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Rapid analysis of paeoniflorin and moisture content in *Paeoniae Radix Alba* by near-infrared diffuse reflection spectroscopy

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ABSTRACT

The aim of our study is to develop a novel method to determine fastly and nondestructively the content of paeoniflorin and moisture in Paeoniae Radix Alba by near-infrared diffuse reflection spectroscopy. Samples with different concentrations were analyzed by HPLC and Loss on drying method; Multivariate calibration models based on PLS algorithm were developed to correlate the spectra and the corresponding values determined by the reference method. The paeoniflorin and moisture correlation coefficient (R^2)of the calibration model were 0.9473 and 0.9509；Internal cross-validation mean square error(RMSECV) were 0.533 and 0.187；The correlation coefficient(R^2) of the true value and prediction value from external validation were 0.9362 and 0.9440 variance of external validation(RMSEP) were 0.092 and 0.253. The results indicated that near-infrared diffuse reflection spectroscopy determination method is rapid, non-destructive, accurate and non-pollution, which can dispose the samples without complicated pretreatment. It could be applied for the rapid determination of large quantities of numbers of Paeoniae Radix Alba samples.

Key words: Near-infrared spectroscopy; Content analysis; *Paeonia lactiflora pallas*; Paeoniflorin; High-performance liquid chromatography(HPLC)

INTRODUCTION

Traditional Chinese Medicine, including Chinese herbal medicine, Chinese Herbal Medicine and Chinese medicine etc., is based on the guidance of traditional Chinese medicine theory and applications[1]. It plays an irreplaceable role in the prevention, diagnosis and treatment of diseases. Due to the complexity of Chinese herbal medicine, limited research methods (both method and instrument), lag of analysis in active ingredients of Chinese herbal medicine, utilizing near-infrared spectroscopy analysis of the quality of traditional Chinese medicine has many advantages[2-3]. For example, the sample does not have to add reagents, do not destroy the sample, do not pollute the environment, enabling rapid measurement and on-line measuremen, which called "green analytical techniques" to drug testing provides a more convenient way. In this paper, using near infrared spectroscopy, combined with chemometrics software determined paeoniflorin and moisture content in *Paeoniae Radix Alba*, then established a quantitative analysis model, which was verified optimization, using chemical HPLC and weightlessness method to measure the true value of unknown samples.[4-6] This study provides a new detection method for the quality control of *Paeoniae Radix Alba*.

EXPERIMENTAL SECTION

1.1 Instruments

Fourier near-infrared spectrometer(FT-NIR):Germany(BRUKER)VECTOR22/N With fiber-optic probe measurements; Sampler Integrating sphere detector OPUS6.5 Chemistry Workstation; Quant 2 quantitative analysis software (NIR spectral scanning area is 12000~4000cm^{-1}; resolution of 8 cm^{-1}; scanning frequency; 32 times, three times an average random scan; HPLC: Agilent 1200LC (Dima C18 column,4.6 mm ×250mm 5 μ m); UV spectrophotometer: 754PC FW-400 ZTE; high-speed grinder: FA2104 HANGPING Analytical Balance

1.2 Reagents

Acetonitrile (HPLC grade); Phosphoric acid (AR);Methanol (AR); Ethanol; Ultrapure water; Paeoniflorin reference was provided by the China Pharmaceutical and Biological Products, batch number 110736-201035

1.3 Herbal medicine

Experiments with traditional Chinese peony Pieces 32 copies, in February 2012 to April purchased from Shaanxi, Shanxi, Hebei, Tianjin and pharmacies around the herbal medicine market. Specific numbers are shown in Table 1-1

Table 1 Paeonia origin and number of samples

Place of origin	Number of sample
Anhui	two,four,five,ten,thirteen,fourteen,fifteen,nineteen,twenty,twenty-one,twenty-three,twenty-five,twenty-seven,twenty-eight,twenty-nine,thirty,thirty-one,thiry-two
Zhejiang	one,six,seven,eleven,twelve,sixteen,senevteen,eighteen,twenty-eight,twenty-two,twenty-four,twenty-six
Hebei	three, nine,
Shaanxi	eight

1.4 Effect of sample thickness of the spectrum

In order to study the effects of different sample loading thickness on the near-infrared spectrum, while maintaining a consistent density premise (by the same person with the same weight of the sample cone bottom compaction).Weigh a sample of different weight (the herbs crushed through a 60 mesh sieve). Thickness of the sample cup 0.5cm, 1cm, 1.5cm, each spectra collected and compared to determine the optimal thickness of the spectral

1.5 Utilizing different pretreatment method

When using near infrared spectrometer collected the original spectrum, inevitably contain various noise outside except the sample composition information, and the presence of noise to a greater or lesser influence the performance of the calibration model and the unknown sample composition or nature of the projections. In order to reduce or even eliminate all noise of the spectrum, the need to adopt an appropriate pretreatment methods to improve the accuracy of the modeling predicted.

In this experiment, the internal cross-validation coefficient of determination R2 and internal cross-validation MSE as an indicator RMSECV. Comparison of eleven different spectral pretreatment model (multiplicative scatter correction, standard normalization constant offset cancellation, the first derivative, etc.) of R^2 and RMSECV, choose R^2 closest 1, RMSECV pretreatment method closest to 0 is the best way.

1.6 Modeling band selection

In the near-infrared spectral calibration model building process, if calculated using the full spectrum, the calculation is great, and due to the nature of its benchmark spectral information between certain spectral region of the sample bias brought about lack of correlation. In order to improve the accuracy of the model, the internal cross-validation test to determine the coefficient R2 and internal cross-validation MSE as an indicator RMSECV to compare the effects of different bands built on the corrected model, choosing the best band for modeling.

1.7 Selecting the main factors

The principal factor number called principal components, the components in a sample number, it is directly related to the actual prediction ability of the model. When you create a quantitative calibration models using PLS method, if the main factor used to build the model number is too small, it can not fully reflect the component being tested sample spectral information generated, resulting in not fully fit, the model prediction accuracy will be reduced. If you use the main number of factors included excessive noise are some of the information is also incorporated into the calculations, it will lead to over-fitting, thereby reducing the predictive power of the model.

In order to eliminate the influence of the improper selection of principal factor number of modeling results, this test uses a cross validation method, influence of principal factor number on the RMSECV, when the RMSECV minimum, can get the most suitable factors.

1.8 NIR quantitative analysis

Comprehensive comparison of different wavelengths, different pretreatment methods and main factor influence on the calibration model, choose the best spectral processing methods, the best band and best master number of factors combined to establish the true value of the chemical Pieces Paeoniflorin TGP and water quantitative calibration models.

1.9 Verifying the calibration model

In order to evaluate the prediction performance of calibration model, prediction correction model to each corresponding set of tested, with external validation coefficient of determination R2 and external validation variance RMSEP as index, to verify the model.

RESULTS

2.1 Effect of sample thickness on the spectrum

The 32 Paeonia samples, 28 samples are collected to buildpaeoniflorin quantitative model, 4 test sets. 27 samples are collected to build a quantitative model of water, 5 samples for test set. The collected samples of different thickness PCS original maps were compared and found that samples of different thickness NIR spectral overlap better, combining NIR diffuse reflectance samples required sample cover bottom of the cup, NIR spectra of the samples collected in the same thickness can reduce experimental error factors as the best choice 1cm thickness. The results shown in Figure 1.

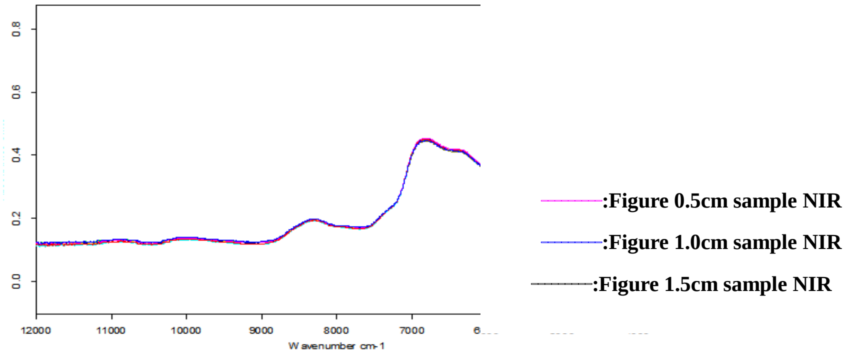

Figure 1. Samples of different thickness near-infrared spectra

2.2 Choose a different pretreatment methods

Internal cross-validation coefficient of determination R^2 and internal cross-validation MSE RMSECV as an index to compare different spectral model after pretreatment (multiplicative scatter correction, standard normalization constant offset cancellation, the first derivative, etc.) of R^2 and RMSECV, Choosing the best pretreatment method. The results are shown in Table 2 and Table 3.

Table 2.Effect of different pretreatment methods for Paeonia samples modeling water

6102.2～4246.9cm^{-1}	RMSECV	R^2（%）
A smooth first derivative +17 points	0.533	94.73
Internal standards	0.578	93.80
Vector normalization	0.593	93.46

Table 3.Effect of different pretreatment methods for modeling Paeoniflorin

7502.4～4246.9cm^{-1}	RMSECV	R^2（%）
Subtract a straight line	0.187	95.09
Multiplicative scatter correction	0.202	94.24
Eliminate the constant offset	0.209	93.19

As can be seen from the above results: In 6102.2~4246.9cm-1 uses a first derivative +17 points smoothing method, the internal cross-validation TGP water can reach better results, RMSECV= 0.533, R^2=94.73. In the 7502.4~4246.9cm^{-1} by subtracting a straight line processing method, Paeonia lactiflora paeoniflorin internal cross validation can achieve better results, RMSECV=0.187, R^2=95.09.

2.3 Modeling band selection

Internal cross-validation coefficient of determination R^2 and internal cross-validation MSE RMSECV as an index to compare different impact on the built-band calibration model, choose the best band. The results are shown in Table 4 and Table 5.

Table 4. Effects of different spectral range of paeoniae RMSECV

A smooth first derivative +17 points	RMSECV	R^2（%）
6102.2～4246.9cm^{-1}	0.533	94.73
5176.5～4246.9cm^{-1}	0.565	94.08
7502.4～5446.5cm^{-1}	0.594	93.45

Table 5. RMSECV affect different spectral ranges peony Paeoniflorin

Subtract a straight line	RMSECV	R^2（%）
7502.4～4246.9cm^{-1}	0.187	95.09
7502.4～6098.4cm^{-1}	0.198	93.19
7502.4～4597.9cm^{-1}	0.258	90.63

2.4 Selecting the main factors

RMSECV curve through the main factors related to the number can be seen: the best peony water main factor correction model number is 11, peony Paeoniflorin best main factor correction model number is 13.The results shown in Figure 2 and Figure 3.

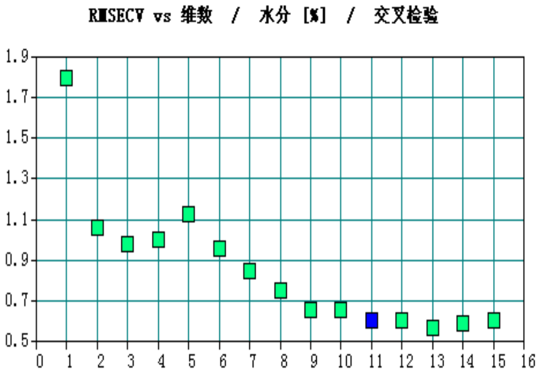

Figure 2. Paeonia main factors to select the optimum moisture

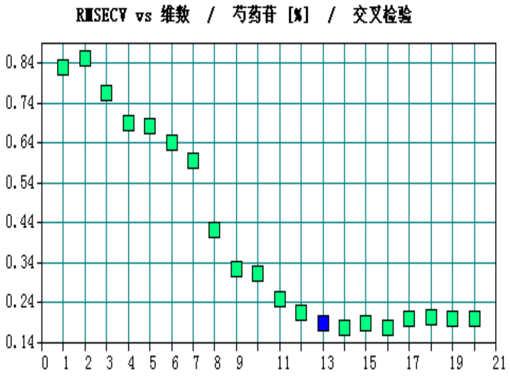

Figure 3. Paeonia lactiflora paeoniflorin best principal factor number

2.5 The calibration models were built by NIR quantitative analysis

Comprehensive comparison of different wavelengths, different pretreatment methods and main factor influence on the calibration model, and choose the best spectral processing method, the best band and best principal factor number, combined with chemical true value correction model establishment of paeoniflorin Radix Paeoniae Alba Decoction and water.

2.5.1 Determination results of samples (true value)

(1)The results of determination of moisture content

Reference to the 2010 edition of Pharmacopoeia measured by loss on drying moisture content Pieces of white peony, the range of 5.99% - 16.70%.

(2)Paeoniflorin assay results

Measured by reference to HPLC peony Paeoniflorin range 0.04 to 3.15%

(3)Determination of recovery

Take the same sample, weighed nine, paeoniflorin reference solution injected different amounts of low high school, Each set of three sample concentration, according to TPG sample preparation method for the test process, carried out by HPLC detection, the average recovery of 91%.

2.5.2 samples of water and Paeoniflorin NIR quantitative analysis to verify calibration model

Paeonia NIR original map as shown in Figure 3-4,To determine the coefficient R2 and internal cross-validation RMSECV and relative mean square deviation RPD were verified as an indicator to compare different spectral pretreatment, different bands, different number of factors corresponding to the model, and ultimately determine the next smallest RMSECV R2 biggest variable as the final construction molding conditions. The resulting correction modeling experimental conditions as shown in Table 3-5,peony water Paeoniflorin correction model shown Table 5 and Table 6.

Table 5. Optimum conditions for each component modeling peony

Correction model	Spectral range cm^{-1}	Pretreatment methods	RMSECV	R^2	Number of main factors	RPD
The moisture content in Paeonia	6102.2~4246.9	A smooth first derivative +17 points	0.533	94.73	11	4.36
	7502.4~4246.9	Subtract a straight line	0.187	95.09	12	4.51

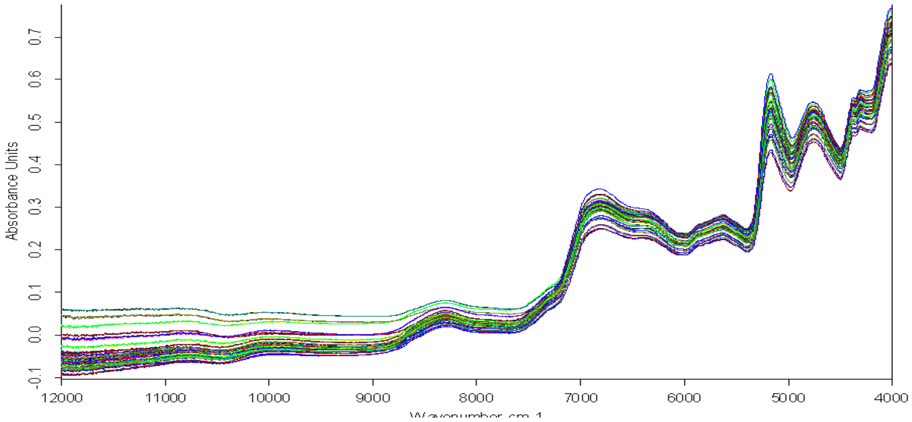

Figure.4 Pieces of the original image peony NIR

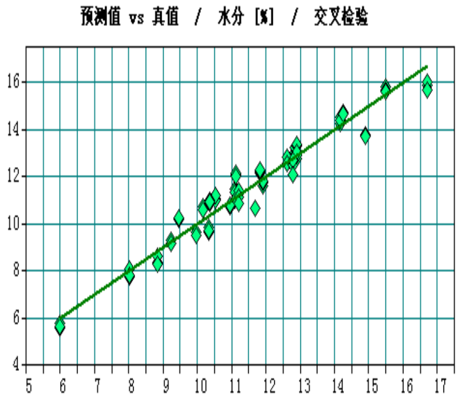

Figure 5. Paeonia moisture correction model diagram

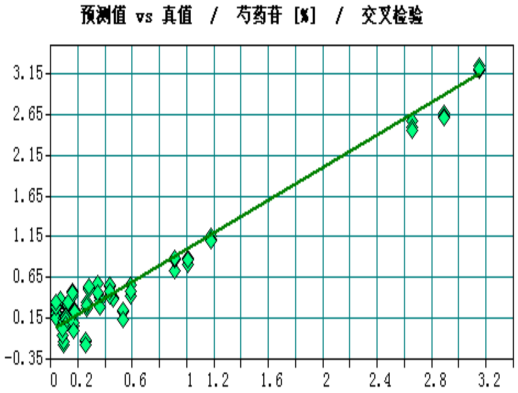

Figure 6. Peony Paeoniflorin correction model diagram

2.5.3 Moisture and Paeoniflorin NIR quantitative analysis

To determine the moisture, paeoniflorin prediction set respectively on the calibration model was validated by the coefficient of determination R2, external validation and external validation variance RMSEP as index, to verify the model, RPD for cross validation of relative standard deviation. The results are shown in Table 6. Validate the model shown in Figure 7 and Figure 8.

Table 6. Peony each component to validate the model results

Validate the model	RMSEP	R^2	RPD
the moisture content in Paeonia	0.215	97.93	10
Peony paeoniflorin	0.0919	93.62	4.32

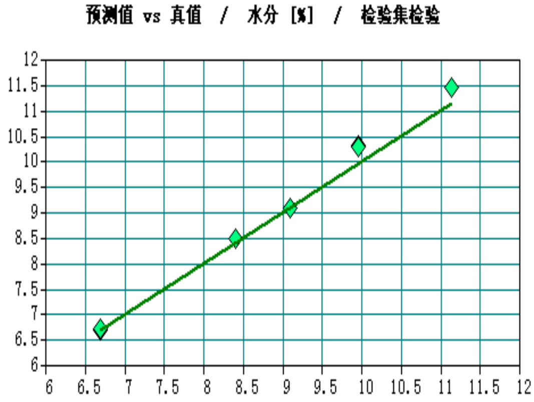

Figure 7. Paeoniae moisture test set validation results

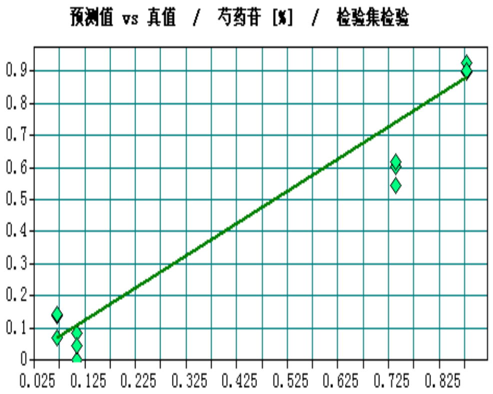

Figure 8. Peony Paeoniflorin test set validation results

Sinnaeve and Park have reported that when RPD is greater than 2.5, thus the model can be used to predict near-infrared, greater than 3.0 model has good predictive capabilities [7-8].

DISCUSSION AND CONCLUSION

The revealed test method has integrated using HPLC and Near-Infrared diffuse reflection spectroscopy, the true value of the chemical results were compared with the NIR prediction. Peony water and Paeoniflorin are decided to validate the model coefficients, which were both above 93%, RPD was greater than 3, which means the model has good predictive performance. Herein, the method does not require tedious sample pre-treatment, with fast speed and no damage analysis, etc. Near-infrared diffuse reflectance technique can be a simple and effective method for the analysis of the quality of traditional Chinese medicine, there are good prospects for further application.

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