-MS/MS; however, quantitation can be challenging as the dynamic range of amino acid concentrations can vary considerably within the same sample. Additionally, reproducibility and robustness for analyses across different matrices can present complications due to variations in ion suppression or enhancement. The new RX source increases reproducibility and robustness while drastically reducing the contributions of unwanted molecules to contamination and matrix effects with the new CoreSpray and improved IonFocus technologies utilizing optimized gas delivery, ESI capillary stabilization, and focusing electrode.

This Application News shows accurate quantitation of amino acids across a large calibration range for a mixture of 17 of the common amino acids.

Method

An Amino Acids Mixture Standard Solution, Type H was purchased from Wako Chemicals (Richmond, VA). 2-Isopropylmalic acid, purchased from Sigma-Aldrich (St. Louis, MO), was utilized as an internal standard (ISTD). The amino acid standard mixture was diluted in LC-MS grade water with 0.1% formic acid to the concentrations shown in Table 1 below with an ISTD concentration of 2.5 µM for each sample.

Table 1: Evaluated Amino Acid Concentrations

5 nM	10 nM	25 nM	100 nM	250 nM	500 nM
1 µM	5 µM	10 µM	50 µM	100 µM	150 µM

For the analysis, 1 µL of each calibration level was injected onto a modular Nexera 40-series HPLC coupled with an LCMS-8060RX triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source.

The LCMS-8060RX was utilized in both positive and negative ionization modes, operating in multiple reaction monitoring (MRM) acquisition mode. Quantitation and confirmation of the targeted analytes were performed through the monitoring of two selective MRM transitions.

The LC-MS/MS method was based on the Shimadzu LC/MS/MS Method Package for Cell Culture Profiling Ver. 3 (item number [225-46320-91](#)) with some voltage adjustments.

Table 2: Method Parameters

Nexera LC	
Column	Cell Culture Profiling Column
	2.1 x 150 mm
	Part Number 220-91581-10
Mobile Phase	A: Water + 0.1% Formic Acid
	B: Acetonitrile + 0.1% Formic Acid
Flow Rate	0.35 mL/min
Oven Temp.	40 °C
Injection Vol.	1 µL
LCMS-8060 RX	
Nebulizing Gas	2.5 L/min
Heating Gas	12 L/min
Interface Temp.	250 °C
DL Temp.	275 °C
Heat Block Temp.	400 °C
Drying Gas	12 L/min

Results and Discussion

Chromatographic separation was achieved using the Cell Culture Profiling Method without modification. Separation of the isomeric amino acids isoleucine and leucine is shown in Figure 1 below.

The calibration range was determined with a quadratic fit, 1/C weighting, with an R^2 value of > 0.99 , and concentration accuracy within $\pm 20\%$. All amino acids could be measured across at least 4 orders of magnitude (OoM), and 13 of the 17 amino acids were measured across 5 to 6 orders of magnitude.

Calibration curves are shown in Figure 2. Figure 3 shows example chromatograms from selected ion monitoring (SIM) at the limits of quantitation. A summary of calibration information is provided in Table 3.

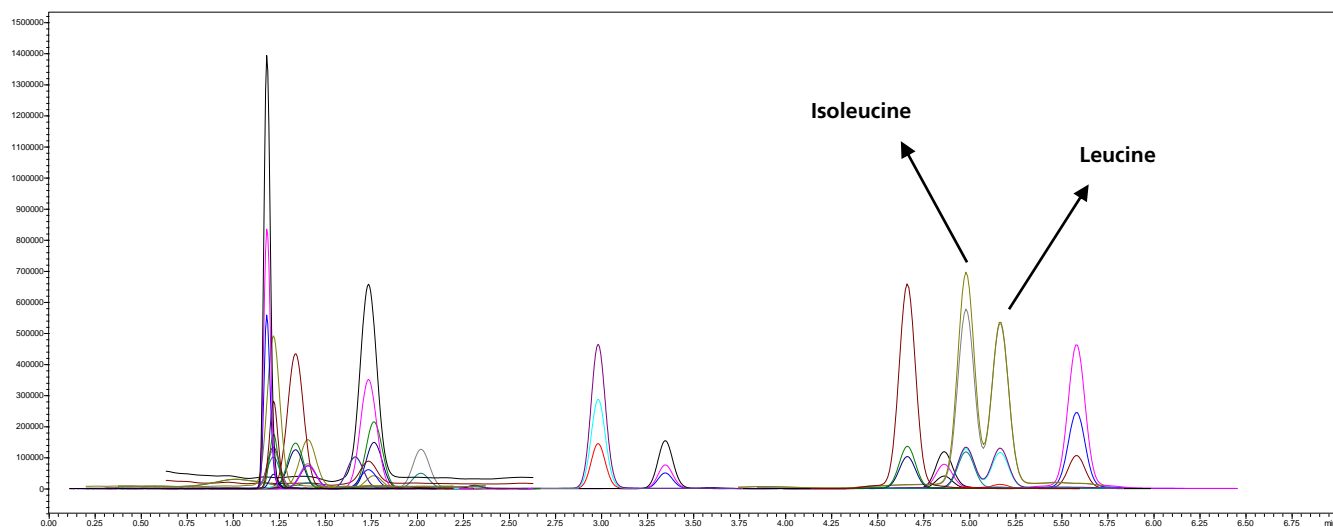


Figure 1: Representative Chromatogram of 1 μ M Amino Acids

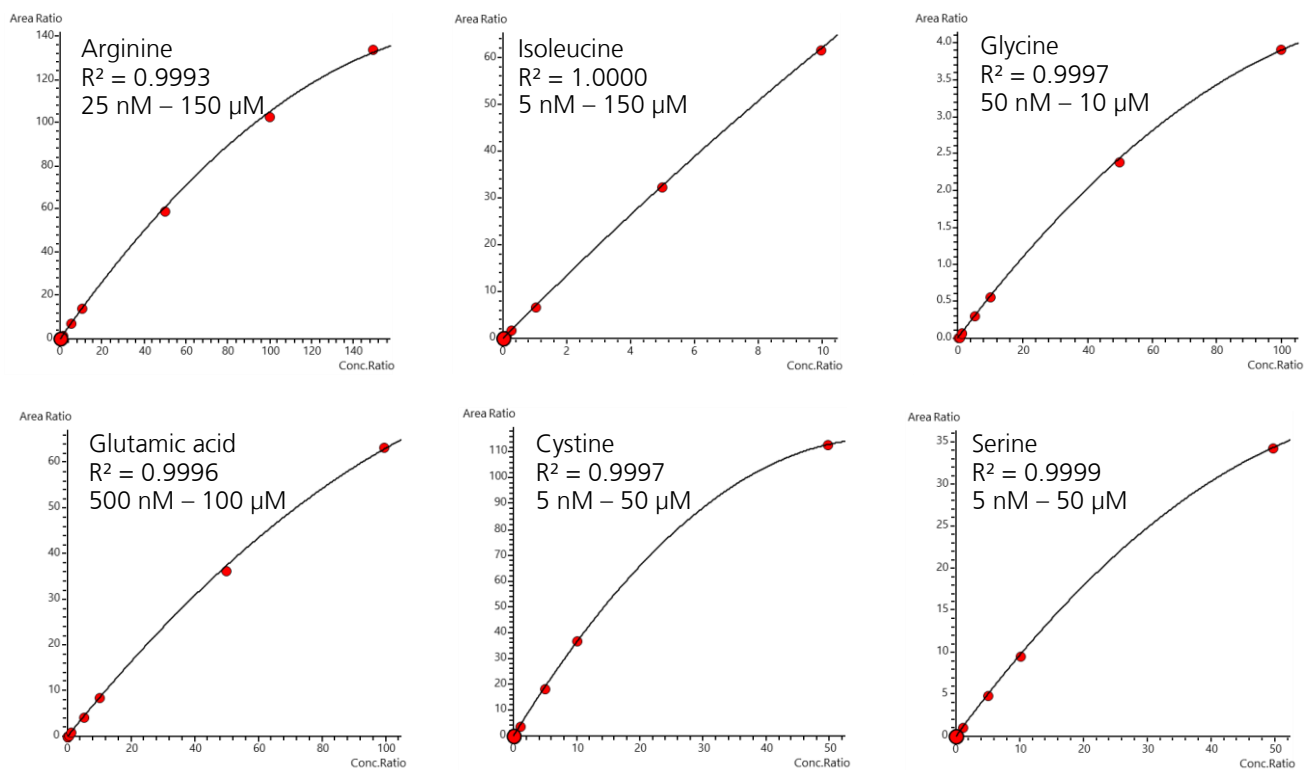


Figure 2: Calibration Curves for Amino Acids

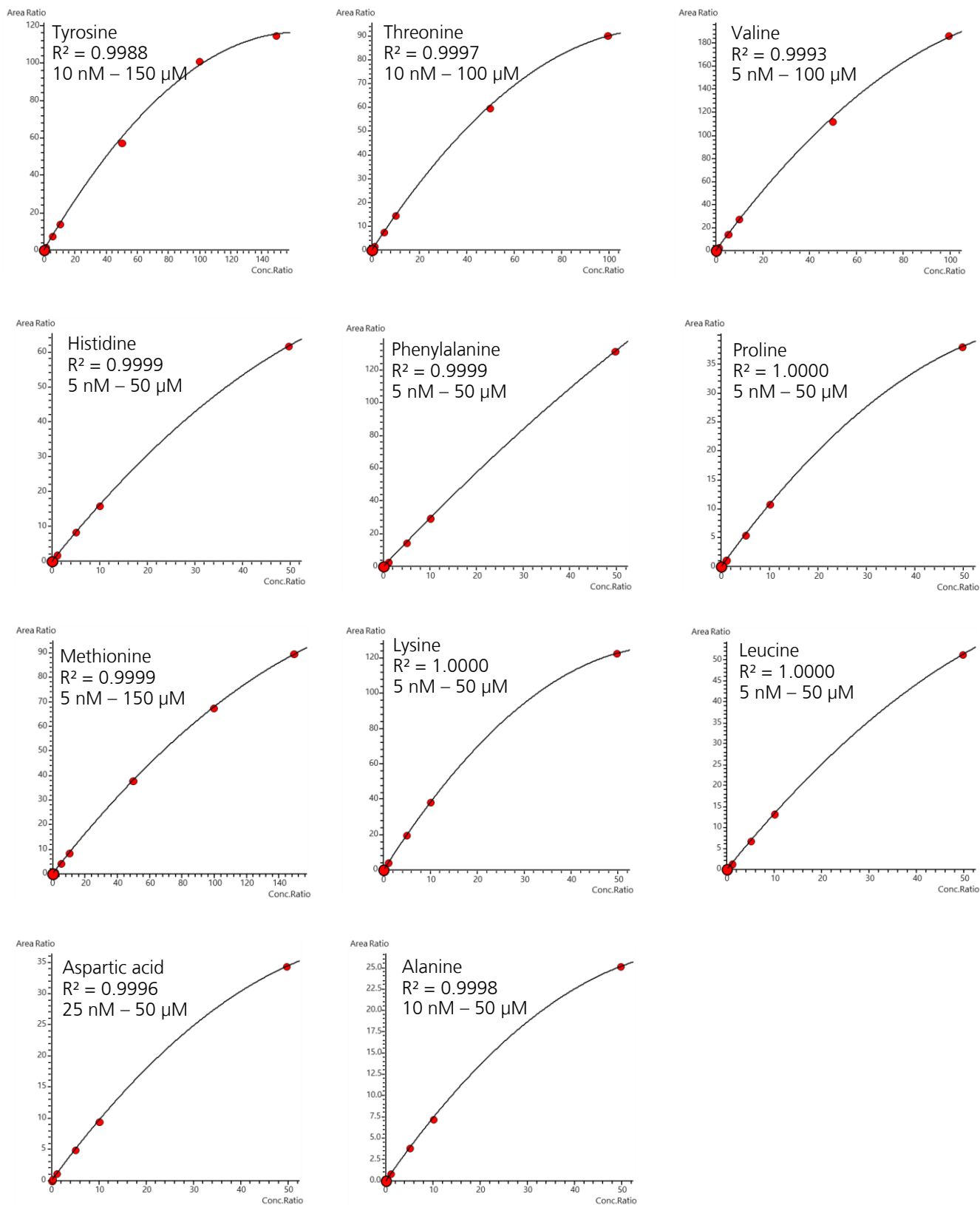


Figure 2 (continued): Calibration Curves for Amino Acids

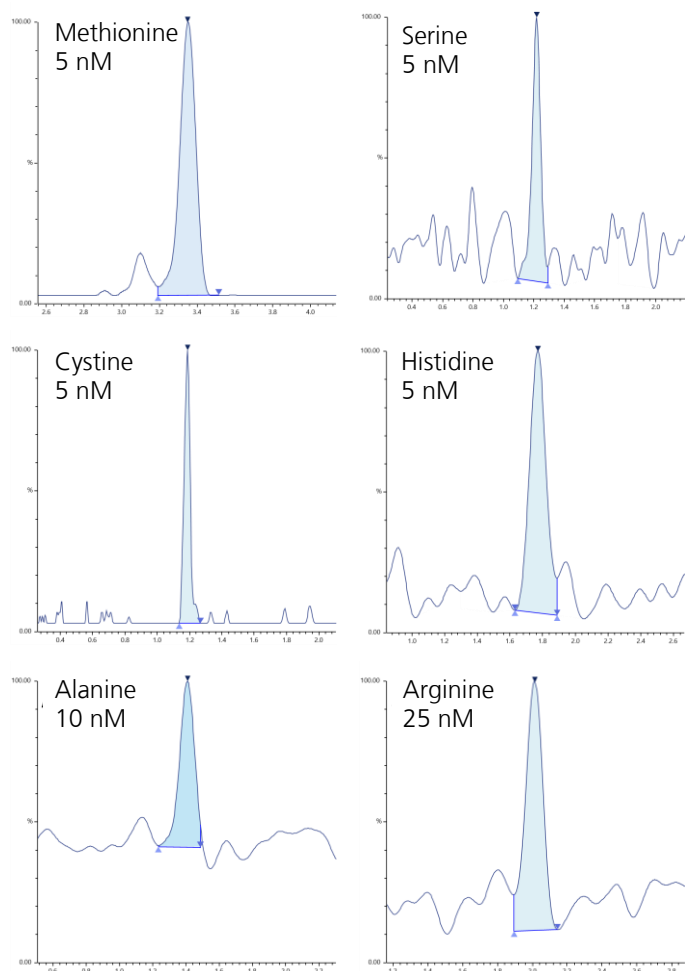


Figure 3: Example SIM Chromatograms at Limits of Quantitation

Conclusion

The calibration range of a mixture of 17 common amino acids was evaluated on a Shimadzu LCMS-8060RX system. Most amino acids demonstrated a calibration range of five or more orders of magnitude, while all amino acids showed a calibration range of at least four orders of magnitude. With an injection volume of only 1 μ L, 11 amino acids could be accurately quantitated at 5 nM.

The high sensitivity from the LCMS-8060RX combined with the CoreSpray and the new IonFocus technologies allow robust amino acid quantitation in different matrices for many applications, including cell culture profiling, food and natural products, protein digests, and supplements. The wide calibration dynamic range simplifies analysis by confidently quantifying the amino acids with a single injection.

Part Numbers

[220-91581-10](#) Column, Cell Culture Profiling, 2.1mm x 15cm

[220-91545-12](#) Solvents, LCMS, B&J Water, >99.9%, 4x4L Case

[220-91545-10](#) Solvents, LCMS, B&J Acetonitrile, >99.9%, 4x4L Case

Table 3: Amino Acids Calibration Summary Table

Amino Acids	R ²	Lower Limit	Upper Limit	OoM
L-Aspartic Acid	0.9996	25 nM	50 μ M	4
L-Alanine	0.9998	10 nM	50 μ M	4
L-Arginine	0.9993	25 nM	150 μ M	5
L-Isoleucine	1.0000	5 nM	10 μ M	5
Glycine	0.9997	50 nM	10 μ M	4
L-Glutamic Acid	0.9973	100 nM	100 μ M	4
L-Cystine	0.9997	5 nM	50 μ M	5
L-Serine	0.9999	5 nM	50 μ M	5
L-Tyrosine	0.9988	10 nM	150 μ M	5
L-Threonine	0.9997	10 nM	100 μ M	5
L-Valine	0.9993	5 nM	100 μ M	6
L-Histidine	0.9999	5 nM	50 μ M	5
L-Phenylalanine	0.9999	5 nM	50 μ M	5
L-Proline	1.0000	5 nM	50 μ M	5
L-Methionine	0.9999	5 nM	150 μ M	6
L-Lysine	1.0000	5 nM	50 μ M	5
L-Leucine	1.0000	5 nM	50 μ M	5

UAFMS

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LCMS-8045RX



LCMS-8050RX



LCMS-8060RX



LCMS-2020



LCMS-2050



Q-TOF LCMS-9030/9050

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