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Chemical Fingerprint and Quantitative Analysis of *Cistanche Deserticola* by HPLC-DAD-ESI-MS

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ABSTRACT

An HPLC-DAD-ESI-MS method was employed for the chromatographic fingerprint analysis of *Cistanche deserticola* (Roucongrong in Chinese). Eleven batches of samples were analyzed to establish the reference fingerprint of *C. deserticola*. Eight peaks in the fingerprint of all the 11 batches of samples were assigned as "characteristic peaks", and identified by comparing their retention time and mass spectra with those of the reference substances. The similarity of the 11 batches of samples was evaluated by a simulative mean chromatogram. The results indicated that the samples from different origins shared similar HPLC fingerprints. In addition, an HPLC-DAD method was developed for simultaneous determination of the contents of the eight compounds in *C. deserticola*. All eight compounds showed good linear regression ($R^2 > 0.9998$) within test ranges and the recovery of the method was in the range of 93.65 - 109.79%, indicating that the developed method combining chromatographic fingerprint with quantification analysis could readily be applied for the quality control of *C. deserticola*.

Key words: chromatographic fingerprint, quantitative analysis, *Cistanche deserticola*, HPLC-DAD-ESI-MS

INTRODUCTION

Cistanche deserticola Y. C. Ma (*C. deserticola*), named "Roucongrong" in Chinese, is a well known traditional Chinese medicine[1]. The dried succulent stems of *C. deserticola* has been used for the treatment of kidney deficiency, female infertility, morbid leucorrhea, neurataxia and senile constipation, *etc*. for a long time[1]. A number of compounds, including phenylethanoid glycosides (PhGs), iridoids, and lignans, have been isolated from *C. deserticola*[2]. So far, it has been reported that the major active components of *C. deserticola* are PhGs, which were reported to have cytostatic[3], cardioactive[4], hepatocyte protective[5], antibacterial[6], antioxidative[6], neuroprotective[7] activities. Among the main PhGs in *C. deserticola*, acteoside exhibits estrogenic/antiestro-tiestrogenic, anti-inflammatory, and antioxidant activities[8-10]; 2'-acetylacteoside exhibits antioxidant, DNA-repair and neuroprotective effects[10-12]; isoacteoside possesses the antioxidant, anti-inflammatory and vasoralaxant activities[13-15]; isocistanoside C has the antioxidant activity[16]; echinacoside exhibits the anti-inflammatory effect and enhances wound healing[17]; cistanoside C possesses the DNA-repair effects[18]; cistanoside A exhibits anti-inflammatory effect[19]; and tubuloside B has the neuroprotective effect[20]. The quantity of PhGs could thus be used as one of the good indicators to comprehensively recognize and evaluate the efficacy of *C. deserticola*.

PhGs have been used as markers for quality evaluation of *C. deserticola* and its formulations. The methodologies currently reported mainly focused on the quantitative determination of single or multiple constituents in *C. deserticola* by HPLC or HPLC-MS[21,22], which may not provide sufficient data for the quality control or profiling of the herb. In view of the wide range of uses of this herb, it is urgent to develop a better method for quality control. In the present study, chromatographic separation of PhGs was developed using a gradient elution on an RP-HPLC-DAD system. The chemical fingerprints of *C. deserticola* harvested in different areas of China were established. To further confirm the chemical structures of the eight common peaks, HPLC-ESI-MS experiments were carried out. In addition, the eight PhGs in every batch of *C. deserticola* were determined quantitatively. The proposed method enables the simultaneous determination of multiple components and provides sufficient chemical fingerprint information in a single run, which could be used as an efficient way for the quality control of *C. deserticola*.

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MATERIALS AND METHODS

I. *Chemicals and Reagents*

HPLC grade methanol was purchased from Aladdin Chemistry Inc. (Shanghai, China). Deionized water was obtained using a Milli-Q Water purification system (Millifore, MA, USA). All other organic solvents used in this study were of analytical grade and purchased from Shanghai Chemical Co. (Shanghai, China). Raw herbal samples were collected from Xinjiang and Inner Mongolia Autonomous Region of China in 2011. Voucher specimens were deposited at College of Pharmacy, Jinan University. The standards of echinacoside, cistanoside A, acteoside, isoacteoside, cistanoside C, 2'-acetylacteoside, isocistanoside C and tubuloside B were isolated and purified from 50% ethanol extracts of *C. deserticola*. Chemical structures of all the standards were confirmed by ^{1}H, ^{13}C NMR and MS, which are shown in Figure 1. Their purities are higher than 95% as determined by HPLC.

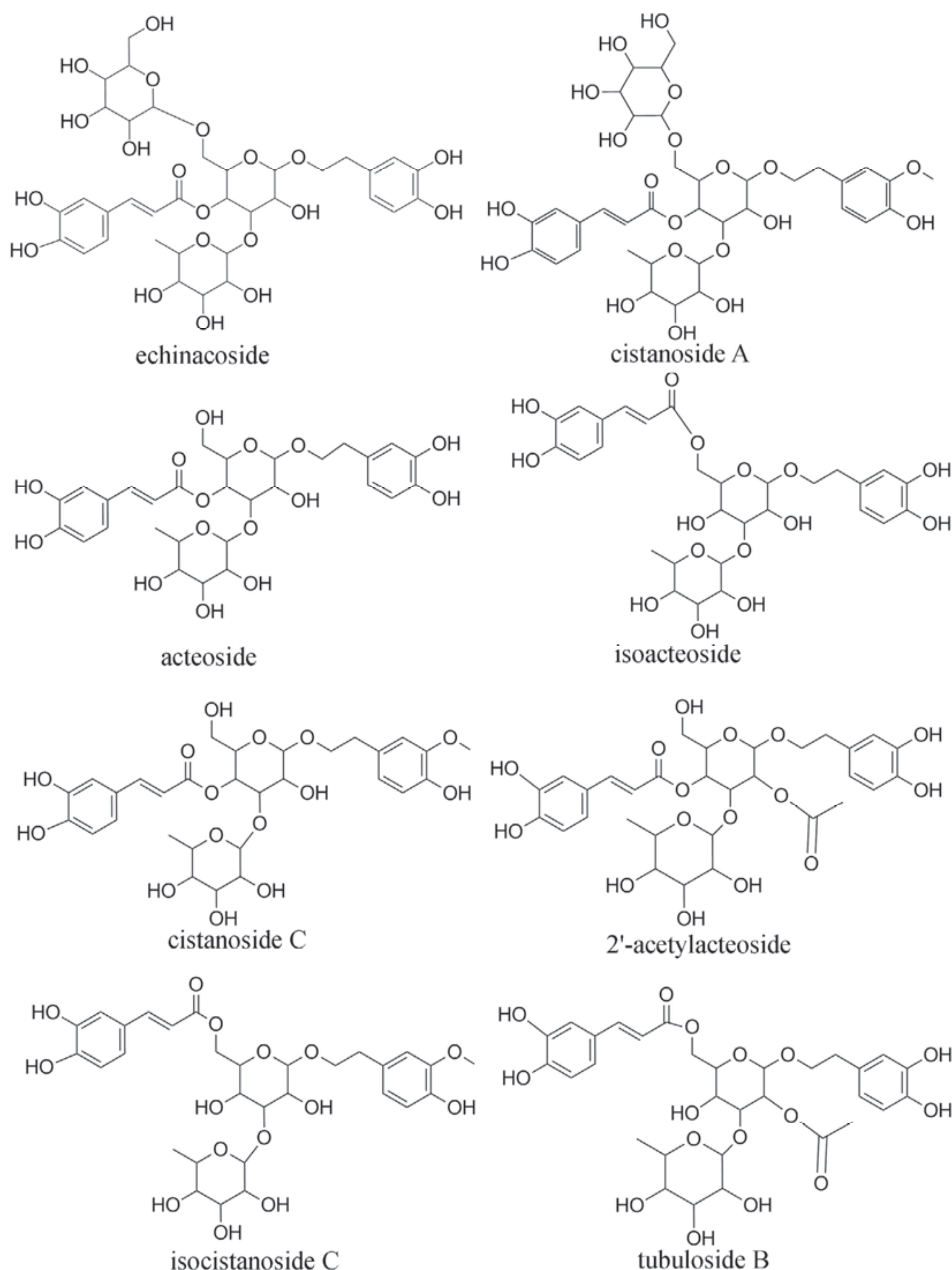

Figure 1. Chemical structures of the eight PhGs in *C. deserticola*.

II. *Instrumentation and Chromatographic Conditions*

Quantitative analysis was performed on an Agilent 1200 liquid chromatography system (Santa Clara, USA), equipped with a diode array detector (190 - 400 nm), a quaternary solvent delivery system, a column temperature controller and an autosampler. Chromatographic data were recorded and processed using Agilent chromatographic work station software. All HPLC experiments were carried out at 30°C on an Agilent eclipse XDB-C_{18} column (5 μm, 4.6 mm × 250 mm), which was protected by a guard column (5 μm, 4.6 mm × 12.5 mm). A linear gradient elution of eluents A (0.1% (v/v) aqueous formic acid) and B (methanol) was used for separation. The elution program was optimized as follows: a linear gradient of 10 - 55% B within 80 min. The detection wavelength was 330 nm, the injection volume 10 μL, and the flow rate 1.0 mL/min.

III. *LC-MS Analysis*

LC-MS analysis was carried on a ThermoFinnigan LCQ DECA XP LC-MS (San Jose, CA, USA). The optimized parameters in the negative ion mode were described as follows: ion spray voltage 3 kV; sheath gas, 15 arbitrary units; auxiliary gas, 0 arbitrary units; capillary temperature, 250°C; capillary voltage, -15 V; tube lens offset voltage, -30 V; collision energy, 25%. For full scan MS analysis, the spectra were recorded in the range of m/z 50 - 1000.

IV. *Preparation of Samples*

After dried out for 4 h in a desiccator, all raw samples were grinded into powder and filtered through a 65-mesh sieve (0.25 mm). Accurately weighed 1.0 g powder sample was placed into a conical flask with stopper and soaked with 50 mL 70% menthol for 30 min, followed by ultrasonic extraction for another 30 min. The resultant mixture was adjusted to the original weight with extraction solvent, and the supernatant was removed through a 0.45 μm membrane filter before HPLC injection.

V. *Preparation of Standard Solutions*

The stock solutions containing all the eight reference standards were prepared by dissolving the reference standards in 30% methanol to yield a final concentration of 1.018 mg/mL for echinacoside, 0.230 mg/mL for cistanoside A, 1.296 mg/mL for acteoside, 1.072 mg/mL for isoacteoside, 0.298 mg/mL for cistanoside C, 0.524 mg/mL for 2'-acetylacteoside, 0.324 mg/mL for isocistanoside C and 0.614 mg/mL for tubuloside B. The stock solution was stored at 4°C. Each stock solution containing the reference compounds was further diluted with 30% methanol to obtain a series of calibration solutions prior to HPLC experiments.

VI. *Validation Procedure*

The calibration curve for each compound was established by plotting the peak area (y) versus the concentration (x) of each analyte. The limit of detection (LOD) and limit of quantification (LOQ) for each analyte were calculated with the corresponding standard solution on the basis of the signal-to-noise ratio (S/N) of 3 and 10, respectively. The injection precision was determined by replicate injection of the same sample solution for five times in one day. Intra-day and inter-day variations were utilized to determine the precision of the developed assay. The intra-day variation was determined by analyzing six independently prepared solutions of sample (1.0 g, S7) within one day, while inter-day variation was determined on three consecutive days. The same sample solution (S7) was stored at 25°C, and analyzed at 0, 3, 6, 12, 18 and 24 h to evaluate the stability of the solution. The recovery test was carried out by standard addition method[23]. Eight components in a mixed standard solution (low, medium and high) were spiked into the samples (0.5 g, S7), and then extracted, processed and quantified in accordance with the established procedures.

VII. *Data Analysis*

Similarity analysis was performed using the professional software of Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Chinese Pharmacopoeia Commission, Ver. 2004A), as recommended by State Food and Drug Administration of China (SFDA). The software was used to calculate the similarity between each chromatographic profile of *C. deserticola* samples and the simulative mean chromatogram. This approach is *via* the calculation of the correlative coefficient of original data, based on the relative peak areas of each major constituent. Furthermore, the relative retention time (RRT) and relative peak area (RPA) of each characteristic peak to reference were calculated in the chromatograms.

RESULTS AND DISCUSSION

I. *Optimization of Extraction Procedure*

Various extraction methods were compared, and the relevent parameters, such as extraction solvent and extraction time, were optimized in order to achieve satisfactory extraction efficiency. The extraction efficiency of 30, 50, 70 and 100% methanol were evaluated and it was found that 70% methanol was the most suitable solvent for extraction. Ultrasonic and reflux extraction were also tested, and the former obtained better extraction. Finally, 1.0 g sample powder were weighed, soaked for 30 min, and then extracted with 50 mL 70% methanol through ultrasonication for 30, 40 and 50 min. It was observed that the complete extraction of the compounds could be achieved within 30 min. Therefore, ultrasonication for 30 min in 70% methanol was chosen as the optimal extraction condition for following experiments.

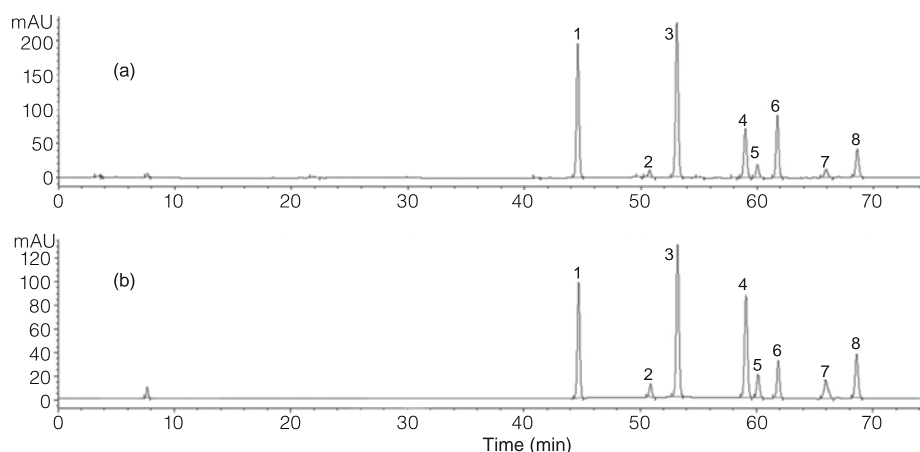

Figure 2. HPLC chromatograms of the extract of *C. deserticola* (a) and standard solution (b), including echinacoside (1), cistanoside A (2), acteoside (3), isoacteoside (4), cistanoside C (5), 2'-acetylacteoside (6), isocistanoside C (7), tubuloside B (8) at 330 nm wavelength. (Agilent eclipse XDB-C18 column (5 μm, 4.6 mm × 250 mm) within 80 min using a gradient elution with 0.1% (v/v) formic acid aqueous solution and methanol as mobile phase)

II. *Optimization of Chromatographic Conditions*

In order to obtain a good resolution within a short analysis time, the composition of mobile phase was optimized. Acidic mobile phase was used in order to suppress the ionization of phenolic hydroxyl groups. This acidification was beneficial, leading to longer retention time and better peak shape. Various mobile phase compositions, such as acetonitrile and 0.1% aqueous ethylic acid (v/v), methanol and 0.1% aqueous ethylic acid (v/v), methanol and 0.1% aqueous formic acid (v/v), were evaluated. Methanol and water containing 0.1% formic acid were chosen as the mobile phases because all components could be resolved under this condition. All the eight compounds are barely separated under an isocratic elution mode, therefore a gradient elution mode was developed for following experiments. A linear gradient elution of eluents A (0.1% (v/v) aqueous formic acid) and B (methanol) was used for separation, and the elution program was as follows: a linear gradient of 10 - 55% B within 0 - 80 min. As shown in Figure 2, all eight PhGs in the real sample or standard mixture could be baseline separated within 80 min, and good peak shapes were observed for all chosen markers. On the basis of the UV spectra of the eight components recorded from 200 to 370 nm, 330 nm was finally selected for monitoring.

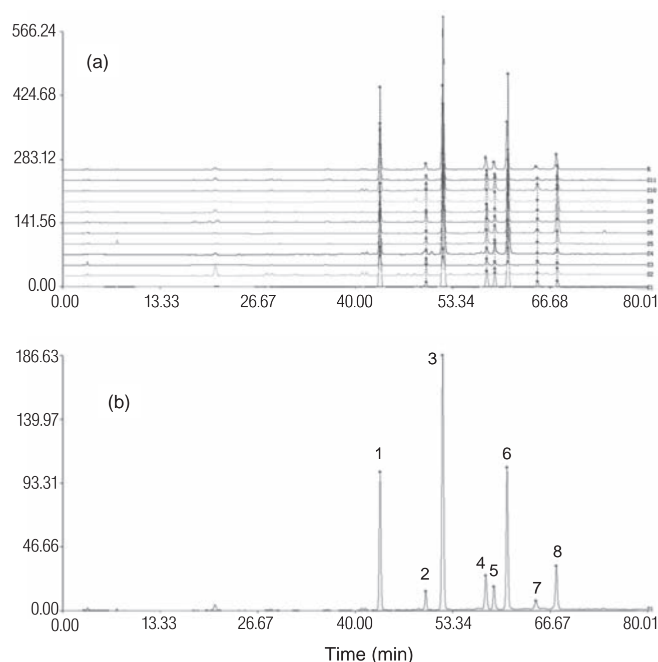

Figure 3. Fingerprint chromatograms of the 11 batches of samples of *C. deserticola* (a) and fingerprint profile of reference (b), including echinacoside (1), cistanoside A (2), acteoside (3), isoacteoside (4), cistanoside C (5), 2'-acetylacteoside (6), isocistanoside C (7), tubuloside B (8). (Other conditions, see Figure 2.)

III. *Chromatographic Fingerprint Analyses of C. deserticola*

The chromatograms of different samples need to be standardized to perform fingerprinting analysis. The process of standardization included the selection of "common peaks" in chromatograms and the normalization of retention times of all common peaks. The extracts of the 11 samples of *C. deserticola* collected from Inner Mongolia and Xinjiang served as the sample set. Their HPLC chromatographic fingerprints were shown in Figure 3. Peak 3 at retention time 52.44 min indicated the highest content in all eight peaks. Therefore, it was selected as the reference peak. Among all the peaks observed, eight of them (denoted from 1 to 8) were defined as common peaks for their existence in all the 11 samples (see Figure 3). The RRT and RPA of the eight peaks with respect to peak 3 in the 11 samples were listed in Table 1. In comparison with standard compounds, the eight peaks were unambiguously identified as echinacoside (1), cistanoside A (2), acteoside (3), isoacteoside (4), cistanoside C (5),

2'-acetylacteoside (6), isocistanoside C (7), and tubuloside B (8).

In addition, similarity analysis was performed for the 11 chosen samples of *C. deserticola*. The similarity indexes were calculated by mean fusion vector method. The correlation coefficients between each chromatogram of *C. deserticola* samples and the simulative mean chromatogram were found to be 0.846, 0.925, 0.981, 0.790, 0.798, 0.938, 0.951, 0.967, 0.961, 0.840 and 0.942 respectively. The similarity indexes of nine samples were all higher than 0.840, which suggested that the samples from different regions shared a similar chromatographic pattern. However, the RSD values of RPA from above 11 batches of samples collected from the different areas were very high (64.72 - 131.10%). Detailed analysis of the HPLC profile of each sample indicated that the common peak abundance variation from each other could be caused by the difference of plant origin, season of collection, drying process and storage conditions, *etc*.

Table 1. The relative retention time (RRT) and relative retention area (RPA) of the eight peaks with respect to peak 3 in 11 *C. deserticola* samples

Peak NO.	Compound	RRT[a]	RPA[a]
1	echinacoside	0.835 ± 0.001	0.773 ± 0.526
2	cistanoside A	0.956 ± 0.001	0.183 ± 0.203
3	acteoside	1.000	1.000
4	isoacteoside	1.114 ± 0.001	0.249 ± 0.212
5	cistanoside C	1.135 ± 0.001	0.154 ± 0.100
6	2'-acetylacteoside	1.169 ± 0.002	0.653 ± 0.476
7	isocistanoside C	1.242 ± 0.011	0.104 ± 0.137
8	tubuloside B	1.283 ± 0.002	0.288 ± 0.343

[a] Data presented as mean±SD of 11 samples.

IV. *Identification of Eight PhGs from C. deserticola*

LC-ESI-MS experiments were performed to further confirm the identity of the eight selected peaks in fingerprints. Negative ionization mode was used for all ESI - MS analysis. MS spectra were dominated by the $[M - H]^-$ ion for eight PhGs (Table 2). The retention times and mass spectra of products matched exactly those of the corresponding standard compounds, as shown in Table 2. Three pairs of isomers, i.e. acteoside and isoacteoside, cistanoside C and isocistanoside C, 2'-acetylacteoside and tubuloside B, exhibited the same molecular mass and similar fragmentation pathway, while they were eluted at different retention times (52.44, 57.70, 58.52, 64.30, 60.21 and 67.88 min, respectively).

Table 2. Compounds identified in *C. deserticola* by LC-MS

Peak NO.	t_R(min)	MS^1	MS^2	Chemical formula	Compound identity [a]
1	44.32	785.3	623.3	$C_{35}H_{46}O_{20}$	echinacoside
2	51.32	799.2	623.2,637.2	$C_{36}H_{48}O_{20}$	cistanoside A
3	52.44	623.2	461.3	$C_{29}H_{36}O_{15}$	acteoside
4	57.70	623.2	461.3	$C_{29}H_{36}O_{15}$	isoacteoside
5	58.52	637.2	461.2	$C_{30}H_{38}O_{15}$	cistanoside C
6	60.21	665.1	503.3,461.3,623.3	$C_{31}H_{38}O_{16}$	2'-acetylacteoside
7	64.30	637.2	461.2	$C_{30}H_{38}O_{15}$	isocistanoside C
8	67.88	665.3	503.3,461.3,623.3	$C_{31}H_{38}O_{16}$	tubuloside B

[a] Compounds identified by comparing the mass spectrum and retention time with corresponding standard compound.

Table 3. Linear equation, LOQs and LODs of eight compounds (n = 6)

Peak NO.	Compound	Linear equation[a]	R^2	Linear range (μg/mL)	LOQ[b] (μg/mL)	LOD[b] (μg/mL)
1	echinacoside	$y = 12.789x – 115.18$	1.0000	50.90 - 4072	0.41	0.12
2	cistanoside A	$y = 8.2154x – 9.8699$	0.9998	11.50 - 920	2.30	0.70
3	acteoside	$y = 14.544x – 269.41$	1.0000	64.80 - 5180	0.52	0.16
4	isoacteoside	$y = 15.166x – 246.30$	1.0000	53.60 - 4228	0.43	0.13
5	cistanoside C	$y = 13.336x – 50.159$	1.0000	14.90 - 1192	1.19	0.36
6	2'-acetylacteoside	$y = 13.166x – 132.70$	0.9999	26.20 - 2096	1.05	0.32
7	isocistanoside C	$y = 12.019x – 42.831$	1.0000	16.20 - 1296	1.30	0.39
8	tubuloside B	$y = 14.295x – 173.92$	0.9999	30.70 - 2456	0.74	0.22

[a] y and x are, respectively, the peak areas and concentrations (μg/mL) of the analytes.
[b] LOD limit of detection (S/N = 3), LOQ limit of quantification (S/N = 10).

V. *Validation of Quantitative Analysis Method*

The calibration curve, LODs, LOQs, precision (inter-day and intra-day), reproducibility and recovery test for the eight PhGs were summarized in Table 3. All calibration curves showed good linear regression (R^2 > 0.9998) within test ranges. The LODs (S/N = 3) and LOQs (S/N = 10) for the analytes were less than 0.70 and 2.30 μg/mL, respectively. Table 4 showed the precision and stability of the tests, which indicated that the RSD values of the overall injection, intra-day and inter-day variations were less than 3.88% for all analytes. Besides, the RSD of stability was not exceeding 3.03% for all analytes. The results of recovery test were summarized in Table 5. The average recoveries of

Table 4. Precision, stability of eight PhGs

Peak NO.	Compound	Precision (RSD, %)[a]			Stability (%)[a] (RSD, n = 6)
		Intra-day (n = 6)	Inter-day (n = 18)	Injection precision (n = 5)	
1	echinacoside	2.89	2.71	0.51	0.70
2	cistanoside A	2.51	2.44	1.23	1.49
3	acteoside	1.39	2.25	0.30	0.63
4	isoacteoside	2.49	2.62	1.21	1.03
5	cistanoside C	2.14	3.28	0.61	0.65
6	2'-acetylacteoside	3.88	3.15	0.29	0.60
7	isocistanoside C	2.66	3.50	0.83	1.46
8	tubuloside B	2.06	2.35	1.18	3.03

[a] RSD (%) = (SD of amount detected/mean of amount detected) × 100.

Table 5. Analysis of the recovery of compounds (n = 3)

Compound	Original (mg)	Addition (mg)	Detection (mg)	Recovery[a] (%)	RSD[b] (%)
		1.532	4.430	97.91	0.44
echinacoside	2.930	3.064	6.023	100.95	0.14
		4.596	7.566	100.87	0.10
		0.252	0.835	103.97	0.64
cistanoside A	0.573	0.504	1.045	93.65	0.33
		0.756	1.403	109.79	1.54
		1.984	5.795	99.34	0.29
acteoside	3.824	3.968	7.811	100.48	0.33
		5.952	10.071	104.96	1.54
		1.370	3.950	105.55	0.10
isoacteoside	2.504	2.740	5.210	98.76	0.22
		4.110	6.606	99.81	0.16
		0.356	0.977	94.38	0.37
cistanoside C	0.641	0.712	1.360	100.98	0.72
		1.068	1.660	95.41	0.80
		0.506	1.640	106.13	0.96
2'-acetylacteoside	1.103	1.012	2.149	103.36	0.42
		1.518	2.747	108.30	1.20
		0.340	1.104	100.00	0.06
isocistanoside C	0.764	0.680	1.427	97.50	0.39
		1.020	1.858	107.25	1.05
		0.602	1.857	103.82	0.17
tubuloside B	1.232	1.204	2.419	98.59	0.35
		1.806	3.139	105.59	2.21

The data was present as average of three experiments.
[a] Recovery (%) =100 × (amount detected – original amount)/addition.
[b] RSD (%) = (SD of amount detected/mean of amount detected) × 100.

Table 6. The content of eight components in 11 batches of *C. deserticola* (n = 3)

Content(mg/g)[a]	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
echinacoside	2.13 ± 0.08	2.29 ± 0.11	4.29 ± 0.15	0.57 ± 0.01	1.93 ± 0.10	4.48 ± 0.04	5.86 ± 0.17	17.70 ± 0.35	4.94 ± 0.06	7.21 ± 0.07	15.68 ± 0.07
cistanoside A	1.24 ± 0.05	2.32 ± 0.10	2.09 ± 0.10	0.17 ± 0.00	0.40 ± 0.01	0.80 ± 0.02	1.15 ± 0.03	3.26 ± 0.06	1.08 ± 0.02	0.73 ± 0.01	1.41 ± 0.00
acteoside	0.92 ± 0.03	3.40 ± 0.12	3.84 ± 0.14	3.25 ± 0.14	8.71 ± 0.38	2.66 ± 0.04	7.65 ± 0.11	31.43 ± 0.64	7.79 ± 0.22	30.65 ± 0.17	25.80 ± 0.10
isoacteoside	0.78 ± 0.03	0.26 ± 0.00	0.40 ± 0.01	0.71 ± 0.02	4.24 ± 0.09	0.37 ± 0.02	5.01 ± 0.12	1.68 ± 0.03	2.69 ± 0.12	0.62 ± 0.03	2.55 ± 0.02
cistanoside C	0.36 ± 0.01	1.00 ± 0.03	0.95 ± 0.04	0.43 ± 0.19	1.12 ± 0.03	0.57 ± 0.02	1.28 ± 0.03	1.67 ± 0.03	0.68 ± 0.03	1.45 ± 0.01	2.25 ± 0.01
2'-acetylacteoside	0.28 ± 0.01	3.21 ± 0.12	3.09 ± 0.12	7.43 ± 0.19	10.51 ± 0.41	0.98 ± 0.04	2.21 ± 0.09	26.48 ± 0.54	4.77 ± 0.11	10.20 ± 0.05	10.72 ± 0.04
isocistanoside C	0.52 ± 0.02	0.33 ± 0.01	0.33 ± 0.01	0.13 ± 0.00	0.80 ± 0.02	0.12 ± 0.00	1.53 ± 0.04	0.20 ± 0.00	0.46 ± 0.01	0.09 ± 0.00	0.85 ± 0.01
tubuloside B	0.29 ± 0.01	0.82 ± 0.03	0.20 ± 0.01	3.32 ± 0.09	9.10 ± 0.44	0.22 ± 0.01	2.46 ± 0.05	1.78 ± 0.05	2.48 ± 0.04	0.32 ± 0.01	0.62 ± 0.01
Total	6.52 ± 0.21	13.61 ± 0.46	15.19 ± 0.57	16.01 ± 0.35	36.81 ± 1.24	10.20 ± 0.15	27.15 ± 0.60	84.20 ± 1.67	24.89 ± 0.25	51.27 ± 0.32	59.88 ± 0.23

[a] Data presented as mean±SD of three experiments.

the eight PhGs were 93.65 - 109.79% with RSD values less than 2.21%. Therefore, the HPLC-DAD method is precise, accurate and sensitive enough for simultaneous quantitative analysis of eight PhGs in *C. deserticola*.

VI. *Quantitative Determination of C. deserticola*

The developed analysis method was successfully applied to the simultaneous quantitative determination of echinacoside, cistanoside A, acteoside, isoacteoside, cistanoside C, 2'-acetylacteoside, isocistanoside C and tubuloside B in the 11 samples of *C. deserticola* which were obtained from Inner Mongolia and Xinjiang in China. Each sample was determined in triplicate. Peaks in the chromatograms were identified by the same retention time and on-line UV spectra with those of the standards and the HPLC profiles were illustrated in Figure 3. Table 6 showed the contents of the eight PhGs in 11 samples of *C. deserticola*. The content of each analyte in different samples varied greatly. Generally, acteoside was the main component in the range from 0.92 to 31.43 mg/g. The variation in contents of the constituents shall certainly lead to the variation of therapeutic effects[24-26].

CONCLUSIONS

Combination of HPLC fingerprint with quantitative analysis of several marker compounds for the identification and quality control of traditional Chinese medicines (TCM) is definitely an improvement over the traditional methodologies. The quantification of a series of marker compounds can better reflect the quality of TCM. In the present study, a simple, accurate and efficient HPLC method was developed to evaluate the quality of *C. deserticola* by establishing the chromatographic fingerprint and simultaneous quantitative analysis of eight PhGs, namely echinacoside, cistanoside A, acteoside, isoacteoside, cistanoside C, 2'-acetylacteoside, isocistanoside C, tubuloside B. The HPLC-ESI-MS experiment was performed to further confirm the identity of compounds. The results demonstrated that the developed method is accurate, reproducible and could be readily employed as a suitable quality control method for the stems of *C. deserticola* and derived extracts. The quantitative analysis on the 11 *C. deserticola* samples suggested that the contents of the eight PhGs varied significantly in the stems of *C. deserticola* from Inner Mongolia and Xinjiang. Therefore, the developed method can be readily used in quality assurance of TCM products, as well as adulteration inspection of the crude drugs.

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