Simultaneous determination of four bioactive compounds in *Verbena officinalis* L. by using high-performance liquid chromatography

Zhen Liu, Zhiwei Xu, Hanyu Zhou, Gang Cao, Xiao-Dong Cong, Yun Zhang, Bao-Chang Cai[1,2,3]

[1]Research Center of TCM Processing Technology, Zhejiang Chinese Medical University, Hangzhou, [2]Engineering Center of State Ministry of Education for Standardization of Chinese Medicine Processing, Nanjing University of Chinese Medicine, Nanjing, [3]National First-Class Key Discipline for Traditional Chinese Medicine of Nanjing University of Chinese Medicine, Nanjing, People's Republic of China

Submitted: 21-10-2011 Revised: 08-11-2011 Published: 23-05-2012

ABSTRACT

Background: *Verbena officinalis* L., called mabiancao in Chinese, is derived from the aerial part of Herba Verbanae. It is a traditional Chinese medicine commonly used in China and northern Europe, which is widely used for clearing away heat and detoxicating, promoting blood circulation, and removing blood stasis. This paper describes a sensitive and specific assay for the determination of four bioactive compounds in *V. officinalis* L. **Materials and Methods:** In this paper, the four components were separated on an Agilent Zorbax Extend C_{18} column (250 mm × 4.6 mm × 5 μm) and detected by a diode array detector. The mobile phase was composed of (a) aqueous phosphoric acid (0.1%, v/v) and (b) acetonitrile using a gradient elution. Analytes were performed at 30°C with a flow rate of 1.0 ml/min and UV detection at 203, 238, and 331 nm. **Results:** All calibration curves showed good linear regression ($r^2 \geq 0.9999$) within tested ranges. Overall intra- and interday variations were less than 1.84%, and the average recoveries were 97.32–102.81% for analytes. **Discussion and Conclusion:** The proposed method would be sensitive enough and reliable for comprehensive quality control for clinical use and modernization of *V. officinalis* L.

Key words: High-performance liquid chromatography-diode array detection, quality control, simultaneous determination, *Verbena officinalis* L.

Access this article online

Website: www.phcog.com

DOI: 10.4103/0973-1296.96575

Quick Response Code:

INTRODUCTION

Verbena officinalis L., called mabiancao in Chinese, is derived from the aerial part of Herba Verbanae. It is a traditional Chinese medicine commonly used in China and northern Europe,[1,2] which is widely used for clearing away heat and detoxicating, promoting blood circulation, and removing blood stasis, inducing diuresis and excreting dampness based on the Chinese medical theory (*National Commission Chinese Pharmacopoeia of the People's Republic of China*, 2010).[3,4] To understand the mechanisms involved in these beneficial effects, a great deal of scientific efforts has been contributed to isolate and identify the active components in the samples of *V. officinalis* L..[5,6]

Modern pharmacological studies of the alcohol extract and decoction of *V. officinalis* L. showed that *V. officinalis* L. has anti-inflammatory and analgesic activities, exciting uterine smooth muscle, immunity reinforcement, and nerve protection effects.[7,8] The VO-3% (3% of the alcohol extract) of *V. officinalis* L. had significant anti-inflammatory activity the same as that of piroxicam and somewhat less analgesic activity than that of methyl salicylate; these effects were concerned with iridoid glycosides.[9] Phenylpropanoid glycosides in *V. officinalis* L. have a significant neuroprotection activity and contribution to the nervous system and the immune system.[10] Up to now, iridoid glycosides and phenylpropanoid glycosides in *V. officinalis* L. mainly contained aucubin, hastatoside, verbenalin, and verbascoside.[11] Two compounds verbenalin and verbascoside showed unstable characteristics, and the contents in the herb *V. officinalis* L. showed a large difference between each sample. So it is essential to develop a method to determine these compounds in the herb material for the purpose of reflecting quality status of *V. officinalis* L. from different resources. In this study, an RP-HPLC-DAD method was first developed for the

Address for correspondence:
Mr. Gang Cao, Research Center of TCM Processing Technology, Zhejiang Chinese Medical University, Hangzhou 310053, People's Republic of China. E-mail: caogang33@163.com

simultaneous determination of the four components (aucubin, hastatoside, verbenalin, and verbascoside) in *V. officinalis* L., which is distributed in China. The method was found to be reliable and sensitive.

MATERIALS AND METHODS

Materials and reagents

V. officinalis L. was collected from six suppliers (Zhejiang, Anhui, Henan, Shanxi, Hunan, and Hubei in China) and identified by Prof. Zhang Yun in Zhejiang Chinese Medical University. Reference standards of aucubin, hastatoside, verbenalin, and verbascoside were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity of each standard compound was greater than 98% determined by using HPLC analysis. The structures of these four compounds are shown in Figure 1. All reagents with high grade were obtained from others. Milli-Q water (Millipore, Bedford, MA) was used throughout the study.

Preparation of sample solutions

The powder of *V. officinalis* L. samples quantitatively (1.0 g) transferred into dark brown calibrated flasks and extracted with 20 ml of 80% methanol in an ultrasonic bath for 45 min and cooled at room temperature; 80% of methanol was added to compensate for the lost weight. The solution was filtered through a 0.45-μm membrane filter before subjecting 10 μl to the HPLC analysis.

Preparation of standard solutions and calibration curve

Each reference standard was dried and accurately weighed and then dissolved in 80% methanol and diluted to appropriate concentration, respectively. A mixed stock solution of standards, containing aucubin (59.4 μg/ml), hastatoside (162.69 μg/ml), verbenalin (99.0 μg/ml), and verbascoside (290 μg/ml), was finally prepared. The standard stock and working solutions were all prepared in calibrated flasks and stored at 4°C. All calibration curves were constructed from peak areas of the reference standards versus their concentrations. The solutions were filtered through a 0.45-μm membrane prior to injection.

Chromatographis analysis

Analyses were performed by using Dionex UltiMate3000 HPLC system (Dionex, Sunnyvale, CA, USA) with a diode array detector. Detection wavelengths were set at 203, 238, and 331 nm. An Agilent Zorbax Extend C_{18} column (250 mm × 4.6 mm × 5 μm) was used with a flow rate of 1.0 ml/min. The injection volume was 10 μL, and the column temperature was maintained at 30°C. The mobile phase was composed of (a) aqueous phosphoric acid (0.1%, v/v) and (b) acetonitrile using a gradient elution of 0--55 min (5--20%).

RESULTS AND DISCUSSION

Calibration curves

The linearity calibration curves were constructed by five concentration assays of each reference compound in

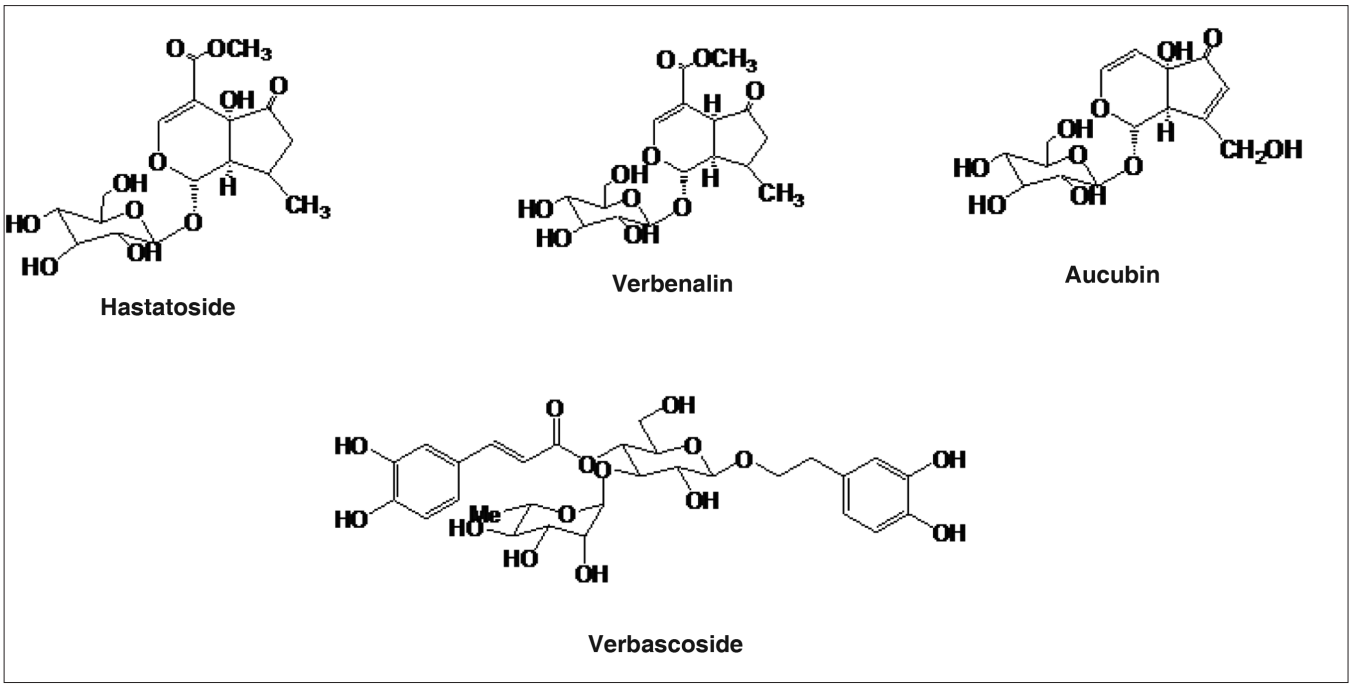

Figure 1: The chemical structures of four active components in *Verbena officinalis* L.

triplicate. An aliquot (10 µl) of each standard solution was subjected to HPLC analysis. The regression equations were calculated in the form of $Y = aX + b$, where Y and X were the concentration of each reference compound and values of the peak area, respectively. The regression equations (linear ranges) were $y = 0.1624x + 1.3346$ (5.94--53.46 µg/ml, aucubin), $y = 0.0952x - 0.9155$ (16.97--169.62 µg/ml, hastatoside), $y = 0.0752x + 0.0837$ (6.6--198.0 µg/ml, verbenalin), $y = 0.0656x + 4.4083$ (14.5--290.0 µg/ml, verbascoside). All the marker substances showed good linearity ($r^2 \geq 0.9999$). The LOD and LOQ of the four analytes were 0.032--0.079 and 0.089--0.238 µg/ml, respectively.

Precision, repeatability and stability

The intra- and interday precision were determined by analyzing calibration samples during a single day and on three different days, respectively. The intraday variation was determined by analyzing the six replicates on the same day, and the interday variation was determined on three consecutive days. The relative standard deviation (RSD) was taken as a measure of precision, and the overall intra- and interday variations were less than 1.84%.

To further evaluate the repeatability of the developed assay, *V. officinalis* L. was analyzed in six replicates as described above. The contents of four compounds in *V. officinalis* L. extracts were calculated from the corresponding calibration curves. The RSDs were taken as measurements of repeatability. The stability was tested with *V. officinalis* L. extracts at room temperature and analyzed at 0, 2, 4, 8, 12, 24, and 48 h within 2 days, respectively. The RSDs of repeatability test and stability were not more than 2.28% for all analytes.

Accuracy

Accuracy was determined by the recovery test. An appropriate amount of *V. officinalis* L. powder was weighed and spiked with the known amount of each standard compound. They were then treated and analyzed as described above. Each sample was analyzed in six replicates. The total amount of each analyte was calculated from the corresponding calibration curve.

$$\text{Recovery (\%)} = \frac{(\text{Amountdetermined} - \text{Amountoriginal})}{\text{Amountspiked}} \times 100\%$$

where Amountdetermined is the determined total of each analyte, Amountoriginal is the original amount of each analyte in *V. officinalis L.* samples measured, and Amountspiked is the spiked amount of each analyte. For comparison, an unspiked sample was prepared and analyzed simultaneously. Mean recoveries of the compounds were 97.32--102.81%, with RSD values ranging from 2.13 to 2.27% ($n = 6$).

Sample analysis

Figure 2 showed the typical separation of a standard mixture and *V. officinalis* L. extracts obtained under the above-mentioned HPLC conditions. The contents were calculated and summarized in Table 1. According to the quantitative analysis results, the total contents of four compounds varied slightly in the same type of samples from different suppliers, which might be due to the differences in soils and climates in each region. Thus, it

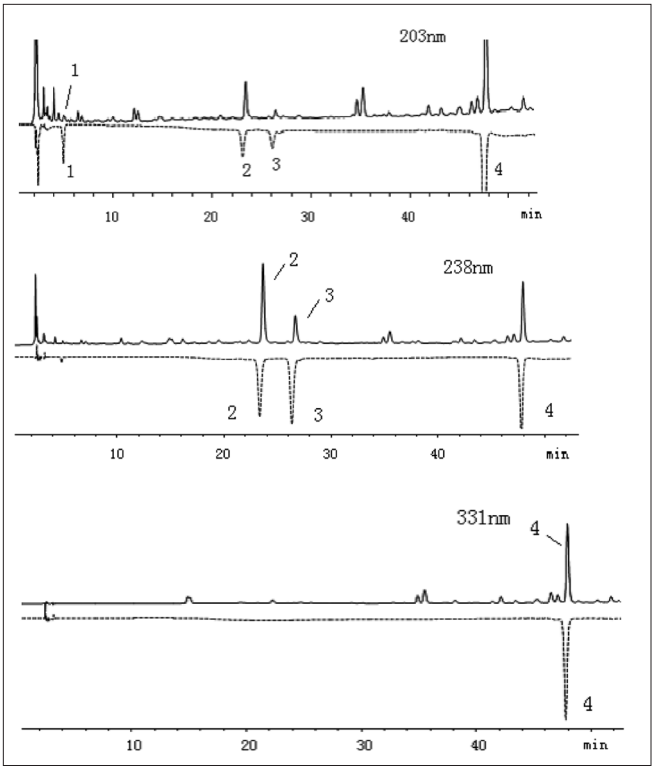

Figure 2: HPLC chromatography comparison of reference substance (above) and extract from *Verbena oficinalis* L. (below). 1. Aucubin, 2. hastatoside, 3. verbenalin, 4. verbascoside

Table 1: Contents of four active compounds in *Verbena oficinalis* L. from different sources (mg/g)

Source	Aucubin	Hastatoside	Verbenalin	Verbascoside
Zhejiang	0.426	7.21	4.98	10.99
Anhui	0.523	4.96	5.01	4.71
Henan	0.311	3.20	4.63	1.32
Shanxi	0.164	2.84	1.21	1.44
Huan	0.309	4.89	2.65	6.83
Hubei	0.296	3.96	2.13	5.22

is necessary to control the main active components in *V. officinalis* L. by good agricultural practice and the norm of Chinese medicinal materials processing.

CONCLUSION

An HPLC-DAD method has been developed to simultaneously determine four active components in *V. officinalis* L. This newly established method is validated as simple, precise, and accurate. It can be used as a valid analytical method for the intrinsic quality control of *V. officinalis* L.

ACKNOWLEDGMENTS

The authors are grateful to the financial support of a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions, PAPD (ysxk-2010) and the Open Project Program of National First-Class Key Discipline for Traditional Chinese Medicine of Nanjing University of Chinese Medicine (2011ZYX2-006) and National Natural Science Foundation of China (No. 30873438) and Science and technology of Chinese medicine of Zhejiang province (No. 2009CB008) and the fund of Zhejiang modernization of traditional Chinese medicine item ([2008]436) and Zhejiang Province Chinese medicine research program (2008ZA002).

REFERENCES

1. Chen GM, Gong RD. Clinical and pharmacological research of *Verbena officinalis* L. Sci Technol Innov Her 2009;31:220-1.
2. Deepak, M, Handa SS. Antiinflammatory activity and chemical composition of extracts of *Verbena officinalis* L. Phytother Res 2000;14:463-5.
3. Chinese Pharmacopoeia Commission. Chinese Pharmacopoeia of the People's Republic of China. Vol.1. Bejing: Chemical Industry Press; 2010. p. 49.
4. Casanova E, Garcia-Mina JM, Jose M, Calvo MI. Antioxidant and Antifungal Activity of *Verbena officinalis* L. Leaves. Plant Foods Hum Nutr 2008;63:93-7.
5. Speroni E, Cervellati R, Costa S, Guerra MC, Utan A, Govoni P, *et al*. Effects of Differential Extraction of *Verbena officinalis* L. on Rat Models of Inflammation, Cicatrization and Gastric Damage. Planta Med 2008;73:227-35.
6. Feng B, Xu CF. Inhibitory effect of 4'-methylether-scutellarein on human choriocarcinoma JAR cells and the possible mechanism. Chin J Cancer Biother 2008;15:444-50.
7. Lai SW, Yu MS, Yuen WH, Chang R. Novel neuroprotective effects of the aqueous extracts from *Verbena officinalis* L. Neoro Pharmacol 2006;50:641-50.
8. Jin WJ, Zhang ZD. The advance on the research of *Verbena officinalis* L. Lishizhen Med Mater Med Res 2007;18:693-4.
9. Calvo MI. Anti-inflammatory and analgesic activity of the topical preparation of *Verbena officinalis* L. J Ethnopharmacol 2006;107:380-2.
10. Xiong Q, Tezuka Y, Kaneko T, Li H, Tran LQ, Hase K, *et al*. Inhibition of nitric oxide by phenylethanoids in activated macrophages. Eur J Pharmacol 2000;400:137-44.
11. Chen XL, Meng Y, Zhang LT. The advance on the research of chemical constituents and pharmacological activities of *Verbena officinalis* L. Hebei Med 2010;32:2089-91.

Cite this article as: Liu Z, Xu Z, Zhou H, Cao G, Cong X, Zhang Y, Cai B. Simultaneous determination of four bioactive compounds in *Verbena officinalis* L. by using high-performance liquid chromatography. Phcog Mag 2012;8:162-5.

Source of Support: Nil, **Conflict of Interest:** None declared.