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Study on HPLC Fingerprint of *Coptis chinensis*

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The aim of this study is to establish the fingerprint of *Coptis chinensis* by HPLC. HPLC analysis was carried out with Kromasil C_{18} column (4.6 × 250 mm) at the wavelength of 300 nm at 30 °C. Acetonitrile-0.01 mol L^{-1} KH_2PO_4 solution (gradient elution) was used as the mobile phase at the flow rate of 1 mL min^{-1}. Different *Coptis chinensis* samples had ten common peaks with the similarity not less than 95 %. The areas of non-common peaks accounted for less than 10 % of the total peak area. This method was highly stable and repeatable and the established HPLC fingerprint provided evidence for the quality control of *Coptis chinensis*.

Keywords: *Coptis chinensis*, HPLC, Fingerprint.

INTRODUCTION

Coptis chinensis is the dry rhizome of *Coptis chinensis* Franch., *Coptis deltoidea* C.Y. Cheng et Hsiao or *Coptis teeta* Wall that are commonly known as "Weilian", "Yalian" and "Yunlian", respectively[1,2]. Since *Coptis chinensis* is widely produced in Chongqing, Sichuan Province and Hubei Province, *etc.*[3,4], this study aimed to primarily study the HPLC fingerprint of *C. chinensis* from different places of origin to ensure the quality of them in practice.

EXPERIMENTAL

Waters 515 HPLC; Empower chromatography work-station; KQ500B ultrasonic instrument (Kunshan Ultrasonic Instruments Co., Ltd.). Reagents: Acetonitrile (HPLC-grade, TEDIA); methanol (HPLC-grade, Hamobile Technology Co., Ltd.); ultra-pure water; other reagents were of analytical grade. Medicinal materials: The sources of *Coptis chinensis* medicinal materials are summarized in Table-1. Reference standard: Berberine hydrochloride (National Institutes for Food and Drug Control, batch No: 110713-200609); palmatine hydrochloride.

HPLC conditions: HPLC analysis was performed by using Kromasil C_{18} column (4.6 × 250 mm) at the wavelength of 300 nm and the temperature of 30 °C with the injection volume of 10 µL. Acetonitrile-0.01 mol L^{-1} KH_2PO_4 solution was used as the mobile phase at the flow rate of 1 mL min^{-1}. The gradient elution conditions are listed in Table-2.

Preparation of reference standard solutions: Berberine hydrochloride and palmatine hydrochloride were accurately weighed and placed in 25 mL volumetric flasks and were dissolved in methanol by sonication. After being cooled down to room temperature, the solutions were set to specific volumes by adding methanol, giving 14.4 and 12.8 µg mL^{-1} reference standard solutions, respectively.

Preparation of sample solutions: *Coptis chinensis* (2 g) were put in a plugged Erlenmeyer flask, into which was added 25 mL of water. After weighing, the materials were extracted for 0.5 h by sonication and cooled down to room temperature. After weight complement, the solution was filtered and 1 mL of the filtrate was collected and transferred in a 10 mL volumetric flask, which was set to specific volume by water, yielding the sample solution.

RESULTS AND DISCUSSION

According to previous literatures[1-4], acetonitrile-0.01 mol L^{-1} KH_2PO_4 solution was used as the mobile phase for gradient elution, which gave rise to ideal peak shape, steady baseline and appropriate retention times. After comparing the chromatograms obtained at the wavelengths of 230, 270, 300, 345 and 300 nm was selected owing to the steady baseline and equal areas of common peaks.

TABLE-1
C. chinensis SAMPLES

No.	Batch No.	Drugstore	Source	Place of origin
1	091111	Xiansheng Drugstore	Bozhou Yonggang Medicinal Herbs Factory Co., Ltd.	Sichuan Province
2	090628	Yifeng Super Pharmacy	Hubei Jingui Chinese Traditional Medicine Electuary Co., Ltd.	Hubei Province
3	091012	Dahua TCM Drugstore	Anhui Huilong Chinese Herbal Slices Co., Ltd.	Sichuan Province
4	090630	Nanjing Dazhong Pharmacy	Anhui Yishengtang Chinese Herbal Medicine Co., Ltd.	Sichuan Province
5	090716	Nanjing Xinyaote Drugstore	Anhui Fengyuan Pharmaceutical Co., Ltd.	Sichuan Province
6	091111	Nanjing Jianjun Pharmacy	Bozhou Yonggang Medicinal Herbs Factory Co., Ltd.	Sichuan Province
7	090620	Nanjing Tongren Pharmacy	Bozhou Yonggang Medicinal Herbs Factory Co., Ltd.	Sichuan Province
8	091019	Laobaixing Pharmacy	Anhui Fengyuan Pharmaceutical Co., Ltd.	Sichuan Province
9	090701	Baixingyuan Great Pharmacy	Bozhou Yonggang Medicinal Herbs Factory Co., Ltd.	Sichuan Province
10	090216	Nanjing Zhilin TCM Drugstore	Xuzhou Pengzu Chinese Traditional Medicine Slices Co., Ltd.	Sichuan Province
11	091117	Nanjing Jinling Pharmacy	Nanjing Lujiang Chinese Traditional Medicine Slices Co., Ltd.	Hebei Province

TABLE-2
GRADIENT ELUTION CONDITIONS

Time (min)	Acetonitrile (%)	KH_2PO_4 solution (%)
	15	85
5	20	80
20	20	80
50	30	70
55	15	85

Precision test: *Coptis chinensis* were randomly selected and prepared into sample solutions. After six times of continuous injection, chromatograms were determined to find out the common peaks. The RSD values of relative retention time and peak area of common peaks were 0.05-0.5 and 0.47-1.34 %, respectively.

Repeatability test: *Coptis chinensis* in the same batch were randomly selected and prepared into five sample solutions. Chromatograms were recorded to determine the common peaks. The RSD values of relative retention time and peak area of common peaks were 0.05-2.02 and 0.62-1.47 %, respectively, indicating that the method was highly repeatable.

Stability test: The solutions of *Coptis chinensis* in the same batch were subjected to HPLC analysis at 0, 1, 2, 4, 8, 12 and 24 h, respectively. The RSD values of relative retention time and peak area of common peaks were 0.09-2.04 and 0.5-2.34 %, respectively, suggesting that the samples remained stable within 24 h.

Selection of reference peak: The peak of berberine that was the main pharmaceutical ingredient of *Coptis chinensis* was selected as the reference due to maximum peak and desirable, stable peak shape.

Calibration of reference standard peaks in the fingerprint: A mixture of reference standard solutions of berberine hydrochloride and palmatine hydrochloride was subjected to HPLC analysis under the conditions described above. The retention times of palmatine hydrochloride and berberine hydrochloride were (38.794 ± 0.039) and (40.294 ± 0.039) min, respectively (Fig. 1).

Parameters of HPLC fingerprints of different *Coptis chinensis*: By using Waters 515 HPLC and Empower chromatography workstation, the fingerprints of different *Copitis chinensis* medicinal materials were plotted by comparing *.cdf files with those in the "SOP of similarity evaluation system for chromatographic fingerprint of traditional Chinese medicine (Version 2004A)" released by Chinese Pharmacopoeia Commission (Fig. 2).

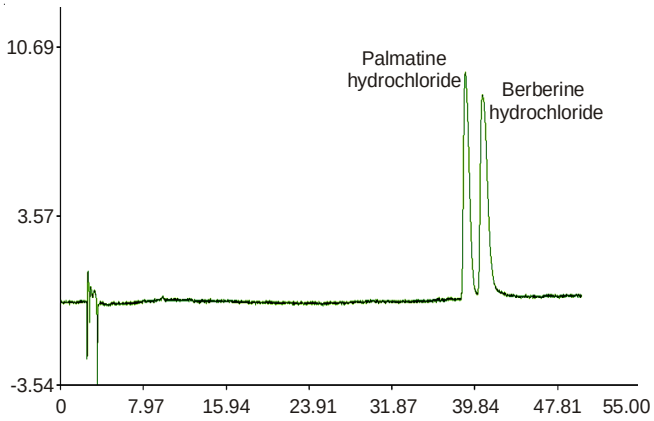

Fig. 1. Chromatogram of mixed berberine hydrochloride and palmatine hydrochloride reference standard solutions

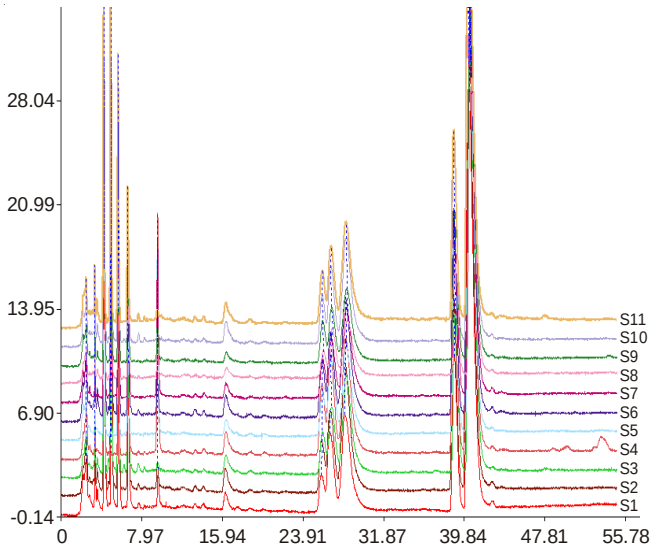

Fig. 2. Matching between different *Coptis chinensis* medicinal materials

Calibration of common peaks: Common peaks were calibrated according to the retention times, *i.e.*, Peak 1 [(4.247 ± 0.013) min], Peak 2 [(4.950 ± 0.015) min], Peak 3 [(5.664 ± 0.016) min], Peak 4 [(6.675 ± 0.023) min], Peak 5 [(9.661 ± 0.045) min], Peak 6 [(25.705 ± 0.031) min], Peak 7 [(26.591 ± 0.045) min], Peak 8 [(28.097 ± 0.041) min], Peak 9 [(38.794 ± 0.039) min] and Peak 10 [(40.294 ± 0.039) min] (Fig. 3).

TABLE-3
RELATIVE PEAK AREAS OF COMMON PEAKS

	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6-8	Peak 9	Peak 10
Sample 1	0.1699	0.1595	0.1190	0.0741	0.0189	0.4045	0.2642	1.0000
Sample 2	0.1548	0.1556	0.1201	0.0602	0.0112	0.4319	0.2627	1.0000
Sample 3	0.1342	0.1409	0.1099	0.0617	0.0040	0.4235	0.2740	1.0000
Sample 4	0.0733	0.0010	0.0566	0.0574	0.1015	0.4778	0.3243	1.0000
Sample 5	0.1194	0.0051	0.0936	0.0734	0.1195	0.4629	0.3073	1.0000
Sample 6	0.1455	0.1236	0.1131	0.0682	0.0165	0.4276	0.2850	1.0000
Sample 7	0.1291	0.0496	0.1066	0.0789	0.0920	0.3788	0.2839	1.0000
Sample 8	0.1680	0.1290	0.1352	0.0730	0.0249	0.3664	0.2852	1.0000
Sample 9	0.1407	0.1229	0.1183	0.0668	0.0265	0.4091	0.3142	1.0000
Sample 10	0.1461	0.1334	0.1177	0.0689	0.0039	0.4020	0.2794	1.0000
Sample 11	0.1467	0.1428	0.1193	0.0683	0.0043	0.4752	0.2938	1.0000

TABLE-4
RELATIVE RETENTION TIMES OF COMMON PEAKS

	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10
Sample 1	0.1062	0.1235	0.1413	0.1657	0.2376	0.6376	0.6583	0.6946	0.9632	1.0000
Sample 2	0.1053	0.1227	0.1405	0.1645	0.2366	0.6407	0.6614	0.6975	0.9644	1.0000
Sample 3	0.1047	0.1225	0.1398	0.1637	0.2359	0.6388	0.6619	0.6961	0.9633	1.0000
Sample 4	0.1060	0.1229	0.1403	0.1643	0.2372	0.6403	0.6610	0.6979	0.9636	1.0000
Sample 5	0.1047	0.1217	0.1394	0.1633	0.2355	0.6388	0.6614	0.6969	0.9616	1.0000
Sample 6	0.1055	0.1225	0.1399	0.1639	0.2364	0.6399	0.6610	0.6995	0.9636	1.0000
Sample 7	0.1051	0.1224	0.1402	0.1637	0.2358	0.6401	0.6607	0.6966	0.9621	1.0000
Sample 8	0.1049	0.1221	0.1398	0.1632	0.2352	0.6402	0.6620	0.6990	0.9618	1.0000
Sample 9	0.1050	0.1223	0.1400	0.1635	0.2352	0.6396	0.6606	0.6981	0.9613	1.0000
Sample 10	0.1055	0.1228	0.1402	0.1642	0.2362	0.6402	0.6609	0.6989	0.9632	1.0000
Sample 11	0.1050	0.1224	0.1402	0.1642	0.2366	0.6410	0.6629	0.6985	0.9628	1.0000
Mean	0.1053	0.1225	0.1401	0.1640	0.2362	0.6397	0.6611	0.6976	0.9628	1.0000
RSD (%)	0.46	0.39	0.35	0.42	0.33	0.16	0.18	0.21	0.10	0.00

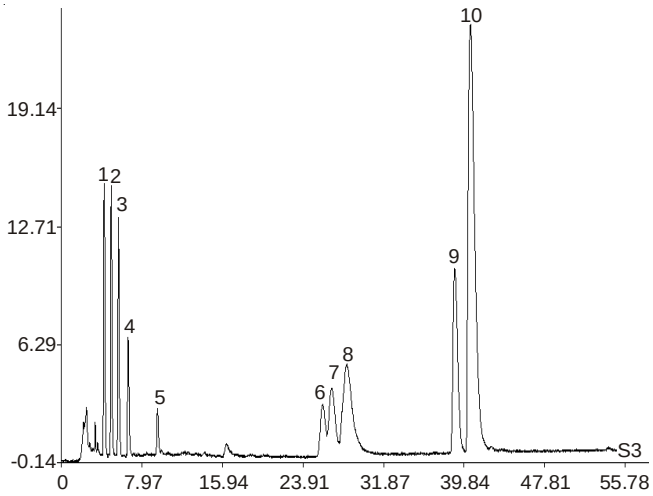

Fig. 3. Calibration of common peaks in HPLC fingerprint

Relative peak areas and relative retention times of common peaks: The relative peak areas and relative retention times of common peaks are listed in Tables 3 and 4.

Common and non-common peak areas: The common and non-common peak areas are summarized in Table-5.

Similarity between fingerprints of different *Coptis chinensis*: Similarity between the fingerprints of different *Coptis chinensis* was calculated by using "SOP of Similarity Evaluation System for Chromatographic Fingerprint of traditional Chinese Medicine (Version 2004A)" based on reference fingerprint (Table-6).

Conclusion

The fingerprints of different *Coptis chinensis* were highly resembled (> 95 %). There were a total of ten common peaks, the areas of which accounted for over 90 % of the total peak area. Moreover, the areas of Peak 9 and Peak 10 exceeded 10 % of the total peak area. This method was highly stable and repeatable and the established HPLC fingerprint provided evidence for the quality control of *Coptis chinensis*.

TABLE-5
COMMON AND NON-COMMON PEAK AREAS

	Total area	Area of common peak	Area of non-common peak	Proportion of non-common peak area to total peak area (%)
Sample 1	3095.118	2958.021	137.097	4.43
Sample 2	3839.978	3692.551	147.427	3.84
Sample 3	2865.707	2759.168	106.539	3.72
Sample 4	3576.892	3432.219	144.673	4.04
Sample 5	2667.279	2575.739	91.54	3.43
Sample 6	3630.735	3485.619	145.116	4.00
Sample 7	2587.461	2503.989	83.472	3.23
Sample 8	1880.271	1824.310	55.961	2.98
Sample 9	2304.302	2228.564	75.738	3.29
Sample 10	3222.030	3106.495	115.535	3.59
Sample 11	3285.354	3101.656	183.698	5.59

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TABLE-6
SIMILARITY BETWEEN FINGERPRINTS OF DIFFERENT *C. chinensis* MEDICINAL MATERIALS

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11
Correlation coefficient	0.996	0.997	0.998	0.985	0.987	0.999	0.994	0.996	0.999	0.998	0.997
Cosine of included angle	0.998	0.998	0.999	0.990	0.993	1.000	0.997	0.998	0.999	0.999	0.998

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