SHIMADZU
Excellence in Science

Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer

Analysis of Ornithine in *Bunashimeji* (Beech mushroom) Using Triple Quadrupole LC/MS/MS

H. Horiike

User Benefits

- The LC/MS/MS method enables highly sensitive analysis (sample conversion concentration: 50 mg/kg or less) of ornithine without derivatization.
- In addition to high sensitivity analysis, high speed analysis (13 min) is also possible with this system.

■ Introduction

Ornithine is a type of free amino acid that exists in large amounts in *shijimi* (Corbicula leana), etc., and has been widely recognized as an effective component for improving liver function in recent years. Ingestion of ornithine is expected to have various positive health benefits, including recovery from fatigue, improvement of the quality of sleep, and enhanced secretion of growth hormones. Ornithine plays an important role in supporting the functioning of the liver, which metabolizes ammonia, a harmful substance formed in the human body.

This article introduces an example of an analysis of the edible mushroom known as *bunashimeji* (beech mushroom), which is said to have an ornithine content at least 5 times greater than that of the *shijimi*. The analysis was carried out in accordance with the Japanese Agricultural Standard (JAS0016)* "Determination of the ornithine in mushroom (*Hypsizygus marmoreus*) — High-performance liquid chromatographic method," which was announced as Ministry of Agriculture, Forestry and Fisheries Notification No. 445 on March 31, 2021, using a Shimadzu LCMS-8060NX.

* Shimadzu Corporation participated in the interlaboratory collaboration in the establishment of this JAS standard.

■ Sample Preparation

Fig. 1 shows the sample preparation protocol. In this experiment, the *bunashimeji* samples were homogenized by uniform pulverization, enabling a comparatively simple extraction operation using dilute hydrochloric acid, and theanine was used as an internal standard.

Homogenization	Remove the hard tip of the mushroom stem. Pulverize uniformly.
Extraction ①	Weigh out 2 g of the sample. Add 15 mL of dilute hydrochloric acid. Shake for 15 min.
Preparation of constant volume	Using a 50 mL flask, prepare a constant volume sample using dilute hydrochloric acid.
Separation/ extraction ②	Transfer 15 mL of the constant volume sample to a centrifugal container. Centrifuge for 10 min (13000 x g). Filter the supernatant.
Addition of internal standard	Dilute the extract uniformly 100 times. Add the internal standard theanine.
Test sample	Measure using LC/MS/MS.

Fig. 1 Sample Preparation Protocol

■ Analysis Conditions

Table 1 shows the analysis conditions conforming to JAS 0016.

Table 1 Analysis Conditions

Analysis column	: Shim-pack™ GIST Amide (100 mm × 2.1 mmI.D., 3.0 µm, P/N: 227-30818-04)	
Mobile phases	: A 0.3% formic acid (aq.) B Acetonitrile containing 0.3% formic acid	
Time program	: B.conc 90% (0 min) - 50% (5 - 8 min) – 90% (8.01 - 13 min)	
Flow rate	: 0.30 mL/min	
Column temperature	: 40 ˚C	
Injection volume	: 0.5 µL	
Ionization	: ESI Positive	
Interface voltage	: +4 kV	
Focus voltage	: +2 kV	
Interface temperature	: OFF	
DL temperature	: 200 ˚C	
Block heater temperature	: 400 ˚C	
Heating gas flow	: 10 L/min	
Nebulizing gas flow	: 2 L/min	
Drying gas flow	: 10 L/min	
ESI position	: +3 mm	
MRM transition	: Ornithine Theanine	(-) *m/z* 133>70 CE -22 V (+) *m/z* 175>84 CE -21 V

■ MRM Chromatogram of Standard Sample

Fig. 2 shows the MRM chromatogram of the standard solution of ornithine to which the internal standard theanine was added. Under these analysis conditions, ornithine and theanine separated and were eluted at around 5 min and 3.5 min, respectively.

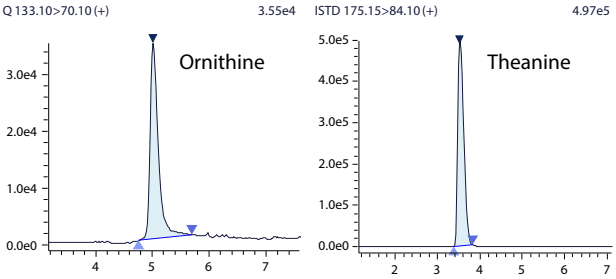


Fig. 2 MRM Chromatogram
(Ornithine, theanine, 20 ng/mL each)

■ Calibration Curve (Internal Standard Method)

Fig. 3 shows the calibration curve obtained by the internal standard method using theanine as the internal standard.

Good linearity was obtained in the calibration curve range of 20 to 2000 ng/mL, as indicated by the coefficient of correlation R>0.9999.

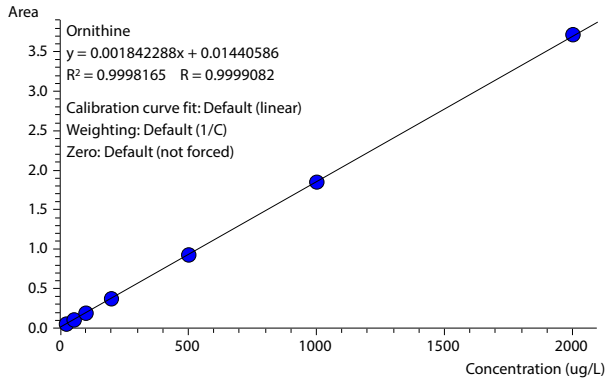


Fig. 3 Calibration Curve (7 Points) of Ornithine by Internal Standard Method (Ornithine: 20 to 2000 ng/mL)

■ Results of Quantitative Analysis of Ornithine in Commercial *Bunashimeji*

Fig. 4 shows the MRM chromatograms of 5 test samples (dilution: 100x) of commercially-available *bunashimeji* obtained by sample preparation by the above-mentioned protocol. The results of the quantitative analysis of the samples are also shown in Table 2.

The ornithine content of the *shijimi* is generally considered to be approximately 100 to 150 mg/kg. In the results of this experiment, the ornithine content in the *bunashimeji* samples was in the range of 779 to 2198 mg/kg.

Thus, it can be understood that *bunashimeji* contains roughly 7 to 14 times more ornithine than *shijimi*.

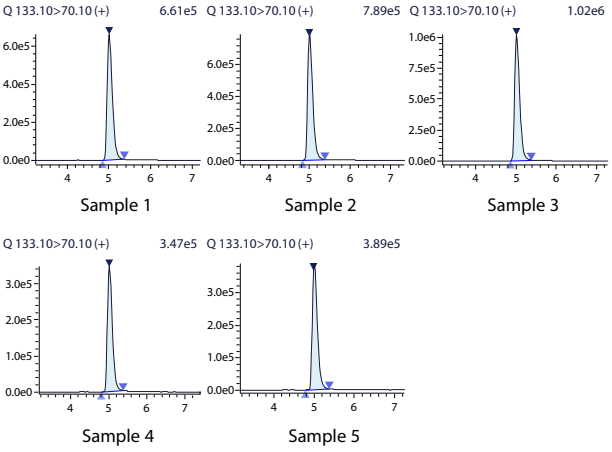


Fig. 4 MRM Chromatograms of 5 Test Samples

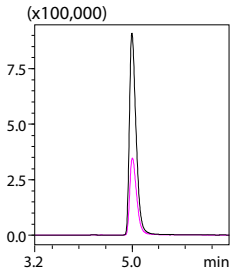
Table 2 Quantitative Values of Test Samples (Conversion to Concentration in *Bunashimeji*)

Unit: mg/kg

Sample No.	1	2	3	4	5
Concentration	1427	1782	2198	779	832

■ Validity Evaluation

A spike-and-recovery test was conducted using the *bunashimeji* test samples. Three of the samples (Nos. 1 to 3) were spiked with the standard solution to obtain an ornithine spiked concentration of 50 ng/mL in each sample. Fig. 5 shows the MRM chromatogram and the spike-and-recovery test results obtained by these measurements.



Unit: ng/mL

Sample No.	Spiked concentration (measured value)	Recovery rate
1	51.4	103%
2	51.5	103%
3	48.6	97%

Fig. 5 MRM Chromatogram (Sample No. 1) and Recovery Rates of Spike-and-Recovery Test Samples and Ornithine-Spiked Test Samples

Table 4 shows the results of QC measurements of the calibration curve sample with the intermediate concentration of 100 ng/mL before, during, and after continuous measurement of the test sample. Good results were obtained, as accuracy was > ±10%.

Table 4 Results of QC Measurements (STD 100 ng/mL)

	Measured concentration	Accuracy (%)
Before measurement of test sample	102.63	103%
During measurement of test sample	106.34	106%
After measurement of test sample	107.63	108%

■ Conclusion

In this experiment, an analysis was carried out using an LCMS-8060NX under appropriate analytical conditions in accordance with the Japanese Agricultural Standard JAS 0016, and the possibility of analyzing ornithine in *bunashimeji* with high sensitivity and good accuracy was confirmed.

Although *shijimi* clam (Corbicula leana) is well known as a food with a high ornithine content, it was found that *bunashimeji* tends to have a substantially higher content than *shijimi*.

In the future, when greater attention is focused on *bunashimeji* and many opportunities for measurement are foreseen, we recommend measurement using a tandem mass spectrometer, which enables simple analysis without derivatizing samples.

<Reference>
Notification of the Ministry of Agriculture, Forestry and Fisheries (MAFF): Establishment of Japanese Agricultural Standard "Determination of the ornithine in mushroom (*Hypsizygus marmoreus*) — High-performance liquid chromatographic method," (MAFF Notification No. 445, March 31, 2021)

[FAMIC] Commentary on JAS 0016 Determination of the ornithine in mushroom (*Hypsizygus marmoreus*)

<YouTube>
https://www.youtube.com/watch?v=yw0tbbABBp0
in Japanese (Sept. 30, 2021)

01-00175-EN First Edition: Nov. 2021

SHIMADZU
Shimadzu Corporation
Analytical & Measuring Instruments Division
Global Application Development Center

www.shimadzu.com/an/