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Simultaneous quantification of 19 diterpenoids in *Isodon amethystoides* by high-performance liquid chromatography–electrospray ionization tandem mass spectrometry

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ABSTRACT

A high-performance liquid chromatography with electrospray tandem mass spectrometry (HPLC–ESI-MS/MS) method was developed to characterize and quantify 19 diterpenoid compounds in *Isodon amethystoides* simultaneously. By employing a Diamonsil C_{18} column, 19 constituents were separated within 15 min using a gradient elution consisted of methanol containing 0.1% formic acid and 0.1% aqueous formic acid. The precursor and product ions of the analytes were monitored on a hybrid quadrupole linear ion trap mass spectrometer equipped with a turbo ion spray interface in positive and negative mode in a single run and quantified by a multiple-reaction monitor (MRM). All standard calibration curves showed good linearity ($r^2 > 0.99$) within the test ranges. The precision was evaluated by intra- and inter-day tests, which revealed relative standard deviation (RSD) values within the ranges of 1.06–3.25% and 1.56–3.84%. The recovery studies for the quantified compounds were between 95.82 and 108.3% with RSD values less than 1.86%. The results indicated that the method is simple, rapid, specific and reliable. This method was successfully applied for identification and quantification of 19 diterpenoids in 11 batches of *I. amethystoides*. The results showed that the contents of diterpenoids in *I. amethystoides* from different sources were widely varied.

1. Introduction

Isodon amethystoides, the dried entire plant of *I. amethystoides* (Benth) Hara, is a well-known traditional Chinese medicine (TCM) that has been used for over 2000 years [1]. This herb has long been used as a folk remedy for stomach pain, sore swollen poison, amenorrhea, wrestling injuries and tumors [2–4].

I. amethystoides is known to contain a large number of compounds, including diterpenoids, flavonoids, phenolic acids, triterpenoids and volatile oils [1]. Among these, diterpenoids are generally considered to be the major components, and many have been found to have biological activities. For example, oridonin and ponicidin were shown to have antitumor properties with very low toxicity. In addition, oridonin and ponicidin were recently found to have significant antiangiogenic activity and to be inhibitors of NF-κB transcription activity and the expression of its downstream targets, COX-2 and inducible nitric oxide synthase [5–7]. Moreover, amethystoidin A can also enhance the non-specific immune function in mice [2]. In recent years, more and more diterpenoids have been isolated from *I. amethystoides*, and they exhibit a variety of bioactivities [8–10].

Quantification of the diterpenoid compounds in *I. amethystoides* is important for quality evaluation of the herb. Simple quantitative analysis of one or two active components in an herb does not represent its integral quality. Consequently, simultaneous quantitative analysis of active components is the most direct method for quality control of TCM.

There are very few reported studies on the quantitative determination of the chemical constituents in *I. amethystoides*. Zhao used GC–MS to determinate volatile oils [11], and Chen employed ultraviolet spectrophotometry (HPLC-UV) to determine the total amount of diterpenoids [12]. Most of the diterpenoids in *I. amethystoides* are in low concentrations and have weak or no UV absorption [1]. Therefore, it is particularly difficult to simultaneously determine these constituents with High Pressure Liquid Chromatography-Ultraviolet (HPLC-UV) or Thin-Layer Chromatography (TLC). By contrast, MS is a sensitive and selective technique that allows for detection of trace amounts of constituents. All of the peaks from the target compounds can be identified by comparison of the retention time and parent and product ions with standards. Mass spectrometry methods are suitable for the analysis of TCM and Chinese herbal prescriptions, especially for low-abundance com-

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Table 1
Isodon amethystoides samples.

Sample no.	Type	Collection date	Sources
1	Wild	2009.10	Anhui-suzhou
2	Wild	2009.10	Anhui-suzhou
3	Wild	2009.10	Zhejiang-hangzhou
4	Wild	2009.10	Zhejiang-jinhua
5	Wild	2009.9	Zhejiang-jiangshan
6	Wild	2009.9	Zhejiang-chunan
7	Wild	2009.10	Zhejiang-lishui
8	Wild	2009.9	Sichuan-chengdu
9	Wild	2009.10	Fujian
10	Cultivated	2009.10	Anhui-suzhou
11	Cultivated	2009.10	Anhui-suzhou

pounds and complex compounds, which are difficult to analyze by conventional isolation.

In the present study, we developed and validated a simple and accurate High Pressure Liquid Chromatography–Electrospray Ionization-Mass Spectrum (HPLC–ESI-MS) method for simultaneous determination of 19 major diterpenoids (effusanin A, enmein, lasiodonin, oridonin, epinodosinol, nervosanin B, serrin B, isodonoiol, sodoponin, shikokianidin, rabdosinate, epinodosin, nodosin, ponicidin, rabdoternin A, enmenol, hebeirubesensin K, lasiokaurin, and lasiokaurinol) in *I. amethystoides*. During the method development, multiple-reaction monitoring (MRM) was employed, and an electrospray ionization source was operated in positive and negative mode in a single run. In addition, 11 batches of *I. amethystoides* from different sources were compared using the developed method (Table 1).

2. Experimental

2.1. Materials and reagents

HPLC-grade methanol (Fisher, USA) was used for HPLC analyses. Redistilled water was prepared in our lab using a Heal Force-PWVF Reagent Water System (Shanghai CanRex Analyses Instrument Corporation Limited, China). Analytical-grade methanol (Tianjin Chemical Corporation, China) was used for sample preparation. HPLC-grade formic acid was purchased from Diamond Technology Incorporation. Eleven batches of raw material sample of *I. amethystoides* were collected from different provinces in China. All of the voucher specimens, which were identified by Professor Zengke Kong, were deposited in the herbarium of the School of Pharmacy, Hebei Medical University.

Effusanin A, enmein, lasiodonin, oridonin, epinodosinol, nervosanin B, serrin B, isodonoiol, sodoponin, shikokianidin, rabdosinate, epinodosin, nodosin, ponicidin, rabdoternin A, enmenol, hebeirubesensin K, lasiokaurin, and lasiokaurinol, which were isolated from different *Isodon* plants and identified based on IR, UV, and NMR spectroscopy and comparisons with literature data [13–17], were generously provided by professor Jixia Zhang, Henan Xinxiang Medical University. The purities of the above ingredients were above 98% according to HPLC analysis. The structures of the 19 compounds are shown in Fig. 1.

2.2. Instrumentation and conditions

2.2.1. Liquid chromatography

An Agilent 1200 liquid chromatography system (Agilent, USA) equipped with a quaternary solvent delivery system, an autosampler and a column compartment was used. The chromatographic separation was performed on a Diamonsil C_{18} column (250 mm × 4.6 mm, 5 μm). A linear gradient elution of eluents A (methanol containing 0.1% formic acid) and B (0.1%, v/v aqueous formic acid) was used for the separation. The elution programmer was optimized and conducted as follows: initial 0–5 min, linear change from A–B (55:45, v/v) to A–B (80:20, v/v); 5–10 min, linear change to A–B (95:5, v/v); and 10–15 min, isocratic elution A–B (95:5, v/v). The flow rate was 0.7 mL/min, and the sample injection volume was 10 μL.

2.2.2. Mass spectrometer

The LC/MS analyses were conducted on a 3200 QTRAP™ system from Applied Biosystems/MDS Sciex (Applied Biosystems, Foster City, CA, USA), a hybrid triple quadrupole linear ion trap mass spectrometer equipped with Turbo V sources and a TurboIonspray interface. The instrument was operated with an electrospray ionization source running in positive and negative modes in a single run, and the ion spray voltage was set to 5500 kV and −4500 kV, respectively. The turbo spray temperature was maintained at 600 °C. The nebulizer gas (gas 1) and heater gas (gas 2) were set at 40 and 50 arbitrary units, respectively. The curtain gas was kept at 25 arbitrary units, and the interface heater was turned on. Nitrogen was used in all cases. Multiple-reaction monitoring mode was employed for quantification. The collision cell exit potential (CXP) and entrance potential (EP) were set at 5.0/−5.0 V and 10.0/−10.0 V, respectively. The precursor-to-product ion pair, declustering potential (DP) and collision energy (CE) for each analyte are described in Table 2. The dwell time of each ion pair was 100 ms. All instrumentation was controlled and synchronized by Analyst software (versions 1.4.2) from Applied Biosystems/MDS Sciex.

2.3. Standard solution preparation

The appropriate amounts of standards were accurately weighed and dissolved in methanol to make 19 standards of stock solutions. All solutions were stored in a refrigerator at 4 °C until analysis.

2.4. Sample solution preparation

The dry plant samples were ground to fine powder by a pulverizer, and 1.0 g of powder was placed in a 50-mL capped conical flask. All crude was added and extracted with 25 mL of methanol in an ultrasonic ice-water bath for 1 h. The extracted solution was adjusted to the original weight by adding methanol. After centrifugation at 12,000 rpm for 10 min, the supernatant was injected into the HPLC system after filtering through a 0.45-μm microporous membrane.

3. Results and discussion

3.1. Optimization of extraction method

To optimize the extraction conditions, the extraction method (ultrasonication, reflux), methanol concentration (50, 70 and 100%, v/v), solvent volume (10, 25 and 30 mL) and extraction time (30 min, 1 and 2 h) were investigated. The sum numbers and areas of characteristic peaks in each chromatogram obtained using different conditions were compared. The optimal condition for extraction of *I. amethystoides* was selected as 1.0 g powder of each dried sample to be extracted with 25 mL of 100% methanol in an ultrasonic ice-water bath for 1 h.

3.2. Optimization of HPLC–MS/MS conditions

The optimization of the mass conditions was achieved by infusion or injection of each analyte separately at a flow rate of 10 μL/min. Full-scan and collision-activated dissociation (CAD) tests were operated to determine the appropriate MRM method.

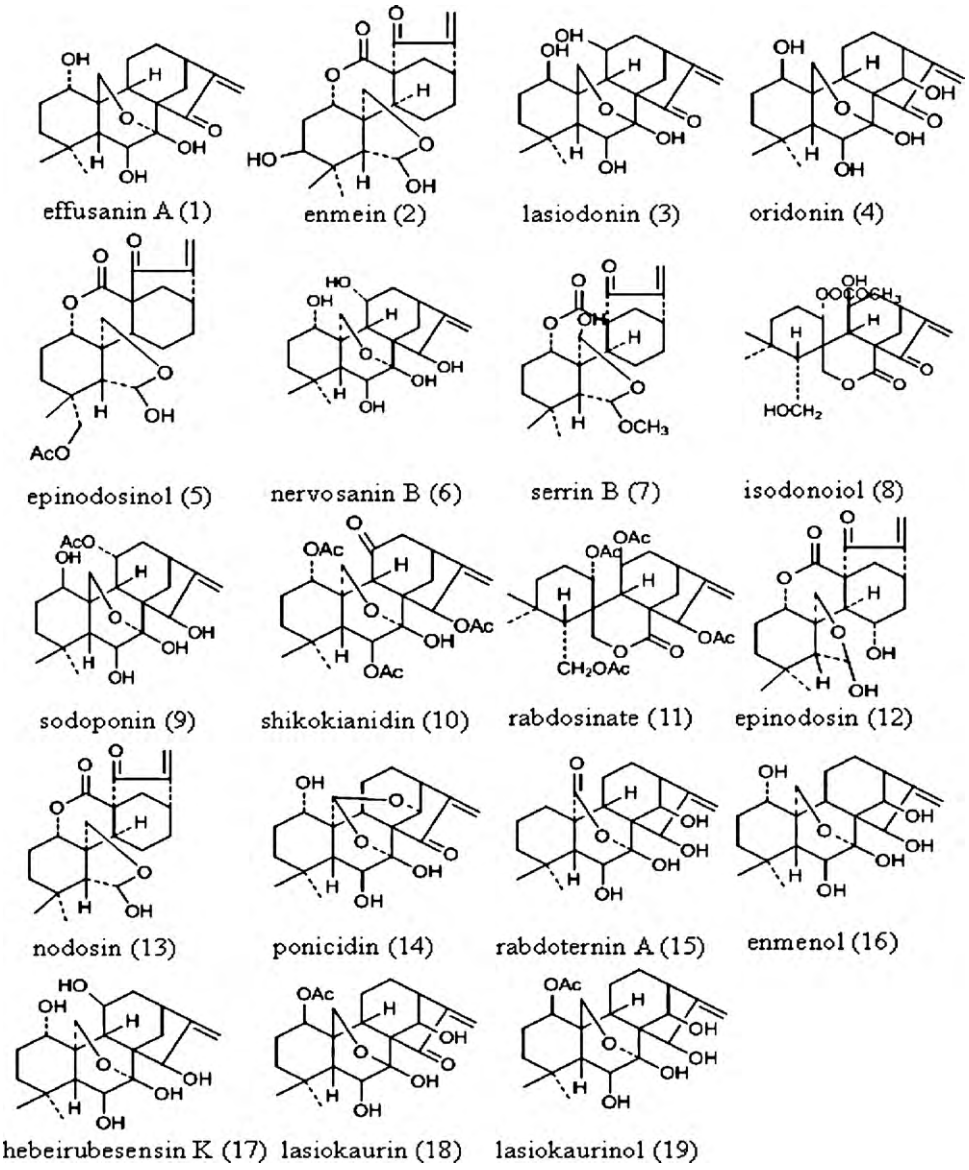

Fig. 1. Chemical structures of the 19 diterpenoid compounds in *Isodon amethystoides.*

The electrospray interface was used, and good sensitivity and fragmentation were obtained. All of the analytes could be ionized under positive and negative electrospray ionization conditions. Therefore, the mass spectral conditions were optimized in both positive- and negative-ion modes. According to the sensitivity and reproducibility of the dominant ions in the full-scan mass spectra, the positive mode was ultimately selected for the detection of compounds 1–11, while the negative mode was used for compounds 12–19. In the full-scan mass spectra, the quasi-molecular ions and fragment ions were observed for all 19 diterpenoid compounds, and most were in good agreement with literature data [18–19]. The protonated molecular ions $[M+H]^+$ and $[M+NH_4]^+$and the deprotonated molecular ions $[M-H]^-$ were considered stable and in higher abundance, so $[M+H]^+$, $[M+NH_4]^+$and $[M-H]^-$ were chosen as the precursor ions for MS/MS fragmentation analysis of compounds 1, 2, 3, 4, 8, 10, and 11; compounds 5, 6, 7, and 9; and compounds 12–19, respectively. Because declustering potential is one of the most important mass spectrometer parameters affecting the ion response, it was optimized to obtain the maximum sensitivity. In MS/MS analysis, only the precursor ion was isolated and then dissociated into the product ions. Several fragment ions of the analytes were observed in the product ion spectra, and the predominant fragment ions were chosen in MRM acquisition for quantification. The most suitable collision energy was also determined by observing the maximum response for the MS/MS monitoring fragment ion.

To obtain higher peak responses and shorter analysis times for the target compound chromatograms, the effect of different mobile phase compositions on chromatographic separation was compared. Our results revealed that there were no differences between methanol–water and acetonitrile–water as the mobile phase. Due to the high toxicity and price of acetonitrile, the binary mixture of methanol–water was chosen. Several mobile phase additives, such as ammonium acetate, formic acid and acetic acid, were used to achieve high sensitivity. We also determined that acidic eluents A (methanol containing 0.1% formic acid) and B (0.1%, v/v aqueous formic acid) were beneficial for enhancing the ionization of compounds detected in positive electrospray interface mode. Although ionization of compounds detected in negative electrospray interface mode was suppressed due to the presence of formic acid in the mobile phase, their quantification was not impaired, as revealed by

Table 2
Retention time, MS/MS fragment ions, declustering potential (DP) and collision energy (CE) of the 19 diterpenoid compounds in *Isodon amethystoides*.

Compound no.	Compounds	MW	Retention times (min)	MS^1 (*m*/*z*)	MS^2 (*m*/*z*)	DP (V)	CE (eV)
1	effusanin A	348	10.38	349.3*	331.3* 313.3 301.3	36	16
2	enmein	362	5.96	363.3*	327.3 281.2* 253.3	42	21
3	lasiodonin	364	8.75	365.3*	347.3* 329.3 311.3	17	17
4	oridonin	364	9.47	365.3*	347.3* 329.4 301.3	17	17
5	epinodosinol	364	8.19	382.3*	347.3* 364.3 329.2	9	16
6	nervosanin B	366	7.82	384.4*	349.3 331.3* 283.4	10	19
7	serrin B	376	10.41	394.4*	377.3 359.3* 327.3	13	17
8	isodonoiol	406	8.77	407.4*	389.3* 331.4 313.3	53	19
9	sodoponin	408	8.88	426.3*	373.3 349.3 331.2*	15	20
10	shikokianidin	490	11.61	491.2*	389.3 371.5 329.2*	42	21
11	rabdosinate	534	10.78	535.4*	433.3 373.4 295.3*	38	28
12	epinodosin	362	7.15	361.2*	331.3 287.1* 243.2	−42	−26
13	nodosin	362	8.43	361.1*	287.2 269.1 257.1*	−50	−31
14	ponicidin	362	10.13	361.2*	343.0 299.1* 255.1	−44	−21
15	rabdoternin A	364	11.62	363.2*	327.1 283.1* 255.1	−52	−32
16	enmenol	366	7.82	365.2*	347.2* 299.1 267.2	−45	−25
17	hebeirubesensin K	366	7.77	365.2*	317.2* 299.2 255.1	−53	−26
18	lasiokaurin	406	10.96	405.2*	387.3 327.0 58.9*	−74	−65
19	lasiokaurinol	408	10.13	407.2*	389.1 329.1* 58.9	−60	−20

* Monitored MRM transitions.

the high sensitivity and accuracy of analysis. Because of the long retention time of some of the late-eluting peaks in the isocratic runs, gradient elution was employed in the HPLC analyses. Satisfactory separation was achieved in 15.0 min by gradient elution using the HPLC conditions described earlier in Section 2.2.1. The typical extract ions chromatograms (XIC) of multiple-reaction monitoring chromatograms of chromatograms of standards and sample 1 are shown in Fig. 2.

3.3. Linearity, limit of detection and limit of quantification

Calibration curves were constructed for at least six concentrations in triplicate. Calibration curves for all the compounds were constructed by plotting the integrated chromatography peak areas (Y) versus the corresponding concentration of the injected standard solutions (X). All of the analytes showed good linearity ($r^2 > 0.99$) over a relatively wide concentration range. The limits of detection (LOD) and quantification (LOQ) under the optimized chromatographic conditions were separately determined at signal-to-noise ratios (S/N) of 3 and 10, respectively. The LOD and LOQ for the 19 compounds were less than 0.0137 and 0.0314 ng, respectively, indicating the high sensitivity of this method with these chromatographic conditions. The results for each compound are given in Table 3.

3.4. Precision, accuracy, repeatability and stability

The precision of the method was validated by determination of intra- and inter-day variance. The intra-day precision was per-

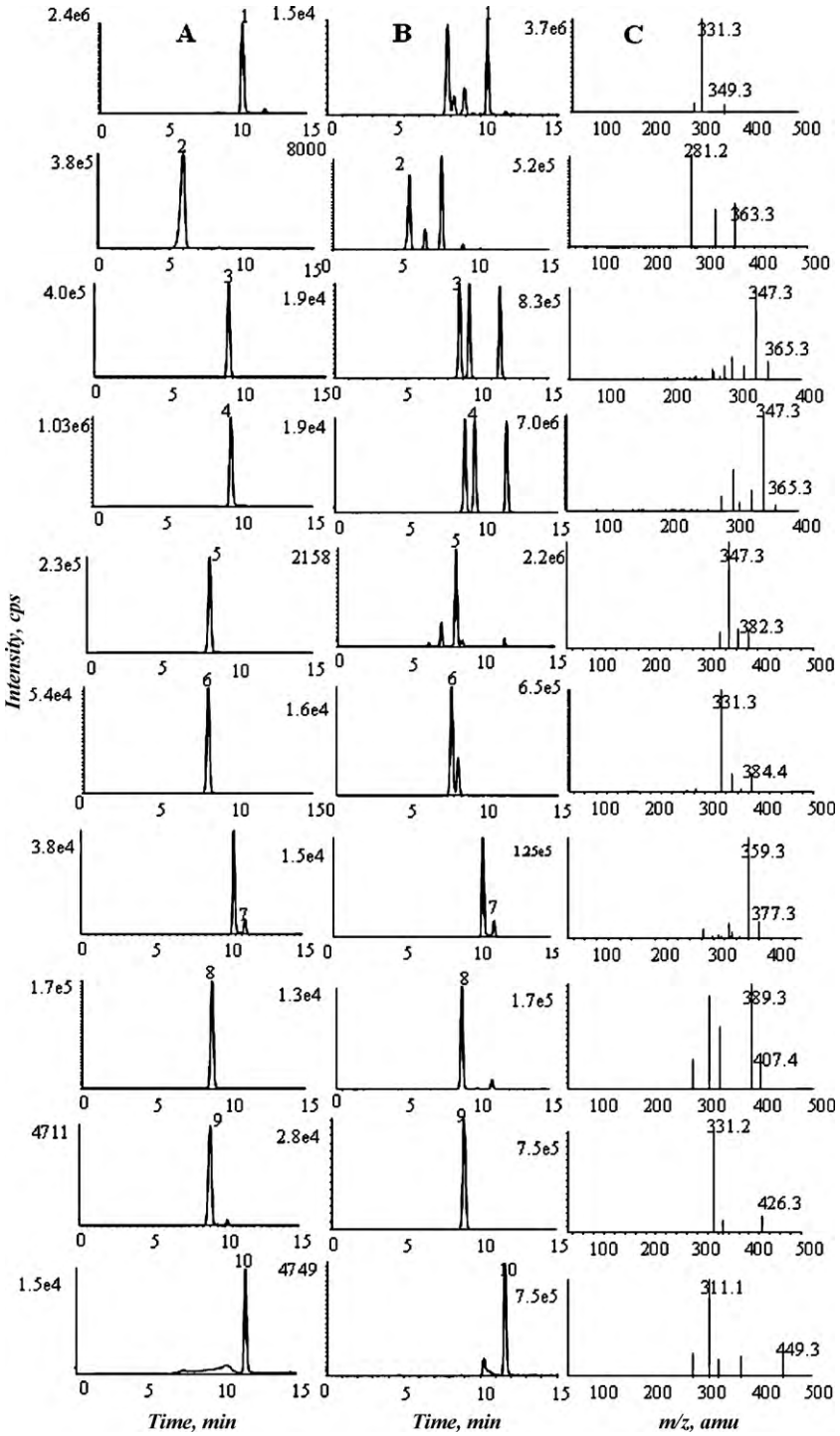

Fig. 2. Representative extract ion chromatograms (XIC) of multiple-reaction monitoring (MRM) chromatograms of effusanin A, enmein, lasiodonin, oridonin, epinodosinol, nervosanin B, serrin B, isodonoiol, sodoponin, shikokianidin, rabdosinate, epinodosin, nodosin, ponicidin, rabdoternin A, enmenol, hebeirubesensin K, lasiokaurin and lasiokaurinol. (A) Standards, (B) *Isodon amethystoides* sample 1 and (C) monitored MRM transitions of 19 standards.

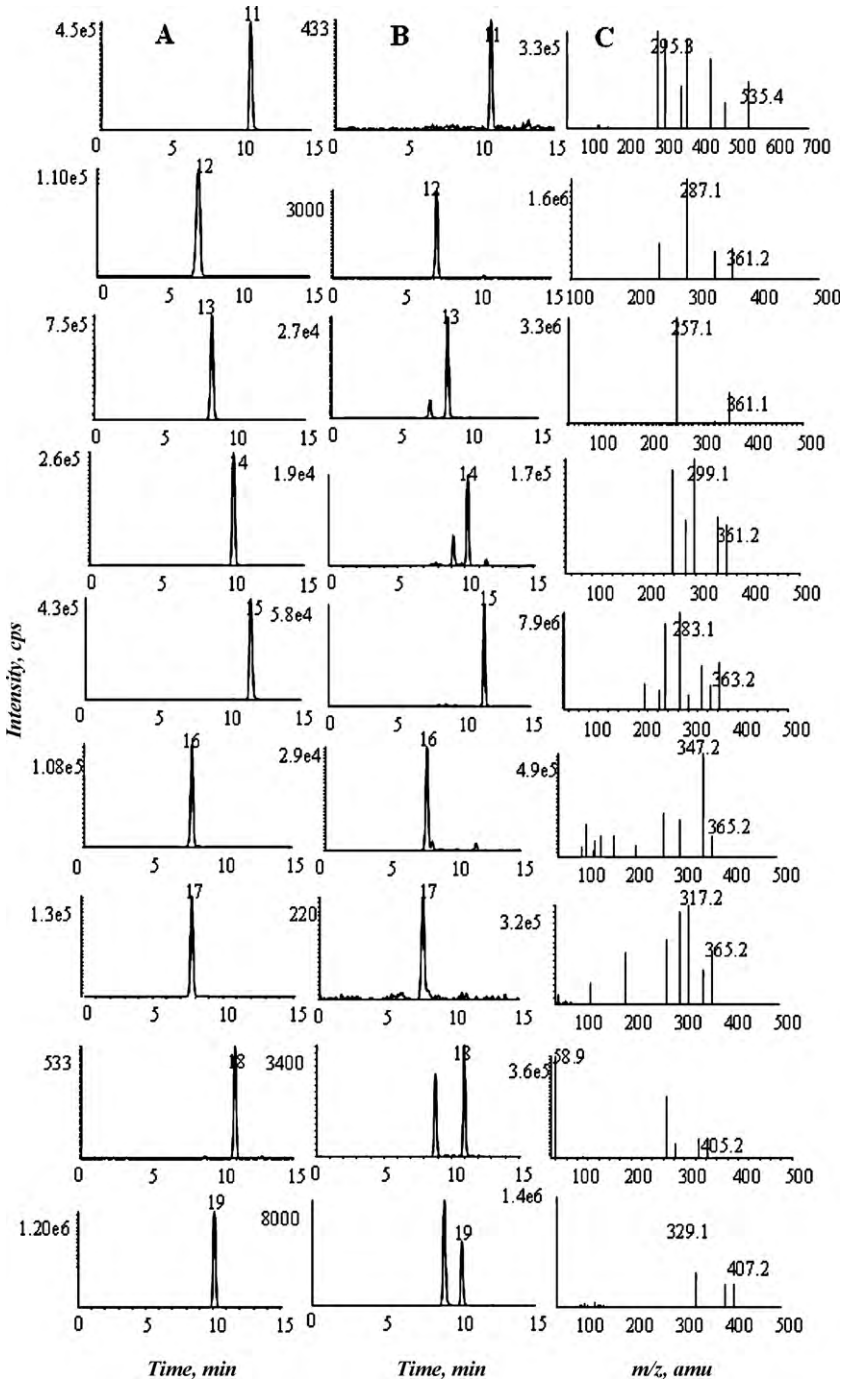

Fig. 2. (*Continued*).

formed with six replications prepared from the *I. amethystoides* sample within one day, while the inter-day precision was performed over three consecutive days. The quantity of each ingredient contained in the *I. amethystoides* sample was determined from the corresponding calibration curve. The relative standard deviation (RSD) was taken as a measure of precision. As shown in Table 4, the intra- and inter-day precisions (RSD) of the investigated components were less than 3.25 and 3.84%, respectively.

Recovery was used to further evaluate the accuracy of the method. A known amount of standard was added to approximately 0.5 g of the *I. amethystoides* sample 1, and then extracted and analyzed with the above-established method. The experiments were repeated three times at each level. The overall recovery rates of these components were in the range of 95.82–108.3% with RSD values from 0.51 to 1.86%, indicating that the method is accurate and reproducible. The concentration levels and detailed results are summarized in Table 4.

Six samples of *I. amethystoides* from the same source were extracted and analyzed using the above-established method. The RSD values were calculated as a measurement of method repeatability. The RSD values of 19 compounds ranged from 1.26 to 2.63%, revealing the high repeatability of the method.

To investigate the stability of the samples, the same sample solution was stored at 4 °C and analyzed every 12 h over 2 days. The solution was found to be rather stable (RSD values of the peak area were lower than 2.25%).

Table 3
Linear regression data, LOD and LOQ of the 19 diterpenoid components in *Isodon amethystoides*.

Analytes	Linear regression data		Linear range (μg/mL)	LOD (ng)	LOQ (ng)
	Regression equation	r^2			
effusanin A	$Y = 3.35e^3X - 4.89$	0.9996	0.0137–0.412	0.0423	0.135
enmein	$Y = 5.84e^4X + 2.82e^3$	0.9923	0.0543–1.63	0.0267	0.116
lasiodonin	$Y = 4.47e^3X - 1.34e^3$	0.9936	0.347–10.4	0.264	0.672
oridonin	$Y = 4.75e^3X - 534.58$	0.9997	0.970–29.1	0.163	0.346
epinodosinol	$Y = 4.25e^4X - 22.10$	0.9987	0.375–11.26	0.0163	0.0346
nervosanin B	$Y = 4.07e^4X - 4.26e^3$	0.9981	0.0103–0.31	0.0262	0.0785
serrin B	$Y = 3.74e^5X + 103.19$	0.9993	0.00847–0.254	0.0234	0.0354
isodonoiol	$Y = 1.31e^5X + 4.89e^3$	0.9977	0.0417–1.25	0.0171	0.0345
sodoponin	$Y = 2.14e^3X + 26.5$	0.9992	0.0607–1.82	0.0542	0.215
shikokianidin	$Y = 3.09e^4X + 120.61$	0.9901	0.039–1.17	0.0175	0.0314
rabdosinate	$Y = 5.81e^3X + 199.3$	0.9990	0.0103–0.310	0.0197	0.0546
epinodosin	$Y = 1.96e^5X - 5.42e^4$	0.9934	0.400–12.01	0.0521	0.195
nodosin	$Y = 2.85e^5X + 273.33$	0.9947	0.00417–0.125	0.0173	0.0453
ponicidin	$Y = 5.12e^3X + 384.55$	0.9979	0.710–21.3	0.0375	0.0756
rabdoternin A	$Y = 1.02e^6X + 1.38e^3$	0.9970	0.0484–1.452	0.0191	0.0354
enmenol	$Y = 2.79e^5X + 666.67$	0.9964	0.0271–0.812	0.0137	0.0521
hebeirubesensin K	$Y = 30.732X + 5.5417$	0.9973	0.104–3.12	0.253	0.604
lasiokaurin	$Y = 2.19e^4X - 1354.2$	0.9992	0.0743–2.23	0.0153	0.0604
lasiokaurinol	$Y = 8.44e^4X + 221.67$	0.9986	0.0229–0.687	0.0342	0.0615

In the regression equation $Y = aX + b$, X refers to the sample injection amount, Y the peak area, and r^2 is the correlation coefficient of the equation. LOD, limit of detection; LOQ, limit of quantification.

3.5. Matrix effect

Evaluation of the matrix effect is a major problem when developing an LC–MS/MS method, even for the analysis of botanical extracts. Co-eluting compounds originating from the matrix can cause signal enhancement or suppression. When matrix compounds and analytes enter the ion source at the same time, the ionization efficiency of the analyte might be influenced. Standard addition is an effective method for providing favorable results even with variable matrices [20]. In this study, the *I. amethystoides* sample 1 was extracted as described in Section 2.2. Next, 12.5 mL of the extract was spiked with a one-fold mixed standard solution at three concentration levels (high, middle and low), and another 12.5 mL of the extract was diluted one fold with 100% methanol. Triplicate samples were prepared at each level. The matrix effect was calculated by the formula: Matrix effect (%) = $(A - B)/C \times 100\%$, where A is the peak area of the analyte in the spiked sample matrix, B is the peak area of the analyte in the unspiked sample matrix and C is the peak area of the standard solution in 100% methanol at the same concentration. No matrix effect is observed when the matrix effect (%) is equal to 100%, values over 100% indicate ionization enhancement, and values lower than 100% suggest ionization suppression [21].

3.6. Specificity

Exact identification of each analyte is a prerequisite for successful quantification. For structural identification, the information-dependent acquisition (IDA) method was employed to trigger the enhanced product ion (EPI) scans by analyzing the MRM signals. All of the peaks of the target compounds in *I. amethystoides* were unambiguously identified by comparison of retention time and parent and product ions in MRM-IDA-EPI spectra of standards.

3.7. Sample analysis

The developed analytical method was applied to analyze 19 diterpenoids in 11 samples of *I. amethystoides* from different places.

Table 4
Intra- and inter-assay and accuracy of the 19 diterpenoid components in *Isodon amethystoides*.

Compounds	Precision ($n = 6$)		Accuracy ($n = 6$)				
	Intra-day RSD (%)	Inter-day RSD (%)	Original (μg)	Spiked (μg)	Found (μg)	Recovery[a] (%)	RSD[b] (%)
effusanin A	3.16	2.46	3.17	3.26	6.38	98.47%	0.58
enmein	2.51	2.12	17.14	18.23	35.41	100.2%	1.23
lasiodonin	3.25	2.58	86.51	88.16	175.03	100.4%	1.15
oridonin	1.45	1.56	210.42	212.43	422.93	100.0%	1.59
epinodosinol	2.56	3.05	150.14	148.34	298.52	100.0%	1.04
nervosanin B	2.42	3.18	1.26	1.48	2.76	101.4%	0.83
serrin B	2.36	3.12	0.32	0.36	0.71	108.3%	0.67
isodonoiol	2.06	3.16	16.67	16.92	33.52	99.59%	0.51
sodoponin	1.93	2.05	17.31	15.26	32.54	99.80%	0.68
shikokianidin	1.06	1.83	2.51	2.64	5.13	99.24%	1.24
rabdosinate	2.63	3.15	3.64	4.12	7.74	99.51%	1.31
epinodosin	1.64	3.08	149.25	150.12	299.35	99.99%	1.07
nodosin	2.18	2.64	1.05	1.11	2.18	101.8%	1.86
ponicidin	3.04	3.84	65.21	70.12	135.36	100.0%	1.54
rabdoternin A	1.83	2.51	3.09	3.11	6.07	95.82%	0.92
enmenol	1.88	1.93	3.72	9.51	13.2	99.68%	0.93
hebeirubesensin K	1.73	1.86	5.61	5.85	11.43	99.49%	1.12
lasiokaurin	2.67	3.02	21.05	20.18	41.26	100.2%	1.34
lasiokaurinol	2.78	3.15	2.09	2.15	4.25	100.5%	1.25

[a] Recovery (%) = (detected amount − original amount)/added amount × 100.
[b] RSD (%) = (SD/mean) × 100.

Table 5
Content (μg/g) of the 19 diterpenoid compounds in *Isodon amethystoides*.

Sample	1 [b]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Total
1[a]	7.121[c]	35.115	152.260	421.056	32.120	3.261	0.625	32.103	35.162	5.137	6.295	301.261	2.160	134.261	3.611	7.362	11.050	42.263	4.126	1236.349
2	2.165	10.261	15.262	453.516	21.562	1.237	±	25.136	30.264	3.615	4.324	235.267	±	135.264	3.546	10.052	13.250	35.162	3.861	1003.744
3	4.287	20.151	132.005	170.250	15.020	3.152	0.536	29.422	25.614	3.416	4.062	185.326	1.305	152.064	2.542	5.164	–	31.028	2.162	787.506
4	9.152	18.613	90.152	106.264	16.034	2.162	0.428	26.152	22.205	3.261	3.924	192.305	2.162	166.325	3.645	6.154	–	16.254	1.364	686.556
5	4.012	21.315	26.782	394.261	–[d]	3.125	0.291	16.451	42.352	±	–	92.215	±	124.260	2.316	4.385	–	11.218	–	742.983
6	2.514	15.621	196.237	364.051	19.380	2.315	0.413	±	35.110	6.126	6.234	264.210	1.511	164.211	2.121	3.305	3.054	2.912	3.745	1093.070
7	0.121	3.125	213.462	463.215	–	2.167	–	11.318	6.135	±	–	60.492	–	176.263	1.304	±	–	2.624	–	940.226
8	0.435	2.897	110.305	387.612	–	0.411	–	–	3.268	–	–	32.051	–	194.326	2.216	±	–	1.235	–	734.756
9	1.542	9.658	195.342	291.021	–	1.362	–	–	29.146	–	–	43.677	–	199.253	2.427	3.151	–	2.064	–	778.643
10	3.628	6.142	104.267	302.164	–	1.924	–	–	31.261	–	–	52.160	–	126.087	2.260	2.521	–	2.354	–	634.768
11	3.615	5.864	112.531	298.126	±[e]	1.528	–	–	26.251	±	–	35.120	±	130.254	2.153	2.761	–	2.568	±	620.771
Mean	3.508	13.524	122.600	331.958	9.465	2.059	0.229	12.780	26.070	1.960	2.258	135.826	0.649	154.779	2.558	4.078	2.487	13.607	1.387	841.761

[a] The samples numbers are the same as in Table 1.
[b] The compounds numbers are the same as in Fig. 1.
[c] Average of duplicates.
[d] Undetected.
[e] Not available for quantification.

The analysis time was reduced to 15 min by switching the ion source polarity between positive and negative modes in a single chromatographic run. Moreover, MRM scanning mode offered good sensitivity as it significantly decreased the levels of noise and accordingly enhanced the response of the analytes. Therefore, some minor constituents in *I. amethystoides* could be accurately measured. The target compounds were identified by comparison of the retention time, parent and product obtained from LC–MS/MS analysis with values of standard compounds and confirmed by the fragment ions produced in MRM-IDA-EPI mode. The quantitative analyses were performed by means of the external standard methods. The data are summarized in Table 5.

The results showed that the content of the total diterpenoids ranged from 620.771 to 1236.349 μg/g. In all plant samples, oridonin was the most prevalent component, with a mean content of 331.958 μg/g, followed by ponicidin, with a mean content of 154.779 μg/g. The results from samples 1–11 revealed that the contents of the 19 compounds, especially epinodosinol, serrin B, isodonoiol, shikokionidin, rabdosinate, nodosin, hebeirubesensin K and lasiokaurinol, varied considerably. From these samples, we can conclude that the amount of diterpenoids was higher in wild plants than cultivated plants. In addition, the wild plant in Suzhou, Anhui Province, contained significantly more diterpenoids (total amount) than the plants from other places of origin.

From the current samples, we determined that the quality of *I. amethystoides* can be assessed, but the place of origin and cultivation methods should be standardized.

4. Conclusion

A novel rapid HPLC–ESI-MS/MS method was established for the simultaneous quantification of 19 constituents in 11 batches of *I. amethystoides*. This method can separate complex constituents in a short time and is also environmentally friendly and inexpensive. The excellent selectivity and sensitivity allowed for identification and quantification of low-level compounds in *I. amethystoides*. The proposed method is promising for improving the quality control of *I. amethystoides* and also provides a model for quantitative analysis of complex chemical systems.

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