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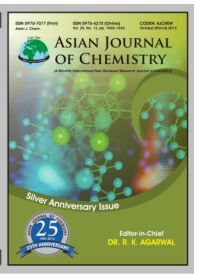

Analysis of Semen Euphorbiae Frostlike Powders by HPLC Fingerprint

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In present work, the analysis method of semen euphorbiae frostlike powders with HPLC fingerprint was established. The high performance liquid chromatography was applied. The chromatographic column was Agilent Zorbax-C_{18} (4.6 × 250 mm, 5 µm) column. The flow phase was acetonitrile:water = 61:39; the flow velocity was: 1 mL min^{-1}, the detection wavelength was 280 nm and the column temperature was 40 °C. The fingerprints of semen euphorbiae frostlike powders were established and 7 common peaks were identified. Fingerprint of semen euphorbiae frostlike powder samples and that of the control group shared a similarity of at least 0.9 and can be used in the qualitative identification of semen euphorbiae frostlike powders.

Key Words: Semen euphorbiae frostlike powders, High performance liquid chromatography, Fingerprint.

INTRODUCTION

Semen euphorbiae is dry and mature seed of *Euphorbia lathyris* L. of *Euphorbiaceae*, showing effects of expelling water retention with drastic purgative and stasis-breaking. It can be externally used to treat tinea and corrode wart[1], as well as obstruction of urination and defecation, edema, phlegm, stagnation, stasis and amenorrhea; in the modern study, semen euphorbiae can inhibit the growth of tumor cells[2], skin whitening and freckles[3], drug tolerance, inflammation, bacteria[4], treatment of leukemia[5] and bladder cancer[6], *etc*. The application of fingerprint in quality control of processed products shows universality and individuality from the overall aspect. It can comprehensively reflect the inherent quality of processed products, which could facilitate the identification of processed products of traditional Chinese medicine (TCM). Many diterpenoid compounds be founded and be regarded as the main effective component in semen euphorbiae frostlike powders, such as *Euphorbia lathyris* A[7], Euphorbia Factor L_1, *Euphorbia Factor* L_2, *Euphorbia Factor* L_3[8-10] *etc.* In this experiment, the fingerprint of semen euphorbiae frostlike powders was explored and analyzed, which can effectively clarify its ingredients and amount. It could also efficiently reflect the impact of processing on traditional Chinese medicine and better control the clinical drug safety.

EXPERIMENTAL

Apparatus included Shimadzu LC-20AHPLC chromatograph (Shimadzu Corporation); BS210S millionth electronic balance(Beijing Sartorius Co., Ltd.); METTLER AE240 hundred thousandth electronic analytical balance (Switzerland); KQ-500DV CNC ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.); DHG-9076A Electro- Thermostatic Blast Oven (Shanghai Jinghong Experimental Equipment Co., Ltd.); HH-S Thermostat Water Bath (Gongyi Yingyu Yuhua Instrument Factory); SZ-93 Automatic Dual Water Distiller (Shanghai Yarong Biochemical Instrument Factory); volumetric flask, round-bottom flask and condenser pipe *etc.* (all from Tianjin Glass Instrument Factory).

Semen euphorbiae sterol controls (China Pharmaceutical and Biological Products Assay Institute, Batch No.: 111789-200901; the purity: 99.3 %), semen euphorbiae L_2 controls (China Pharmaceutical and Biological Products Assay Institute, Batch No.: 111790-200901; the purity: 98.5 %), semen euphorbiae L_3 controls (China Pharmaceutical and Biological Products Assay Institute, Batch No.: 111791-200901; the purity: 98.6 %); Acetonitrile (Dikma Technologies Inc., chromatographically pure); methanol and other reagents were analytically pure; distilled water.

Ten batches of semen euphorbiae frostlike powders of the medicinal material samples were produced as follows: different batches of semen euphorbiae were peeled, heated in the microwave at high temperature for 1 min and suppressed by hot-press intelligent frost-like power device (100 g of feeding amount, 75 °C of preheating temperature, 45 MP of pressure). Te products were sieved through the 24-mesh sieve after the smashing.

Selection of mobile phase and mobile phase proportion: With experimental comparison, acetonitrile and water (61:39) were treated with isocratic elution, which efficiently separated chromatographic peaks of samples with relatively large amount of peaks. After 1 h of the analysis time, no characteristic peak occurred. In this research, four systems were compared, including the acetonitrile-water, acetonitrile-0.1 % phosphoric acid solution, acetonitrile-0.1 % glacial acetic acid solution and acetonitrile-0.1 % formic acid solution. Ultimately, the fingerprint of four systems did not show remarkable differences. But the spike of acetonitrile-water was relatively sound and the preparation was simple. Thus, the acetonitrile-water solution was taken as the mobile phase system and the result was shown in Table-1.

TABLE-1
MOBILE PHASE PROPORTION OF FINGERPRINT OF SEMEN EUPHORBIAE FROSTLIKE POWDERS

Mobile Phase	0 to 60min
Acetonitrile	61
Water	39

Selection of detection wavelength: In this experiment, semen euphorbiae sterols, semen euphorbiae L_2 and L_3 were taken as index peaks. Effects of different wavelengths (230, 280 and 300nm) were compared. The wavelength of 280 nm showed proper chromatographic peak proportion and good spike. Thus, the wavelength was identified as 280 nm.

Selection of column temperature: Column temperatures (35, 40, and 45 °C) were compared. The experiment showed that at 40 °C, the peak time, spike and resolution were satisfying. Thus, in this research, the column temperatures was set as 40 °C.

Chromatographic conditions: Chromatographic column: Agilent Zorbax-C_{18} (4.6 × 250 mm, 5 μm); mobile phase: acetonitrile-water = 61:39; flow velocity: 1.0 mL min^{-1}; the wavelength: 280 nm; column temperature: 40 °C; sample size:10 μL; sampling time: 60 min.

Preparation of reference solutions: 0.00106 g of semen euphorbiae sterols, 0.00045 g of semen euphorbiae L_2 and 0.00080 g of L_3 were accurately weighed and placed in 10 mL volumetric flasks, respectively. Methanol was added for salvation. The solution was shaken up so as to obtain the semen euphorbiae mixing controls. Their quality concentration was 0.106, 0.045 and 0.080 mg mL^{-1}, respectively.

Preparation of the test solution: About 0.2 g of the semen euphorbiae frostlike powder was accurately weighed and 20 mL of methanol was added. Through 2 h of the bath reflux, the solution was chilled, filtered and dried by water bath and distillation. A certain amount of methanol was added for dissolution. Later, the solution was placed in the 5 mL volumetric flask and diluted with methanol to the scale. Then, the solution was shaken up and filtered through the millipore filter (0.45 μm), so as to obtain the test fluid.

RESULTS AND DISCUSSION

Precision test: About 0.2 g of the semen euphorbiae frostlike powder was accurately weighed and operated according to the above mentioned preparation method. The sample size was 10 μL and the sample was consecutively fed for 5 times and its fingerprint was recorded. Chromap 1.5 was used to process data. The data showed that the relative retention time of each chromatographic peak was RSD < 3 % and the relative peak area RSD < 5 %. This indicated that under this condition, the fingerprint precision of semen euphorbiae frostlike powders was sound.

Stability test: About 0.2 g of the semen euphorbiae frostlike powders were accurately weighed and operated according to the above mentioned preparation method. At 0, 2, 8, 12, 24 and 48 h, its fingerprint was recorded. Chromap 1.5 was used to process data. The data showed that the relative retention time of each chromatographic peak was RSD < 3.0 % and the relative peak area RSD < 5.0 %. This indicated that samples remained relatively stable in 48 h.

Repeatability test: Five samples of semen euphorbiae frostlike powders were taken and each was about 0.2 g. Samples were accurately weighted and operated according to the above mentioned preparation method. Samples were respectively fed and their respectively fingerprint was recorded. Chromap1.5 was used to process data. The data showed that the relative retention time of each chromatographic peak was RSD < 3.0 % and the relative peak area RSD < 5.0 %. This indicated that under this condition, the fingerprint of semen euphorbiae frostlike powders showed good repeatability, which was consistent with the requirements of the fingerprint.

Standardization of common peaks: The fingerprint of 10 batches of semen euphorbiae frostlike powders was analyzed. 7 common peaks were identified. Among them, peak 2 was semen euphorbiae sterol. Peak 4 was semen euphorbiae L_3; peak 7 was semen euphorbiae L_2. The semen euphorbiae sterol was taken as the reference peak. Its relative retention time and relative peak area was set as 1 to calculate the two data of other characteristic peaks. Chromap1.5 was used to process data. Results were shown in Fig. 1, Tables 2 and 3.

TABLE-2

Sample No.	Relative retention time (min)						
	1	2	3	4	5	6	7
1	0.6390	1.000	1.056	1.612	1.852	1.941	2.666
2	0.6386	1.000	1.056	1.611	1.852	1.941	2.666
3	0.6382	1.000	1.056	1.612	1.852	1.942	2.667
4	0.6382	1.000	1.056	1.612	1.853	1.942	2.668
5	0.6378	1.000	1.056	1.612	1.853	1.941	2.667
6	0.6384	1.000	1.056	1.612	1.852	1.941	2.665
7	0.6389	1.000	1.057	1.612	1.852	1.941	2.667
8	0.6380	1.000	1.056	1.612	1.853	1.942	2.668
9	0.6389	1.000	1.057	1.612	1.852	1.942	2.668
10	0.6382	1.000	1.056	1.612	1.852	1.942	2.668

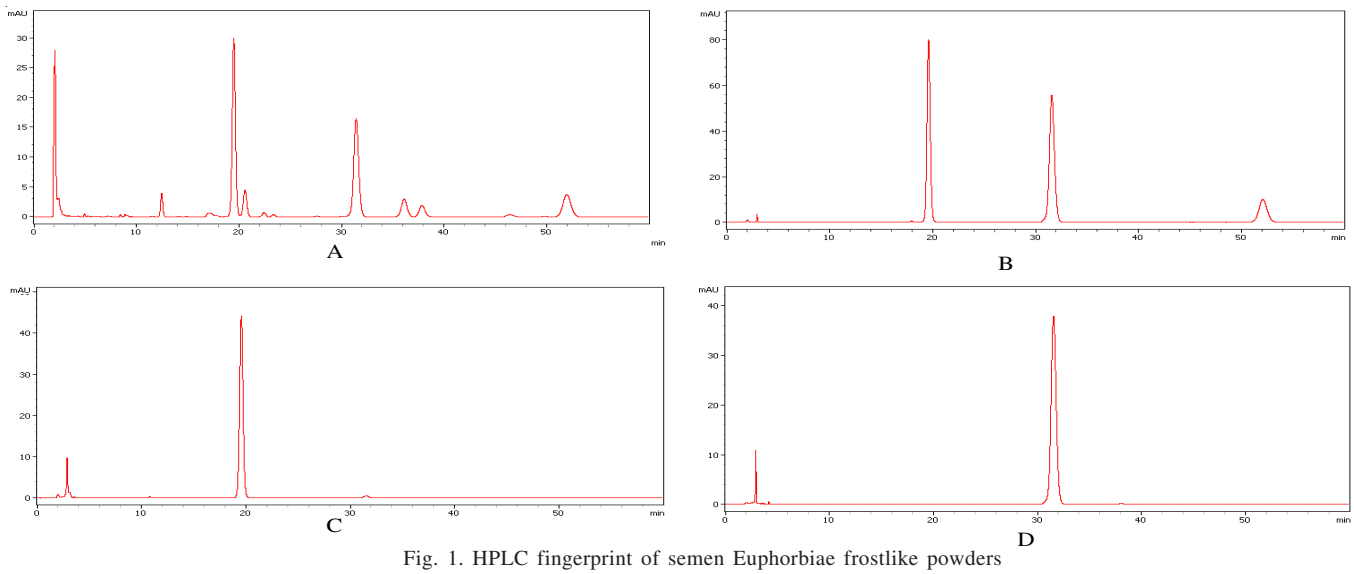

Fig. 1. HPLC fingerprint of semen Euphorbiae frostlike powders

A. semen Euphorbiae frostlike powders, B. mixing controls, C. semen Euphorbiae element L_1, D. semen Euphorbiae element L_3

TABLE-3
RELATIVE PEAK AREA OF COMMON PEAKS

Sample No.	Relative retention peak area						
	1	2	3	4	5	6	7
1	0.0888	1.000	0.1577	0.8401	0.1554	0.0943	0.2783
2	0.8887	1.000	0.1583	0.8409	0.1568	0.0962	0.2805
3	0.1057	1.000	0.1865	1.0129	0.1960	0.1113	0.3467
4	0.0867	1.000	0.1637	0.8528	0.1630	0.1092	0.2909
5	0.0903	1.000	0.1660	0.8985	0.1663	0.1051	0.2918
6	0.1098	1.000	0.2096	1.2093	0.2358	0.0819	0.4020
7	0.1141	1.000	0.2093	1.1040	0.2237	0.1043	0.3513
8	0.0848	1.000	0.1596	0.9292	0.1717	0.0799	0.2844
9	0.1088	1.000	0.2063	1.1704	0.2292	0.1001	0.3930
10	0.1018	1.000	0.1958	1.1324	0.2100	0.0898	0.3589

Calculation of fingerprint similarity: The fingerprint processing software was used to process the fingerprint of semen Euphorbiae frostlike powders so as to obtain the fingerprint overlay chart of semen Euphorbiae frostlike powders and the similarity analysis result. The similarity was no less than 0.9. The results were shown in Table-4, Figs. 2-4.

Conclusion

Semen *Euphorbiae* frostlike powders are commonly used as clinical medicine and no studies on semen *Euphorbiae* frostlike powders with its fingerprint were reported. In this research, HPLC fingerprint of semen *Euphorbiae* frostlike powders was established so that 7 chromatographic peaks can be simultaneously identified. Overall, it displayed the characteristics of semen *Euphorbiae* frostlike powders and systematically reflected a complete perspective of components of semen *Euphorbiae* frostlike powders. This method laid a solid foundation for the quality control of semen *Euphorbiae* frostlike powders.

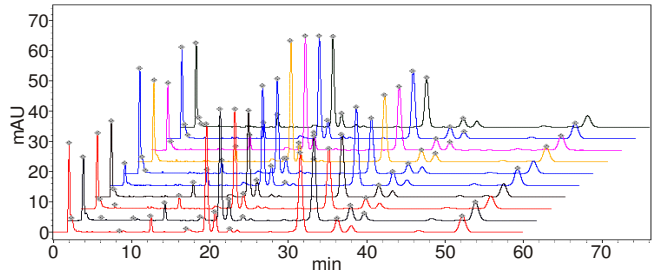

Fig. 2. 2D fingerprint overlay chart of 10 batches of semen *Euphorbiae* frostlike powders

TABLE-4
FINGERPRINT SIMILARITY ANALYSIS RESULTS OF SEMEN EUPHORBIAE FROSTLIKE POWDERS

Sample No.	correlation index	included angle cosine
1	0.9451	0.9616
2	0.9922	0.9949
3	0.9803	0.9875
4	0.9777	0.9858
5	0.9413	0.9619
6	0.9599	0.9735
7	0.9597	0.9728
8	0.9605	0.9716
9	0.9855	0.9907
10	1.000	1.000

With the analysis of 10 batches of semen *Euphorbiae* frostlike powders, it was found that the similarity of its common peaks was no less than 0.9, indicating that different batches of semen *Euphorbiae* frostlike powders did not have significant fingerprint changes. However, the relative peak area of the chromatographic peaks showed that certain differences were

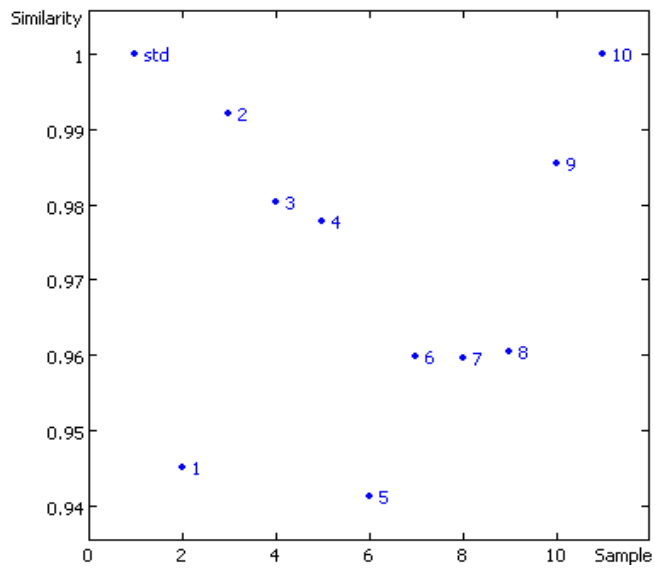

Fig. 3. Similarity of semen *Euphorbiae* frostlike powders (correlation index)

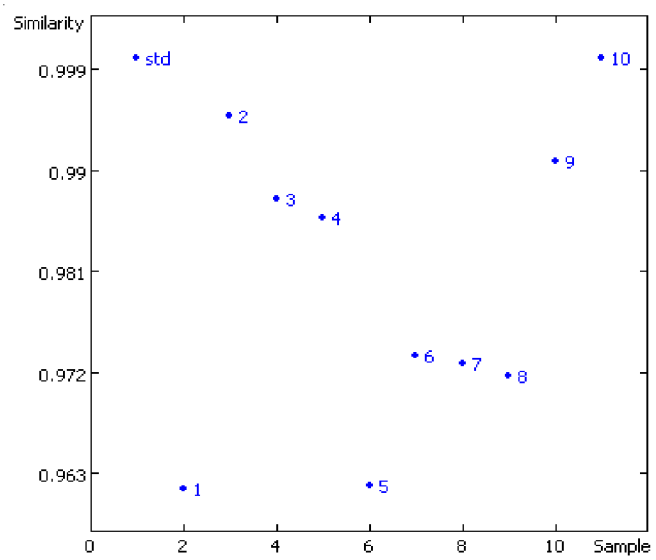

Fig. 4. Similarity of semen *Euphorbiae* frostlike powders (included angle cosine)

discovered among the ingredient levels in different batches. This may be correlated with the place of origin of samples and the processing degrees.

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