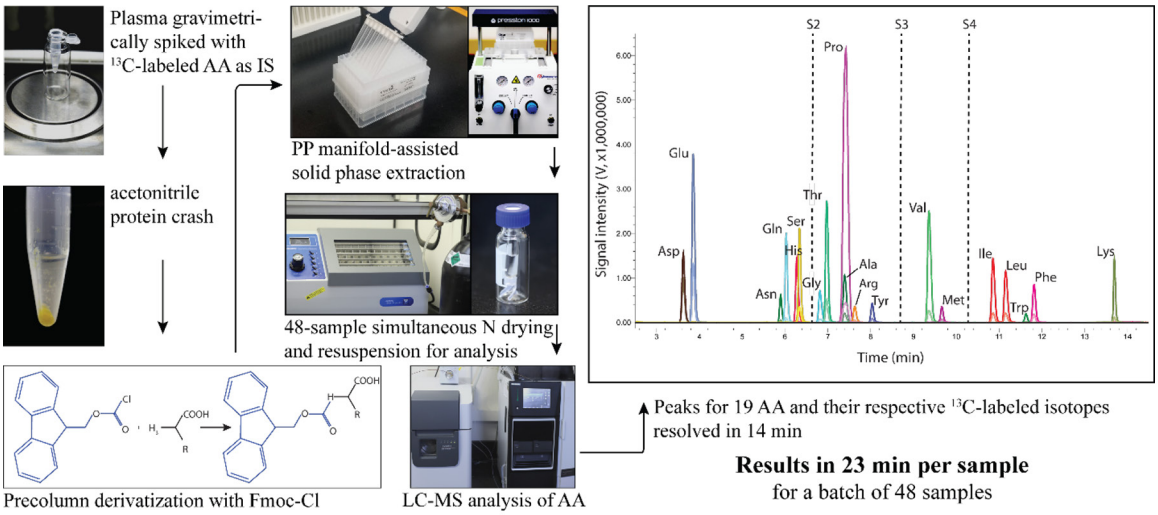
JDS Communications®
2024; 5:745–750

https://doi.org/10.3168/jdsc.2024-0546
Technical Note
Physiology

Fast and reliable method for analysis of derivatized plasma amino acids by liquid chromatography-single quadrupole-mass spectrometry

August Hoppmann and Sebastian I. Arriola Apelo*

Graphical Abstract



Summary

We developed and validated a high-throughput method for the quantification of 19 amino acids (AA) in bovine plasma using liquid chromatography–single quadrupole-mass spectrometry (LC-MS). For a fully quantitative approach, samples or standards and internal standards consisting of 13C isotopes of each AA, are gravimetrically mixed. Plasma proteins are precipitated on acetonitrile, and AA are derivatized with 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl) to enhance resolution. Precolumn derivatization allows for a single strong-anion solid-phase extraction step, performed on a 96-well format aided by a positive-pressure (PP) manifold. After drying under a stream of N2, eluates are reconstituted in mobile phase and injected into the LC-MS, which is programmed with a 17.5-minute run time method divided into 4 segments. Sample preparation and analysis are accomplished in 23 minutes per sample when run in batch. Upper limits of quantitation are beyond the physiological range (except Gly) and $R^2 \geq 0.999$ for every AA.

Highlights

- A method is described to measure concentrations of 19 encoded AA (except Cys) in bovine plasma.
- Results are obtained in 23 minutes per sample for a batch of 48 samples.
- Derivatization and strong-anion solid phase extraction are used to maximize analyte resolution.
- Minimal preparation and instrument error and $R^2 > 0.999$ were observed for every AA.

Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI 53706. *Corresponding author: arriolaapelo@wisc.edu. Received January 16, 2024. Accepted July 12, 2024.

The list of standard abbreviations for JDSC is available at adsa.org/jdsc-abbreviations-24. Nonstandard abbreviations are available in the Notes.

JDS Communications®
2024; 5:745–750

https://doi.org/10.3168/jdsc.2024-0546
Technical Note
Physiology

Fast and reliable method for analysis of derivatized plasma amino acids by liquid chromatography-single quadrupole-mass spectrometry

August Hoppmann and Sebastian I. Arriola Apelo*

Abstract: The pool of free, genetically encoded AA in plasma plays an essential role not only as substrate for every protein synthesized in the body but also as signaling molecules that regulate a wide range of physiological processes. Here we present a method for the analysis of 19 of the 20 encoded AA (except Cys) in dairy cow plasma. Isolated plasma or standards for the 19 AA were gravimetrically mixed with an internal standard mix consisting of ^{13}C isotopes of each AA. Plasma proteins were precipitated on acetonitrile and supernatants transferred to glass vials. For precolumn derivatization, plasma supernatants were buffered with sodium borate (pH 9.5–10), and AA were derivatized with 9-fluorenylmethoxycarbonyl (Fmoc) chloride. Analytes were isolated by solid-phase extraction using a strong-anionic ion exchange column and dry eluates were reconstituted in mobile phase consisting of 70% water solution of ammonium formate and 30% acetonitrile. Amino acid derivatives were separated by reverse-phase liquid chromatography over 17.5 min with a C18 column in which acetonitrile increased to 80% over the first 11 min of the method, before returning to initial levels. Electrospray ionization on negative mode was used for most AA, except Arg and Pro, for which positive mode yielded superior results. Single or double (only Lys) derivatives were measured by single quadrupole-mass spectrometry. We hypothesized that precolumn Fmoc derivatization would yield optimal resolution for quantitative analysis of the 19 targeted AA and their respective ^{13}C internal standards, with limits of quantitation beyond physiological ranges. All 19 AA were detected with minimal background noise. An 11-point standard curve was developed for each AA. Limits of quantitation were beyond concentrations observed in plasma samples of lactating dairy cows, except for Gly where upper curve points had to be removed to maintain linearity, limiting quantitation to the upper range of physiological concentration. After removing the 4 highest concentrations from the Gly standard curve, coefficients of determination were greater than 0.999 for all of the AA. Recovery of spiked AA from plasma samples ranged from 89.9% for Phe to 100.3% for Trp. Instrument repeatability averaged 0.91 and ranged from 0.33 for Val to 2.29 for Arg. Meanwhile, sample preparation method repeatability averaged 2.02 and ranged from 1.14 for Tyr to 3.34 for Arg. Although robust methods have been developed, they depend on either availability of sophisticated instruments, mostly limited to core facilities (i.e., tandem MS methods), long and expensive chromatography without specific internal standards for each AA (i.e., HPLC-ultraviolet and HPLC-fluorescence detector), or unstable derivatization (GC-MS). Here we describe a method with high throughput, stable derivatization, high precision and recovery, and potentially more affordable than most existing methods. This method could help dairy nutritionists to consider plasma AA information for diet formulation strategies, potentially reducing feeding costs and N emissions.

The pool of circulating free AA is the main substrate for protein synthesis in every cell of a mammalian organism. However, circulating AA are not only substrates for protein synthesis, but they are also signaling molecules that regulate a wide range of physiological and cellular functions (Pszczolkowski and Arriola Apelo, 2020). In the mammary glands, specific AA signal on several transduction pathways, including the mechanistic target of rapamycin, integrated stress response, and unfolded protein response, to not only regulate protein synthesis but also fatty acid and lactose synthesis (Cant et al., 2018; Pszczolkowski and Arriola Apelo, 2020). Beyond the mammary glands, AA have been shown to regulate insulin sensitivity, glucose and lipid metabolism, liver function, immune response, reproductive functions, and host-microbiome interactions (Koh et al., 2018; Yu et al., 2021; Chandler et al., 2022; Toledo et al., 2023). Amino acids have also been shown to be important biomarkers of performance and diseases associated with some of those functions (Sturmey et al., 2010; Masoodi et al., 2021). Hence, an affordable, reliable, rapid, and quantitative method for the analysis of free AA in circulation will contribute to the progress on several areas of dairy science research. Furthermore, with progress in modeling individual essential AA requirements (NASEM, 2021), timely information about circulating AA concentrations could help dairy nutritionists and producers to maximize production performance, improve profitability, reduce environmental footprint, and potentially prevent metabolic disorders.

There are several reliable and robust analytical methods to measure free AA in circulation. Colorimetric methods include the classical AA analyzer based on ninhydrin reaction (Le Boucher et al., 1997; Friedman, 2004) and o-phthalaldehyde 3-mercaptopropionic acid (OPA) derivatization (Frank and Powers, 2007). Alternatively, AA can be measured with a fluorometric detection of fluorenylmethoxycarbonyl (**Fmoc**) derivatives (Jámbor and Molnar-Perl, 2009). However, colorimetric/fluorometric-based methods cannot

Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI 53706. *Corresponding author: arriolaapelo@wisc.edu. Received January 16, 2024. Accepted July 12, 2024.

The list of standard abbreviations for JDSC is available at adsa.org/jdsc-abbreviations-24. Nonstandard abbreviations are available in the Notes.

differentiate co-eluting AA, requiring long chromatography separation (Schwarz et al., 2005) and, in the case of liquid chromatography (**LC**), high analysis cost. Furthermore, colorimetric methods typically relay on a single or limited number of internal standards (**ISTD**) that elute at a different time than the target analyte, depending on the assumption that the ISTD will have a similar linear range and response as the analyte.

Mass spectrometry identifies co-eluting analytes based on mass-to-charge ratio (***m*/*z***), overcoming the limitations indicated above. Using selected ion monitoring (**SIM**), the MS can change in milliseconds the target *m*/*z* measured, allowing for accurate quantification of co-eluting signals (Björkhem and Lawson, 1979). Therefore, MS reduces chromatography time and allows use of isotopes of target analytes as ISTD, which is critical for absolute quantitation as molecules of different chemical structure behave differently through ionization and quadrupole selection (Giovannini et al., 1991; Calder et al., 1999). However, accurate quantitation of a large panel of AA still requires some separation to reduce the number of target ions per segment, maintain monitoring frequency of each ion, and not compromise resolution and sensitivity. Gas chromatography is an older and more affordable separation method than LC, but it requires prior derivatization of AA for volatilization and thermal stability. N-(tert-Butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) has been extensively used as derivatization agent for AA analysis by GC-MS (Jiménez-Martín et al., 2012). However, derivatization of positive charged EAA Arg, His, and Lys is difficult and time consuming, and Trp is poorly detected. Liquid chromatography does not require derivatization because AA are dissolved in the mobile phase, but derivatization can improve chromatographic separation and ionization, enhancing sensitivity (Toledo et al., 2021). Recently, a LC-MS method based on 3-aminopyridyl-N-hydroxysuccinimidyl carbamate derivatization has been reported for the analysis of free AA in human plasma (Nakayama et al., 2019). Although the method has good repeatability and recovery, the quantitative range was not reported, and it is unclear if sensitivity is enough for dairy cow plasma.

Derivatization can be avoided with more sophisticated and expensive tandem mass spectrometers (**MS/MS**). In quadrupole MS/MS with multiple reaction monitoring, the ion selected in the first MS is fragmented in a collision cell and a fragment is targeted by the third MS for detection. Other tandem MS systems such as time of flight and Q-trap have been developed more recently but are beyond this discussion. Multiple reaction monitoring significantly reduces background, improving sensitivity of underivatized analytes and further reducing separation time. Although robust LC-MS/MS methods for analysis of free AA in plasma have been established, including with Fmoc derivatization (Ziegler et al., 2019), instrument availability and analysis cost per sample restrict its use to limited number of samples, mostly in research settings. To overcome the current limitations, the objective of this study was to develop a LC-MS method based on Fmoc-Cl derivatization, previously used for fluorometric detection, followed by ion exchange extraction of derivatized AA. Sample preparation is limited to 5 min per sample, plus 30 min of drying time for a batch of 48 samples, and 17.5 min of run time between injections, completing sample preparation and analysis in less than 25 min per sample. Nineteen of the 20 encoded AA (except Cys) can be detected with linear ranges beyond physiological concentrations in dairy cow plasma, except for Gly that can show saturation, requiring rerun of at high-concentration samples at lower injection volume. The R^2 is greater than 0.999 for all the AA measured, and recoveries ranging from 86.2 for Asp to 102.1% for Met, making it not only a rapid and affordable method, but also reliable for quantification.

For method development, we prepared standard stock solutions from individual dry AA (Sigma-Aldrich, St. Louis, MO) dissolved in ultra-pure water (18 MΩ.cm; used for every solution), at approximate proportions to a reference plasma profile in lactating dairy cows (Martineau et al., 2017). An algal AA mixture containing 17 universally labeled ^{13}C AA (CLM-1548–0.5) was combined with individually purchased $^{13}C_{11}$-Trp (CLM-4290-H-PK), $^{13}C_4$-Asn (CLM-8699-H-PK), and $^{13}C_5$-Gln (CLM-1822-H-PK), all from Cambridge Isotope Laboratories (Tewksbury, MA), for use as ISTD. Quality control (**QC**) plasma was pooled from samples obtained from lactating dairy cows consuming between 15% and 16.5% CP diets, aliquoted to avoid repeated freezes and thaws, and stored at −80°C. Plasma samples were collected for separate projects, all approved by the University of Wisconsin–Madison College of Life Sciences Institutional Animal Care and Use Committee (**IACUC**). Therefore, this study did not require IACUC approval.

For sample preparation, 85 mL of plasma or ^{12}C standard mix was spiked with 12 mL of ^{13}C labeled ISTD mix in a 1.5-mL microcentrifuge tube, and volumes were recorded gravimetrically. Plasma proteins were precipitated with 2.5 sample volumes of HPLC-grade acetonitrile (part no. 34967, Honeywell, Charlotte, NC), and pelleted by centrifugation at 14,000 × *g* for 5 min at 4°C. The supernatant was transferred to a 12 × 75 mm glass tube and buffered with 5 sample volumes of chilled 50 mmol/L sodium borate buffer (SXO355–1, Sigma-Aldrich) at pH 9.5 to 10. Derivatization was performed with the addition of 2.5 sample volumes of 8 mmol/L Fmoc-Cl (23186; Sigma-Aldrich) in acetonitrile. The solution was mixed gently on a shaker for 5 min. Solid-phase extraction was performed in 96-well plates containing 60 mg of 33 mmol/L strong anion exchange polymer (Strata X-A, #8E-S123-TGB, Phenomenex, Torrance, CA) using positive pressure (Phenomenex Part no. AH1–7033). Anion exchange columns were conditioned with 1.0 mL of HPLC-grade methanol (Honeywell 34966) and equilibrated with 1.0 mL of water, before loading samples onto the column. Subsequently, columns were washed with 1.0 mL of water and 1.0 mL of methanol before AA were eluted with 1.0 mL of 5% (vol/vol) formic acid (no. 85178, Fisher Scientific, Waltham, MA) in methanol. Eluates were dried under a stream of nitrogen at 37°C for 25 min or until completely dry, reconstituted in 80 µL of mobile phase, and transferred into a LC-vial insert for injection into the LC-MS for analysis.

The analytical instrument consisted of a Nexera-i-2040C LC coupled to a single quadrupole MS (LC-MS 2020, Shimadzu, Kyoto, Japan) equipped with electrospray ionization (**ESI**). A C18 reversed-phase column of 250 × 3 mm dimensions (Gemini NX-C18, 110 Å, particle size 3 µm; no. 00G-4453-Y0, Phenomenex) was used for analyte separation. Mobile phase A was a water solution of 10 mmol/L ammonium formate (no. CAS:540–69–2, Fisher Scientific), whereas pure acetonitrile was used for mobile phase B. Flow rate was set at 0.5 mL/min and oven temperature at 46°C. Chromatography conditions consisted of an initial mobile phase with 70% A and 30% B for 3.5 min, followed by a linear increase of B for 6.5 min to 40%; further increase of B to 80% over 1 min, to then decrease to 45% over 3 min, and finish with an

Table 1. Parameters for quantification of derivatized (deriv.) AA and their internal standards (ISTD) via liquid chromatography-MS

AA	AA MW[1]	^{12}C deriv. *m/z*	^{13}C deriv. *m/z* ISTD	Retention time (min)	Ionization mode[2]
Asp	133.1	354	358	3.6	–
Glu	147.1	368	373	4.126	–
Asn	132.1	353	357	6.1	–
Gln	146.2	367	372	6.235	–
His	155.2	376	382	6.5	–
Ser	105.1	326	329	6.581	–
Gly	75.1	296	298	7.05	–
Thr	119.1	340	344	7.24	–
Ala	89.1	310	313	7.35	–
Arg[3]	174.2	397	403	7.68	+
Pro[3]	115.1	338	343	7.68	+
Tyr	181.2	402	411	8.39	–
Val	117.1	338	343	9.3	–
Met	149.2	370	375	9.6	–
Ile	131.2	352	358	10.75	–
Leu	131.2	352	358	11.2	–
Trp	204.2	425	436	11.6	–
Phe	165.2	386	395	11.75	–
Lys[4]	146.2	589	595	13.775	–

[1]MW = molecular weight.

[2]Positive (+) or negative (–) electrospray ionization.

[3]Peaks for Arg and Pro produced higher signal and lower noise in positive ionization.

[4]Lys is doubly derivatized by Fmoc-Cl.

immediate drop to 30% B for 2.5 min to re-equilibrate the column. Mass spectrometer conditions included nebulizing gas, 1.5 L/min; drying gas, 15 L/min; desolvation line, 250°C; heat block, 400°C; detector voltage, 1.9 kV; and interface voltage, −3.5 kV.

Table 1 shows *m/z* of target analytes, including ^{13}C-labeled ISTD, retention time, and ionization mode. Retention times for each prospective peak were determined under SIM, targeting selected *m/z* values for each AA. The potential for multiple derivatizations was expected for AA with more than one amino group, and *m/z* corresponding to double derivatization were investigated for Arg, Asn, and Lys. Only Lys was found to produce better signal in the doubly derivatized form, and this *m/z* was subsequently used for analysis. Initial testing was performed in negative ESI and scan mode, as Fmoc derivatives were expected to form negatively charged ions for most AA. Low signal and poor peak shape prompted a shift to positive ESI mode for Arg and Pro, which improved both metrics. Peak identity was confirmed by analyzing linearity of peak areas for each AA across a range of concentrations, and further confirmed with the addition of co-eluting ^{13}C isotopes (Figure 1). Finally, the method was divided into 4 segments to limit the time between scans for a selected ion, and optimize the number of scans for each AA (Figure 1).

Limits of detection (not shown) and quantitation, as well as response curve parameters for each AA, were estimated using an 11-point standard curve with a range of ~0.005× to 5× reference dairy cow plasma concentration (Table 2). Plasma QC samples were prepared alongside ^{12}C standards to ensure that peak areas fell well within the linear range of each AA in plasma. If necessary, AA concentrations were adjusted in the ^{12}C standards to best fit within their respective limits of quantitation and QC concentrations. Injection volume for standards, QC, and experimental samples was set at 3 µL. However, for specific samples with very high or low concentration of specific AA due to the treatment interventions (e.g., abomasal infusion), injection volumes ranged from 1 to 5 µL to fit signal within the limits of quantitation of those AA. In optimal conditions, the signal for the ^{13}C ISTD would roughly match the signal seen in ^{12}C standards. The use of a more affordable universally labeled ^{13}C algal AA mix limited our ability to adjust ISTD for 16 of the 19 AA, except Asn, Gln, and Trp, resulting in an imperfect matching of ^{12}C to ^{13}C signal. However, we were able to ensure the ^{13}C signal fell within the linear range of signal for every AA.

The method showed excellent linearity within the range tested for each AA ($R^2 > 0.999$) except for Glu and Gly, which exhibited some loss of linearity. For Glu, the highest (366.8 µ*M*) and lowest (1.91 µ*M*) points were removed, leaving a 9-point curve that still covers the physiological range for that AA. For Gly, the 4 highest points (1,640.08 to 657.19 µ*M*) were removed, leaving a 6-point curve that fits tight with the physiological range, potentially requiring reruns at lower injection volume for high-concentration samples. Following the removal of the indicated standard curve points, Glu and Gly curves exhibited comparable fit to the other AA ($R^2 > 0.999$). Method accuracy was evaluated in 6 QC samples that were gravimetrically spiked with 2 different amounts of a standard containing the 19 AA plus the respective nonspiked controls that were used to determine the background plasma signal of each QC. A standard curve run along with those samples was applied to the spiked and nonspiked QC samples for the recovery calculation. Average recovery for individual AA ranged from 102.1% for Met to 86.2% for Asp. A deuterated ISTD for Met was also tested ($^2H_8$-Met, DLM-6797-PK) as a cheaper alternative to universally labeled ^{13}C AA. Although this ISTD exhibited comparable signal and peak shape to what was seen for other AA, recovery was substantially lower (data not shown). This is likely a result of losses due to the replacement of deuterium by hydrogen in the isotope during sample preparation or analysis.

Table 2. Individual AA quantitative range, R^2, qualitive control signal, instrument and method repeatabilities, and recovery

AA	Standard curve linear range (μmol/L)	Standard curve signal range (V/min)	R^2	QC[1] signal range (V/min)	QC (μmol/L)	Instrument repeatability (%)	Method repeatability (%)	Total repeatability (%)	Recovery (%)
Asp	0.27–52.6	12,592–2,150,700	0.9999	76,042–114,554	4.8	1.11	2.56	2.72	86.2
Glu[2]	3.81–292.89	70,562–8,592,941	0.9999	707,437–1,037,916	58.8	0.56	2.52	2.56	97.1
Asn	1.64–315.52	4,473–2,906,556	0.9999	87,995–147,824	53.6	1.53	1.94	2.31	94.5
Gln	4.45–855.74	16,060–7,262,293	0.9999	602,215–934,404	273.7	0.50	2.39	2.42	95.4
His	0.93–179.12	28,189–3,697,592	0.9998	446,655–568,939	41.1	1.13	1.99	2.19	97.6
Ser	2.8–538.34	25,254–5,854,739	0.9999	485,735–751,310	110.8	0.66	1.89	1.97	97.6
Gly[2]	8.24–328.35	13,932–457,164	0.9995	156,011–213,717	296.6	1.18	2.12	2.33	93.7
Thr	2.9–558.25	36,551–10,799,604	0.9998	203,194–373,786	85.9	0.77	2.43	2.51	99.7
Ala	6.14–1,180.91	35,808–4,506,686	0.9999	53,657–71,804	281.9	1.00	1.89	2.06	92.6
Arg	3.08–592.55	8,784–1,190,030	0.9999	56,582–81,641	62.2	2.29	3.34	3.83	90.4
Pro	2.65–508.63	140,316–14,267,622	0.9996	4,258,770–4,647,082	104.6	0.63	1.67	1.75	96.6
Tyr	2.1–403.09	7,743–1,578,059	0.9999	109,304–128,470	97.9	0.53	1.14	1.22	97.0
Val	6.57–1,262.37	57,661–5,073,884	0.9998	1,169,823–1,359,375	224.7	0.33	1.79	1.81	98.6
Met	0.48–93.18	7,212–670,589	0.9998	43,071–53,907	21.3	0.40	1.51	1.54	102.1
Ile	3.27–628.04	34,063–2,536,612	0.9998	819,336–908,762	116.2	0.49	1.68	1.72	98.6
Leu	4.06–779.37	25,759–1,893,897	0.9997	867,042–959,339	199.5	0.55	1.25	1.33	96.1
Trp	1.24–238.14	26,114–341,176	0.9993	98,759–137,102	43.4	1.68	1.65	2.15	91.2
Phe	1.34–258.36	17,766–1,588,804	0.9999	489,834–600,854	49.2	0.38	1.64	1.67	95.7
Lys	2.68–515.99	21,588–592,032	0.9994	46,301–102,596	84	1.62	2.94	3.22	96.7

[1]QC = quality control samples pooled from plasma samples from lactating dairy cows consuming 15% to 16.5% CP diets. Signal range and average concentration of 5 independent aliquots.

[2]Curve points were removed from Glu (highest and lowest: 366.8 and 1.91 μ*M*, respectively) and Gly (4 highest: 1,652.67 to 664.06 μ*M*) to preserve linearity.

The precision of the assay was evaluated based on the repeatability of the instrument, of the sample preparation method, and of the overall assay precision, as described by Chesher (2008). We calculated the instrument repeatability from 5 aliquots of the QC, each injected thrice into the LC-MS. Method repeatability was calculated as the standard deviation among the 5 independently prepared aliquots. The overall assay precision, defined as intralaboratory variation by Chesher (2008), was calculated from the combination of both sources of variation, instrument and method. The 3 precision variables were expressed as CV, relative to the overall mean of the 15 runs (5 aliquots in triplicate). The average instrument repeatability among the 19 AA was 0.91%. Arginine had the highest instrument repeatability (2.29%), followed second by Trp (1.68%), whereas 11 AA had instrument repeatability

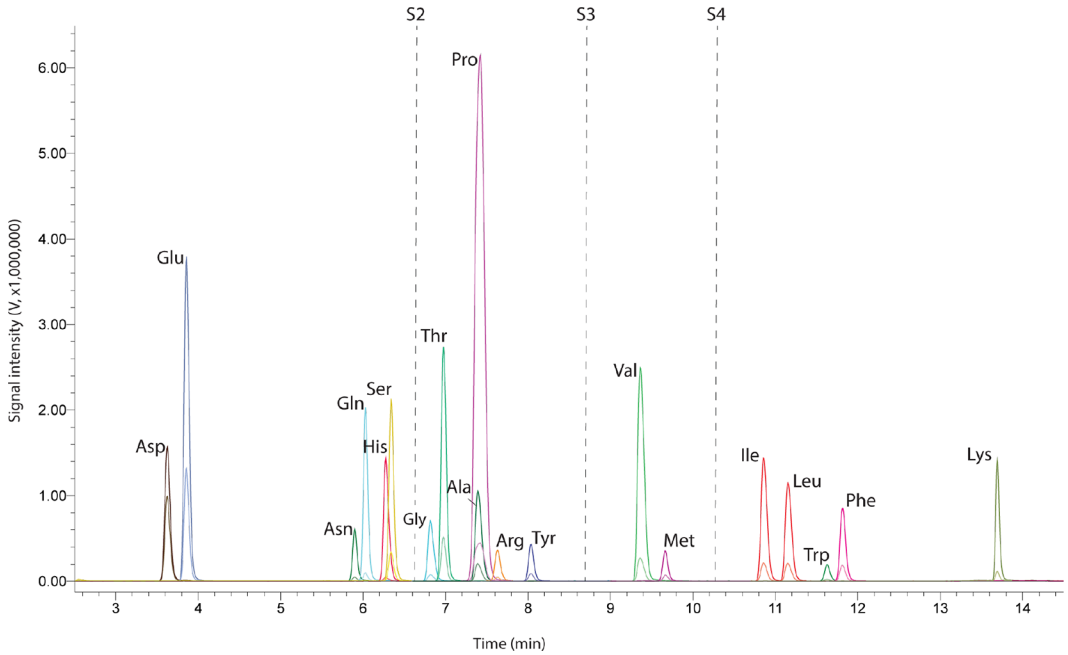


Figure 1. Reference chromatogram of 19 AA standards and their ^{13}C isotopes analyzed using selected ion monitoring. Co-eluting peaks of a similar color correspond to the ^{13}C isotopes. S2, S3, S4 = scanning segments.

≤1.00%, with Val having the lowest value (0.33%, i.e., highest precision). For sampling preparation method repeatability, we first removed the instrument error by averaging the 3 injections, and the error among the 5 aliquots was expressed as percent of the mean of the 5 aliquots. Arginine also had the largest method repeatability (3.34%), followed by Lys (2.94%), whereas Tyr, Leu, and Phe had the lowest (1.14%, 1.25%, and 1.64%, respectively), with an average method repeatability among the 19 AA of 2.02%.

In summary, we developed a robust and accurate method to measure AA in plasma with limits of quantitation exceeding physiological ranges of concentrations in dairy cows. Active, hands-on sample preparation can be completed in less than 5 min per sample when working on a batch of 48 samples, and running time is 17.5 min. Although several robust methods to measure free AA in biological samples are available, this method fills an existing gap in AA analysis for a more extended use, combining accuracy, precision, quantitation range, and speed. Importantly, this method can be run on a more ubiquitously available and affordable instrument than faster and more comprehensive methods, making it potentially available not only for research, but also for commercial settings.

References

Björkhem, I., and A. M. Lawson. 1979. Selective ion monitoring in clinical chemistry. CRC Crit. Rev. Clin. Lab. Sci. 11:53–105. https://doi.org/10.3109/10408367909105854.

Calder, A. G., K. E. Garden, S. E. Anderson, and G. E. Lobley. 1999. Quantitation of blood and plasma amino acids using isotope dilution electron impact gas chromatography/mass spectrometry with U-13C amino acids as internal standards. Rapid Commun. Mass Spectrom. 13:2080–2083. https://doi.org/10.1002/(SICI)1097-0231(19991115)13:21<2080::AID-RCM755>3.0.CO;2-O.

Cant, J. P., J. J. M. Kim, S. R. L. Cieslar, and J. Doelman. 2018. Symposium review: Amino acid uptake by the mammary glands: Where does the control lie? J. Dairy Sci. 101:5655–5666. https://doi.org/10.3168/jds.2017-13844.

Chandler, T. L., T. A. Westhoff, A. S. Sipka, T. R. Overton, and S. Mann. 2022. Lipopolysaccharide challenge following intravenous amino acid infusion in postpartum dairy cows: II. Clinical and inflammatory responses. J. Dairy Sci. 105:4611–4623. https://doi.org/10.3168/jds.2021-21227.

Chesher, D. 2008. Evaluating assay precision. Clin. Biochem. Rev. 29(Suppl. 1):S23–S26.

Frank, M. P., and R. W. Powers. 2007. Simple and rapid quantitative high-performance liquid chromatographic analysis of plasma amino acids. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 852:646–649. https://doi.org/10.1016/j.jchromb.2007.01.002.

Friedman, M. 2004. Applications of the ninhydrin reaction for analysis of amino acids, peptides, and proteins to agricultural and biomedical sciences. J. Agric. Food Chem. 52:385–406. https://doi.org/10.1021/jf030490p.

Giovannini, M. G., G. Pieraccini, and G. Moneti. 1991. Isotope dilution mass spectrometry: definitive methods and reference materials in clinical chemistry. Ann. Ist. Super. Sanita 27:401–410.

Jámbor, A., and I. Molnar-Perl. 2009. Quantitation of amino acids in plasma by high performance liquid chromatography: Simultaneous deproteinization and derivatization with 9-fluorenylmethyloxycarbonyl chloride. J. Chromatogr. A 1216:6218–6223. https://doi.org/10.1016/j.chroma.2009.06.083.

Jiménez-Martín, E., J. Ruiz, T. Perez-Palacios, A. Silva, and T. Antequera. 2012. Gas chromatography-mass spectrometry method for the determination of free amino acids as their dimethyl-tert-butylsilyl (TBDMS) derivatives in animal source food. J. Agric. Food Chem. 60:2456–2463. https://doi.org/10.1021/jf2052338.

Koh, A., A. Molinaro, M. Stahlman, M. T. Khan, C. Schmidt, L. Manneras-Holm, H. Wu, A. Carreras, H. Jeong, L. E. Olofsson, P. O. Bergh, V. Gerdes, A. Hartstra, M. de Brauw, R. Perkins, M. Nieuwdorp, G. Bergstrom, and F. Backhed. 2018. Microbially produced imidazole propionate impairs insulin signaling through mTORC1. Cell 175:947–961.e17. https://doi.org/10.1016/j.cell.2018.09.055.

Le Boucher, J., C. Charret, C. Coudray-Lucas, J. Giboudeau, and L. Cynober. 1997. Amino acid determination in biological fluids by automated ion-exchange chromatography: Performance of Hitachi L-8500A. Clin. Chem. 43:1421–1428. https://doi.org/10.1093/clinchem/43.8.1421.

Martineau, R., D. R. Ouellet, E. Kebreab, R. R. White, and H. Lapierre. 2017. Relationships between postruminal casein infusion and milk production, and concentrations of plasma amino acids and blood urea in dairy cows: A multilevel mixed-effects meta-analysis. J. Dairy Sci. 100:8053–8071. https://doi.org/10.3168/jds.2016-11813.

Masoodi, M., A. Gastaldelli, T. Hyotylainen, E. Arretxe, C. Alonso, M. Gaggini, J. Brosnan, Q. M. Anstee, O. Millet, P. Ortiz, J. M. Mato, J. F. Dufour, and M. Oresic. 2021. Metabolomics and lipidomics in NAFLD: Biomarkers and non-invasive diagnostic tests. Nat. Rev. Gastroenterol. Hepatol. 18:835–856. https://doi.org/10.1038/s41575-021-00502-9.

Nakayama, A., A. Imaizumi, and H. Yoshida. 2019. Methods for absolute quantification of human plasma free amino acids by high-performance liquid chromatography/electrospray ionization mass spectrometry using precolumn derivatization. Methods Mol. Biol. 2030:207–218. https://doi.org/10.1007/978-1-4939-9639-1_16.

NASEM (National Academies of Sciences, Engineering, and Medicine). 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. National Academies Press, Washington, DC.

Pszczolkowski, V. L., and S. I. Arriola Apelo. 2020. The market for amino acids: understanding supply and demand of substrate for more efficient milk protein synthesis. J. Anim. Sci. Biotechnol. 11:108. https://doi.org/10.1186/s40104-020-00514-6.

Schwarz, E. L., W. L. Roberts, and M. Pasquali. 2005. Analysis of plasma amino acids by HPLC with photodiode array and fluorescence detection. Clin. Chim. Acta 354:83–90. https://doi.org/10.1016/j.cccn.2004.11.016.

Sturmey, R. G., P. Bermejo-Alvarez, A. Gutierrez-Adan, D. Rizos, H. J. Leese, and P. Lonergan. 2010. Amino acid metabolism of bovine blastocysts: A biomarker of sex and viability. Mol. Reprod. Dev. 77:285–296. https://doi.org/10.1002/mrd.21145.

Toledo, M. Z., C. Nienow, D. Luchini, S. I. Arriola Apelo, and M. C. Wiltbank. 2021. Quantification of bovine plasma amino acids via liquid chromatography-electrospray ionization-mass spectrometry: Comparison of underivatized and precolumn derivatized methods. JDS Commun. 2:227–232. https://doi.org/10.3168/jdsc.2020-0060.

Toledo, M. Z., M. L. Stangaferro, R. Caputo Oliveira, P. L. J. Monteiro Jr., R. S. Gennari, D. Luchini, R. D. Shaver, J. O. Giordano, and M. C. Wiltbank. 2023. Effects of feeding rumen-protected methionine pre- and postpartum in multiparous Holstein cows: Health disorders and interactions with production and reproduction. J. Dairy Sci. 106:2137–2152. https://doi.org/10.3168/jds.2022-21950.

Yu, D., N. E. Richardson, C. L. Green, A. B. Spicer, M. E. Murphy, V. Flores, C. Jang, I. Kasza, M. Nikodemova, M. H. Wakai, J. L. Tomasiewicz, S. E. Yang, B. R. Miller, H. H. Pak, J. A. Brinkman, J. M. Rojas, W. J. Quinn 3rd, E. P. Cheng, E. N. Konon, L. R. Haider, M. Finke, M. Sonsalla, C. M. Alexander, J. D. Rabinowitz, J. A. Baur, K. C. Malecki, and D. W. Lamming. 2021. The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine. Cell Metab. 33:905–922.e6. https://doi.org/10.1016/j.cmet.2021.03.025.

Ziegler, J., H. Hussain, R. H. H. Neubert, and S. Abel. 2019. Sensitive and selective amino acid profiling of minute tissue amounts by HPLC/electrospray negative tandem mass spectrometry using 9-fluorenylmethoxycarbonyl (Fmoc-Cl) derivatization. Methods Mol. Biol. 2030:365–379. https://doi.org/10.1007/978-1-4939-9639-1_27.

Notes

August Hoppmann https://orcid.org/0009-0009-2223-926X

Sebastian I. Arriola Apelo https://orcid.org/0000-0003-0274-5367

The authors thank Phenomenex Inc. (Torrance, CA) for product support and Adisseo USA Inc. (Alpharetta, GA) for partial financial support for the development of this method.

The authors also thank Zachary Woodward and Sesha Raghavan Krishnamachari (Phenomenex Inc., Torrance, CA) for assistance with method development and Alison Snyder, Seth Frishman, Greg Robinson, and Caleb Nienow

(Shimadzu Scientific Instruments Inc., Columbia, MD) for input on instrument conditions and technical support.

Plasma samples were collected for separate projects, all approved by the University of Wisconsin–Madison College of Life Sciences IACUC. Therefore, this study did not require IACUC approval.

The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: ESI = electrospray ionization; Fmoc = 9-fluorenylmethoxycarbonyl; IACUC = Institutional Animal Care and Use Committee; ISTD = internal standard; LC = liquid chromatography; MS/MS = tandem mass spectrometers; *m*/*z* = mass-to-charge ratio; QC = quality control; SIM = selected ion monitoring.