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Qualitative and quantitative determination of ten alkaloids in traditional Chinese medicine *Corydalis yanhusuo* W.T. Wang by LC–MS/MS and LC–DAD

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Abstract

An analytical method incorporating high-performance liquid chromatography (HPLC) with MS and UV-detection was developed for the qualitative and quantitative determination of alkaloids in *Corydalis yanhusuo*. Ten alkaloids, including seven tertiary alkaloids and three quaternary alkaloids, were identified by comparing their retention times, UV and MS spectra with those of authentic compounds. Furthermore, the collision-induced dissociations of the $[M+H]^+$ and $[M]^+$ ions were studied to clarify the MS behavior of the different types of alkaloids. In positive ion electrospray ionization tandem mass spectrometry (ESI-MS) all the tertiary alkaloids yielded prominent $[M+H]^+$ ions and quaternary alkaloids yielded prominent $[M]^+$ ions in the first order mass spectra. Fragments involving losses of H, CH_3, CO, H_2O and OCH_3 were observed in the MS/MS spectra. In addition, quantification of the 10 alkaloids in *Corydalis yanhusuo* from methanol and ethyl acetate extract of different origins were performed by this method, which provides a new tool for the assessment of quality of *Corydalis yanhusuo* preparations. The method provides the best sensitivity and specificity for characterization and quantitative determination of the alkaloids in *Corydalis yanhusuo* so far.

Keywords: LC–MS/MS; LC–DAD; Alkaloid; *Corydalis yanhusuo* W.T. Wang; Identification; Quantification

1. Introduction

Corydalis yanhusuo also called *Rhizoma corydalis* is well known as a traditional Chinese herbal medicine, prepared from the dried tubers of *Corydalis yanhusuo* W.T. Wang [1,2]. It has gained ever-increasing popularity in today's world because of its therapeutic effects for promoting blood circulation, reinforcing vital energy and alleviating pain such as headache, chest pain, epigastric pain, abdominal pain, backache, arthralgia or trauma [1,3]. Also it has been shown to be a traditional Chinese medicine in the treatment of cardiac arrhythmia disease, gastric and duodenal ulcer and menorrhalgia [4,5].

The chemical constituents of *Corydalis yanhusuo* have been investigated in some detail like tertiary and quaternary alkaloids [2,3]. Nearly 20 alkaloids of these two types have been isolated from *Corydalis yanhusuo* so far, which may be responsible for the biological activities of this drug [6]. Previous phytochemical studies have shown that alkaloids have been identified as the active secondary metabolites of the plant. Tetrahydropalmatine, the major active alkaloid in the plant, has been found to be a neuroactive alkaloid with analgesic and hypnotic action. Tetrahydropalmatine, corydaline and protopine have been found to be effective on allevitating pain, and the efficiency decreases in order [6–9]. Therefore, their rapid and accurate identification is of great significance in the quality control of this natural medicine and its formulations [10]. High-performance liquid chromatography (HPLC) had been used to analysis alkaloids in *Corydalis yanhusuo* in some previous studies [6,11–19]. However, none of the above analyses dealt with tertiary alkaloids and quaternary alkaloids simultaneously in one run process. Meanwhile, little reference concerning the simultaneous characterization of both tertiary alkaloids and

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quaternary alkaloids could be found. It is obvious that they are not sufficient for authenticating this herb since the two types of alkaloids are pharmacologically active components in *Corydalis yanhusuo*.

In this paper, the HPLC–ESI–MS/MS technique in positive ion mode was used to identify 10 alkaloids including seven tertiary alkaloids and three quaternary alkaloids in the extract of *Corydalis yanhusuo*, based on direct comparison with authentic standards. The proposed fragmentation rules could facilitate the convenient and rapid identification of *Corydalis yanhusuo* and also adjust to other alkaloids in *Corydalis* species. In addition, quantification of the 10 alkaloids in *Corydalis yanhusuo* from the crude extract and ethyl acetate extract was performed and samples from 10 different origins have been analyzed, which provides a new tool for the assessment of extraction procedure of *Corydalis yanhusuo.*

2. Experimental

2.1. Material and reagents

Acetonitrile for HPLC analysis was of chromatographic grade (Merck, Germany); acetic acid, ethanol, HCl, NaOH, ethyl acetate and triethylamine were of AR grade from WuLian Chemical Factory (Shanghai, China); 0.45 μm membrane filter was purchased from Millipore Corporation (USA); Water for HPLC analysis was purified by a Milli-Q academic water purification system (Millipore, USA). The *Corydalis yanhusuo* was purchased from a local drug store and identified by Dr. Luping Qin (Department of Pharmacognosy, College of Pharmacy, the Second Military Medical University, Shanghai, China). Reference compounds, protopine, tetrahydrocolumbamine, glaucine, palmatine, berberine, dehydrocorydaline, tetrahydropalmatine, canadine, corydaline, and tetrahydrocoptisine (see Fig. 1) were isolated from plants "*Corydalis yanhusuo W.T. Wang*" by ourselves, and their purity was checked by reversed-phase HPLC. The identities of the isolates were characterized by ^{1}H-NMR, MS spectra and the comparison with the literature data [10]. Samples of *Corydalis yanhusuo W.T. Wang* were purchased from 10 different provinces of China.

2.2. Sample preparation

About 5 g of dried tuber of *Corydalis yanhusuo* was chopped and extracted 3 times by reflux with 100 ml volumes of 70% ethanol in a round bottom flask for 1 h. After filtration, the extract was combined and evaporated to 10 ml by rotary evaporation at 50 °C under reduced pressure to yield the crude extract. The pH of the solution was set to 2 with HCl and filtered. Then the pH value of the filtrate was raised to 12 with NaOH and extracted with ethyl acetate. The extract was collected and evaporated to dryness by rotary evaporation at 50 °C under reduced pressure to obtain the ethyl acetate extract. The ethyl acetate extract was then dissolved in 10 ml methanol. The crude extract and the ethyl acetate extract were filtered through 0.45-μm membrane filter before HPLC analysis.

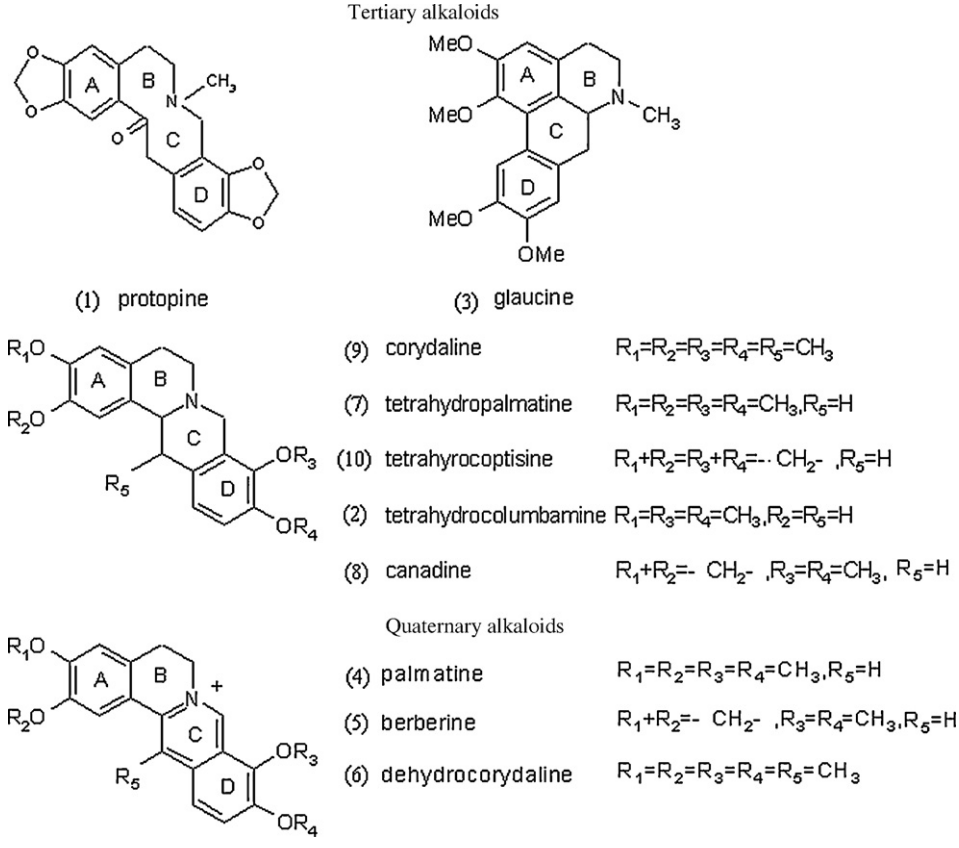


Fig. 1. Chemical structures of alkaloids 1–10.

2.3. HPLC–DAD–ESI–MS/MS system

A Varian ProStar HPLC instrument (Varian Corporation, USA), equipped with a ProStar 210 solvent delivery module, a ProStar 410 autosampler and a Prostar 330 photodiode array detector (DAD) was used. For HPLC–MS analysis, a Varian 1200 l triple quadrupole mass spectrometer was connected to the HPLC instrument via an ESI source in a post-column splitting ratio of 4:1. Data acquisition and analyses were performed using the Varian 1200 l workstation software.

The mass spectrometer was run in the positive ion mode (ESI+) and set up in the multiple reaction monitoring (MRM) mode. The electrospray capillary potential was set to −40 V. Nitrogen was used as a drying gas for solvent evaporation. The API housing and drying gas temperatures were kept at 50 and 350 °C, respectively. The full-scan mode over the mass range of *m/z* 100–400 spectra of the ethanolic extract solution was obtained. Argon was used as the collision gas. The scan time was 1 s and the detector multiplier voltage was set to 1090 V.

DAD detection was achieved in the range of 190–400 nm. A Diamonsil^{TM} C_{18} column (4.6 mm × 200 mm, 5 μm, Dikma Technologies, Beijing, China) was used along with a pre-column filled with the same stationary phase. A linear gradient elution of A (0.2% acetic acid solution, adjusted with triethylamine to pH 5.0) and B (acetonitrile) was used according to the following profile: 0–15 min, 20% B; 15–35 min linear increase to 80% B; 35–37 min linear decrease to 20% B. The mobile phase was filtered through a 0.45 μm membrane filter and degassed before delivered into the system. The solvent flow rate was 1.0 ml/min and the column temperature was set at 25 °C. The injection volume was 20 μl. The HPLC chromatogram was monitored at 280 nm.

2.4. Validation of the quantitative analysis

2.4.1. Calibration curves

A stock solution containing 10 analytes was prepared by dissolving the reference compounds in methanol and then diluted with methanol to appropriate concentrations for establishing calibration curves. Solutions containing different concentrations of the 10 analytes were injected in triplicate. Calibration curves were peak area versus concentration for each analyte.

2.4.2. Limits of detection and quantitation

Stock solution containing 10 reference compounds was diluted to a series of appropriate concentrations with methanol and an aliquot of the diluted solutions was injected into HPLC for analysis, the limit of detection (LOD) and limit of quantification (LOQ) for each analyte was calculated with corresponding standard solution on the basis of signal-to-noise ratio (S/N) of 3 and 10, respectively.

2.4.3. Precision, stability and accuracy

To assess the precision, stability and accuracy of the developed method, Batch No. 2 was selected randomly to be extracted and injected for HPLC analysis.

Intra- and inter-day variations were determined for precision. Certain concentrations of standard and sample solutions were tested. For intra-day variability test, the standard solution was analyzed 6 times within 1 day ($n = 6$), and the inter-day reproducibility was determined with six individual sample solutions for three consecutive days ($n = 6$).

Stability study was performed with sample solution on two consecutive days ($n = 8$). Variations were expressed by relative standard deviations (R.S.D.).

To determine the recovery, the contents of the 10 analytes in a sample were calculated according to their respective calibration curves. The same volume of each analyte presented in the sample was spiked into the sample for 6 times. Then the fortified samples were extracted, disposed as described above, and analyzed with the procedure. The average recoveries were estimated by the formula: recovery(%) = (amount found − original amount)/amount spiked × 100%, and R.S.D. (%) = (S.D./mean) × 100%.

3. Results and discussion

3.1. HPLC–DAD–MS/MS analysis

The chemical structures of the 10 alkaloids were characterized based on their retention behavior and their UV spectra obtained on-line (Table 1). Different types of compounds showed different UV absorption characteristics. Alkaloids exhibited maximum absorption at 260–300 nm. This characteristic facilitated their preliminary identification, and 10 peaks with suitable maximum absorptions were selected as the major alkaloids in *Corydalis*.

Table 1
Retention times (t_R),MS data and UVλ_{max} values for the main alkaloids present in *Corydalis yanhusuo* W.T. Wang

No	Retention time (min)	Identification	UV λ_{max} (nm)	$[M+H]^+$ *m/z*	$[M]^+$ *m/z*	ESI-MS/MS *m/z*
1	11.13	Protopine	286.6	354	–	206,188,149
2	12.46	Tetrahydrocolumbamine	279.8	342	–	178,165,151
3	13.51	Glaucine	280.2	356	–	324,294,279
4	17.05	Palmatine	272.0	–	352	337,336,322,308
5	18.23	Berberine	277.3	–	336	321,306,292,278
6	19.28	Dehydrocorydaline	263.3	–	366	351,350,334,322
7	21.17	Tetrahydropalmatine	279.9	356	–	192,165,151
8	28.28	Canadine	271.3	340	–	176,165,149
9	31.67	Corydaline	280.6	370	–	192,165,151
10	34.18	Tetrahydrocoptisine	287.5	324	–	176,149

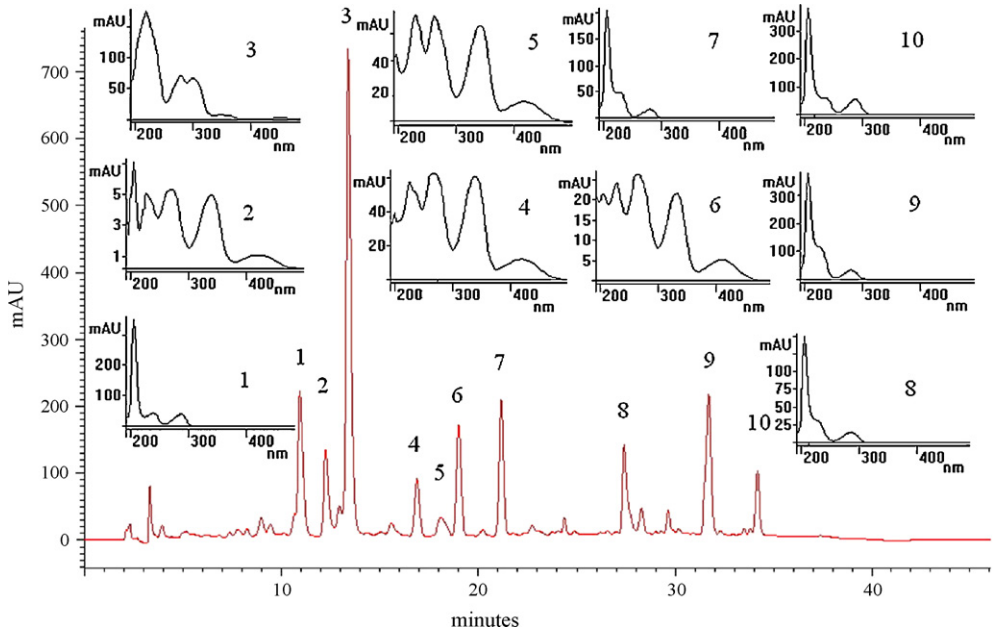


Fig. 2. HPLC–DAD chromatogram of extract of *Corydalis yanhusuo* (gradient elution); diode array UV-detection (λ = 280 nm).

For MS analysis the positive ion mode of ESI was selected in the present study, as it easily provided extensive information via collision-induced dissociation (CID) fragmentations. Under the optimized HPLC and MS/MS conditions all 10 alkaloids could be unequivocally identified in *Corydalis yanhusuo* W.T. Wang by comparing their retention times and their UV and MS data with those of the reference standards. The structures were further characterized mainly based on their MS fragmentation behavior (see Table 1, Figs. 2 and 3).

Tertiary alkaloids gave $[M+H]^+$ ions. The $[M+H]^+$ ions of tetrahydrocolumbamine, tetrahydropalmatine, canadine, corydaline and tetrahydrocoptisine eluting at 12.46, 21.17, 28.28, 31.67 and 34.18 min were observed to undergo the Retro–Diels–Alder (RDA) fragmentation reaction. Since there was a double bond in the C-ring, which was opposite to the B-ring, the C-ring was opened. Two fragment ions were obtained from the part of tetrahydroisowuinolin and the part of benzene ring, respectively. As an example, the RDA pathway proposed for tetrahydropalmatine is shown in Scheme 1. The same fragmentation behavior can also be applied to tetra-proberberine alkaloids.

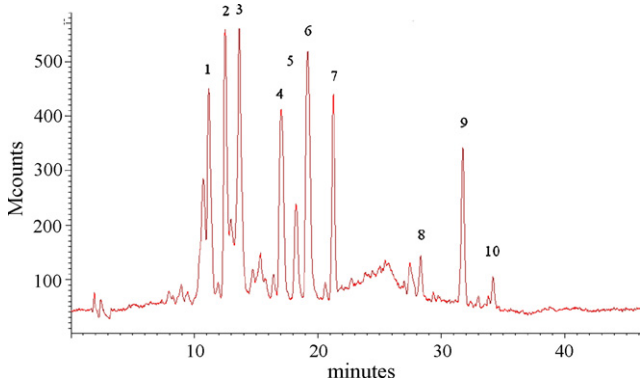
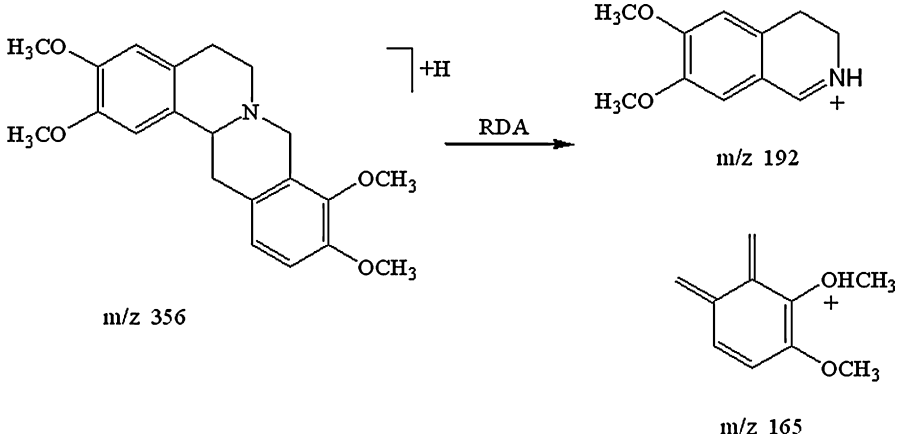
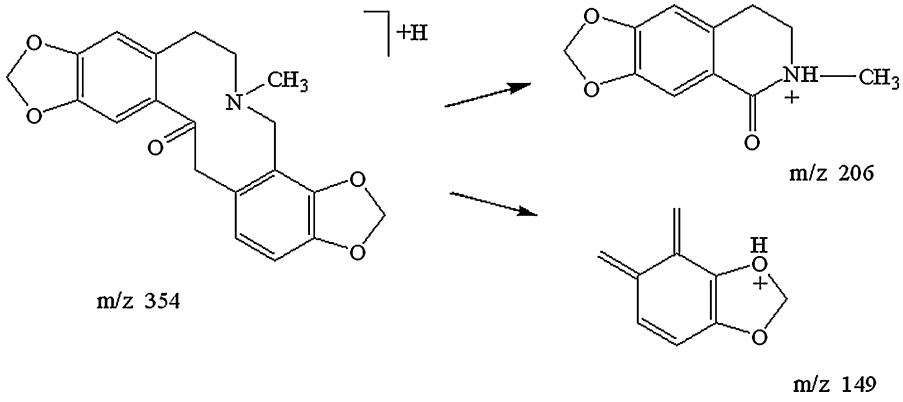


Fig. 3. HPLC–MS total ion chromatogram of the extract of *Corydalis yanhusuo*.

The $[M+H]^+$ ion at *m/z* 354 eluting at 11.13 min was observed to undergo similar Retro-Diels-Alder (RDA) fragmen-



Scheme 1. Proposed retro-Diels-Alder (RDA) pathway for tetrahydropalmatine.

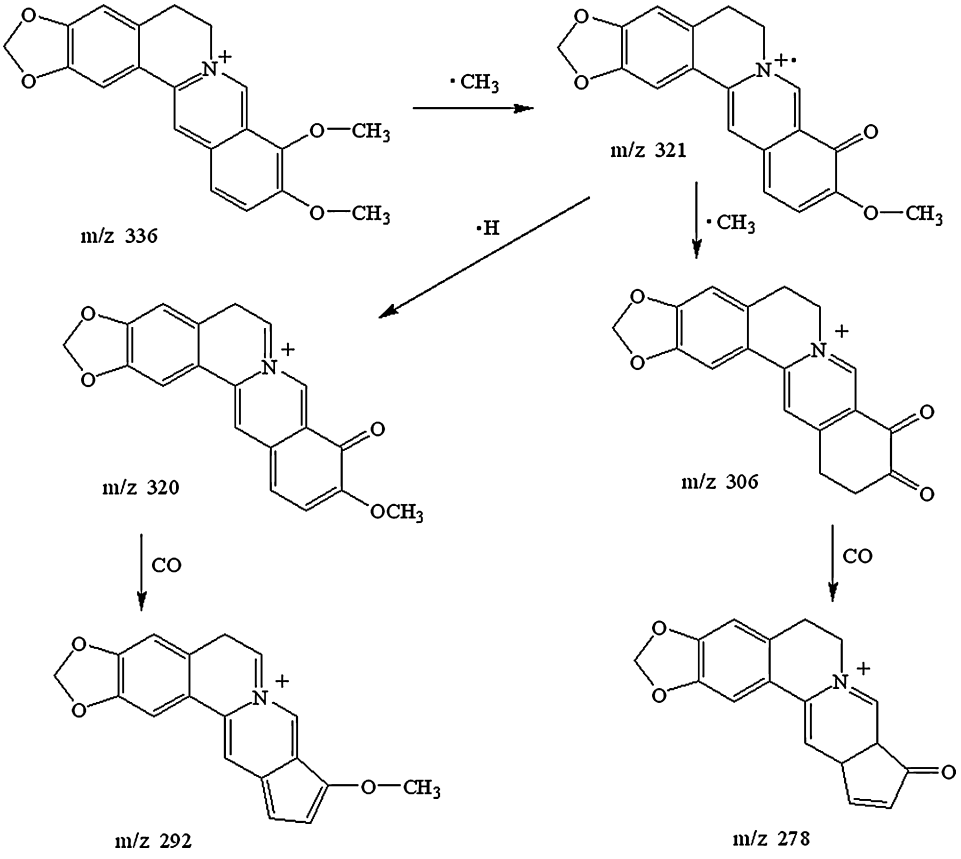


Scheme 2. Proposed fragmentation pathway for protopine.

tation reaction; the fragment was here observed at *m/z* 206 and 149. Furthermore, the neutral loss of H_2O (*m/z* 206 → 188) was also observed, and the ion at *m/z* 188 was significant in the MS/MS spectra. The loss of H_2O could be adopted to identify the presence of a carbonyl group in *ortho*-positions, and hence the $[M+H]^+$ ion was identified as protopine. The fragmentation pathway proposed for protopine is shown in Scheme 2.

The $[M+H]^+$ ion at *m/z* 356 eluting at 13.51 min gave an ion $[M+H–2OCH_3]^+$ at *m/z* 294 as significant ion with a further fragment $[M+H–2OCH_3–CH_3]^+$. However, $[M+H–OCH_3]^+$ and $[M+H–OCH_3–CH_3]^+$ were observed without any further fragmentations. It was remarkable that no ring cleavage occurred and the loss of CH_3 could be adopted to identify the presence of methyl connected to nitrogen. The $[M+H]^+$ ion at *m/z* 356 was identified as glaucine.

Three quaternary alkaloids eluting at 17.05, 18.23 and 19.28 min, giving $[M]^+$ ions, were identified as palmatine, berberine, and dehydrocorydaline. The CID spectra of the $[M]^+$ ions were studied using MS/MS. They provided significant $[M–CH_3]^+$ fragments with characteristic *m/z* values as the base peak in the product-ion spectra, in agreement with previous reports [20,21]. In addition, $[M–CH_3–H]^+$ and $[M–2CH_3]^+$ fragments were also observed in the product-ion spectra. Furthermore, these $[M–CH_3–H]^+$ and $[M–2CH_3]^+$ fragments could undergo subsequent CO loss. As an example, the fragmentation pathway proposed for berberine is shown in Scheme 3



Scheme 3. Proposed fragmentation pathway for berberine.

Table 2
Linear regression, precision of the 10 analytes in *Corydalis yanhusuo*

Analytes	Linear regression				Precision	
	Calibration curves	Correlation coefficient	Linear range (μg/ml)	LOD (μg)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)
1	$y = 69.49x + 266.97$	0.9981	20–500	0.10	1.25	2.56
2	$y = 56.63x + 185.00$	0.9977	20–500	0.10	1.69	2.23
3	$y = 65.66x + 160.50$	0.9997	50–1000	0.10	1.76	3.24
4	$y = 56.03x + 201.75$	0.9994	20–500	0.08	1.03	2.31
5	$y = 68.21x + 76.50$	0.9980	20–500	0.10	2.36	3.75
6	$y = 62.95x + 42.99$	0.9975	20–500	0.08	1.53	2.30
7	$y = 56.03x + 201.75$	0.9997	20–500	0.04	2.37	2.97
8	$y = 46.07x + 105.30$	0.9996	10–100	0.05	1.89	3.12
9	$y = 57.66x + 202.48$	0.9988	20–500	0.10	1.34	2.84
10	$y = 55.39x + 261.50$	0.9977	10–100	0.05	2.43	3.66

y and x stand for the peak area and the concentration (μg/ml) of the analytes, respectively.

Table 3
Recovery of alkaloids determined by standard addition method ($n = 6$)

Analytes	Original amount (mg)	Added amount (mg)	Detected amount mean (mg)	Recovery mean (%)	R.S.D. (%)
1	1.42	1.39	2.81	99.76	4.62
2	1.37	1.35	2.68	96.91	4.31
3	4.92	4.86	9.49	94.17	4.50
4	2.14	2.06	4.20	99.59	4.14
5	0.50	0.57	1.07	100.58	4.22
6	2.42	2.40	4.82	100.14	4.13
7	3.33	3.31	6.50	95.82	4.85
8	0.36	0.28	0.64	101.19	3.65
9	0.89	0.86	1.75	101.20	4.72
10	0.25	0.22	0.45	93.19	2.67

and its fragmentation behavior could be extended to other alkaloids.

3.2. Validation results

The experimental results showed that the peak area of each standard was linearly correlated to the injected concentration within a particular range (Table 2). As shown in Table 2, all calibration curves showed good linear regression ($R^2 > 0.9975$) and the LOD was less than 0.10 μg per injection, indicating that this method is precise and sensitive for the quantitative evaluation of major active alkaloids in *Corydalis yanhusuo*. Validation studies of the method proved that this assay had good reproducibility, and the overall intra-day and inter-day variations were less than 5% for all analytes. It was also found that the analytes in the sample solution were stable for 2 days with a relative standard deviation less than 5%. As illustrated in Table 3, the method is accurate with the overall recovery of more than 93%.

3.3. Quantitative determination of Corydalis yanhusuo

The results for the contents of the 10 alkaloids in crude extract and ethyl acetate extract from six batches in Tables 4 and 5 show that the sample preparation method is reliable.

Table 4
Contents of 10 alkaloids in the crude extract (mg/g) ($n = 3$)[a]

Batches						
Analytes	1	2	3	4	5	6
1	0.493 ± 0.015	0.497 ± 0.021	0.48 ± 0.008	0.503 ± 0.008	0.486 ± 0.006	0.487 ± 0.004
2	0.415 ± 0.005	0.423 ± 0.006	0.418 ± 0.018	0.424 ± 0.006	0.423 ± 0.007	0.416 ± 0.006
3	1.353 ± 0.015	1.379 ± 0.028	1.315 ± 0.038	1.323 ± 0.025	1.369 ± 0.024	1.348 ± 0.015
4	0.523 ± 0.015	0.533 ± 0.021	0.537 ± 0.015	0.517 ± 0.015	0.537 ± 0.021	0.523 ± 0.015
5	0.183 ± 0.006	0.177 ± 0.007	0.191 ± 0.007	0.189 ± 0.009	0.192 ± 0.010	0.181 ± 0.008
6	1.147 ± 0.032	1.133 ± 0.015	1.119 ± 0.005	1.147 ± 0.025	1.133 ± 0.021	1.111 ± 0.037
7	0.807 ± 0.015	0.823 ± 0.025	0.827 ± 0.021	0.807 ± 0.015	0.823 ± 0.012	0.797 ± 0.021
8	0.075 ± 0.003	0.078 ± 0.003	0.083 ± 0.004	0.080 ± 0.004	0.078 ± 0.003	0.082 ± 0.003
9	0.221 ± 0.009	0.206 ± 0.010	0.223 ± 0.006	0.235 ± 0.005	0.212 ± 0.012	0.224 ± 0.005
10	0.071 ± 0.003	0.072 ± 0.005	0.077 ± 0.003	0.073 ± 0.004	0.071 ± 0.002	0.077 ± 0.004

[a] The value referred to the amount of certain alkaloid per crude *Corydalis yanhusuo*.

Table 5
Contents of 10 alkaloids in the ethyl acetate extract (mg/g) ($n = 3$)

Batches						
Analytes	1	2	3	4	5	6
1	20.92 ± 0.33	20.30 ± 0.34	20.60 ± 0.52	21.18 ± 0.30	20.72 ± 0.37	20.43 ± 0.77
2	18.59 ± 0.55	19.59 ± 0.73	18.68 ± 0.59	19.60 ± 0.54	20.23 ± 0.73	18.60 ± 0.41
3	69.14 ± 1.18	70.21 ± 0.89	68.96 ± 1.31	68.39 ± 1.17	68.68 ± 1.72	69.08 ± 0.92
4	30.21 ± 1.31	30.64 ± 1.19	30.42 ± 0.81	30.12 ± 0.79	30.54 ± 0.68	30.47 ± 0.68
5	7.27 ± 0.37	7.08 ± 0.09	7.09 ± 0.19	7.31 ± 0.24	7.03 ± 0.14	7.17 ± 0.22
6	34.84 ± 1.50	34.58 ± 1.50	34.64 ± 1.49	33.71 ± 1.10	34.02 ± 1.47	33.39 ± 0.82
7	46.49 ± 0.59	47.58 ± 1.57	46.17 ± 0.69	45.92 ± 1.99	46.73 ± 1.39	47.49 ± 2.37
8	5.12 ± 0.12	5.16 ± 0.27	5.06 ± 0.27	5.07 ± 0.09	4.99 ± 0.16	4.98 ± 0.18
9	12.73 ± 0.33	12.68 ± 0.55	12.92 ± 0.15	12.94 ± 0.10	12.84 ± 0.12	12.73 ± 0.83
10	3.61 ± 0.07	3.58 ± 0.15	3.58 ± 0.08	3.56 ± 0.10	3.59 ± 0.14	3.59 ± 0.15

Table 6
Contents of 10 analytes in samples of *Corydalis yanhusuo* ($n = 3$)

Sample origin	Contents (mg/g)									
	1	2	3	4	5	6	7	8	9	10
Zhejiang	0.48	0.42	1.34	0.54	0.19	0.69	0.78	0.10	0.88	0.08
Hebei	0.32	0.45	0.98	0.72	0.22	1.05	0.61	0.09	0.71	0.07
Fujian	0.38	0.43	0.96	0.33	0.18	0.75	0.53	0.12	0.75	0.09
Anhui	0.45	0.42	1.23	0.74	0.23	0.87	0.71	0.09	0.94	0.10
Jiangsu	0.38	0.36	1.05	0.72	0.14	0.74	0.77	0.10	0.86	0.06
Shandong	0.37	0.25	0.67	0.21	0.14	0.66	0.67	0.08	1.06	0.07
Hubei	0.41	0.36	0.76	0.31	0.17	0.46	0.58	0.10	1.11	0.08
Shanxi	0.45	0.39	0.98	0.43	0.21	0.54	0.09	0.06	0.05	0.07
Jilin	0.53	0.32	0.87	nd[a]	0.05	nd[a]	0.14	0.04	0.19	0.05
Liaoning	0.35	0.39	0.76	nd[a]	0.17	nd[a]	0.45	0.08	0.33	0.07

[a] Not detected.

The current method was also utilized to analyze the 10 alkaloids in samples purchased from 10 different locations (Table 6). It was found that there were remarkable differences, in terms of concentrations of the 10 alkaloids from different places. From provinces of Zhejiang, Hebei, Fujian, Anhui, Jiangsu, Shandong and Hubei in China, the content of the 10 alkaloids was found to be similar in *Corydalis yanhusuo*. In sample from province Shanxi, contents of compound 7 and compound 9 were found to be lower than those in other samples. Compound 4 and compound 6 were not found in the samples from provinces of Jilin and Liaoning, while other 8 compounds were found to be much lower than those in other samples.

4. Conclusions

A rapid and efficient method for simultaneous identification and quantification of 10 alkaloids in *Corydalis yanhusuo* by LC–MS/MS and LC–DAD was established, which can facilitate the convenient and rapid quality control of the production procedure of *Corydalis yanhusuo* preparations. In addition, samples from 10 different origins have been analyzed. The specific MS and MS/MS fragmentation rules based on comparisons with authentic standards could facilitate the rapid screening and structural characterization of alkaloid extracts by LC/ESI-MS. The analytical liquid chromatographic separation of 10 alkaloids of *Corydalis yanhusuo* with UV-detection demonstrated an accurate and reproducible quantitation of these alkaloids.

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References

[1] F.Y. Tang, A.G. Nie, J. Clin. Exp. Med. 5 (2006) 185–186.
[2] Sh.Q. Tong, J.Zh. Yan, J. Liq. Chromatogr. Related Techol. 28 (2005) 2979–2989.
[3] W.C. Lenung, H. Zheng, Prog. Neuropsychopharmacol. Biol. Psychiatry 27 (2003) 775–779.
[4] A.P. Sagare, Y.L. Lee, T.C. Lin, C.C. Chen, Plant Sci. 160 (2000) 139–147.
[5] L.Z. Zhang, J. Guiyang Med. Coll. 31 (2006) 280–282.
[6] Z.H. Cheng, T.L. Guo, H.Y. Wang, G.Q. Chen, Chin J. Nat. Med. 2 (2004) 99–102.
[7] C.J. Xie, Z.Q. Zhang, F.Q. Zhang, J. Shanxi. Nor. Univ. 33 (2005) 82–85.
[8] C.K. Lai, Y.W. Chan, Clin. Chem. 45 (1999) 229–236.
[9] F.Y. Tang, A.G. Nie, Y.L. Li, J. Clin. Exper. Med. 5 (2006) 185–187.
[10] X.H. Xu, Zh.G. Wang, J. Chin. Pharm. Univ. 33 (2002) 483–486.
[11] Y.F. Yuan, Z.L. Liu, X.L. Li, Biomed. Chromatogr. 10 (1996) 11–14.
[12] X.Y. Fu, W.Z. Liang, G.S. Tu, Acta Pharmaceutica Sinica 21 (1986) 527–531.

[13] J.M. Cheng, S.L. Jing, Lishizhen Med. Mater. Res. 17 (2006) 1236–1237.
[14] K. Sagra, Y. Ito, M. Qjima, Chem. Pharm. Bull. 33 (1985) 5369–5374.
[15] J.J. Qu, L. Kong, C.S. Pan, Chromatogr. A 1117 (2006) 163–169.
[16] Z.D. Zhai, Y.P. Shi, X.M. Wu, X.P. Luo, Anal. Bioanal. Chem. 384 (2006) 939–945.
[17] X.H. Xu, G.D. Yu, Z.T. Wang, Zhong Guo Zhong Yao Za Zhi 9 (2004) 399–401.
[18] Z.H. Cheng, T.L. Guo, H.Y. Wang, G.Q. Chen, Anal. Chim. Acta. 555 (2006) 269–277.
[19] Q. Yu, G.J. Xu, R.Y. Jin, J. Chin. Pharm. Univ. 19 (1988) 4–7.
[20] L.J. Li, A. Zeper, X. Yun, A.G. Wang, J. Chin. Mass Spectrom. Soc. 21 (2000) 81–82.
[21] S.M. Liu, P. Hu, G.A. Luo, Acta Pharmaceutica Sinica 40 (2005) 846–849.