Journal of Pharmaceutical and Biomedical Analysis 92 (2014) 82–89

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

A pre-classification strategy for identification of compounds in traditional Chinese medicine analogous formulas by high-performance liquid chromatography–Mass spectrometry

Shun Xiao, Kedi Luo, Xuexun Wen, Xiaohui Fan, Yiyu Cheng*

Pharmaceutical Informatics Institute, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

Article history:
Received 5 November 2013
Received in revised form
23 December 2013
Accepted 30 December 2013
Available online 18 January 2014

Keywords:
Traditional Chinese Medicine
Analogous formulas
Structure classification
Chemical differentiation
HPLC-MS

ABSTRACT

Compound identification is the essential step in the mechanistic research of traditional Chinese medicine (TCM). As the most common mode of practice in the clinic, TCM formula (Fangji or FJ in Chinese) is often utilized to treat diseases. With the proved therapeutic efficacies by century-long clinical applications, these TCM formulas have been the valuable resource of drug discovery and it is important to understand their mechanisms of action systemically in order to broaden their utilizations in modern system of medicine. Structure elucidation of compounds in FJs has been a very difficult and time-consuming task due to the extremely complex composition. In this work we developed a pre-classification strategy on multiple spectrums (high-performance liquid chromatography–mass spectrometry) of TCM analogous formulas to assist compound identification from FJs. Here Ma Huang decoction group (Herba Ephedrae, MHs, widely used in respiratory diseases) were analyzed as an example of TCM analogous formulas to demonstrate the accuracy and speed of our method. The results showed this strategy is very effective and powerful for this kind of complex sample analysis. Additionally, we carried out the chemical differentiation study of this formula group, which was reported for the first time and would be useful to further pharmacological studies.

1. Introduction

As an important component of healthcare system in China and other Asian countries, traditional Chinese medicine (TCM) has been widely used to treat many common diseases, such as cardiovascular diseases, respiratory diseases and infectious diseases. Unlike the chemical drugs, the chemical composition of TCM is quite complex, which make it difficult to analyze even with the advanced separation and detection techniques [1,2]. Compound identification is essential to the research of the TCM from multiple perspectives, including the discovery of active constituents in mechanistic study, quality control for raw material and final product, as well as toxicological and other aspects [3,4]. Many significant progresses have been made in this field with the help of modern hyphenated analysis technologies, which results in the clarification of many herbs and active compounds [5–9]. Most researchers focused on analyzing single plant and using several high content compounds to represent the whole herb. However, the signature of TCM is the combinatorial use of herbs (sometimes minerals and animals are involved), e.g., TCM formulas, which also is common mode of application for TCM in the clinic. [10]. Therefore, it is important to analyze the components in these TCM formulas (Fangjis or FJs in Chinese) systemically [11]. Due to molecular complexity and limitations on the analytic techniques, only a few relatively simple or famous FJs have been studied [12–14]. More works are needed to elucidate chemical compositions of FJs in order to gain thorough understanding on their pharmacological effects, including unique compatibility rules of herb combinations and possible mechanisms of action. It is interesting to note that most FJs can be grouped into certain categories according to the shared herbs and/or similar indications, which are normally named analogous formulas. Comparisons between analytical spectrums of the analogous formulas would shed some light on the chemicals leading to the differences and their originating herbs, which offers an advantage in systematic compound identification through complexity reduction. Liquid chromatography–mass spectrometry (LC-MS) is one of the most commonly used technologies in TCM analysis [15]. The advantages of LC-MS include highly efficient separation capability, applicability for both qualitative and quantitative analysis, structural information deduction for chemicals, etc.

In this paper, we proposed a pre-classification strategy to facilitate the compound identification through multi-spectrum

* Corresponding author at: Zhejiang University, College of Pharmaceutical Sciences, 866 Yuhangtang Road, Hangzhou, Zhejiang 310058, China.
Tel.: +86 571 88208596; fax: +86 571 88208426.
E-mail addresses: fanxh@zju.edu.cn (X. Fan), chengyy@zju.edu.cn (Y. Cheng).

0731-7085/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jpba.2013.12.042

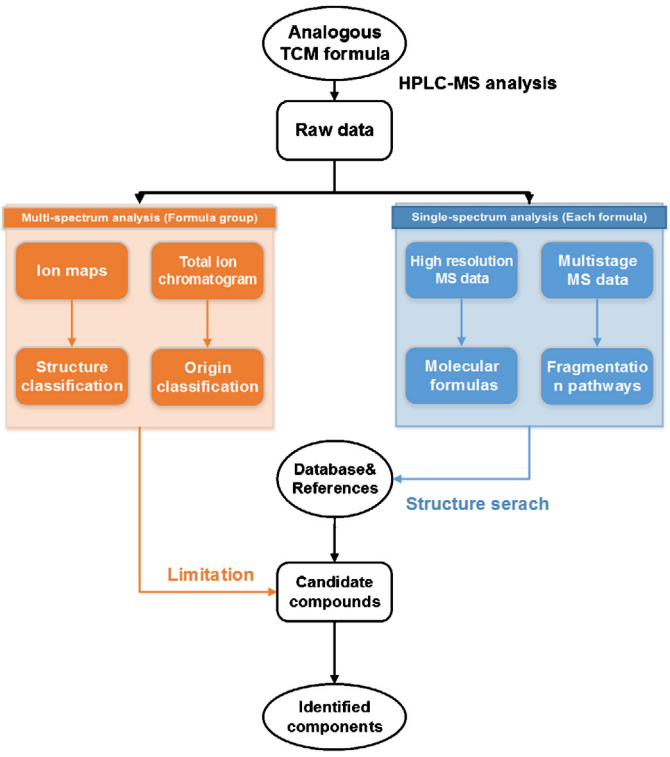

Fig. 1. Analysis scheme for analogous TCM formulas.

comparison of TCM analogous formulas and the traditional 'single-spectrum' analysis on one formula. This novel strategy would improve the accuracy and speed of identifying compounds that meet the spectroscopic properties from chemical databases and certain literatures (Fig. 1). We applied this strategy to five formulas of Mahuang decoction group (*Herba Ephedrae*, MHs, commonly used in respiratory diseases, also been clinical used in Japan as Kampo medicine) and identified components from these analogous formulas [16–18], which demonstrated the usefulness of our method. In addition, the systemic analysis and chemical differentiation study of these five formulas is first reported and can be used for further studies.

2. Materials and methods

2.1. Chemicals and materials

All herbs and mineral materials in the MHs were obtained from the Hangzhou traditional Chinese herbal medicine factory (Hangzhou, Zhejiang province, P. R. China). HPLC-grade acetonitrile, methanol (Merck, Darmstadt, Germany), formic acid (ROE Scientific Inc., Newark, DE, USA), and ultrapure water (Milli-Q Plus, Millipore Co. Ltd., Billerica, MA, USA) were used in all the experiments. All other chemicals and solvents were of analytical grade.

2.2. Sample preparation

The herbs compositions of MHs including Xiao-Qing-Long decoction (XQL), Mahuang-Xingren-Shigao-Gancao decoction (MXSG), Mahuang-Fuzi decoction (MF), Houpu-Mahuang decoction (HM) and Da-Qing-Long decoction (DQL) were shown in Table 1. These traditional TCM decoctions were prepared according to the information of ancient Zhang Zhongjing's herbal formulae (treatise on cold damage and concise essential of the golden cabinet). Air-dried herbs and mineral materials of

Table 1
Composition of different MHs formulas.

TCMs	TCM formula (g)				
	XQL	MXSG	MF	HM	DQL
Radix Glycyrrhizae	24	21	57		24
Rhizoma Zingiberis Recens	24			11	24
Fructus Jujubae					64
Herba Ephedrae	24	41	57	23	48
Ramulus Cinnamomi	24				16
Radix Paeoniae Alba	24				
Pinellia Tuber	26			19	
Plaster ($CaSO_4 \cdot 2H_2O$)		83		34	48
Semen Armeniacae Amarum		55		28	32
Herba Asari	24			11	
Fructus Schisandrae Chinensis	32			23	
Radix Aconiti Lateralis Preparata			86		
Cortex Magnoliae Officinalis				28	
Triticum aestivum Linn.				57	

MHs were extracted twice together with pure water for 1 h under reflux (the amount of the solvent were six and four times of the total weight of herbs, respectively). Combined extracts were first dried under 70 °C and then concentrated to total dryness by vacuum freeze-drying. Samples for LC-MS analysis were prepared by dissolving the extracts in 50% methanol-water to obtain a final concentration of 5 mg mL-1. The solutions were centrifuged at 10000 rpm for 10 min before analysis.

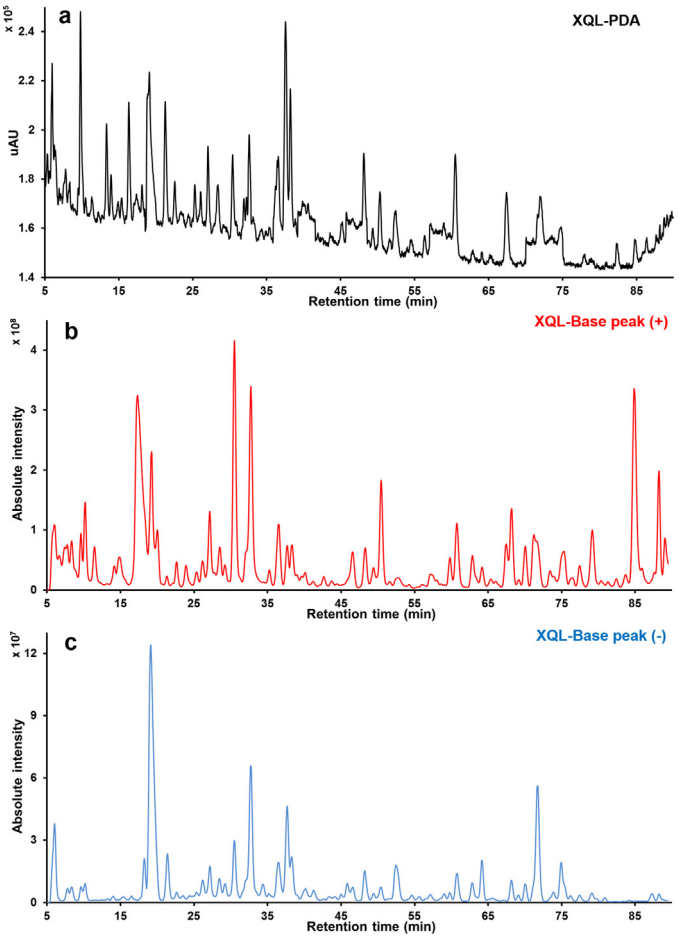

Fig. 2. Spectrum of XQL (a: PDA 190–400 nm, b: positive base peak MS, c: negative base peak MS).