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

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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 ($R^2 \geq 0.9991$), desirable intra- and inter-day precisions (RSD $\leq$ 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.

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1. Introduction

Menispermi Rhizoma (MR), the dried rhizome of *Menispermum 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, menisporphine, *N*-demethyl-*N*-formyldehydronuciferine, 6-*O*-demethylmenisporphine and dauriporphine, were separated and purified from MR in our laboratory, whose structures were elucidated by their spectra data (MS, ^{1}H NMR and ^{13}C 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, menisporphine, *N*-demethyl-*N*-formyldehydronuciferine, 6-*O*-demethylmenisporphine 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