SHORT COMMUNICATION

Fingerprint analysis and multi-component determination of Zibu Piyin recipe by HPLC with DAD and Q-TOF/MS method

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ABSTRACT
Zibu Piyin recipe (ZBPYR), a traditional Chinese medicine formula, is used for curing dementia caused by diabetes. For quality control of ZBPYR, fingerprint analysis and qualitative analysis using high-performance liquid chromatography (HPLC) with a diode-array detector, and confirmation using HPLC coupled with electrospray ionisation quadrupole time-of-flight tandem mass spectrometry (HPLC-Q-TOF-MS) were undertaken. HPLC fingerprint consisting of 34 common peaks was developed among 10 batches of ZBPYR, in which 7 common peaks were identified in comparison with the authentic standards and detected simultaneously. Furthermore, these seven compounds were verified by HPLC-Q-TOF-MS methods. The method can be applied to the quality control of ZBPYR.

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1. Introduction
ZiBu PiYin recipe (ZBPYR) was developed by Professor Libin Zhan from Zicheng soup according to clinical experiences and therapeutic principles, which is a classic formula of traditional Chinese medicine to enhance the effects of tonifying spleen, benefiting intelligence and...
soothing the nerves (Zhu et al. 2014). The formula for ZBPYR consists of 12 crude drugs: Panax ginseng C.A.Mey. (30.0 g), Dioscorea opposite Thunb. (15.0 g), Poria cocos (Schw.) Wolf (15.0 g), Paronia lactiflora Pall. (15.0 g), Salvia miltiorrhiza Bge. (12.0 g), Dolichos lablab L. (15.0 g), Nelumbo nucifera Gaertn. (20.0 g), Acorus tatarinowii Schott (10.0 g), Polygonum tenuifolia Wildl. (10.0 g), Santalum album L. (4.5 g), Citrus reticulate Blanco. (9.0 g) and Glycyrrhiza uralensis Fisch. (9.0 g) (Chen et al. 2014). Our previous research has shown that ZBPYR exhibits excellent pharmacological activity against diabetes-associated cognitive decline (Shi et al. 2011; Zhu et al. 2014). Although the analysis of chemical constituents from individual herbs has previously been reported (Wen et al. 2013; Zhang et al. 2013; Jia et al. 2014; Salminen et al. 2014), there was no report about ZBPYR, especially fingerprint analysis and simultaneous determination of multi-components from different herbs.

In the present work, for the first time, a qualitative and quantitative analysis of components from ZBPYR can be achieved through HPLC with diode-array detector (DAD) and Q-TOF/MS method, which is more rapid and effective. The analytical method of HPLC fingerprint was established and the common pattern of fingerprint contained 34 co-possessing peaks, in which 7 peaks that were considered to be the main active compounds in ZBPYR due to their specific pharmacological activity were identified to be liquiritin (1), naringin (2), 3,6′-disinapoylsucrose (3), 3,4,5-trimethoxycinnamic acid (4), rosmarinic acid (5), isoliquiritin apioside (6), and salvianolic acid B (7) by comparing with authentic standards (chemical structure shown in Fig. S1). They were also confirmed by HPLC-Q-TOF-MS method and simultaneously determined in 10 samples from different regions of China.

2. Results and discussion

2.1. Selection of chromatographic conditions

Almost the same resolution among the peaks was found when methanol and acetonitrile were used as organic solvent of mobile phase. To consider cost analysis, methanol was finally chosen. 0.1% (v/v) formic acid was chosen as additive in the aqueous phase so as to enhance the resolution and eliminate peak-tailing of most components. Finally, methanol (A)–0.1% formic acid (B) system with a linear gradient of 5–28% (A) in 0–50 min, 28–30% (A) in 50–60 min, 30–35% (A) in 60–80 min, 35–50% (A) in 80–110 min, 50–80% (A) in 110–118 min, was chosen for its good baseline resolution and suitable analysis duration. The column temperature was maintained at 30 °C with satisfactory retention times (RTs) and peak shapes. The typical chromatograms of ZBPYR are shown in Fig. S2.

2.2. HPLC with DAD and Q-TOF/MS method

Firstly, the method of HPLC-DAD used for fingerprint analysis and qualitative analysis was established. As a result, 7 compounds from 34 co-possessing peaks were identified and determined. In consideration of the complex components from ZBPYR, it is inadequate to identify target analytes only by comparison of their RT and UV absorption with reference standards using HPLC-DAD method. Thus, HPLC-Q-TOF-MS method was applied to validate the above speculations combining with the information of exact molecular weight. The mass spectra of 7 compounds from ZBPYR are shown in Figure 1.
2.3. Application of the method

In fingerprint analysis, 34 peaks were selected as the characteristic peaks to describe a chromatographic fingerprinting of ZBPYR and evaluate the similarity of different samples (Fig. S3). All samples from 10 different regions were determined in triplicate. The content variations of 7 components in ZBRYR are shown in Fig. S4.

3. Conclusions

HPLC with DAD and Q-TOF/MS methods were established and applied to the quality of ZBPYR. The method for content determination was validated for good accuracy, intra-day and inter-day precision. It could also be used for the study of other prescriptions.

Supplementary material

Experimental details relating to this article are available online, along with Figure S1–S4 and Tables S1–S6.

Disclosure statement

No potential conflict of interest was reported by the authors.
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