Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1121 (2006) 114–119
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

High-performance thin-layer chromatographic fingerprints of isoflavonoids for distinguishing between *Radix Puerariae Lobate* and *Radix Puerariae Thomsonii*

Si-Bao Chen [a], He-Ping Liu [b], Run-Tao Tian [b], Da-Jian Yang [a], Shi-Lin Chen [a,*], Hong-Xi Xu [c], Albert S.C. Chan [a], Pei-Shan Xie [b,**]

[a] *State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Hong Kong, China*
[b] *Zhuhai Chromap Institute of Herbal Medicine Research, Zhuhai 519085, China*
[c] *Hong Kong Jockey Club Institute of Chinese Medicine Limited, Kowloon, Hong Kong, China*

Received 1 October 2005; received in revised form 28 March 2006; accepted 5 April 2006
Available online 22 May 2006

Abstract

The roots of *Pueraria lobata* (Wild.) Ohwi and *Pueraria thomsonii* Benth have been officially recorded in all editions of *Chinese Pharmacopoeia* under the same monograph 'Gegen' (*Radix Puerariae*, RP). However, in its 2005 edition, the two species were separated into both individual monographs, namely 'Gegen' (*Radix Puerariae Lobatae*, RPL) and 'Fenge' (*Radix Puerariae Thomsonii*, RPT), respectively, due to their obvious content discrepancy of puerarin, the major active constituent. In present paper, the fingerprint of high-performance thin-layer chromatography (HPTLC) combining digital scanning profiling was developed to identify and distinguish the both species in detail. The unique properties of the HPTLC fingerprints were validated by analyzing ten batches of *Pueraria lobata* and *P. thomsonii* samples, respectively. The common pattern of the HPTLC images of the roots of *Pueraria* spp. and the respective different ratios of the chemical distribution can directly discern the two species. The corresponding digital scanning profiles provided an easy way for quantifiable comparison among the samples. Obvious difference in ingredient content and HPTLC patterns of the two species questioned their bio-equivalence and explained that recording both species separately in the current edition of *Chinese Pharmacopoeia* (2005 edition) is reasonable due to not only the content of major constituent, puerarin, but also the peak-to-peak distribution in the fingerprint and integration value of the total components. Furthermore, the HPTLC fingerprint is also suitable for rapid and simple authentication and comparison of the subtle difference among samples with identical plant resource but different geographic locations.

Keywords: High-performance thin-layer chromatography; Digital scanning profile; Fingerprint; *Radix Puerariae*; *Pueraria lobata* (Wild.) Ohwi; *Pueraria thomsonii* Benth

1. Introduction

The roots of *Pueraria lobata* (Wild.) Ohwi and *Pueraria thomsonii* Benth (Fabaceae family), two commonly used Chinese herbal medicines, were officially included in *Chinese Pharmacopoeia* (CP) till 2000 edition under the same name 'Gegen' (*Radix Puerariae*, RP) [1]. It is employed to relieve fever and dysentery, promote the production of body fluid, facilitate eruption, lessen stiffness and pain of the nape [2], and for the treatment of cardiovascular diseases, e.g. hypertension, myocardial infarction and arrhythmia [3]. *P. thomsonii* was also used as a soup resource in southern China and dietary supplements in North America [4,5]. Isoflavonoids, such as puerarin, daidzin, genistin, genistein and daidzein have been demonstrated to be the major efficient components of RP as phytoestrogen in pharmacology and clinical use [6–9]. However, the great disparity in content of puerarin, the major phytoestrogen isoflavonoid in RP (2.4% in the root of *P. lobata* and 0.3% in the root of *P.*

* Corresponding author. Tel.: 852 27665606; fax: 852 23649932.
** Corresponding author. Tel.: +86 756 3326296; fax: +86 756 3326961.
E-mail addresses: bcslchen@inet.polyu.edu.hk (S.-L. Chen), psxie163@163.com (P.-S. Xie).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.04.082

thomsonii) [10] has arisen doubt about the 'bio-equivalence' of the two species with the same dose. Therefore, the current CP (2005 edition) recognizes the root of *P. lobata* as independent herb, namely "Gegen" (*Radix Puerariae Lobatae*, RPL) as well as that of *P. thomsonii* as another one, namely "Fenge" (*Radix Puerariae Thomsonii*, RPT). The therapeutic efficacy of herbal medicine is always attributed to its multi-components but not to any single ingredient. So, besides quantification of single compound, a comprehensive multi-ingredient analytical method is necessary to be developed for more effective species differentiation and quality assessment of the two herbal drugs.

Concerning quality assessment of herb medicine, conventional analysis based on GC, HPLC and TLC always focus on qualitative and quantitative determination of individual or several known components but fail to evaluate chemical properties comprehensively and effectively. In recent years, chromatographic fingerprint profiling has shown to be more convenient and effective for quality assessment of herbal materials, especially when there is a lack of authentic standard substances for the identification of the entire active component present in these complex natural products [11–15]. Although HPLC dominated the chromatographic fingerprint literature, the unique feature of picture-like image of HPTLC coupled with digital scanning profile is more and more attractive to the herbal analysts to construct the herbal chromatographic fingerprint by means of HPTLC [13]. In the present study, the fluorescent HPTLC fingerprint of isoflavonoids (glycosides and aglycones) in RPL and RPT was developed with advanced instrumental planar chromatographic facilities, and the corresponding digital scanning chromatographic profiles were generated with self-developed software. This HPTLC fluorescence image coupling with the scanning profile provided adequate information and parameters for comprehensive identification and assessment of the two close-related species in order to use the herbal drug properly.

2. Experimental

2.1. Apparatus and reagents

HPTLC was carried out with a Camag TLC system (Camag, Muttenz, Switzerland) fitted with a WinCATS 1.2.3 software. Samples were applied with a Camag automatic TLC sampler 4 (ATS 4) and developed in twin-trough glass chamber (24.5 cm × 8 cm × 22.5 cm). A ReproStar 3 with VideoStore 2 documentation software (Camag, Muttenz, Switzerland) was used for the imaging and archiving the TLC chromatograms. TLC digital scanning software used to transfer plate image to digital scanning plot was developed by our research group. HPTLC precoated plates, silica gel Merck 60, 20 cm × 10 cm were used (Merck, Darmstadt, Germany, code: OB456076). All chemicals and solvents were of analytical grade and used as obtained.

2.2. Materials

Puerarin and daidzein were purchased from the Chinese National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Daidzin and genistin were obtained from Tauto Biotech (Shenzhen, China). Ten roots samples of *Pueraria lobata* and ten of *P. thomsonii* were collected from eight RP planting location in China (Table 1). All samples were authenticated by Dr. Jing Y. Song (Institute of Medicinal Plant development, Peking Union Medical College, Chinese Academy of Medical Science, where voucher specimens were deposited) according to morphological characteristics.

Table 1
A summary of the tested samples

No.	Latin name	Collected location
1[a]	*Radix Puerariae Lobatae*	Beijing, China
2	*Radix Puerariae Lobatae*	Nanjing, Jiangsu, China
3	*Radix Puerariae Lobatae*	Huoshan, Anhui, China
4	*Radix Puerariae Lobatae*	Shenyang, Liaoning, China
5	*Radix Puerariae Lobatae*	Bozhou, Anhui,
6	*Radix Puerariae Lobatae*	Yichuan, Henan, China
7	*Radix Puerariae Lobatae*	Hengyang, Hunan, China
8	*Radix Puerariae Lobatae*	Hengyang, Hunan, China
9	*Radix Puerariae Lobatae*	Hengyang, Hunan, China
10	*Radix Puerariae Lobatae*	Hengyang, Hunan, China
11[a]	*Radix Puerariae Thomsonii*	Guangxi, China
12	*Radix Puerariae Thomsonii*	Guangzhou, Guangdong, China
13	*Radix Puerariae Thomsonii*	Guangzhou, Guangdong, China
14	*Radix Puerariae Thomsonii*	Chengdu, Sichuan, China
15	*Radix Puerariae Thomsonii*	Nanjing, Jiangsu, China
16	*Radix Puerariae Thomsonii*	Guangzhou, Guangdong, China
17	*Radix Puerariae Thomsonii*	Guangzhou, Guangdong, China
18	*Radix Puerariae Thomsonii*	Chengdu, Sichuan, China
19	*Radix Puerariae Thomsonii*	Chengdu, Sichuan, China
20	*Radix Puerariae Thomsonii*	Zigong, Sichuan, China

[a] Reference samples purchased from National Institute for the control of Pharmaceutical and Biological Products of China, Beijing.

2.3. Preparation of standard substances solution

Dissolve puerarin, daidzin, genistin and daidzein reference substances in methanol to prepare the solution containing 1 mg/mL each. This is the standard substances solution.

2.4. Preparation of sample solution

0.2 g powder of RPL and 3 g of RPT were accurately weighed into a conical flask, respectively, and refluxed with 80 ml absolute ethanol for 1 h. This extraction process was repeated two times. The extract solution was filtered, and then the residues were rinsed with 20 ml absolute ethanol for two times. The extracts and washings were combined and concentrated to dryness under vacuum. The dry residue was dissolved with 2 ml methanol and then filtrated through a 0.45-μm-membrance filter.

2.5. Chromatography

Standard and sample solutions were applied bandwise (bandlength 6 mm, 70 nL/s delivery speed, track distance 6 mm, distance from left edge 12 mm and low edge 8 mm) to the HPTLC plates. Then, the plate was desiccated in a vacuum trunk con-