Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(8):347-356

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Development of a novel method combining multi-wavelength HPLC fingerprint and quantitative analysis of multi-component by single marker for quality control of *Houttuynia cordata*

Liu Yan[1], Xiong Wei[2], Tian Ji[1], Li Chun Hong[1], Yang Shi Yan[3], Zeng Guo Guo[4] and He Bin[1]*

[1]*Department of Research Center for Drug and Function Food, Luzhou Medical College, Sichuan, China*
[2]*School of Public Health, Luzhou Medical College, Sichuan, China*
[3]*Department of Ultrasonic Image, Affiliated TCM hospital of Luzhou Medical College, Sichuan, China*
[4]*Department of library, Luzhou Medical College, Sichuan, China*

ABSTRACT

A novel method combining multi-wavelength high-performance liquid chromatographic (HPLC) fingerprints and quantitative analysis of multi-component by single marker (QAMS) was developed for quality evaluation of Houttuynia Cordata. For QAMS, Chlorogenic acid was selected as markers to work out the relative correction factors (RCF) of other six components including new chlorogcnic acid, cryptochlorogenic acid, rutin, hyperin, isoquercitrin, quercitrin. The forecast conents of six components were caculated by RCF. No significant difference of the conents was found in comparison with the determined data by external standard method in 20 batches, indicating that the RCF have high reliability within their linear ranges and could be used in quality control of Houttuynia Cordata. An enhanced fingerprints was constructed at two wavelengths (326 and 254 nm). All tested samples contained the 12 common peaks, 7 of which were verified, and the similarity of chromatographic fingerprints was from 0.912 to 0.991. The above new method is to be a new quality evaluation pattern for Traditional Chinese medicine.

Key words: Multi-wavelength high-performance liquid chromatographic (HPLC) fingerprint; Quantitative analysis of multi-components by single marker (QAMS); *Houttuynia* Cordata; chlorogenic acid; Relative correction factor(RCF)

INTRODUCTION

Houttuynia cordata Thunb, documented in the Chinese Pharmacopoeia (2005 edition) [1, 2], is a well-known traditional Chinese medicinal material widely used in China and Japan. There were abundant previous research works related to H. cordata. More than 30 compounds, which belong to various structural types, were isolated. Among these compounds, several components belonging to the classes of essential oils, flavonoids and organic acids were found to be pharmacologically active. The flavonoid components in H. cordata possess anti-neoplastic, anti-oxidant, anti-mutagenic and free radical scavenging capacity [3–5]. Similarly, chlorogenic acid possessed significant antipyretic and antibiotic properties [6]. It is generally believed that these compounds all contributed to the therapeutic effects of the medicinal herb.

Up to now, the H. cordata.'s quality control approach for simultaneous determination of multiple components has been developed in recent years. Analytical techniques like HPLC, GC-MS, and HPLC-MS were used [7–10]. However, the limitation of this method is that it requires sufficient chemical purity standards or chemical reference substances. In addition, high purity chemical standards or chemical reference substances of herbal medicines are expensive and insufficient, especially when the component is of low content level and hard to be purified from the

plant. Moreover, some constituents of herbal medicines become unstable when they are purified from a complicated matrix. In considering of the above reasons, factory has many difficulties to apply this type of quality control in manufacture. It is urgently necessary to develop a convenient and low-cost approach for controlling the quality of herbal medicines [11].

Herein, we propose a method combining quantitative analysis of multi-components by single marker (QAMS) for quality control of H. cordata and multi-wavelength HPLC fingerprint. Among QAMS, one component was determined with external standard method, while the amounts of the other components are calculated by their UV relative correction factors (RCFs) at specific wavelength [12-14]. Compared with conventional analytical approaches, Chromatographic fingerprint can give an overall view of all components in TCM and demonstrates both the 'sameness' and 'differences' among various samples successfully. However, one drawback is that it can only show results of similarity calculated based on the relative value using pre-selected marker compound as a reference standard, and minor differences between very similar chromatograms might not be distinguishable [15-17]. Thus, the chemical pattern recognition methods such as QAMS should be taken into consideration for reasonable definition of the class of H. cordata [18].

Hence, seven compounds: neochlorogenic acid, chlorogenic acid, cryptochlorogenin Acid, rutin, hyperin, isoquercitrin, and quercitrin were selected for analysis and evaluation of H. cordata. To our knowledge, no QAMS method is available for the simultaneous quantification of these seven components by HPLC. We have strategically established chromatographic fingerprinting profile and QAMS determination of seven compounds for the assessment of the quality of H. cordata by HPLC.

EXPERIMENTAL SECTION

1.1. Chemical and reagents

Chlorogenic acid, new chlorogcnic acid, cryptochlorogenic acid, rutin, hyperin, isoquercitrin, quercitrin (all with **>** 99% purity index)were purchased from the Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and Must Bio-technology Co.Ltd. (Chengdu, China).

Acetonitrile (HPLC- grade) were purchased from Dima Technology Inc. (USA). Phosphoric acid and methanol (Analytical-reagent grade) was provided by ChengDu Kelong Chemical Reagent Company (Chengdu, China). Water was produced by Milli-Q Advantage A10 system (Millipore, China).

1.2. Plant samples

Twenty samples of *Houttuynia Cordata* were acquired during a single growing season (January 2013) in three different citys of Sichuang Province, China (S1-S10 were wild, S11-S20 were cultivation). The samples were authenticated by corresponding author and voucher specimens deposited in the herbarium of Luzhou medical college.

1.3. Preparation of standard solutions and sample solutions

The reference standards stock solutions of the seven compounds were prepared in methanol–water (9:1, v/v) and stored in brown vials at 4℃. All solutions were diluted to the desired concentration with methanol–water (9:1, v/v) prior to use.

All plant samples were dried at 60 °C for 12 h and then were pulverized to fine powder. 0.5 gram of each sample was accurately weighed into a 100mL conical flask, and then 25mL of methanol-water (9:1, v/v) was added. After the weight of the whole conical flask was recorded, sample was extracted with ultrasonic for 30 minutes. The original solvent weight was restored after sample was cooled to room temperature. Above solutions were filtered with filter paper and sample solutions were prepared.

1.4. Instrumentation

Analysis was performed on two HPLC systems with a Dionex series, including a P680A quaternary pump, a PDA-100 detector, TCC-100 column compartment, Chromeleon work station, and an Agilent 1100 series, including an G1311A quaternary pump, an G1315B diode array detector, a vacuum degasser, a thermostated autosampler, G1316A column compartment, G1313A autosampler and a data system (Agilent Chem Station).

The chromatographic separation was performed on AkzoNobel Kromasil $C_{18,}$ Lubex Kromasil $C_{18,}$ PhenomenexLuna C_{18} (two), and Dikma Platisil ODS column (250 mm × 4.6 mm, 5 μm).

1.5. Gradient chromatographic condition

The UV absorbance was monitored at 326 nm from 0~25 min and 254 nm from 25~40 min. The mobile phase was acetonitrile (A) and 0.1%phosphoric acid aqueous solution (B) with a gradient program as follows: 0~10min, linear gradient 6%~8%A; 10~35min, linear gradient 8%~27%A; 35~37min, linear gradient 27%~6%A; 37~40min, linear gradient 6%A at a flow rate of 1ml/min. All injection volumes of samples and standard solutions were 10 μL.

1.6. Method validation.

Standard linear calibrations were established at seven data points covering the concentration range of each compound according to the level estimated in the samples. Working solutions were prepared by accurately measuring 0.1ml, 0.5ml, 1 ml, 2 ml, 3 ml, 4 ml, 5 ml of above stock solutions and diluted with the methanol-water (9:1, v/v) in 25ml volumetric flask respectively. Triplicate analyses with 10 μl for each were performed for each concentration. Calibration curves were constructed from peak areas versus compound concentrations. The LOD and LOQ for each marker compound under the present chromatographic conditions were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively.

To assess the intraday precision of the method, the standard solution of assayed compounds was injected six times within a day. The inter-day precision was determined with the same standard solution over 6 days by one injection per day. To confirm the repeatability, six different sample solutions prepared from the same batch (NO.: S_1) were analyzed. The stability of sample solutions was tested at room temperature. The sample solution was analyzed every 4 hour within 48 hours period. The relative standard deviation (RSD) was taken as a measure of precision, repeatability and stability.

Recovery tests were carried out to investigate the accuracy of the method by adding three concentration levels of the mixed standard solutions to known contents of the sample (NO.: S_1). The nine samples were then extracted and analyzed with the described method. Average recoveries were calibrated by the following formula: recovery (%) = {(amount found-original amount)/amount spiked} × 100% [relative standard deviation or RSD (%) = (SD/mean) × 100%]. Amount found represented the detected amount of analytes. Original amount represented the content of the sample solutions. Amount spiked was the added amount of standard.

1.7. Computational method of relative correction factor

Slope correction (SC) was applied to calculate the relative correction factor (RCF). According to the principle that within a concentration range, the absorption of analyte was linearly proportional to sample concentration and their relations could be shown with the formula $W = f \times A$ [19], where W is the sample concentration, A is the response value, and f is the correction factors (CF). The value of CF is a constant related with the detected substance and the sensitivity of the detector. Supposed several components coexisting in Chinese materia medica sample, every component could be shown as formula (1).

$$W_i / A_i = f_i \ (i\ 1,2,...,k\ ,...,m) \quad (1)$$

If components was used as an internal standard, the RCFs between components *s*(internal standard) and *k* ($f_{k/s}$) is established through formula (2):

$$f_{k/s} = f_k / f_s = W_s \times A_k / W_k \times A_s \quad (2)$$

Standard curve method was usually applied in quantitative analysis, calibration equations could be shown as formula (3)

$$A = a \times C + b \quad (3)$$

C is the sample concentration, *A* is the response value, *a* is the slope, and *b* is the intercept.

The sample concentration could be shown as formula (4).

$$C = A/a - b/a \quad (4)$$

If b/a >100, the sample concentration could be shown as formula (5).

$$C = A/a \quad (5)$$

According to the formula (1) (2) and formula (5), slope correction is established through formula (6).

$$f_{k/s} = f_k / f_s = W_s \times A_k / W_k \times A_s = a_k / a_s \quad (6)$$

a_k is the slope of determinand sample, a_s is the slope of internal standard. The determinand sample concentration (C_k) could be shown as formula (7).

$$C_k = A_k / (a_s \times f_{k/s}) \quad (7)$$

a_k is the slope of determinand sample, a_s is the slope of internal standard, A_k is the peak area of determinand sample.

2.8. Fingerprint Analysis.

Date analysis for chromatographic fingerprint was performed by use of the professional software 'Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine' (Version 2004A), which is recommended by SFDA. Using this software the correlative coefficient for samples was calculated and the similarities of different chromatograms were compared with the average chromatogram among the samples tested.

The accepted similarity was >0.9 according to the technical guideline of chromatographic fingerprint of Traditional Chinese Medicine injection [20].

RESULTS AND DISCUSSION

3.1 HPLC method validation.

The Calibration Curves, LOD and LOQ calculated results were given in Table 1. All of the analytes showed good linearity (r > 0.999) in a wide range of concentrations.

The precision, stability and repeatability %RSD values of the seven compounds were all <2% (Table 2) indicating the method is precise for the analysis of *Houttuynia* Cordata.

The recoveries of the analytes were 98.43-99.36% with RSD < 2.0 % as shown in Table 3. Above results exhibited the reliability and accuracy for the measurement of these constituents.

3.2 Quantitative analysis of multi-component by single marker

3.2.1 Relative correction factor (RCF)

Chlorogcnic acid was selected as the reference component. According to the section of "Computational method of relative correction factor", the RCF of other six compounds, which caculated by formula (7), were 1.002 (new chlorogcnic acid), 0.996 (cryptochlorogenic acid), 0.598 (rutin), 0.855 (hyperin), 0.867 (isoquercitrin) and 0.863 (quercitrin).

3.2.2 The reproducibility of RCF

In order to test the reproducibility of RCF , two HPLC systems including Dionex series and Agilent 1100 series in different laboratory, several separation columns including Kromasil C_{18}(Akzonobel, Switzerland), Kromasil C_{18} (Lubex, China), Luna C_{18} (Phenomenex , China)， Platisil (Dikma, China), specification of which were all 5μm and 250 mm × 4.6 mm, were evaluated and compared. The obtained results (Table 4) indicated that the method has good reproducibility.

3.2.3 Identification of target chromatographic peaks

It is essential to find a convenient means to identify correctly the determinand target components from the sample. The parameter of relative retention time (RRT) was usually used to locate the target peaks. In this research, chlorogcnic acid was used as the external standard, four separation columns including Kromasil C_{18}(Akzonobel, Switzerland), Kromasil C_{18}(Lubex, China), Luna C_{18}(Phenomenex , China)， Platisil (Dikma, China), specification of which were all 5μm and 250 mm × 4.6 mm, were evaluated and compared. The results listed in Table 5 clearly indicated that the relative retention time and RSD was affected by the chromatographic packings and it was difficult to exactly locate the target peaks with relative retention time.

According to the principle that different HPLC instruments or different columns were applied to analysis the same sample with the same method, the rentention time of compounds has simple linear relation. In this research, the peak of chlorogcnic acid and quercitrin were selected as marker to deduce the linear correction equation. Calibration curves were plotted by applying linear regression method according to the equation $y = ax + b$, where "x" represents the retention time of chlorogcnic acid and quercitrin measuring by Kromasil C_{18} (Akzonobel, Switzerland) columns and Dionex HPLC system, "y" is measured retention time of the chlorogcnic acid and quercitrin using other

separation columns mentioned above, "a" is the slope of the regression line, and "b" is the intercept. Table 6 listed the correction equation of different columns or HPLC systems and the speculated retention time of the seven compounds. The RSD between speculated and Measured retention time were all **<** 2%, which indicated that linear regression method was feasible and reliable. Then the target peaks in the HPLC profile of Houttuynia Cordata were quickly located by the speculated retention time.

However, the HPLC profile usually varied, such as the number of peaks increased or other peaks appeared next to the target peaks, when using different batches or geographical origin of Houttuynia Cordata. In order to more accurately locate the target peaks, the components UV absorption characteristics(Fig. 2), peak shapes combined the speculated retention time were applied to identify.

3.2.4 Comparision of quantitative analysis of multi-component by single marker and external standard method

A total of 20 batches samples were determined. In this paper, in order to validate this method, two routes have been arranged for quantifying the seven target components. The first is to determine the content of chlorogcnic acid, then to calculate the other six contents according to their RCFs. The second is to determine the seven target components by external standard method. These two group results are compared in Table 7, and analysed by the correlation coefficient and t – test. No significant difference was found between the two groups of data (correlation coefficient>0.9999, P>0.05), indicating that this proposed method has potential for developing a pattern for quality control of herbal medicines.

3.3 HPLC fingerprints analysis

The process of standardization included the selection of "common peaks" in chromatograms and the normalization of retention times of all common peaks. Furthermore, the total area of the common peaks must be more than 90% of the whole area in one chromatogram. Using the proposed method, HPLC-DAD chromatograms of different *Houttuynia* Cordata samples were acquired. The first chromatogram was regarded as the standardized characteristic fingerprint. Among these components, chlorogcnic acid represents as a high-level and stable content, therefore it was chosen as the reference peak. As shown in Fig. 3 and Fig. 4, there were 12 common peaks shown in all samples. All common peaks' relative retention time (RRA) and relative peak area (RPA) were obtained with reference to this substance (Table 8). The relative standard deviation (RSD) values of the RRA were less than 2.0%, which demonstrated good stability and reproducibility of the fingerprint analysis by HPLC. The similarity indexes of twenty samples were all **>** 0.9, which meaned that the common peaks were in good correlation.

Table 1 Calibration Curve, Limits of Detection (LOD) and Quantification (LOQ) for 7 components of Houttuynia Cordata

Analyte	Calibration Curve	Linear range/μg	*r*	LOD/ng	LOQ/ng
Neochlorogenic acid	$A=52.6851C-0.3647$	0.077~3.825	0.9999	0.23	0.76
Chlorogcnic acid	$A=52.5764\ C-0.2658$	0.038~1.900	0.9999	0.26	0.86
Cryptochlorogenic acid	$A=52.3595\ C-0.3015$	0.009~0.433	0.9997	0.29	0.97
rutin	$A=31.4356\ C-0.2672$	0.023~1.150	0.9998	0.37	1.25
hyperin	$A=44.9662\ C-0.3254$	0.052~2.575	0.9998	0.27	0.91
isoquercitrin	$A=45.5787\ C-0.4176$	0.027~1.354	0.9998	0.31	1.04
quercitrin	$A=45.3579\ C-0.3741$	0.069~3.450	0.9999	0.26	0.88

Limit of Detection, as ~3 S/N.
Limit of Quantitation, as ~10 S/N.

Table 2 The Results of Precision, Repeatability and Stability

RSD/%	Neochlorogenic acid	Chlorogcnic acid	cryptochlorogenic acid	rutin	hyperin	Iso quercitrin	quercitrin
intraday precision	0.23	0.27	0.46	0.41	0.35	0.46	0.31
interday precision	0.35	0.41	0.59	0.52	0.48	0.62	0.44
Stability	1.3	1.1	1.4	1.4	1.0	1.6	1.1
Repeatability	0.72	0.86	0.90	1.0	0.95	1.2	0.79

relative standard deviation or RSD(%)= (SD/mean) × 100%].

Table 3 The Results of recovery test of 7 components

Components	Content/mg	Added amount/mg	Amount found /mg	Recovery/%	Average recovery/%	RSD/%
Neochlorogenic acid	2.015 3	1.596 0	3.592 4	98.82	99.36	1.0
	2.012 1	1.995 0	3.992 9	99.29		
	2.018 5	2.394 0	4.411 5	99.96		
Chlorogcnic acid	0.600 3	0.472 0	1.063 1	98.05	98.59	1.1
	0.599 4	0.590 0	1.179 9	98.40		
	0.601 3	0.708 0	1.304 5	99.33		
cryptochlorogenic acid	0.337 5	0.264 0	0.594 8	97.46	98.09	1.4
	0.337 0	0.330 0	0.660 2	97.93		
	0.338 1	0.396 0	0.729 6	98.88		
rutin	0.197 8	0.154 0	0.348 1	97.61	98.43	1.2
	0.197 5	0.192 5	0.387 1	98.53		
	0.198 1	0.231 0	0.427 1	99.15		
hyperin	0.695 9	0.550 0	1.236 0	98.21	99.05	1.1
	0.694 8	0.687 5	1.376 2	99.12		
	0.697 0	0.825 0	1.520 4	99.81		
isoquercitrin	0.149 3	0.116 0	0.262 7	97.74	98.63	1.3
	0.149 1	0.145 0	0.291 8	98.43		
	0.149 5	0.174 0	0.323 0	99.72		
quercitrin	1.424 6	1.128 0	2.534 4	98.39	99.16	1.1
	1.422 3	1.410 0	2.821 3	99.22		
	1.426 8	1.692 0	3.116 4	99.86		

Recovery (%)={(amount found —original amount)/amount spiked} ×100% , where n=9

Table 4 Relative correction factors determined by different lab, instruments and columns

Lab	instruments	columns	relative correction factor					
			Neochlorogenic acid	cryptochlorogenic acid	rutin	hyperin	isoquercitrin	quercitrin
Lab1	Dionex	AkzoNobel Kromasil	1.002	0.996	0.598	0.855	0.867	0.863
		LubexKromasil	1.023	0.997	0.588	0.868	0.859	0.869
		PhenomenexLuna	1.020	1.008	0.607	0.873	0.865	0.873
		DikmaPlatisil	0.977	0.983	0.603	0.830	0.848	0.844
Lab2	Agilent	AkzoNobelKromasil	1.004	0.998	0.601	0.859	0.869	0.867
		PhenomenexLuna	1.000	0.990	0.597	0.853	0.847	0.856
Mean			1.004	0.995	0.599	0.856	0.859	0.862
RSD/%			1.65	0.84	1.04	1.73	1.10	1.22

Table 5 Relative retention time determined by different columns

column	Neochlorogenic acid	chlorogcnic acid	crypto chlorogenic acid	rutin	hyperin	isoquercitrin	quercitrin
AkzoNobel Kromasil	0.64	1.00	1.08	1.58	1.63	1.65	1.83
Lubex Kromasil	0.71	1.00	1.06	1.48	1.53	1.55	1.71
Phenomenex Luna	0.70	1.00	1.05	1.48	1.53	1.55	1.70
Dikma Platisil	0.76	1.00	1.02	1.40	1.45	1.46	1.60
RSD / %	6.85	0	2.35	4.92	4.80	4.94	5.41

Table 6 Speculated retention time in different lab with different instruments and columns

instruments	columns	t_R	A	B	C	D	E	F	G
Dionex	AkzoNobel Kromasil	t_R	11.650	18.133	19.533	28.567	29.500	29.933	33.167
	Lubex Kromasil	Measured t_R	14.325	20.242	21.417	29.992	30.992	31.408	34.583
		Speculated t_R (a)	14.058	20.242	21.577	30.195	31.085	31.498	34.583
	PhenomenexLuna	Measured t_R	14.358	20.583	21.625	30.450	31.392	31.817	35.075
		Speculated t_R (b)	14.334	20.583	21.933	30.641	31.540	31.958	35.075
	Dikma Platisil	Measured t_R	18.117	23.842	24.267	33.300	34.508	34.892	38.217
		Speculated t_R (c)	17.643	23.842	25.181	33.819	34.711	35.125	38.217
Agilent	AkzoNobel Kromasil	Measured t_R	11.751	18.239	19.606	28.705	29.626	30.064	33.321
		Speculated t_R (d)	11.735	18.239	19.643	28.706	29.642	30.076	33.321
	PhenomenexLuna	Measured t_R	14.641	20.866	21.908	30.733	31.675	32.100	35.358
		Speculated t_R (e)	14.616	20.866	22.215	30.923	31.823	32.240	35.358

a, b, c, d, e represent the correction equation respectively.

a: Y=0.954X+2.945, b: Y=0.964X+3.104, c: Y=0.956X+6.504, d: Y=1.003X+0.049, e: Y=0.964X+3.386

A: Neochlorogenic acid B: chlorogcnic acid C: cryptochlorogenic acid D: rutin E: hyperin F: soquercitrin G: quercitrin

Table 7 Determination results of 7 components in Houttuyniae Herba by two methods

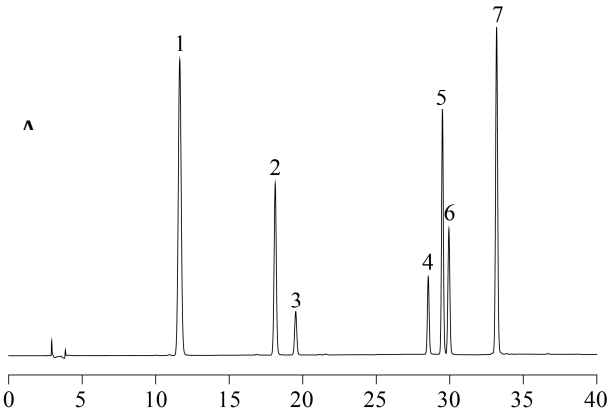
Sample	A		B		C		D		E		F		G	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
S_1	0.799	0.798	0.238	0.238	0.134	0.135	0.078	0.079	0.276	0.276	0.059	0.059	0.565	0.564
S2	0.716	0.715	0.248	0.248	0.022	0.022	0.295	0.296	1.047	1.045	0.215	0.216	1.179	1.178
S3	0.563	0.562	0.291	0.291	0.026	0.026	0.096	0.096	0.356	0.355	0.093	0.093	0.324	0.323
S4	0.359	0.358	0.336	0.336	0.026	0.026	0.103	0.104	0.254	0.254	0.096	0.096	0.235	0.235
S5	0.606	0.606	0.249	0.249	0.073	0.074	0.109	0.109	0.482	0.481	0.129	0.129	0.965	0.964
S6	0.827	0.826	0.126	0.126	0.035	0.035	0.227	0.228	0.534	0.533	0.175	0.176	0.661	0.660
S7	0.513	0.512	0.258	0.258	0.018	0.018	0.133	0.133	0.347	0.346	0.092	0.092	0.332	0.332
S8	0.708	0.707	0.307	0.307	0.024	0.024	0.081	0.081	0.260	0.259	0.072	0.072	0.192	0.192
S9	0.747	0.746	0.302	0.302	0.035	0.036	0.073	0.073	0.230	0.230	0.070	0.070	0.289	0.289
S10	0.859	0.858	0.267	0.267	0.020	0.021	0.193	0.194	0.375	0.374	0.158	0.158	0.714	0.713
S11	0.423	0.422	0.419	0.419	0.015	0.015	0.103	0.103	0.374	0.374	0.135	0.135	0.462	0.461
S12	0.288	0.287	0.187	0.187	0.014	0.014	0.076	0.076	0.393	0.393	0.150	0.151	0.347	0.347
S13	0.286	0.286	0.087	0.087	0.093	0.094	0.046	0.046	0.230	0.230	0.041	0.041	0.596	0.596
S14	0.388	0.388	0.252	0.252	0.009	0.009	0.122	0.123	0.522	0.521	0.120	0.121	0.337	0.337
S15	0.312	0.311	0.135	0.135	0.011	0.011	0.069	0.069	0.344	0.343	0.072	0.072	0.250	0.249
S16	0.497	0.496	0.171	0.171	0.075	0.076	0.103	0.103	0.236	0.235	0.107	0.107	0.730	0.729
S17	0.196	0.196	0.048	0.048	0.036	0.036	0.056	0.057	0.157	0.157	0.051	0.051	0.291	0.290
S18	0.260	0.260	0.147	0.147	0.012	0.012	0.115	0.116	0.391	0.390	0.099	0.099	0.326	0.325
S19	0.421	0.421	0.266	0.266	0.019	0.019	0.109	0.109	0.296	0.296	0.057	0.057	0.471	0.470
S20	0.597	0.596	0.254	0.254	0.025	0.025	0.058	0.058	0.105	0.105	0.022	0.022	0.264	0.264

a: contents were determined by the traditional external standard method
b: contents were calculated by the proposed method
A: Neochlorogenic acid B: chlorogcnic acid C: cryptochlorogenic acid D: rutin E: hyperin F: soquercitrin G: quercitrin
Values in %, n = 3, RSD <2%.

Table 8 Technical parameter for HPLC fingerprint of Houttuyniae Herba

NO.	Retention time /min	Relative retention time	Peak area	Peak area /%	Relative peak area	Remark
1	10.988±0.115	0.605±0.002	1.3523±0.7138	0.67±0.30	0.057±0.033	
2	11.705±0.160	0.645±0.004	54.9509±21.9324	28.03±7.40	2.534±1.242	A
3	17.002±0.136	0.936±0.001	1.8705±1.2436	0.96±0.57	0.086±0.050	
4(S)	18.160±0.143	1.000±0.000	24.2830±9.4542	12.91±5.29	1.000±0.000	B
5	19.512±0.214	1.074±0.009	3.6905±3.5154	2.03±1.88	0.213±0.281	C
6	28.281±0.088	1.557±0.008	2.0422±1.4166	1.00±0.42	0.100±0.074	
7	28.536±0.083	1.571±0.008	7.1835±3.8796	3.59±0.96	0.347±0.237	D
8	29.455±0.090	1.622±0.009	32.5638±17.7753	16.58±5.67	1.577±0.952	E
9	29.888±0.086	1.646±0.009	9.2187±4.4933	4.71±1.72	0.445±0.278	F
10	31.766±0.089	1.749±0.010	1.0965±0.6797	0.56±0.24	0.057±0.050	
11	33.107±0.086	1.823±0.011	43.4690±24.0604	21.79±7.47	2.241±1.646	G
12	36.561±0.089	2.013±0.013	1.7687±1.4967	0.89±0.65	0.085±0.065	
	Peak area of common peak		183.4898	93.72		
	Peak area of uncommon peak		12.4118	6.28		
	Total Peak area		195.9016	100.00		

A: Neochlorogenic acid B: chlorogcnic acid C: cryptochlorogenic acid D: rutin E: hyperin F: soquercitrin G: quercitrin
Values in mean ±SD.



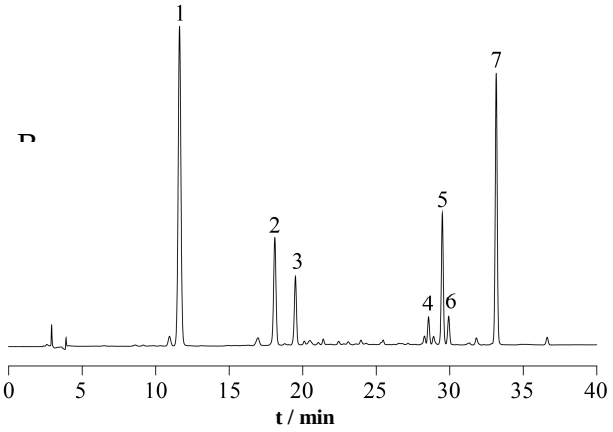


Fig. 1 HPLC chromatograms of mixed reference substances（A） and Houttuyniae Herba（B）
A-mixed reference substances; B-Houttuyniae Herba; 1-neochlorogenic acid；2-chlorogenic acid; 3-cryptochlorogenic acid；4-rutin; 5-hyperin; 6-soquercitrin; 7 -quercitrin

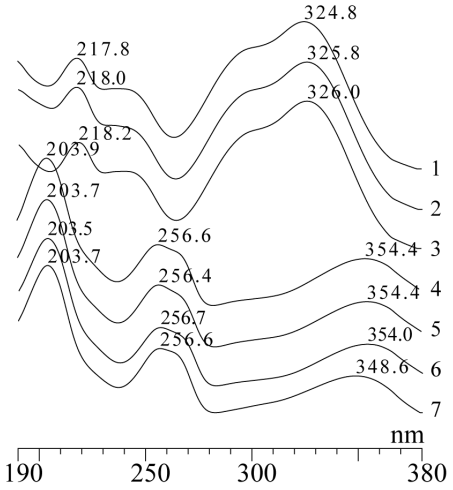


Fig.2 Ultraviolet absorption spectrum of reference substances
1-neochlorogenic acid; 2-chlorogenic acid; 3-cryptochlorogenic acid; 4-rutin; 5-hyperin; 6-soquercitrin; 7-quercitrin

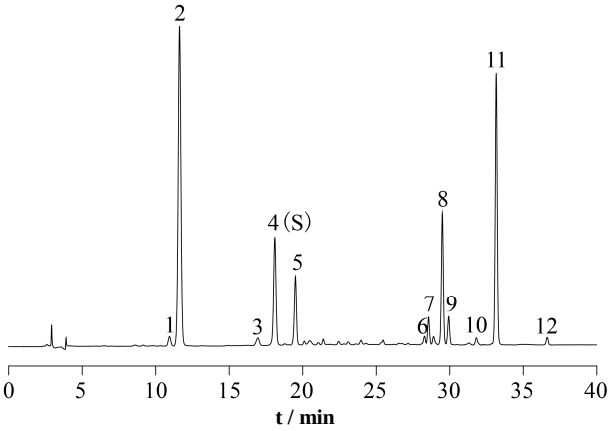


Fig. 3 HPLC fingerprint of Houttuyniae Herba
4(s) - reference peak

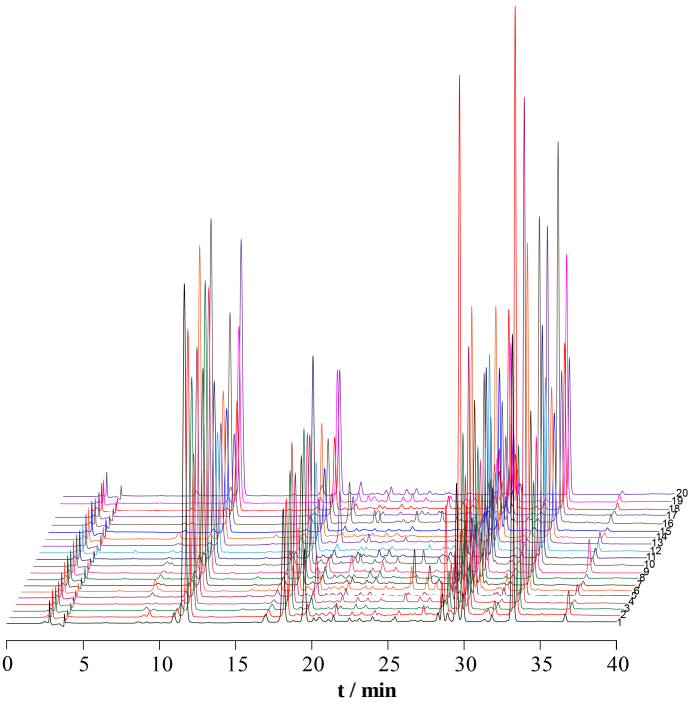


Fig. 4 HPLC fingerprint of 20 batches of Houttuyniae Herba

CONCLUSION

To the best of our knowledge, this is the first report on multi-wavelength HPLC fingerprints and quantitative analysis of multi-component by single marker (QAMS) in *Houttuynia* Cordata analysis. This novel evaluation approach can overcome the deficiencies of previously described methods revealing the complexity and synergistic effects of samples' constituents. It provides much more qualitative information than any other singular evalution.

Data analysis on the conents of 20 batches sample indicated that the RCF have high reliability within their linear ranges and could be used in quality control of *Houttuynia* Cordata. The proposed method is a simple and low-cost quality control pattern for herbal medicines, which is especially suitable for determination of the unstable constituents.

Acknowledgements

This paper was supported by science and technology project of Luzhou【2011】108; Luzhou Medical college youth project, 2010108.

REFERENCES

[1] The Pharmacopoeia Committee of China. "The Chinese pharmacopoeia", vol. I. Beijing, China: Chemical Industry Publishing House; **2005**; 155.
[2] Meng J, Leung KS, Dong XP, Zhou YS, Jiang ZH. *Fitoterapia* **2009**; 80: 468-474.
[3] Wang LQ, Zhao YX, Zhou J, *Chinese Herbal Medicines*, **2007**; 12: 1788-1790.
[4] Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, Park SH, Kim SK. *Plant Sci* **2002**; 163: 1161–8.
[5] Wu WH, Kang Z, Ou YDS. *Natural product research and development* **2006**; 18: 691-694
[6] Kim SK, Ryu SY, No J, Choi SU, Kim YS. *Arch Pharm Res* **2001**; 24:518–21.
[7] Zhang TT, Wu Y, Hang TJ. *Journal of chinese medicinal materials* **2009**; 05: 687-690.
[8] Huang QW, Zhang WT, Zhao WL. *Chinese Herbal Medicines* **2007**; 37： 1253-1255
[9] Meng J, Zhou YS, Zhao ZZ. *Journal of china pharmaceutical university* **2007**; 38: 516-518
[10] He B, Liu Y, Li CH, Yang SY, Zhang Y. *Chinese Herbal Medicines* **2013**; 43: 2160-2164
[11] Zhu JJ, Wang ZM, Ma XY , Feng WH, Zhang QW. *Chinese Herbal Medicines* **2012**; 4: 157-163
[12] Wang LX, Xiao HB, Liang XM. *Chinese journal of analytical chemistry* **2003**; 31: 1232-1236
[13] Chen J, Wang SM, Meng J, Sun F, Liang SW. *J Chinese material medica* **2013**; 09: 1406-1410.
[14] Lin Q, Kuang YH, Huang L, YaoXH, Wang ZM, WangDQ. *Chinese Herbal Medicines* **2012**; 12: 2406-2411.
[15] P.S. Xie, S.B. Chen, Y.Z. Liang, X.H. Wang, R.T. Tian, R. Upton. *J Chromatogr*. A **2006**; 1112: 171–180.
[16] J.H. Zeng, G.B. Xu, X. Chen. *Med. Chem. Res*. **2009**; 18: 158–165.

[17] C.M. Xiong, J.L. Ruan, Y. Tang, Y.L. Cai, W. Fang, Y. Zhu, D.N. Zhou. *Chromatographia* **2009**; 70: 117–124.
[18] H. Zhu, Y. Wan g, H. Liang, Q. Chen, P. Zhao, J. Tao. *Talanta* **2010**; 81: 129–135.
[19] Yu DH, **1992**. Determination of impurity samples by chromatography correction factors. *Chem World* 7: 816-820.