Journal of Pharmaceutical and Biomedical Analysis 88 (2014) 391–400

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

A high performance liquid chromatography fingerprinting and ultra high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry chemical profiling approach to rapidly find characteristic chemical markers for quality evaluation of dispensing granules, a case study on Chuanxiong Rhizoma

Xiao-Lin Zhang[a,b,1], Li-Fang Liu[b,1], Ling-Ying Zhu[a], Ying-Jia Bai[a,b], Qian Mao[a], Song-Lin Li[a,*], Shi-Lin Chen[d], Hong-Xi Xu[c,**]

[a] Department of Pharmaceutical Analysis and Metabolomics, Jiangsu Province Academy of Traditional Chinese Medicine, Nanjing 210028, PR China
[b] Department of Chinese Medicines Analysis, China Pharmaceutical University, Nanjing 210009, PR China
[c] School of Chinese Medicines, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, PR China
[d] The Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, PR China

ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form
21 September 2013
Accepted 24 September 2013
Available online 6 October 2013

Keywords:
HPLC-PDA fingerprinting
UHPLC-PDA-QTOF-MS/MS
Dispensing granule
Characteristic chemical marker
Chuanxiong Rhizoma

ABSTRACT

A high performance liquid chromatography-photodiode array detector (HPLC-PDA) fingerprinting and ultra high performance liquid chromatography-photodiode array detector coupled with quadrupole time-of-flight mass spectrometry (UHPLC-PDA-QTOF-MS/MS) based chemical profiling approach was developed to rapidly find characteristic chemical markers for quality control of dispensing granules, taking Chuanxiong Rhizoma (CR) as a model herb. Firstly, CR crude drugs, their traditional decoctions and CR dispensing granules were analyzed by HPLC-PDA to rapidly establish the fingerprints and thereby generate the simulative median chromatograms of CR crude drugs, decoctions and dispensing granules, and by comparing the simulative median chromatograms, major characteristic peaks of CR decoctions and dispensing granules could be determined. Secondary, UHPLC-PDA-QTOF-MS/MS was used to identify the major characteristic peaks of CR decoctions and dispensing granules. The identities of three major peaks were elucidated and confirmed to be ferulic acid (**1**), senkyunolide I (**2**) and senkyunolide H (**3**) by comparing the mass/UV spectra and retention times with that of the reference compounds. Thirdly, an HPLC-PDA method was validated to quantify the three characteristic components in commercial CR dispensing granules. The average contents of ferulic acid and senkyunolide H were found to be less than 1.0 mg/g, whereas that of senkyunolide I was 4.40 mg/g in CR dispensing granules, which indicated that senkyunolide I might be chosen as a suitable quantitative marker, while ferulic acid and senkyunolide H as qualitative markers for the quality evaluation of CR dispensing granules. It is suggested that this newly established approach could be used to practically and rapidly find suitable marker compounds for quality control of dispensing granules derived from other medicinal herbs.

1. Introduction

In the past two decades, dispensing granules have been increasingly used instead of traditional decoctions for prescription of oriental medicines such as traditional Chinese medicine (TCM) in China and Kampo in Japan [1]. Dispensing granules are products of water extracts of individual herbs in the form of granule, which are manufactured and quantitatively packed by Good Manufacturing Practice (GMP) qualified pharmaceutical companies [2]. Patients can directly dissolve different dispensing granules with hot water in accordance with the prescription of combinatorial formulae to get dispensing granule decoction. Comparing to traditional decoction,

* Corresponding author. Tel.: +86 25 8563 9640.
** Corresponding author at: School of Chinese Medicines, Shanghai University of Traditional Chinese Medicine, No. 1200 Cai Lun Road, Shanghai 201203, PR China. Tel.: +86 15000775800.
E-mail addresses: songlinli64@126.com (S.-L. Li), xuhongxi88@gmail.com (H.-X. Xu).
[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.jpba.2013.09.023

often boiled by patients themselves, with boiling time and amount of water difficult to control, the major advantages of dispensing granules are better quality control and easier for administration [3,4]. However, one of the major problems still challenged the quality control of dispensing granules is the selection of suitable chemical markers.

Currently, in most circumstances, the chemical markers used for quality control of one herb (crude drug) are selected for quality evaluation of its dispensing granule [5]. However, accumulating studies show that decocting could qualitatively or quantitatively induce chemical changes of medicinal herbs or combinatorial formula [6–8]. During decocting, some original major components that are used as chemical markers for quality control of the crude drug may be transformed into new components, which may mainly contribute to the efficacy of the decoction, thus the transformed components should be the potential chemical markers for the quality evaluation of dispensing granules. Therefore, rapidly finding the major characteristic bioactive components including the transformed ones is very important for the selection of suitable chemical markers for the quality control of dispensing granules.

Conventional phytochemical strategies such as extraction, isolation and structure elucidation are tedious and time-consuming for finding characteristic components of dispensing granules. An effective and efficient approach for rapidly finding the characteristic bioactive components of dispensing granules is therefore needed.

Ultra high performance liquid chromatography coupled with photodiode array detector and quadrupole time-of-flight mass spectrometry (UHPLC-PDA-QTOF-MS/MS) has been embraced by researchers as a powerful tool for chemical profiling studies of medicinal herbs [9]. However, owing to its relatively high cost of operation, this technique is currently still unavailable for routine analysis. On the other hand, with relatively lower cost, HPLC-PDA fingerprinting has been introduced and accepted by WHO as a strategy for the holistic quality assessment of herbal medicines [10]. This technique has been recommended by the State Food and Drug Administration of China for routine analysis of injections derived from traditional Chinese medicines [11].

Chuanxiong Rhizoma (CR), the dried rhizome of *Ligusticum chuanxiong* Hort., is a well known traditional Chinese medicinal herb commonly prescribed in combinatorial formula for the treatment of menstrual, cardiovascular and cerebrovascular disorders [12]. In current version of China Pharmacopeia, ferulic acid is employed as chemical marker for quality control of CR crude drug. Recently, Yang et al. used ferulic acid as marker compound to evaluate the quality of CR dispensing granules [13]. Nevertheless, it is well known that ferulic acid is not a characteristic component of CR. Our previous studies found that phenolic acids (such as ferulic acid and coniferyl ferulate) and phthalides (such as senkyunolide A and (*Z*)-ligustilide) are main components of CR crude drug [14,15]. Coniferyl ferulate could be transformed to ferulic acid, and (*Z*)-ligustilide to senkunolide I and senkyunolide H under hot-processing of this herb [16]. Therefore, these thermo-labile components of CR crude drug may undertake chemical transformation during decocting of CR, consequently lead to the difference of chemical profiles of CR decoction from CR crude drug. Understanding the major characteristic components of CR decoction and dispensing granules would be helpful in selecting suitable chemical markers for the quality control of CR dispensing granules.

Gas chromatography–mass spectrometry (GC–MS) [17], HPLC-UV [18] and HPLC-PDA–MS [19] methods had been used to investigate the ingredients of CR crude drugs. However, as mentioned above, some of the volatile phthalides such as (*Z*)-ligustilide is unstable at high temperature, and the dimeric phthalides are also thermolabile and retro-Diels–Alder reactions can easily take place below 100 °C [20]. So GC–MS is not suitable to analyze the chemical compounds of CR. Furthermore, no methods have been developed for chemical profiling of CR decoction and dispensing granules.

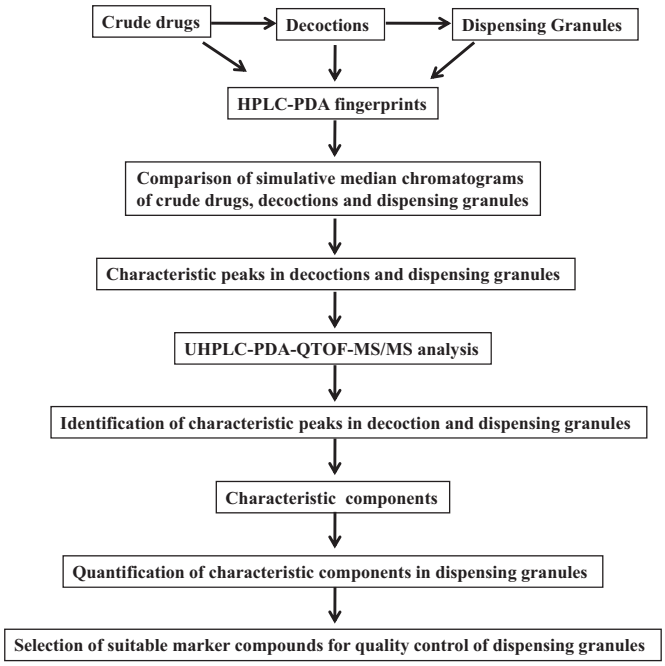

Fig. 1. Strategy proposed for rapidly finding characteristic chemical markers for quality evaluation of dispensing granules by HPLC-PDA fingerprinting coupled with UHPLC-QTOF-MS/MS chemical profiling approach.

In the present study, using CR as an example, an HPLC-PDA fingerprinting coupled with UHPLC-PDA-QTOF-MS/MS based chemical profiling approach was proposed for the first time to rapidly find major characteristic components, which might be potential chemical markers for quality evaluation of dispensing granules. Briefly, CR crude drugs, their traditional decoctions and dispensing granules were comparatively analyzed by HPLC-PDA fingerprinting method to rapidly find the major characteristic peaks of traditional decoctions and dispensing granules, then the identities of the characteristic peaks were elucidated by UHPLC-PDA-QTOF-MS/MS chemical profiling method, and finally, a quantitative method was validated to analyze the contents of the selected characteristic bioactive components in commercial CR dispensing granules, so that the suitable quantitative and qualitative marker compounds could be determined. The proposed strategy is demonstrated in Fig. 1.

2. Experimental

2.1. Chemicals and reagents

HPLC grade acetonitrile from Merck (Darmstadt, Germany), MS-grade formic acid from Sigma–Aldrich (Steinheim, Germany), HPLC grade methanol from Hanbon Sci. & Tech. Co. Ltd (Jiangsu Province, China) were purchased. Ultra-pure water was prepared from a Milli-Q system (Millipore, Bedford, MA, USA).

Reference compounds of ferulic acid (**1**), senkyunolide I (**2**), senkyunolide H (**3**), senkyunolide A (**4**), (*Z*)-ligustilide (**5**), coniferyl ferulate (**6**), butylidenephthalide (**30**), riligustilide (**36**) and levistolide A (**37**) were prepared by our research team, their identities were confirmed by HR-MS, and ^{1}H, ^{13}C NMR analysis [15,16]. The purities of ferulic acid, senkyunolide I and senkyunolide H were determined to be higher than 98% by HPLC analysis, and used