文章编号:1001-6880(2014)Suppl-0067-05

HPLC 法快速测定川芎药材中阿魏酸的含量

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摘　要:本文建立了快速测定川芎药材中阿魏酸含量的方法。以阿魏酸为标准品,采用 Agilent 1100 高效液相色谱仪和 Waters Symmetry C_{18} 色谱柱,以乙腈和1% 冰乙酸(32∶68)为流动相,检测波长为 321 nm。经测定阿魏酸浓度在 0.1 ~ 160 μg/mL 范围内呈良好的线性关系(r = 0.9999),平均回收率在 96.88 ~ 99.75% 之间,检测限和定量限分别为 0.0043 μg/mL 和 0.0128 μg/mL。该方法简便、准确、重现性好,可作为快速检测川芎药材中阿魏酸含量的方法。

关键词:阿魏酸;HPLC;川芎;含量;方法建立

中图分类号:R284.2　　**文献标识码**:A

Rapid Determination of Ferulic Acid in *Ligusticum chuanxiong* Rhizomes using High-Performance Liquid Chromatography

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Abstract: We developed a simple and rapid method for determining ferulic acid in *Ligusticum chuanxiong* rhizomes. Ferulic acid was extracted from the samples using methanol and analyzed using reversed-phase high performance liquid chromatography (HPLC). The extract was chromatographically separated using an Agilent 1100 series HPLC system with a Waters Symmetry C_{18} column and isocratically eluted with a mixture of acetonitrile and 1% aqueous acetic acid (32:68, v/v). The effluent was monitored using a VWD detector set at 321 nm. The average recovery rates ranged from 96.88% to 99.75% (n = 3). The limit of detection was 0.0043 μg/mL and the limit of quantification was 0.0128 μg/mL. The method has been successfully applied to analyze *L. chuanxiong* samples. The ferulic acid content of 38 samples varied from 0.41 mg/g to 3.12 mg/g.

Key words: ferulic acid; HPLC; *Ligusticum chuanxiong*; quantitative determination; method validation

Introduction

Ligusticum chuanxiong Hort. is widely used as a traditional medicine and food in China. The essential biological active ingredients of this herb include ferulic acid, alkaloids and volatile oil[1]. This herb facilitates blood circulation and disperses blood stasis; hence, it is commonly prescribed for treating angina pectoris, cardiac arrhythmias, hypertension and stroke [2].

Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) is an ubiquitous phenolic compound in plant tissues and the sole quality control indicator for *L. chuanxiong*[2,3]. The determination of the ingredients of herbs has focused on speed, low-cost, and high reproducibility[2]; therefore increasing the need for a rapid and accurate methods for determining ferulic acid.

Many HPLC analytical methods have been developed for determining ferulic acid in Chuanxiong[3-10]. However, these methods are time consuming and require organic solvents, and the ferulic acid peak time ranges from 10 min to 30 min, thereby requiring an entire measurement time of 30 min to 60 min. Therefore, developing a simple and rapid HPLC method for determi-

Received: October 12, 2013; Acceppted: May 6, 2014

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ning ferulic acid in *L. chuanxiong* rhizomes is necessary would be valuable for further studies.

This study aims to develop a simple and rapid method for quantitatively analyzing ferulic acid in *L. chuanxiong* rhizomes. The method has been successfully utilized for determining ferulic acid content and investigating the range of ferulic acid content in *L. chuanxiong* rhizome samples.

Materials and Methods

Materials, reagents and apparatus

Eight commercial samples were purchased from markets, which were designated as YP01 to YP08. Thirty other samples, YP09 to YP38, were obtained from the cultivated fields in a major production zone for *L. chuanxiong* rhizomes (Du jiang yan County, Chengdu City, Sichuan Province, China) during harvest time. All samples were sundried and grounded into powder before analysis, and then were shifted through a 60-mesh sieve and kept in an air-tight container until being used.

Ferulic acid (standard sample) was obtained from National Institutes for Food and Drug Control (Beijing, China). Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Fisher Scientific, Inc. Acetic acid (AC grade) was purchased from the Chengdu Kelong Chemical Factory (Chengdu, China). Deionized water was prepared using a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA).

HPLC analysis was carried out using Agilent 1100 LC system (Agilent, USA), which consisted of a quaternary pump, an ALS auto injector, a column oven, and a VWD detector, that was connected to an LC ChemStation.

HPLC conditions

Waters Symmetry® C_{18} column (250 mm × 4.6 mm, 5 μm) (Milford, MA, USA) was used. The column temperature was maintained at 35.4 ℃. The standards and samples were separated using an isocratic mobile phase that consisted of 1% aqueous acetic acid and acetonitrile (68∶32, v/v). The flow rate was set to 1 mL/min and the injection volume was 10 μL. The detection wavelength was set to 321 nm. Ferulic acid was identified based on retention time when co-injected with the standards.

Sample preparation

Ferulic acid extraction was performed by adding 0.5 g of the powdered sample in 25 mL of methanol into a 50 mL tube. The samples were extracted using an ultrasonic extractor (Model No.: SB-5200 DTD, Ningbo Science Biotechnology Co., Ltd, Zhejiang, China), with a working frequency of 40 kHz, and a bath power rating of 200 W, and the temperature was 40 ℃ for 60 min. After extraction, this solution was filtered using a 0.45 μm membrane filter and collected into a 1.5 mL vial prior to HPLC analysis.

Standard preparation

A stock solution of ferulic acid standard (0.32 mg/mL) was prepared by dissolution in methanol. The working standard solutions for linear calibration were prepared by diluting the stock solution to produce a concentration sequence of 0, 6.4, 16, 32, 48, 64, 80, 96, and 160 μg/mL.

Method validation

The analytical method was validated for linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) in accordance with the International Conference on Harmonization guidelines[11].

Linearity

The linearity of the method was determined by injecting nine known concentrations of the standard (0 μg/mL to 160 μg/mL) in triplicate. The calibration curves were obtained by plotting the peak area versus the amount/concentration of the standards.

Precision

Intra-day precision and inter-day precision were measured by analyzing five samples extracted from the solution. The intra-day precision (repeatability) was examined by analyzing three times a day, whereas the inter-day precision (reproducibility) was examined for three consecutive days by the proposed method. Both values were expressed as percent relative standard deviation (%RSD).

Accuracy

The recovery rate of ferulic acid was determined via

standard addition to measure the accuracy of the method. Known amounts of ferulic acid were added into five samples extracted from the solution, and a standard concentration of 3.98 μg/mL. The spiked samples were prepared in triplicate. The recovery rate was calculated as follows: recovery (%) = (found amount -original amount)/amount spiked × 100.

Limit of detection (LOD) and limit of quantification (LOQ)

The signal-to-noise ratio was determined under the proposed chromatographic condition. The LOD was set to 3 : 1 and the LOQ was set to 10: 1.

Quantification of ferulic acid

Quantification was based on the external standard. The standard calibration curve was obtained, and the ferulic acid content of each sample was calculated and expressed as milligram equivalent per gram of the sample (mg/g, Section 2.5.1).

Results and Discussion

Method development

The proposed HPLC method was established for quantitative analyzing ferulic acid in *L. chuanxiong* rhizome extracts. The various mobile phase trials revealed the enhancement in separation selectivity, the increase efficiency, and the elimination of peak tailing of ferulic acid. Hence, a mobile phase containing aqueous acetic acid was selected together with acetonitrile to provide a stable baseline, a symmetric peak, and the most efficient separation rate and speed. Finally, a mobile phase consisting of acetonitrile and 1% aqueous acetic acid (32: 68, v/v) was chosen to determine the ferulic acid in *L. chuanxiong* rhizomes. Fig. 1A shows the ultraviolet spectrum of the ferulic acid reference revealed a maximum absorbance of 321 nm. Thus, it was selected as the detection wavelength. The method differs from previously reported methods[3-10], and is simple and rapid. The peak time was 5.7 min (Fig. 1 B), whereas the control time was 7 min, and that of the Chinese pharmacopoeia method was 21.1 min (Fig. 1 C). Hence, the ferulic acid peak time ranged from 10 min to 30 min, and the entire measurement time using the Chinese pharmacopoeia method or other reported methods ranges from 30 min to 60 min[4-10]. The HPLC method achieved a rapid, high reproducible, efficient, and low-cost quantitative determination of the ferulic acid content of *L. chuanxiong* rhizomes.

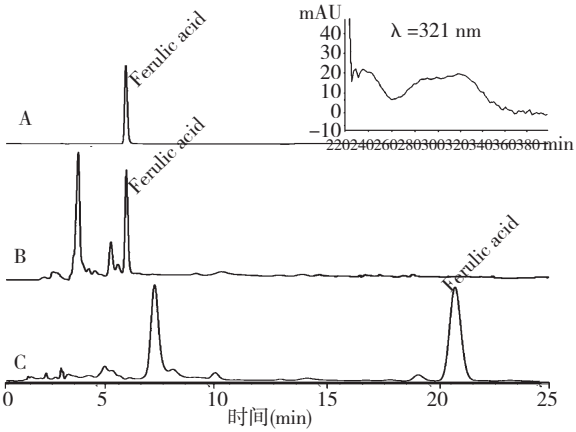

Fig. 1 (A-C) show the typical HPLC chromatograms of the ferulic acid standard, the actual samples under the study conditions and the Chinese pharmacopoeia method

Method validation

The developed method was validated for linearity, precision, accuracy, LOD, and LOQ. The calibration plots for ferulic acid the linear relationship is described by the best-fit curve $Y = 5997.6X + 5.0497$, where X and Y are the peak area and concentration of the standard solution (μg/mL), respectively. Linear regression analysis showed good linearity ranging from 0.1 μg/mL to160 μg/mL, with a correlation coefficient of 0.9999. This finding allows the determination of ferulic acid over a wide range of concentrations. The precision of the method was determined using the five sample solutions. The results show that the method has acceptable precision with %RSD lower than 2% (Table 1). The recovery rate of ferulic acid, which represents the accuracy of the method, ranged from 96.88 to 99.75 (Table 2). The LOD for ferulic acid was 0.0043 μg/mL and the LOQ was 0.0128 μg/mL, which indicated the high sensitivity of the method. Hence, the method is accurate and precise, as evidenced by the high recovery rate and low %RSD.

Table 1 Intra-day and inter-day precisions for ferulic acid determination, where the results are shown as RSD%

Samples	inter-day			intra-day
	Day 1	Day 2	Day 3	
1	1.71	1.93	1.68	0.59
2	0.05	1.24	1.53	0.07
3	1.91	1.54	1.48	0.36
4	1.16	1.41	1.30	0.82
5	0.87	1.29	0.71	0.58

Table 2 Recovery rate of ferulic acid (n = 3) expressed as mean ± SD

Samples	Theoretical (mg)	Found (mg)	Recovery (%)
1	0.395	0.395 ± 0.001	99.688 ± 0.027
2	0.372	0.371 ± 0.002	96.875 ± 0.006
3	0.364	0.364 ± 0.001	99.750 ± 0.002
4	0.377	0.377 ± 0.000	99.359 ± 0.024
5	0.380	0.379 ± 0.000	99.062 ± 0.034

Application of the method

The proposed HPLC method was used to quantify the ferulic acid content in 38 samples. The ferulic acid content ranged from 0.41 mg/g to 3.12 mg/g. The details are summarized in Table 3. According to the results, some *L. chuanxiong* samples were rich in ferulic acid, but their ferulic acid content varied widely.

Conclusions

A HPLC method has been established for the quantitative analysis of ferulic acid in *L. chuanxiong* rhizomes. The proposed method is simple and rapid, highly precise, accurate, and reliable, which is appropriate for detection purposes. Up to 38 *L. chuanxiong* samples were analyzed for ferulic acid content. We found a substantial variation among different samples upon comparison. Further studies will analyze the factors that lead to the variations in ferulic acid content of different *L. chuanxiong* samples.

Table 3 Content of ferulic acid (mg/g) in actual rhizomes of *L. chuanxiong* samples (n = 3) and expressed as mean ± SD

Sample No.	Content	Sample No.	Content,	Sample No.	Content
YP01	1.72 ± 0.21	YP14	2.59 ± 0.06	YP27	2.38 ± 0.04
YP02	1.53 ± 0.18	YP15	2.59 ± 0.03	YP28	2.27 ± 0.02
YP03	0.41 ± 0.05	YP16	2.4 ± 0.03	YP29	2.07 ± 0.04
YP04	0.43 ± 0.10	YP17	2.39 ± 0.01	YP30	2.35 ± 0.13
YP05	0.42 ± 0.09	YP18	2.24 ± 0.01	YP31	2.34 ± 0.21
YP06	0.43 ± 0.01	YP19	2.12 ± 0.03	YP32	2.47 ± 0.25
YP07	0.76 ± 0.04	YP20	2.53 ± 0.09	YP33	2.67 ± 0.06
YP08	0.85 ± 0.01	YP21	2.82 ± 0.24	YP34	2.55 ± 0.04
YP09	2.02 ± 0.01	YP22	3.12 ± 0.32	YP35	2.33 ± 0.11
YP10	2.71 ± 0.03	YP23	2.67 ± 0.01	YP36	2.35 ± 0.14
YP11	2.89 ± 0.02	YP24	2.58 ± 0.02	YP37	2.47 ± 0.03
YP12	3.08 ± 0.08	YP25	2.36 ± 0.01	YP38	2.23 ± 0.02
YP13	2.54 ± 0.02	YP26	2.72 ± 0.19		

Acknowledgements

The authors wish to thank the College of Agronomy, Sichuan Agricultural University and Key Laboratory of Crop Ecophysiology and Farming System in Southwest China, Ministry of P. R. China for providing the necessary facilities.

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