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Determination of the Fingerprint Chromatogram of Sanhuang Tablet by HPLC

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The establishment of fingerprint of Sanhuang tablets by HPLC offers an effective way for the quality control of Chinese patent medicine and its production. The chromatographic conditions are as follows: the column for the VP ODS C_{18} (4.6 mm × 150 mm, 5 μm), temperature is 30 ºC, a flow rate of 1.0 mL/min, detection at 280 nm, with methanol and 0.2 % aqueous solution of phosphate acid for mobile phase gradient elution. The results showed that 19 peaks were indicated on the HPLC fingerprint of Sanhuang tablet. According to the computer software-assisted analysis of similarity, the similarity of 10 batches of Sanhuang tablet was not lower than 0.98. The method was simple and reliable.

Key Words: San Huang tablet, HPLC, Fingerprints.

INTRODUCTION

The quality control for traditional Chinese medicine is one of the key technologies for its modernization. Nowadays, the high performance liquid chromatographic (HPLC) fingerprint techniques have been increasingly used in the quality control of traditional Chinese medicine[1-6]. Sanhuang tablets mainly consist of radix et, rhizome rhei, berberine hydrochloride and extractum scutellariae. It can clear away the heat and toxic material, purge intense heat and relax the bowel[7]. There are more than a dozen manufacturers of Sanhuang tablets, among which Handan pharmaceutical company is the best one. At present HPLC research for Sanhuang tablets mainly concentrates on the ingredient content determination. In order to reflect its real intrinsic quality, this experiment has conducted the HPLC fingerprint research to Sanhuang tablets, which is the basis for its quality appraisal.

EXPERIMENTAL

Sanhuang tablet was purchased from Handan pharmaceutical Co. Ltd. Methanol were HPLC grade and purchased from Damao Chemicals Co. Ltd., Tianjin, China. Water were HPLC grade. The reference substance of berberine hydrochloride was homemade. The other chemicals and solvents used in this experiment were analytical grade.

HPLC method was performed on a Shimadzu 2010A HPLC system. A VP ODS C_{18} column (150 mm × 4.6 mm, 5 μm size) was used.

Preparation of reference solution: 4 mg berberine hydrochloride was weight precisely, then put into 10 mL volumetric flask and set constant volume by adding methanol, finally made as reference solution with 0.4 mg mL^{-1}.

Preparation of sample solution: The sample of Sanhuang was obtained from Handan pharmaceutical company, Lot Number is 1512, 109286, 109486, 109488, 109606, 109609, 109557, 110003, 110012 and 110048. Two tablets were taken, removed coating and crushed. Then 0.25 g powder was put into 10 mL of volumetric flask, dissolved with methanol-phosphate (100:1) and set the volume, under ultrasonic extraction at 35 ºC for 45 min, then filtered by 0.45 μm membrane, directly introduced as the test sample[8-10].

Chromatographic conditions: Chromatographic column: VP ODS C_{18} (150 mm × 4.6 mm, 5 μm); volume flow: 1.0 mL min^{-1}; column temperature: 30 ºC, 280 nm of detection wavelength; injection volume: 10 μL; mobile phase: methanol-0.2 % phosphoric acid water solution. The gradient condition was shown in Table-1. Validation of the method was done.

Under the above chromatographic conditions and methods, the same test samples (lot number:1512)were continuously analyzed 5 times. Results shown in 5 determination atlases the RSD of retention time in 19 common peaks is 0.26-1.48 %, peak area's RSD is 0.51-1.76 %. In the fingerprint chromatogram, the peak relative retention time is consistent with the peak area, its similarity is bigger than 97 %, the instrument precision is good.

TABLE-1
GRADIENT ELUTION OF SANHUANG TABLET HPLC

Time (min)	Methanol (%)	0.2 % phosphoric acid water solution (%)
0	5	95
5	15	85
7	30	70
22	43	57
24	43	57
31	50	50
33	50	50
44	58	42
50	78	22
60	85	15
65	90	10

Five different samples (lot number:1512)were taken and made into 5 test solution. Results indicated that the RSD of retention time in 19 common peaks is 0.14-1.45 %, peak area's RSD is 0.58-1.75 %, the similarity of 5 samples' chromatogram is bigger than 98 %, this method had high stability and good repeatability.

The same samples (lot number:1512)were tested under 0, 2, 4, 8, 10, 15, 20, 24 and 36 h. The RSD of retention time and peak area in 19 common peaks are separately 0.27-1.34 % and 0.37-1.98 %, through 9 analysis, the similarity of chromatogram is bigger than 97 %, the sample is stable within 36 h.

RESULTS AND DISCUSSION

Similarity calculation of chromatogram fingerprint: In accordance with the selected chromatographic conditions, Sanhuang tablets of 10 lot numbers were conducted fingerprint detection and record chromatograph chart. The data was input into the calculation software of traditional medicine fingerprint similarity (2004A)[11-13], 0-65 min chromatograms were intercepted and the reference chromatogram was generated by using average value methods, then its similarity was calculated and there were 19 common peaks (Fig. 1), the retention time of the common peak and peak area are shown in Table-2 and the similarity in Table-3. The sum of peak area of the 19 common peaks accounted for more than 90 % of the total peak area, the RSD of the retention time was 0.42 %, similarity is bigger than 96 %, which meet the technical requirements of fingerprint. Based on Fig. 1, the reference solution of berberine hydrochloride was determined, the characteristic peak of No. 9 was obtained by the retention time.

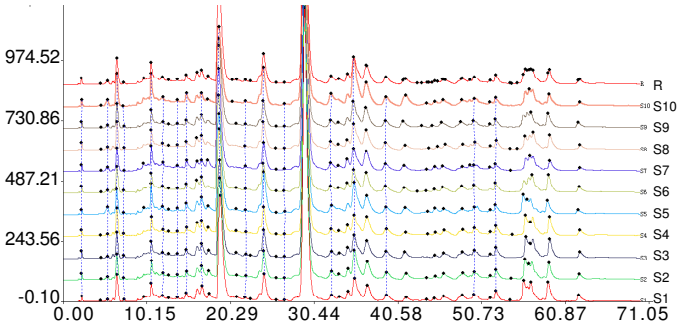

Fig. 1. HPLC fingerprint pattern of Sanhuang tables of 10 lot numbers

TABLE-2
RETENTION TIME OF COMMON PEAKS AND PEAK AREA

Sample No.	Peak number								
	1	2	3	4	5	6	7	8	9
S1	239474.1	165007.9	2191663	140203.8	2570132	867184.3	634299.3	1604508	3314180
S2	289120.1	238247.3	2035225	153317	3909388	1001897	1136666	1244325	3069629
S3	305924.8	160390.5	2092031	231841	3379555	868636.1	676182.4	1608663	3006380
S4	249707.8	168204.8	2257415	168952	3727018	853243.2	661042.1	1738212	1796986
S5	271967.9	1095069	2163534	195250.3	3759341	1311566	984296.6	2101357	3699634
S6	272309.3	200665.1	2167681	199914.6	3803043	983288.1	697159.4	1849649	3427602
S7	273653.1	398954.4	1883698	60019.98	3493397	2230515	941147.9	1953562	1263679
S8	320867.3	240230	2334137	202555.1	3886898	1171916	719330.6	1803071	3284907
S9	268556.4	228670.6	1992027	147176.8	3759109	952516.9	765529.9	1587870	4363785
S10	394411.8	747930.1	2867945	0	6188142	2659533	1188885	2402226	3134072
Retention time	2.227	5.377	6.505	7.352	10.67	12.076	13.837	14.931	16.77
Reference fingerprint	288599.3	364336.9	2198536	149923.1	3847602	1290030	840453.8	1789344	3036085
RSD of peak area (%)	15.34	85.76	12.24	47.2	23.71	49.11	24.53	17.78	29.43

	10	11	12	13	14	15	16	17	18	19
S1	16989190	537328.4	4587648	264449.9	45864340	1258777	4290070	1442642	1843600	1406053
S2	18278680	250393.2	4796268	361387.2	47409510	980007.7	5330834	1650478	2027783	1284971
S3	17881530	176440.7	4287078	197097.4	43252020	907736.3	3799947	1317744	1853436	1328499
S4	20439130	237020.2	4761597	308457.3	46659740	889882.4	3822604	1448133	1808992	1249110
S5	17593250	1161931	7040948	612886.7	45635360	1622805	7345790	1740809	2322270	1486247
S6	19023990	235363.3	5161355	324365.9	46979150	961153.1	4084955	1512788	1883481	2107991
S7	19358460	313918.2	4819292	382945.8	50010740	1107828	4093036	1272906	1473467	1067599
S8	20462630	222403.2	4906296	369427.9	45289120	944284.1	4959238	1490933	2296844	1300189
S9	17927400	233074.9	4546649	310979.1	44501430	916664.3	6371736	1492923	1933007	1151951
S10	17136880	329037.1	6998212	578739.4	48791660	2285600	10364190	2141466	2542780	1182937
Retention time	18.897	22.099	24.282	26.819	29.199	32.471	35.229	39.135	49.809	52.423
Reference fingerprint	18509114	369691	5190534	371073.7	46439306	1187474	5446240	1551082	1998566	1356555
RSD of peak area (%)	6.82	80.04	19.1	35.16	4.29	37.66	38.35	16.04	15.51	21.42

TABLE-3
SIMILARITY OF HPLC FINGERPRINT

Lot Number	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Similarity	0.990	0.993	0.992	0.992	0.987	0.991	0.993	0.993	0.987	0.984

Selection of chromatographic conditions: The test solution was prepared by ultrasonic extraction based on the components of Sanhuang tablets and relevant literatures. The extracting solvents include: methanol, methanol-water, methanol-phosphoric acid, ethanol, ethanol-water, ethanol-phosphoric acid, acetone, acetone-water, acetone-phosphoric acid. There are the most peaks and the highest peak in the test solution of methanol-phosphoric acid (100:1), so the solution finally was considered as extracting solution. The extracting time includes: 35, 45 and 60 min, among which the fingerprints of 45 and 60 min are similar, peak number and peak height are greater than that of 35 min, consideration of time and energy savings, 45 min was the extracting time.

The selection of mobile phase: the experiment adopted gradient elution, gradient elution was conducted by using acetonitrile-water, acetonitrile-phosphoric acid, acetonitrile-acetic acid, methanol-water, methanol-phosphoric acid, methanol-acetic acid and taking the effect of acetic acid and phosphoric acid concentration, the results show the chromatogram of methanol-0.2 % phosphate acid aqueous solution mobile phase was stable, has most peaks and shaper peak and the test solution is easy to absorb and separate under the condition, so it was adopted as the mobile phase. Through further optimized gradient conditions under Table-1, each peak is well-distributed, the overall separation is good.

Choice of detection wavelength: considering the coloured substances in Sanhuang tablets, the detection wavelength was tested in UV and visible region separately. The fingerprint under the wavelength of visible region appeared low peak height, less peak number and the larger the wavelength the more obvious, so the experiment adopted UV-light with high absorption. Comparing peak number, peak height and shape characteristics of the overall map and the wavelength of 280 nm was selected.

Choice of column temperature: Comparing the fingerprints of column temperature under 30, 35 and 40 ºC, the peak height under 35 and 40 ºC became small and the diminishing under 40 ºC was significant, so the column temperature was selected as 30 ºC.

Under the selected conditions, the liquid chromatogram shows the largest number of peaks, good resolution and main peak of berberine hydrochloride was distinct. Methodological study indicates that the experimental method is stable, reliable and has some practical value on the evaluation of the intrinsic quality of Sanhuang tablets.

By the calculation software of traditional medicine fingerprint similarity and the reference of the average value chromatogram of Sanhuang tablets of 10 lot numbers, each similarity of the common peaks of 10 lot numbers is bigger than 96 %, which shows the stable quality of Sanhuang tablets. From the fingerprint, there were corresponding component peaks, but peak area ratios different, which indicates Sanhuang tablets of different lot number has significantly different content, the reasons may be lie in the raw material and unstable extracting technique, so for the manufacturers of Sanhuang tablets, it is important to fix origin raw herbs and stabilize the technique in order to secure the quality of traditional Chinese injection[14-17]. The production process control by fingerprint might monitor medicinal materials, intermediate product and finished product during the whole process timely and entirely, so as to improve the intrinsic quality of the finished product.

Conclusion

The establishment of fingerprint of Sanhuang tablets by HPLC offers an effective way for the quality control of Chinese patent medicine and its production. The chromatographic conditions are as follows: the column for the VP ODS C_{18} (4.6 mm × 150 mm, 5 μm), the temperature for 30 ºC, a flow rate of 1.0 mL/min, detection at 280 nm, with methanol and 0.2 % phosphate acid aqueous solution for mobile phase gradient elution. The results showed that 19 peaks were indicated on the HPLC fingerprint of Sanhuang tablet. According to the computer software-assisted analysis of similarity, the similarity of 10 batches of Sanhuang tablet was not lower than 0.98. The method was simple and reliable.

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