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A Universal HPLC Method for the Determination of Phenolic Acids in Compound Herbal Medicines

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A universal method to separate and quantify 13 phenolic acids (gallic acid, chlorogenic acid, gentsic acid, vanillic acid, caffeic acid, syringic acid, sinapic acid, *p*-coumaric acid, ferulic acid, anisic acid, rosmarinic acid, salicylic acid, and cinnamic acid) in some compound herbal medicines was established by liquid chromatographic (HPLC). On an Agela XBP-C18 (5 μm, 4.6 mm $\times$ 150 mm) column, a multistep binary gradient elution program and a simplified sample pretreatment approach were used in the experiment. For all of the phenolic acids, detection limits ranged around 0.01 mg/L. Linear ranges of higher than 2 orders of magnitude were obtained with a correlation coefficient of 0.9991 to 1. Repeatability was 0.39−2.24% (relative standard deviation, RSD) for intraday, 1.17−3.96% (RSD) for interday, and 0.14−5.33% (RSD) for drug sample analysis. Recovery, tested by a standard addition method, ranged from 83.3% to 104.9% for various trace phenolic acids.

KEYWORDS: Phenolic acid; compound herbal medicines; traditional Chinese medicine (TCM); high-performance liquid chromatography.

1. INTRODUCTION

Phenolic acids, known as a kind of multipurpose bioactive agent, frequently occur in herbal plants (*1*, *2*). Besides the pharmacological actions of antibacterial and antifungal activity of most species, phenolic acids were considered in recent years as potentially protective compounds against cancer and heart disease, in part because of their potent antioxidative properties (*3*−*5*). Furthermore, the research regarding their effect on signal transduction and gene expression has progressed in the past decade (*6*, *7*).

Concentrated on the remarkable therapeutic and health protection effects of phenolic acids, much literature focused on the analytical methods for phenolic acids in various kinds of food or other natural sources. Some elaborate reviews summarized those publications (*8*−*10*). Most of the analytical approaches were typically based on reversed-phase high-performance liquid chromatography (RP-HPLC) method. For example, Robbins and Bean developed a procedure to separate and determine 16 different phenolic compounds (including 12 natural phenolic acids, three aromatic aldehydes, and an internal standard) in wine by using HPLC with diode array detection (DAD) (*11*). Similarly, Mattila and Kumpulainen reported a method for the determination of 11 different phenolic acids and positional isomers of hydroxybenzoic acid and coumaric acid in plant-derived foods (*12*).

On the other hand, herbal drugs, particularly time-honored traditional Chinese medicines (TCMs), have become more and more popular in the world. With the development of advanced analytical techniques, the modernization of traditional medicine has become a hot area in recent years, and more and more herbal medicines have been increasingly accepted in western countries. The separation and determination of the active components in medicinal plant extracts represent an advisable method to achieve standardization and quality control of TCMs. Some separation methods of a few individual phenolic acids in corresponding natural plant drugs appear mature and have been summarized in a pertinent review (*13*). But the research on a universal method for the quantitation of multifarious phenolic acids in compound herbal drugs has not been developed. Hence, this work is an attempt to establish a universal method that can be used as a quality control procedure on antibacterial, antiinflammatory, and some other kinds of compound TCMs.

The aim of this study is to establish a procedure for the analysis of 13 phenolic acids (gallic acid, chlorogenic acid, gentsic acid, vanillic acid, caffeic acid, syringic acid, sinapic acid, *p*-coumaric acid, ferulic acid, anisic acid, rosmarinic acid, salicylic acid, and cinnamic acid) in TCM samples. Compound herbal drug samples used in the experiment are two kinds of TCMs: Yinhuang powder and Chenxiang-Shuqi pill. Yinhuang powder comprises Huangqin (root of *Scutellaria baicalensis* Georgi.) and Jinyinhua (flower of *Lonicera japonica* Thunb.) (*14*). Chlorogenic acid contained abundantly in Jinyinhua gives Yinhuang powder the antibiosis and antiinflammation activity. In contrast, Chenxiang-Shuqi pill, which consists of 15 kinds of medicinal herbs, is a prescription often used for treating patients with dyspepsia, abdominal pain, and hyperchlorhydria (*14*). Phenolic acids in Chenxiang-Shuqi pill were at trace concentrations but have their therapeutic effect with other components integrally. In this study, 13 phenolic acids in these drugs were successfully separated and determined, and the optimization of analytical conditions was also discussed.

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2. EXPERIMENTAL PROCEDURES

2.1. Reagent and Materials. Sinapic, rosmarinic, *p*-coumaric, gentsic, and syringic acid were purchased from Fluka (Buchs, Switzerland). Vanillic, *trans*-cinnamic, and ferulic acid were from Acros (Geel, Belgium). Gallic, chlorogenic, caffeic, anisic, and salicylic acid were provided by the Institute of Medicine Plant Development (Beijing, P. R. China).

Trifluoroacetic acid (TFA), purchased from Acros (Geel, Belgium), was of analytical grade. Methanol of HPLC grade was purchased from Fisher (Fairlawn, NJ). Deionized water (10^{-18} Ω) was repurified by using an Aquapro purification system (Aquapro, Chongqing, China).

Chenxiang-Shuqi pill was produced by Tongrentang co., Ltd (Beijing, China). Yinhuang powder was from Jimin Pharmaceutical Company (Jiangxi, China).

2.2. Sample Preparation. For Chenxiang-Shuqi pill and Yinhuang powder, 1 g of each desiccated drug sample was triturated and dissolved in a measuring flask in methanol/water/TFA (50:50:0.1) mixed solvent, and the volume of the turbid fluid was adjusted to 10 mL accurately. The mixture was sonicated (B3200S-T, Branson, China) for 30 min at room temperature, then centrifuged at 3000 rpm for 5 min, and filtered through a 0.45 μm nylon filter (Agilent, Palo Alto, CA) to obtain a clear solution of hydrophilic constituents. For the analysis of chlorogenic acid, 50 mg of desiccated Yinhuang powder sample was extracted by the aforementioned procedure. All of the solution was injected into the HPLC system directly for the analysis.

2.3. Preparation of Standard Solutions and Method Validation. *2.3.1. Standard Solutions and Calibration Curves.* For quantitation, an external standard method was utilized. Peak areas from the HPLC chromatogram were plotted against the known concentrations of stock solutions at varying concentrations. Equations generated by linear regression were used to establish concentrations for herbal medicines and standard solutions.

About 10 mg of a standard of each kind of phenolic acid weighed accurately was dissolved into a 10 mL volumetric flask in 1:1 MeOH/water to obtain stock solutions.

For calibration curves, the stock solution was diluted with 1:4 MeOH/water to obtain the concentration sequence. The linear range and the equations of linear regression were obtained through such a sequence of 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, and 0.05 mg/L. Mean areas ($n = 6$) generated from the standard solutions were plotted against concentration to establish calibration equations.

2.3.2. Detection Limits. For the evaluation of detection limits, all the phenolic acids were dissolved separately in water at the concentration of 100 mg/L as stock solutions, and a concentration sequence of 50, 25, 10, and 5 mg/L of the standards was obtained by diluting these stock solutions. The mean value of the signal-to-noise ratio ($n = 4$) generated from the solution that just caused more than 3 times S/N ratio was used to calculate the detection limit (based on S/N = 3) of the corresponding phenolic acid.

2.3.3. Repeatability. The standard solutions of 1 mg/L, which were near the concentration of those constituents in the two kinds of TCM, were used to achieve repeatability testing for intraday and interday ($n = 5$). The data used to calculate relative standard deviation (RSD) percent of interday repeatability was the mean value of three injections in succession. And the repeatability of peak area of drug extract was also validated in the experiment.

2.3.4. Recovery. A standard additional method was utilized to assess recovery behavior. For the recovery at low concentration, stock solutions that amounted to 200 mg standards were added into 10 g of desiccated and triturated Chenxiang-Shuqi sample. After evaporation of solvent, the solid sample was extracted with 100 mL of solvent using the method in section 2.2. For the recovery of the main constituent, chlorogenic acid, in Yinhuang powder, stock solution containing 200 mg of chlorogenic acid was added into 0.5 g of desiccated and triturated Yinhuang powder sample and, after evaporation of solvent, was extracted with 100 mL ofsolvent by the method in section 2.2.

2.4. Apparatus and Conditions. The separation of phenolic acids was performed with an Agilent 1100 series HPLC system equipped with on-line degasser (G 1322A), quatpump (G 1311A), autosampler (G 1313A), column heater (G 1316A), and photodiode array detector (G 1315A). Instrument control and data analysis was carried out using Agilent HPLC Chemstation 10.1 editon through Windows 2000.

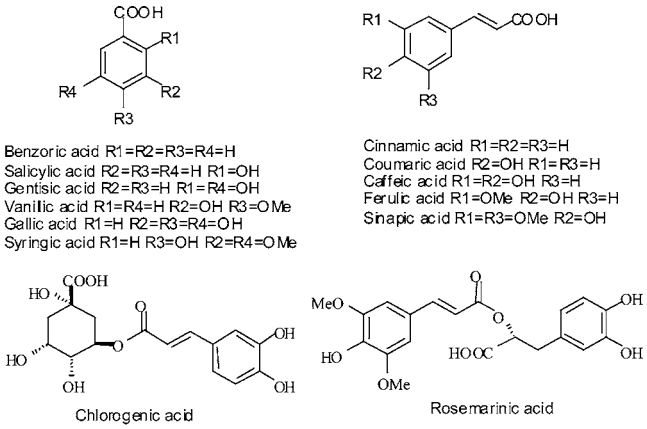

Figure 1. The structures of 13 phenolic acids.

Five kinds of C18 and one kind of C8 columns were utilized for the initial attempt: Agela XBP-C18 (5 μm, 4.6 mm × 150 mm, Agela, Newark, DE), Agela XBP-C18 (5 μm, 4.6 mm × 250 mm, Agela), Zorbax Extend-C18 (5 μm, 4.6 mm × 150 mm, Agilent), Waters Puresil C18 (5 μm, 4.6 mm × 150 mm, Waters, Milford, MA), Zorbax Hypersil-ODS (5 μm, 4.0 mm × 250 mm, Agilent), and Zorbax Eclipse XDB-C8 (5 μm, 4.6 mm × 150 mm, Agilent). During some tentative isocratic and plain gradient elution procedures, considering the tradeoff between separation efficiency and time, the Agela XBP-C18 column (5 μm, 4.6 mm × 150 mm, Agela) was chosen for succeeding optimization.

The flow rate of the mobile phase was kept at 0.5 mL/min. Mobile phase A was water containing 0.02% TFA, and phase B was methanol containing 0.02% TFA.The gradient conditions were as follows: 0−5 min, 25% B; 5−10 min, 25−30% B; 10−16 min, 30−45% B; 16−18 min, 45% B; 18−25 min, 45−80% B; 25−30 min, 80% B; 30−40 min, 80−25% B. The temperature of column was controlled at 25 °C. Injection volume was 10 μL. The detection wavelengths of DAD were set at four selected positions: 254, 275, 305, and 320 nm. Prior to each run, the HPLC-DAD system was allowed to warm, and the baseline was monitored until it was stable before sample analysis.

3. RESULTS AND DISCUSSION

3.1. Optimization of Analytical Conditions. All of the structures of phenolic acids are indicated in **Figure 1**. A chromatogram demonstrating the separation of 13 phenolic acids is shown in **Figure 2**, and retention times of the analytes are listed in **Table 1** with their repeatability. **Figure 3** shows typical chromatograms of two real samples, showing that the resolution is satisfactory. The concentration of mixed standard solution was about 10 mg/L for each phenolic acid.

Table 1. Retention Time and RSD% of Phenolic Acid Standards ($n = 5$)

analyte	retention time (min)	RSD% ($n = 5$)	analyte	retention time (min)	RSD% (interday)
gallic acid	4.69	0.56	*p*-coumaric acid	21.01	0.08
chlorogenic acid	11.27	0.19	ferulic acid	21.34	0.08
gentsic acid	13.97	0.16	anisic acid	25.31	0.04
vanillic acid	15.62	0.14	rosmarinic acid	26.16	0.04
caffeic acid	16.10	0.15	salicylic acid	26.77	0.04
syringic acid	16.52	0.12	cinnamic acid	28.32	0.02
sinapic acid	20.75	0.09			

Typically, sulfuric, phosphoric, formic, acetic, and trifluoroacetic acids are additives in the mobile phase for the analysis of phenolic acids to suppress ionization in reversed-phase HPLC. Considering the pK_a's of formic acid (3.75) and acetic acid (4.76), which are both higher than those of some analytes

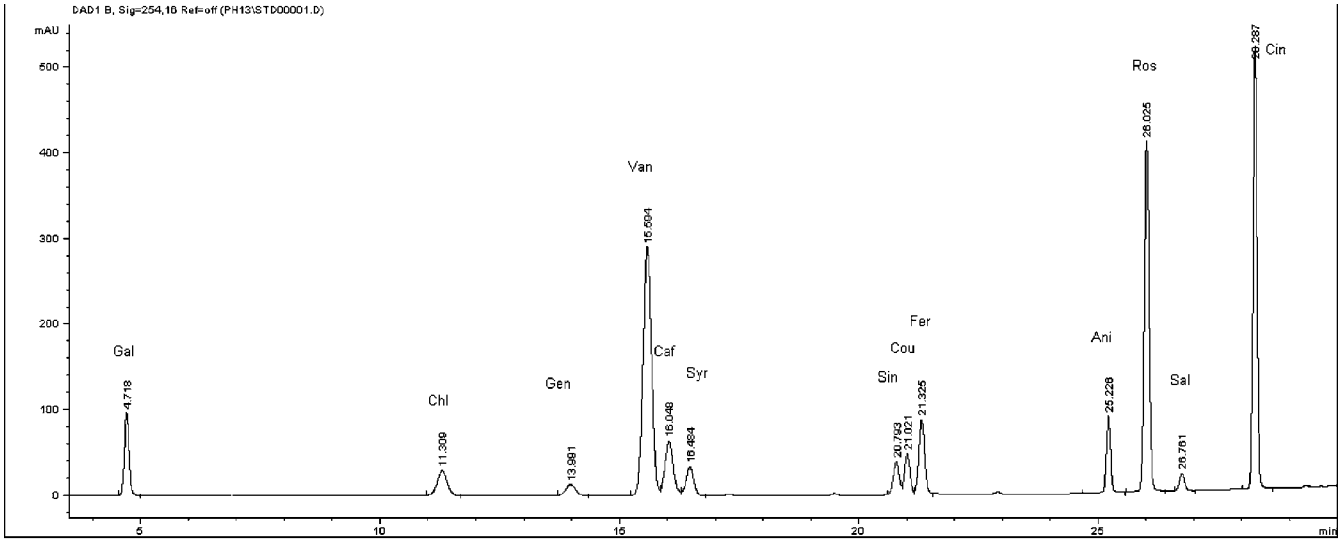

Figure 2. Typical chromatogram of 13 phenolic acid standards. Separation conditions were as follows: column, Agela XBP-C18 (4.6 mm × 150 mm, 5 μm); mobile phase A = water containing 0.02% TFA; mobile phase B = methanol containing 0.02% TFA. Gradient program was as follows: 0–5 min, 25% B; 5–10 min, 25–30% B; 10–16 min, 30–45% B; 16–18 min, 45% B; 18–25 min, 45–80% B; 25–30 min, 80% B; 30–40 min, 80–25% B. Flow rate was 0.5 mL/min; temperature was 25 °C; injection volume was 10 μL; detection was at 254 nm. Peaks are indicated as follows: Gal = gallic acid; Chl = chlorogenic acid; Gen = gentsic acid; Van = vanillic acid; Caf = caffeic acid; Syr = syringic acid; Sin = sinapic acid; Cou = *p*-coumaric acid; Fer = ferulic acid; Ani = anisic acid; Ros = rosmarinic acid; Sal = salicylic acid; Cin = *trans*-cinnamic acid.

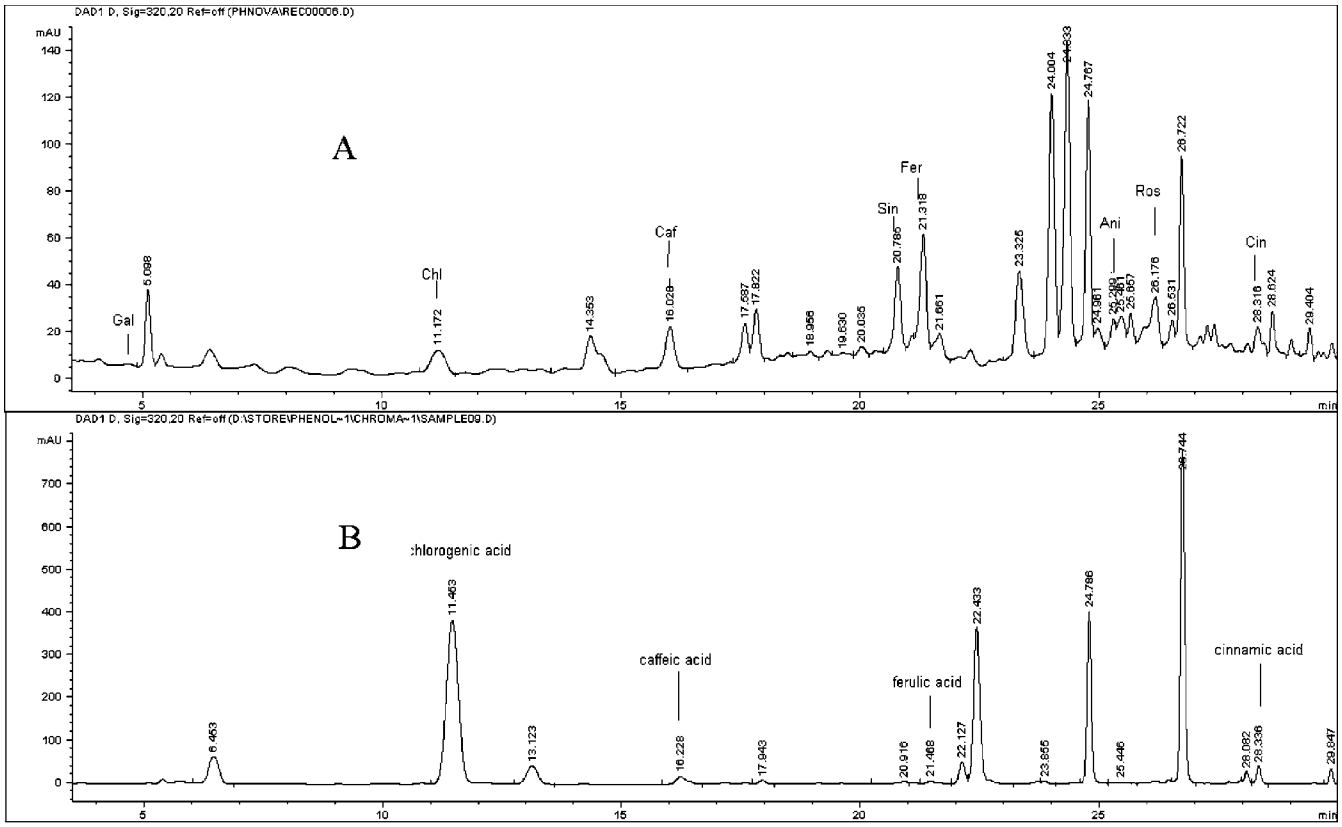

Figure 3. Chromatograms of TCM samples: (**A**) Chenxiang-Shuqi pill; (**B**) Yinhuang powder. Signal was collected at 320 nm. Separation conditions are the same as those in Figure 2 with the exception of detection at 320 nm.

(particularly salicylic acid, pK_a = 2.98) and the inaptness of injecting nonvolatile inorganic anions to the MS detector, TFA was chosen to be the preferred additive for the experiments. Meanwhile, in view of the potential for detection of the analytes by LC-ESI-MS in negative mode, formic acid was also tried as an additive for eliminating the interference from TFA anion. Both 0.02% (v/v) TFA and 0.05% (v/v) formic acid were added into both water and methanol, resulting in symmetric peaks.

The gradient program of the elution was optimized at 25 °C, and the results indicated that with use of the gradient described in section 2.4, 13 analytes can be baseline separated within 30 min. As for column temperature, that was tested from 15 to 40 °C (5 °C increment); either higher (≥30 °C) or lower (15 °C) temperature can cause a change in selectivity, leading to the coelution of several analytes (peaks 4 and 5 and 6, 8, and 9 at high temperature; peaks 3 and 4 at low temperature). The

Table 2. Detection Limit and Linear Relation of 10 Phenolic Acids

acid	detection limit (mg/L)	linear range (mg/L)	equation of linear regression (concn–peak area)	r^2	equation of linear regression (log–log)	r^2 (log–log)
gallic acid	0.012	0.096–48	$y = 62.566x + 5.8199$	0.9997	$y = 0.9957x + 1.8104$	0.9996
chlorogenic acid	0.018	0.049–49	$y = 46.523x - 0.6877$	1	$y = 0.9893x + 1.6738$	0.9999
gentsic acid	0.020	0.21–52	$y = 23.831x + 0.7035$	0.9999	$y = 1.0025x + 1.3774$	0.9995
vanillic acid	0.013	0.050–50	$y = 75.364x + 0.2242$	0.9999	$y = 0.9853x + 1.8769$	0.9997
caffeic acid	0.007	0.048–48	$y = 91.806x + 7.5795$	1	$y = 0.9945x + 1.9795$	0.9988
syringic acid	0.018	0.051–20.2	$y = 61.509x - 0.9518$	0.9999	$y = 0.9847x + 1.7971$	0.9998
sinapic acid	0.012	0.051–20.2	$y = 62.234x - 0.4684$	1	$y = 1.0008x + 1.7911$	1
p-coumaric acid	0.006	0.048–48	$y = 139.76x - 2.0619$	0.9999	$y = 1.0262x + 2.1178$	0.9996
ferulic acid	0.007	0.048–48	$y = 97.604x + 0.6081$	1	$y = 0.9895x + 2.0014$	0.9999
anisic acid	0.006	0.050–20	$y = 139.94x + 1.5979$	0.9999	$y = 0.9551x + 2.185$	0.9994
rosmarinic acid	0.015	0.105–52.5	$y = 44.266x - 0.6832$	0.9999	$y = 1.0243x + 1.616$	0.9999
salicylic acid	0.021	0.192–49	$y = 30.699x + 3.1897$	0.9991	$y = 0.9942x + 1.5061$	0.9995
cinnamic acid	0.010	0.050–50	$y = 194.81x + 3.4951$	0.9999	$y = 0.997x + 2.2898$	0.9996

Table 3. Intraday and Interday Precision of Phenolic Acids (Based on the Peak Areas of 1 mg/L Samples, $n = 5$)

analyte	RSD% (intraday)	RSD% (interday)	analyte	RSD% (intraday)	RSD% (interday)
gallic acid	0.45	1.32	*p*-coumaric acid	1.25	2.75
chlorogenic acid	2.05	2.18	ferulic acid	1.61	2.57
gentsic acid	2.24	3.30	anisic acid	0.39	1.64
vanillic acid	1.01	1.96	rosmarinic acid	0.86	2.43
caffeic acid	0.73	2.62	salicylic acid	2.10	3.96
syringic acid	0.78	2.09	cinnamic acid	0.60	1.17
sinapic acid	1.14	1.87			

separation efficiency stayed the same from 20 to 25 °C, but the analytical time at 25 °C was shorter than that at 20 °C. To abridge analysis time, 25 °C was selected for subsequent experiments.

Determination of the analytes was based on peak area. Because more than a hundred kinds of ultraviolet absorptive organic components would be contained in a compound herbal drug, which may interfere with the analysis of these phenolic acids, choosing suitable detection wavelengths should be seriously considered. Three rules were followed in our study: first, UV detection wavelength should be set at the maximum absorbance wavelength or absorbance band of each phenolic acid; second, higher wavelength was preferred to obtain clear chromatograms for phenolic acids; third, for the simplification of data processing, detection wavelengths for different analytes were merged as much as possible. The conditions were selected as follows: 254 nm for anisic and vanillic acid, 275 nm for gallic, *trans*-cinnamic, and syringic acid, 305 nm for salicylic acid, and 320 nm for chlorogenic, gentsic, caffeic, sinapic, *p*-coumaric, ferulic, and rosmarinic acid.

3.2. Method Validation. Equations of linear regression relating the concentration to peak area listed in **Table 1**, together with their correlation coefficients for the determination of phenolic acids, indicate that the linearity is acceptable.

In TCMs, particularly in various compound herbal drugs, the concentrations of different phenolic acids vary dramatically. Therefore a calibration curve was established in a range of more than 3 orders of magnitude for each acid, and both unweighted linear regression and log−log plot equations were generated to validate the linearity of the calibration curve, showing that the correlation coefficients (r^2) ranged from 0.9991 to 1 for the unweighted linear regression and from 0.9988 to 1 for log−log plot equations. Additionally, the detection limit of each analyte was calculated on the basis of three times of signal-to-noise, which is also listed in **Table 2**. For most of the analytes, detection limits were around 0.01 mg/L, and the limits of quantitation were around 0.1 mg/L.

Table 4. Concentration of 10 Phenolic Acids in TCM Samples and Its Precision

sample name	Yinhuang powder (μg/g)	RSD% ($n = 5$)	Chenxiang-Shuqi bolus (μg/g)	RSD% ($n = 5$)
gallic acid	*a*		4.11	2.12
chlorogenic acid	5554	0.15	27.05	0.74
gentsic acid	*a*		*a*	
vanillic acid	18.21	1.96	6.85	2.11
caffeic acid	106.9	1.92	5.01	2.31
syringic acid	*b*		6.83	4.57
sinapic acid	3.36	5.33	13.74	2.90
p-coumaric acid	7.71	3.76	2.36	3.89
ferulic acid	20.44	1.19	25.24	0.52
anisic acid	6.11	0.98	1.71	3.92
rosmarinic acid	*a*		15.80	2.34
salicylic acid	*a*		*a*	
cinnamic acid	173.7	0.14	6.08	1.76

[a] Not detected. [b] Trace.

Repeatability of the method was evaluated by the intraday and interday data from standards and the intraday data from some real extract samples including Chenxiang-Shuqi pill and Yinhuang powder, in which the concentrations of trace phenolic acids were around 1 mg/L (see **Table 4**). Three injections were carried out for each sample. As illustrated in **Table 3**, for standards, the repeatability of intraday analysis ranged from RSD 0.39% to 2.24% ($n = 5$), and interday analysis ranged from RSD 1.17% to 3.96% ($n = 5$). For real drug samples, RSD of 0.14−5.33% were obtained for trace constituents (see also **Table 4**). In addition, chlorogenic acid, a main bioactive constituent in Yinhuang powder, exhibited the repeatability of RSD 0.15% ($n = 5$), which was obviously better than that of any other trace

Table 5. Recovery of Phenolic Acids in Chenxiang-Shuqi Pill

acid	recovery (%)	mean value (%)	RSD%
gallic acid	86.46, 96.92, 98.04, 89.91	92.8	6.00
chlorogenic acid	101.13, 97.65, 100.40, 94.37	98.4	3.12
gentsic acid	86.19, 87.36, 88.13, 83.25	86.2	2.48
vanillic acid	104.10, 106.61, 105.38, 103.42	104.9	1.34
caffeic acid	91.79, 95.13, 94.57, 87.93	92.4	3.56
syringic acid	91.46, 87.72, 89.36, 83.90	88.1	3.63
sinapic acid	81.22, 92.02, 84.15, 78.17	83.9	7.09
p-coumaric acid	99.44, 106.46, 105.11, 101.10	103.0	3.20
ferulic acid	90.27, 102.55, 99.81, 93.53	96.5	5.83
anisic acid	102.23, 100.91, 104.29, 97.67	101.3	2.74
rosmarinic acid	96.21.105.00,101.17,95.76	99.5	4.41
salicylic acid	90.07, 95.34, 91.53, 84.21	90.3	5.11
cinnamic acid	82.61, 83.03, 84.84, 82.63	83.3	1.27

compound. Hence, a preeminent precision for a highly concentrated constituent could be expected by using this analytical approach.

3.3. Analysis of TCM Samples. It was commonly apparent that the complexity of natural products made it extremely difficult to separate and determine the active constituents in crude drugs and their medical preparations. To identify the attribution of peaks, three criteria were adopted: retention time, ultraviolet absorption spectrum, and observation of an increase of peak area after the addition of the corresponding phenolic standard. A schematic chromatogram is demonstrated in **Figure 3**. Because of the low concentrations of phenolic acids in Chenxiang-Shuqi pills, the chromatogram (**Figure 3B**) was obtained by adding suitable amounts of standards to make the peaks more evident. **Figure 3A** is a chromatograph of Yinhuang powder extract without any modification.

Concentration and RSD% of phenolic acid contained in the two kinds of TCMs are listed in **Table 4**. Each concentration is a mean value of five parallel analyses.

3.4. Recovery. In most of the literature related to the analysis of phenolic acids, liquid−liquid extraction (with or without pH adjustment) was applied as a typical approach for food analysis. In general, when ethyl acetate or diethyl ether was used as extractant, recovery of various phenolic acids ranged from 80% to 100% (*11*, *12*, *15*−*17*). Though liquid−liquid extraction is a mature method for sample pretreatment in this case, some disadvantages, such as tough procedure, laborious workload, wastage of time and solvent, should be noticed.

After a series of tentative analyses using similar extract approaches as reported in the aforementioned literature, the concentrations of phenolic acids in real samples can be successfully determined by DAD without enrichment; as a result, a simple and convenient extract procedure (as described in section 2.2) was adopted.

Evaluation of the performance of this extract method resorted to the standard addition method. Chenxiang-Shuqi pill was used as a substrate for recovery test of trace phenolic acid, while Yinhuang powder was used to validate the recovery of a main constituent, chlorogenic acid. As mentioned in section 2.3.4, the amounts of added standard (equivalent to 2 mg/L) were approximated to the concentration of the corresponding phenolic acids. The recoveries of trace phenolic acids, laid out in **Table 5**, ranged from 83.3% to 104.9%. For Yinhuang powder, the mean value of recovery of chlorogenic acid was 97.1% (89.25%, 95.57%, 104.16%, 99.44%).

3.5. Conclusion. We have established a universal reversed-phase HPLC method for the separation and determination of phenolic acids, widely contained in compound herbal drugs, particularly in traditional Chinese medicines. The method is based on an Agela XBP-C18 column (150 mm × 4.6 mm, 5 μm) with a binary multistep gradient program of methanol and water, each containing 0.02% TFA. Thirteen phenolic acids were separated by this method. Detection limits ranged from 60 to 210 ng for different phenolic acids. External calibration was employed for all acids with the linear range from 0.02 to 50 mg/L. Squares of correlation coefficients were in the range of 0.9991−1 (concentration−peak area), and 0.9988−1 (log). The repeatability of the method was 0.39−2.24% (RSD) for intraday, 1.17−3.96% (RSD) for interday, and 0.14−5.33% (RSD) for real drug sample analysis.

A facile extraction procedure without liquid−liquid extraction was adopted in the experiment, resulting in sample pretreatment time of less than 1 h. Acceptable recoveries for phenolic acids (ranging from 83.3% to 104.9%) were achieved. It is indicated that the extraction procedure is suitable for most of the hydrophilic dosage forms of herbal drugs.

For Yinhuang powder, pharmacological effect of antibacterial activity and antiinflammation is directly related to the concentration of chlorogenic acid. The aforementioned analytical procedure provides a universal method for quality control and standardization of herbal drugs.

For Chenxiang-Shuqi pill, all kinds of phenolic acids are trace constituents (**Table 4**), but they can act as references for the identification of other chromatography peaks and can be used for the confirmation of similarity between fingerprintings of standards and actual commercial samples. Furthermore, because the concentrations of important biologically active components in herbal medicines are influenced by the conditions of the implantation and the planting process, the concentrations of these phenolic acids could be utilized as potential criteria for the quality control of TCMs.

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