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Application of a new validated HPLC-DAD method for simultaneous determination of ten active components in Xiedu San

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Abstract: In this research, a sensitive high-performance liquid chromatography-diode array detector (HPLC-DAD) method was established and validated for simultaneous detection of ten active constituents (gallic acid, catechin, berberine, palmatine, baicalin, baicalein, wogonin, rhein, emodin and chrysophanol) in Xiedu San, a traditional Chinese medicine compound preparation with effects of clearing away heat and toxic materials. The analysis was achieved on Agilent ZORBAX SB-C_{18} column (5μm, 250mm×4.6mm) with the temperature of 30℃. Gradient elution was applied using methanol (A)-0.1% phosphoric acid solution (B) as mobile phase at the flow rate of 1.0mL· min^{-1}. The determination was performed at the wavelength of 225, 245, 278 and 348 nm along with the sample volume of 5μL. The tested constituents demonstrated good linear relationships within their respective determination ranges (r>0.9995). Average recoveries varied from 99.97% to 101.12% with RSDs of 0.71% to 1.92%. The contents of tested constituents ranged from 0.970 to 24.602 mg·g^{-1}. The developed method was proved to be simple, accurate and sensitive, which can provide a quantitative analysis method for the quality evaluation and quality control of Xiedu San.

Keywords: Xiedu San (XS), HPLC-DAD, simultaneous determination, active components.

INTRODUCTION

Xiedu San (XS) is a compound preparation (Chinese Pharmacopoeia Commission, 1993) composed of *Rhei Radix Et Rhizoma* (RRER), *Coptidis Rhizoma* (CR) and *Scutellariae Radix* (SR), which is included in "Departmental Standards of Traditional Chinese Medicine" (Standard No. WS3-B-2359-97). XS can be used in the initial stage of swelling and ulcer on the body surface, red swelling and hot pain due to its effects of clearing away heat and toxic materials. According to literature, two methods were used for the quality control of XS at present. Thin layer chromatography (TLC) was used to the qualitative identification of CR and SR in XS (Gao JF *et al*., 2008). Besides, high performance liquid chromatography (HPLC) was used for the detection of berberine, emodin and chrysophanol in XS (Fu XJ *et al*., 2002; Jin PF *et al*., 2001). However, these two methods cannot reflect the contents of the effective components in the preparation comprehensively and systematically. At present, HPLC-DAD is widely used as an effective method in order to control the quality of traditional Chinese medicine and western medicine preparations (Vieiraa ES and Elenara LS, 2020).

The free anthraquinones (rhein, emodin and chrysophanol), quality control indexes of RRER (Jin LX *et al*., 2020; Chinese Pharmacopoeia Commission, 2015), can play the part of purgation, diuresis, cooling blood and detoxification together with gallic acid and catechin (Sun HQ *et al*., 2018; Wang YJ *et al*., 2018). Berberine and palmatine in CR have anti-inflammatory, anti-bacterial, sedative and analgesic effects (Sun J and Yan GH, 2018; Gai XH *et al*., 2018). In addition, flavonoids (baicalin, baicalein and wogonin) in SR are considered as the substance basis of anti-bacterial, anti-viral, anti-inflammatory and other pharmacological actions of SR (Li EZ *et al*., 2018; Hao C *et al*., 2018). Therefore, an accurate HPLC-DAD method for the determination of ten active constituents (gallic acid, catechin, berberine, palmatine, baicalin, baicalein, wogonin, rhein, emodin and chrysophanol) simultaneously was established in this paper. The proposed method can provide a more comprehensive and accurate approach for the quality evaluation and quality control of XS and provide guarantee for its wider clinical application. The chemical structure of these ten constituents are displayed in fig. 1.

MATERIALS AND METHODS

Materials

XS was made by laboratory. RRER, CR and SR with proportion of 2:1:1 were smashed into fine powder, sieved and mixed fully to obtain XS (batch No. 20180101, 20180102 and 20180103). RRER, CR and SR were all purchased from Jilin pharmacy (Changchun, China). These medicines were in accord with the requirements in "Pharmacopoeia of the People's Republic of China (2015 edition)".

Gallic acid (batch No. 110831-200302), berberine hydrochloride (batch No. 110713-201212), palmatine chloride (batch No. 110732-200907), baicalin (batch No.

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110715-201318), baicalein (batch No. 111595-201306), emodin (batch No. 110756-200110), chrysophanol (batch No. 110796-201118) and catechin (batch No. 877-200001) were obtained from China Pharmaceutical and Biological Products Calibration Institute (Beijing, China). Wogonin (batch No. ASB-00023510-025) was obtained from CHROMADEX of USA. Methanol (chromatographic grade) was acquired from Thermo Fisher Scientific (Massachusetts, America). Phosphoric acid was of excellent purity and was purchased from Guangfu Technology Development Co., Ltd (Tianjin, China). Besides, Other reagents (analytical grade) were obtained from Beijing Chemical Industry Factory (Beijing, China). Ultra pure water was acquired from Wahaha Co., Ltd (Hangzhou, China).

Instrumentation

The quantitative analysis was accomplished on Agilent 1260 HPLC system (containing quaternary low pressure pumping system, 1100 diode array detector, auto-sampler, column heater system and Chemstation work station). Electronic balance (AB135-S) was obtained from Mettler Toledo International Co., Ltd. Heating and drying oven (DHG-912A) was obtained from Shanghai Precision Experimental Equipment Co., Ltd. Ultrasonic cleaner (KQ-250) was purchased from Kunshan Ultrasonic Instrument Co., Ltd. R series rotatory vacuum evaporator was acquired from Shanghai Shensheng Technology Co., Ltd.

Chromatographic Conditions

The simultaneous determination was performed on Agilent ZORBAX SB-C_{18} column (5μm, 250mm× 4.6mm). The mobile phase was composed of methanol (A) and 0.1% phosphoric acid solution (B). The detailed gradient elution conditions were as below: 0～12min, 19%A→19%A; 12～15min, 19%A→32%A; 15～51min, 32%A→32%A; 51～54min, 32%A→45%A; 54～63min, 45%A→45%A; 63～66min, 45%A→60%A; 66～77min, 60%A→60%A; 77～80min, 60%A→75%A; 80～89min, 75%A→75%A; 89～92min, 75%A→80%A; 92～106min, 80%A→80%A. The detective wavelengths were monitored at 225 nm (gallic acid and catechin), 245 nm (berberine and palmatine), 278 nm(baicalin, baicalein and wogonin) and 348nm (rhein, emodin and chrysophanol). The column temperature was maintained at 30℃. The flow rate was set as 1.0mL· min^{-1}. The sample volume was 5μL.

Preparation of standard solutions

Moderate amount of gallic acid, catechin, berberine hydrochloride, palmatine chloride, baicalin, baicalein, wogonin, rhein, emodin and chrysophanol were weighted precisely and put into ten different volumetric flasks (10 mL). Subsequently, the volumetric flasks above were dissolved and adjusted to volume scale with methanol to acquire stock standard solutions with the concentrations of 0.514mg·mL^{-1} (gallic acid), 2.17mg·mL^{-1} (catechin), 2.141mg·mL^{-1} (berberine), 0.537mg·mL^{-1} (palmatine), 2.314mg·mL^{-1} (baicalin), 0.479mg·mL^{-1} (baicalein), 0.466mg·mL^{-1} (wogonin), 0.521mg·mL^{-1} (rhein), 1.48 mg·mL^{-1} (emodin) and 0.980mg·mL^{-1} (chrysophanol), respectively. Then appropriate amount of stock standard solutions were taken, placed in the same 10mL volumetric flask, followed by dissolved and adjusted to volume scale with methanol to acquire the mixed standard solutions containing 51.4μg (gallic acid), 21.7μg (catechin), 214.1μg (berberine), 53.7μg (palmatine), 231.4μg (baicalin), 47.9μg (baicalein), 46.6μg (wogonin), 52.1μg (rhein), 14.8μg (emodin) and 9.8μg (chrysophanol) per mL.

Preparation of test solutions

Approximate 1.2g of XS was weighed and put into the stoppered conical flask and methanol (10 times) was added and was treated with ultrasound for 30 min. The XS sample was extracted two times in total. Then the methanol extracts were mixed up and evaporated to dry via rotary evaporator. At last, the residue was dissolved with methanol (adjusted to 25mL scale) and was diluted 5 times to obtain test solutions.

Preparation of negative control solutions (NCS)

On the basis of the prescription proportion of XS and preparation method of test solutions, gallic acid NSC (without RRER-CR), berberine-palmatine NCS (without RRER-CR), catechin NCS (without RRER-SR), baicalin-baicalein-wogonin NCS (without RRER-SR) and rhein-emodin-chrysophanol NCS (without RRER) were prepared, respectively.

Method validation

Validation experiments of the proposed method was carried out in this study, which included the following parameters: specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, stability and accuracy.

Specificity was performed by comparing the chromatograms obtained so as to confirm the possible interference of the prescription factors in HPLC-DAD analysis. Standard solutions, test solutions and negative control solutions were sucked and injected into HPLC to obtain chromatograms at different wavelengths.

Linearity was assessed by constructing standard curves with six points. Appropriate amount of each reference substance was weighed accurately and prepared into mixed stock standard solutions with the concentration of gallic acid 514μg·mL^{-1}, catechin 217μg·mL^{-1}, berberine 2141μg·mL^{-1}, palmatine 537μg·mL^{-1}, baicalin 2314 μg·mL^{-1}, baicalein 479μg·mL^{-1}, wogonin 466μg·mL^{-1}, rhein 521μg·mL^{-1}, emodin 148μg·mL^{-1} and chrysophanol

98μg·mL^{-1}, respectively.

Then the mixed stock standard solutions above (exact 0.2mL, 0.4mL, 0.8mL, 1.0mL, 5.0mL and 10.0mL) were put into a 10mL volumetric flask, dissolved and adjusted to scale with methanol, respectively, to acquire the standard serial solutions. Subsequently, the serial solutions above were injected into HPLC for determination, respectively. In addition, the mixed standard solutions was diluted using methanol gradually. The limits of detection (LOD) and limits of quantification (LOQ) were confirmed by 3/1 and 10/1 of the signal to noise ratio, respectively.

Precision was evaluated through HPLC analysis of standard solutions within one day. The standard solutions were determined via six consecutive injections based on the developed chromatographic conditions, respectively. Besides, the chromatographic peak areas (CPAs) and relative standard deviations (RSDs) of each constituent were recorded and computed separately. Repeatability of the method was checked by measuring ten components in six different test solutions. Six copies of same XS sample (batch No. 20180101) were weighed and made into six duplicate test solutions, followed by analyzed through HPLC, respectively. Besides, content RSDs of these ten components were calculated, respectively.

Stability was investigated by analyzing the changes of CPAs of ten components within 24 hour. The same test solutions were taken and injected into chromatographic system at 0, 2, 4, 8, 12 and 24 hour for determination, respectively. The CPAs of each analyte were recorded and RSDs of CPAs in different point of time were calculated.

Accuracy of this method was estimated by the recovery experiment. Six copies of XS samples (0.6 g) with known levels were precisely weighed and put into six various stoppered conical flasks, respectively. Then the standard solutions (table 1) were added to these six conical flasks, followed by made into test solutions, separately. Finally, the test solutions were sucked and injected into chromatographic system for testing, separately.

Determination of active components

Three batches of XS samples were taken and made into test solutions, separately. Then the solutions above were filtered through a millipore filter (0.22μm) and continuing filtrate were injected into HPLC for determination, respectively. In addition, CPAs were recorded and contents of ten various constituents were calculated via external standard method.

STATISTICAL ANALYSIS

Microsoft Excel (2020 version) was used for calculating mean and RSD.

RESULTS

Method validation for simultaneous determination

The chromatograms obtained from the specificity test are displayed in fig. 2-5. As displayed in these figs., no interference peaks were found in the corresponding location of each analyte at different detective wavelength. In addition, the separation degrees between measured chromatographic peaks and their adjacent peaks were all more than 1.5, indicating that the developed method was of strong specificity.

The calibration curve was built taking the concentration of standard solutions (X) as abscissa and the chromatographic peak area (Y) as the ordinate. Furthermore, the LOD (S/N=3) and LOQ (S/N=10) were calculated. The calculation results are exhibited in table 2, indicating that ten constituents showed good linear relationships within their detection ranges, as well as good sensitivity under the developed chromatographic conditions.

The RSDs (precision test) of CPAs of gallic acid, catechin, berberine, palmatine, baicalin, baicalein, wogonin, rhein, emodin and chrysophanol were 1.52%, 1.20%, 1.59%, 1.69%, 0.50%, 1.45%, 1.45%, 1.51%, 1.97% and 1.68%, respectively. The RSD values were all less than 2.00%, which illustrated that the method was of high precision.

The RSDs (stability test) of CPAs of gallic acid, catechin, berberine, palmatine, baicalin, baicalein, wogonin, rhein, emodin and chrysophanol were 0.96%, 0.57%, 0.86%, 1.64%, 0.44%, 1.15%, 0.70%, 1.60%, 1.37% and 1.41%, respectively. The RSD values were all not more than 2.00%, indicating that test solutions was stable within 24 hour.

The average contents of gallic acid, catechin, berberine, palmatine, baicalin, baicalein, wogonin, rhein, emodin and chrysophanol in repeatability test were 4.844 mg·g^{-1}, 1.968 mg·g^{-1}, 9.745 mg·g^{-1}, 5.758 mg·g^{-1}, 24.559 mg·g^{-1}, 4.800 mg·g^{-1}, 4.516 mg·g^{-1}, 5.061 mg·g^{-1}, 1.057 mg·g^{-1} and 1.011 mg·g^{-1}. Besides, the RSDs of mean contents of those ten components were 1.56%, 1.79%, 1.66%, 1.69%, 1.41%, 1.85%, 1.83%, 1.41%, 1.83% and 1.41%, respectively, suggesting that the proposed method had good repeatability.

The contents of ten constituents were detected and the recovery rates (table 3) were calculated, respectively. The mean recoveries of ten analytes ranged from 99.97% to 101.12% with RSDs of 0.71% to 1.92%. The results above suggested this analytical method was of high accuracy.

Simultaneous determination of ten active components in XS

The developed HPLC-DAD quantitative method was used for simultaneous determination of ten active constituents

in XS. The contents of measured constituents ranged from 0.970 to 24.602 mg·g^{-1} and the determination results are summed up in table 4. The contents of berberine and baicalin were higher in the tested samples compared to those of other components. The contents of ten active components in XS with the same batch number were relatively stable (RSD ≤1.93%). While there were some differences in the contents of different batches of samples, which may be related to the quality difference of TCM. Therefore, the quality of TCM should be strictly controlled to ensure the quality of the compound preparation.

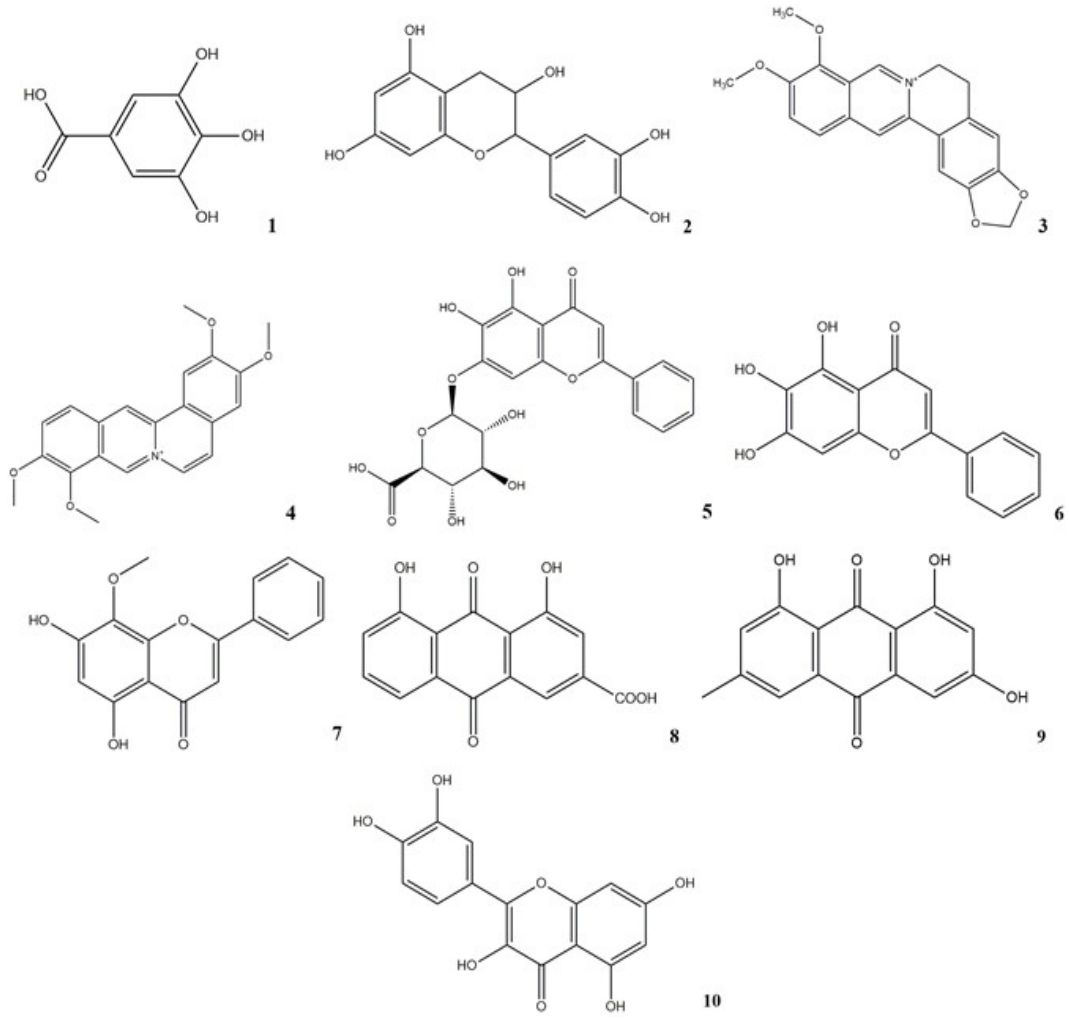

Fig. 1: Chemical structure of ten active components.(1. gallic acid; 2. catechin; 3. berberine; 4. palmatine; 5. baicalin; 6. baicalein; 7. wogonin; 8. rhein; 9. emodin; 10. chrysophanol)

Table 1: Addition of standard solutions

Constituent	Concentration (mg·mL^{-1})	Added amount (mL)
Gallic acid	3.080	1
Catechin	1.102	1
Berberine	3.000	2
Palmatine	3.010	1
Baicalin	2.966	5
Baicalein	2.965	1
Wogonin	2.970	1
Rhein	3.030	1
Emodin	0.651	1
Chrysophanol	0.650	1

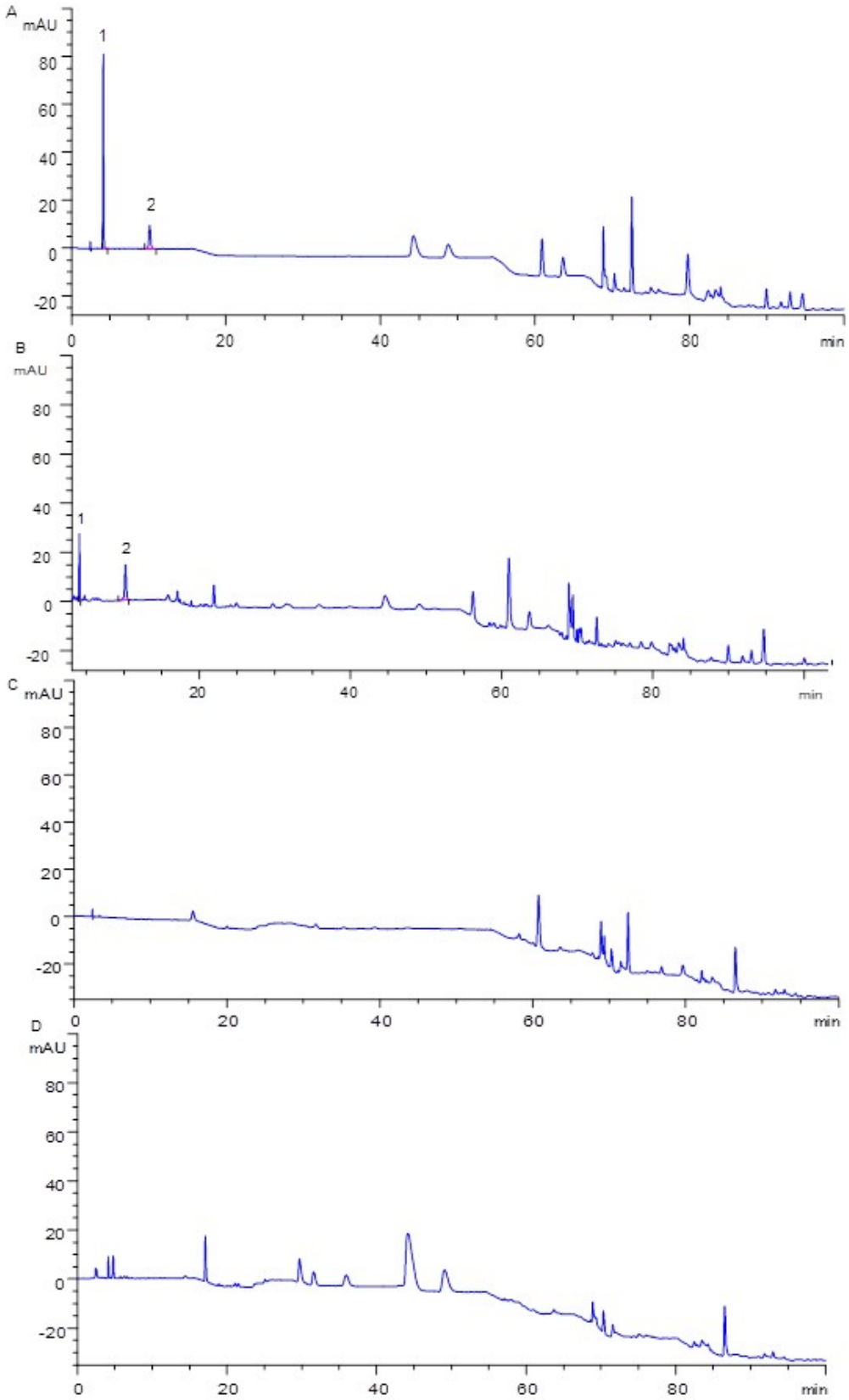

Fig. 2: Chromatograms of mixed standard solutions (A), test solutions (B), gallic acid NCS (C), catechin NCS (D) at 225nm. 1. gallic acid 2. catechin

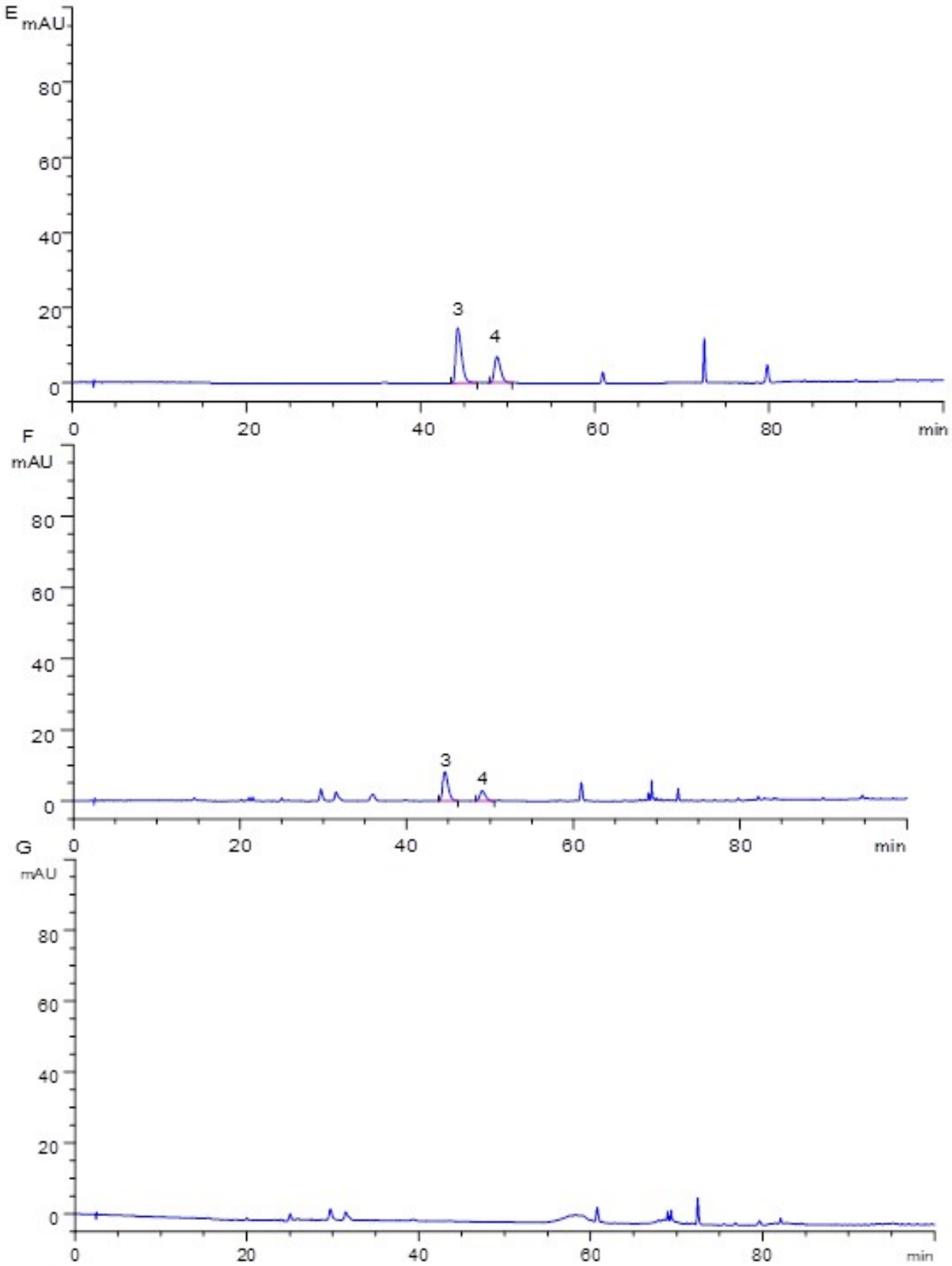

Fig. 3: Chromatograms of mixed standard solutions (E), test solutions (F) and berberine-palmatine NCS (G) at 348nm. 3. berberine 4. palmatine

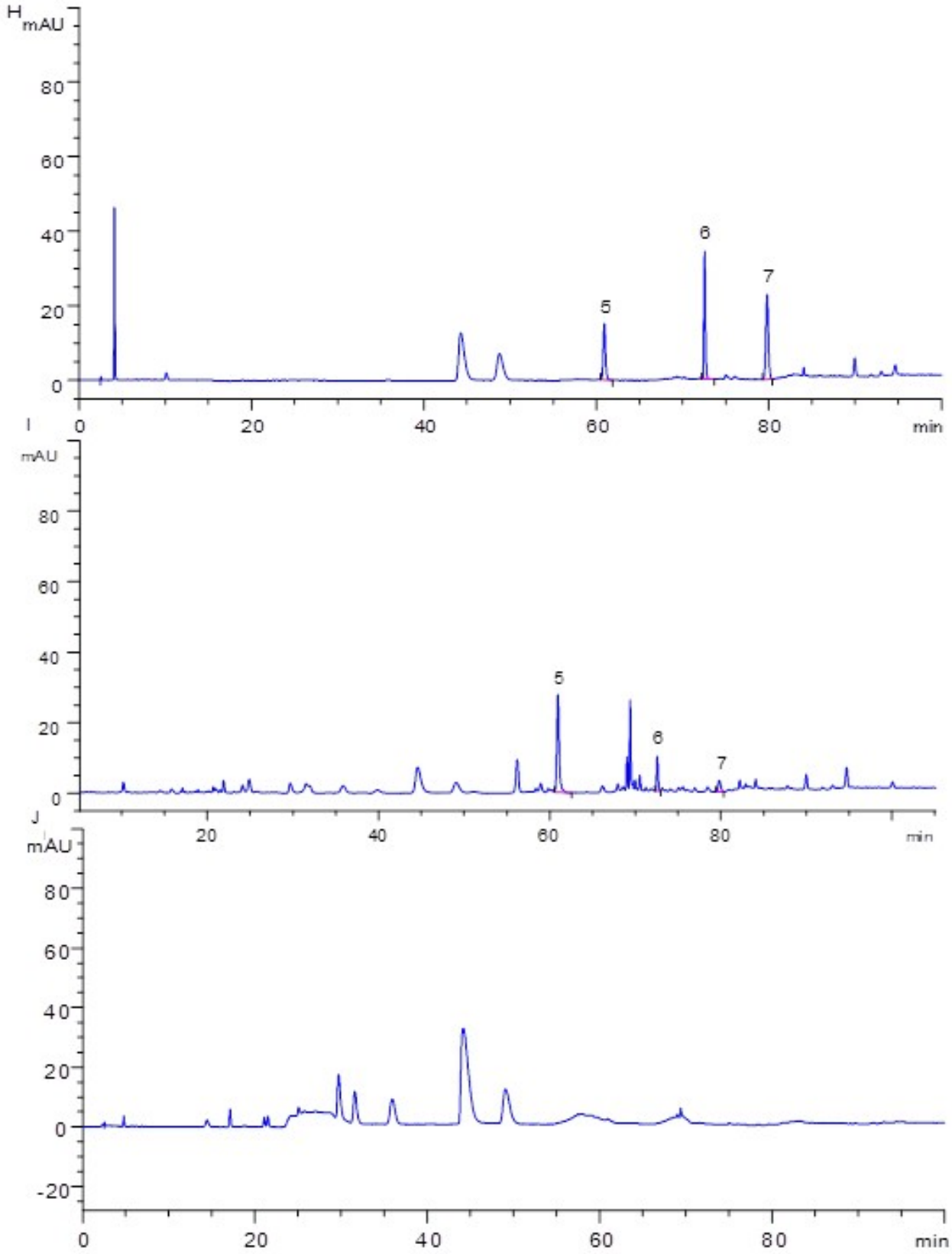

Fig. 4: Chromatograms of mixed standard solutions (H), test solutions (I) and baicalin-baicalein-wogon in NCS (J) at 278nm. 5. baicalin 6. baicalein 7. wogonin

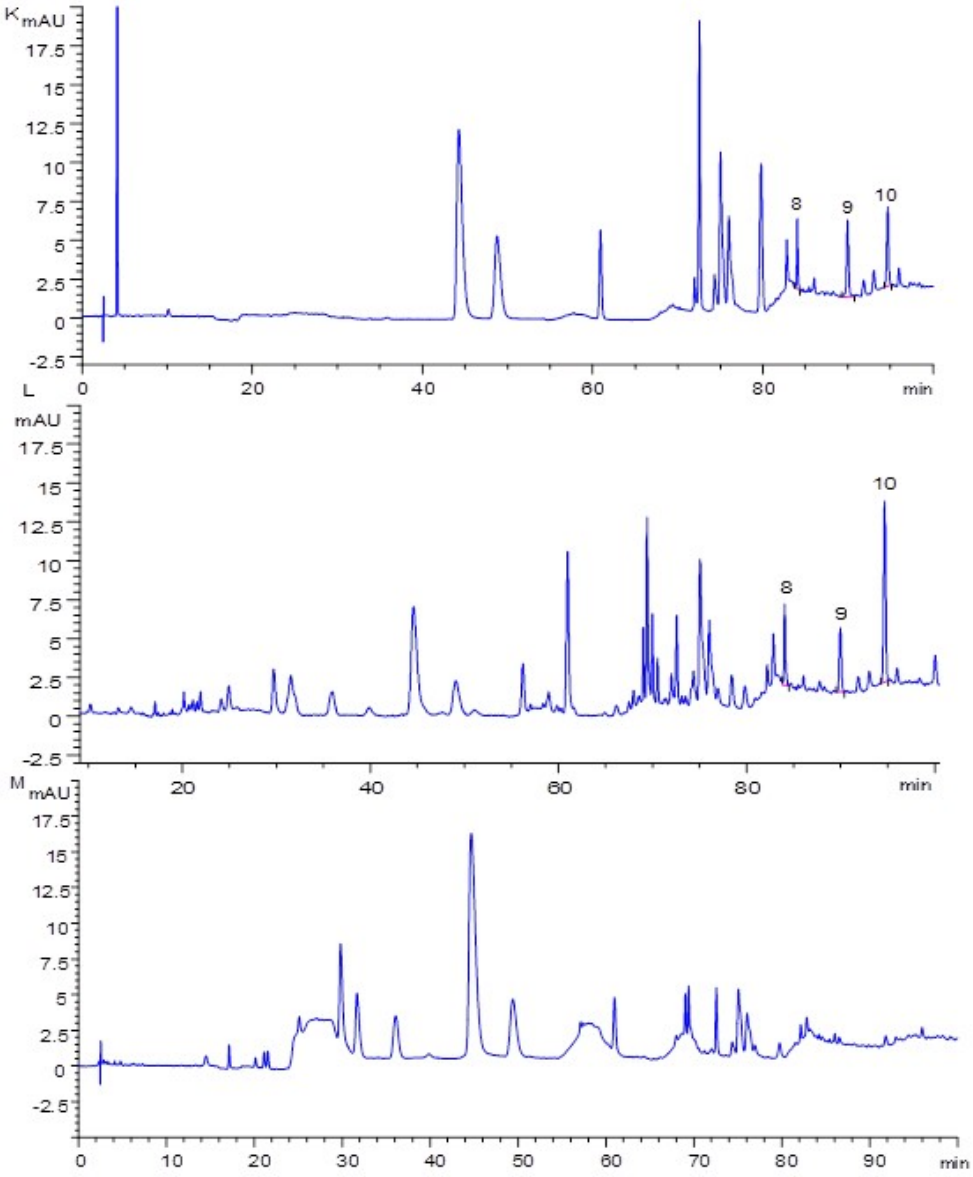

Fig. 5: Chromatograms of mixed standard solutions (K), test solutions (L), rhein-emodin-chrysophanol NCS (M) at 254nm. 8. rhein 9. emodin 10. chrysophanol

Table 2: Linear relationships of various constituents

Constituent	Standard curve	Linearity range ($\mu g \cdot mL^{-1}$)	*r*	LOD ($\mu g \cdot mL^{-1}$)	LOQ ($\mu g \cdot mL^{-1}$)
Gallic acid	y = 10.757x +6.0275	10.28~514.00	0.9997	0.39	1.27
Catechin	y = 7.1809x - 1.5261	4.34~217.00	0.9999	0.76	2.51
Berberine	y =3.1776x + 14.684	42.82~2141.00	0.9999	0.71	2.35
Palmatine	y =6.6357x + 1.5034	10.74~537.00	0.9999	1.07	3.54
Baicalin	y = 1.2546x + 1.5413	46.28~2314.00	0.9998	1.55	5.15
Baicalein	y = 9.1941x - 0.6891	9.58~479.00	0.9999	0.73	2.41
Wogonin	y = 10.565x - 12.421	9.32~466.00	0.9999	0.59	1.95
Rhein	y = 0.8606x + 4.025	10.42~521.00	0.9997	0.46	1.51
Emodin	y = 5.3722x - 1.5073	2.96~148.00	0.9997	0.29	0.95
Chrysophanol	y = 8.1311x + 0.8794	1.96~98.00	0.9999	0.25	0.82

Table 3: Results of recovery test of ten active constituents

Constituent	Known content (mg)	Added amount (mg)	Measured amount (mg)	Recovery rate (%)	Average recovery (%)	RSD (%)
Gallic acid	2.983	3.080	6.001	97.99	99.98	1.12
	2.919	3.080	5.999	100.00		
	2.862	3.080	5.942	100.00		
	3.047	3.080	6.155	100.91		
	2.944	3.080	6.060	101.17		
	2.859	3.080	5.933	99.81		
Catechin	1.091	1.102	2.207	101.27	101.06	1.40
	1.145	1.102	2.263	101.45		
	1.117	1.102	2.257	103.45		
	1.001	1.102	2.113	100.91		
	1.06	1.102	2.157	99.55		
	1.162	1.102	2.261	99.73		
Berberine	6.033	6.000	12.032	99.98	100.28	1.20
	5.952	6.000	11.953	100.02		
	6.000	6.000	12.014	100.23		
	6.027	6.000	12.185	102.63		
	5.964	6.000	11.922	99.30		
	5.982	6.000	11.952	99.50		
Palmatine	3.005	3.010	6.021	100.20	101.12	1.39
	2.947	3.010	5.947	99.67		
	2.872	3.010	5.961	102.62		
	2.903	3.010	6.007	103.12		
	3.011	3.010	6.038	100.56		
	3.065	3.010	6.091	100.53		
Baicalin	14.888	14.830	29.739	100.14	100.07	1.79
	14.412	14.830	29.075	98.87		
	15.325	14.830	29.998	98.94		
	13.039	14.830	27.71	98.93		
	14.446	14.830	29.801	103.54		
	14.899	14.830	29.726	99.98		
Baicalein	2.992	2.965	5.966	100.30	100.02	0.71
	3.005	2.965	5.945	99.16		
	2.832	2.965	5.778	99.36		
	2.955	2.965	5.943	100.78		
	2.799	2.965	5.787	100.78		
	2.944	2.965	5.901	99.73		
Wogonin	2.969	2.970	5.926	99.56	100.03	1.21
	2.831	2.970	5.816	100.51		
	2.959	2.970	5.989	102.02		
	2.799	2.970	5.773	100.13		
	2.995	2.970	5.918	98.42		
	2.952	2.970	5.908	99.53		
Rhein	3.037	3.030	6.049	99.41	99.97	1.91
	2.998	3.030	6.046	100.59		
	3.055	3.030	5.997	97.10		
	3.017	3.030	6.055	100.26		
	2.894	3.030	6.014	102.97		
	3.053	3.030	6.067	99.47		
Emodin	0.656	0.651	1.311	100.61	100.84	1.92
	0.602	0.651	1.283	104.61		
	0.631	0.651	1.281	99.85		
	0.694	0.651	1.341	99.39		
	0.602	0.651	1.251	99.69		
	0.675	0.651	1.332	100.92		
Chrysophanol	0.646	0.650	1.301	100.77	100.36	0.79
	0.646	0.650	1.294	99.69		
	0.603	0.650	1.251	99.69		
	0.698	0.650	1.347	99.85		
	0.644	0.650	1.297	100.46		
	0.645	0.650	1.306	101.69		

Table 4: Results of content determination of various constituents ($mg \cdot g^{-1}$, n=3)

Batch number	20180101		20180102		20180103	
	Content	RSD (%)	Content	RSD (%)	Content	RSD (%)
Gallic acid	4.917±0.052	1.05	4.273±0.016	0.38	4.531±0.065	1.44
Catechin	1.903±0.027	1.43	1.889±0.021	1.12	1.973±0.027	1.37
Berberine	9.994±0.180	1.81	9.427±0.138	1.47	9.393±0.128	1.36
Palmatine	5.113±0.080	1.56	5.683±0.102	1.80	4.935±0.072	1.46
Baicalin	24.730±0.252	1.02	24.046±0.236	0.98	25.030±0.100	0.40
Baicalein	4.967±0.037	0.75	5.053±0.076	1.51	4.437±0.066	1.48
Wogonin	4.872±0.063	1.30	4.515±0.039	0.86	4.432±0.045	1.02
Rhein	5.013±0.017	0.34	5.255±0.073	1.39	5.836±0.038	0.65
Emodin	1.051±0.019	1.78	1.005±0.013	1.26	1.996±0.020	0.99
Chrysophanol	1.022±0.016	1.61	0.978±0.009	0.95	0.911±0.018	1.93

DISCUSSION

Firstly, mixed standard solutions was scanned under the wavelength of 190～400 nm to obtain the wavelength range with absorption maximum of each component. The detection wavelengths were confirmed as 225 nm (gallic acid and catechin), 245 nm(berberine and palmatine), 278 nm (baicalin, baicalein and wogonin) and 348nm (rhein, emodin and chrysophanol) referring to "Pharmacopoeia of the People's Republic of China" and related literature (Zhao YB and Xu DQ, 2020; Wang FC *et al*., 2020; Liu L *et al*., 2019).

Secondly, different extraction methods(refluxing extraction, ultrasonic extraction, solvent extraction and soxhlet extraction) and different extraction time (10min, 20min, 30min, 40min, 50min and 60min) were investigated (Liang *et al*., 2018; Li *et al*., 2018), respectively. Finally, the test samples were extracted for 30min via ultrasound taking extraction rate and economic factors into consideration.

At last, mobile phase systems with various ratios of acetonitrile-water, methanol-water, methanol-phosphoric acid-water were inspected considering separation effects between each analyte and other components as indexes in this experiment (Shui D *et al*., 2018; Li ZY *et al*., 2018; Shi YL *et al*., 2017). Gradient elution of methanol(A)-0.1% phosphoric acid solution(B) was selected ultimately.

Through consulting a large number of literatures, two methods were used for the quality control of XS at present. TLC was used to the qualitative identification of CR and SR in XS. Besides, HPLC was used for the detection of berberine, emodin and chrysophanol in XS. However, these methods cannot reflect the contents of effective components in the preparation comprehensively and systematically. In this study, a novel HPLC-DAD method was established for simultaneous detection of ten active constituents (gallic acid, catechin, berberine, palmatine, baicalin, baicalein, wogonin, rhein, emodin and chrysophanol) in XS. The developed method was proved to be simple, stable and accurate after method validation including precision, repeatability, stability and recovery experiments, which can provide a scientific basis for the qualitative and quantitative analysis of active ingredients in XS. This study can not only comprehensively reflect the quality of XS, but also conform to the characteristics of working together of multi-component combination of TCM. The proposed method provide reference for quality control, drug research and development, preparation processing and stability research of XS as well.

CONCLUSIONS

Compound preparation of traditional Chinese medicine (TCM) possesses the characteristics of complex composition and multiple effective components, which is the same as TCM. With the advance of the combination of TCM and western medicine (Liu SH *et al*., 2020; Lee MS, 2020; Yang L and Zhao XM, 2020), quantification of active constituents plays a crucial role in the development, application and quality control of TCM compound preparation. Thus, a simple, stable and rapid HPLC-DAD method was established and applied for the simultaneous detection of ten active constituents (gallic acid, catechin, berberine, palmatine, baicalin, baicalein, wogonin, rhein, emodin and chrysophanol)) in XS. The proposed method can offer methodological basis and reference for quality control of XS.

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REFERENCES

Chinese Pharmacopoeia Commission (1993). Departmental Standards of Traditional Chinese

Medicine. Chinese Materia Medica preparation (12 volumes), The Medicine Science and Technology Press of China, Beijing, China, p.101.

Chinese Pharmacopoeia Commission (2015). Pharmacopoeia of the People's Republic of China, Volume I, The Medicine Science and Technology Press of China, Beijing, China, p.1124.

Fu XJ, Zhu CW and Wang XG (2002). Determination of dissociation emodin and chrysophanol in xiedusan by RP-HPLC. *Chin. Pharm. J.,* **37**(04): 66-68.

Gai XH, Liu SX, Ren T, Liu Y and Tian CW (2018). Research progress on chemical constituents *of Coptidis rhizoma* and its pharmacological activities. *Chin. Tradit. Herb. Drugs,* **49**(20): 4919-4927.

Gao JF, Zhang ZH and Wu YZ (2008). Study on the quality standard of xiedu san. *China Pharm.,* **17**(18): 41-42.

Hao C, Zhao HQ, Cui L, Liu W, Liu FQ, Wang X and Mu Y (2018). Quality control of huangqin dispensing granules. *Chin. J. Hosp. Pharm.,* **38**(7): 723-727.

Jin LX, Jin LJ, Luan ZQ, Liu C and Pan C (2020). Research progress on chemical constituents and pharmacology of rhubarb. *Inf. Tradit. Chin. Med.,* **37**(01): 121-126.

Jin PF, Mou HR, Qian YP and Cui B (2001). Determination of berberine hydrochloride in xiedusan by HPLC. *Chin. J. Tradit. Med. Sci. Technol.,* **8**(2): 102.

Lee MS (2020). The constant progression of integrative medicine research. *Integr. Med. Res.,* **9**(1): 33-34.

Li EZ, Liu YF, Liu YJ, Jiang S and Wang YN (2018). Simultaneous determination of six components in *Scutellaria baicalensis* Georgi from different districts by HPLC. *Chin. J. Hosp. Pharm.,* **38**(9): 946-948.

Li MM, Zhang HT, Bai WR, Wang ZZ and Xiao W (2018). Simultaneous determination of six constituents in Yitai capsules by HPLC. *Chin. Tradit. Pat. Med.,* **40**(6): 1316-1319.

Li ZY, Chen XT, Wang LL and Xu WJ (2018). Study on the content of main active components in sanhuang xiexin dispensing granules and standard decoction. *J. Jiangxi Univ. Tradit. Chin. Med.,* **30**(04): 79-81+85.

Liang L, Chen JL, Liu Z, Liu L and Song HP (2018). Simultaneous determination of nine constituents in jiangtangjia tablets by HPLC. *Chin. Tradit. Pat. Med.,* **40**(6): 1312-1315.

Liu L, Chen T, Cheng XD, Meng SS and Tang L (2019). Simultaneous determination of the content of five ingredients in sanhuang lotion by HPLC-DAD. Asia-*Pac. Tradit. Med.,* **15**(12): 25-28.

Liu SH, Wang YL, Lan Y, Lu Z, Zhang S and Long K (2020). Clinical practice of the combination of chinese and western medicine in the treatment of a patient with the coronavirus disease. *China Pharm.,* **29**(06): 38-40.

Shi YL, Bao YQ, Ling Y and Zhou CX (2017). Component content difference of dahuang huanglian xiexin decoction by HPLC under conditions of different dipping. *Liaoning J. Tradit. Chin. Med.,* **44**(05): 1011-1013.

Shui D, Wang YQ and Geng RH (2018). Determination of thirteen active constituents in sanhuang xiexin decoction by HPLC. *Chin. J. Hosp. Pharm.,* **38**(21): 2235-2239.

Sun HQ, Li JP, Liu LK, Yang CF, Zuo WM and Li CH (2018). progress in research of chemical constituents and pharmacological actions of rhubarb. *Qinghai Prataculture,* **27**(1): 47-51.

Sun J and Yan GH (2018). Research on the progress of chemical composition and active ingredient pharmacological activity of chinese herbal medicine *Rhizoma coptidis*. *China Contin. Med. Educ.,* **10**(8): 140-143.

Vieiraa ES and Elenara LS (2020). Application of a new validated HPLC-PDA method for simultaneous determination of curcumin and melatonin in hyaluronic acid-coated nanoemulsions. *J. Braz. Chem. Soc.,* **31**(3): 467-475.

Wang FC, Li DH, Guo WB, Feng XR, Ma X and Yang PR (2020). Simultaneous determination of ten components in zhizi jinhua pills by HPLC-DAD. *China J. Pharm. Anal.,* **40**(01): 145-154.

Wang YJ, Feng SH, Cheng JT and Chen S (2018). Research progress on chemical constituents and pharmacological action of anthraquinone in rhei radix. *Chin. J. Exp. Tradit. Med. Formulae.,* **24**(13): 227-234.

Yang L and Zhao XM (2020). Integrated Chinese and Western medicine for acute guillain-barré syndrome treatment. *De Gruyter.,* **11**(1): 38-47.

Zhao YB and Xu DQ (2020). Simultaneous determination of multi-components in jianwei xiaoyan granules by HPLC with Wavelength Switching. *China Pharm.,* **23**(03): 581-585.