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Rapid Authentication of *Gastrodiae Rhizoma* by Direct Ionization Mass Spectrometry

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Abstract

In this study, direct ionization mass spectrometry (DI-MS) for rapid authentication of *Gastrodiae rhizoma* (known as *Tianma* in Chinese), a popular herbal medicine, has been developed. This method is rapid, simple and allows direct generation of characteristic mass spectra from the raw herbal medicines with the application of some solvents and a high voltage. The acquired DI-MS spectra showed that gastrodin, parishin B / parishin C and parishin, the major active components of *Gastrodiae rhizoma*, could be found only in genuine *Gastrodiae rhizoma* samples, but not in counterfeit samples, thus allowing rapid authentication of *Gastrodiae rhizoma*. Moreover, wild and cultivated *Gastrodiae rhizoma* could be classified and *Gastrodiae rhizoma* from different geographical locations could be differentiated based on their different intensity ratios of characteristic ions or principal component analysis (PCA). This method is simple, rapid, reproducible, and can be extended to analyze other herbal medicines.

Keywords: Mass spectrometry; Direct ionization; *Gastrodiae rhizoma*; Active components; Authentication.

1. Introduction

Herbal medicines are plant-derived materials or preparations that contain either raw or processed ingredients from one or more plants and can provide therapeutic or other health benefits to humans [1]. Many scientific studies have been conducted to indicate the therapeutic effects and health benefits of herbal medicines [2-4]. The increased global demands for herbal medicines and the profitability of trading herbal medicines have given rise to the problem of herbal medicine counterfeiting which compromises the safety and efficacy in the use of herbal medicines [5]. Therefore, authentication of herbal medicines is an important issue. Various methods such as morphological method [6], taxonomic method [7], microscopic method [8, 9], fingerprint chromatography [10-12] and DNA molecular marker technology [13-16] have been used for authentication of herbal medicines. Fingerprint chromatography is usually used for assessing the quality of herbal medicine as it can provide more comprehensive information, both qualitative and quantitative. However, this method typically involves sample homogenization and extraction, separation of components using capillary electrophoresis (CE) [17], micellar electrokinetic chromatography (MEKC) [18], thin-layer chromatography (TLC) [19], gas chromatography (GC) [20] and liquid chromatography (LC) [21], and detection using techniques such as mass spectrometry (MS) [22], near infrared (NIR) spectroscopy [23], electrochemical detection (ECD)

[24], diode array detection (DAD) [25] and nuclear magnetic resonance (NMR) spectrometry [26], which are usually labor-intensive and time-consuming. Development of simple and rapid analytical methods for authentication of herbal medicines is thus an important and necessary task.

Gastrodiae rhizoma (also known as *Tianma* in Chinese) is one of the most popular herbal medicines in China and it has been used as medicine for thousands of years. *Gastrodiae rhizoma* belongs to the Orchidaceae family. The most commonly used species of *Gastrodiae rhizoma* is called *Gastrodia elata* Bl. Tuber of *Gastrodiae rhizoma* is usually harvested from early winter to late spring, and is used as herbal medicine after washed and dried. *Gastrodiae rhizoma* is mainly found in Sichuan province and Shaanxi province of China [27], and is a valuable medicine widely used in China, Japan, and Korea. Studies have revealed that *Gastrodiae rhizoma* could produce pharmacological effects such as neuroprotection, memory improvement, anti-depressant activity, anti-oxidation, anti-inflammation and could be used for treatment of mental disorder, headache, convulsion, dizziness and paralysis [28-31]. Gastrodin (p-hydroxy-methylphenyl-β-D-glucopyranoside), parishin B / parishin C (bis-[4-(β-D-glucopyranosyloxy)benzyl] citrate) and parishin (tris-[4-(β-D-glucopyranosyloxy)benzyl] citrate) are the major active components of *Gastrodiae*

rhizoma [32-35] that contribute to the pharmacological effects [36-42]. Gastrodin, also called gastrodia elata glycosides, is specified as marker for quality control of *Gastrodiae rhizoma* [39], with the requirement that the level of gastrodin in *Gastrodiae rhizoma* should not be less than 0.20 % by weight [43]. Detection of gastrodin in *Gastrodiae rhizoma* is typically carried out by high-performance liquid chromatography (HPLC)-based methods [44]. On the other hand, *Gastrodiae rhizoma* is easily confused with two counterfeit species, *Cacalia davidii* (Franch.) Hand.-Mazz. [45] and *Canna edulis* Ker [46], which look very much like the genuine species (see Fig. S1 in the Supporting Information for photos of genuine and counterfeit *Gastrodiae rhizoma*) but differ greatly in prices and pharmacological values. These two counterfeit species lack the active components of the genuine species and have no known or remarkable pharmacological activities [47-50]. It is difficult to distinguish between genuine and counterfeit species of *Gastrodiae rhizoma* visually because of their very similar appearances, and thus it is essential to establish an effective method for the authentication.

Gastrodiae rhizoma is available in wild and cultivated types. Wild *Gastrodiae rhizoma* grows in natural environments, especially in mountains while cultivated *Gastrodiae rhizoma* is grown in farms and is the major source of *Gastrodiae rhizoma* in commercial

markets. Pharmacological activities between wild and cultivated *Gastrodiae rhizoma* are different due to their different growing conditions such as soil, climate, and growth time [51]. Studies have revealed that wild *Gastrodiae rhizoma* has better pharmacological effects than the cultivated one [52-54]. Wild *Gastrodiae rhizoma* is thus more expensive than cultivated *Gastrodiae rhizoma* due to its better quality and limited availability. Moreover, herbal medicines originating from different geographical locations also differ in selling prices because of their different growth environments and physiological efficacies [55]. Therefore, it is worth developing a reliable, simple and rapid analytical method to differentiate the types (wild or cultivated) and discriminate the origins of *Gastrodiae rhizoma* in addition to authenticating the genuine species from counterfeit.

In the past few years, we have been developing new mass spectrometric techniques for facilitating analysis of raw samples [56-61]. Direct ionization mass spectrometry (DI-MS) [62] is such a technique developed for direct analysis of raw bulk samples [62-64]. This technique is simple, fast and requires no, or only minimal, sample preparation. In this technique, a high voltage is applied to a small piece of raw sample cut in a sharp triangular shape and placed in front of the mass spectrometer inlet; with solvent loaded onto the sample surface, spray ionization could be induced at the tip of the sample to

generate corresponding mass spectra. The analysis of one sample can be completed within minutes and no specialized device or pneumatic assistance is needed. It is a fast and efficient method for analysis of herbal medicines [62]. In the present study, DI-MS was applied and developed for rapid authentication of *Gastrodiae rhizoma*. We demonstrated that DI-MS could unambiguously distinguish between genuine and counterfeit *Gastrodiae rhizoma* samples based on their mass spectra. Meanwhile, *Gastrodiae rhizoma* of the wild type and cultivated type as well as of different geographical origins could be differentiated based on their different intensity ratio of characteristic ions or principal component analysis (PCA) of the spectra.

2. Experimental

2.1. Chemicals and materials

Genuine samples from different geographical origins and counterfeit samples (*Cacalia davidii* (Franch.) Hand.-Mazz. and *Canna edulis* Ker) of *Gastrodiae rhizoma* used in this study were provided by the local farmers or manufactures [65] or purchased from Chinese herbal medicine markets (see Table S1 for the sample information), and confirmed by Mr. Jifeng Zhao and Mr. Songyun Qin, who are experts in authentication of herbal medicines. All samples were sealed and stored in an electronic dry cabinet before analysis. Methanol and all the other solvents were of HPLC grade and purchased

from Tedia (Fairfeild, OH, USA). Sodium iodide was purchased from Panreac Química (Barcelona, Spain). All chemicals were used directly without further purification.

2.2. Setup for direct ionization mass spectrometry of Gastrodiae rhizoma

The schematic diagram of experimental setup for DI-MS analysis of *Gastrodiae rhizoma* samples is shown in Fig. 1. A small piece of raw tissue sample was cut into a sharp triangular shape (about 0.5 cm for the base width and about 1 cm for the height) and placed orthogonal to the mass spectrometer inlet by using a clip, with distances of 0.5 cm in vertical (y-coordinate) and 1 cm in horizontal (x-coordinate) from the sample tip to the MS inlet (see Fig. S2 for photo of the experimental setup). The high voltage supply from the mass spectrometer was then connected to the clip. With the application of a high voltage (3.8 kV) and some solvents (15 μL) to the center of the tissue sample, spray ionization could be induced from the sharp end of the herbal medicine to generate mass spectra.

2.3. Mass spectrometric measurements

Mass spectra were acquired on a QToF II mass spectrometer (Waters, Milford, MA) using positive ion mode. Data acquisition and instrumental control were conducted by using MassLynx 4.1 software. Instrumental parameters, including sample cone voltage, source temperature and voltage of microchannel plate (MCP) detector, were set at 30 V,

40 ºC and 2.1 kV, respectively. Capillary voltage applied to the samples was typically set at 3.8 kV, a voltage obtained after optimization. Mass spectra were acquired in a *m/z* range of 100 – 1100 Da with a scan time of 1 s and an interscan time of 0.1 s. Typically, data from the first minute was accumulated to generate the spectra. Sodium iodide was used for *m/z* calibration of the instrument before DI-MS analysis. Tandem mass spectrometry (MS/MS) experiments were performed for identification of the major active components of *Gastrodiae rhizoma*.

2.4. Principal component analysis (PCA)

Principal component analysis (PCA) was carried out using Umetrics SIMCA 13. For each DI-MS spectrum, the normalized intensities (absolute intensity of the peak observed / total absolute intensity of all peaks observed in the mass spectrum) of those monoisotopic peaks with signal intensities higher than 5% were input for the analysis.

3. Results and discussion

3.1. Optimization for direct ionization mass spectrometry of Gastrodiae rhizoma

Three important factors, i.e. solvent used for extraction and ionization of compounds from the sample, the high voltage used to induce spray ionization from the sample and the configuration of the experimental setup used for the DI-MS analysis, were

optimized for DI-MS of *Gastrodiae rhizoma*.

In DI-MS, solvent loaded onto the surface of sample serves for extraction of compounds from the sample and ionization of the extracted compounds, and is thus crucial for the observation of desired ion signals. Solvents of different polarities and acidities, including ethyl acetate, dichloromethane, acetone, acetonitrile, ethanol, methanol, methanol with 0.1% formic acid, acetonitrile/water (1/1) with 0.1% formic acid, methanol/water (1/1) with 0.1% formic acid and water, were tested for DI-MS analysis of *Gastrodiae rhizoma*. The resulting mass spectra are shown in Fig. S3, and the detectability of gastrodin, parishin B / parishin C and parishin, the three major active components of *Gastrodiae rhizoma,* and the signal intensities of the DI-MS spectra for the genuine *Gastrodiae rhizoma* are summarized in Table 1. The spectra varied significantly with the solvents used. Detection of all the three major active components could be achieved with methanol, ethanol, acetonitrile or acetone as the solvent, but not with solvents of lower polarity i.e., dichloromethane and ethyl acetate, or of higher polarity, i.e., acetonitrile/water (1/1), methanol/water (1/1) and water, indicating that solvents of middle polarity were more suitable for the extraction and ionization of gastrodin, parishin B/C and parishin. Methanol with 0.1% formic acid was eventually chosen as the solvent for DI-MS analysis of *Gastrodiae rhizoma* because the

corresponding mass spectrum allowed detection of all the three major active components with the highest signal intensity.

Different voltages were tested for DI-MS analysis of *Gastrodiae rhizoma* and it was found that ion signals could be observed only when the applied voltage was higher than 3.1 kV. Increasing the voltage could enhance the intensity of ion signals. However, if the applied voltage was higher than 3.8 kV, undesirable electrical discharge could be easily induced on the sample tip. Therefore, a high voltage of 3.8 kV, which gave relatively intense and stable ion signals, was employed for the subsequent DI-MS analysis of *Gastrodiae rhizoma*.

Moreover, configuration of the experimental setup used for the DI-MS analysis, i.e. the distance between and the orientation of the tissue sample and MS inlet, was also optimized. When the tissue sample was getting close to the mass inlet, ion signals would be increased accordingly, but when the tissue sample was too close to the MS inlet, undesirable electrical discharge could be easily induced on the sample tip. A configuration with distances of 0.5 cm in y-coordinate and 1 cm in x-coordinate and an angle of 90^{o} between the tissue tip end and MS inlet (see Fig. S1) was found to allow relatively stable and intense signals and thus used for the analysis.

3.2. DI-MS spectra of Gastrodiae rhizoma samples

Typical DI-MS spectra of wild and cultivated genuine *Gastrodiae rhizoma* are shown in Fig. 2. Gastrodin, parishin B / parishin C and parishin, three active components used for characterization of *Gastrodiae rhizoma* [66], could be observed in the DI-MS spectra. These compounds were detected as potassium adduct ions at *m/z* 325, 767 and 1035, respectively in the spectra with the masses consistent with the literatures [27, 39, 67-70], and further confirmed with MS/MS in this study (see Fig. S4 for the MS/MS structural confirmation). The CID of the ion at *m/z* 325 produced fragment ions at *m/z* 219 and 145, corresponding to the two moieties obtained by cleavage of the glycosidic linkage of the potassium adduct ion of gastrodin (Fig. S4a). MS/MS spectrum of the ion at *m/z* 767 showed fragment ions at *m/z* 499, 393 and 231 (Fig. S4b), which corresponded to loss of one 4-(β-D-glucopyranosyloxy)benzyl group ($C_{13}H_{16}O_6$, 268 Da), further loss of another 4-(β-D-glucopyranosyloxy)benzyl group, and loss of the benzyl alcohol (C_7H_6O, 106 Da) through the cleavage of the glycosidic linkage of 4-(β-D-glucopyranosyloxy)benzyl group of potassium adduct ion of bis-[4-(β-D-glucopyranosyloxy)benzyl] citrate, respectively (Fig. S4b). Parishin B and parishin C are two isomers of bis-[4-(β-D-glucopyranosyloxy)benzyl] citrate, which are co-existed in *Gastrodiae rhizoma* [66, 68-70], and are overlapped in the DI-MS spectrum. The

MS/MS spectra of the ion at *m/z* 1035 gave fragment ions at *m/z* 767, 661, 499, 393 and 231, among which the ions at *m/z* 767, 499 and 231 were produced by loss of one, two and three 4-(β-D-glucopyranosyloxy)benzyl group(s) from potassium adduct ion of parishin, respectively, and the ions at *m/z* 661 and 393 were formed by loss of benzyl alcohol through the cleavage of the glycosidic linkage of 4-(β-D-glucopyranosyloxy)benzyl group of ions *m/z* 767 and 499, respectively (Fig. S4c). In addition to potassium adduct ions, sodium adduct ions of the three major active components could also be observed in the DI-MS spectra of some *Gastrodiae rhizoma* samples that were mainly from Hubei and Shaanxi. These sodium adduct ions were also confirmed with MS/MS (Table S2). The MS/MS fragmentation patterns of these sodium adduct ions were similar to those of the corresponding potassium adduct ions, however, higher collision energy was required for the MS/MS fragmentation of the sodium adduct ions. The predominance of sodium or potassium adduct ions in the DI-MS spectra was believed to be related to the presence of alkali metal salts in the herbal samples and that the use of relatively polar solvent (methanol with 0.1% formic acid) favored the extraction of these salts. As shown in Fig. 2, potassium adduct ions of sucrose and its dimer and trimer were also observed in the DI-MS spectra, and were confirmed with MS/MS (Table S2).

The reproducibility of the DI-MS spectra was tested by performing three independent experiments with different pieces of the same herb sample for method validation in this study. Taking sample 2 (wild type) and sample 27 (cultivated type) as examples, similar peaks were detected with similar signal intensities in the DI-MS spectra obtained from three independent experiments (see Fig. S5), indicating a high level of reproducibility of the present DI-MS method.

3.2.1. Wild and cultivated Gastrodiae rhizoma

To quantitatively compare the contents of the active components in each sample, mannose, an endogeneous coumpound with intensities similar to those of gastrodin, parishin B/C and parishin, was chosen as the internal reference compound for the comparison. Similar to gastrodin, parishin B/C and parishin, mannose was observed as both sodium and potassium adduct ions in the DI-MS spectra. In this study, the relative abundances of both sodium and potassium adduct ions of each of these compounds were summated as the intensity of the compound for comparison. The intenstity ratios of each active component to mannose, i.e. $I_{Gastrodin}$ / $I_{Mannose}$, $I_{Parishin\ B/C}$ / $I_{Mannose}$ and $I_{Parishin}$ / $I_{Mannose}$, for wild and cultivated *Gastrodiae rhizoma* are summarized in Table S3 and Fig. 3. It could be found that the ratio of active components to mannose for the wild types were significantly higher than for the cultivated types. The ratio of total ion

intensity of the three active components to the ion intensity of mannose ($I_{Gastrodin,\ Parishin\ B/C,\ Parishin}$ / $I_{Mannose}$) in the wild types were about four times as those in the cultivated types (Table S3), allowing unequivocal differentiation of the wild and cultivated *Gastrodiae rhizoma*. These results were consistent with previous results obtained by using conventional LC-MS [71, 72], and agreed with the general belief that wild-type *Gastrodiae rhizoma* has higher contents of active components than the cultivated type and can have better pharmacological effects. The relative standard deviations obtained by three independent measurements for the above ratios were generally below 10 % (see Tables S3), demonstrating usefulness of the use of the endogenous compound as the internal reference compound and that good reproducibility could be achieved by the DI-MS method. Quantification has been always a problem for direct analysis techniques such as DI-MS [62, 73]. The above results indicated that by choosing a suitable endogenous compound as internal reference, relative quantification could be achieved with acceptable accuracy.

To further compare the DI-MS spectra obtained from different *Gastrodiae rhizoma* samples, the normalized intensities of mass spectrometric peaks obtained from the mass spectra of each sample in three independent experiments were averaged and input for principal component analysis (PCA). A score plot (Fig. 4) was generated from the first

and second principal components based on the DI-MS data, and the two-component PCA model accounted for 78% of total variance of data. From the PCA plot, it could be found that clusters of wild and cultivated *Gastrodiae rhizoma* samples were well separated in the PCA plot, allowing unequivocal differentiation between them.

3.2.2. Gastrodiae rhizoma from different sources

It could be found in the PCA plot (Fig. 4) that cultivated *Gastrodiae rhizoma* samples of the same geographical origin were closely clustered and located in the same circle region in the PCA plot. Although for some sources, there were only limited numbers of samples available for study, the PCA results clearly showed that samples from different origins could be differentiated. The variance of gastrodin, parishin B/C and parishin, the three active components of *Gastrodiae rhizoma*, dominated in quadrant III, IV and I of the PCA plot, respectively (see Fig. S6 in the Supporting Information for PCA loading scatter plot showing the locations of the variance). The *Gastrodiae rhizoma* samples from Shaanxi and Henan were located in quadrant III of the PCA plot (Fig. 4) since they had a higher level of gastrodin than parishin B/C and parishin (see Table S4). The *Gastrodiae rhizoma* samples from Gansu were located in quadrant IV of the PCA plot since they had a higher level of parishin B/C than gastrodin and parishin. The cultivated *Gastrodiae rhizoma* samples from Yunnan were located in quadrant I of the

PCA plot since they had a higher level of parishin than gastrodin and parishin B/C. The cultivated *Gastrodiae rhizoma* samples from Sichuan had similar level of the three active components thus were located in the middle of the PCA plot. The cultivated *Gastrodiae rhizoma* sample from Hubei were located in quadrant II of the PCA plot since it had similar level of gastrodin and parishin. On the other hand, as shown in Table S4, cultivated *Gastrodiae rhizoma* samples from different sources had very similar $I_{Gastrodin}$ / $I_{Mannose}$, while samples from Gansu and Sichuan exhibited significantly higher $I_{Parishin\ B/C}$ / $I_{Mannose}$ than samples from Hubei, Henan, Shaanxi and Yunnan, and samples from Hubei, Sichuan and Yunnan showed significantly higher $I_{Parishin}$ / $I_{Mannose}$ than samples from Gansu, Henan and Shaanxi. For $I_{Gastrodin,\ Parishin\ B/C,\ Parishin}$ / $I_{Mannose}$, the cultivated samples from Henan were the lowest one while the cultivated samples from Sichuan were the highest one.

The wild *Gastrodiae rhizoma* samples from Sichuan, Liaoning, Heilongjiang and Yunnan were clustered differently in the PCA plot (Fig. 4), indicating that the sources of wild *Gastrodiae rhizoma* samples might also be distinguished by the method. However, due to the difficulty in obtaining the wild *Gastrodiae rhizoma* samples, only 3 wild-type samples from Sichuan, and 1 wide-type sample each from Liaoning, Heilongjiang and Yunnan were investigated in this study. Data from more wild-type

samples were needed to confirm the differentiation of sources for wild *Gastrodiae rhizoma*.

3.2.3. Genuine and counterfeit Gastrodiae rhizoma

Two frequently found counterfeit *Gastrodiae rhizoma* species, *Cacalia davidii* (Franch.) Hand.-Mazz. [45] and *Canna edulis* Ker [46], were investigated using DI-MS and the obtained spectra were shown in Fig. 2. Significantly different spectral patterns were observed with the two counterfeit *Gastrodiae rhizoma* samples, compared to those from the genuine samples. Particularly, characteristic peaks of the major active components of *Gastrodiae rhizoma*, i.e. gastrodin, parishin B/C and parishin, could not be detected with *Cacalia davidii* (Franch.) Hand.-Mazz. and *Canna edulis* Ker, (Fig. 2), enabling rapid authentication of the herbal samples.

4. Conclusions

In this study, a DI-MS-based analytical method was developed for rapid authentication of *Gastrodiae rhizoma*. Genuine and counterfeit *Gastrodiae rhizoma* species could be unambiguously differentiated based on the detection of the major active components that present in *Gastrodiae rhizoma.* Differentiation between wild and cultivated *Gastrodiae rhizoma* could also be achieved based on the intensity ratio of characteristic

ions and PCA analysis. Cultivated *Gastrodiae rhizoma* from different geographical origins could be distinguishable based on the DI-MS spectra and PCA analysis. The results also suggested that by using a suitable endogenous compound as the internal reference compound, DI-MS could be efficiently used for quantitative comparison of active ingredients in herbal samples. The overall method is simple, rapid, and can be further extended to analyze other herbal medicines.

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Table 1.

Summary of the detectability of the major active components and signals intensity of the DI-MS spectrum for genuine *Gastrodiae rhizoma* with the application of different solvents.

Solvent	Major active components			Signal intensity of DI-MS spectrum
	Gastrodin	Parishin B/C	Parishin	
Methanol with 0.1% formic acid	√	√	√	Strong
Methanol	√	√	√	Strong
Ethanol	√	√	√	Medium
Acetonitrile	√	√	√	Weak
Acetone	√	√	√	Weak
Water	√	x	√	Medium
Methanol/water (1/1) with 0.1% formic acid	√	x	√	Medium
Acetonitrile/water (1/1) with 0.1% formic acid	√	x	√	Weak
Dichloromethane	√	x	√	Weak
Ethyl acetate	√	x	x	Weak

FIGURE CAPTIONS

Fig. 1. Schematic diagram of DI-MS analysis of *Gastrodiae rhizoma* samples.

Fig. 2. DI-MS spectra of genuine and counterfeit *Gastrodiae rhizoma*: (a) wild *Gastrodiae rhizoma*, (b) cultivated *Gastrodiae rhizoma*, (c) *Cacalia davidii* (Franch.) Hand.-Mazz. and (d) *Canna edulis* Ker.

Fig. 3. A chart showing the ratio of $I_{Gastrodin}$ / $I_{Mannose}$, $I_{Parishin\ B/C}$ / $I_{Mannose}$, $I_{Parishin}$ / $I_{Mannose}$ and $I_{Gastrodin,\ Parishin\ B/C,\ Parishin}$ / $I_{Mannose}$ for differentiation of wild-type and cultivated-type *Gastrodiae rhizoma* samples as observed in their DI-MS spectra.

Fig. 4. PCA plot for *Gastrodiae rhizoma* samples from different geographical origins based on their DI-MS data. Samples 1-6 are wild-type samples and samples 7-50 are cultivated-type samples from different provinces of China.

Fig. 1.

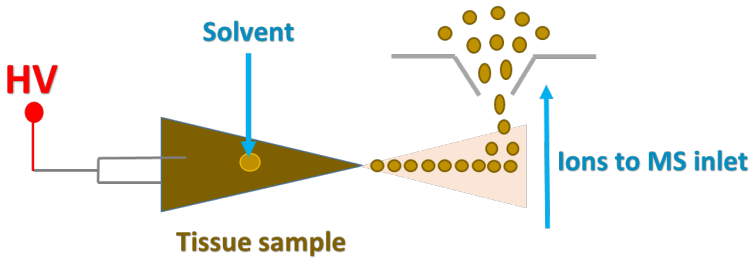


Fig. 2.

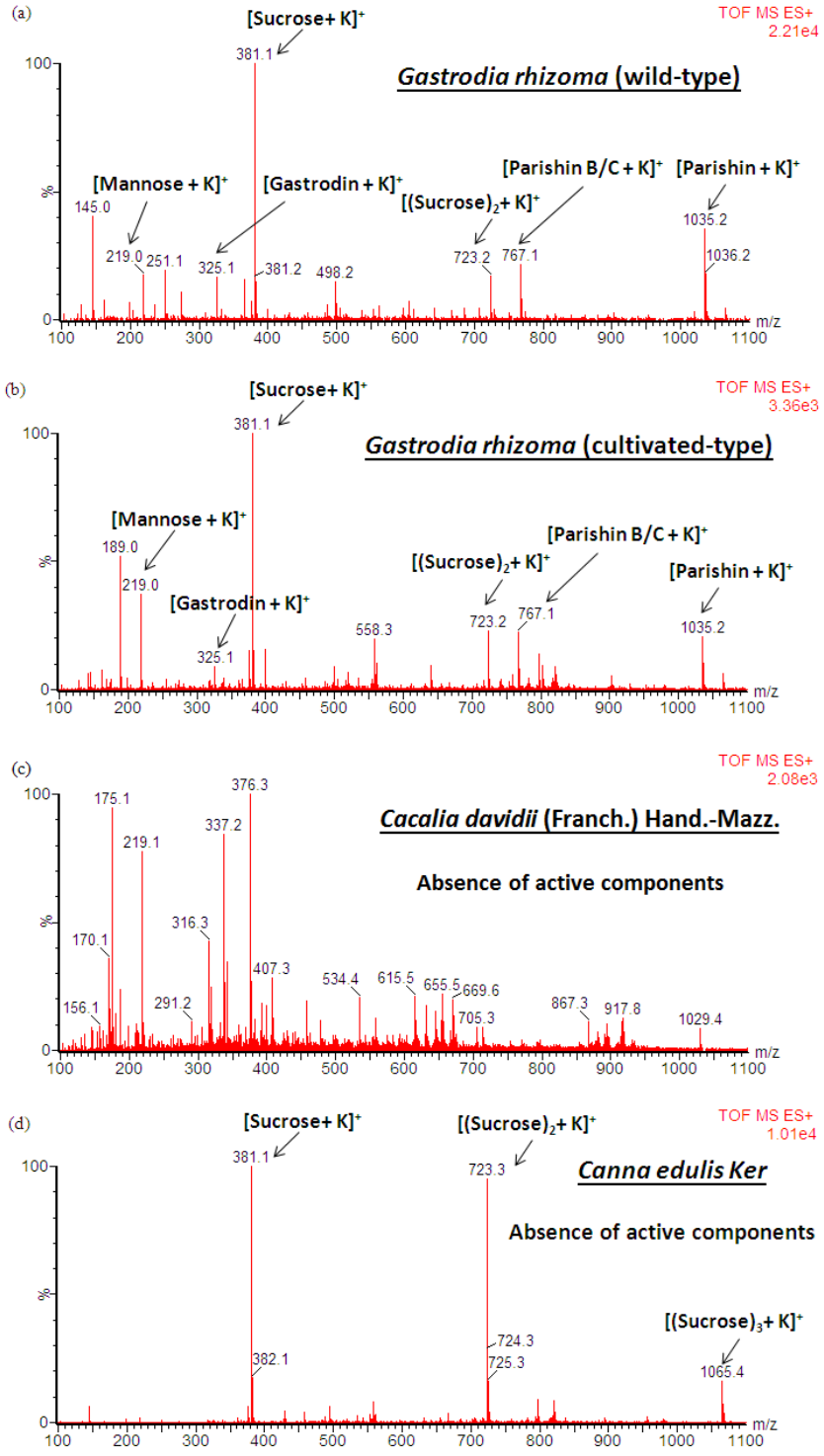

(a)
TOF MS ES+
2.21e4
[Sucrose+ K]+
381.1
Gastrodia rhizoma (wild-type)
[Mannose + K]+
[Gastrodin + K]+
[(Sucrose)2+ K]+
[Parishin B/C + K]+
[Parishin + K]+
145.0
219.0
251.1
325.1
381.2
498.2
723.2
767.1
1035.2
1036.2
(b)
TOF MS ES+
3.36e3
[Sucrose+ K]+
381.1
Gastrodia rhizoma (cultivated-type)
[Mannose + K]+
189.0
219.0
[Gastrodin + K]+
325.1
558.3
[(Sucrose)2+ K]+
[Parishin B/C + K]+
723.2
767.1
[Parishin + K]+
1035.2
(c)
TOF MS ES+
2.08e3
376.3
175.1
337.2
219.1
Cacalia davidii (Franch.) Hand.-Mazz.
Absence of active components
316.3
170.1
407.3
534.4
615.5
655.5
669.6
705.3
156.1
291.2
867.3
917.8
1029.4
(d)
TOF MS ES+
1.01e4
[Sucrose+ K]+
381.1
[(Sucrose)2+ K]+
723.3
Canna edulis Ker
Absence of active components
[(Sucrose)3+ K]+
724.3
725.3
382.1
1065.4
m/z
100
200
300
400
500
600
700
800
900
1000
1100

Fig. 3.

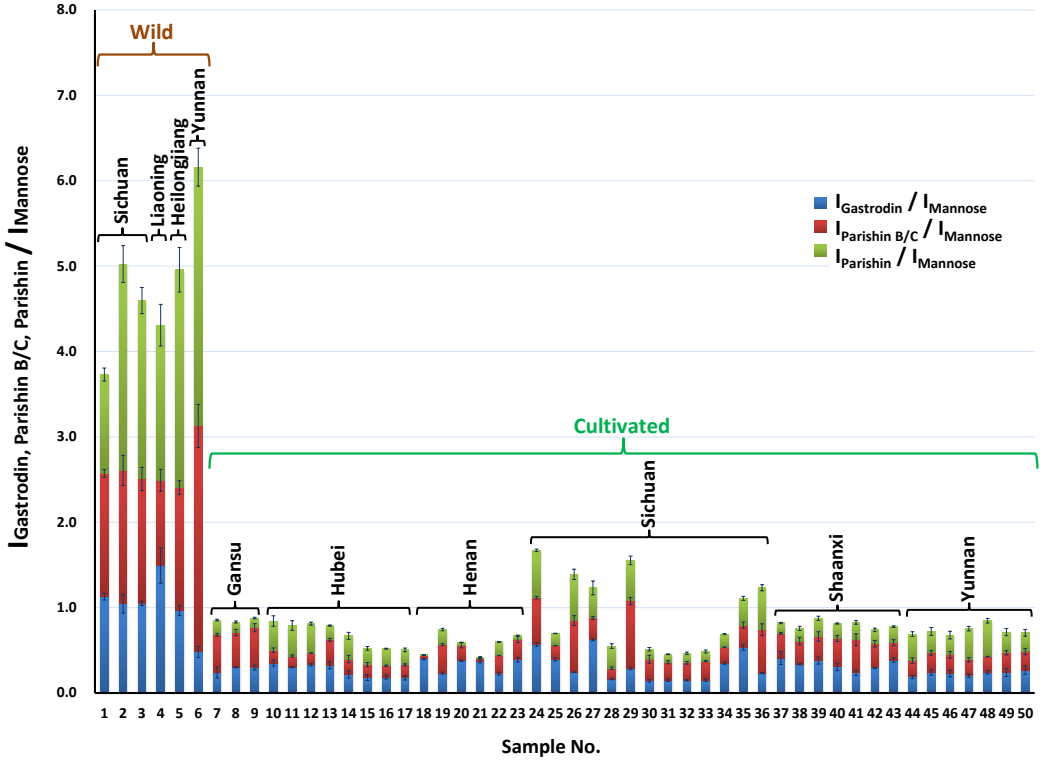
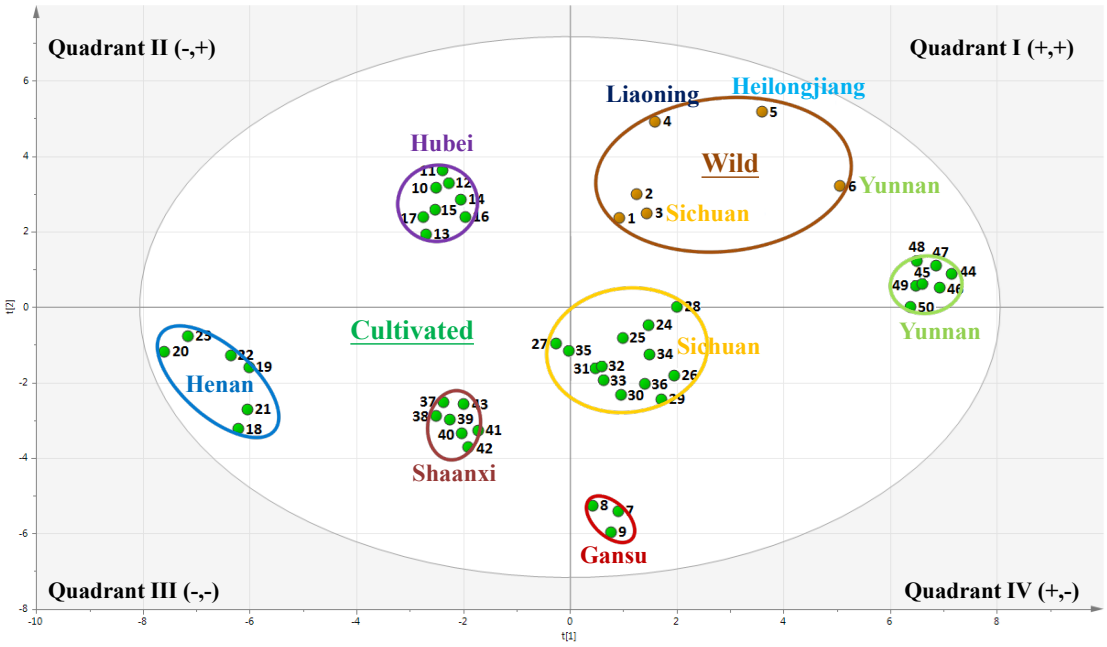

Wild
Cultivated
Sichuan
Liaoning
Heilongjiang
Yunnan
Gansu
Hubei
Henan
Sichuan
Shaanxi
Yunnan
I Gastrodin / I Mannose
I Parishin B/C / I Mannose
I Parishin / I Mannose
I Gastrodin, Parishin B/C, Parishin / I Mannose
Sample No.
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50

Fig. 4.


Quadrant II (-,+)
Quadrant I (+,+)
Quadrant III (-,-)
Quadrant IV (+,-)
Liaoning
Heilongjiang
Hubei
Wild
Yunnan
Sichuan
Cultivated
Henan
Shaanxi
Gansu
t[1]
t[2]