Study on UPLC Fingerprint of Alpinia officinarum Hance

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Abstract. Objective: The UPLC fingerprint of Alpinia officinarum Hance was established to provide reference for evaluating the quality of Alpinia officinarum Hance on the market. Method: Agilent ZORBAX Eclipse Plus C18 (2.1 mm ×100 mm,1.8μm) chromatographic column, mobile phase 0.01% phosphoric acid solution (A) - acetonitrile (B), flow rate 0.1ml·min-1, detection wavelength 254 nm, column temperature 30℃. The fingerprints of 10 batches of Alpinia officinarum Hance were determined, and the similarity of fingerprints was compared by using the "similarity evaluation system of chromatographic fingerprints of traditional Chinese medicine" (2004 A). Results: The specific UPLC fingerprint of Alpinia officinarum Hance was established, 17 common peaks were established, and the similarity was between 0.922-0.989. Conclusion: The method is simple, efficient and reliable, which provides an effective and rapid evaluation method for the quality control of Alpinia officinarum Hance.

1. Introduction

Alpinia officinarum Hance is derived from the dried rhizome of the Zingiberaceae plant Alpinia officinarum Hance. In late summer and early autumn, the roots and scales were removed, washed, cut into sections and dried in the sun [1]. There are many reports on the chemical constituents and pharmacological activities of Alpinia officinarum Hance. A large number of diarylheptane compounds and flavonoids were isolated from Alpinia officinarum Hance [2]. It also contains volatile oils, phenylpropanoids, glycosides, terpenes, organic acids and esters, sterols and their glycosides [3-6]. As a medicinal and edible homologous variety, Alpinia officinarum Hance can be used as a flavoring agent and antioxidant in the food industry to adjust the freshness of food and extend the shelf life of food [7]. Modern pharmacological studies have shown that Alpinia officinarum Hance plays an important role in regulating the function of mitochondrial sodium pump and calcium pump, anti-Alzheimer’s disease, anti-oxidation, anti-ulcer, anti-allergy, anti-liver damage and cirrhosis, anti-vitiligo and other pharmaceutical effects[8]. The volatile component of Alpinia officinarum Hance is known to have good anti-tumor, anti-oxidation, anti-inflammatory and analgesic effects, and the effect is significant [9]. Currently, HPLC, C-MS, capillary electrophoresis and other analytical methods have been used to control the quality of Alpinia officinarum Hance, among which HPLC fingerprint has...
been widely studied [10-12]. Due to build the HPLC fingerprint analysis for a long time, so the research on the market different pharmacy galangal UPLC fingerprint contrast research, with the high efficiency, fast analysis Alpinia officinarum Hance composition change, on the basis of the digital processing, combined with similarity evaluation method of quality analysis and evaluation, in order to more comprehensive to control the quality of the Alpinia officinarum Hance medicinal materials.

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 2004A).

2.2. Reagents and Plant materials
Methanol, acetonitrile for chromatographic pure (Merck stock Lianghe company, Germany), phosphoric acid analysis pure (Chinese medicine group chemical reagent Co., Ltd.), water for wow ha pure water. Alpinia officinarum Hance is from various pharmacies in Guiyang. Lerentang Pharmacy, Yipin Pharmaceutical, Tongrentang Pharmacy, Yishu Pharmaceutical, Siyuan Pharmacy, Fuankang Pharmaceutical, Tianyi Pharmacy, Zhilin pharmacy, Tongji Tong Pharmaceutical Industry, Dechangxiang Pharmaceutical Co., Ltd. The sample was identified by Professor Wang Xianpei of Guizhou University of traditional Chinese Medicine as the dried rhizome of Alpinia officinarum Hance of Zingiberaceae.

2.3. Chromatographic conditions
Agilent ZORBAX Eclipse Plus C18(2.1 mm ×100 mm,1.8μm) chromatographic column; flow rate 0.1 mL·min⁻¹; detection wavelength 254 nm; column temperature 30°C; injection volume 1μL; mobile phase:0.1% phosphoric acid water (A)-acetonitrile (B). Gradient elution procedure: 0~5min, 95 ~ 79% (A); 5 ~ 15min, 79 ~ 50% (A); 15 ~ 23min, 50 ~ 40% (A).

2.4. Preparation of sample solutions
Take 10 batches of samples, crush, pass the No.4 sieve, take about 1.0 g each, accurately weigh, add 50% methanol 25 mL, weigh the quality, ultrasonic extraction (power 250W, frequency 35kHz) 45min, take out, let out cold, weigh the quality again, use methanol to complement the lost quality, shake well, filter, take the filtrate over 0.22 micro porous filter membrane, that is, get the sample solution.

2.5. Methodological investigation of liquid fingerprint

2.5.1. 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.

2.5.2. 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.

2.5.3. 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. Results

3.1. Establishment of liquid fingerprint
Using 10 batches of samples of UPLC color atlas into the "Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System 2004A Edition" to establish the fingerprint of *Alpinia officinarum* Hance (Fig.1) and the common pattern (Fig.2). A total of 17 common peaks were identified and selected as the characteristic peaks were those with common, good stability, large peak area and good separation degree. The peak 12 has moderate retention time, good separation effect and the largest peak area. The relative retention time and relative peak area of each common peak were calculated respectively (Tables 1, 2).

![Figure 1. Fingerprint of 10 batches of Alpinia officinarum Hance](image1)

![Figure 2. Common pattern of fingerprint of 10 batches of Alpinia officinarum Hance](image2)
ranged from 0.922 to 0.989, indicating that there was no difference and the quality was good.

The results of calculating the UPLC similarity of 10 batches of

Table 1. Relative retention time of common peaks

| NO. | S1   | S2   | S3   | S4   | S5   | S6   | S7   | S8   | S9   | S10  |
|-----|------|------|------|------|------|------|------|------|------|------|
| 1   | 0.1257| 0.1257|0.1257|0.1258|0.1258|0.1258|0.1257|0.1258|0.1259|0.1260|
| 2   | 0.1524| 0.1524|0.1527|0.1526|0.1526|0.1521|0.1524|0.1521|0.1525|0.1516|
| 3   | 0.4120| 0.4110|0.4117|0.4108|0.4116|0.4111|0.4109|0.4107|0.4110|0.4117|
| 4   | 0.4252| 0.4242|0.4249|0.4240|0.4246|0.4244|0.4243|0.4242|0.4242|0.4251|
| 5   | 0.5273| 0.5265|0.5272|0.5265|0.5266|0.5270|0.5263|0.5271|0.5267|0.5281|
| 6   | 0.6905| 0.6896|0.6907|0.6902|0.6900|0.6904|0.6899|0.6909|0.6899|0.6914|
| 7   | 0.7489| 0.7481|0.7487|0.7486|0.7483|0.7490|0.7483|0.7493|0.7481|0.7491|
| 8   | 0.7648| 0.7642|0.7646|0.7645|0.7643|0.7651|0.7643|0.7653|0.7640|0.7650|
| 9   | 0.8796| 0.8805|0.8810|0.8805|0.8808|0.8813|0.8798|0.8815|0.8792|0.8809|
| 10  | 0.9024| 0.9027|0.9024|0.9025|0.9022|0.9027|0.9026|0.9029|0.9024|0.9027|
| 11  | 0.9228| 0.9230|0.9231|0.9229|0.9228|0.9231|0.9231|0.9234|0.9230|0.9233|
| 12(S)|1.0000|1.0000|1.0000|1.0000|1.0000|1.0000|1.0000|1.0000|1.0000|1.0000|
| 13  | 1.0571| 1.0566|1.0591|1.0566|1.0570|1.0565|1.0568|1.0568|1.0567|1.0570|
| 14  | 1.1903| 1.1894|1.1904|1.1897|1.1901|1.1893|1.1896|1.1902|1.1897|1.1903|
| 15  | 1.2189| 1.2180|1.2190|1.2184|1.2187|1.2178|1.2180|1.2189|1.2182|1.2191|
| 16  | 1.2528| 1.2518|1.2530|1.2522|1.2525|1.2520|1.2519|1.2529|1.2519|1.2532|
| 17  | 1.6041| 1.6039|1.6046|1.6043|1.6039|1.6042|1.6039|1.6054|1.6036|1.6057|

Table 2. Relative peak area of common peaks

| NO. | S1   | S2   | S3   | S4   | S5   | S6   | S7   | S8   | S9   | S10  |
|-----|------|------|------|------|------|------|------|------|------|------|
| 1   | 0.0129| 0.0160|0.0172|0.0180|0.0166|0.0090|0.0125|0.0161|0.0138|0.0179|
| 2   | 0.0569| 0.0203|0.0117|0.0125|0.0112|0.0022|0.0538|0.0192|0.0161|0.0179|
| 3   | 0.1839| 0.1976|0.1429|0.3953|0.0579|0.1730|0.2435|0.1912|0.1499|0.2329|
| 4   | 0.0893| 0.0930|0.1842|0.0189|0.0248|0.1092|0.0965|0.0917|0.0772|0.0178|
| 5   | 0.1353| 0.1307|0.2005|0.4908|0.0518|0.1989|0.3301|0.2301|0.2735|0.1861|
| 6   | 0.0203| 0.0227|0.0452|0.0455|0.0150|0.0296|0.0217|0.0221|0.0274|0.0292|
| 7   | 0.0247| 0.0191|0.0269|0.0271|0.0209|0.0223|0.0208|0.0195|0.0224|0.0259|
| 8   | 0.0535| 0.0667|0.0864|0.0802|0.0554|0.0595|0.0593|0.0672|0.0669|0.0752|
| 9   | 0.0209| 0.0184|0.0272|0.0239|0.0412|0.0247|0.0216|0.0208|0.0265|0.0224|
| 10  | 0.0824| 0.0866|0.0759|0.0761|0.0646|0.0910|0.0821|0.0861|0.0690|0.0750|
| 11  | 0.4143| 0.4563|0.3563|0.3546|0.3313|0.4146|0.4112|0.4569|0.3426|0.3513|
| 12(S)|1.0000|1.0000|1.0000|1.0000|1.0000|1.0000|1.0000|1.0000|1.0000|1.0000|
| 13  | 0.1335| 0.1136|0.1155|0.1167|0.1013|0.1114|0.1321|0.1167|0.1320|0.1165|
| 14  | 0.0425| 0.0333|0.0505|0.0510|0.0556|0.0367|0.0420|0.0335|0.0527|0.0507|
| 15  | 0.1888| 0.1469|0.2249|0.2261|0.2429|0.1491|0.1865|0.1479|0.2425|0.2284|
| 16  | 0.3482| 0.3870|0.3127|0.3144|0.3089|0.3380|0.3429|0.3845|0.2801|0.3093|
| 17  | 0.1470| 0.1185|0.2009|0.2013|0.2028|0.1207|0.1463|0.1178|0.1851|0.2053|

3.2. Similar fingerprint evaluation analysis
The results of calculating the UPLC similarity of 10 batches of *Alpinia officinarum* Hance were shown in Table 3. The similarity between 10 batches of *Alpinia officinarum* Hance and control fingerprints ranged from 0.922 to 0.989, indicating that there was no difference and the quality was good.
4. Discussion

*Alpinia officinarum* Hance is widely used in many fields, such as medicine, food and so on. In this study, 10 batches of *Alpinia officinarum* Hance purchased by different pharmacies in Guiyang market were used as raw materials to establish the uplc fingerprint of 10 batches of *Alpinia officinarum* Hance, thus evaluating the quality consistency of *Alpinia officinarum* Hance between different pharmacies. The research shows that the quality of *Alpinia officinarum* Hance in the market is stable and controllable at present, and the consistency is high. In 10 batches of *Alpinia officinarum* Hance, the chemical information was abundant, the fingerprint confirmed 17 Common Peaks, the information of each chromatographic peak was uniform, and the chemical composition was the same. The results of similarity evaluation showed that the similarity between 10 batches of *Alpinia officinarum* Hance was more than 0.9, and the quality was stable and reliable. Therefore, the UPLC fingerprinting method established in this study can evaluate the quality of *Alpinia officinarum* Hance efficiently, quickly and reliably, and provide the basis for the clinical application of *Alpinia officinarum* Hance.

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