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Chemical Fingerprint and Metabolic Fingerprint Analysis of the Medicinal Extract of *Corydalis yanhusuo* by HPLC-UV and HPLC-MS Methods

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HPLC-UV and HPLC-MS techniques were used in fingerprint analysis of the medicinal extract of *Corydalis yanhusuo*. An HPLC profile of medicinal extract of *Corydalis yanhusuo* was established as a characteristic fingerprint and employed to assess the consistency and differences between extracts. To determine the major constituents of medicinal extract of *Corydalis yanhusuo* for quality control, 12 major chromatographic peaks were characterized. To explore the *in vivo* metabolism of the major active constituents' rat plasma was analyzed by HPLC-MS following oral administration of medicinal extract of *Corydalis yanhusuo* at different time intervals. These results indicate that the quaternary alkaloids are significant for the biological activity of medicinal extract of *Corydalis yanhusuo*. It is concluded that the chemical fingerprint combined with the metabolic fingerprint is a useful means of controlling the quality of medicinal extract of *Corydalis yanhusuo* and clarifying the possible mechanism of action.

Key Words: Fingerprint, HPLC-MS technique, Alkaloids, *Corydalis yanhusuo*.

INTRODUCTION

Traditional Chinese medicine (TCM) has played an indispensable role in the prevention and treatment of diseases in China. *Corydalis yanhusuo* W.T. Wang, also known as *Rhizoma corydalis*, is one of the important crude drugs in TCMs. For centuries it has been employed in TCMs as an analgesic agent for treating spastic, abdominal and menstrual pain, as well as pain due to injuries[1]. In addition, it has been widely used to promote blood circulation and treat coronary heart diseases[2,3]. Alkaloids are acknowledged to be the major active constituents in *Corydalis yanhusuo*[4]. The chemical constituents of *Corydalis yanhusuo* adhere to two chemical types: tertiary and quaternary alkaloids. Tertiary alkaloids have been reported to be effective at alleviating pain, while some quaternary alkaloids such as dehydrocorydaline were found to be more active than tertiary ones in increasing the tolerance of mice to monobaric and hypobaric hypoxia[5-7]. Previous analysis of the quaternary alkaloids of *Corydalis yanhusuo* revealed limited information. We have carried out preliminary studies on the constituents in *Corydalis yanhusuo*, indentifying the medicinal extracts of *Corydalis yanhusuo* (MEC) and proven its efficacy in increasing the tolerance of rats to monobaric and hypobaric hypoxia as well as shown the protective effect against myocardial necrosis induced by isoproterenol. In total eleven alkaloids, including two tertiary alkaloids and nine quaternary alkaloids were isolated from MEC. Among the alkaloids identified, two are new protoberberine quaternary alkaloids, named 13-methylpalmatrubine and 13-methyldehydrocorydalmine, in addition noroxyhydrastinine, corunine and dehydrocorybulbine were also isolated from *Corydalis yanhusuo* for the first time[8-11].

Pharmacological studies have shown that quaternary alkaloids possess more potent biological activities than tertiary ones in antimyocardial ischemia. However, in the quality standard of *Corydalis yanhusuo*, only tetrahydropalmatine was quantified and used as a marker compound for the quality control of medicinal materials and preparation. Hence, this current quality control standard does not reflect a true and comprehensive list of active constituents of MEC, therefore it is inadequate for controlling the quality of MEC.

Analytical fingerprinting is a method for identifying the chemical components of medicines using spectrograms, chromatograms and other graphical techniques. Both the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) clearly denote that the appropriate fingerprint chromatogram should be applied to botanical drugs to assess their consistency. Currently, fingerprint analysis has been used successfully as a powerful tool for the quality control

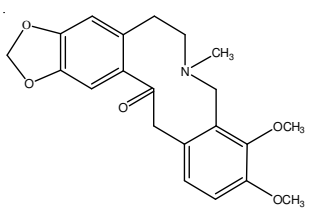
of multi-component herbal extracts and medicine and is accepted by the WHO as a strategy for assessing herbal medicines. There have been several studies regarding the use of HPLC, GC, CE, HPTLC fingerprints on the quality assessment of some herbal medicines and their raw materials[12-14].

Recently, electrospray ionization tandem mass spectrometry (ESI-MSn) has been used for structural analysis of constituents in herb extracts and has demonstrated a superior advantage over other methods due to its high sensitivity, rapidity and low sample consumption. In addition, liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography coupled with sequential mass spectrometry ($LC\text{-}MS^n$) have been extensively applied to the on-line structure elucidation of herb constituents[15-17]. Tandem mass spectrometry techniques also play an important role in metabolism analysis, by elucidating structure of drug metabolites *via* biotransformation[18,19].

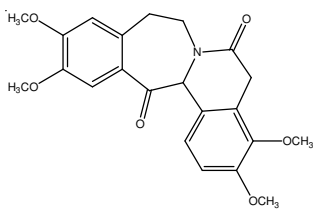
The present study aims to develop the HPLC-UV fingerprint of MEC and to characterize its major active constituents based on mass spectra. The fingerprint model can be used to accurately reflect the quality of and guarantee clinical efficacy of MEC. Metabolic MS-TLC chromatogram fingerprint profiles were developed after oral administration of MEC to rats at different time intervals in order to clarify the mechanism of clinical efficacy of MEC. A chemical fingerprint combined with a metabolic fingerprint will be helpful for clarifying the differences between the *in vitro* and *in vivo* alkaloids in MEC. This will be potentially useful in establishing a suitable quality control model for MEC.

EXPERIMENTAL

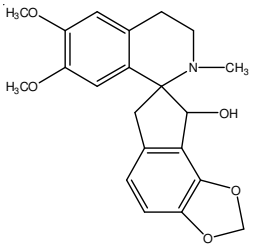
Acetonitrile (HPLC grade) was from Honeywell Burdick & Jackson Company. Acetic acid, ethanol and triethylamine were of analytical grade from Beijing Chemical Factory (Beijing, China); Milli-Q water (Millipore, Bedford, MA) was used throughout the study. All the standard reference chemical compounds were extracted, isolated and purified from *Corydalis yanhusuo* in our laboratory. The chemical structures of alkaloids identified from MEC are shown in Fig. 1. The tubes of *Corydalis yanhusuo* W.T. Wang were obtained from Zhejiang, China. MEC samples were prepared by our laboratory.



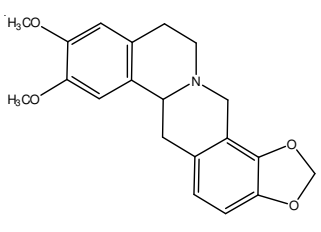
Allocryptopine



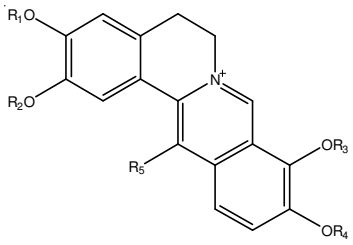
Saulatin



Fumaricine



Tetrahydroberberrubine



Compounds	R_1	R_2	R_3	R_4	R_5
Columbamine	CH_3	H	CH_3	CH_3	H
Coptisine	-CH_2-		-CH_2-		H
13-Methyldehydrocorydalmine	CH_3	CH_3	CH_3	H	CH_3
Dehydrocorybulbine	CH_3	H	CH_3	CH_3	CH_3
13-Methylpalmatrubine	CH_3	CH_3	H	CH_3	CH_3
Palmatine	CH_3	CH_3	CH_3	CH_3	H
Berberine	-CH_2-		CH_3	CH_3	H
Dehydrocorydalmine	CH_3	CH_3	CH_3	CH_3	CH_3
Corysamine	-CH_2-		-CH_2-		CH_3

Fig. 1. Structures of alkaloids identified from medicinal extracts of *Corydalis yanhusuo*

HPLC-UV instrument and conditions: A Waters HPLC system [consisting of a Waters 600 pump, a 2996 diode array detector (DAD) and a LC workstation equipped with Empower TM software for data collection] was used for quantitative determination. The chromatographic separation was performed on a Diamonsil C_{18} analytical column (4.6 mm × 250 mm, 5 μm, Dikma Corporation) with a column temperature set at 30 ºC. The UV Detection wavelength was set at 335 nm. The flow rate during analysis was constant at 1.0 mL/min and the injection volume was 10 μL. The mobile phase was water (containing 0.2% acetic acid, 0.1 % triethylamine)-acetonitrile (24:76, v/v).

HPLC-MS instrument and conditions: An Angilent HPLC system (Angilent, USA) coupled to an API 3000 Triple Quadrupole LC-MS/MS mass spectrometer from MDS Sciex/ Applied Biosystems (CA, USA) was used. Data acquisition and processing were performed using Analyst software from Applied Biosystems (CA, US). The HPLC conditions were the same as above. An electrospray ionization (ESI) interface with in positive mode was employed. The ESI conditions were as follows: capillary voltage = 3800 V, nebulizer gas = 8 (arbitrary units), curtain gas = 12 (arbitrary units), collision gas = 6 (arbitrary units), nebulizer current = 3 (arbitrary units) and temperature = 450 ºC, declustering potential = 20 (arbitrary units), focusing potential = 375 (arbitrary units), entrance potential = 10 (arbitrary units), collision energy = 50 eV.

Animal and biological sample collection: Eighteen male Wister rats (200 ± 20 g body weight) were provided by the Animal Center of the Chinese Academy of Medical Sciences and the Peking Union Medical College. The rats were maintained in an air-conditioned animal quarter at a temperature of 22 ± 2 ºC and a relative humidity of 50 ± 10 %, having free access to water and fed with a laboratory rodent chow. The animals were acclimatized to the facilities for 5 days and then fasted, with free access to water for 12 h prior to the experiment. Medicinal extracts of *Corydalis yanhusuo* was dissolved in an adequate volume of water and was administered to 15 rats (0.4 g extract/kg, body weight) by oral gavage. Physiological saline was orally administered to three control rats.

Blood samples were obtained from the oculi chorioideae vein before dosing and subsequently at 15, 30, 60, 120, 150, 180 min following administration. Blood was transferred to a clean heparinized eppendorf tube and centrifuged at 3500 rpm for 10 min. Plasma was frozen at -20 ºC until analysis.

Sample preparation: 20.0 mg of each MEC sample was dissolved in 100.0 mL 80 % methanol producing a concentration of 0.2 mg/mL. All samples were filtered through 0.45 µm filer for HPLC-UV and HPLC-MS analysis.

A 1.0 mL sample of plasma was mixed with 0.5 mL phosphate buffer (pH 7.2; 0.2 M) and vortexed for 30 s. The solution was then loaded and drawn through by gravity on an SPE cartridge, which was pretreated by first passing through 1 mL acetonitrile, 1 mL water and 1mL phosphate buffer (pH 7.2; 0.2 M) successively before loading. The solid-phase cartridge was then washed with 1 mL water and 1 mL 20 % methanol successively. Finally, the cartridge was eluted with 1 mL hydrochloric acid-acetonitrile (0.5:99.5, v/v) and the elution was evaporated until dry under a nitrogen stream without heating. The residue was dissolved in 100 µL of 2 % acetic acid-acetonitrile (1:9, v/v). After centrifugation at 10000 rpm for 15 min at 4 ºC, the supernatant was ready for HPLC-MS analysis.

RESULTS AND DISCUSSION

Optimization of chromatographic separation conditions: The selection of a mobile phase was a critical factor in achieving good chromatographic behaviour (peak shape and resolution) and appropriate ionization. Optimized chromatographic conditions were achieved following several trials with elution systems of methanol-water, acetonitrile-water and acetonitrile-acetate buffer in various proportions. It was found that the presence of 0.2 % acetic acid and 0.1 % triethylamine in the mobile phase leads to a significant improvement of the retention behaviour of alkaloids in MEC. However, the peaks were rather broad and had poor separation. Therefore, the optimum mobile phase was achieved with water (0.2 % acetic acid, 0.1 % triethylamine, v/v)-acetonitrile (24:76, v/v). The spectra of all peaks in the chromatogram of the sample were investigated using photodiode array detection (DAD). Column temperature did not obviously affect separation, therefore the column temperature was set at 30 ºC throughout analysis. DAD detection was employed at the wavelength range of 200-400 nm and the UV spectra of MEC were investigated. The chromatogram at 335 nm properly represented the profile of the target constituents in MEC and showed good separation as well as high sensitivity.

HPLC-UV fingerprint analysis of MEC: The HPLC-UV profiles of MEC are presented in Fig. 2. The constituents in MEC were well separated under the established HPLC conditions. In order to obtain a stable and repeatable chromatographic fingerprint of MEC for quality control validation of, the method of HPLC fingerprint analysis was performed on the basis of the retention time and the peak area. The sample solution of MEC was successively injected into the HPLC system six times. The precision of relative retention times (RRT) and relative peak areas (RPA) of all peaks did not exceed 0.38 and 2.67 %, respectively. The fingerprint data of 9 sample batches was processed in order to analyze the similarity between these samples using two different mathematic methods including correlation coefficient and the included angle cosine calculated using Microsoft Excel 2003 software[20]. All of the correlation coefficient values and the included angle cosine of the samples were more than 0.998. The method reproducibility was studied through six-replicated sample solutions from a single batch of the extract. The corresponding RSDs of RRT and RPA were reported as less than 0.25 and 0.50 %. The stability test was performed with a sample solution of MEC for 24 h. The RSDs of the RRT and RPA were found to be less than 0.50 and 2.19 %, respectively. The results indicate that the method is valid and applicable for sample analysis.

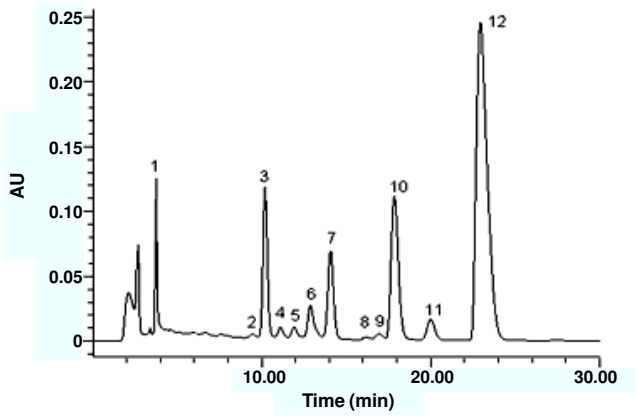


Fig. 2. HPLC-UV profiles of medicinal extracts of *Corydalis yanhusuo,* Peaks represented: 3 = columbamine; 5 = coptisine; 6 = 13-methyldehydrocorydalmine; 7 = dehydrocorybulbine; 9 = 13-methylpalmatrubine; 10 = palmatine; 11 = berberine; 12 = dehydrocorydalmine

To establish the representative chromatographic fingerprint, 9 MEC preparation batches were analyzed under the established HPLC-UV method. Peaks that appeared in all HPLC profiles from all 9 batches were assigned as "common peak" which represented the characteristics of MEC. There are 12 "common peaks" in HPLC fingerprint (Fig. 3). The whole HPLC profile and the relative retention time and relative areas of the common peaks of the 12 "common peaks" (Tables 1-2) can be used to characterize the quality of MEC and assess the consistency from batch to batch.

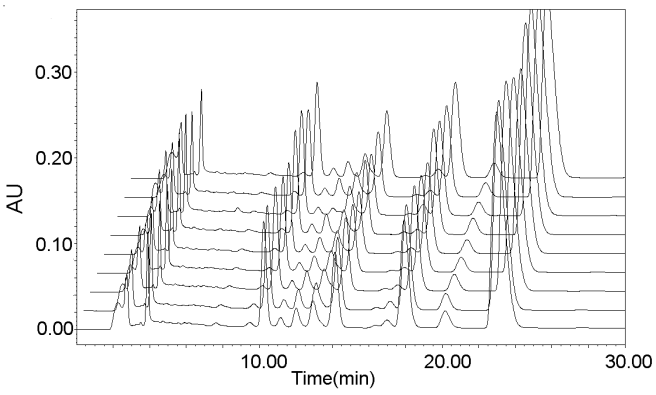


Fig. 3. HPLC-UV chromatograms of 9 batches of medicinal extracts of *Corydalis yanhusuo*.

TABLE-1
RELATIVE RETENTION TIME IN HPLC FINGERPRINTS OF MEDICINAL EXTRACT OF *Corydalis yanhusuo* (MEC)

Common peak no.	Sample number									RSD (%)
	1#	2#	3#	4#	5#	6#	7#	8#	9#	
1	0.1632	0.1630	0.1649	0.1637	0.1653	0.1689	0.1690	0.1670	0.1684	1.48
2	0.4103	0.4103	0.4130	0.4111	0.4106	0.4119	0.4139	0.4135	0.4135	0.36
3	0.4428	0.4435	0.4454	0.4443	0.4452	0.4455	0.4472	0.4454	0.4465	0.31
4	0.4827	0.4829	0.4850	0.4839	0.4849	0.4855	0.4863	0.4852	0.4857	0.26
5	0.5176	0.5179	0.5201	0.5187	0.5208	0.5227	0.5217	0.5210	0.5218	0.35
6	0.5602	0.5598	0.5622	0.5607	0.5616	0.5629	0.5628	0.5628	0.5625	0.21
7	0.6123	0.6118	0.6139	0.6128	0.6140	0.6147	0.6147	0.6144	0.6139	0.17
8	0.7060	0.7050	0.7074	0.7059	0.7257	0.7113	0.7073	0.7090	0.7083	0.89
9	0.7385	0.7370	0.7397	0.7383	0.7386	0.7386	0.7380	0.7389	0.7378	0.10
10	0.7775	0.7765	0.7785	0.7780	0.7785	0.7795	0.7792	0.7794	0.7784	0.12
11	0.8725	0.8709	0.8732	0.8719	0.8730	0.8771	0.8722	0.8753	0.8744	0.22
12	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.00

TABLE-2
RELATIVE AREAS OF THE COMMON PEAKS IN HPLC FINGERPRINTS OF MEDICINAL EXTRACT OF *Corydalis yanhusuo* (MEC)

Common peak no.	Sample number									RSD (%)
	1#	2#	3#	4#	5#	6#	7#	8#	9#	
1	0.0927	0.0885	0.0882	0.0883	0.0869	0.0929	0.0949	0.0857	0.0936	3.70
2	0.0081	0.0071	0.0071	0.0067	0.0075	0.0080	0.0081	0.0071	0.0072	6.95
3	0.1988	0.2000	0.1950	0.1812	0.2018	0.2016	0.2046	0.1989	0.2039	3.57
4	0.0179	0.0175	0.0182	0.0159	0.0146	0.0189	0.0194	0.0194	0.0199	9.79
5	0.0422	0.0377	0.0409	0.0418	0.0187	0.0416	0.0377	0.0416	0.0385	19.61
6	0.0709	0.0684	0.0640	0.0679	0.0769	0.0699	0.0707	0.0595	0.0631	7.55
7	0.1849	0.1828	0.1868	0.1806	0.1533	0.1851	0.1858	0.1828	0.1832	5.76
8	0.0071	0.0064	0.0065	0.0072	0.0066	0.0065	0.0055	0.0065	0.0058	8.34
9	0.0255	0.0196	0.0146	0.0205	0.0303	0.0229	0.0287	0.0175	0.0153	26.01
10	0.3307	0.3324	0.3283	0.3102	0.2988	0.3319	0.3360	0.3305	0.3333	3.86
11	0.0549	0.0540	0.0538	0.0513	0.0448	0.0551	0.0542	0.0551	0.0543	6.23
12	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.00

HPLC-MS analysis of MEC and identification of chemical compounds in fingerprint chromatograms: In order to identify the structures of the main constituents in MEC, the samples were analyzed by HPLC-MS. ESI in both negative and positive mode was tried. Results indicated that ESI in positive mode was sensitive to alkaloids and major constituents in MEC were well detected. Through careful analysis of the mass spectra of the compounds and comparisons with standards and reference data, 11 common peaks in MEC were designated and identified (Table-3) as described below.

Peaks 6-7 and 9-12, eluted at 12.75, 13.93, 16.78, 17.68, 19.84 and 22.72 min respectively and could be unambiguously assigned as 13-methyldehydrocorydalmine, dehydrocorybulbine, 13-methylpalmatrubine, palmatine, berberine and dehydrocorydaline through retention time comparison, $[M]^+$ ions and the characteristic fragment ions corresponding with authentic standards. A poor abundance of the ion at m/z 366 in the HPLC-ESI-MS of peak 9 was also detected. Its fragment ions in ESI-MS^2 were similar to that of molecular ions at m/z 352, such as 13-methylpalmatrubine and palmatine, indicating that the ion at m/z 366 might be a methylated derivative of the ion at m/z 352, which has not previously been reported in the literature. Hence the ion at m/z 366 in peak 9 is thought to be a new quaternary alkaloid.

Peak 3 eluted at 10.12 min and corresponds to the ions at m/z 338 and m/z 398 in the HPLC-ESI-MS. For the ion at m/z 338, the retention time and the fragment ions obtained by ESI-MS-MS are consistent with the columbamine standard. For the ion at m/z 398, the specific ion $[M\text{-}CH_4\text{-}CO]^+$ at m/z 354 was obtained, which is the characteristic ion of isoquinoline alkaloids., The ion at m/z 398 was identified as saulatin according to the literature[21]. Peak 5 eluted at 11.81 min and yields the ions at m/z 320 and m/z 338 in the HPLC-ESI-MS. For the ion at m/z 320, the retention time and the fragment ions obtained by ESI-MS-MS are consistent with the coptisine standard. The ion at m/z 338 was observed to undergo Retro-Diels-Alder (RDA) fragmentation reaction and showed fragment ions m/z 190 and 149. The ion at m/z 338 is identified as tetrahydroberberrubine according to the literature[22].

Peaks 2 and 4 eluted at 9.37, 11.02 min respectively and exhibited the same $[M]^+$ ion at m/z 370 in the HPLC-ESI-MS. They were observed to undergo Retro-Diels-Alder fragmentation reaction, the fragment was observed at m/z 206. Furthermore, the neutral loss of H_2O (m/z 206→188) was also observed for peak 2 and the ion at m/z 188 was significant in the MS-MS spectra. The loss of H_2O was used to identify the presence of a carbonyl group in *ortho*-position, in agreement with the literature[7] and hence peak 2 was identified as allocryptopine. For peak 4, the neutral loss of H_2O (m/z 206→188) was not observed, but the ion $[206\text{-}CH_4]^+$ at m/z 190 was detected. Therefore, peak 4 is identified as fumaricine according to the literature[23]. Peak 8 eluted at 16.06 min, which corresponds to the ion at m/z 334 in the HPLC-ESI-MS. The ion at m/z 334 showed the ion 306 $[M\text{-}CO]^+$ and corresponded

TABLE-3
ON-LINE DETECTED CHROMATOGRAPHIC AND SPECTROMETRIC DATA OF 13 CHARACTERISTIC, RETENTION TIMES (TR) AND MS DATA FOR THE MAIN ALKALOIDS PRESENT IN MEDICINAL EXTRACT OF *Corydalis yanhusuo*

Common peak no.	Retention time (min)	Major ions (m/z)	ESI-MS/MS (m/z)				Identification	Other ions (m/z)	ESI-MS/MS (m/z)	Identification
			$-CH_4$	-C=O	RDA	Other fragments				
1	3.76	492, 407, 307, 140	–	–	–	–	Unkonwn compound	–	–	–
2	9.37	370			206, 165	192, 188, 178	Allocryptopine	–	–	–
3	10.12	338	322	–	–	323, 308, 307, 294, 280, 279, 265, 250	Columbamine	398	382, 354, 322	Saulatin
4	11.02	370	354		206, 165	190	Fumaricine	–	–	–
5	11.81	320	–	292	–	277, 262	Coptisine	338	322, 280, 190, 188, 149	Tetrahydro-berberrubine
6	12.75	352	336	–	–	337, 322, 321	13-Methyldehydro-corydalmine	–	–	–
7	13.93	352	336,	–	–	321, 320, 308, 294, 279	Dehydrocorybulbine	–	–	–
8	16.06	334	–	306	–	304, 291, 276, 263, 248	Corysamine		–	–
9	16.78	352	336	–	–	320, 308, 292	13-Methyl-palmatrubine	366	320, 292	Unkown alkaloids
10	17.68	352	336	–	–	320, 308, 292	Palmatine	–	–	–
11	19.84	336	320	–	–	306, 293, 292, 278	Berberine	–	–	–
12	22.72	366	350	–	–	336, 322, 308, 306, 292, 278	Dehydrocorydalmine	–	–	–

to a loss of [-C=O]. It also exhibited the presence of the similar substitution group [-O-CH_2-O-] but had no methoxy group substitutions in benzene rings, in agreement with the literature[24,25]. Based on the comparison of its molecular ion and MS-MS spectrum with published data, $[M]^+$ ion at m/z 334 is proposed to be corysamine.

Peak 1 eluted at 3.76 min, corresponds to a complicated multi-component in the HPLC-ESI-MS making identification difficult.

Metabolic fingerprint analysis of MEC: To clarify the active constituents responsible for the pharmacological action and guarantee the clinical efficacy of MEC, it is necessary to know the metabolic changes and the *in vivo* chemical constituents profile in a biological system. Therefore, rat plasma samples after oral administration of MEC at different time intervals, were analyzed by the same HPLC-MS method used for MEC. A comparison of the MS-TLC chromatogram from blank plasma, showed no interference around the main active constituents of MEC. It was established that plasma preparation method and HPLC-MS analytical method for MEC are suitable for the analysis of active constituents in rat blood (Fig. 4).

In order to identify the structure of the main constituents in rat plasma, samples obtained 60 min after administration of MEC were analyzed using the established HPLC-MS method. A comparison between the main components in the chemical fingerprint of MEC and rat plasma showed that allocryptopine, saulatin, coptisine and corysamine were identified in MEC only, suggesting that these constituents are not absorbed or are quickly transformed to other forms during metabolism. However, with prolonged administration time other predominantly quaternary alkaloids such as dehydrocorydaline, palmatine, berberine and colubamine, slowly. In addition, an unknown ion at m/z 366 was detected since it presented in high concentration in plasma, indicating that this constituent was easily absorbed after oral administration to rats. This could be due to differences in binding affinity of constitutes with plasma protein or to the difference in metabolic rate in blood. 10 original alkaloids were detected and identified in plasma samples (Table-4). These results suggested that quaternary alkaloids may be important for the pharmacological activity of MEC.

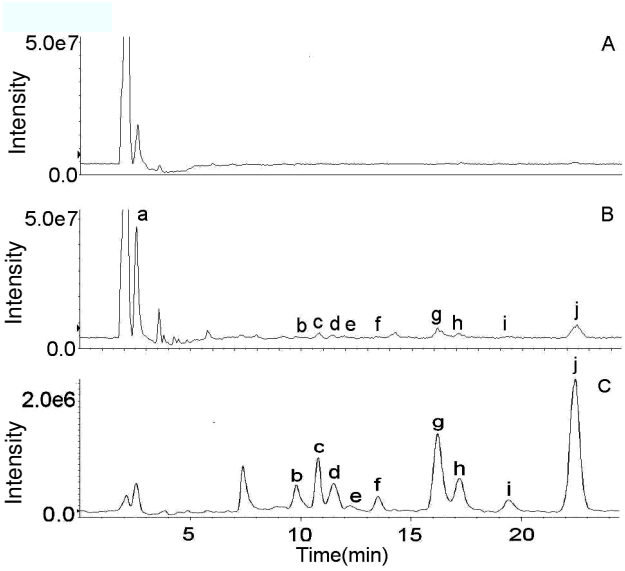


Fig. 4. MS-TLC chromatograms of blank rat's plasma (A), plasma after 60 min administration (B) and alkaloids in plasma marked in chemical fingerprint (C)

TABLE-4
MS DATA AND IDENTIFICATION OF MAJOR CONSTITUTES IN RAT PLASMA

Peak No.	TR (min)	Major ions (m/z)	m.w.	Identification	Consistent with the common peak no. of MEC
a	2.55	514 518 528 532 546	514 518 528 532 546	Glucuronide conjugates of alkaloids	–
b	10.12	338	338	Columbamine	3
c	11.02	370	369	Fumaricine	4
d	11.81	338	338	Tetrahydroberberrubine	5
e	12.75	352	352	13-Methyldehydro-corydalmine	6
f	13.93	352	352	Dehydrocorybulbine	7
g	16.33	366 352	366 352	Unknown alkaloids 13-Methylpalmatrubine	9
h	17.20	352	352	Palmatine	10
i	19.44	336	336	Berberine	11
j	22.62	366	366	Dehydrocorydaline	12

Molecular ions $[M]^+$, m/z 514, 518, 528, 532 and 546 were detected in the peak eluting at 2.55 min of the metabolic HPLC fingerprint. They produced a characteristic protonated ion $[M-176]^+$, which was identified as a molecular ion fragment of glucuronic acid. The ions shown above are proposed to be glucuronide conjugates of alkaloids and are part of the metabolic pathway of alkaloids in MEC. As previous studies have demonstrated the relative complexity of the metabolites of protoberberine alkaloids[26], not surprisingly, in this study, not all the metabolites or their original alkaloids in MEC were able to be fully identified. However, the inability to identify some components does not explain why so few protoberberine alkaloids were detected in plasma of rats and human in the previous study. Our results provide useful information regarding the active constituents of *Corydalis yanhusuo* and provide marked compounds for quality control of MEC. Based on this study, fingerprint analysis can be used to improve the preparation and quality control of MEC.

Conclusion

In this paper HPLC-UV and HPLC-MS techniques were used in chemical fingerprint and metabolic fingerprint analysis of the medicinal extract of *Corydalis yanhusuo* (MEC). A chemical fingerprint of MEC was established and 13 alkaloids of MEC were identified by comparing their mass spectra and retention behaviour with reference standards or literature data. Most of the major quaternary alkaloids in MEC were detected and identified in rat plasma after oral administration. Furthermore, several generated constitutes were observed and are proposed to be glucuronide conjugates of alkaloids. This study offers scientific data to clarify active constituents responsible for the pharmacological effects of MEC. It is also useful for better understanding the *in vivo* metabolism of MEC constituents.

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