UPLC-ELSD Fingerprint Research of *Dipsaci Radix*

Xiaosong Yang¹, Juan Kong¹, Xulong Huang¹, Lingling Zhang¹, Xiaofen Li¹, Fenqiu Yan¹, Xiangpei Wang¹, Hongmei Wu¹*

¹Department of Pharmacognosy, Guiyang University of Chinese Medicine, 50, Nanming District, Guiyang City, Guizhou Province, Guiyang 550002, PR China;
*Corresponding author’s e-mail: whm0425@126.com

**Abstract:** Objective: To establish the UPLC-ELSD fingerprint of *Dipsaci Radix* and provide reference for the quality control of *Dipsaci Radix*. Methods: Agilent ZORBAX RRHD Eclipse Plus C18 (2.1mm×100mm, 1.8μm) column was used, the mobile phase was composed of acetonitrile-water, gradient elution, the flow rate was 0.2 mL/min, atomization temperature of evaporative light scatter detector was 60°C, the column temperature was 30°C, nitrogen carrier gas flow rate was 1.5 L/min. Similarity evaluation was used to adjust 17 batches of *Dipsaci asper*. Results: The UPLC-ELSD fingerprint of *Dipsaci Radix* Aspergillus was established. Seven common peaks were identified. The similarity of 17 batches of *Dipsaci Radix* was between 0.659-1.000, which indicated that there were great differences among batches. Conclusion: The method is simple, efficient, rapid and reliable, it can provide reference for the quality evaluation of *Dipsaci Radix*.

1. Introduction

*Dipsaci Radix*, which is the dried root of *Dipsacus asper* Wall, ex Henry, is commonly used as a traditional Chinese medicine for the treatment of bone diseases, functions in strengthening bone and healing bone fractures [1]. It is widely distributed in China, mainly in Hubei, Sichuan, Hunan, Guizhou and other regions[2]. The chemical constituents of *Dipsaci Radix* are complex, including volatile oils, alkaloids, iridoid terpenes and triterpenes saponins[3]. The Chinese Medicine Fingerprint is based on the whole information of chromatogram, which is used to evaluate the quality of Traditional Chinese Medicine as a whole, to ensure the uniformity and stability of its internal quality, and to play an important role in controlling the authenticity and quality of raw materials and standardizing the production process and product quality [4]. At present, it has been reported that the fingerprint of *Dipsaci Radix* was established by HPLC, but it was established by HPLC fingerprint[5-8]. Jiao Tao [9] established the HPLC-ELSD fingerprint of *Dipsaci Radix* of processing with salt and rice wine. *Dipsaci Radix* mainly contains saponins, ELSD is a universal detector, the response value to saponins is large, and it can overcome the shortcomings of maximum absorption of saponins around 200nm, but baseline drift and noise. UPLC can greatly shorten the analysis time, significantly improve the chromatographic peak separation and detection sensitivity with the advantages of ultra-high efficiency, and it makes up for the shortcomings of HPLC, such as long analysis time, low resolution and big solvent consumption. Therefore, in this study, 17 batches of *Dipsaci Radix* were analyzed by UPLC-ELSD, and compared the similarity. In order to provide reference for the quality control of *Dipsaci Radix*. 
2. Materials and methods

2.1. Instruments and reagents
Chromatographic analysis was performed on a UPLC (Waters company, USA), including evaporative light scattering detector (Waters), and Empower workstation. It was useful to analytical balance (Shanghai Anting Instruments Ltd), KQ-100E ultrasonic cleaner (Kunshan Ultrasonic Instruments Ltd), and Digital constant temperature water bath (model: HH-6, Changzhou Aohua Instruments Ltd). Data analysis was performed by professional software named Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine composed by Chinese Pharmacopoeia Committee (Version 2004A), The reagent was methanol and acetonitrile for the chromatographic purity, and water was the redistilled water. The sample was identified as the dry root of *Dipsaci Radix* by Professor Xiang-pei Wang of Guizhou University of Traditional Chinese Medicine. Specific sources are shown in table 1.

2.2 chromatographic conditions
The Chromatographic column was Agilent ZORBAX RRHD Eclip Plus C18 (2.1mm × 100mm, 1.8μm) C18 column. A binary gradient elution system consisting of water (A)-acetonitrile (C) was used with the following gradient programs: 0-2min, 90-88%; 2-5min, 88-80%; 5-10min, 80-72%; 10-14min, 72-67%; 14-15min, 67-57%; 15-25min, 57-48%. The column temperature was 30℃, the volume flow rate was 0.2ml/min; and the injection volume was 3.0 μL. The drift tube temperature was 60℃, the N gas pressure was 45kpa, and the flow rate was 1.5L/min.

2.3 Preparation of samples
All the samples were crushed into fine powder, accurately weighed 1.0g, and placed into round bottom flask, respectively. The extraction method was added 50mL of methanol and ultrasonic treatment 45 min, and made up the lost methanol, it was filtered, the filtrate was passed through 0.22μm microporous membrane as sample solution.

2.4. UPLC-ELSD method validation

2.4.1. Precision test. The same samples(S16) was injected for 6 times by the chromatographic method under 2.1 and 2.2 item, and the chromatogram was recorded. Using the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A) to evaluate the fingerprint data. The similarity of chromatographic fingerprint was 0.099, 1.000, 1.000, 1.000 and 1.000, respectively, it was more than 0.900. The precision of the instrument was good.

2.4.2. Stability test. The same sample (S16) was determined by chromatographic method under 2.1 and 2.2 item at 0, 2, 4, 8, 12, 24, respectively. The similarity soft (2004 A version) of TCM the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A) fingerprint was used to process the fingerprint data, and the similarity was calculated. The similarity of chromatographic fingerprints was 1.000, 1.000, 1.000, 1.000, 0.917, 1.000 and 0.997 respectively. The results show that the chemical composition of the sample was stable within 24 hours.

2.4.3. Repeatability test. the same six samples were prepared and determined by the method of 2.1 and 2.2 item. The chromatogram was recorded, and the fingerprint data was processed by the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A). The similarity of chromatographic fingerprints was 1.000, 1.000, 1.000, 1.000, 0.973 and 0.999 respectively. The results show that the method had good reproducibility.
3. Results

3.1 Establishment of fingerprint of Dipsaci Radix

17 batches of *Dipsaci Radix* were determined by chromatographic methods of "2.2" and "2.1". The chromatographic data were processed by the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A) for similarity analysis, and seven common peaks were obtained, such as figure 1. The relative retention time and relative peak area of each common peak were calculated. The results are shown in table 2 and 3.

![UPLC-ELSD fingerprint of 17 batches of Dipsaci Radix](image)

Table 1 Source of 17 batches of *Dipsaci Radix*

| No. | species         | producing regions                        | Similarity |
|-----|-----------------|------------------------------------------|------------|
| S1  | *Dipsaci Radix* | Guiyang City, Guizhou Province           | 0.933      |
| S2  | *Dipsaci Radix* | Guiyang City, Guizhou Province           | 0.996      |
| S3  | *Dipsaci Radix* | Guiyang City, Guizhou Province           | 0.993      |
| S4  | *Dipsaci Radix* | Guiyang City, Guizhou Province           | 0.659      |
| S5  | *Dipsaci Radix* | Guiyang City, Guizhou Province           | 0.743      |
| S6  | *Dipsaci Radix* | Guiyang City, Guizhou Province           | 0.994      |
| S7  | *Dipsaci Radix* | Guiyang City, Guizhou Province           | 0.997      |
| S8  | *Dipsaci Radix* | Guiyang City, Guizhou Province           | 0.995      |
| S9  | *Dipsaci Radix* | Kaili city, guizhou province             | 0.950      |
| S10 | *Dipsaci Radix* | Kaili city, guizhou province             | 0.998      |
| S11 | *Dipsaci Radix* | Kaili city, guizhou province             | 0.991      |
| S12 | *Dipsaci Radix* | Anshun city, guizhou province            | 0.987      |
| S13 | *Dipsaci Radix* | Anshun city, guizhou province            | 0.629      |
| S14 | *Dipsaci Radix* | Anshun city, guizhou province            | 1.000      |
| S15 | *Dipsaci Radix* | Chengdu, sichuan province               | 1.000      |
| S16 | *Dipsaci Radix* | Chengdu, sichuan province               | 0.993      |
| S17 | *Dipsaci Radix* | Chengdu, sichuan province               | 0.991      |

Table 2 Relative retention time of common peaks in UPLC-ELSD fingerprint of 17 batches of *Dipsaci Radix*

| Common peak | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 |
|-------------|----|----|----|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1           | 0.064 | 0.065 | 0.063 | 0.064 | 0.065 | 0.065 | 0.064 | 0.065 | 0.065 | 0.066 | 0.065 | 0.066 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 |
| 2           | 0.082 | 0.096 | 0.094 | 0.091 | 0.090 | 0.091 | 0.079 | 0.091 | 0.091 | 0.101 | 0.089 | 0.095 | 0.097 | 0.091 | 0.091 | 0.091 | 0.091 |
| 3           | 0.626 | 0.654 | 0.614 | 0.620 | 0.626 | 0.633 | 0.620 | 0.617 | 0.620 | 0.628 | 0.627 | 0.622 | 0.626 | 0.626 | 0.626 | 0.626 | 0.626 |
| 4           | 0.092 | 0.090 | 0.087 | 0.084 | 0.089 | 0.074 | 0.098 | 0.097 | 0.091 | 0.091 | 0.091 | 0.098 | 0.074 | 0.091 | 0.091 | 0.091 | 0.091 | 0.091 |
4. Conclusion
In this study, UPLC-ELSD was used to determine the fingerprint of 17 batches of *Dipsaci Radix*, and the similarity was between 0.659 and 1.000. According to the similarity results, the similarity of the samples of S4, S5, S12 was worse than others. The similarity of other 14 batches of *Dipsaci Radix* was between 0.933-1.000, indicating that there was correlation the quality of 14 batches of *Dipsaci Radix*. Because *Dipsaci Radix* is widely used in clinic, it is difficult to ensure its quality effectively. In this study, the fingerprint of 17 batches of *Dipsaci Radix* was established by UPLC-ELSD, it can provide a further basis for the selection, purchase and quality control of *Dipsaci Radix*.

5. Discuss
In this experiment, ultrasonic and reflux extraction methods were compared, and the results showed that ultrasonic extraction and reflux extraction were the same. So the ultrasonic extraction method was chosen as the best extraction method. It were reviewed that the methods were different solvents such as ethanol, 50% ethanol, methanol and 50% methanol, as well as different extraction time, reflux and ultrasonic extraction in this study. The results showed that the extraction was complete with methanol, the chromatographic peaks were more; compared the mobile phase acetonitrile-water and methanol-water, acetonitrile-water was selected as the mobile phase for gradient elution, and the chromatogram had good separation degree and peak shape.

Acknowledgements
This work was supported by the The Guizhou First-Class Course Construction Construction Project [2017], the authors thank the government of China for their financial support.

References
[1] National Pharmacopoeia Commission. Pharmacopoeia of the people's Republic of China(2015 Edition. A [S]. Beijing: China Medical Science and Technology Press, 2015, 6
[2] Liu HB. Investigation on the provenance of genuine medicinal material Chuanxuantian[J]. Journal of Panzhihua University, 2005, 22 (6): 118-119.
[3] Wang Q, Liu EW, Han LF, et al. Studies on the chemical constituents of *Dipsaci Radix* [J]. Acta Pharmacologica Sinica, 2013, 48 (7): 1124-1127.
[4] Zhu M, Chen BL, Shi SM, et al. Application of fingerprint Technology of Chinese Materia Medica in Chinese Pharmacopoeia 2015 Edition [J]. Modern Applied Pharmacy of China, 2016, 33 (5): 611- 614.
[5] Zhu JG, Li LY, Ma P, et al. Study on HPLC fingerprint of *Dipsaci Radix*[J]. China Pharmacy, 2012,
23(11): 1012 -1014.
[6] Wei SH, Tian HH, Yang WL, et al. Study on HPLC fingerprint of Dipsaci Radix [J]. Guizhou Agricultural Science, 2014, 42(10): 45-47.
[7] Liu EW, Wu Sh A, Wang JL. Study on HPLC fingerprint of Dipsaci Radix [J]. Drug Evaluation study, 2010, 33 (6): 432-435.
[8] Cao Y, Peng W, Su WW. Study on fingerprint of Dipsaci Radix [J]. New Chinese Medicine and Clinical Pharmacology, 2010, 21 (1): 50-54.
[9] Jiao T, Wu CL. Comparative study on HPLC-ELSD fingerprinting of Dipsaci Radix before and after processing [J]. Shi Zhen, Chinese Medicine, 2017, 28 (8): 1895-1896.