A ultrahigh performance liquid chromatography coupled with quadrupole-time of flight mass spectrometry (UPLC-Q-TOF/MS) method was developed for simultaneous determination of 8 endogenous alkaloid compounds in 

Boletus samples were extracted by 50% (V/V) methanol-water solution, then separated by CORTECS UPLC HILIC column using a binary solvent system by gradient elution. The analytes were determined by Q-TOF/MS in TOF MS and information dependent acquisition (IDA)-MS/MS mode. The results showed that mass accuracy error of the 8 endogenous alkaloids were lower than $5.0 \times 10^{-6}$, good linear relationship was got in range of 0.2–500 μg/L, and correlation coefficient was higher than 0.9990. The limit of detection was in the range of 0.002–0.100 mg/kg and the limit of quantification was in the range of 0.004–0.200 mg/kg. Recovery of the method was in range of 80.1%–101.5% with spike levels of 0.004–2.00 mg/kg, relative standard deviations were lower than 10%. The method was simple, specific, and reliable. It could be used for the rapid screening and quantitative analysis of 8 endogenous alkaloids in 

Boletus.

1. Introduction

The 

Boletus is an edible fungus belonging to the 

Fungi, Basidiomycotina, Hymenomycetes, Agaricaceae [1]. It is mainly distributed in Yunnan Province of China. Due to a wide variety of secondary metabolites from fungus and their biological activities, extensive attention has been paid to the researches of fungus both in China and abroad [2]. 

Boletus is popular because of its delicious taste, appreciated nutrition value [3], and a variety of medicinal effects [4]. The chemical composition of the 

Boletus was mainly terpenes, flavonoid [5], phenols [6], and alkaloids, which possessed comprehensive biological activities, such as antioxidant, antifatigue, antitumor, and anti-inflammatory [7, 8]. Generally, the species for which a particular alkaloid structure is characteristic are closely related and can be comprised within the next higher taxon, genus, or family.

Alkaloids have been reported as a group of basic organic substances of 

Boletus, containing at least one nitrogen atom in a ring structure in the molecule [9]. A number of alkaloids were discovered and reported with biological activities in 

Boletus. Nicotine is an important and most well-known alkaloid, and it is the most important alkaloid in tobacco. In addition, nicotine is also widely present in 

Boletus and other plants. Some countries restricted the nicotine content in some foods. For example, the European Commission stipulated that the maximum limit of nicotine in edible fungus fresh and dry products is 0.036 mg/kg and 1.17 mg/kg, while the maximum limit in 

Boletus dried product is 2.3 mg/kg. Besides nicotine, 

Boletus contains some important and
pharmacologically secondary alkaloids. Choline is an indispensible substance for normal metabolism, which has the functions of promoting brain development and improving memory. *Boletus* is rich in amino acids. Arginine and ornithine produce putrescine under the decarboxylation of bacterial amino acid decarboxylase. Tryptamine is a monoamine alkaloid, based around the indole ring structure. It is regarded as the backbone for tryptamines, a group of compounds that include many pharmacologically active compounds, including serotonin (neurotransmitters), melatonin (hormone), and psilocybin (psychedelic drugs) [10]. Du et al. [11] isolated nicotinamide from the *Boletinus pictus*. Zhang et al. [12] found that anabasine, cotinine, and muscarine exist in *Boletus*. According to references, nicotine, anabasine, choline, tryptamine, putrescine, nicotinamide, muscarine, and cotinine can be detected in *Boletus*. However, due to a lack of research on the detection of alkaloids in *Boletus*, the inconsistency of the species, and the maturity of the tested samples, there are certain differences in the data.

At present, there are few studies on the detection of endogenous alkaloids in *Boletus*. The analytical methods of these alkaloids mainly include gas chromatography [13–15], high-performance liquid chromatography [16–21], and liquid chromatography-tandem mass spectrometry [22–28]. However, gas chromatography requires derivation and determination, and the process is relatively cumbersome; high-performance liquid chromatography has poor anti-interference ability [29]. Liquid chromatography-tandem mass spectrometry has a limited ability to identify isomers, and the ion dwell time is limited due to the scan rate. UPLC-Q-TOF/MS has the characteristics of high resolution, high sensitivity, high accuracy, and wide scanning range [30]. It has been widely used in quantitative analysis of pesticide residues, veterinary drug residues, and other research fields [31, 32]. In this paper, UPLC-Q-TOF/MS was first used for fast and accurate determination of endogenous alkaloids in the *Boletus*. It can obtain accurate and stable test results and provide reliable technical support for enterprises and regulatory authorities. This study lays the foundation for the development and establishment of quality standards in *Boletus* resources, provide a reference for comprehensive quality control and evaluation of *Boletus* and its products, and promote sustainable development of the industry.

2. Materials and Methods

2.1. Materials and Reagents. Methanol, acetonitrile, ethanol, and formic acid were HPLC grade and were purchased from Merck (Darmstadt, Germany). Water was purified using a Milli-Q System (Millipore, Guyancourt, France). Ammonium acetate (NH₄OAc) was purchased from Beijing Chemical Reagent Factory (Beijing, China). Formic acid was purchased from Duksan Pure Chemicals (Ansan, Korea). Eight alkaloids reference materials with purity ≥95% were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

2.2. Instruments and Equipment. The high-speed refrigerated centrifuge (CR22N, HITACHI, Germany), the vortex mixer (Vortex Genius 3, IKA, Germany), and the ultrasonic cleaner (Elmasonic P300H, Elma, Germany) were used in the procedure of extraction. The separation of compounds was carried out on a LC-30AD UPLC system equipped with a binary solvent manager, sample manager, and column manager (Shimadzu, Japan). Quantitative analysis of target compounds was conducted on a TripleTOFTM 5600+ quadrupole/time of flight mass spectrometry (AB Sciex, USA).

2.3. Sample Preparation. The sample was pulverized uniformly on a small pulverizer, and one gram of pulverized sample was weighed and transferred to a 50 mL centrifuge tube. Following spiking 20 mL of 50% methanol-water solution, vortexing for 1 min, and sonication for 20 min, the sample was centrifuged at 8000 r/min for 5 min. After centrifugation, the supernatant was filtered through a 0.22 μm nylon membrane before UPLC-Q-TOF/MS detection.

2.4. Chromatographic Conditions. The chromatographic separation was performed on a CORTECS UPLC HILIC column (100 mm × 2.1 mm, 1.6 μm; Waters, USA). The column temperature was 40°C. The injection volume was 5.0 μL. The flow rate was 300 μL/min. 20 mmol/L NH₄OAc solution containing 0.1% (v/v) formic acid (phase A) and acetonitrile containing 0.1% (v/v) formic acid (phase B) were used as mobile phase. The consecutive program was as follows: 0–3.00 min, 85% B; 3.0–6.0 min, 85% to 60% B; 6.0–11.0 min, 60% B; 11.0–12.0 min, 60% to 85% B; 12.0–15.0 min, 85% B.

2.5. Mass Spectrometry Conditions. The MS analysis was performed using an electrospray ion source (ESI) in positive ionization mode. The optimized parameters of ion source were as follows: the ionization voltage was 5.5 kV, the source temperature was 400°C, the pressure of curtain gas was 35 psi, the pressure of nebulizer gas was 50 psi, and the pressure of auxiliary gas was 55 psi; TOF MS conditions were as follows: scan range was 50–300 m/z, duration time was 15 min, and accumulation time was 0.15 s. IDA-MS/MS conditions were as follows: accumulation time was 0.05 s, high sensitivity mode was set, exclude isotopes were within ±50 m/z, duration time was ∼500 m/z, and accumulation time was 0.15 s. IDA-MS/MS conditions were as follows: accumulation time was 0.05 s, high sensitivity mode was set, exclude isotopes were within ±50 m/z, duration time was ∼500 m/z, and accumulation time was 0.15 s.

2.6. Optimization of Sample Extraction. To find out an optimum extraction method for samples, this experiment investigated the extraction effect when acetonitrile, methanol, ethanol, water, 50% acetonitrile-water solution, 50% methanol-water solution, and 50% ethanol-water solution were used as the extraction solvent, and the extraction time of 10, 15, 20, and 30 min was compared.
2.7. Optimization of Liquid Chromatography and Mass Spectrometry Conditions. Three analytical columns were tested prior to the selection of any additional chromatographic parameters, the Acquity BEH C18 column (100 mm × 2.1 mm, 2.5 μm), the Acquity UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm), and the Cortecs UPLC HILIC column (100 mm × 2.1 mm, 1.6 μm) were used. The mobile phase was evaluated. First, methanol and acetonitrile were tested as organic solvents in the mobile phase. In relation to the aqueous phase, an aqueous solution with various concentrations of NH₄OAc (0, 5 mmol/L, 10 mmol/L, 15 mmol/L, 20 mmol/L) were tested.

Syringe injection was used to inject 8 endogenous alkaloids directly into the mass spectrometer. The experiment investigated the response of target compounds under positive and negative ionization modes. For this method, TOF MS and IDA MS/MS modes were adopted for the detection of a target analyte. Under TOF MS mode, the experiment investigated the response of target compounds under different declustering potentials (50–300 V) and found the declustering potentials with the highest response.

2.8. Method Validation. Validation of the whole analytical method was performed with linearity, the limit of detection (LOD), the limit of quantification (LOQ), precision, accuracy, and recovery. The linearity of the method was evaluated by constructing calibration curves with different concentrations of 8 alkaloids. The LOD and LOQ under the present chromatographic conditions were calculated on the basis of the response and slope of each regression equation at signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively. The recoveries of analytes were evaluated by adding the standard solutions with three different concentration levels (1x LOQ, 3x LOQ, 10x LOQ) to the known amounts of fungus samples, and each level was repeated six times. The precision of the method was expressed by a relative standard deviation (RSD).

### Table 1: Mass parameters for the 8 endogenous alkaloids.

| Compound name | Formula      | Adduct/charge | Theoretical mass (m/z) | Experimental mass (m/z) | Mass error (×10⁻⁶) | Retention time (min) |
|---------------|--------------|---------------|------------------------|-------------------------|---------------------|----------------------|
| Nicotine      | C₁₀H₁₄N₂     | [M + H]⁺      | 163.123                | 163.1231                | 0.9                 | 4.69                 |
| Anabasine     | C₁₀H₁₂N₂     | [M + H]⁺      | 161.107                | 161.1069                | −2.5                | 2.97                 |
| Choline       | C₆H₆N₂      | [M + H]⁺      | 104.107                | 104.1071                | 1.5                 | 4.30                 |
| Tryptamine    | C₁₀H₁₂N₂     | [M + H]⁺      | 161.107                | 161.1075                | 0.9                 | 1.80                 |
| Putrescine    | C₆H₁₂N₂     | [M + H]⁺      | 89.107                 | 89.1073                 | −0.1                | 6.55                 |
| Nicotinamide  | C₉H₂ₐNO₂⁺    | [M + H]⁺      | 123.055                | 123.0554                | 0.8                 | 1.04                 |
| Muscarine     | C₁₀H₂ₐNO₂⁺   | [M + H]⁺      | 174.149                | 174.1491                | 0.6                 | 3.79                 |
| Cotinine      | C₁₀H₁₂N₂O    | [M + H]⁺      | 177.102                | 177.1025                | 1.4                 | 1.34                 |

### Figure 1: Effect of different solvent on the recoveries of 8 endogenous alkaloids.

### Figure 2: Comparison of extraction efficiency at extraction times.
Figure 3: Continued.
3. Results and Discussion

3.1. Optimization of Sample Extraction

3.1.1. Optimization of Extraction Solvents. As Figure 1 shows, the extraction efficiency of extraction solvents for various endogenous alkaloids was different. 50% methanol-water solution yielded the best reproducibility and recovery of the 8 alkaloids. Therefore, a 50% methanol-water solution was selected as the extraction solvent.

3.1.2. Optimization of Extraction Times. As Figure 2 shows, when the ultrasonic extraction time was increased from 10 min to 20 min, the extraction efficiency of alkaloids was greatly improved. After 20 min, the growth trend decelerates, and the extraction efficiency of alkaloids changes little. Considering the extraction efficiency of the experiment and time cost, 20 min was chosen as extraction time.

3.2. Optimization of Liquid Chromatography and Mass Spectrometry Conditions

3.2.1. Optimization of Liquid Chromatography Conditions. The results showed that the nicotine, anabasine, choline, tryptamine, putrescine, and muscarine were not retained on the Acquity BEH C18 column and the Acquity UPLC HSS T3 column. They can be retained on the CORTECS UPLC HILIC column, and the separation effect was good. Because they are strong polar hydrophilic compounds, which were weakly retained on the C18 and T3 columns, so it is necessary to replace hydrophilic columns for separation. Therefore, the CORTECS UPLC HILIC column (100 mm × 2.1 mm, 1.6 μm) was selected as the analytical columns.

The mobile phase was evaluated. First, methanol and acetonitrile were tested as organic solvents in the mobile phase, observing that methanol and acetonitrile as mobile phases have little effect on the signal intensity, but when methanol was used as the mobile phase for gradient elution, column pressure varies widely, and equilibration takes
Figure 4: Continued.
longer. In relation to the aqueous phase, the results showed 20 mmol/L NH₄OAc as the aqueous solution showed better separation and elution capabilities. Considering that under the positive mode, the addition of formic acid could increase the ionization of the compounds, which improved the separation efficiency and the intensity of the mass spectrometry signal. Therefore, 20 mmol/L NH₄OAc solution containing 0.1% (v/v) formic acid and acetonitrile containing 0.1% (v/v) formic acid were selected as the mobile phase for subsequent experiments.

3.2.2. Optimization of Mass Spectrometry Parameters.

The TripleTOF™ 5600® instrument has a CDS automatic calibration infusion system. The reference spray of the DuoSpray™ ion source was used to input the calibration solution for automatic system calibration. The DuoSpray™ ion source has two types: ESI and atmospheric pressure chemical ionization source (APCI). In this experiment, the ESI was selected as the detection ion source, and the APCI was used as the calibration ion source. Through the automatic calibration system, automatic batch calibration was performed to ensure the accurate mass of the system was stable for a long time. The results showed that compounds have a higher response in positive ionization mode, so the positive ionization mode was used for detection. Under TOF MS mode, the experiment investigated the response of target compounds under different declustering potentials (50–300 V), with the findings that at the fragmentor voltage of 80 V, compound response was the highest; relatively low fragmentor voltage was unfavorable for ion transmission, and overly high fragmentor voltage would cause the compound to fragment within the source. The accurate mass, retention time, and isotope ratio was obtained. Figure 3 shows the extracted ion chromatograms of 8 alkaloids (500 ng/mL). The accurate mass deviations of target compounds were less than $5.0 \times 10^{-6}$ (Table 1). The MS/MS spectra of the target were used for the final confirmation of the initial screening results of the accurate mass (Figure 4).

3.3. Method Validation

3.3.1. Linearity and Sensitivity. As Table 2 shows, the linear range was studied by preparing a calibration curve with a concentration range of 0.2–500 μg/mL for each compound,
and a good linear relationship with correlation coefficients ($R^2$) higher than 0.9990 was achieved for 8 endogenous alkaloids in their respective linear range. The LODs of 8 endogenous alkaloids were in the range of 0.002–0.100 mg/kg. The LOQs of 8 endogenous alkaloids were in the range of 0.004–0.200 mg/kg. LOD and LOQ for the methods of determination of 8 endogenous alkaloids in the tea are shown in Table 2.

### Table 2: Linearity, LODs, and LOQs of the 8 endogenous alkaloids.

| Compound       | Linear equation                      | Linear range (μg/L) | $R^2$    | LOD (mg/kg) | LOQ (mg/kg) |
|----------------|--------------------------------------|---------------------|----------|-------------|-------------|
| Nicotine       | $Y = 10091.33111X + 3329.87164$      | 0.5–500             | 0.99866  | 0.005       | 0.010       |
| Anabasine      | $Y = 11037.04373X + 23487.04699$     | 0.2–500             | 0.99844  | 0.002       | 0.004       |
| Choline        | $Y = 14658.46420X - 5.77788 \times 10^4$ | 0.5–500             | 0.99785  | 0.005       | 0.010       |
| Tryptamine     | $Y = 483.76213X + 2103.10951$        | 5.0–500             | 0.99876  | 0.050       | 0.100       |
| Putrescine     | $Y = 483.76213X + 2103.10951$        | 10.0–500            | 0.99876  | 0.100       | 0.200       |
| Nicotinamide   | $Y = 2672.35197X - 3753.02170$       | 0.5–500             | 0.99899  | 0.005       | 0.010       |
| Muscarine      | $Y = 28863.69968X - 3.26291 \times 10^5$ | 0.2–500             | 0.99932  | 0.002       | 0.004       |
| Cotinine       | $Y = 6622.74425X + 3.13607 \times 10^4$ | 0.5–500             | 0.99928  | 0.005       | 0.010       |

### Table 3: Linearity, LODs, and LOQs of the 8 endogenous alkaloids.

| Compound       | Background (mg/kg) | Added (mg/kg) | Recovery (%) | RSD (%) |
|----------------|-------------------|---------------|--------------|---------|
| Nicotine       | 0.160             | 0.010         | 87.6         | 1.93    |
|                |                   | 0.030         | 85.2         | 3.90    |
|                |                   | 0.100         | 90.4         | 1.62    |
|                |                   | 0.004         | 101.5        | 4.15    |
| Anabasine      | 0.111             | 0.012         | 85.9         | 0.30    |
|                |                   | 0.040         | 81.1         | 1.63    |
|                |                   | 0.010         | 89.2         | 1.45    |
| Choline        | 301               | 0.030         | 92.3         | 0.44    |
|                |                   | 0.100         | 88.7         | 0.13    |
|                |                   | 0.100         | 83.3         | 2.10    |
| Tryptamine     | ND                | 0.300         | 82.4         | 6.82    |
|                |                   | 1.00          | 82.3         | 1.89    |
| Putrescine     | ND                | 0.200         | 80.1         | 7.59    |
|                |                   | 0.600         | 89.2         | 3.09    |
|                |                   | 2.00          | 88.3         | 4.21    |
| Nicotinamide   | 7.01              | 0.030         | 80.1         | 2.58    |
|                |                   | 0.100         | 91.7         | 1.43    |
|                |                   | 0.004         | 97.5         | 3.45    |
| Muscarine      | ND                | 0.012         | 91.3         | 1.68    |
|                |                   | 0.040         | 88.8         | 0.06    |
|                |                   | 0.010         | 85.4         | 4.54    |
| Cotinine       | ND                | 0.030         | 87.3         | 7.58    |
|                |                   | 0.100         | 90.5         | 2.19    |

*ND means not detected.

### Table 4: Content of the 8 endogenous alkaloids in actual samples.

| Compound       | Boletus albus peck | Boletus rubellus krombh | Boletus impolitus |
|----------------|--------------------|--------------------------|------------------|
| Nicotine (mg/kg) | 0.491              | 1.45                     | 3.41             |
| Anabasine (mg/kg) | ND                 | ND                       | ND               |
| Choline (mg/kg)  | 147                | 131                      | 142              |
| Tryptamine (mg/kg) | 115               | 9.15                     | 45.0             |
| Putrescine (mg/kg) | 108              | 90.6                     | 82.6             |
| Nicotinamide (mg/kg) | 9.34           | 6.68                     | 3.94             |
| Muscarine (mg/kg) | 0.215              | 0.0329                   | 0.0483           |
| Cotinine (mg/kg)  | ND                 | ND                       | ND               |
3.3.2. Recovery and Precision. The recoveries of analytes were evaluated by adding the standard solutions with three different concentration levels to the known amounts of fungus samples. The data of recovery and precision are given in Table 3; the average recoveries of 8 endogenous alkaloids were in the range between 80.1% and 101.5%. The RSDs were in the range of 0.06%–7.59%.

3.4. Application to Actual Samples. In order to investigate the content of 8 endogenous alkaloids in *Boletus*, three *Boletus* samples from the local supermarket were analyzed using the developed method in this study. Their detection results are shown in Table 4. The compositions and contents of alkaloids were different in 3 *Boletus* samples. Anabasine and cotinine were not detected. The contents of muscarine in 3 *Boletus* samples were the lowest, which were 0.215, 0.0329, and 0.0483 mg/kg, respectively. The contents of choline in 3 *Boletus* samples were the highest, which were 147, 131, and 142 mg/kg, respectively.

4. Conclusions

In this experiment, a rapid and sensitive UPLC-Q-TOF/MS method was developed to analyze 8 endogenous alkaloids in the *Boletus* sample. The analytes were determined by Q-TOF/MS in TOF MS and IDA-MS/MS mode. In TOF MS mode, the target compounds qualified by the retention time, accurate mass, isotope distribution, and isotope abundance ratio of the target, and quantitated by the peak area of the excimer ion peak. In IDA-MS/MS mode, the target compounds were further confirmed by the ion fragment information under the corresponding collision energy. The linearity, sensitivity, accuracy, and precision of the method were investigated. The method has simple sample processing, high sensitivity, and high analysis efficiency. It is suitable for the rapid detection of alkaloids in batches of *Boletus* samples and can be used for quality control and formulation experiments of *Boletus* production. This method also provides a reference for the determination of various alkaloids in tobacco, tea, and other samples.

**Data Availability**

The data used to support the findings of this study are included within the article.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

YZ was responsible for the conceptualization of the study; JM, QL, SF, LH, LS, DW, and HZ investigated the study; JM, QL, LH, LS, and HZ reviewed the study; JM, SF, and DW were involved in the discussion; JM and YZ were responsible for writing, reviewing, and editing the original draft; YZ was involved in the project administration and funding acquisition.

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**References**

[1] A. Choma, K. Nowak, I. Komaniecka et al., “Chemical characterization of alkali-soluble polysaccharides isolated from a *Boletus edulis* (Bull.) fruiting body and their potential for heavy metal biosorption,” *Food Chemistry*, vol. 266, no. 15, pp. 329–334, 2018.

[2] L. Zhang, Y. Hu, X. Duan et al., “Characterization and antioxidant activities of polysaccharides from thirteen *Boletus* mushrooms,” *International Journal of Biological Macromolecules*, vol. 113, no. 1, pp. 1–7, 2018.

[3] Y. Li, L. You, F. Dong, W. Yao, and J. Chen, “Structural characterization, antiproliferative and immunoregulatory activities of a polysaccharide from *Boletus Leccinum rugosiceps*,” *International Journal of Biological Macromolecules*, vol. 157, no. 15, pp. 106–118, 2020.

[4] N.-N. Yang, S.-Z. Huang, Q.-Y. Ma et al., “A new pyroyle alkaloid from *leccinum extremsioriente*,” *Chemistry of Natural Compounds*, vol. 51, no. 4, pp. 730–732, 2015.

[5] M. Gasecka, P. Pﬁszerski, M. Mleczek et al., “The relationship between metal composition, phenolic acid and flavonoid content in *Imelia badia* from non-polluted and polluted areas,” *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes*, vol. 52, no. 3, pp. 171–177, 2017.

[6] I. K. Jedidi, I. K. Ayoub, T. Philippe, and N. Bouzouita, “Chemical composition and nutritional value of three *Tunisian wild edible mushrooms*,” *Journal of Food Measurement and Characterization*, vol. 11, no. 4, pp. 2069–2075, 2017.

[7] P. Ginterová, B. Sokolová, P. Ondra et al., “Determination of mushroom toxins ibotenic acid, muscimol and muscarine by capillary electrophoresis coupled with electrospay tandem mass spectrometry,” *Talanta*, vol. 125, pp. 242–247, 2014.

[8] S. Vidovic, I. O. Mujic, Z. Zekovic, and V. T. Saponjic, “Antioxidant properties of selected *boletus* mushrooms,” *Food Biophysics*, vol. 5, no. 1, pp. 49–58, 2010.

[9] P. Kittakoop, C. Mahidol, and S. Ruchirawat, “Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation,” *Current Topics in Medicinal Chemistry*, vol. 14, no. 2, pp. 239–252, 2013.

[10] Z. A. Mahmood, S. W. Ahmed, L. Azhar et al., “Bioactive alkaloids produced by fungi. I. Updates on alkaloids from the species of the genera *Boletus, Fusarium* and *psilocybe*,” *Pakistan Journal of Pharmaceutical Sciences*, vol. 23, no. 3, pp. 349–357, 2010.

[11] Z. W. Wu, J. K. Liu, C. Xiang, and G. Wang, “Chemical constituents of *Boletinus pictus*,” *Natural Product Research and Development*, vol. 24, no. 5, pp. 618–621, 2012.

[12] H. F. Zhang, Y. B. Luo, X. Y. Li et al., “Determination of nicotine and secondary alkaloids in edible plants using modified QuEChERS procedure coupled with gas chromatography/mass spectrometry,” *Journal of Analytical Science*, vol. 34, no. 2, pp. 239–244, 2018.

[13] M. Woniakiewicz, A. Wóżniakiewicz, P. M. Nowak et al., “‘Green’ and complementary methods for the analysis of biogenic amines in wine,” *Food Analytical Methods*, vol. 11, pp. 2614–2627, 2018.
[14] A. Meshram, A. Kumar, and N. Srivastava, “Gas chromatography-mass spectrometry (GC-MS) analysis of alkaloids isolated from *Epipremnum aureum* (linden and andre) bunting,” *International Journal of Pharma Sciences and Research*, vol. 6, no. 2, pp. 337–342, 2015.

[15] L. R. Tembrock, C. D. Broeckling, A. L. Heuberger, M. P. Simmons, F. R. Stermitz, and J. M. Uvarov, “Employing two-stage derivatisation and GC-MS to assay for cathine and related stimulant alkaloids across the celastraceae,” *Phytochemical Analysis*, vol. 28, no. 4, pp. 257–266, 2017.

[16] K. László and V. Szke, “HPLC-ESI-MS/MS of brain neurotransmitter modulator lobeline and related piperidine alkaloids in *Lobelia inflata* L.” *Journal of Mass Spectrometry*, vol. 50, no. 5, pp. 727–733, 2015.

[17] I. Rhee and K.-J. Paeng, “Simultaneous determination of betaine and choline using derivatization by HPLC with UV detection,” *Analytical Science and Technology*, vol. 28, no. 2, pp. 112–116, 2015.

[18] U. A. Attar and S. G. Ghane, “In vitro antioxidant, antiabetic, antiacetylcholinesterase, anticancer activities and RP-HPLC analysis of phenolics from the wild bottle gourd (*Lagenaria siceraaria* (Molina) Standl.),” *South African Journal of Botany*, vol. 125, pp. 360–370, 2019.

[19] U. G. Spizzirri, D. Restuccia, M. Curcio, O. I. Parisi, F. Jemma, and N. Picci, “Determination of biogenic amines in different cheese samples by LC with evaporative light scattering detector,” *Journal of Food Composition and Analysis*, vol. 29, no. 1, pp. 43–51, 2013.

[20] Y.-X. Yang, C.-L. Mu, J.-F. Zhang, and W.-Y. Zhu, “Determination of biogenic amines in digesta by high performance liquid chromatography with precolumn dansylation,” *Analytical Letters*, vol. 47, no. 8, pp. 1290–1298, 2014.

[21] A. Herrero, S. Sanllorente, C. Reguera, M. C. Ortiz, and L. A. Sarabia, “A new multiresponse optimization approach in combination with a D-Optimal experimental design for the determination of biogenic amines in fish by HPLC-FLD,” *Analytica Chimica Acta*, vol. 945, pp. 31–38, 2016.

[22] F. Martin, C. Gimenez, P. Fontannaz, T. Kilinc, E. Campos-Gimenez, and D. Dowell, “Choline in infant formula and adult/pediatric nutritional formula by ultra high-performance liquid chromatography/tandem mass spectrometry: AOAC first action 2012.18,” *Journal of AOAC International*, vol. 96, no. 6, pp. 1396–1399, 2013.

[23] W. Jing, J. J. Q&_h Thompson, W. A. Jacobs, and L. M. Salvati, “Determination of free and total carnitine and choline in infant formulas and adult nutritional products by UPLC/MS/MS: single-laboratory validation, first action 2014.04,” *Journal of AOAC International*, vol. 98, no. 5, pp. 1395–1406, 2015.

[24] D. J. Ellingson, J. J. Shippar, and J. M. Gilmore, “Determination of free and total choline and carnitine in infant formula and adult/pediatric nutritional formula by liquid chromatography/tandem mass spectrometry (LC/MS/MS): single-laboratory validation, first action 2015.10,” *Journal of AOAC International*, vol. 99, no. 1, pp. 204–209, 2016.

[25] G. H. Gu, D. J. Yang, S. Y. Wang et al., “Simultaneous determination of eleven alkaloids in *Corydalis decumbens* by HPLC with diode-array detection,” *Acta Chromatographica*, vol. 29, no. 1, pp. 121–134, 2017.

[26] Z. Xun, D. Liu, R. Huang et al., “Simultaneous determination of eight alkaloids and oleandrin in herbal cosmetics by dispersive solid-phase extraction coupled with ultra high performance liquid chromatography and tandem mass spectrometry,” *Journal of Separation Science*, vol. 40, no. 9, pp. 1966–1973, 2017.

[27] M.-x. Su, M. Song, D.-s. Yang, J.-f. Shi, B. Di, and T.-j. Hang, “Simultaneous LC–MS/MS determination of five tripterygium pyridine alkaloids in dog plasma and its application to their pharmacokinetic study after oral administration of tripterygium glycosides tablets,” *Journal of Chromatography B*, vol. 990, pp. 31–38, 2015.

[28] A. Lin, X. Su, D. She, K. Qiu, Q. He, and Y. Liu, “LC-MS/MS determination and comparative pharmacokinetics of strychnine, brucine and their metabolites in rat plasma after intragastric administration of each monomer and the total alkaloids from *Semen Strychni*,” *Journal of Chromatography B*, vol. 1008, pp. 65–73, 2016.

[29] W. W. Zhang, X. R. Wang, S. P. Yang et al., “Simultaneous quantification of five biogenic amines based on LC-MS/MS and its application in honeybee venom from different subspecies,” *Biomedical Chromatography*, vol. 34, p. 4740, 2020.

[30] N. Yoshioka, S. Akamatsu, T. Mitsuhashi, C.Todo, M. Asano, and Y. Ueno, “A simple method for the simultaneous determination of mushroom toxins by liquid chromatography-time-of-flight mass spectrometry,” *Forensic Toxicology*, vol. 32, no. 1, pp. 89–96, 2014.

[31] J.-H. Huo, X.-W. Du, G.-D. Sun, W.-T. Dong, and W.-M. Wang, “Identification and characterization of major constituents in *Juglans mandshurica* using ultra performance liquid chromatography coupled with time-of-flight mass spectrometry (UPLC-ESI-Q-TOF/MS),” *Chinese Journal of Natural Medicines*, vol. 16, no. 7, pp. 525–545, 2018.

[32] L.-W. Chen, Q. Wang, K.-M. Qin et al., “Chemical profiling of Qixue Shuangbu tincture by ultra-performance liquid chromatography with electrospray ionization quadrupole-time-of-flight high-definition mass spectrometry (UPLC-QTOF/MS),” *Chinese Journal of Natural Medicines*, vol. 14, no. 2, pp. 141–146, 2016.