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DETERMINATION OF PAEONIFLORIN FROM RADIX PAEONIAE RUBRAE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

Radix Paeoniae rubrae is a medicinal herb used in traditional medicine as well as in the manufacture of medicinal products for the treatment of chest pain, anti-inflammatory, anti-coagulant, vasodilatation However, the quality of Radix Paeoniae rubrae is suspected because of not tight controlled. The main chemical component of this medicinal herb is paeoniflorin, a monoterpen glycoside. We conducted this study with the aim of developing the quantitative procedures of paeoniflorin in Radix Paeoniae rubrae by HPLC, which can detect cases of counterfeit or exhausted pharmaceuticals, this will help improve the quality control of this medicinal plant.

The optimized conditions included isocratic mobile phase of 18% of acetonitrile and 82% of phosphate buffer 0.05M pH 4.5. Chromatographic system used Phenomenex Lunar C18 column (250 x 4.6 mm; 5 μm) at 25 ^{0}C, injection volume was 20 μL, flow rate at 1.0 mL/minute, as detector a spectrophotometer set at 230 nm. The procedures enables determination of paeoniflorin in Radix Paeoniae rubrae within 22 minutes. The

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analytical procedures was validated the suitability of the system, specificity, linearity ($\hat{y}$ = 26.5702x, R = 0.9999), repeatability with RSD = 0.8%, intermediate precision, accuracy with recovery rate 96.6 - 103.7% and range 6.4 - 25.6 μg/mL.

1. Introduction

Radix Paeoniae rubrae is a medicinal herb used in traditional medicine as well as in the manufacture of medicinal products for the treatment of chest pain, anti-inflammatory, anti-coagulant, vasodilatation However, the quality of Radix Paeoniae rubrae is suspected because of not tight controlled. The main chemical component of this medicinal herb is paeoniflorin, a monoterpen glycoside. The Chinese Pharmacopoeia 2005 evaluated the quantitative criteria of Radix Paeoniae rubrae based on the content of paeoniflorin, but using the test method of Chinese Pharmacopoeia to quantify, we found that paeoniflorin peak was not separated from other peaks. Vietnamese Pharmacopoeia IV also has Radix Paeoniae rubrae monographs but not included the assay, so it is impossible to acurately assess the quality when this medicinal herb has been extracted active ingredients. From that fact, we conducted this study with the aim of developing the quantitative procedures of paeoniflorin in Radix Paeoniae rubrae by HPLC method, which can detect cases of counterfeit or exhausted pharmaceuticals, help improve the quality control of this medicinal plant.

2. Materials and Methods

2.1. Instrumentation

All experiments were performed on a HPLC system of Agilent 1260 (USA) equipped with an auto sampler and Phenomenex Lunar C18 column (250 x 4.6 mm, 5μm) coupled with temperature control system for column (25 ^{0}C) in the Laboratory of analytical chemistry and drug quality control, pharmacy faculty, Lac Hong University.

2.2. Chemical and materials

Acetonitrile (Merck), methanol (Merck), KH2PO4 (Merck). Paeoniflorin (Sigma Aldrich), purity of 99,0% was used as chemical reference substance (CRS). Radix Paeoniae rubrae was purchased in Ho Chi Minh city, August 2017.

2.3. Solution preparation

Phosphate buffer 0.05M pH 4.5: Dissolve 6.8 g of KH2PO4 into 1000 mL of distiled water. Reference solution: Dissolve a quantity of paeoniflorin CRS,

accurately weighed, in mobile phase to produce a solution containing 16 μg per mL.

Test solution: Accurately weigh 80 mg of the Radix Paeoniae rubrae powder to a 50 mL volumetric flask, add 35 mL methanol and ultrasonicate for 30 minutes. Allow to cool, add methanol to 50 mL, filter, 10 mL of filter fluid is diluted to 25 mL with the mobile phase in a volumetric flask. This solution was then filtered through filter paper with 0.45 m before injected.

Solution used for validation: Accurately weigh 8.0 mg paeoniflorin CRS, dissolve in mobile phase to produce a solution containing 80 μg per mL (solution A).

Test solution add paeoniflorin: Accurately weigh 80 mg of the Radix Paeoniae rubrae powder to a 50 mL volumetric flask, add 5 ml solution A, produce in the same manner as the test solution.

2.4 Optimization of chromatographic conditions

Different compositions of mobile phases were investigated:

Methanol - phosphate buffer 0.05M pH 4.5 (40:65) (mobile phases 1)

Acetonitrile - phosphate buffer 0.05M pH 4.5 (18:82) (mobile phases 2)

Phenomenex Lunar C18 column (250 x 4.6 mm, 5 m) at 25 ^{0}C.

Injection volume: 20 μL.

Wavelengh of detector: 230 nm.

Flow rate: 1.0 mL/min.

The optimization was obtained when chromatographic parameters meet requirement such as theoretical plate number ($N \geq 3000$), resolution ($Rs \geq 1.5$), symmetrical factor ($0.8 \leq As \leq 1.5$) and peak purity.

2.5. Validation of analytical procedures

This analytical procedures was subsequently validated according to the ICH guideline Q2 (R1) (ICH 2005) with respect to the system suitability, specificity, linearity, repeatability, intermediate precision, accuracy and range.

Results and Discussions

3.1 Optimization of the mobile phase conditions

With the mobile phase 1 condition, which was written in the Chinese Pharmacopoeia 2005, paeoniflorin peak was not separated from other peaks. The second mobile phase by isocratic of Acetonitrile - phosphate buffer 0.05M pH 4.5 (18:82, v/v) gave good parameters for paeoniflorin peak in test solution, theoretical plate number $N = 14333$, $Rs = 4.41$, $As = 0.88$ and paeoniflorin peak reached purity. Thus, it was chosen as optimum chromatography condition.

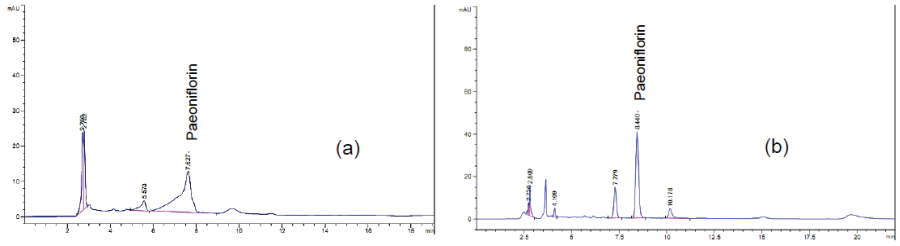

Figure 1. Chromatograms of the test solution when analyzed by mobile phase 1 (a) and mobile phase 2 (b).

3.2. Validation of analytical procedures

3.2.1. System suitability

System suitability was tested by performing six replicate injections reference solution and test solution, determining capacity factor (k'), symmetrical factor (As), theoretical plate number (N), resolution (Rs), selectivity factor (α) and repeatability (RSD of retention times and peak areas) for the analytes of interest. The %RSD values of peak area and retention time for all peaks were less than 2% indicating the precise analysis of paeoniflorin by this system. All the results showed that the proposed analytical procedures met the requirements.

		t_R (min)	k' (1 - 8)	S (mAU.s)	A_s (0.8 - 1.5)	N (≥ 3000)	Rs (≥ 1.5)	α (1.05 - 2.0)
Refrence solution	*Average*	8.465	2.40	430.81347	0.88	14570		
	RSD	0.1%	0.2%	0.3%	0.6%	0.9%		
Test solution	*Average*	8.433	2.38	438.32476	0.88	14333	4.41	1.24
	RSD	0.1%	0.2%	0.3%	0.0%	1.4%	0.6%	0.0%

3.2.2. Specificity

The chromatogram of the test solution obtained the peak has same retention time as the peak of paeoniflorin in chromatogram of reference solution. The chromatogram of the test solution add paeoniflorin obtained the peak of paeoniflorin has an increase in peak area compared to before the addition. The chromatogram of dissolving solvent wasn't obtained the peak has same retention time with peak of paeoniflorin. The UV-Vis spectra of the active substance in the chromatogram obtained with reference solution and test solution were similar. Peak of the paeoniflorin in the chromatogram obtained with test solution reached peak purity. Thus, the analytical procedures met the specificity

requirements.

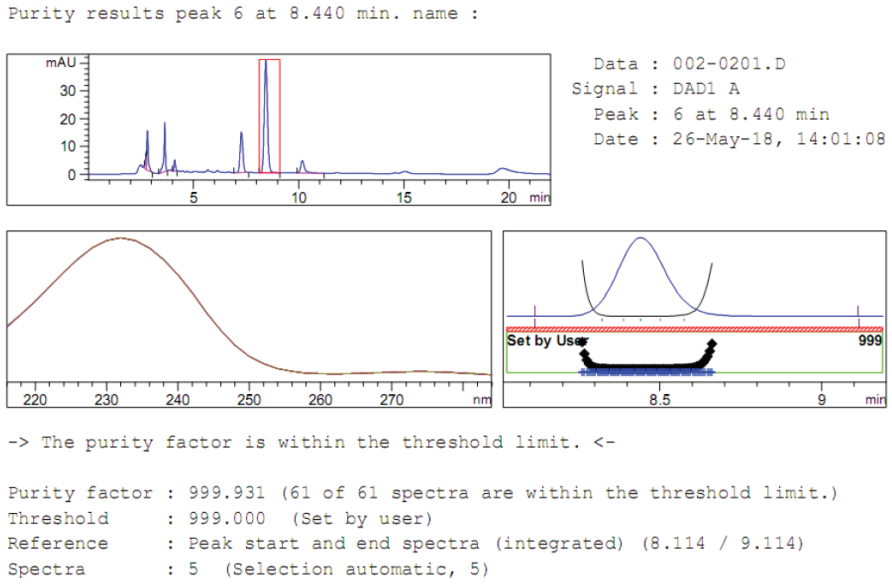

Figure 2. Test results for the purity of Paeoniflorin peak in the chromatogram of the test solution.

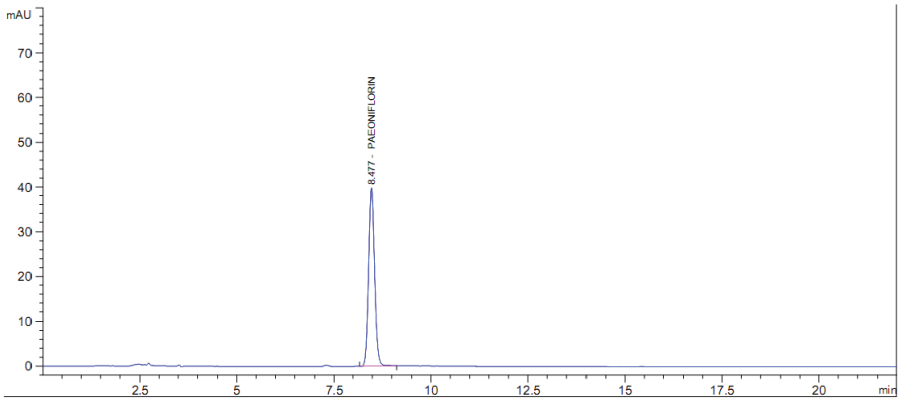

Figure 3. Chromatogram obtained with the reference solution.

3.2.3. Linearity

From solution A, dilute to 8 solutions at a concentration of 1.6 - 51.2 μg/mL (equivalent to 10 - 320% of the quantitative concentration) as described in Table 2. Carry out analyzes according to chromatography conditions described,

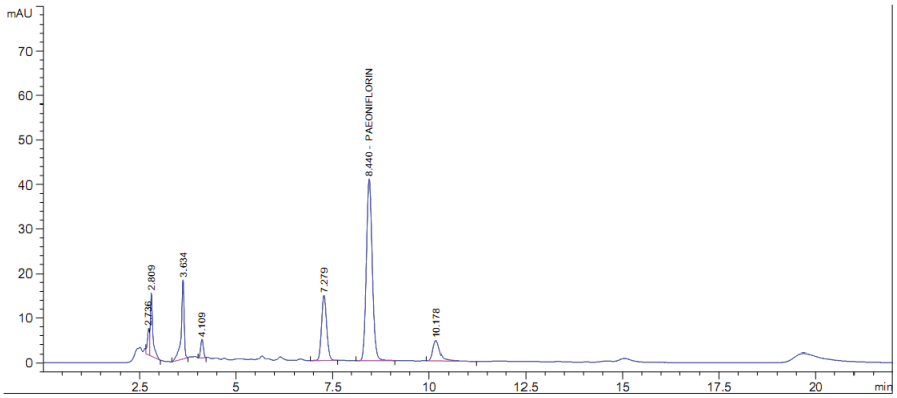

Figure 4. Chromatogram obtained with the test solution.

record retention time, peak area of paeoniflorin. Establish a linear regression equation for the peak area according to the paeoniflorin concentration. Using the regression function "Regression" in MS - Excel 2013 with the F-test to test the suitability of the regression equation $\hat{y} = ax + b$ and the t-test to test the significance of the coefficients a, b.

Table 2. Peak area of the reference solutions

Concentration (µg/mL)	Peak area (mAu.s)
1.6	49.19891
6.3	177.83865
11.1	295.15924
15.8	428.08017
20.6	546.54468
25.3	677.56598
38.0	1018.84497
50.7	1352.1908

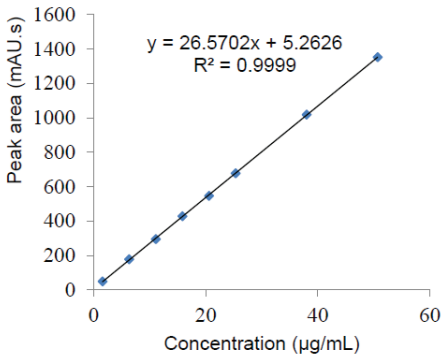

Figure 5. Linear line of paeoniflorin in rage of concentration 1.6 – 50.7 µg/mL.

Significance F = 1.09584 x 10^{-13} < α = 0.05 the regression equation is compatible.

P-value (b) = 0.0697 > α = 0.05 the coefficient b is not statistically significant.

P-value (a) = 1.09584 x 10^{-13} < α = 0.05 the coefficient a is statistically significant.

Linear equation: $\hat{y}$ = 26.5702x with R^2 = 0.9999.

3.2.4. Repeatability

Prepare six different test solution, analyze according to chromatography

conditions described. Record retention time, peak area, calculate content (%) of paeoniflorin according to the following formula:

$$C(\%) = \frac{S_T \times m_c \times C_c \times D_T \times 100}{S_c \times D_C \times m_T}$$

With S_T, S_C is the peak area of paeoniflorin in chromatogram obtained with test solution and reference solution; m_C : weight of paeoniflorin to make reference solution; C_C : purity of paeoniflorin CRS; D_C, D_T : the dilution of the reference solution and test solution, m_T : weight of the Radix Paeoniae rubrae powder to make test solution.

According to the results in Table 3, the content (%) of paeoniflorin in Radix Paeoniae rubrae is 2.67, RSD of the content over the six tests is 0.8% not to exceed 2.0%. The procedures met repeatability requirements.

Table 3. The results of the repeatability test

Test solution	Weight (mg)	Peak area (mAU.s)	Content (%)
1	79.6	476.38306	2.69
2	79.8	475.85651	2.68
3	80.0	473.77292	2.66
4	80.1	467.82379	2.63
5	80.0	472.48288	2.66
6	79.8	472.73694	2.67
Average			2.67
RSD			0.8%

3.2.5. Intermediate precision

Two testers, independently analyzed on two different days, each tested 6 samples. Carry out the analysis according to the described chromatography conditions.

The quantitative RSD value of each tester and of both testers was less than 2.0%. Anova analysis showed that the quantitative results between the two testers were not statistically significant difference (P-value = 0.095 > α = 0.05). The analytical procedures met intermediate precision requirements.

3.2.6. Accuracy

Applying the method of adding paeoniflorin to the test solution, the amount of added paeoniflorin corresponding to 5 levels of 40%, 70%, 100%, 130%, 160% of the quantitative concentration. Each level contains 3 samples. Carry

Table 4. The results of the intermediate precision test

Tester 1 Khanh Duy Tran Date of analysis 26-05-2018 HPLC system of Agilent 1260				Tester 2 Duy Tai Trinh Date of analysis 04-06-2018 HPLC system of Agilent 1260		
Test solution	Weight (mg)	Peak area (mAU.s)	Content (%)	Weight (mg)	Peak area (mAU.s)	Content (%)
1	79.6	476.38306	2.69	80.6	499.73010	2.79
2	79.8	475.85651	2.68	80.5	483.23383	2.70
3	80.0	473.77292	2.66	80.2	471.46167	2.65
4	80.1	467.82379	2.63	80.2	480.81674	2.70
5	80.0	472.48288	2.66	79.8	476.68704	2.69
6	79.8	472.73694	2.67	79.8	476.59033	2.69
Average			2.67	*Average*		2.70
RSD			0.8%	*RSD*		1.8%
The average content (%) of 12 samples of both testers was 2.68; RSD: 1.4%.						

out analyzes according to the described chromatography conditions, record retention time, peak area of paeoniflorin, calculate concentration of paeoniflorin was found, recovery rate at each concentration level.

Table 5. The results of the accuracy test

Concentration levels	Concentration of paeoniflorin added (µg/mL)	Peak area of paeoniflorin (mAU.s)	Concentration of paeoniflorin was found (µg/mL)	Recovery rate (%)	Average	RSD
40%	6.34	672.56946	6.58	103.8%	103.7%	0.7%
	6.34	671.04242	6.52	103.0%		
	6.34	673.51190	6.61	104.3%		
70%	11.09	797.76288	10.96	98.8%	98.8%	0.1%
	11.09	797.27118	10.94	98.7%		
	11.09	797.95630	10.97	98.9%		

	15.84	922.13593	15.31	96.7%		
100%	15.84	924.12970	15.38	97.1%	96.9%	0.2%
	15.84	922.90631	15.34	96.8%		
	20.59	1055.00256	19.96	96.9%		
130%	20.59	1057.72205	20.06	97.4%	97.2%	0.3%
	20.59	1057.25366	20.04	97.3%		
	25.34	1183.39612	24.46	96.5%		
160%	25.34	1184.10303	24.48	96.6%	96.6%	0.1%
	25.34	1184.35266	24.49	96.6%		

Recovery rates at all concentration levels ranged 96.6 - 103.7%. The analytical procedures met the requirements of correctness

3.2.7. Range

Based on the results of linearity, precision and accuracy, figure out the analytical procedures has a range 6.4 - 25.6 mug/mL (corresponding to 40 - 160% of the quantitative concentration).

Conclusions

By using high-performance liquid chromatography with diode array detector, we have developed the quantitative procedures of paeoniflorin in Radix Paeoniae rubrae as follows: isocratic mobile phase acetonitrile - phosphate buffer 0.05M pH 4.5 (18:82), as detector a spectrophotometer set at 230 nm,

Phenomenex Lunar C18 column (250 x 4.6 mm; 5 m) at 25 ^{0}C, injection volume was 20 μL, flow rate at 1.0 mL/minute. Procedures validated system suitability, specificity, linearity, repeatability, intermediate precision, accuracy and range. This is the basis for providing test methods for the assay of Radix Paeoniae rubrae monographs, which improve the quality control of this medicinal plant.

References

[1] Chinese Pharmacopoeia commision (2005), *Pharmacopoeia of the People's Republic of China,* Vol. 1, Beijing China, pp. 223.

[2] Huber, Ludwig (2007), "Validation and qualification in analytical laboratories" (second edition), United States of America, pp. 125 - 154.

[3] ICH. 2005. Q2 (R1). "Validation of analytical procedures: Text and Methodology".

[4] Liu Yang et al. (2009), *A Simple Thin-Layer Chromatographic Fingerprint Method for Distinguishing Between Radix Paeoniae Rubra and Radix Paeoniae Alba,* Journal of Liquid Chromatography and Related Technologies, **32**, pp. 2893 - 2905.

[5] WHO (1999), "Monographs on selected medicinal plants", Vol. 1, Geneva, pp. 195 - 201.