Development and validation of a UPLC-DAD-MS method for characterization and quantification of alkaloids in Menispermi Rhizoma and its preparations

Yanan Liu a,b,c,d, Xiao Song a,b,d, Ruiqing Yan a,b, Tianxiang Li c, Xin Chai a,b, Aidi Qi c, Yuefei Wang a,b,* Zhenzuo Jiang a

a Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, PR China
b Tianjin Key Laboratory of TCM Chemistry and Analysis, Tianjin University of Traditional Chinese Medicine, Tianjin, PR China
c College of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, PR China

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Abstract
Menispermi Rhizoma (MR), a well known traditional Chinese medicine, is widely used to prevent and treat sore throat, enteritis, dysentery and rheumatoid arthralgia clinically. However, many rhizomes of Chinese herbal medicines are mistaken as MR due to their similar appearance, which could affect MR quality and cause serious consequences for patients. To guarantee the quality of MR products, an ultra-high-performance liquid chromatography–diode array detector–tandem mass spectrometry (UPLC-DAD-MS) method was established for the characterization of major active ingredients in MR and its preparations. By comparing their retention times and characteristic fragmentations with those of authentic compounds, nine alkaloids in MR were unequivocally identified as acutumidine, acutumine, magnoflorine, menisperine, dauricine, menisporphine, N-demethyl-N-formyldehydronuciferine, 6-O-demethylmenisporphine and dauriporphine. Quantitative analysis of the nine alkaloids in MR and its preparations was accomplished by UPLC-DAD. A UPLC C18 column was employed for the chromatographic separation which was effected by a gradient elution with acetonitrile and 0.1% aqueous formic acid solution containing 5 mM ammonium acetate at a flow rate of 0.3 mL/min. This quantitative method was validated with good linearity ($R^2 > 0.9991$), desirable intra- and inter-day precisions (RSD $< 3.32\%$), and acceptable recoveries (97.90–106.8%). The method was also successfully applied to quantify nine alkaloids in eight batches of MR, six batches of MR capsules and two batches of MR pills. A counterfeit MR sample from Henan province was identified by the validated method, followed by further verification by appearance and microscopic identification. The developed UPLC-DAD-MS method overcame the shortcomings of other quality control methods, such as scant chemical marker, long analytical time, consumption of large amounts of organic solvents and limitation to MR or its single dosage form.
1. Introduction

Menispermi Rhizoma (MR), the dried rhizome of Menispernum dauricum DC, with the function of clearing away heat and toxic material, expelling wind and removing dampness, is widely used to prevent and treat sore throat, enteritis, dysentery and rheumatoid arthralgia clinically [1]. On account of facilitating clinical use, MR pills and capsules, as two modern pharmaceutical dosage forms, have been developed and recorded in the Chinese Pharmacopoeia [1]. Modern pharmacological studies have reported that alkaloids are the major active ingredients in MR and its preparations [2], which have been demonstrated to have activities of anti-inflammation [3], anti-bacterial [4], preventing in vitro cancer cell proliferation [5], inhibiting platelet aggregation [6], neuroprotection [7], and protecting against myocardial ischemia and cerebral ischemia [8,9].

In recent years, many rhizomes of Chinese herbal medicines such as Sophora tonkinensis Gagnep [10] and Aristolochia mollissima Hance [11] have been mistaken for MR due to their similar appearance, which could affect MR quality and cause serious consequences for patients. To guarantee the quality of MR and its preparations, several analytical methods have been employed, such as high-performance liquid chromatography with ultraviolet detector (HPLC–UV) [12,13], HPLC with tandem mass spectrometry (HPLC–MS) [14], thin-layer chromatography micellar fluorometry system [15], spectrophotometric method [16], and capillary electrophoresis (CE) [17]. However, these methods are confined by scant quantitative markers [18], long analytical time, consumption of large amounts of organic solvents and limitation to MR or its single dosage form [13,19]. Thus, we were prompted to develop a comprehensive analytical method to evaluate more bioactive compounds in MR and its preparations to ensure their safety and efficacy.

In this paper, an ultra performance liquid chromatography-diode array detector-tandem mass spectrometry (UPLC-DAD-MS) method was established for the characterization of alkaloids in MR and its preparations. The validated UPLC-DAD method was successfully applied to quantify nine alkaloids in eight batches of MR purchased from different origins, six batches of MR capsules and two batches of MR pills. A counterfeit MR sample, identified using the validated method, was further verified by appearance and microscopic identification. Compared to previously reported methods, the newly developed analytical method was more comprehensive, selective, sensitive and efficient for quality control of MR products.

2. Methods

2.1. Materials

Eight batches of MR were obtained from various drug stores throughout China and labeled as MD1–4 (Xinzhou, Shanxi, China), MD5 (Taiyuan, Shanxi, China), MD6 (Zhengzhou, Henan, China), MD7 (Nankai District, Tianjin, China) and MD8 (Mongolia, China). All samples were authenticated by Dr. Tianxiang Li from the College of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine in Tianjin, China. The voucher specimens were deposited in the Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine. Six batches of MR capsules were collected from three pharmaceutical factories in China, which were labeled C-YC1-3, C-JFK1-2 and C-WSLJ. Two batches of MR pills labeled P-AS and P-YCWJS were purchased from two pharmaceutical factories in China.

2.2. Chemicals and reagents

Reference standards, acutumidine, acutumine, magnoflorine, menisperine, dauricine, menisperphine, N-demethyl-N-formyldehydronuciferine, 6-O-demethylmenisperphine and dauriporphine, were separated and purified from MR in our laboratory, whose structures were elucidated by their spectra data (MS, 1H NMR and 13C NMR). N-demethyl-N-formyldehydronuciferine was separated from this plant for the first time. The purities of those standards were all above 97% using UPLC-UV analysis. Their structures are shown in Fig. 1.

LC-grade water was obtained from a Milli-Q system (EMD Millipore, Billerica, MA, USA). LC-grade acetonitrile and methanol were purchased from Scharlau (Scharlab, S.L., Sentmenat, Spain). Formic acid and ammonium acetate were supplied by DAMAO chemical reagent factory (Tianjin, China).

2.3. Instrumentation and UPLC-DAD-MS analytical conditions

Chromatographic separation was achieved on an Acquity UPLC BEH C18 (2.1 × 100 mm, 1.7 μm) column by employing the Waters Acquity UPLC system consisting of a column oven, sample manager, binary solvent manager and DAD detector (Waters Corp., Milford, MA, USA). The mobile phase was composed of acetonitrile (A) and 0.1% formic acid aqueous solution (B) using a gradient elution of 6–20% A in the first 11 minutes, 20–40% A during 11–16 minutes, 40–50% A during 16–19 minutes, 50–52% A during 19–21 minutes, then a linear increase to 90% A in the last minute. To attain better resolution of the detected alkaloids, solution B was supplemented with 5 mM ammonium acetate. The column temperature was fixed at 50 °C and the flow rate was set at 0.3 mL/min. Two different wavelengths were employed to monitor and quantify the targeted compounds: 254 nm for acutumidine, acutumine, menisperphine, N-demethyl-N-formyldehydronuciferine, 6-O-demethylmenisperphine and dauriporphine; 282 nm for magnoflorine, menisperine and dauricine. The injection volume was 2 μL.

A Waters Quattro Premier XE™ triple quadrupole mass spectrometer (Waters Corp.) equipped with an electrospray ionization (ESI) source was used for the identification of alkaloids in MR. The mass spectrometer was operated in positive ionization mode. The mass spectrometric conditions were optimized as follows: cone gas (nitrogen) flow rate, 50 L/h; desolvation gas (nitrogen) flow rate, 600 L/h; capillary voltage, 3200 V; source temperature, 120 °C; desolvation temperature, 350 °C; cone voltage, 30 V; collision activation dissociation gas...
(argon), 0.20 mL/min. Spectra were recorded in the range of $m/z$ 50–950 for full-scan MS analysis.

2.4. Preparation of standard solutions

Stock solutions were prepared by dissolving the accurately weighed reference standards individually in methanol containing a smaller part of dimethyl sulfoxide. A certain amount of stock solution was then mixed to obtain the combined solution at a concentration of 25.75 μg/mL for acutumidine, 5.130 μg/mL for acutumine, 354.5 μg/mL for magnoflorine, 234.7 μg/mL for menisperine, 168.5 μg/mL for dauricine, 1.760 μg/mL for menisporphine, 0.709 μg/mL for $N$-demethyl-$N$-formyldehydronuciferine, 1.655 μg/mL for 6-O-demethylmenisporphine

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**Fig. 1** — Chemical structures of the nine alkaloids in Menispermi Rhizoma.

**Fig. 2** — Chromatograms acquired using different mobile phase systems included: (A) acetonitrile and 0.1% formic acid aqueous solution without ammonium acetate; (B) containing 5 mM ammonium acetate; (C) containing 10 mM ammonium acetate; (D) containing 15 mM ammonium acetate with a gradient elution.
Table 1 – Results of methodological validation of the nine marker compounds.

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Compound</th>
<th>Regression equation</th>
<th>$r^2$</th>
<th>Linear range (µg/mL)</th>
<th>LOQ (µg/mL)</th>
<th>Intra-day (RSD%, n = 6)</th>
<th>Inter-day (RSD%, n = 3)</th>
<th>Repeatability (n = 6)</th>
<th>Stability RSD%</th>
<th>Recovery (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acutumidine</td>
<td>$Y = 20901X - 9917.6$</td>
<td>0.9991</td>
<td>0.800–25.75</td>
<td>0.040</td>
<td>0.17</td>
<td>0.85</td>
<td>1.190</td>
<td>1.50</td>
<td>0.52</td>
</tr>
<tr>
<td>2</td>
<td>Acutumine</td>
<td>$Y = 20326X - 330.44$</td>
<td>0.9999</td>
<td>0.160–5.130</td>
<td>0.032</td>
<td>0.42</td>
<td>1.14</td>
<td>0.180</td>
<td>0.88</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>Magnoflorine</td>
<td>$Y = 6782X + 2524.8$</td>
<td>0.9999</td>
<td>2.216–354.5</td>
<td>0.111</td>
<td>0.19</td>
<td>0.60</td>
<td>17.42</td>
<td>0.89</td>
<td>0.55</td>
</tr>
<tr>
<td>4</td>
<td>Menisperine</td>
<td>$Y = 8725X + 1753.3$</td>
<td>0.9999</td>
<td>1.467–234.7</td>
<td>0.073</td>
<td>0.25</td>
<td>0.52</td>
<td>11.03</td>
<td>0.86</td>
<td>0.46</td>
</tr>
<tr>
<td>5</td>
<td>Dauricine</td>
<td>$Y = 4518X - 15543.0$</td>
<td>0.9999</td>
<td>5.260–168.5</td>
<td>0.526</td>
<td>0.24</td>
<td>0.84</td>
<td>5.950</td>
<td>0.85</td>
<td>0.62</td>
</tr>
<tr>
<td>6</td>
<td>Menisporphine</td>
<td>$Y = 54739X + 261.64$</td>
<td>0.9999</td>
<td>0.055–1.760</td>
<td>0.011</td>
<td>1.95</td>
<td>3.32</td>
<td>0.078</td>
<td>2.28</td>
<td>1.77</td>
</tr>
<tr>
<td>7</td>
<td>N-demethyl-N-formyldehydrodronuciferine</td>
<td>$Y = 65110X + 303.69$</td>
<td>0.9999</td>
<td>0.022–0.709</td>
<td>0.011</td>
<td>1.01</td>
<td>0.31</td>
<td>0.018</td>
<td>0.58</td>
<td>0.49</td>
</tr>
<tr>
<td>8</td>
<td>6-O-demethyl-menisporphine</td>
<td>$Y = 43622X + 47.025$</td>
<td>0.9999</td>
<td>0.052–1.655</td>
<td>0.010</td>
<td>0.53</td>
<td>0.58</td>
<td>0.074</td>
<td>2.56</td>
<td>0.80</td>
</tr>
<tr>
<td>9</td>
<td>Dauriporphine</td>
<td>$Y = 36429X + 128.63$</td>
<td>0.9999</td>
<td>0.031–0.996</td>
<td>0.016</td>
<td>0.24</td>
<td>0.30</td>
<td>0.051</td>
<td>2.33</td>
<td>1.00</td>
</tr>
</tbody>
</table>

LOQ = limit of quantification; RSD = relative standard deviation.
Fig. 3 – UPLC-ESI-MS total ion current chromatogram in the positive ion mode of Menispermi Rhizoma. 1 = acutumidine; 2 = acutumine; 3 = magnoflorine; 4 = menisperine; 5 = dauricine; 6 = menisporphine; 7 = N-demethyl-N-formyldehydronuciferine; 8 = 6-O-demethylmenisporphine; 9 = dauriporphine.

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>TR (min)</th>
<th>Positive ions (m/z)</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.04</td>
<td>[M+H]$^+$ (56), [M+HCl]$^+$ (30), [M+H-HCl-HO]$^+$ (34), 367 [M+H-NH$_2$CH$_3$]$^+$ (6)</td>
<td>Acutumidine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100), 352 [M+H-NH=O$^+$] (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>305 [M+H-NCH$_3$CH$_2$-H-CH$_3$OH-HCl-H$_2$O$^+$] (45), 273 [M+H-NCH$_3$CH$_2$-CH$_2$OH-HCl-H$_2$O$^+$] (25), 58$^+$ [M+H-SH] (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>265 [M-NCH$_2$CH$_2$-CH$_3$OH] (15), 264 [M-NCH$_2$CH$_2$-CH$_3$OH-HCl$^+$] (45), 251 [M-NCH$_2$CH$_3$-CH$_2$OH-HCl-H$_2$O$^+$] (20), 236 [M-NCH$_2$CH$_3$-CH$_2$OH-H$_2$O$^+$] (8), 58$^+$ [M+H-SH] (100)</td>
<td></td>
</tr>
</tbody>
</table>

- CE = collision energy.
- a M$^+$. 
- b m/z 58 = \( \text{N} + \text{H} \).
- c m/z 58 = \( \text{N}^+ \).
- d m/z 206 = \( \text{O} \).
- e C$_8$H$_{14}$O$_2$ = HO–C=C–OCH$_3$. 

Table 2 – Characterization of the nine alkaloids in Menispermi Rhizoma.

- Peak no. = peak number.
- TR (min) = retention time (min).
- Positive ions (m/z) = positive ions measured at m/z.
- Identification = identification of the alkaloid.
demethylmenisporphine and 0.996 μg/mL for dauriporphine. The combined solutions were further diluted to give a series of concentrations for linear validation.

2.5. Preparation of sample solutions

The dried rhizome of *Menispermum dauricum* DC was pulverized into a fine herb powder (100 mesh). The MR pills and contents of the MR capsules were ground into preparation powder (100 mesh). Then, 200 mg of herb powder or 50 mg of preparation powder was accurately weighed and extracted with 20 mL of 75% methanol in an ultrasonic bath for 40 minutes at room temperature. Additional 75% methanol was added to make up the lost weight. Next, 1 mL of the sample solution was diluted to 2 mL with 50% methanol. The solution was used for UPLC analysis after centrifugation at 14,000g for 15 minutes.

3. Results and discussion

3.1. Optimization of UPLC-DAD-MS conditions

To optimize UPLC conditions, preliminary experiments were conducted to test the chromatographic column, column temperature, flow rate and mobile phases. The Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm) was selected for UPLC-DAD-MS analysis based on its good separation ability and short analytical time. With the aim of obtaining

![Fig. 4 - The MS² spectra and fragmentation patterns of acutumidine, menisperine, dauricine, N-demethyl-N-formyldehydronuciferine and dauriporphine.](image-url)
Fig. 4 — (continued).
excellent chromatographic separation and pretty peak shape, a small amount of ammonium acetate was added to 0.1% formic acid aqueous solution, which markedly improved the separation effect of the investigated alkaloids on the UPLC BEH C18 column. Various final concentrations (5 mM, 10 mM, 15 mM) of ammonium acetate in 0.1% formic acid aqueous solution were examined. The chromatograms obtained (Fig. 2) indicated that the most satisfactory effect was achieved when 5 mM ammonium acetate was added, especially for the chromatographic separation of N-demethyl-N-formyldehydronuciferine, menisporphine, 6-O-demethylmenisporphine and dauriporphine.

Under these optimized MS conditions, the alkaloids gave stronger signal responses in positive ion mode than negative ion mode. To obtain higher sensitivity, the cone voltage and capillary voltage were optimized to generate protonated molecules of the analytes. The fragment energy was optimized to achieve maximum response of the compound fragment ion peaks.

3.2. Optimization of sample preparation

According to the comparative results of different methods for sample preparation, ultrasonic extraction was demonstrated to be simpler and more efficient than reflux extraction. The crucial parameters, including ultrasonic time (20, 30, 40 and 50 minutes), solvent for extracting (50%, 75% and 100% methanol), and the ratio of solvent to sample weight (25, 50, 100 and 150 mL per gram of herbs), were then optimized individually. The final conditions for sample preparation were set as follows: 200 mg herb powder or 50 mg preparation powder extracted with 20 mL of 75% methanol in an ultrasonic bath for 40 minutes at room temperature.

3.3. Methodological validation of determining nine alkaloids by UPLC-DAD

Methodological validation for quantification of nine alkaloids in MR and its preparations was carried out by UPLC-DAD with regard to linearity, intra- and inter-day precision, limit of quantification, stability, repeatability and recovery. The linearity with a correlation coefficient value above 0.9991 was approved for the nine alkaloids. The limit of quantification of the tested compounds expressed as a signal-to-noise ratio of 10:1 was in the range of 0.010–0.526 μg/mL. The appraisal of the intra- and inter-day precision of this method was independently performed by
The UPLC-DAD-ESI-MS/MS method was employed to identify and characterize major active ingredients in MR and its preparations. Comparing retention times and MS characteristic fragmentations with those of reference standards, nine compounds were unambiguously identified as acutumidine, acutumine, magnoflorine, menisperine, dauricine, menisporphine, N-demethyl-N-formylethohydrinuciferine, 6-O-demethylmenisporphine and dauriporphine in MR. Only six alkaloids were detected in the MR preparation: acutumidine, acutumine, magnoflorine, menisperine, dauricine and 6-O-demethylmenisporphine. The total ion current chromatogram of MR is shown in Fig. 3. The retention time, protonated molecules or molecular ions and fragmentation information of the nine alkaloids are displayed in Table 2. According to their chemical structures, the nine alkaloids can be classified into five categories as follows: morphinan alkaloids (acutumidine and acutumine), aporphine quaternary ammonium alkaloids (magnoflorine and menisperine), oxoisoporphine alkaloids (menisporphine, 6-O-demethylmenisporphine and dauriporphine), dehydroporphine alkaloids (N-demethyl-N-formylethohydrinuciferine), and bisbenzylisoquinoline alkaloids (dauricine).

As shown in Fig. 4, the protonated molecule [M+H]+ of acutumidine yielded three dominant fragment ions at m/z 367 [M+H-NH3]+, 348 [M+H-HCl]+ and 341 [M+H-C2H5N]+ in (+) ESI-MS/MS, which could be attributed to the consecutive loss of one NH3, one HCl and one NH2CH2CH2. In (+) ESI-MS/MS spectrum, menisperine displayed the base peak at m/z 58 [M-298]+, which embodied the characteristic cleavage of aporphine quaternary ammonium alkaloids. In addition, fragment ions at m/z 313 [M-C2H7NH]+, m/z 311 [M-C2H5N]+ and m/z 279 [M-C2H7N-C2H5OH]+ were also observed. Dauricine, a bisbenzylisoquinoline alkaloid, exhibited characteristic fragment ions at m/z 594 [M+H-C2H5NH]+, m/z 582 [M+H-C2H5NH]+ and m/z 206 [M+H-419]+. N-demethyl-N-formylethohydrinuciferine gave fragment ions at m/z 280 [M+H-CO]+ and m/z 278 [M+H-CH2O]+, which indicated the neutral loss of CO and HCHO. Different from other alkaloids, dauriporphine, as an oxoisoporphine alkaloid that has relatively stable mother nucleus structures, tended to give fragment ions at m/z 322 [M+H-CH2O]+ and m/z 320 [M+H-CH2O]+, suggesting the loss of sextuplicate analysis of one sample within 1 day and analysis of samples in the consecutive 3 days, the relative standard deviations (RSDs) of which were below 3.32%. Repeatability was evaluated by six samples prepared in parallel; the results were able to satisfy the quantitative analysis of the nine alkaloids in the samples. The targeted analytes were certified to be stable with RSD values below 1.77% by replicate analysis of one sample at 0, 2, 4, 8, 10 and 12 hours. The recoveries were investigated by adding authentic standards to the sample, the amount of which were generally equal to those contained in the sample. The samples in sextuplet were prepared as mentioned above. Satisfactory recoveries between 97.90% and 106.8% were achieved, with RSD values less than 3.46%. The detailed results of methodological validation are listed in Table 1.
formaldehyde and methanol, respectively. These five alkaloids (acutumidine, menisperine, dauricine, N-demethyl-N-formyldehydronuciferine and dauriporphine) possessing their own characteristic fragmentation patterns were chosen as typical examples, whose MS² spectra and detailed fragmentation patterns are displayed in Fig. 4 [20–22].

3.5. Quantitative analysis of the nine alkaloids in MR from different origins

The validated UPLC-DAD method was applied to simultaneous quantitative analysis of the nine alkaloids in MR collected from different drug stores distributed in different provinces in China.

From the analytical results listed in Table 3, it was found that MD6 (Zhengzhou, Henan, China) was different from the other samples in that most of the important alkaloids could not be detected. Therefore, it was tentatively identified as a counterfeit drug. To confirm our conjecture about MD6, further appearance and microscopic identification were performed. The detailed descriptions of MR (MD2) and counterfeit (MD6) are listed in Table 4 [23]. The pictures of microscopic identification are shown in Fig. 5.

In another seven MR samples, the contents of the nine alkaloids varied greatly from 0.008 mg/g to 17.42 mg/g. Acutumidine, acutumine, magnoflorine, menisperine and dauricine with retention time less than 8 minutes (16% acetonitrile in mobile phase at 8 minutes) were considered to be readily soluble in water, the contents of which were in the range of 0.17 mg/g to 17.42 mg/g. It may be deduced that these alkaloids might be the major bioactive compounds in MR. Menisperphine, N-demethyl-N-formyldehydronuciferine, 6-O-demethylmenisporphine and dauriporphine with low contents in MR could not be detected in any of these eight preparations, while 6-O-demethylmenisporphine was assayed with a content of 0.044–0.068 mg/g in the preparations. As for the four fat-soluble components, menisporphine, N-demethyl-N-formyldehydronuciferine and dauriporphine with retention time more than 19 minutes (50% acetonitrile at 19 minutes) were considered as fat-soluble components, whose contents were below 0.13 mg/g. The total contents of the nine alkaloids varied from 24.05 mg/g to 36.05 mg/g. The discrepancies among the samples of different origins were obvious. Accordingly, it is necessary to pay close attention to the quality evaluation of MR in clinical use.

3.6. Quantification of the nine alkaloids in MR preparations from different pharmaceutical factories

The established analytical method was also employed to determine the nine alkaloids in preparations manufactured from MR, including six batches of capsules from three manufacturers and two batches of pills from different pharmaceutical factories. Compared with the analytical results of MR, the most obvious variation was that dauricine, as the chemical marker for MR preparation (capsules and pills) in the Chinese Pharmacopoeia, had the highest content from 50.13 mg/g to 103.1 mg/g. The contents of the other four water-soluble components (acutumidine, acutumine, magnoflorine and menisperine) were still relative high. As for the four fat-soluble components, menisporphine, N-demethyl-N-formyldehydronuciferine and dauriporphine with low contents in MR could not be detected in any of these eight preparations, while 6-O-demethylmenisporphine was assayed with a content of 0.044–0.068 mg/g in the preparations. According to the Chinese Pharmacopoeia, MR preparations (capsules and pills) are manufactured from standardized MR extracts, which are obtained by acid-base treatment of MR using dauricine as a chemical marker. Therefore, water-soluble components are well reserved in MR preparations (capsules and pills), especially for dauricine, while fat-soluble components are not well transferred from the raw material to the standardized MR extracts.

The different batches of MR preparations from one manufacturer had a relatively stable content of targeted compounds. However, the individual content of detected compounds varied significantly in MR preparations with different dosage forms or single dosage form from different manufacturers. This may be due to the different manufacturing procedures and the different weights of each unit (average weight for each unit: 0.30 ± 0.0057 g for C-YC, 0.20 ± 0.0095 g for C-JFK, 0.18 ± 0.0031 g for C-WSLJ, 0.11 ± 0.027 g for P-AS, 0.18 ± 0.0035 g for P-YCWJS). On account of regulations released by the Chinese Pharmacopoeia, the contents of dauricine in MR pills and capsules used in our study must exceed 6 and 12 mg per unit, respectively. The analytical results indicated that all of the samples collected from the different manufacturers fulfilled these standards. The

| Table 4 – Detailed descriptions of certified and counterfeit herbs. |
|-----------------|------------------|
| **Appearance**  | **Microscopic characteristics** |
| Menispermi Rhizoma (MD2) | Powder: pale brownish-yellow. Vessels bordered pitted. Stone cells singly scattered. Crystals of calcium oxalate minute and gritty centered in tegumental cells and ray cells. Epidermal cells are numerous. Cork consists of several layers of cells. Wood fibers and phloem fibers bunchy or scattered [23]. |
| Counterfeit (MD6) | Powder: pale brownish-yellow. Vessels reticulate and bordered pitted, pale yellowish or golden yellowish. Stone cells clumped or singly scattered. Crystals of calcium oxalate square, rhombic or irregularly shaped. Crystal fiber, xylem parenchyma and cuticle masses are numerous. Non-glandular hairs existent. |

<table>
<thead>
<tr>
<th><strong>Microscopic characteristics</strong></th>
<th><strong>Appearance</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Covered with brownish-yellow or dark brown cuticle outside, which is easy to be flaked. Flexible texture, not easy to break, irregular cross-section. Xylem is pale yellowish and medullated [23].</td>
<td>Slim, cylindrical and branched, 0.3–0.8 cm in diameter.</td>
</tr>
<tr>
<td>Cylindrical and multi-branched, 0.5–1.5 cm in diameter. Covered with light gray to reddish-brown cuticle outside. Hard texture, not easy to break, relatively flat cross-section, shows evident concentric rings and radial striation. Xylem is pale yellowish.</td>
<td>Covered with light gray to reddish brown cuticle outside.</td>
</tr>
</tbody>
</table>

From the different origins were obvious. Accordingly, it is necessary to pay close attention to the quality evaluation of MR in clinical use.
Fig. 5 – Pictures of microscopic identification. 1 = Menispermi Rhizoma (MD2); 2 = counterfeit (MD6); A = vessels; B = stone cells; C = cork cells; D = epidermal cells; E = crystals of calcium oxalate; F = starch granules; G = phloem fiber; H = wood fiber; I = crystal fibers; J = non-glandular hair; K = horny block; L = xylem parenchyma.
total contents of the alkaloids showed marked diversities in the eight samples from 67.62 mg/g to 119.72 mg/g. This validated method was successfully applied to the quality control of MR preparations including capsules and pills, which provided particularly important information for production and application. The UPLC-UV chromatograms of the standard solution, MR, MR capsules and pills are shown in Fig. 6.

4. Conclusion

In our study, nine alkaloids in MR were ambiguously identified and characterized by UPLC-DAD-MS, which were further quantified by UPLC-DAD. The established method described herein for the qualitative and quantitative analyses of the nine alkaloids was successfully applied in MR, MR capsules and pills; the method has also overcome the shortcomings of other quality control methods, such as scant chemical marker, long analytical time, consumption of large amounts of organic solvents and limitation to MR or its single dosage form.

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