A simple dilute-and-shoot LCMS method for the determination of free and modified amino acids in dietary supplements

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1. Overview

We present here the direct determination of free and modified amino acids typically found in commercially available dietary supplement formulations such as tablets, capsules, and powders using liquid chromatography-mass spectrometry (LCMS).

2. Introduction

Dietary protein is composed of 20 different amino acids. The consumption of amino acids individually has been proposed to enhance performance and prevent mental fatigue. Amino acids are among the top five most popular sports supplements. Different formulation types are currently available in the market such as tablets, capsules, and powders. We report here a LCMS method that can analyze all 20 L-amino acids and modified amino acids in a single analytical run in various formulations of dietary supplements.

Figure 1. Graphical representation of easy sample preparation and analysis by LCMS-2020 Single Quadrupole Mass Spectrometer

3. Method

The amino acids standards were purchased from Sigma Aldrich (St. Louis, MO). Various dietary supplement formulations- powder, capsule, and tablet were obtained from online store. Samples were prepared by extraction with 0.1N HCl followed by dilution in initial mobile phase composition. A Nexera-i LC coupled to a single-quadrupole LCMS-2020 instrument was used to analyze the samples. The samples were eluted using a binary gradient with the LCMS operating in scan and SIM mode. Both positive and negative polarity were used.

Table 1. Instrument parameters used for the analysis of amino acids in dietary supplements.

Nexera –i 2040-C 3D		LCMS-2020	
Column:	Sigma Aldrich Discovery column (150 × 2.1 mm; 5 µm)	Nebulizing Gas:	1.5 L/min
Mobile phase:	A: 0.1 % Formic acid in water B: 0.1 % Formic acid in acetonitrile	Interface Temp.:	250 °C
Flow Rate:	0.2 mL/min	DL Temp.:	250 °C
Oven Temp.:	40°C	Heat Block Temp.:	400 °C
Injection volume	0.5 µL	Ionization mode	ESI positive and negative SIM

4. Results and Discussion

A SIM method was developed to monitor free and four modified amino acids listed in the table of contents for the amino acid dietary supplement tablet, capsule, and powder. Additionally, taurine and caffeine listed in the contents were also monitored. Table 1 shows the names, mode of analysis, m/z monitored for the SIM analysis and the retention time of each analyte.

Table 1. List of unmodified and modified amino acids, their ionization mode, SIM m/z, calibration range with r value and retention times used in this method.

S.No.	Analyte	Mode	m/z	R_t (~min)	Calibration range (µg/mL)	r value
1	L-Alanine	ESI-Pos	90.1	2.040	0.1-25	0.996
2	L-Arginine	ESI-Pos	175.1	1.971	0.1-5	0.988
3	L-Asparagine	ESI-Pos	133.1	1.981	0.1-5	0.995
4	L-Aspartic Acid	ESI-Pos	134.0	2.010	0.1-5	0.983
5	L-cysteine	ESI-Pos	122.0	2.110	0.5-10	0.997
6	L-cystine	ESI-Pos	241.0	1.922	0.1-10	0.986
7	L-Glutamic Acid	ESI-Pos	148.1	2.089	0.1-10	0.988
8	L-Glutamine	ESI-Pos	147.1	2.018	0.1-10	0.995
9	Glycine	ESI-Pos	76.0	1.958	0.1-25	0.993
10	L-Histidine	ESI-Pos	156.1	1.927	0.1-25	0.995
11	L-Hydroxyproline	ESI-Pos	132.1	2.048	0.1-10	0.997
12	L-Isoleucine	ESI-Pos	132.1	4.039	0.1-25	0.999
13	L-Leucine	ESI-Pos	132.1	4.427	0.1-25	0.999
14	L-Lysine	ESI-Pos	147.1	1.849	0.1-25	0.999
15	L-Methionine	ESI-Pos	150.1	3.053	0.1-25	0.999
16	L-Phenylalanine	ESI-Pos	166.1	8.363	0.1-25	1.000
17	L-Proline	ESI-Pos	116.1	2.321	0.1-25	0.997
18	L-Serine	ESI-Pos	106.0	1.966	0.5-25	0.970
19	L-Threonine	ESI-Pos	120.1	2.037	0.5-10	0.980
20	L-Tryptophan	ESI-Pos	205.1	11.076	0.1-25	0.999
21	L-Tyrosine	ESI-Pos	182.1	4.732	0.1-25	0.999
22	L-Valine	ESI-Pos	118.1	2.710	0.1-25	0.999
23	Caffeine	ESI-Pos	195.1	11.695	0.1-25	0.997
24	Taurine	ESI-Pos	126.0	1.986	0.5-10	0.908
25	DL-α-hydroxymethionine	ESI-Neg	149.2	8.640	*	*
26	Ketoisoleucine	ESI-Neg	129.1	9.983	*	*
27	Ketoleucine	ESI-Neg	129.1	10.882	*	*
28	α-ketovaline	ESI-Neg	115.1	5.770	*	*

*Reference calibration curve was used for quantification of these compounds.

- A one-step sample preparation was employed for extraction of the amino acids from the formulations.
- Samples were analyzed by simple dilute-and-shoot method.
- Minimal sample manipulation allowed for maintaining sample integrity and reliable quantitation.
- Analytes with similar m/z values such as hydroxyproline, leucine and isoleucine or ketoisoleucine and ketoleucine were chromatographically separated.
- Minimal sample manipulation allowed for maintaining sample integrity and reliable quantitation.
- For SIM chromatograms where more than one peak was observed, the amino acid peak was confirmed based on the retention time observed for the standard.
- Table 2 shows example data obtained for one capsule sample and compared with label claims. Figure 2 shows an example overlay SIM chromatogram for capsule, powder, and tablet samples for α-ketovaline.

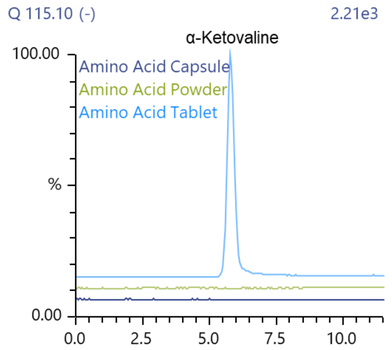

Figure 2. Representative SIM chromatogram capsule, powder, and tablet formulation demonstrating the presence of α-Ketovaline in tablet but not in capsule and powder formulations.

Table 2. Quantitation and comparison of amino acid ingredients reported in capsule formulation.

S.No.	Analyte	Reported (mg)	Calculated (mg)
2	L-Arginine	12.5	17.5
4	L-Aspartic Acid	35.0	37.0
5	L-cysteine	7.5	9.5
6	L-cystine	10.0	11.0
7	L-Glutamic Acid	45.0	72.5
9	Glycine	17.5	19.0
10	L-Histidine	12.5	17.5
12	L-Isoleucine	32.5	34.5
13	L-Leucine	40.0	44.0
14	L-Lysine	24.0	36.0
15	L-Methionine	15.0	10.5
16	L-Phenylalanine	27.5	28.0
17	L-Proline	20.0	21.5
18	L-Serine	40.0	49.5
19	L-Threonine	25.0	30.5
21	L-Tyrosine	22.5	22.0
22	L-Valine	37.5	41.0
24	Taurine	12.5	13.0

5. Conclusions

- A rapid, simple, and easy to execute SIM method was developed on the Shimadzu LCMS-2020 single quadrupole mass spectrometer for the analysis of dietary supplements.
- The method utilizes fast polarity switching of the LCMS-2020 to analyze all the free amino acids, modified amino acids, and excipients listed in the contents.
- The greatest advantage of SIM method lies in the fact that it allows for correct identification of the analyte even if they are not fully chromatographically resolved. Moreover, the analytes with similar m/z values such as in the case of hydroxyproline, leucine and isoleucine chromatographic method provided good resolution to distinguish each amino acid.
- All the reported components in the tablet, capsule, and powder formulation were extracted and successfully identified.
- The rapid extraction and chromatography, coupled with simplicity of execution on LCMS-2020 makes this analytical method easy to execute in any analytical laboratory.

Suggested Reading

Chitranshi, P. and Gilles, C.T. Direct determination of free and modified amino acids in dietary supplements using the LCMS-2020 single quadrupole mass spectrometer. SSI-LCMS-111 Application News.