Study on the Fingerprint of Stellarlae Radix by UPLC

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Abstract. Objective: The UPLC fingerprint of Stellarlae Radix was established to provide reference for quality control and evaluation of Stellarlae Radix. Method: The chromatographic separation was performed on a Agilent ZORBAX Eclipse Plus C18 (2.1mm×100mm, 1.8μm) with a gradient elution program using a mixture of acetonitrile and 0.1% phosphoric acid water as mobile phase at 225 nm wavelength, the flow rate was 0.1mL/min and the column temperature was 30℃. The Injection volume 1μL. 10 batches of Stellarlae Radix were measured under the same chromatographic conditions, and the similarity evaluation of 10 batches of Stellarlae Radix was carried out by using the software traditional Chinese medicine chromatographic fingerprint similarity evaluation system (version 2004). Results: The method for UPLC fingerprint analysis of 10 batches of Stellarlae Radix was established, and the similarity was between 0.941 and 0.980, and 13 common peaks were calibrated. Conclusion: The fingerprint method established in this study has good precision, repeatability, stability, short analysis time and strong specificity, which can provide scientific basis for the quality evaluation and control of Stellar Radix.

1. Introduction
Stellarlae Radix is derived from the dried roots of the Stellaria dichotoma L.var. Lanceolata Bge. Nature sweet, slightly cold. Return liver, stomach classics. It has the effect of clearing vacuity heat and eliminating fever in infantile malnutrition. For yin deficiency fever, steaming bone taxation fever, infantile chancre fever, children gan fever, etc [1]. Modern pharmacological studies have shown that Stellarlae Radix has anti-inflammatory and antipyretic effects, anti-allergic effects, anti-atherosclerosis and other pharmacological effects, etc [2-4]. In recent years, scholars at home and abroad have carried out a lot of research on the chemical composition of Stellarlae Radix, which mainly contains sterols, cyclic peptides, alkaloids, phenolic acids, volatiles and other chemicals [5]. Some studies have shown that carboline alkaloids in Stellarlae Radix show good anti-inflammatory activity [6], and polypeptide H and I in cyclic peptide compounds show good antitumor activity in vitro [7]. At present, there are few reports on the quality control of Stellarlae Radix by liquid chromatography fingerprinting, so it is necessary to establish a complete and comprehensive quality evaluation method. Traditional Chinese medicine fingerprint technology has the characteristics of whole, macro and fuzzy analysis, and is often used as a means of quality control of traditional Chinese medicine [8]. Based on this, the
fingerprint of 10 batches of traditional Chinese medicine Stellarlae Radix collected from the market was established by UPLC-PDA method, and the similarity analysis of Stellarlae Radix medicinal materials purchased by each drugstore was carried out in order to further ensure the quality and safety of Chinese medicinal material Stellarlae Radix in the market, and to provide scientific basis for the quality control of its preparation development.

2. Materials and methods

2.1. Instrument
ACQUITY UPLC (American Waters Company, including quaternion high pressure gradient pump, automatic sampler, column temperature box, PDA detector, Empower chromatographic workstation); AB265-S Electronic Balance (Metler-Toledo Instrument Co, Ltd.); KQ-100E Ultrasound Cleaner (Kunshan Ultrasound Instrument Co, Ltd.); 800Y Multifunctional Crusher (Yongkang Platinum Ou Hardware Co, Ltd.); “Similarity Evaluation system of chromatographic fingerprint of traditional Chinese Medicine” (National Pharmacopoeia Commission, version 2004).

2.2. Reagents and Plant materials
Methanol and acetonitrile are chromatographic purity (TEDIA Company, USA), phosphoric acid is analytical purity (Chongqing Chuandong Co., Ltd., Cina), and purified water (Hangzhou Wahaha Co., Ltd., Cina). The Stellarlae Radix was purchased in various drugstores in Guiyang City, Guizhou Province. The specific source is shown in Table 1. The sample was identified by professor xiangpei Wang of Guizhou University of Traditional Chinese Medicine as the plant of *Stellaria dicifuoma* L. var. *Lanceolata* Bge. And a dry root. The samples were sealed and stored in a cool and dry place.

| NO. | source | NO. | source |
|-----|--------|-----|--------|
| S1  | Le Ren Tang         | S6  | Fuankang Pharmacy |
| S2  | Yi Pin Pharmaceutical industry | S7  | Tianyi Pharmacy |
| S3  | Tongrentang         | S8  | Zhilin pharmacy |
| S4  | Yi Shu Pharmaceutical industry | S9  | Guiyang TongJitang |
| S5  | Hesiyuan Pharmacy   | S10 | Guiyang Dchang-xiang Pharmaceutical Co., Ltd. |

2.3. Chromatographic conditions
Agilent ZORBAX Eclipse plus C18 (2.1mm×100 mm, 1.8 μm) was used, the detection wavelength was set at 225 nm and column temperature was 30°C. The injection volume was 1 μL and flow rate was 0.8 mL/min; Mobile phase: acetonitrile (A)-0.1% phosphoric acid water (B). The gradient elution procedure is shown in table 2.

| Time(min) | acetonitrile(A) | 0.1% phosphoric acid water(B) |
|-----------|-----------------|-------------------------------|
| 0~6       | 5~18            | 95~82                         |
| 6~7       | 18~21           | 82~79                         |
| 7~17      | 21~45           | 79~55                         |
| 17~25     | 45~63           | 55~37                         |

2.4. Preparation of sample solutions
Take 10 batches of samples of Stellarlae Radix, silver pieces, and the 4 screen, each about 1.0 g, precision said, adding 50% methanol 50mL, according to quality of ultrasonic extraction (power 250W, frequency 35 kHz) 45 min, remove and cool off, then according to quality, make up the quality
loss reduction with methanol, shake well, filtering. The filtrate was filtered by 0.22 μm membrane for reserve.

2.5. Data analysis
The data were processed and analyzed by "the Similarity Evaluation System Software for Chromatographic Fingerprints of Traditional Chinese Medicine" (2004 edition), and the control fingerprints were generated.

3. Results

3.1. Precision
Injection precision was assessed by repetitive injections of the same sample solution for six time. The RSD of retention time and peak area of each common peak were lower than 3.0%, which indicated that the instrument had good precision.

3.2. Sample stability
Stability was evaluated by analysis of the same sample solution at 0, 2, 4, 8, 12 and 24h, respectively. The RSD of relative retention time and relative peak area of each common peak were calculated to be lower than 3.0%, indicated that the sample solution remained stable for 24h.

3.3. Repeatability
Repeatability was determined by analyzing six independently prepared samples of Stellarlae Radix using the same method, and RSD of the relative retention time and relative peak area of each common peak were calculated to be less than 3.0%, which showed that the method had good repeatability.

3.4. Sample determination
The Stellarlae Radix medicinal materials were taken, the sample solution was prepared, and the UPLC chromatogram of each batch of samples was obtained according to the determined chromatographic conditions. According to the measurement result of the sample, a fingerprint spectrum is established by using the similarity evaluation system of the traditional Chinese medicine chromatographic fingerprint spectrum (National Pharmacopoeia Committee, 2004A edition), the S1 is a reference peak, the automatic matching is carried out, a control map is generated by adopting a median method. The fingerprints of 10 batches of Stellarlae Radix and their common pattern were obtained. As shown in figs.1 and 2.

Figure 1. 10 batches of Stellarlae Radix UPLC fingerprint
3.5. Establishment of Common Peak and Analysis of fingerprint

In the fingerprint of Stellariae Radix established in this study, the similarity evaluation system software of chromatographic fingerprint of traditional Chinese medicine (2004 edition) was used for data processing and analysis, and the similarity among the 10 batches of Stellariae Radix was 0.949, 0.975, 0.965, 0.957, 0.971, 0.969, 0.961, 0.941 and 0.980, respectively. As shown in Table 3. A total of 13 common chromatographic peaks were identified in the fingerprint, among which 12 chromatographic peaks were taken as the reference peak to calculate the relative retention time and relative peak area of each common peak, as shown in table 4 and table 5. By comparing the similarity between different batches of samples, the similarity of Stellariae Radix purchased by each drugstore is better, which indicates that the quality of Stellariae Radix in each drugstore is more stable.

Table 3. Evaluation of the similarity of the fingerprint of the Stellariae Radix

| NO. | S1    | S2    | S3    | S4    | S5    | S6    | S7    | S8    | S9    | S10   | R    |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|------|
| S1  | 1.000 | 0.917 | 0.858 | 0.861 | 0.890 | 0.985 | 0.903 | 0.849 | 0.831 | 0.913 | 0.949|
| S2  | 0.917 | 1.000 | 0.931 | 0.951 | 0.920 | 0.924 | 0.950 | 0.927 | 0.920 | 0.953 | 0.975|
| S3  | 0.858 | 0.931 | 1.000 | 0.936 | 0.963 | 0.861 | 0.969 | 0.994 | 0.915 | 0.934 | 0.965|
| S4  | 0.861 | 0.951 | 0.936 | 1.000 | 0.912 | 0.867 | 0.904 | 0.932 | 0.964 | 0.944 | 0.957|
| S5  | 0.890 | 0.920 | 0.963 | 0.912 | 1.000 | 0.883 | 0.954 | 0.959 | 0.930 | 0.967 | 0.971|
| S6  | 0.985 | 0.924 | 0.861 | 0.867 | 0.883 | 1.000 | 0.905 | 0.855 | 0.825 | 0.909 | 0.950|
| S7  | 0.903 | 0.950 | 0.969 | 0.904 | 0.954 | 0.905 | 1.000 | 0.965 | 0.873 | 0.927 | 0.969|
| S8  | 0.849 | 0.927 | 0.994 | 0.932 | 0.959 | 0.855 | 0.965 | 1.000 | 0.910 | 0.930 | 0.961|
| S9  | 0.831 | 0.920 | 0.915 | 0.964 | 0.930 | 0.825 | 0.873 | 0.910 | 1.000 | 0.958 | 0.941|
| S10 | 0.913 | 0.953 | 0.934 | 0.944 | 0.967 | 0.909 | 0.927 | 0.930 | 0.958 | 1.000 | 0.980|
| R   | 0.949 | 0.975 | 0.965 | 0.957 | 0.971 | 0.950 | 0.969 | 0.961 | 0.941 | 0.980 | 1.000|
Table 4. Relative retention time of shared peaks

| NO. | S1   | S2   | S3   | S4   | S5   | S6   | S7   | S9   | S10  |
|-----|------|------|------|------|------|------|------|------|------|
| 1   | 0.3479 | 0.3474 | 0.3473 | 0.3480 | 0.3480 | 0.3459 | 0.3491 | 0.3477 | 0.3468 |
| 2   | 0.4753 | 0.4756 | 0.4734 | 0.4755 | 0.4741 | 0.4752 | 0.4741 | 0.4743 | 0.4754 |
| 3   | 0.5177 | 0.5183 | 0.5184 | 0.5158 | 0.5183 | 0.5165 | 0.5190 | 0.5167 | 0.5172 |
| 4   | 0.5859 | 0.5789 | 0.5665 | 0.5848 | 0.5868 | 0.5852 | 0.5880 | 0.5859 | 0.5865 |
| 5   | 0.6106 | 0.6109 | 0.6106 | 0.6095 | 0.6108 | 0.6096 | 0.6122 | 0.6103 | 0.6108 |
| 6   | 0.6392 | 0.6392 | 0.6390 | 0.6388 | 0.6387 | 0.6387 | 0.6406 | 0.6390 | 0.6394 |
| 7   | 0.6746 | 0.6748 | 0.6743 | 0.6741 | 0.6741 | 0.6739 | 0.6756 | 0.6749 | 0.6744 |
| 8   | 0.7176 | 0.7179 | 0.7171 | 0.7174 | 0.7170 | 0.7169 | 0.7176 | 0.7188 | 0.7177 |
| 9   | 0.7256 | 0.7258 | 0.7252 | 0.7250 | 0.7252 | 0.7248 | 0.7262 | 0.7263 | 0.7255 |
| 10  | 0.7694 | 0.7701 | 0.7687 | 0.7693 | 0.7690 | 0.7688 | 0.7692 | 0.7705 | 0.7694 |
| 11  | 0.8065 | 0.8071 | 0.8055 | 0.8068 | 0.8061 | 0.8062 | 0.8057 | 0.8079 | 0.8066 |
| 12(S)| 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 13  | 1.0379 | 1.0375 | 1.0380 | 1.0379 | 1.0378 | 1.0375 | 1.0378 | 1.0382 | 1.0376 |

Table 5. Relative peak area of common peaks

| NO. | S1   | S2   | S3   | S4   | S5   | S6   | S7   | S8   | S9   |
|-----|------|------|------|------|------|------|------|------|------|
| 1   | 0.9614 | 0.2970 | 0.4157 | 0.3936 | 0.3244 | 0.8039 | 0.3052 | 0.3638 | 0.4134 |
| 2   | 3.4588 | 0.4932 | 0.5513 | 0.5818 | 0.7570 | 3.1056 | 0.5247 | 0.4774 | 0.6032 |
| 3   | 5.8959 | 1.9136 | 1.9252 | 1.9460 | 2.5000 | 5.2691 | 1.9984 | 1.6758 | 2.0195 |
| 4   | 0.5440 | 1.0725 | 0.9673 | 1.1924 | 1.1997 | 1.9976 | 1.1157 | 0.8394 | 1.2372 |
| 5   | 2.7147 | 3.6239 | 3.6623 | 5.0770 | 6.0965 | 2.5202 | 1.9512 | 3.1064 | 5.3546 |
| 6   | 1.3473 | 0.5512 | 0.5360 | 0.6416 | 0.6817 | 1.2207 | 0.5269 | 0.4513 | 0.6061 |
| 7   | 0.7100 | 0.3343 | 0.3891 | 0.4008 | 0.4872 | 0.8541 | 0.4600 | 0.3435 | 0.5462 |
| 8   | 5.4648 | 1.7331 | 0.8280 | 0.7123 | 1.4555 | 4.8394 | 1.8187 | 0.7279 | 0.7554 |
| 9   | 5.4648 | 1.7331 | 0.8280 | 0.7123 | 1.4555 | 4.8394 | 1.8187 | 0.7279 | 0.7554 |
| 10  | 0.1819 | 0.0859 | 0.1729 | 0.1347 | 0.2348 | 0.1424 | 0.0911 | 0.1532 | 0.1425 |
| 11  | 0.1339 | 0.0796 | 0.1071 | 0.0852 | 0.1100 | 0.1121 | 0.0822 | 0.0970 | 0.0903 |
| 12(S)| 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 13  | 0.3024 | 0.2229 | 0.1782 | 0.3245 | 0.2392 | 0.2982 | 0.2297 | 0.1642 | 0.4124 |

4. Discussion
In this study, the UPLC fingerprint of Stellarae Radix was established for the first time to reflect the chemical composition information of Stellarae Radix comprehensively in order to control the overall quality of Stellarae Radix in the market. In order to compare the effects of different solvent extraction on the fingerprint of Stellarae Radix. The extraction solvents of 50% ethanol, 70% methanol, 50% ethanol and 50% ethanol were investigated respectively. The results showed that the extraction rate of Stellarae Radix was higher when 50% methanol was extracted, and the chemical information of the map was abundant, so 50% methanol was selected as extraction solvent. The extraction methods were investigated by ultrasound and reflux. The results showed that there was no significant difference in chromatographic peak information between the two methods, so the simple ultrasonic method was selected as the extraction method. The mobile phase was used to investigate methanol-water, acetonitrile-water and acetonitrile-0.1% phosphoric acid water. The results showed that acetonitrile-0.1% phosphoric acid water was used as mobile phase, and the separation degree of each chromatographic peak was good, which was conducive to the analysis of fingerprint.

As an effective quality control mode of traditional Chinese medicine, the fingerprint of traditional Chinese medicine can still give sufficient and reliable information to control the quality of traditional Chinese medicine when the chromatographic peak is not clear about what composition. The results
showed that there were 13 common peaks in samples from different sources, and the similarity was higher than 0.9, indicating that the quality of Stellarlae Radix from various pharmacies in the market was stable and could ensure the correct clinical use. There are also some differences, as can be seen from the peak area of fingerprint, which may be influenced by the content of chemical components in Stellarlae Radix. In summary, the analytical method established in this study is accurate, reliable and specific, which can reflect the quality of Stellarlae Radix on the whole and provide theoretical basis for the analysis and evaluation of the quality of Stellarlae Radix.

5. Conclusion
Through literature research, it is found that there are few reports on the quality control methods of Stellarlae Radix. Therefore, the UPLC fingerprint of Stellarlae Radix established in this study can characterize the quality information of Chinese medicinal materials in different pharmacies more quickly and comprehensively, and can also provide reference basis and significance for the control of quality standards of Stellarlae Radix. There are many research methods for quality control of traditional Chinese medicine. If we need to establish a more scientific, effective and accurate quality control standard to control the quality of traditional Chinese medicine, we should combine more quantitative determination with multiple components or other methods to reflect the consistency and stability of the quality of Stellarlae Radix, and have more positive significance for the quality comparison and evaluation of Stellarlae Radix. This is also the deficiency of this study, which needs to be further studied in the later stage.

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