Simultaneous Determination of Eight Bioactive Constituents in Shensong Yangxin Capsule by UPLC

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Abstract: **Objective** To develop a ultra performance liquid chromatography (UPLC) method for the quality evaluation of Shensong Yangxin Capsule (SYC). **Methods** The Waters Acquity UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm) was used. Acetonitrile and water containing 0.1% phosphoric acid were used as mobile phases of UPLC with gradient elution. The detection wavelengths were set at 203 (ginsenoside Rb_1), 286 (salvianolic acid B), 230 (paeoniflorin), 221 (schisantherin A), 280 (sodium danshensu), 327 (chlorognenic acid), 335 (spinosin), and 345 nm (berberine hydrochloride), respectively. The flow rate was set at 0.4 mL/min and column temperature was 35 ℃. **Results** The contents of paeoniflorin, salvianolic acid B, schisantherin A, sodium danshensu, chlorognenic acid, spinosin, berberine hydrochloride, and ginsenoside Rb_1 were determined from 10 batches of SYC. **Conclusion** The method of the quality evaluation of SYC has acceptable precision, reproducibility, and stability, which could be used as a new method for the quality control of SYC.

Key words: Shensong Yangxin Capsule; simultaneous determination; sodium danshensu; spinosin; ultra performance liquid chromatography

DOI: 10.3969/j.issn.1674-6348.2013.03.006

Introduction

Shensong Yangxin Capsule (SYC), a traditional patent medicine consisting of *Ginseng Radix* et *Rhizoma* (*Renshen*), *Ophiopogonis Radix* (*Maidong*), *Corni Fructus* (*Shanzhuyu*), *Salviae Miltiorrhizae Radix* et *Rhizoma* (*Danshen*), *Ziziphi Spinosae Semen* (*Suanzaoren*), *Taxilli Herba* (*Sangjisheng*), *Paeoniae Radix Rubra* (*Chishao*), *Eupolyphaga* (*Tubiechong*), *Nardostachyos Radix* et *Rhizoma* (*Gansong*), *Coptidis Rhizoma* (*Huanglian*), *Schisandrae Sphenantherae Fructus* (*Nanwuweizi*), and *Os Draconis* (*Longgu*), has been widely used to treat arrhythmias in clinic (Zou *et al*, 2011).

In previous reports, paeoniflorin, salvianolic acid B, schisantherin A, loganin, berberine, and deoxy-schizandrin in SYC have been analyzed by HPLC (Chen, Huang, and Wang, 2010; Xin *et al*, 2011; Chen *et al*, 2010; Xin *et al*, 2011). Unfortunately, these methods could only be used to determine single, two, or four components and to evaluate the quality of Chinese materia medica (CMM). In recent years, ultra performance liquid chromatography (UPLC) has been widely applied to controlling or evaluating the quality of CMM (Zhang *et al*, 2011; 2012), because the UPLC method was faster and produced the data of equal or better quality than the HPLC method (Chen *et al*, 2008).

In this study, UPLC method was developed to establish a method for the determination of multi-components in SYC with good separation and repeatability. Meanwhile, the contents of paeoniflorin, salvianolic acid B, schisantherin A, sodium danshensu, chlorognenic acid, spinosin, berberine hydrochloride, and ginsenoside Rb_1 (Fig. 1) have been determined under the same chromatographic conditions. It could provide more scientific basis for assessing quality of SYC. Up to now, this is the first report on the simultaneous determination of eight bioactive components in SYC.

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Received: February 12, 2013; Revised: April 18, 2013; Accepted: May 12, 2013
Fund: the National Technology Research Program for Creating New Drugs (2011ZX09401-020) and National Basic Research Program of China (973 Program 2012CB518606)
Online time: July 17, 2013 Online website: http://www.cnki.net/kcms/detail/12.11410.R.20130717.1427.004.html

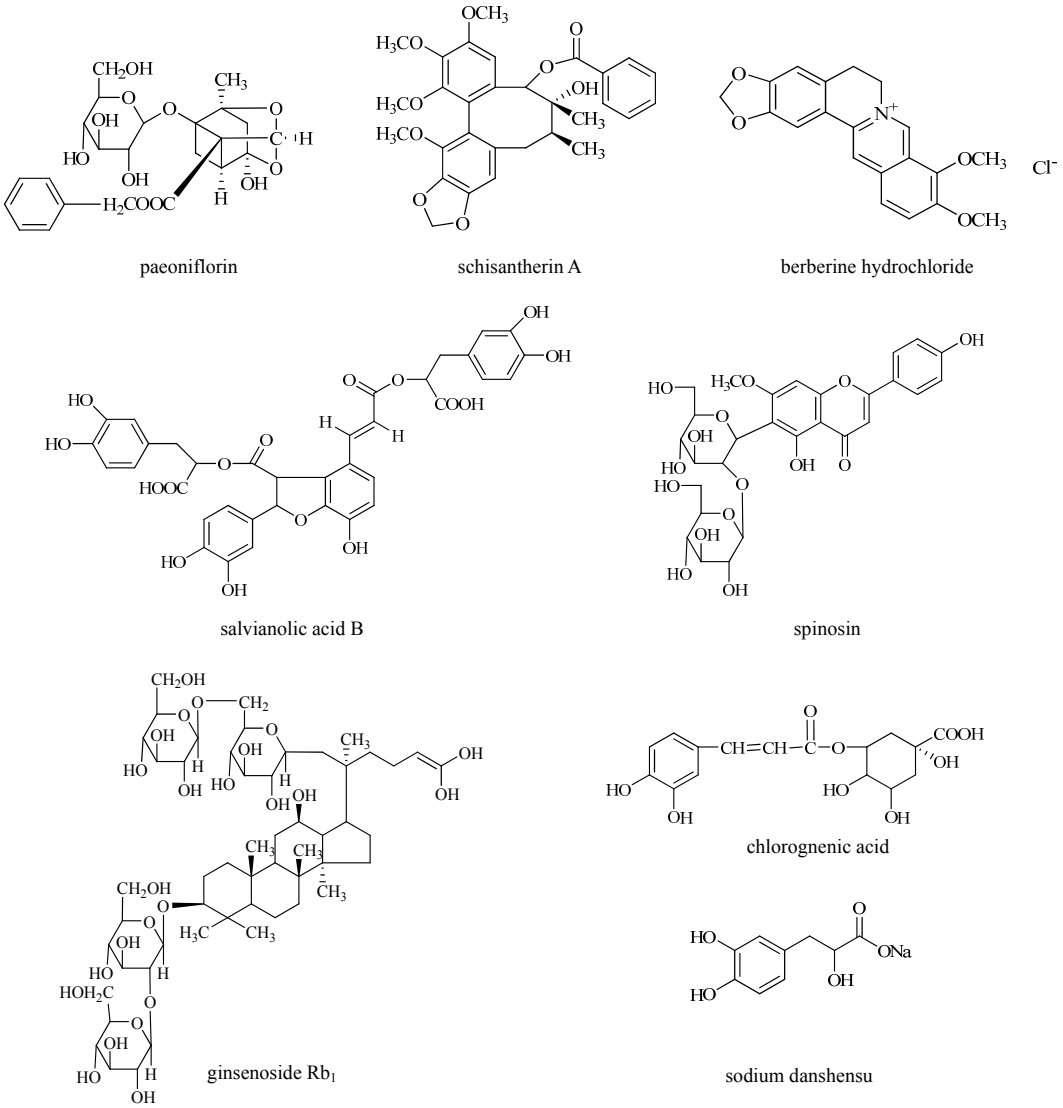

Fig. 1 Chemical structures of eight bioactive constituents

Materials and methods

Instruments

UPLC analysis was carried out with a Waters Acquity UPLC™ H CLASS system (Waters, USA), including high-pressure gradient quaternary pump, auto sampler manager, column compartment, and photo diode array (PDA) detector, connected to Empower 3.0 software. Separation was achieved on an Acquity UPLC™ HSS T3 Column (100 mm× 2.1 mm, 1.8 μm, Waters Corporation, Ireland) coupled with a Acquity UPLC™ HS-S T3 VanGuard™ Pre-column (2.1 mm × 5 mm, 1.8 μm, Waters Corporation, Ireland); KQ300VDB Ultrasonic Instrument (Kunshan, China); Mettler AE240 Analytic Balance (Mettler Toledo, Switzerland); TGL-16G Centrifuge (Shanghai Anting Scientific Instrument Factory, China).

Chromatographic conditions

The mobile phase consisted of acetonitrile (A) and 0.1% aqueous phosphoric acid (B) in a gradient elution mode as follows: 0—3 min, 94% B—89.3% B; 3—3.5 min, 86% B; 3.5—17 min, 74% B; 17—22 min, 60% B; 22—23 min, 50% B; 23—34 min, 20% B. The column temperature was maintained at 35 ℃ and flow rate was 0.4 mL/min. The injection volume was 2 μL. The detection wavelengths were set at 203 nm for ginsengoside Rb_1, 286 nm for salvianolic acid B, 230 nm for paeoniflorin, 280 nm for sodium danshensu, 327 nm for chlorognenic acid, 335 nm for spinosin, 345 nm for berberine hydrochloricde, and 221 nm for schisantherin A.

Chemicals and materials

Salvianolic acid B, paeoniflorin, schisantherin A, chlorognenic acid, spinosin, sodium danshensu,

berberine hydrochloride, and ginsenoside Rb_1 (Batch Nos.: 111562-201111, 110736-201136, 111529-200604, 110753-200413, 111869-201102, 110855-201210, 110713-200911, and 110704-201122) were purchased from National Institute for Food and Drug Control (Beijing, China). Acetonitrile of HPLC grade was from Fisher Chemicals (Pittsburg, USA). Other chemicals of analytical grade were purchased from Beijing Chemical Factory (Beijing, China). Water was purified by using a Milli-Q water purification system (Millipore, USA). Ten batches of SYC were obtained from Shijiazhuang Yiling Pharmaceutical Co., Ltd. (Batch Nos.: 1201005, 1203031, 1101042, 1201002, 1106042, 1107006, 1106047, 1103003, 1111018, and 1109017).

Reference substance solution preparation

Quantitative analysis was carried out using external standard method. The stock solution of salvianolic acid B reference substance (0.1229 mg/mL) was prepared by accurate weighing and dissolving in 100 mL water. The stock solution of paeoniflorin reference substance (64.56 μg/mL) was prepared by accurate weighing and dissolving in 100 mL 25% methanol. The stock solution of schisantherin A reference substance (4.325 μg/mL) was prepared by accurate weighing and dissolving in 500 mL methanol. The stock solution of chlorogenic acid reference substance (5.429 μg/mL) was prepared by accurate weighing and dissolving in 500 mL acetonitrile-0.4%phosphoric (3:97). The stock solution of spinosin reference substance (7.612 μg/mL), sodium danshensu reference substance (41.80 μg/mL), berberine hydrochloride reference substance (100.20 μg/mL), ginsenoside Rb_1 reference substance (10.11 μg/mL) was prepared by accurate weighing and dissolving in 500, 100, 100, 500 mL 80% methanol, respectively.

Sample preparation

Each sample (0.5 g) was accurately extracted with 50 mL of 80% methanol aqueous solution in an ultrasonic water bath for 50 min, allowed to stand to cool. The sample was weighed again and the loss was complemented with 80% methanol aqueous solution, then, it was shaked up and centrifuged at 4500 r/min, and the supernatant was filtrated through a 0.22 μm membrane filter unit.

Results and discussion

Validation of method

Calibration curves Chlorogenic acid reference substance (5.429 μg/mL), spinosin reference substance (7.612 μg/mL), sodium danshensu reference substance (41.80 μg/mL), berberine hydrochloride reference substance (100.20 μg/mL), ginsenoside Rb_1 reference substance (10.11 μg/mL), paeoniflorin reference substance (64.56 μg/mL), salvianolic acid B reference substance (0.1229 mg/mL), and schisantherin A reference substance (4.325 μg/mL) (0.5, 1.0, 2.0, 2.5, 3.0, 4.0 μL) were injected into the UPLC to establish calibration curves, respectively. Eight calibration curves were constructed from peak areas (Y) of the reference standards versus their weight (X) (Table 1).

Table 1 Regression equations and linear range

Reference substances	Regression equation	Correlation coefficients (r)	Ranges / μg
paeoniflorin	Y = 3 673 578.9963 X−6973.2300	0.9999	0.0323—0.2582
salvianolic acid B	Y = 1 651 330.0244 X−2593.3300	1.0000	0.0615—0.4916
schisantherin A	Y = 14 176 767.6301 X + 2542.0400	0.9999	0.0022—0.0173
chlorogenic acid	Y = 6 163 805.5353 X−905.0100	1.0000	0.0027—0.0220
spinosin	Y = 6 025 775.0920 X + 1881.2000	0.9996	0.0038—0.0304
sodium danshensu	Y = 1 483 666.5072 X−962.7300	1.0000	0.0209—0.1672
berberine hydrochloride	Y = 9 045 361.8762 X−21 797.2300	1.0000	0.0501—0.4008
ginsenoside Rb_1	Y = 616 104.8467 X + 27.8900	0.9998	0.0051—0.0404

Precision test The stock solutions of chlorogenic acid reference substance (5.429 μg/mL), spinosin reference substance (7.612 μg/mL), sodium danshensu reference substance (41.80 μg/mL), berberine hydrochloride reference substance (100.20 μg/mL), ginsenoside Rb_1 reference substance (10.11 μg/mL), paeoniflorin reference substance (64.56 μg/mL), salvianolic acid B reference substance (0.1229 mg/mL), and schisantherin A reference substance (4.325 μg/mL) were measured by UPLC for five times in a day. The RSD values of five successive peak areas of chlorogenic acid (327 nm), spinosin (335 nm),

sodium danshensu (280 nm), berberine hydrochloricde (345 nm), ginsenoside Rb_1 (203 nm), paeoniflorin (230 nm), salvianolic acid B (286 nm), and schisantherin A (221 nm) were 0.22%, 0.18%, 0.19%, 0.41%, 0.21%, 0.35%, 0.31%, and 0.49%, respectively. The results showed that the method had a good precision.

Stability tests The sample (1201005) solution was measured by UPLC at 0, 6, 12, 24, 36, and 48 h. The RSD values of the peak areas of chlorogenic acid (327 nm), spinosin (335 nm), sodium danshensu (280 nm), berberine hydrochloricde (345 nm), ginsenoside Rb_1 (203 nm), paeoniflorin (230 nm), salvianolic acid B (286 nm), and schisantherin A (221 nm) were 0.98%, 0.87%, 1.23%, 1.41%, 1.12%, 1.54%, 1.43%, and 1.67%, respectively. The results showed that the sample solution was stable at 48 h.

Repeatability tests The repeatability was assessed by analyzing six independently prepared samples (1201005). The RSD values of the peak area of chlorogenic acid (327 nm), spinosin (335 nm), sodium danshensu (280 nm), berberine hydrochloricde (345 nm), ginsenoside Rb_1 (203 nm), paeoniflorin (230 nm), salvianolic acid B (286 nm), and schisantherin A (221 nm) were 1.02%, 0.89%, 0.65%, 1.06%, 0.85%, 1.13%, 0.76%, and 0.98%, respectively. The results showed that the repeatability was good.

Recovery tests The recovery assays of paeoniflorin, salvianolic acid B, and schisantherin A were carried out by adding the reference substances to the sample powders (six copies, 0.25 g per copy, 1201005), which were treated according to the procedure of the sample preparation described above. As shown in Table 2, the recoveries of chlorogenic acid (327 nm), spinosin (335 nm), sodium danshensu (280 nm), berberine hydrochloricde (345 nm), ginsenoside Rb_1 (203 nm), paeoniflorin (230 nm), salviaolic acid B (286 nm), and schisantherin A (221 nm) were 101.08% (RSD = 2.20%), 99.83% (RSD = 2.14%), 100.67% (RSD = 2.24%), 99.83% (RSD = 1.94%), 100.42% (RSD = 1.77%), 99.80% (RSD = 1.74%), 100.58% (RSD = 1.18%), and 100.78% (RSD = 1.53%), respectively.

Table 2 Recovery of eight ingredients for quantitative analysis (n = 6)

Compounds	Original / mg	Spiked / mg	Found / mg	Average recovery / %	RSD / %
chlorogenic acid	0.17	0.2	0.372	101.08	2.20
spinosin	0.06	0.1	0.160	99.83	2.14
sodium danshensu	0.94	1.0	1.947	100.67	2.24
berberine hydrochloride	0.95	1.0	1.948	99.83	1.94
ginsenoside Rb_1	0.17	0.2	0.371	100.42	1.77
paeoniflorin	0.84	0.85	1.688	99.80	1.74
salvianolic acid B	2.66	2.60	5.275	100.58	1.18
schisantherin A	0.11	0.15	0.261	100.78	1.53

Simultaneous determination of eight bioactive components

The sample was prepared by sample preparation item and determinated by UPLC under chromatographic condition item (Fig. 2). The contents of sodium danshensu, chlorognenic acid, paeoniflorin, spinosin, salvianolic acid B, berberine hydrochloride, ginsenoside Rb_1, and schisantherin A were calculated by external standard method (Table 3).

Conclusion

In the present study, the influence of extraction solvents, extraction solvent amount, and extraction time were evaluated. The analysis conditions including formic acid system, phosphoric acid system, eluant proportion, and detection wavelength were evaluated.

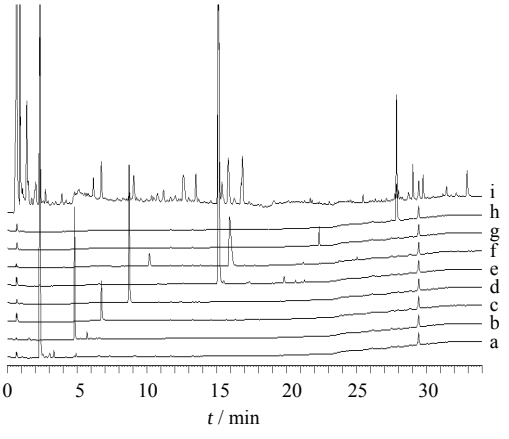

Fig. 2 UPLC chromatograms of SYC (i:1106042) and reference substances

a: sodium danshensu b: chlorognenic acid c: paeoniflorin d: spinosin e: salvianolic acid B f: berberine hydrochloride g: ginsenoside Rb_1 h: schisantherin A

Table 3 Determination of eight bioactive constituents in 10 batches of SYC

Batch No.	Contents / ($mg \cdot g^{-1}$)							
	Paeoniflorin	Salvianolic acid B	Schisantherin A	Sodium danshensu	Chlorognenic acid	Spinosin	Berberine hydrochloride	Ginsenoside Rb_1
1101042	3.39	11.52	0.34	3.79	0.32	0.26	3.45	0.79
1103003	3.15	10.31	0.38	3.94	0.29	0.26	3.79	0.77
1106042	3.09	11.38	0.30	3.09	0.30	0.32	3.33	0.56
1106047	3.22	12.95	0.27	3.53	0.28	0.26	3.35	0.64
1107006	3.65	11.27	0.25	3.19	0.31	0.28	3.04	0.56
1109017	2.99	10.20	0.45	3.56	0.35	0.27	3.22	0.67
1111018	3.09	9.57	0.52	3.49	0.55	0.28	3.43	0.72
1201002	3.07	10.00	0.42	3.54	0.52	0.28	3.70	0.67
1201005	3.37	10.65	0.45	3.74	0.67	0.25	3.81	0.66
1203031	3.10	9.25	0.40	3.87	0.56	0.27	3.51	0.83

In summary, a specific, rapid, and reliable UPLC method was successfully developed for the quantification of sodium danshensu, chlorognenic acid, paeoniflorin, spinosin, salvianolic acid B, berberine hydrochloride, ginsenoside Rb_1, and schisantherin A in SYC. This assay could be readily utilized as a suitable method for more complete and stringent quality control of SYC. As a result of this experiment only using a diode array detector, the components of no UV absorption could not be detected. More comprehensive analysis should be done with evaporative light scattering detector or mass spectrometry in future experiments.

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