Application Note

Food Testing & Agriculture

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# Analysis of Amino Acids in Animal Feed Matrices Using the Ultivo Triple Quadrupole LC/MS System

**Figure 1.** Ultivo integrated into LC stack.


## Authors

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## Abstract

This Application Note demonstrates a fast and simple analytical method developed for the quantitation of underivatized amino acids using an Agilent Ultivo triple quadrupole LC/MS system.

Ultivo was designed to address many challenges faced by laboratories, and this study was conducted to assess how this novel LC/MS could perform with typical endogenous analytes of interest. Innovative technologies within Ultivo allowed for a reduced physical footprint while generating an analytical performance similar to that of physically larger MS systems.

This study outlines a typical confirmation performance of free amino acids using the Ultivo LC/MS in combination with HILIC chromatography. Lower limits of quantitation (LLOQ), chromatographic precision, linear dynamic range, and accuracy are discussed.

## Introduction

Amino acids are the building blocks of proteins, and are necessary components of a balanced diet. For pets and farm animals, the diet must supply the essential amino acids, which includes cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine. To ensure the quality of their food in providing a balanced diet, pet food and animal feed nutrition companies monitor their products for the presence of these amino acids, along with the remaining nonessential ones.

Historically, amino acids have been analyzed using a labor-intensive hydrolysis and derivatization for sample preparation, which can limit the speed of analysis. However, it has been found that underivatized amino acids can be analyzed with excellent sensitivity and accuracy using hydrophilic interaction chromatography (HILIC) with low pH solvents and positive ion mode MS detection.

The Ultivo is designed to address many of the challenges faced by labs performing routine analyses. Innovative technologies housed within Ultivo created a reduced overall footprint while conserving the performance found in traditional systems (Figure 1). Innovations such as the Cyclone Ion Guide, Vortex Collision Cell, and the Hyperbolic Quads maximize quantitative performance in a small package, enhancing instrument reliability, robustness, and uptime.

In this application, we demonstrate the sensitive and precise quantitation of 17 underivatized amino acids in animal feed using the novel Ultivo triple quadrupole LC/MS (Figure 1).

## Experimental

### Reagents and chemicals

All reagents used were HPLC or LC/MS grade. Acetonitrile was purchased from Honeywell (Morristown, NJ, USA), and ultrapure water was sourced from a Milli-Q Integral system with an LC-Pak Polisher and a 0.22-µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid and ammonium acetate were purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA). Chemical standards were purchased from Pierce (Thermo Scientfic, Waltham, MA, USA).

### Sample preparation

Standards were prepared through serial dilution into water. Animal feed was obtained from local suppliers, and ground up. A sample of 0.1 g was weighed into a 250-mL Erlenmeyer flask, and hydrolyzed using 25 mL of 6 N HCl with phenol. The flasks were placed under nitrogen flow for 23 hours, then removed and allowed to cool to room temperature. The samples were transferred to 50-mL volumetric flasks that had been rinsed with 0.1 N HCl prior to sample introduction. The flasks were filled to the line with 0.1 N HCl, and mixed. Aliquots were transferred to sample vials for introduction into the LC/TQ system.

### Data analysis

System control and data acquisition were performed by Agilent MassHunter Acquisition Software (C.01.00). To determine optimal precursor and product ions, fragmentor voltages, and collision energies upon injection of a neat solution of the compounds, MRM transitions were obtained using MassHunter Acquisition Optimizer software. Data were analyzed using Agilent MassHunter Quantitative Analysis Software (B.09.00) and Qualitative Analysis Software (B.08.00).

### Instrumentation

Agilent 1260 Infinity II Bio-Inert HPLC

- 1260 Infinity II Bio-Inert pump (G5654A)
- 1260 Infinity II Bio multisampler (G5668A)
- 1260 Infinity II multicolumn thermostat (G7116A)

Agilent Ultivo triple quadrupole LC/MS system

- Agilent Jet Stream electrospray ionization source

### Method

Table 1 summarizes the 1260 Infinity II Bio-Inert HPLC conditions. Table 2 summarizes the Ultivo triple quadrupole parameters and Agilent Jet Stream ESI source parameters. Analysis was carried out with positive ionization and dynamic multiple reaction monitoring (dMRM). Data were evaluated using MassHunter Quantitative Analysis Software B.09 with the Quant-My-Way tool.

## Results and discussion

### Chromatography

With the Poroshell HILIC-Z column and inert chromatography system, the 17 amino acids were well separated within a seven-minute window (Figures 2 and 3). A baseline separation of 0.3 minutes was also achieved for leucine and isoleucine (Figure 4), and the retention times demonstrated excellent stability, with %RSDs of less than 0.5 % for each compound.

**Table 1.** 1260 Infinity II Bio-Inert HPLC parameters.

| Parameter | Value |
|---|---|
| Column | Agilent Poroshell 120 HILIC-Z, 2.1 × 150 mm, 2.7 µm (p/n 6x3775-924) |
| Column temperature | 25 °C |
| Injection volume | 2 µL |
| Mobile phase | A) 20 mM ammonium acetate + 0.1 % formic acid in water, pH 3<br>B) 20 mM ammonium acetate in 90 % acetonitrile, pH 3 |
| Flow rate | 0.50 mL/min |
| Gradient | Time %B<br>0 100<br>11.5 70<br>12.0 100<br>15.0 100 stop time<br>19.0 100 post time |

**Table 2.** AJS source and Ultivo triple quadrupole parameters.

| Parameter | Value |
|---|---|
| Drying gas temperature | 150 °C |
| Drying gas flow | 15 L/min |
| Sheath gas temperature | 400 °C |
| Sheath gas flow | 12 L/min |
| Nebulizer pressure | 20 psi |
| Capillary voltage | 2,000 V(+) |
| Nozzle voltage | 0 V(+) |
| Cycle time | 500 ms |

**Table 3.** Transitions for amino acid detection in dMRM mode.

| Compound | Precursor (*m/z*) | Product (*m/z*) | RT (min) | RT Window (min) | Frag. (V) | CE (V) | Polarity |
|---|---|---|---|---|---|---|---|
| Alanine | 90.1 | 62.1 | 6.83 | 2 | 75 | 0 | Positive |
| Alanine | 90.1 | 44.1 | 6.83 | 2 | 75 | 0 | Positive |
| Arginine | 175.1 | 116.1 | 10.43 | 2 | 105 | 2 | Positive |
| Arginine | 175.1 | 70.1 | 10.43 | 2 | 105 | 8 | Positive |
| Arginine | 175.1 | 60.1 | 10.43 | 2 | 105 | 4 | Positive |
| Aspartic acid | 134.1 | 88.0 | 9.03 | 2 | 75 | 0 | Positive |
| Aspartic acid | 134.1 | 74.0 | 9.03 | 2 | 75 | 4 | Positive |
| Aspartic acid | 134.1 | 70.0 | 9.03 | 2 | 75 | 6 | Positive |
| Cystine | 241.0 | 241.0 | 11.16 | 2 | 105 | 0 | Positive |
| Cystine | 241.0 | 152.0 | 11.16 | 2 | 105 | 0 | Positive |
| Cystine | 241.0 | 120.0 | 11.16 | 2 | 105 | 0 | Positive |
| Cystine | 241.0 | 74.1 | 11.16 | 2 | 105 | 25 | Positive |
| Glutamic acid | 148.1 | 148.1 | 8.27 | 2 | 85 | 0 | Positive |
| Glutamic acid | 148.1 | 84.0 | 8.27 | 2 | 85 | 6 | Positive |
| Glutamic acid | 148.1 | 56.1 | 8.27 | 2 | 85 | 22 | Positive |
| Glutamic acid | 148.1 | 41.0 | 8.27 | 2 | 85 | 18 | Positive |
| Glycine | 76.0 | 48.0 | 7.36 | 2 | 65 | 0 | Positive |
| Glycine | 76.0 | 30.0 | 7.36 | 2 | 65 | 0 | Positive |
| Histidine | 156.1 | 110.1 | 9.81 | 2 | 95 | 4 | Positive |
| Histidine | 156.1 | 95.1 | 9.81 | 2 | 95 | 6 | Positive |
| Isoleucine | 132.1 | 86.1 | 4.90 | 2 | 85 | 0 | Positive |
| Isoleucine | 132.1 | 44.1 | 4.90 | 2 | 85 | 16 | Positive |
| Isoleucine | 132.1 | 41.0 | 4.90 | 2 | 85 | 18 | Positive |
| Isoleucine | 132.1 | 30.0 | 4.90 | 2 | 85 | 6 | Positive |
| Leucine | 132.1 | 86.1 | 4.62 | 2 | 85 | 0 | Positive |
| Leucine | 132.1 | 44.1 | 4.62 | 2 | 85 | 14 | Positive |
| Leucine | 132.1 | 41.0 | 4.62 | 2 | 85 | 25 | Positive |
| Leucine | 132.1 | 30.0 | 4.62 | 2 | 85 | 4 | Positive |
| Lysine | 147.1 | 130.1 | 11.12 | 2 | 85 | 0 | Positive |
| Lysine | 147.1 | 84.1 | 11.12 | 2 | 85 | 6 | Positive |
| Methionine | 150.1 | 104.1 | 5.16 | 2 | 75 | 0 | Positive |
| Methionine | 150.1 | 61.0 | 5.16 | 2 | 75 | 14 | Positive |
| Methionine | 150.1 | 56.1 | 5.16 | 2 | 75 | 6 | Positive |
| Methionine | 150.1 | 28.0 | 5.16 | 2 | 75 | 26 | Positive |
| Phenylalanine | 166.1 | 120.1 | 4.23 | 2 | 85 | 4 | Positive |
| Phenylalanine | 166.1 | 103.1 | 4.23 | 2 | 85 | 22 | Positive |
| Phenylalanine | 166.1 | 91.1 | 4.23 | 2 | 85 | 32 | Positive |
| Phenylalanine | 166.1 | 77.0 | 4.23 | 2 | 85 | 36 | Positive |
| Proline | 116.1 | 70.1 | 6.01 | 2 | 85 | 6 | Positive |
| Proline | 116.1 | 43.1 | 6.01 | 2 | 85 | 25 | Positive |
| Serine | 103.1 | 88.1 | 7.63 | 2 | 65 | 0 | Positive |
| Serine | 103.1 | 60.1 | 7.63 | 2 | 65 | 0 | Positive |
| Threonine | 120.0 | 74.1 | 6.98 | 2 | 75 | 0 | Positive |
| Threonine | 120.0 | 56.1 | 6.98 | 2 | 75 | 6 | Positive |
| Tyrosine | 182.1 | 136.1 | 5.53 | 2 | 95 | 0 | Positive |
| Tyrosine | 182.1 | 119.1 | 5.53 | 2 | 95 | 10 | Positive |
| Tyrosine | 182.1 | 91.1 | 5.53 | 2 | 95 | 22 | Positive |
| Tyrosine | 182.1 | 77.0 | 5.53 | 2 | 95 | 34 | Positive |
| Valine | 118.1 | 72.1 | 5.84 | 2 | 75 | 0 | Positive |
| Valine | 118.1 | 55.1 | 5.84 | 2 | 75 | 14 | Positive |

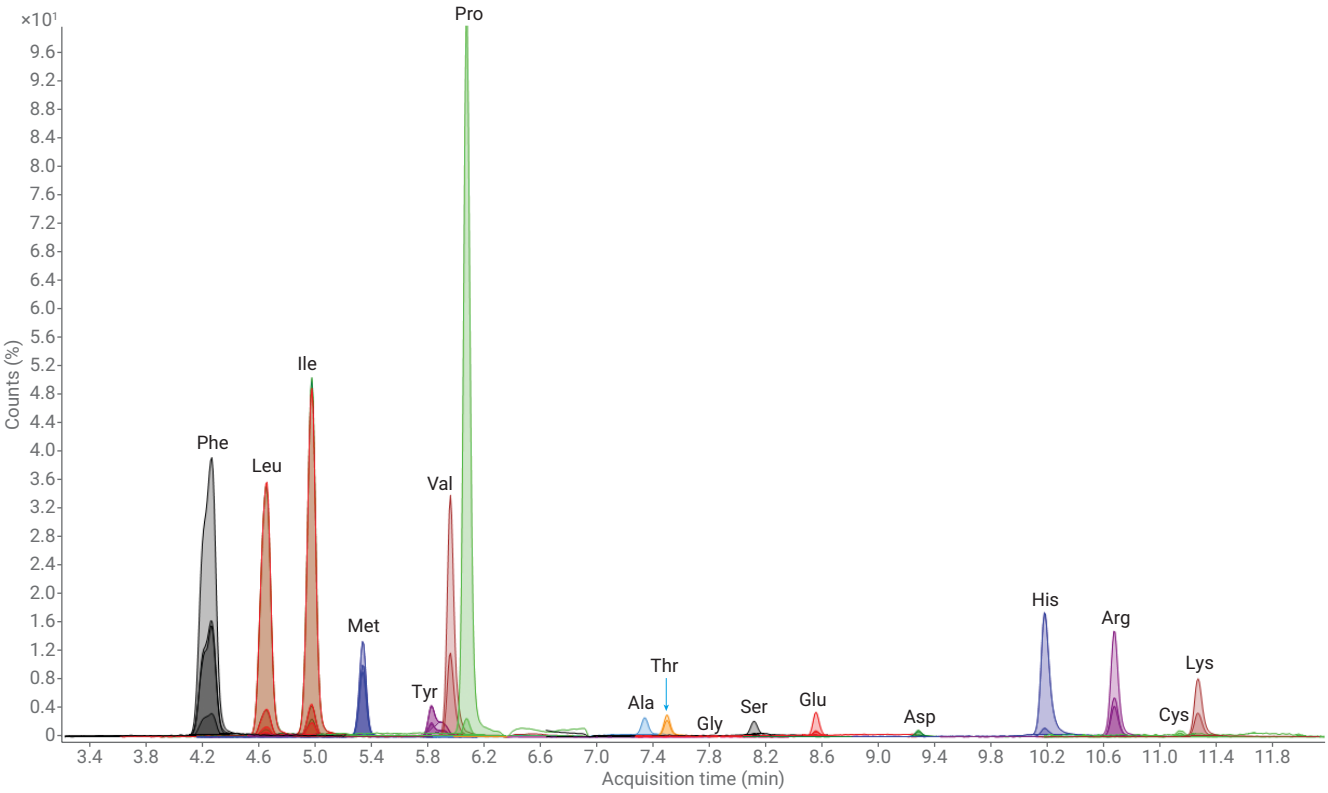


**Figure 2.** Composite dMRM chromatogram of amino acids, 10 ppb spike.

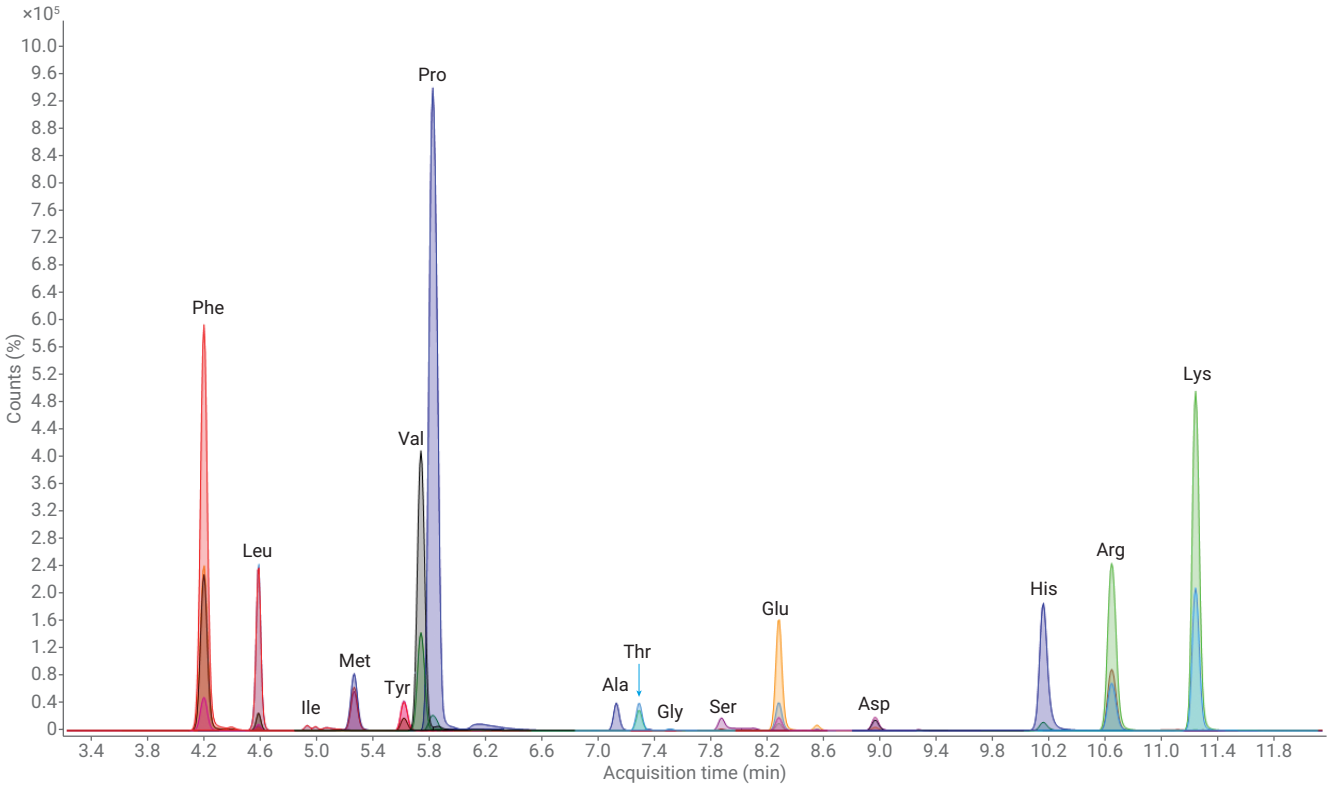


**Figure 3.** Composite dMRM chromatogram of amino acids in an animal feed sample.

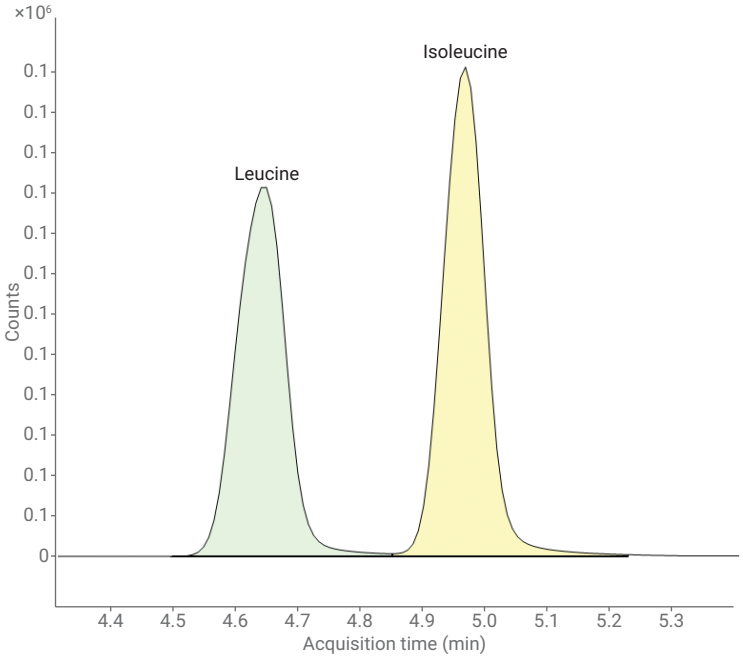


**Figure 4.** Separation of isobars leucine and isoleucine.

## Linearity, accuracy, and reproducibility

The calibration concentrations ranged from 1.12 to 18,844 ppb for the various analytes. Table 4 gives the limits of quantitation (LOQs), and curve fit parameters. Each curve had an $R^2$ value greater than 0.992, and responses showed excellent reproducibility from run to run. Calibration curve accuracies were within 11.5 % of the expected concentration at the lowest level, and demonstrated RSDs within 20 % at the LOQs, and within 5 % at the higher levels. Figure 5 shows examples of calibration curves for six selected compounds, while six replicate injections of three selected compounds in matrix are shown in Figure 6, demonstrating excellent precision.

**Table 4.** Calibration curve fit, LOQs, and S/N.

| Compound | Curve fit | $R^2$ | LOQ (ppb) | S/N at LOQ |
|---|---|---|---|---|
| Alanine | Linear | 0.9997 | 46.33 | 4.35 |
| Arginine | Quadratic | 0.9920 | 8.71 | 10.75 |
| Aspartic acid | Linear | 0.9971 | 13.31 | 5.76 |
| Cystine | Linear | 0.9969 | 61.04 | 13.51 |
| Glutamic acid | Quadratic | 0.9998 | 7.65 | 5.37 |
| Glycine | Linear | 0.9986 | 75.07 | 3.51 |
| Histidine | Quadratic | 0.9998 | 7.76 | 15.92 |
| Isoleucine | Linear | 0.9961 | 1.36 | 5.88 |
| Leucine | Linear | 0.9978 | 1.36 | 4.03 |
| Lysine | Quadratic | 0.9934 | 7.60 | 18.94 |
| Methionine | Linear | 0.9977 | 1.55 | 14.57 |
| Phenylalanine | Linear | 0.9974 | 1.72 | 4.82 |
| Proline | Linear | 0.9954 | 1.15 | 6.03 |
| Serine | Quadratic | 0.9987 | 10.51 | 5.43 |
| Threonine | Linear | 0.9995 | 11.91 | 4.35 |
| Tyrosine | Linear | 0.9943 | 1.88 | 3.86 |
| Valine | Linear | 0.9972 | 1.12 | 6.19 |

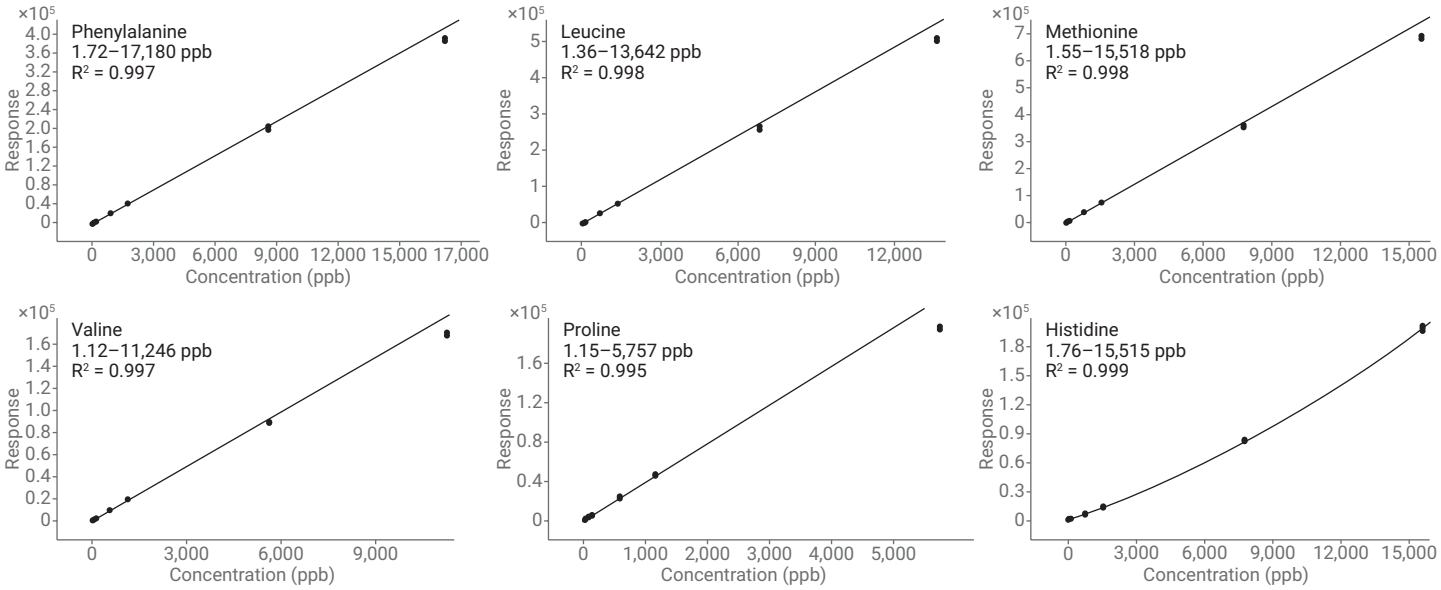


**Figure 5.** Calibration curves for selected compounds.

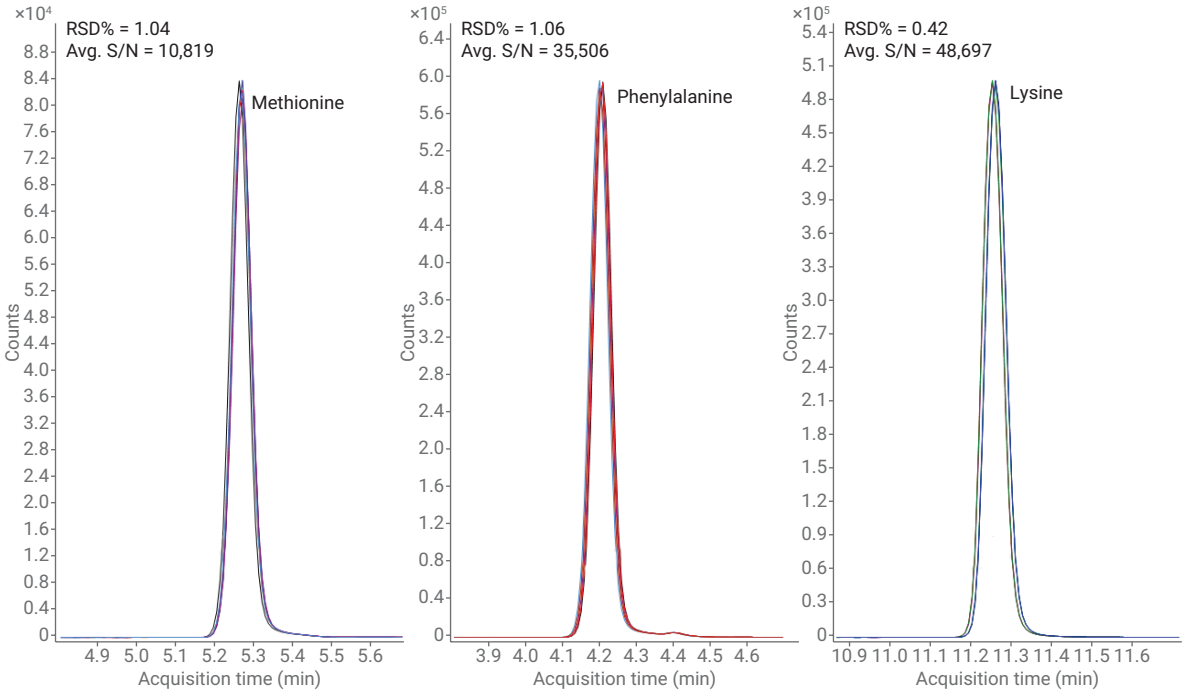


**Figure 6.** Excellent precision demonstrated for six replicate injections of three amino acids in sample matrix.

## Conclusions

Ultivo is an innovative triple quadrupole mass spectrometer that can minimize laboratory workspace requirements and reduce maintenance challenges. The LC/MS system demonstrated the accurate and sensitive detection of commonly monitored amino acids in an animal feed matrix, while the use of the Poroshell 120 HILIC-Z column enabled analytes to remain underivatized, resulting in a simplified workflow.

## References


1. Methods for the Analysis of Underivatized Amino Acids by LC/MS, *Agilent Technologies Application Note*, publication number 5991-8582EN.
2. Quantitative Analysis of Underivatized Amino Acids in Plant Matrix by Hydrophilic Interaction Chromatography (HILIC) with LC/MS Detection, *Agilent Technologies Application Note*, publication number 5991-8922EN.
3. Analysis of Underivatized Amino Acids by LC/MS for Bioreactor Cell Culture Monitoring, *Agilent Technologies Application Note*, publication number 5991-8816EN.
4. Otter, D. E. Standardised methods for amino acid analysis of food. *British Journal of Nutrition* **2012**, *108*, S230-S237.
5. Wu, G. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. *Journal of Animal Science and Biotechnology* **2014**, *5*, 34-45.


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