

Simultaneous Determination of Paeoniflorin and Albiflorin in Radix Paeoniae Rubra by HPLC-DAD-ELSD

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Summary. High-performance liquid chromatography coupled with photodiode array detection and evaporative light scattering detection (HPLC-DAD-ELSD) was established to determine paeoniflorin and albiflorin simultaneously in Radix Paeoniae Rubra. The assay was performed on a Diamonsil C₁₈ (4.6 mm × 250 mm, 5 μm) column by a gradient elution program with acetonitrile and aqueous formic acid (0.05% v/v) as mobile phase at a flow rate of 1.0 mL min⁻¹. The detection wavelength of DAD was 230 nm, and the evaporator tube temperature of ELSD was set at 110 °C with the nebulizing gas flow rate of 3 L min⁻¹. The temperature of column was kept at 30 °C. The linear ranges of paeoniflorin and albiflorin were within 0.050–1.510 mg mL⁻¹ and 1.007–5.035 mg mL⁻¹. The recoveries of paeoniflorin and albiflorin were 96.2–102.9% and 95.0–102.4%, respectively, while the relative standard deviation (RSD) of them was 0.2–2.5%. This method was quick, simple, accurate, and specific. It could be used for the quality control of Radix Paeoniae Rubra. The proposed approach was expected as a powerful tool for the quality control of Radix Paeoniae Rubra.

Key Words: HPLC-DAD-ELSD, Radix Paeoniae Rubra, paeoniflorin, albiflorin

Introduction

Radix Paeoniae Rubra (RPR), the dried root of *Paeonia lactiflora* Pall or *Paeonia veitchii* Lunch [1], has been widely used by Chinese medicine practitioners to treat cardiovascular, inflammation, and female reproductive diseases [2]. Based on the principle of Chinese medicine, historical literatures described RPR with the functions of tonifying blood, cooling blood, cleansing heat, and invigorating blood circulation [3].

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In recent years, plenty of studies [4] showed that Radix Paeoniae Rubra had effects on coagulant, atherosclerosis, and thrombo, protects the heart and liver, etc., and it had broad application prospects. The main active components [5, 6] of Radix Paeoniae Rubra include paeoniflorin, albiflorin, hydroxy paeoniflorin, benzoyl paeoniflorin, and other monoterpene glycosides, which are considered as total paeony glycoside (TPG) collectively. The most abundant and active components in RPR are identified as paeoniflorin (PF) and albiflorin (AF) [7, 8], which are reported to have many biological properties including antipyretic, antiallergic, antioxidative, anti-inflammatory, and anxiolytic activities [9–12]. As for the Radix Paeoniae Rubra material quality control method, high-performance liquid chromatography (HPLC) is used to determine paeoniflorin alone; albiflorin and paeoniflorin; or paeoniflorin, albiflorin, and benzoylpaeoniflorin together [13–16]. However, simultaneous determination of paeoniflorin and albiflorin by HPLC coupled with photodiode array detection and evaporative light scattering detection (HPLC-DAD-ELSD) has not been reported. It could establish the foundation for further research and development of Radix Paeoniae Rubra.

Experimental

Instrument

A Dikma Diamonsil C₁₈ (4.6 m × 250 mm, 5 μm) column in Agilent 1200 HPLC system (Agilent Technologies Co. Ltd., USA) equipped with a quaternary pump, a column compartment, an automatic injection system with a 100-μL loop, and an Alltech ES2000 ELSD (Alltech Corporation, USA) was used for analysis. The QE-200 g Chinese medicine grinder was purchased from Chinese Stands Tools Co., Ltd., and the AB265-S one hundred-thousandth analytical balance was purchased from Swiss Mettler Toledo Company.

Chemicals and Reagents

The standards of paeoniflorin and albiflorin were isolated in our laboratory, and their structures were elucidated according to their spectral data (ultraviolet [UV], infrared [IR], mass spectrometry [MS], proton nuclear magnetic resonance [¹H-NMR], carbon nuclear magnetic resonance [¹³C-NMR]). The purities of paeoniflorin and albiflorin were both greater than 98.0% by

HPLC. HPLC grade acetonitrile, methanol, and formic acid were purchased from Yuwang Chemical Corporation (Shandong, P.R. China). Other reagents were of analytical grade.

Standard Solutions

Stock standard solutions of paeoniflorin and albiflorin were prepared in aqueous methanol (70% *v/v*) at concentrations of 10.070 and 3.020 mg mL⁻¹, respectively, and stored at -20 °C. The stock standard solutions of paeoniflorin were diluted in aqueous methanol (70% *v/v*) immediately before preparation of calibration curve to concentrations of 1.007, 1.510, 2.014, 3.021, 4.028, and 5.035 mg mL⁻¹. The calibration curve of albiflorin was prepared at concentrations of 0.050, 0.151, 0.302, 0.906, 1.208, and 1.510 mg mL⁻¹. The solutions were filtered through a 0.45- μ m membrane filter before HPLC analysis.

Sample Solutions

Radix Paeoniae Rubra samples (marked as 1-9) were collected from nine sources. The dried Radix Paeoniae root samples were crushed and then passed through a 20-mesh sieve. A 1.00 g Radix Paeoniae Rubra powder sample with 10 mL aqueous methanol (70% *v/v*) was soaked for 1 h and extracted at a micro-boiling state for 1 h. The extracted solutions were then cooled to room temperature, weighed, and added aqueous methanol (70% *v/v*) for lost weight. The extracted solutions were filtered through filter paper and then filtered through 0.45 μ m microporous membrane for HPLC analysis.

Chromatographic Conditions

The mobile phase consisted of acetonitrile (A) and aqueous formic acid (0.05% *v/v*) (B). The proportion of solvent A and B was 14:86 (*v/v*), and the mobile phase flow rate was 1.0 mL min⁻¹. The detection wavelength of DAD was 230 nm. The temperature of column was kept at 30 °C, and the injection volume was 5 μ L. The evaporator tube temperature of ELSD was set at 110 °C with the nebulizing gas flow rate of 3 L min⁻¹, and the striker was in a closed state. Under the condition of the chromatographic analysis, the chromatogram of standards and sample of Liaoning Xifeng were showed as Fig. 1 and Fig. 2.

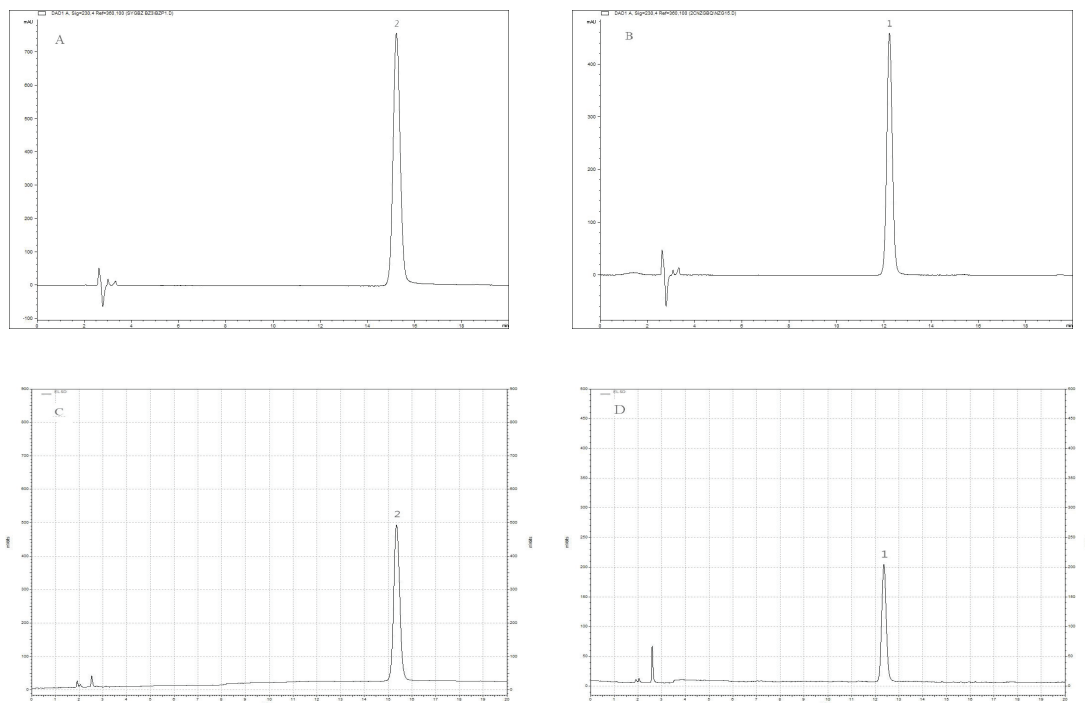


Fig. 1. The chromatogram of standard paeoniflorin (2) and albiflorin (1). A: HPLC-DAD chromatogram of standard paeoniflorin; B: HPLC-DAD chromatogram of standard albiflorin; C: HPLC-ELSD chromatogram of standard paeoniflorin; D: HPLC-ELSD chromatogram of standard albiflorin

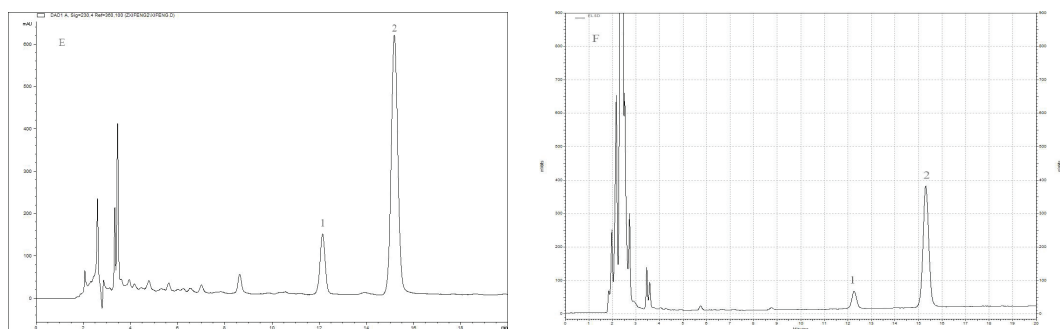


Fig. 2. The chromatogram of Liaoning Xifeng sample (paeoniflorin – 2, albiflorin – 1). E: HPLC-DAD chromatogram of Liaoning Xifeng sample; F: HPLC-ELSD chromatogram of Liaoning Xifeng sample

Calibration Curves

The calibration curves were constructed by analyzing six different concentrations of standard solutions. The regression equations of standards were calculated in the form of $Y = A \times X + B$, where Y and X were peak area and sample concentration, respectively. The stock solutions of paeoniflorin and albiflorin were diluted to a lower concentration and then gradually diluted and detected, repeatedly. Signal and noise ratios (S/N) of 10:1 and 3:1 were used to detect the limits of quantification and detection, respectively. The calibration curves, and quantification and detection limits of paeoniflorin and albiflorin were showed in *Table I*.

Table I. Calibration data of paeoniflorin and albiflorin standards

Standards	Detector	Linear range (mg mL ⁻¹)	Regression equation	Correlation (r)	LOQ (mg mL ⁻¹)	LOD (mg mL ⁻¹)
Paeoniflorin	DAD	1.007~5.035	$Y = 5503.60X + 241.84$	0.9955	7.049	3.524
Paeoniflorin	ELSD	1.007~5.035	$Y = 4244294.98X - 2270943.69$	0.9967	1.510	7.049
Albiflorin	DAD	0.050~1.510	$Y = 5219.60X + 12.46$	0.9991	1.510	5.043
Albiflorin	ELSD	0.050~1.510	$Y = 1825446.78X - 124110.96$	0.9976	45.30	15.10

Y : peak area; X : concentration.

Method Validation

Method validations including precision, repeatability, intra- and inter-day stability, and sample recovery were evaluated. Precision and repeatability were assessed by the successive analysis of six injections of the same sample solution and six replicates of the same patch sample, separately. One of the sample solutions mentioned above was injected into the apparatus at 0, 2, 4, 8, 12, and 24 h, respectively, to evaluate the intra-day stability of the solution. The analysis of one sample during four-day periods (1, 2, 3, and 4 days) was used to evaluate the inter-day stability of the solution. Recovery was determined by adding known quantities of standards to the tested samples and calculated by comparing the added and obtained quantities through external standard linear regression.

Quantification

Radix Paeoniae Rubra from nine different sources was prepared in accordance with section 'Sample Solutions' for the next test solution operations. The sample of each source was operated in paralleled three copies, and chromatographic conditions were conducted according to section 'Chromatographic Conditions'. Quantification was based on the calibration curves fitted by linear regression analysis. The contents of paeoniflorin and albiflorin in various sources were demonstrated in *Table II*. The contents of paeoniflorin and albiflorin in various sample distributions were shown in *Figs. 3 and 4*.

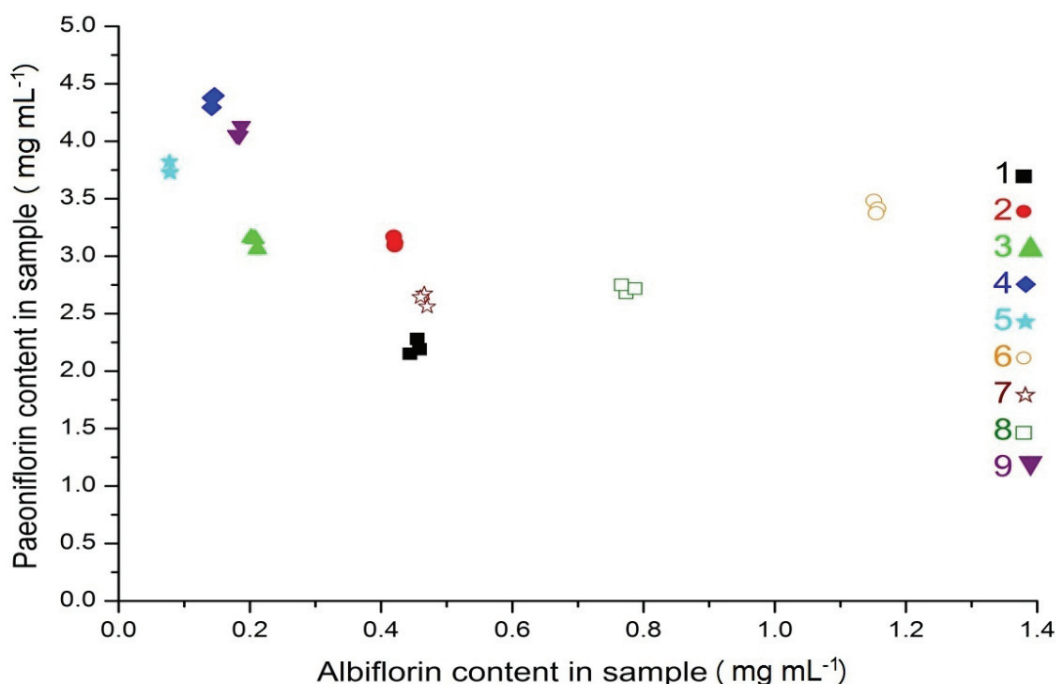


Fig. 3. The distribution of paeoniflorin and albiflorin contents in various sources samples by DAD (1: Liaoning Xifeng, 2: Hebei Tangshan, 3: Shandong, 4: Heilongjiang, 5: Inner Mongolia, 6: Zhejiang, 7: Anhui, 8: Liaoning Dalian, 9: Liaoning Huludao)

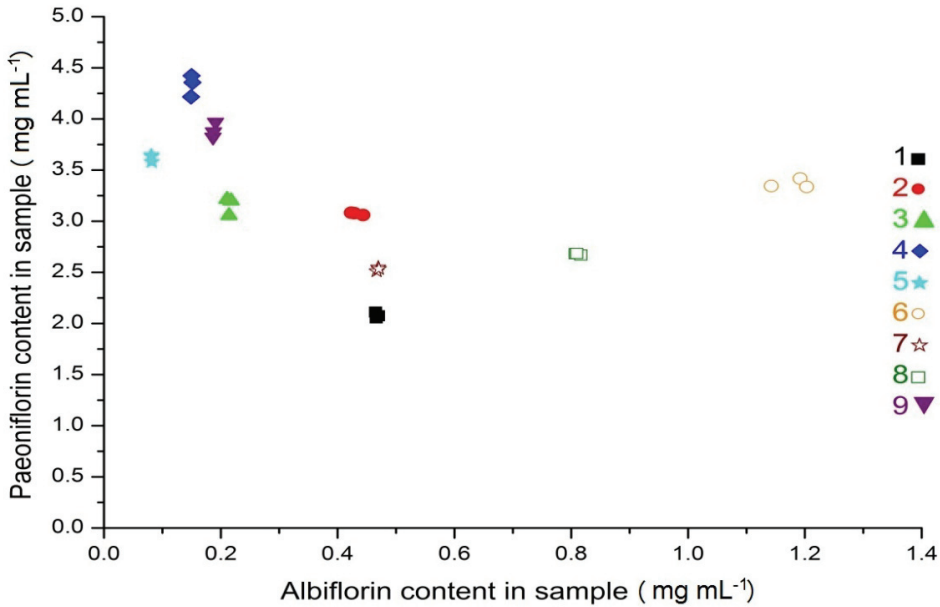


Fig. 4. The distribution of paeoniflorin and albiflorin contents in various sources samples by ELSD (1: Liaoning Xifeng, 2: Hebei Tangshan, 3: Shandong, 4: Heilongjiang, 5: Inner Mongolia, 6: Zhejiang, 7: Anhui, 8: Liaoning Dalian, 9: Liaoning Huludao)

Table II. Quantitative analytical results of various sources samples

Sample	Mean content of paeoniflorin (mg mL ⁻¹)				Mean content of albiflorin (mg mL ⁻¹)			
	DAD	RSD (%)	ELSD	RSD (%)	DAD	RSD (%)	ELSD	RSD (%)
1	2.2064	2.4	2.1105	1.1	0.4526	1.4	0.4672	0.1
2	3.1270	1.0	3.0737	0.3	0.4204	0.2	0.4317	2.0
3	3.1238	1.4	3.1564	2.3	0.2072	1.8	0.2142	1.3
4	4.3565	1.0	4.3324	2.0	0.1434	1.4	0.1503	0.7
5	3.7600	1.2	3.6208	0.8	0.0780	0.6	0.0815	0.4
6	3.4231	1.3	3.3656	1.1	1.1543	0.2	1.1792	2.2
7	2.6260	2.1	2.5316	0.6	0.4653	0.9	0.4691	0.4
8	2.7157	1.0	2.6776	0.3	0.7757	1.1	0.8111	0.5
9	4.0775	0.9	3.8875	1.6	0.1835	1.6	0.1885	1.0

1: Liaoning Xifeng, 2: Hebei Tangshan, 3: Shandong, 4: Heilongjiang, 5: Inner Mongolia, 6: Zhejiang, 7: Anhui, 8: Liaoning Dalian, 9: Liaoning Huludao.

Results and Discussion

Select the Mobile Phase

The mobile phases of methanol–water, acetonitrile–water, and acetonitrile–aqueous formic acid (0.05% *v/v*) were examined. The results indicated that the chromatogram of paeoniflorin and albiflorin in samples had a good separation and a better peak shape when the portion of acetonitrile (A) and aqueous formic acid (0.05% *v/v*) (B) was 14:86 (*v/v*).

Method Validation

The values of relative standard deviation (RSD) of peak area of analytes for method precision, repeatability, stability, and intra-day precision were from 0.30% to 2.7% (see *Table III*). The results of sample recovery indicated that the average recovery of analytes was from 95.0% to 102.9% (see *Table IV*). All these results implied that the established method of determination was valid and feasible.

Table III. Method validation

Test	Paeoniflorin (RSD %)		Albiflorin (RSD %)	
	DAD	ELSD	DAD	ELSD
Precision (<i>n</i> = 6)	0.9	0.6	1.2	0.6
Repeatability (<i>n</i> = 6)	1.3	0.3	2.3	2.6
Intra-day Stability (<i>n</i> = 6)	1.1	2.6	0.8	2.7
Inter-day Stability (<i>n</i> = 4)	0.6	1.4	1.5	2.3

Table IV. Recovery of paeoniflorin and albiflorin in Xifeng samples ($n = 3$)

Concentration	Paeoniflorin				Albiflorin			
	DAD Recovery (%) (means \pm SD)	RSD (%)	ELSD Recovery (%) (means \pm SD)	RSD (%)	DAD Recovery (%) (means \pm SD)	RSD (%)	ELSD Recovery (%) (means \pm SD)	RSD (%)
Low	102.9 \pm 0.5	0.2	101.9 \pm 2.7	2.3	96.8 \pm 0.4	0.3	96.3 \pm 2.5	2.5
Medium	100.1 \pm 2.3	1.1	102.5 \pm 0.6	0.4	102.4 \pm 0.5	1.4	101.3 \pm 0.4	0.4
High	96.2 \pm 1.8	0.8	99.9 \pm 1.9	0.8	97.6 \pm 0.5	0.7	95.0 \pm 0.5	1.3

Analysis of the Results

In this study, samples from nine different sources were determined. The content range of paeoniflorin was 2.2064–4.3565 mg mL⁻¹ and that of albiflorin was 0.0780–1.1543 mg mL⁻¹ by DAD. The content range of paeoniflorin was 2.1105–4.3324 mg mL⁻¹ and that of albiflorin was 0.0815–1.1792 mg mL⁻¹ by ELSD. Since the content range of paeoniflorin from the nine sources was 2.11–4.36% (*w/w*), these medicinal materials were qualified, according to 2015 Chinese Pharmacopoeia [1], in which the content of Paeoniflorin in *Radix Paeoniae Rubra* should not be less than 1.8% (*w/w*).

The paeoniflorin content of Heilongjiang sample was 4.3565 mg mL⁻¹ (DAD) and 4.3324 mg mL⁻¹ (ELSD), which was the highest among the nine samples, while the paeoniflorin content of Liaoning Xifeng sample was 2.2064 mg mL⁻¹ (DAD) and 2.1105 mg mL⁻¹ (ELSD), which was the lowest. The albiflorin content of Zhejiang sample was 1.1543 mg mL⁻¹ (DAD) and 1.1572 mg mL⁻¹ (ELSD), which was the highest among the nine samples, while the albiflorin content of Inner Mongolia sample was 0.0780 mg mL⁻¹ (DAD) and 0.0815 mg mL⁻¹ (ELSD), which was the lowest. The contents of paeoniflorin and albiflorin in nine sources samples varied, which might be associated with *Radix Paeoniae* growth pattern, growth years, soil conditions, harvest time, etc. The paeoniflorin contents in Shandong, Hebei Tangshan, and Zhejiang samples were close. The albiflorin contents in Liaoning Xifeng, Anhui and Hebei Tangshan samples were quite similar, which were consistent with DAD and ELSD. The paeoniflorin and albiflorin

contents of Zhejiang sample were higher than the others, so its quality should be better.

Chinese herbs are used more compatibly in clinic and therapeutic effects. Traditional Chinese Medicine (TCM) is based on the synergic effect on its complex components, which is different from western medicine [17–20]. The quality control methods of herbal medicine almost depend on selecting one or some of components as reference indexes, which cannot represent the whole property of herbal medicine just by several so-called index components [16]. With the improvement of quality standards and quality control, a variety of active ingredients of traditional Chinese medicine can be detected for better quality control.

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