SHORT COMMUNICATION

Simultaneous determination of seven bioactive components in Guizhi Fuling capsule by microwave-assisted extraction combined with ultra performance liquid chromatography tandem mass spectrometry

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A simple, rapid and reliable microwave-assisted extraction (MAE) combined with ultra performance liquid chromatography tandem mass spectrometry method was developed for simultaneous determination of the seven bioactive constituents in Guizhi Fuling capsule (GFC), namely gallic acid, amygdalin, albiflorin, paeoniflorin, paeonol, cinnamic acid and pachymic acid, respectively. The operation of MAE optimised through orthogonal array design experiment was performed at 80°C for 10 min with methanol–water (70:30, v/v) as the extracting solvent. The method was validated including intra- and inter-day precision, repeatability and stability, with relative standard deviation less than 3.9%, 3.3%, 4.4% and 3.1%, respectively. All analytes showed the good linearity ($r > 0.999$), and their average recoveries varied between 98.2% and 101.2%. The results indicated that this method was simple, effective and suitable for the quality control of GFC.

Keywords: MAE; UPLC-MS/MS; quantitative analysis; GFC

1. Introduction

Guizhi Fuling capsule (GFC) was originated from an ancient Chinese medical book \textit{JinKuiYaoLue}, which has been clinically used in China for almost 2000 years in the remedy of women’s diseases such as dysmenorrhoea, hysteromyoma, endometriosis and other gynaecological diseases. The quality control of GFC is mainly conducted according to the official criterion recorded in Pharmacopoeia of the people's Republic of China (Commission 2010) in which amygdalin, paeoniflorin and paeonol were designated as markers.
for the quantitative analysis of GFC by ultrasound-assisted extraction combined with HPLC-UV detection with isocratic elution, respectively.

Conventional extraction methods were often time consuming and require large amount of extraction solvent, which would result in lower extraction yield, increasing cost and breaking environmental sustainability. Therefore, microwave-assisted extraction (MAE) (Pellati et al. 2013), being an innovative extraction technique with shorter extraction time, higher extraction yield and less solvent expenditure, had greatly improved the reproducibility and recovery of the analytes compared to traditional extraction methods above. Compared to the previous HPLC methods, ultra-high performance liquid chromatography coupled with tandem mass spectrometry could obtain higher peak capacity and higher sensitivity with shorter analysis time and better resolution (Wang et al. 2011).

A simple and rapid analysis method based on MAE combined with ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) technique was developed for the simultaneous determination of seven bioactive components in GFC, including gallic acid,
amygdalin, albiflorin, paeoniflorin, paeonol, cinnamic acid and pachymic acid (Kim et al. 2014), the structures of which are shown in Figure 1. Moreover, the MAE conditions were optimised through orthogonal array design (OAD) experiment. The proposed method provided a promising approach for the quality control of GFC.

2. Results and discussion
2.1. Optimisation of MAE conditions
A four-factor, three-level OAD L9 (3^4) was selected as a chemometric method for investigating the extraction efficiency of GFC. To indicate whether the effects of individual factors on MAE efficiency were statistically significant, an analysis of variance was used to explain the experimental data obtained from the OAD optimisation. On the basis of this analysis results and the feasibility of experiment, the optimum conditions of extraction were determined as follows: extraction solvent 70% methanol, extraction time 10 min, extraction temperature 80°C, solvent-to-solid ratio 1:100, microwave power 400 W and extraction cycle one.

2.2. Optimisation of chromatographic and mass spectrometric conditions
Different mobile phase compositions, additives, column temperature and flow rate were investigated. It was found that the presence of acetonitrile – 0.1% formic acid solution by gradient elution at a flow rate of 0.2 mL/min with the column temperature of 20°C led to the better resolution of adjacent peaks within a short analysis time (11 min) of the different components in GFC. For MS condition, both positive and negative ionisation modes were optimised. It was observed that the signal intensity of paeonol and verapamil (internal standard) in positive ionisation mode was much better than that in negative ionisation mode, which was able to form a stable protonated ion of [M + H]^+ in the positive full-scan mass spectra. The other analytes were able to form a stable deprotonated ion of [M – H]^- in the negative full-scan mass mode, and the signal intensity in negative ionisation mode was much better than that in positive ionisation mode.

2.3. Sample analysis
The established analytical method was successfully applied to the simultaneous analysis of the seven active components (gallic acid, amygdalin, albiflorin, paeoniflorin, cinnamic acid, paeonol and pachymic acid) of GFC. The contents of the seven analytes in the samples were quantified with the mean content of three replicate analysis (n = 3).

3. Conclusion
Based on MAE combined with UPLC-MS/MS technique, a simple, fast and reliable method was developed and validated for the simultaneous analysis of the seven bioactive compounds (gallic acid, amygdalin, albiflorin, paeoniflorin, cinnamic acid, paeonol and pachymic acid) in GFC for the first time. This study provided some useful guidance to the quality control and clinical application of GFC.

Supplementary material
Experimental details relating to this article are available online at http://dx.doi.org/10.1080/14786419.2015.1052068, alongside Figures S1 and S2 and Tables S1–S6.
Disclosure statement
No potential conflict of interest was reported by the authors.

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