

Simultaneous Determination of Four Index Polar Constituents by HILIC-ESIMS/MS for the Quality Control of Guizhi Fuling Wan

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A rapid and accurate method was developed and validated for the analysis of four index polar constituents in Chinese medicinal preparation Gui-zhi-fu-ling Wan by microwave-assisted extraction coupled with hydrophilic interaction liquid chromatography-tandem mass spectrometry (MAE-HILIC - MS/MS). The chromatographic separation was performed on a Waters HILIC column (100 mm × 1.7 mm, 2.1 μm) with gradient elution using 0.1% formic acid aqueous solution (A) and acetonitrile (B) at a flow rate of 0.2 mL/min. All the compounds showed good linearity. The method provided good accuracy with perfect recoveries and precision, which has been successfully applied to the simultaneous identification and determination of 4 index compounds in a single run for the quality control of Guizhi-fu-ling Wan. Gui-zhi-fu-ling Wan (GFW) has been used in China for centuries. It is a traditional Chinese medical (Kampo) formulation that composed of five kinds of medicinal plants, Cinnamomum cassia BLUME (Cinnamomi Cortex), Paeonia lactiflora PALL (Peonies Radix), Paeonia suffruticosa ANDREWS (Moutan Cortex), Prunus persica BATSCH (Persicae Semen), and Poria cocos WOLF (Hoelen). GFW, prescript for treating blood disorders was compiled by Zhang Zhongjing, an outstanding physician in the Eastern Han Dynasty (150-219 AD). Based on a large number of pharmacological researches, the polar active constituents of pachymic acid, albiflorin, amygdalin and cinnamic acid, were found to be responsible for the biological activities of treating gynecological diseases, uterine fibroids, pelvic inflammatory disease, ovarian cyst, dysmenorrhea, endometriosis, cervical cancer, hot flashes, improvement of blood circulation. In addition, GFW was reported to prevent the progression of atherosclerosis in cholesterol-fed rabbits in vivo by limiting oxidative LDL modification. Only a few analytical methods has been reported to determine the active components in GFW, including Reverse phase highperformance liquid chromatography (RP-HPLC) and gas phase chromatography coupled with mass spectrometry (GC-MS), high-performance liquid chromatography-diode array detection tandem mass spectrometry (HPLC-DAD-MS/MS). However, up to now, HILIC-MS/MS coupled with MAE has rarely been employed for the determination of these four polar active constituents contained in TCM samples. Appropriate amount of paeoniflorin, cinnamic acid, amygdalin, pachymic acid, accurately weighed, dissolved with 70% methanol in a 25 mL volumetric flask to produce a reference standard stock solution. A precision volume of 0.1, 2.0, 4.0, 6.0, 8.0, 10.0 ml of the mixture standard stock solution were transferred to a 10 mL volumetric flask, diluted with acetonitrile to produce mixed reference solution with series concentration. All solutions above were prepared in volumetric flasks and stored at 4°C. The relative standard deviations (RSD) were determined as inter- and intra-day precisions and accuracy. The intra-day precision was

investigated for a mixed standard solution of seven analytes using five replicates within one day, and inter-day precision was determined in duplicates over consecutive three days. The results indicated that the intra- and inter-day precision was less than 2.81% for all 4 analytes. Recovery of all 4 tested bioactive constituents was within the range of 95.81-104.77%, with an RSD of between 0.65% and 2.81% (n=5). From the results of precision test and recovery test, it was known that the method manifested good precision and accuracy. For stability test, the same sample solution was analyzed every 12 h in 3 days at the room temperature. The RSD values of the peak area and retention times were no more than 5.12% and 2.0%, respectively. The solution was therefore considered to be stable within 72 h. An accurate and reliable HPLC method to simultaneously determine multiple active components in traditional Chinese medicinal preparation GFW was developed. This is the first report for the simultaneous determination of 4 major active components in GFW by using HILIC-MS/MS. High extraction efficiency was achieved by an optimized MAE procedure, with shorter extraction time and less solvent consumption obtained. This method provides useful experience and shows promising perspective in analysis of other similar TCM samples, especially concerning the efficient extraction and convenient simultaneous determination of multiple target analytes contained in TCM samples. The mass spectrometer was tuned as follows in the final methods. The capillary voltage was set to 3 kV in negative ion mode and 1 kV in positive ion mode. The source and desolvation temperatures, desolvation gas flow were 110 and 400°C, 450 L/h, respectively. The cone gas flow was 30 L/h. The RF Lens was set to 0.3 V in negative ion mode and 0.1 V in positive ion mode. Nitrogen was used as the nebulizer, desolvation and cone gas. Argon gas was used for collision induced dissociation. Data acquisition was performed in MRM mode, and the dwell time was 0.2 s. The system was controlled by Mass Lynx™ NT 4.1 software with QuanLynx™ program (Waters Corp., Milford, MA, USA). The standards of albiflorin, amygdalin and cinnamic acid were obtained from Chengdu Must Bio-technology Co., Ltd. (Sichuan, China), while pachymic acid was isolated and purified in our laboratory, and the structure was characterized by comparing the ACQUITY Ultra Performance LCTM UPLC was gifts from Waters (USA). The separation was carried out on a Waters HILIC column (100 mm × 1.7 mm, 2.1 μm). The mobile phase consisted of aqueous solution (A) and 0.1% formic acid acetonitrile (B). A gradient programmer was used according the following profile: 0-3 min, linear gradient 3% A; 3-5 min, linear gradient 3-90% A; 5-8 min, the system returned to initial condition. The flow rate was 0.2 mL/min, and the injection volume was 20 μL. chemical and spectroscopic (UV, NMR and MS) data. GFW was purchased from ShanXi Zheng yuan sheng bang pharmaceutical Co., Ltd. (Shanxi, China). Acetonitrile

(HPLC-grade) was purchased from Tedia Company Inc., Formic acid (HPLC-grade) was obtained from DIMA Technology Inc., (USA). Acetonitrile and methanol of HPLC grade were purchased from Tedia Company Inc., (Fairfield, USA). Formic acid of HPLC grade was purchased from Dikma (Richmond Hill, NY, USA). All other reagents were of analytical grade. The detector used was a Quattro Micro API mass spectrometer (Waters Corporation, Milford, MA, USA) equipped with an ESI working in either positive (ESI+) or negative (ESI-) ion mode. The MS parameters were manually optimized by the direct infusion of the compounds (20 μ L) into the MS interface. The mass spectrometer was tuned as follows in the final methods. The capillary voltage was set to 3 kV in negative ion mode and 1 kV in positive ion mode. The source and desolvation temperatures, desolvation gas flow were 110 and 400°C, 450 L/h, respectively. The cone gas flow was 30 L/h. The RF Lens was set to 0.3 V in negative ion mode and 0.1 V in positive ion mode. Nitrogen was used as the nebulizer, desolvation and cone gas. Argon gas was used for collision induced dissociation. Data acquisition was performed in MRM mode, and the dwell time was 0.2 s. The system was controlled by Mass Lynx™ NT 4.1 software with QuanLynx™ program (Waters Corp., Milford, MA, USA). The method provided good accuracy with perfect recoveries and precision, which has been successfully applied to the simultaneous identification and determination of 4 index compounds in a single run for the quality control of Gui-zhi-fu-ling Wan. Gui-zhi-fu-ling Wan (GFW) has been used in China for centuries. It is a traditional Chinese medical (Kampo) formulation that composed of five kinds of medicinal plants, Cinnamomum cassia BLUME (Cinnamomi Cortex), Paeonia lactiflora PALL (Peonies Radix), Paeonia suffruticosa ANDREWS (Moutan Cortex), Prunus persica BATSCH (Persicae Semen), and Poria cocos WOLF (Hoelen). GFW, prescript for treating blood disorders was compiled by Zhang Zhongjing, an outstanding physician in the Eastern Han Dynasty (150-219 AD). Based on a large number of pharmacological researches, the polar active constituents of pachymic acid, albiflorin, amygdalin and cinnamic acid, were found to be responsible for the biological activities of treating gynecological diseases, uterine fibroids, pelvic inflammatory disease, ovarian cyst, dysmenorrhea, endometriosis, cervical cancer, hot flashes, improvement of blood circulation. In addition, GFW was reported to prevent the progression of atherosclerosis in cholesterol-fed rabbits in vivo by limiting oxidative LDL modification. Only a few analytical methods have been reported to determine the active components in GFW, including Reverse phase highperformance liquid chromatography (RP-HPLP) and gas phase chromatography coupled with mass spectrometry (GC-MS), high-performance liquid chromatography-diode array detection tandem mass spectrometry (HPLC-DAD-MS/MS). However, up to now, HILIC-MS/MS coupled with MAE has rarely been employed for the determination of these four polar active constituents contained in TCM samples. Based on a large

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