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Original Article

Quality assessment of Fritillariae Thunbergii Bulbus sold in Taiwan markets using a validated HPLC-UV method combined with hierarchical clustering analysis

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ABSTRACT

The present paper describes the development of a high performance liquid chromatography-ultraviolet (HPLC-UV) detection method for quantitative determination of peimine and peiminine in Fritillariae Thunbergii Bulbus (FTB). Separation was achieved using a conventional XBridge™ Shield RP 18 column (250 mm × 4.6 mm, internal diameter 3.5 μm) with photodiode array detection at 190–400 nm for UV spectra and 220 nm for quantification. The mobile phase consisted of (A) 0.03% diethylamine aqueous solution and (B) acetonitrile eluted by an isocratic procedure at 45:55 (A:B) over 25 minutes. The method was validated for linearity, limits of detection (LOD) and quantification (LOQ), inter- and intra-day precisions, repeatability, stability, and recovery. All the validation results were satisfactory. The developed method was then applied to assay the contents of the two chemical markers in all the FTB samples collected. Based on the contents of the two analytes, hierarchical clustering analysis (HCA) was performed to reveal the similarities and differences of the samples.

1. Introduction

Fritillariae Thunbergii Bulbus (FTB), the dried bulb of *Fritillaria thunbergii* Miq., is one of the traditional Chinese medicines commonly used for detoxification, to eliminate phlegm, and to relieve cough [1,2]. Pharmacological studies indicated that FTB showed good effects in the treatment of hyperthyroidism [3] and prostatitis [4] and could enhance the multidrug resistance (MDR) reversal effect of cisplatin (DDP) on A549/DDP cells *in vitro* and *in vivo* as well as downregulate MDR1 mRNA

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and P-glycoprotein (P-gp) expression in A549/DDP cells [5]. Peimine and peiminine are regarded as the main bioactive compounds in FTB. Intraperitoneal administration of peimine and peiminine resulted in a significant antitussive effect in mice [6]. Peimine was found to reverse MDR in the A549/DDP cell line [7]. According to Chinese Pharmacopeia (2010 edition) and Taiwan Herbal Pharmacopeia (2nd edition), the total content of peimine and peiminine in qualified FTB should not be less than 0.08% [1,2]. However, the quantification of peimine and peiminine is difficult and challenging because the chemical structures of the two compounds are similar. There is also a lack of UV-conjugated systems (Fig. 1). In the Chinese Pharmacopeia analysis, an evaporative light scattering detector (ELSD) was used for quantification of the two analytes in FTB samples [1,2]; however, the expensiveness, poor repeatability, and poor stability of the detector make it be unacceptable in routine analyses.

Therefore, in the present study, we established and validated a high performance liquid chromatography coupled with ultraviolet (HPLC-UV) detector for quantitative determination of peimine and peiminine. FTB samples from different pharmacies and markets in Taiwan were analyzed. The data enabled us to ascertain the stability and homogeneity of the herb in Taiwan markets.

2. Methods

2.1. *Chemicals, solvents, and herbal materials*

Peimine and peiminine were purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, People's Republic of China). LC-grade methanol and acetonitrile were purchased from Merck (Taipei, Taiwan). Purified water was prepared with the Milli-Q system (Millipore, Milford, MA, USA). All other reagents used in the present study were of analytical grade. Herbal materials of FTB were

added and each sample was soaked for 1 hour, after which 50 mL of dichloromethane-methanol (4:1, v/v) was added to

Alltima C18) were tested before the Waters XBridge C18 column (250 mm × 4.6 mm, internal diameter 3.5 μm) was finally selected as the column of choice. To obtain a sufficiently large number of detectable peaks on the chromatographic finger-

3.4. *Quantitative determination of peimine and peiminine in FTB samples*

The developed HPLC-UV analytical method was applied for

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