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SIMULTANEOUS DETERMINATION OF CONTENTS OF THREE ACTIVE COMPONENTS IN JIEJIA TINCTURE BY HPLC METHOD

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Abstract

The objective of the study was to determine the contents of three active components in Jiejia tincture by establishing HPLC method. Test articles were prepared by ultrasonic extraction. Separation was performed using a Kromasil C18 (250 mm × 4.6 mm, 5 μm) chromatographic column, and gradient elution was performed with acetonitrile-0.3% phosphoric acid solution as the mobile phase at a volumetric flow rate of 0.80 mL/min. The contents of catechin, baicalin and berberine in Jiejia tincture were determined at the wavelength of 276 nm and a column temperature of 30 □. The results revealed that catechin showed a good linear relationship at the range of 100~800 μg/mL (r=0.9997); baicalin showed a good linear relationship at the range of 15~120 μg/mL (r=0.9996), and berberine at the range of 7~56 μg/mL (r=0.9995). Their average recovery rates were 99.67% (RSD 1.01%, n=6), 98.7% (RSD 1.93%, n=6) and 100.5% (RSD 2.88%, n=6) respectively. The study concluded that the high-performance liquid chromatography established in this study was simple, accurate and reproducible, and can also be used in the determination of catechin, baicalin and berberine contents in Jiejia tincture.

Key words: Jiejia tincture, catechin, baicalin, berberine, HPLC

Introduction

The Jiejia tincture is composed of 100 g of Acacia catechu, *Scutellaria baicalensis* and *Cortex Phellodendri* each, and 30-50 g of borneol (Hu, 1989). It has the heat-clearing and astringing, bleeding-arresting and sore-closing, granulation-promoting and pain-relieving efficacies. And it is commonly used in the clinical treatment of burns and scalds. Acacia catechu is a plant in genus Acacia, family Fabaceae. Its pharmacological effects are astringing dampness, promoting granulation and closing sores, and is mainly used in the clinic for treatment of ulcers, skin eczema, traumatic bleeding, etc. (Chinese Pharmacopoeia Commission, 2010). In recent years, it is also commonly used for anti-oxidation (Osaman, 2011) and anti-bacteria (Li et al, 2001). *Scutellaria baicalensis* is a plant in the Lamiaceae family, which has the antipyretic, anti-inflammatory, anti-allergic, anti-endotoxin, and anti-anxiety efficacies (Liao et al, 2003; Mi et al, 2010). *Phellodendron* is a Rutaceae family plant. Its pharmacological effects include clearing heat and drying dampness, purging fire and removing toxin, pathogenic microorganism, anti-inflammation and anti-allergy. It is also relatively effective on eczema, burns, bedsores and insect dermatitis (Shen, 2011; Bai, 2008). In recent years, Jiejia tincture is commonly used in the clinic for skin abrasions and severe pressure sores (Gao et al, 2012; He, 2009). However, even with all these efficacies, the method for determination of catechin, baicalin and berberine contents in Jiejia tincture has never been reported. This study established HPLC method for simultaneous determination of catechin, baicalin and berberine contents in Jiejia tincture.

Instruments and reagents

Instruments

The instruments used for the study include the following: high performance liquid chromatograph-UV3000 UV-Vis detector-P3000 high pressure constant flow pump (Beijing Chuangxintongheng Science & Technology Co., Ltd.); FA1004 analytical balance (Shanghai Balance Instrument Factory); KQ-600DE numerical control ultrasonic cleaner (Kunshan Ultrasonic Instruments Co., Ltd.); and 2100A rotary evaporator (Shanghai Yarong Biochemical Instrument Factory).

Reagents

The reagents include thus: acetonitrile (HPLC grade, Sigma); phosphoric acid (analytical grade, Tianjin Kemiou Chemical Reagent Co., Ltd.); methanol (analytical grade, Tianjin Kemiou Chemical Reagent Co., Ltd.); catechin standard (batch No. 110877-200001, National Institute for Pharmaceutical and Biological Products Inspection); baicalin standard (batch No. 110715-201016, National Institute for Pharmaceutical and Biological Products Inspection); and berberine standard (batch No. 110713-200911, National Institute for Pharmaceutical and Biological Products Inspection)

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Methods and results

Chromatographic conditions

Kromasil C18 (250 mm × 4.6 mm, 5 μm) chromatographic column was gradient eluted with acetonitrile-0.3% phosphoric acid solution as the mobile phase. The gradient elution mode is shown in Table 1 (collection time was 45 min). Volumetric flow rate was 0.80 mL/min, column temperature was 30 □, and detection wavelength was 276 nm. Determination was performed under the above chromatographic conditions. The peak retention time of catechin, baicalin and berberine was consistent with the chromatographic peak retention time of corresponding reference substances. Other components were not interferential on the determination. The chromatogram is shown in Fig.1.

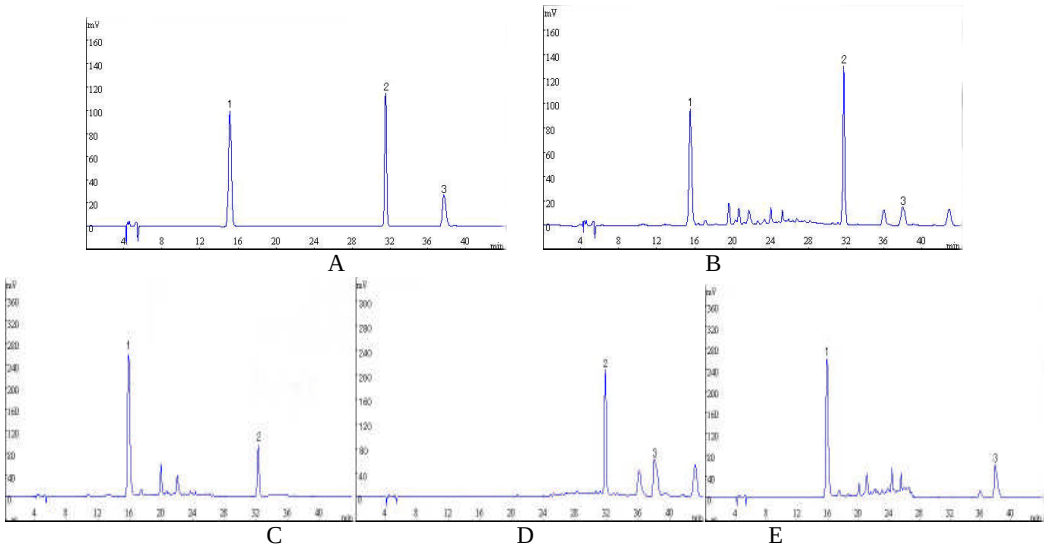

Figure 1: HPLC chromatograms of reference substances (A), samples (B), blank samples without phellodendron amurense Rupr (C), without Acacia catechu (D),without *Scutellaria baicalensis* Georgi (E)

Table 1: Gradient elution mode of mobile phase

Time (min)	Acetonitrile (%)	0.3% phosphoric acid solution (%)
0	10	90
5	10	90
15	18	82

Preparation of reference solutions

Adequate amount of Jiejia tincture powder i.e. catechin, baicalin and berberine were precisely weighed respectively, dissolved with 10% acetonitrile and diluted to volume to prepare into mixed solutions with concentrations of 3 mg/mL, 45 mg/mL and 0.21 mg/mL respectively as the mixed stock solutions of reference substances. A certain volume of mixed reference substances were accurately weighed, stored in volumetric flasks, and added with the mobile phase to dilute into mixed reference solutions with a series of mass concentrations.

Preparation of test solutions

5 g of ground Acacia catechu, *Scutellaria baicalensis* and *Cortex Phellodendri* were weighed respectively, added with 10-fold amount of 80% ethanol, and ultrasonically extracted at 30 □ for 45 min once. The power was 100 kwh. After suction filtration, the filtrates were evaporated to dryness in water bath. Then, a certain amount of the powders was weighed and prepared into a concentration of 2 mg/mL with 10% acetonitrile. Before injection, the samples were filtered with 0.45 μm Millipore membrane, and the test solutions were thereby obtained.

Preparation of negative control solutions

According to the proportion of prescriptions in the test solutions, medicinal materials in addition to the Acacia catechu, *Scutellaria baicalensis* and *Cortex Phellodendri*, were weighed and made into samples. Negative sample solutions were obtained according to the method for preparation of test solution.

Plotting of standard curves

The pre-prepared mixed reference solutions were precisely weighed, where 1.0, 2.0, 4.0, 5.0, 7.0 and 8.0 mL were aspirated respectively and diluted to the volume of 10 mL. After filtration through a 0.45 μm Millipore membrane, 10 μL were taken and injected in accordance with the chromatographic conditions described in 2.1 respectively. Peak areas were

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measured. Standard curves were plotted with peak area A as the ordinate, and mass concentration C as the abscissa to obtain the regression equation of catechin to be A=5140.7C+97290 (r=0.9997); regression equation of baicalin to be A=24579C+38949 (r=0.9995); and regression equation of berberine A=24579C+38949 (r=0.9995), indicating that the catechin, baicalin and berberine were in good linear relationship with the peak areas in the mass concentration ranges of 100~800 μg/mL, 15~120 μg/mL and 7~56 μg/mL respectively.

Precision test

The mixed reference solutions were taken and continuously and repeatedly injected six times to measure the peak areas respectively. The calculation showed that the RSD of peak areas of catechin, baicalin and berberine was 1.41%, 0.78% and 1.52% respectively, indicating that the instruments were of good precision.

Repeatability test

The Jiejia tincture powder of the same batch was accurately weighed in 6 copies, prepared in accordance with the method in 2.3, and injected and measured in accordance with the chromatographic conditions in 2.1. The RSD of peak areas of catechin, baicalin and berberine was calculated to be 1.4%, 1.9% and 2.6% respectively, indicating that this method has good repeatability.

Stability test

The same test solution was taken, injected and measured once every 2 h. The injection was repeated a total of 6 times. Peak areas were measured, and RSD of peak areas of catechin, baicalin and berberine was calculated to be 2.4 %, 1.7% and 1.3% respectively, indicating that the test solution was basically stable within 10 h.

Recovery test

The Jiejia tincture powders were accurately weighed in 6 copies and prepared in accordance with the method in 2.3. Each copy was precisely added with a certain amount of catechin, baicalin and berberine reference stock solutions, and then measured according to the chromatographic conditions in 2.1, followed by calculation of contents. The sample recovery rates (n=6) of catechin, baicalin and berberine were 99.67% (R=1.01%), 98.7% (R=1.93%) and 100.5% (R=2.88%) respectively. The results of recovery test are shown in Table 2.

Table 2: Results of recovery test

Component	Original amount/ μg	Added amount/ Mg	Measured amount/ Mg	Recovery/ %	Average recovery/ %	RSD/ %
	203.3	200	404.39	100.55		
	202.8	200	401.02	99.11		
	201.6	200	399.56	98.98		
Catechin	200.4	200	397.13	98.37	99.67	1.01
	203.7	200	403.88	100.09		
	200.1	200	401.96	100.93		
	77.65	75	158.92	108.36		
	75.89	75	157.35	108.61		
	76.55	75	156.76	106.95		
Baicalin	75.42	75	155.49	106.76	106.7	1.93
	77.31	75	157.52	106.95		
	75.12	75	152.28	102.88		
	15.17	14	29.18	100.07		
	15.42	14	30.08	104.71		
	14.53	14	28.52	99.93	100.5	2.88
Berberine	15.64	14	29.16	96.57		
	14.36	14	28.22	0.9900		
	14.02	14	28.43	1.0293		

Sample determination

The samples were accurately weighed in triplicate respectively, and prepared into solutions according to the method for preparation of test solutions described in 2.3. 10 μL were precisely aspirated and determined under the above chromatographic conditions. The peak areas were recorded, and were then substituted into the regression equations to calculate the catechin, baicalin and berberine contents in the samples. The results are shown in Table 3.

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Discussion

During the experiment, two mobile phase systems of phosphoric acid-methanol (Zhang & Zhou, 2009) and phosphoric acid-acetonitrile (Li, 2004) were investigated. It was found that in the phosphoric acid-methanol system, the peak of berberine was relatively wide and tailing, and the retention time of baicalin was relatively long. While in the phosphoric acid-acetonitrile system, not only can each test component be effectively separated but the retention time was also shortened, and this had the stable, efficient and economical advantages.

Table 3: Determination results of samples (n=3)

Sample ID	Catechin content/ (mg/g)	RSD/%	Baicalin content/ (mg/g)	RSD/ %	Berberine content/ (mg/g)	RSD/ %
1	89.7289±0.0253	1.35	16.3256±0.0156	1.67	4.5733±0.0112	1.55
2	86.8819±0.0172	1.94	16.9610±0.0092	2.01	4.3490±0.0098	1.32
3	87.6386±0.0204	1.73	16.0018±0.0103	1.82	4.145±0.01085	1.47

In the 2010 edition of Pharmacopoeia, the detection wavelength for catechin and baicalin is recorded to be 280 nm, and the detection wavelength for verberine 265 nm. Relevant literatures have reported that baicalin and berberine can be detected simultaneously at 275 nm (Jin et al, 2009); catechin and berberine can be detected simultaneously at 270 nm (Ruan & Yuan, 2010), and catechin and baicalin at 278 nm (Tan et al, 2008). Above all, 200~500 nm were selected for wavelength scanning in this experiment, and the results revealed that catechin, baicalin and berberine had maximum absorptions at 276 nm.

The Jiejia tincture is regarded as a good drug for the treatment of burns, scalds and wounds for its widespread medicinal resource, simple preparation, cost-effectiveness, safety and ease of use. It had once been frequently used in hospitals. However, its active components are unknown, and its quality is difficult to control, bringing about problems in clinical medication. This paper established HPLC method for simultaneous determination of the contents of three effective components in the Jiejia tincture. This method has not been reported previously, and is simple in operation, reliable in results, and can be used in the quality control of Jiejia tincture. At the same time, it also laid the foundation for the safe and rational use of the medicine in the clinic.

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