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Study on HPLC Fingerprint of *Clematis manshurica* Rupr from Different Areas

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Abstract. Objective: To establish HPLC fingerprint of *Clematis manshurica* Rupr from different areas. Method: 10 batches of *Clematis manshurica* Rupr was analyzed by RP-HPLC that the methods were used Odyssil-C_{18} chromatographic column, with the mobile phase of acetonitril-0.1% phosphoric acid solution, the flow rate was 0.8mL/min, the wavelength was 210 nm, and column temperature was 30℃. And the similarity was evaluated by similarity evaluation software. Result: The HPLC-UV fingerprint of *Clematis manshurica* Rupr was established, and 20 common peaks were determined, the similarity was between 0.974-0.823, indicating that the differences of *Clematis manshurica* Rupr from different regions were significant. Conclusion: The analytical method has good separation degree and feasibility, and it can provide a reliable basis for the quality control of *Clematis manshurica* Rupr.

1. Introduction

Clematis manshurica Rupr is the dried roots and rhizomes of *Clematis manshurica* Rupr of ranunculaceae. It is one of the varieties of *Clematis chinensis* Osbeck recorded in Chinese pharmacopoeia. *Clematis manshurica* Rupr of flavor is acrid and salty, property is warm and have a little toxic in traditional Chinese medicine(TCM). It has the effect of dispel wind and eliminate dampness, dredging collaterals and relieving pain[1-2]. Modern research shows that it mainly contains triterpenoid saponins, flavonoids, polysaccharides, coumarins, lignans and other chemical components, and these components have analgesic and anti-inflammatory, anti-tumor, anti-oxidation, anti-bacterial, releases spasms, inhibition of melanin, immunosuppression and other pharmacological effects[3-6]. There are three varieties of *Clematis chinensis* Osbeck, which are widely used in clinical practice. And it is very similar with the fake of the plant morphology and microscopic characteristics, which is the main reason for the confusion of the medicinal materials of *Clematis chinensis* Osbeck. So it is necessary to identify the species of *Clematis manshurica* Rupr and control its quality[7]. Though the chemical composition of *Clematis manshurica* Rupr is very complex, the integrality and systematicness of TCM fingerprint provide a new method for quality evaluation, and there is no report on the study of fingerprint of *Clematis manshurica* Rupr by HPLC. Therefore, in this study, 10 batches of *Clematis manshurica* Rupr were determined by HPLC, and their similarity was evaluated. In order to discuss the influence of areas on the quality of *Clematis manshurica* Rupr from different origins, and to provide references for the quality control and clinical application of *Clematis manshurica* Rupr.

2. Materials and methods

2.1. Instruments and reagents

Chromatographic analysis was performed on the Agilent1260 series of HPLC (Agilent Technology of USA, including four elements gradient pump, automatic injector, diode array detector, chemstation workstation). Data analysis was performed by professional software named Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine composed by Chinese Pharmacopoeia Committee (Version 2012A). The reagent was methanol and acetonitrile for the chromatographic purity, and water was redistilled water.

2.2. Medicinal Materials

10 batches of *Clematis mandshurica* Rupr were identified by Professor Wang Xiangpei of Guiyang University of Chinese Medicine as the dried roots and rhizomes of *Clematis manshurica* Rupr of Ranunculaceae. And the origin of *Clematis manshurica* Rupr were listed in table 1.

Table 1. Origin of *Clematis mandshurica* Rupr from different areas

No.	species	producing area	Similarity
S1	*Clematis mandshurica* Rupr	Heilongjiang Province	0.936
S2	*Clematis mandshurica* Rupr	Heilongjiang Province	0.974
S3	*Clematis mandshurica* Rupr	Heilongjiang Province	0.959
S4	*Clematis mandshurica* Rupr	Hebei Province	0.847
S6	*Clematis mandshurica* Rupr	Liaoning Province	0.950
S7	*Clematis mandshurica* Rupr	Jilin Province	0.956
S8	*Clematis mandshurica* Rupr	Hebei Province	0.823
S9	*Clematis mandshurica* Rupr	Jilin Province	0.957
S10	*Clematis mandshurica* Rupr	Jilin Province	0.901

2.3. chromatographic conditions

The chromatographic column was Dikma Odyssil C18(250mm × 4.6mm, 5μm); A binary gradi-ent elution system consisting of acetonitrile(A) and water containing 0.1% phosphoric acid(C)was used with the following gradient programs: 0-6min, 98-95%C; 6-24min, 95-85%C; 24-32min, 85-82%C; 32-34min, 82-82%C; 34-41min, 82-80%C; 41-51min, 80-78%C; 51-57min, 78-76%C; 57-62min, 76-70%C; 62-75min, 70-67.5%C; 75-85min, 67.5-65%C; 85-90min, 65-62.5%C; 90-105min, 62.5-10%C; 105-120min, 10-0%C; 120-140min, 0-0%C. The detection wavelength was 210nm, and the flow rate was 0.8mL/min. The column temperature was maintained at 30°C.

2.4. Preparation of samples

All the samples were crushed into fine powder, accurately weighed 1.0g, and placed into round bottom flask, respectively. Under70°C, the samples were refluxed twice with 50mL of methanol, the first time was refluxed 2h, and the second was refluxed 1h. The filtrate was concentrated to 10 mL, then the filtrate passes through the 0.45μm microporous membrane and the filtrate is obtained.

2.5. HPLC method validation

2.5.1. Precision test. The sample solution of *Clematis manshurica* Rupr(S3) was injected for 6 times by the chromatographic method under 2.1 and 2.2 item, and the retention time and peak area of each common peak were recorded. Taking No.20 peak as the reference peak, the relative retention time and relative peak area of each common peak were calculated to be less than 3.0%, which indicated that the precision of the instrument was good.

2.5.2. Stability test. The sample solution of *Clematis manshurica* Rupr(S3) was determined by chromatographic method under item of 2.1 and 2.2 at 0, 2, 4, 8, 12, 24, respectively. No.20 peak was identified as the reference peak, the relative retention time and relative peak area of each common peak were calculated to be less than 3.0%, indicating that the sample solution was stable in 24 hours.

2.5.3. Repeatability test. Six samples of *Clematis manshurica* Rupr(S3) were prepared and determined by the method of 2.1 and 2.2, and the retention time and peak area of each common peak were recorded. The reference peak was No.20 peak, the relative retention time and relative peak area of each common peak were calculated to be less than 3.0%, which it showed that the method had good reproducibility.

3. Results

3.1. Establishment of HPLC fingerprint
According to the chromatographic methods of "2.2" and "2.1", 10 batches of *Clematis manshurica* Rupr from different habitats were detected, and the peak, peak number, peak position and other parameters of HPLC fingerprint were compared and analyzed to determine the fingerprint of *Clematis manshurica* Rupr. The results are shown in figure 1 and 2.

3.2. Establishment of Common fingerprint Peak
The HPLC fingerprints of 10 batches of *Clematis manshurica* Rupr were studied by using the peak of 117 min as reference, and 20 common peaks were identified. The results are shown in tables 2 and 3. The relative retention time and relative peak area of each peak are calculated, the results are shown in figure 1 and 2.

3.3. Similarity evaluation of Fingerprint
The determination data of 10 batches of *Clematis manshurica* Rupr samples were imported into the"Chinese medicine chromatographic fingerprint similarity evaluation system 2012A edition". After selecting peaks and setting matching templates, the peaks were automatically matched to generate the control fingerprints, and then the standard templates were set up, and the parameters such as peak value, peak number and peak position were compared and analyzed to determine the HPLC fingerprint . The results are shown in table 1.
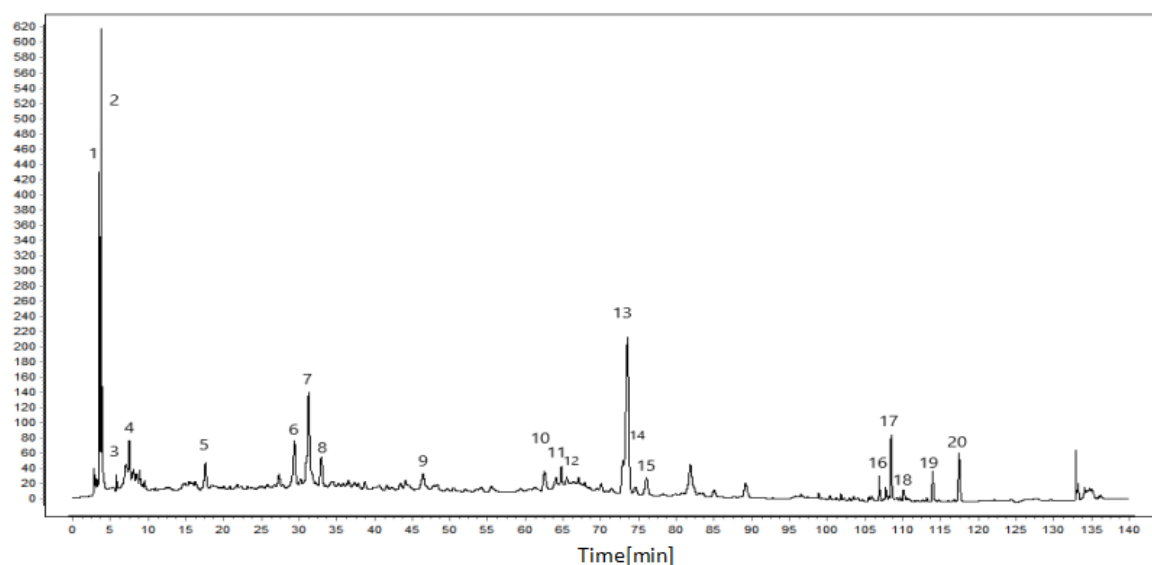

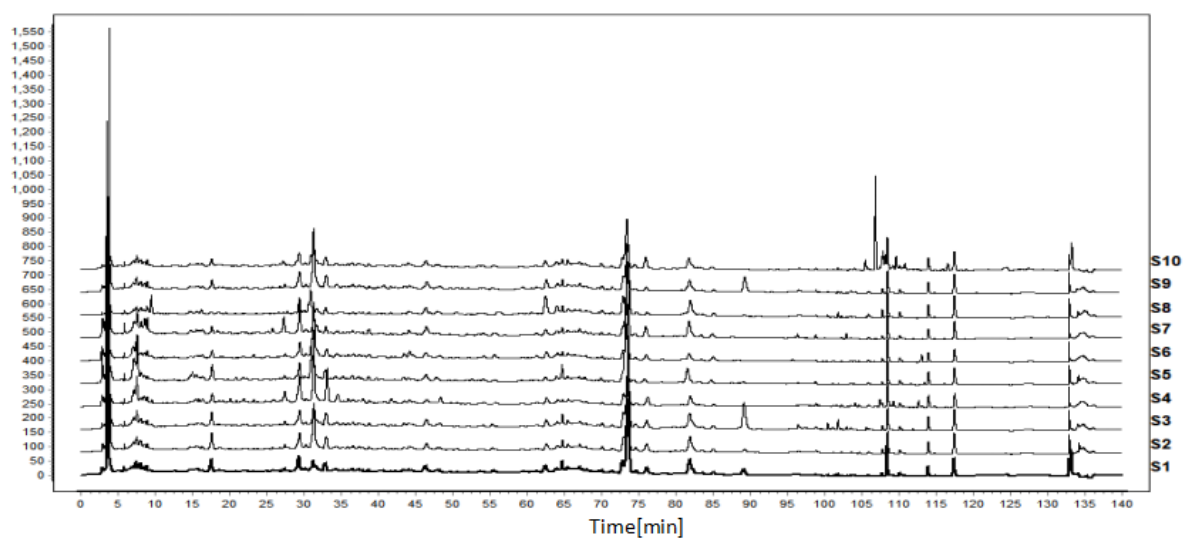Fig 1. Common pattern of HPLC fingerprint of *Clematis manshurica* Rupr

Fig 2. HPLC fingerprint of 10 batches of *Clematis manshurica* Rupr

Table 2. The relative retention time of common peaks in HPLC fingerprints of 10 batches
of *Clematis manshurica* Rupr

Common peak	1	2	3	4	5	6	7	8	9	10	RSD%
1	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.59
2	0.032	0.033	0.032	0.033	0.032	0.032	0.032	0.032	0.032	0.032	0.33
3	0.050	0.050	0.050	0.049	0.049	0.050	0.049	0.049	0.049	0.050	0.20
4	0.064	0.064	0.064	0.063	0.064	0.064	0.064	0.064	0.064	0.064	0.45
5	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.149	0.150	0.150	0.27
6	0.250	0.251	0.250	0.250	0.251	0.251	0.250	0.250	0.250	0.250	0.12
7	0.257	0.258	0.257	0.26	0.258	0.257	0.257	0.256	0.257	0.257	0.16
8	0.280	0.281	0.281	0.281	0.282	0.280	0.280	0.280	0.281	0.280	0.25
9	0.395	0.396	0.396	0.400	0.395	0.396	0.396	0.395	0.396	0.395	0.12
10	0.545	0.546	0.545	0.541	0.545	0.545	0.545	0.545	0.546	0.545	0.26
11	0.552	0.552	0.551	0.551	0.551	0.551	0.551	0.551	0.551	0.551	0.06
12	0.558	0.558	0.558	0.561	0.557	0.557	0.557	0.558	0.557	0.557	0.18
13	0.627	0.626	0.627	0.626	0.625	0.624	0.625	0.627	0.626	0.625	0.16
14	0.636	0.635	0.635	0.638	0.634	0.633	0.634	0.636	0.635	0.634	0.21

15	0.648	0.648	0.648	0.648	0.647	0.645	0.647	0.648	0.647	0.647	0.15
16	0.917	0.918	0.918	0.917	0.917	0.917	0.917	0.917	0.918	0.917	0.03
17	0.923	0.924	0.924	0.923	0.923	0.923	0.923	0.923	0.924	0.923	0.02
18	0.937	0.937	0.937	0.938	0.937	0.937	0.937	0.937	0.938	0.937	0.02
19	0.970	0.970	0.970	0.970	0.970	0.970	0.970	0.970	0.970	0.970	0.00
20	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.00

Table 3. The relative peak area of common peaks in HPLC fingerprints of 10 batches of *Clematis manshurica* Rupr

Common peak	1	2	3	4	5	6	7	8	9	10
1	6.164	4.829	2.977	1.150	2.349	5.148	3.637	0.734	3.176	3.620
2	3.792	2.992	2.884	3.129	3.900	3.936	5.596	1.837	3.400	2.652
3	0.147	0.137	0.178	0.139	0.140	0.215	0.310	0.120	0.159	0.109
4	0.706	0.744	0.866	1.891	2.670	1.952	1.442	0.404	0.748	0.667
5	0.906	1.025	0.946	1.045	1.340	0.849	0.597	0.181	0.628	0.650
6	1.614	1.561	1.641	3.835	1.983	2.472	2.350	0.958	1.830	1.575
7	0.489	0.501	0.449	0.757	0.619	0.587	0.668	0.293	0.528	0.413
8	0.906	1.072	1.107	3.502	1.032	1.179	0.707	0.439	1.228	0.883
9	1.071	0.923	0.983	1.202	1.207	1.415	1.287	0.810	1.059	0.981
10	0.504	0.557	0.581	0.840	0.685	1.071	0.824	0.790	0.750	0.648
11	0.848	0.657	0.829	0.612	1.160	0.896	0.800	0.545	0.851	0.670
12	0.630	0.664	0.656	0.966	0.878	1.235	1.063	0.727	0.666	0.641
13	6.747	6.653	5.967	3.757	8.963	4.959	7.931	3.853	4.449	4.129
14	0.339	0.339	0.359	0.359	0.343	0.399	0.426	0.344	0.212	0.355
15	0.571	0.727	0.639	1.053	0.427	0.793	1.125	0.426	0.281	0.903
16	0.134	0.138	0.140	0.221	0.128	0.220	0.124	0.249	0.158	0.404
17	1.203	0.800	0.750	1.213	1.001	1.316	0.991	0.805	0.865	1.290

18	0.224	0.163	0.147	0.304	0.257	0.336	0.216	0.252	0.177	0.337
19	0.472	0.475	0.522	0.722	0.643	0.616	0.469	0.506	0.468	0.647
20	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

4. Discuss

In this study, it was reviewed that the methods were different solvents such as ethanol, 70% ethanol, methanol and 70% methanol, as well as different extraction time, reflux and ultrasonic extraction in this study. The results were the extraction with methanol reflux for 2h and 1h that was relatively complete, this method was stable, there was many peaks and good repeatability. And it was examined for the mobile phase system for instance methanol and water, acetonitrile-water and acetonitrile-0.01%phosphate water, acetonitrile-0.1% phosphate water, the column temperature was 25℃, 30℃, and 35℃, and the wavelength was 210nm, 254 nm, 270nm, and 280nm. The results showed when the mobile phase system was acetonitrile-0.1% phosphate water, the column temperature was 30℃, and the wavelength was 210nm, which the separation degree of each peak was better, the baseline was stable, and it had good repeatability. It was propitious to the analysis of HPLC fingerprint of *Clematis manshurica* Rupr.

5. Conclusion

In this paper, the HPLC fingerprint of *Clematis manshurica* Rupr from different habitats was established. The results showed that the similarity was significantly different among 10 batches of *Clematis manshurica* Rupr, which the main differences were in chemical composition and content. The similarity of *Clematis manshurica* Rupr was above 0.900 in Heilongjiang Province, Jilin Province and Liaoning Province, but was above 0.800 in Hebei Province. The results indicated that the quality of *Clematis manshurica* Rupr was affected greatly by the environment of soil, climate, temperature, light etc. Therefore, it was very important to establish the genuine medicinal materials of *Clematis chinensis* Osbeck. It is beneficial to the quality control of *Clematis manshurica* Rupr, and the quality of *Clematis manshurica* Rupr should be paid attention to when it is used in clinic.

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