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Short communication

Simultaneous determination of triptolide, tripterifordin, celastrol and nine sesquiterpene alkaloids in *Tripterygium* preparations using high-performance liquid chromatography–triple quadrupole mass spectrometry

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ABSTRACT

Tripterygium wilfordii tablet (TWT) and *Tripterygium hypoglaucum* tablet (THT), the preparations of the two *Tripterygium* herbs, are well known for the treatment of rheumatoid arthritis and other related inflammatory diseases clinically. In the present study, a high performance liquid chromatography (HPLC) coupled with electrospray ionization (ESI) tandem triple quadrupole mass spectrometry (QQQ/MS) method was developed for simultaneous quantification of 12 chemical components in *Tripterygium* preparations. The fragmentation patterns of analytes using ESI and collision-induced dissociation (CID) techniques were reported. This assay method was validated with respect to linearity ($r^2 > 0.9991$), precision, repeatability, and accuracy (recovery rate between 97.2 and 104.2%). The proposed method was successfully applied for simultaneous quantification of the 12 compounds in *Tripterygium* preparations from the different manufactures. In addition, to evaluate the quality of *Tripterygium* preparations, partial least square discrimination analysis (PLS-DA) was performed to differentiate the contents of 12 compounds. In conclusion, the established HPLC/QQQ/MS method was proven to be useful and efficient for quality control of *Tripterygium* preparations.

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1. Introduction

Tripterygium wilfordii (Leigongteng in Chinese) and *Tripterygium hypoglaucum* (Kunming Shanhaitang in Chinese), two morphologically similar herbs from *Tripterygium* genus, are woody twining vines belonging to the family Celastraceae [1,2]. They are native to Southern and Eastern China, Korea, and Japan, and have been used in traditional Chinese medicine for over 2000 years [3]. *Tripterygium* preparations, such as "*Tripterygium* glycosides tablet" (TGT), "Leigongteng tablet" (*T. wilfordii* tablet, TWT) and "Kunming Shanhaitang tablet" (*T. hypoglaucum* tablet, THT), have been widely used in treatingchronic inflammatory disease [4] and rheumatic arthritis [5,6] in Chinese clinics. A number of diterpenoids, triterpenoids and sesquiterpene alkaloids have been isolated from herbs, and some of them showed significant biological activities (anti-HIV/antineoplastic and immunomodulating activities) [7–9]. Accumulating documentary records have indicated that sesquiterpenoid alkaloids were the mainly active components of two *Tripterygium* preparations(TWT and THT) [10]. However, most of the compounds in *Tripterygium* genus have a narrow therapeutic index and some serious side effects [11]. From a safety point of view, it is essential to make an accurate measurement of contents of these components in *Tripterygium* preparations. Reciprocally, the lack of strict quality control contributes to the marketing of *Tripterygium* preparations of questionable quality. Therefore, a strictly quality control method for *Tripterygium* preparations is urgently needed, and quantitative analysis of multiple components should be taken for the most direct and important method for quality control of *Tripterygium* preparations.

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In recent years, a few analytical methodsfocused on diterpenes or triterpenes, including HPLC [12], HPLC/MS [13] and GC/MS [14], have been reported as quality assessment for *Tripterygium* herbs or preparations. However, the contents of diterpenes or triterpenes cannot represent the real quality of *Tripterygium* herbs or preparations. Recently, two studies using high liquid chromatography coupled with a triple quadruple electrospray tandem mass spectrometry (HPLC/MS/MS) method determining multiple active components of *Tripterygium* herbs [15,16] has been developed, but methods for the simultaneous quantitative determination of multiple sesquiterpene alkaloids in a single running for *Tripterygium* preparations are still not available. Therefore, a universal method for the quantitative determination of multiple components in *Tripterygium* preparations is necessary and convenient for their quality control.

In this study, a simple, accurate, and reliable HPLC–MS/MS method was developed for the first time for quantitative determination of *Tripterygium* preparations. A total of 12 main compounds, including triptolide, tripterifordin, celastrol, triptonine B, wilfornine A, wilfordine, wilfortrine, wilforine, wilforgine, peritassine A, euonine and wilfordinine E, have been quantitatively detected in 29 samples from different manufactures, and the results were further analyzed by PLS-DA to provide more information about the chemical difference, as well as to evaluate the qualityof each sample. To the best of our knowledge, this is the most comprehensive report on the quantitative analysis of *Tripterygium* preparations.

2. Experimental

2.1. Chemicals reagents and materials

Triptolide, and carbamazepine (IS) were purchased from National Institute for Food and Drug Control (Beijing, China). Tripterifordin, celastrol, triptonine B, wilfornine A, wilfordine, wilfortrine, wilforine, wilforgine, euonine, peritassine A and wilfordinine E (Fig. 1), were extracted from *T. wilfordii* and purified in our laboratory. These compounds were identified using ESI/MS, ^{1}H and ^{13}C NMR techniques, and by comparing their experimental and reported spectroscopic data. Their purity of all constituents exceeds 98% by HPLC analysis.

Twenty-nine batches of commercially available TWT and THT, obtained from different retail pharmacies in Chengdu, Sichuan province, China. HPLC-grade formic acid and ammonium acetate were purchased from Tianjin Kermel Chemical Reagents Development Centre (Tianjin, China). HPLC-grade methanol and acetonitrile were purchased from Fisher Scientific (Fair lawn, New Jersey, USA). Ultrapure water was prepared on a TCEH-RO/40 Reagent Water System (Beijing Aisitaike Technology Development Co., Ltd., Beijing, China) for the preparation of samples and buffer solutions.

2.2. Instrumentation and conditions

Chromatographic analysis was performed on an Agilent Rapid Resolution HPLC system, 1200 series (Agilent Technologies,Santa Clara, CA, USA) equipped with a binary pump, micro degasser, an auto plate-sampler, and thermostatic column compartment. Separation was performed on an Agilent ZORBAX Eclipse XDB-C_{18} (2.1×50 mm, 1.8 μm) column. The column temperature was maintained at 30 °C. The mobile phase consisted of 5 mM ammonium acetate −0.1% acetic acid aqueous solution (A) and acetonitrile (B) using a gradient elution of 43–48% B at 0–3 min, 48–58% B at 3–5 min, 58–90% B at 5–8 min, 90–90% B at 8–12 min. The flow rate was kept at 0.25 mL/min. Mass spectrometry was performed using an Agilent 6410 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. The MS conditions were as follows: drying gas temperature, 350 °C; drying gas flow, 10 L/min; nebulizer pressure, 40 psi; capillary voltage, 4000 V. Monitoring reaction mode (MRM) was applied for quantitative analysis using precursor/product ion information. Data acquisition and processing were performed with MassHunter Workstation (Agilent Technologies, Santa Clara, CA, USA).

2.3. Preparation of standard solutions

Each accurately weighted standard was dissolved in methanol to give the stock solutions. Working standard solutions containing 12 reference standards and IS were prepared by diluting the stock solutions with methanol–water (4:1, v/v) to a series of proper concentrations with the ranges: triptolide, 15.4–3860 ng/mL; tripterifordin, 8.136–4068 ng/mL; triptonine B, 5.212–5212 ng/mL; wilfornine A, 5.026–5026 ng/mL; wilfordine, 4.304–4304 ng/mL; wilfortrine; 4.119–4119 ng/mL, wilforine, 4.880–4880 ng/mL; wilforgine, 4.272–4272 ng/mL; euonine, 5.220–5220 ng/mL; wilfordinine E, 5.566–5566 ng/mL; peritassine A, 6.468–6468 ng/mL; celastrol, 8.380–4190 ng/mL. The solutions were stored at 4 °C.

2.4. Preparation of samples for analysis

Ten tablets of TWT or THT were grounded into powder individually and passed through a 50-mesh sieve. 0.3 g of sample powder was accurately weighed and transferred to a 50 mL glass-stoppered conical flask, after adding18 mL methanol, the filled flask was weighed with a precision of ±0.01 g. The sample solution was refluxed for 60 min in a 60 °C water bath, cooled to room temperature, and then adjusted to the initial weight by adding methanol as needed. A mixture consisted of 0.5 mL of methanol extract (or standard solutions for calibration curve) and 0.5 mL of the IS working solution were diluted to 5 mL by using methanol–water (4:1, v/v). After filtered by a 0.22 μm membrane filter, 10 μL of each solution was injected into the HPLC/MS instrument for analysis. A total of 21 batches of *Tripterygium* preparations as well as 8 expired preparations were subjected to HPLC/QQQ/MS analysis after being prepared.

3. Method validation

For the calibration curves, each concentration of standard solutions was analyzed in triplicate. All calibration curves were constructed from the peak area ratio of the tested reference peak to that of the internal standard versus their concentrations. Limit of detection (LOD; S/N > 3), limit of quantification (LOQ; S/N > 10), precision, repeatability, and recovery were studied respectively as described below: The precision of the developed method was determined by the intra- and inter-day variations. For intra-day variability test, a sample solution prepared as the method described in Section 2.4 was analyzed for six replicates within one day, while for inter-day variability test, one sample was examined in duplicates for consecutive three days. The relative standard deviation (RSD) for peak area was calculated as the measure of precision. Repeatability was confirmed with six independent analytical sample solutions prepared from the same batch of sample (TWT1, Huangshifeiyun, Hubei) and variations were expressed by RSD. One of the sample solutions mentioned above was stored at 25 °C, and injected into the apparatus at 0, 2, 4, 8, 12 and 24 h, respectively, to evaluate the stability of the solution. Recovery was determined by analyzing spiked samples. A known amount of the 12 standards at low (80% of the known amounts), medium (the same as the known amounts) and high (120% of the known amounts) were added into a certain amount of samples (0.15 g), and then extracted and analyzed with the same procedures. Three replicate extractives at each level were used to calculate the extraction recovery rates for evaluating the

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