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Research Article

Rapid Determination of Pachymic Acid Content by Near-Infrared Spectroscopy

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In this paper, a rapid model for the determination of pachymic acid content in *Poria* was established by partial least squares (PLS) regression and near-infrared spectroscopy (NIR). During the research, a total of 108 batches of *Poria* samples from different producing regions were used, while their corresponding pachymic acid contents by high-performance liquid chromatography (HPLC) were adopted as reference. These samples were divided randomly into calibration sets for model establishment and validation sets for model validation. The test results from the calibration set showed that the best preprocessing method of the NIR spectra model was the standard normal variate (SNV) + second derivatives (SD), and the most suitable number of principal factors was 9. In this model, the coefficient of determination of the calibration set (r_c^2) and validation set (r_v^2) was 0.915 and 0.917, respectively. Meanwhile, the root mean square error of calibration (RMSEC) and the root mean square error of validation (RMSEP) with the calibration set were 0.051 mg/g and 0.054 mg/g, respectively. These results indicated this model could rapidly and reliably predict the pachymic acid content in *Poria* and increase the determination efficiency of pachymic acid in *Poria*. This is conducive to promote the development of industry.

1. Introduction

Poria cocos, one important medicinal fungus, has been used in China for a long time [1, 2]. Pachymic acid, a rich component in *Poria*, is the key active ingredient of *Poria*, with the effects of anti-inflammatory [3], antitumor [4], and hypoglycemic [5]. Usually, pachymic acid was used as the mark component to reflect the quality of *Poria* [6]. Nowadays, the common determination methods for pachymic acid include the colorimetric method and the HPLC method. For its high accuracy and stability, HPLC is usually taken as a reference method, but its analysis process is labor-intensive and time-consuming, which has hampered its wide applicability in the market. Moreover, the 2020 edition of the "Chinese Pharmacopoeia" has not included one quantitative determination method for this component. Therefore, a method with high efficiency and throughput for the determination of pachymic acid content in *Poria* is necessary.

NIR spectroscopy is one rapid determination method widely used in the research field of traditional Chinese medicine recently for the advantages of fast speed [7, 8], high efficiency, high sensitivity, simple sample processing, and convenient instrument operation [9–11]. NIR spectroscopy has been widely used in food [12, 13], medicine [14–16], and other industries [17, 18]. Gao Hongbin et al. [19] used vector normalization and first derivative to process the NIR spectrum data of Glycyrrhizae Radix et Rhizoma Yinpian and established the corresponding rapid NIR analysis model for the determination of glycyrrhizic acid and glycyrrhizin with the PLS method. The correlation coefficients of the

models were 0.981 and 0.919, and the mean square errors of cross-validation were 0.184 μg/mL and 0.144 μg/mL, respectively. Ying Liang [20] used the PLS method combined with cross-validation methods with regression analysis and MSC + SD data processing methods to develop a NIR analysis model for rapid determination of naringin content. The model has good indexes and can rapidly evaluate the content of naringin in *Exocarpium Citri Grandis*.

According to references, it can be concluded that the NIR spectrum majorly reflects the doubling- and combination-frequency absorption of the movement of some characteristic groups (such as C-H, O-H, C=O, -CH_2, and -CH_3), and these groups have a fixed vibration frequency which can excite to generate resonance when irradiated by infrared rays [21]. The quantity and connection of these groups of pachymic acid could form a specific NIR spectrum in the instrument, and the amount of energy in the near-infrared light absorbed by the pachymic acid, which can be mined in the NIR spectrum by the *Poria* sample, could reflect the quantity of pachymic acid. In this study, NIR were used with PLS regression to establish a rapid quantitative analysis model to detect pachymic acid, which was expected to provide technical reference for quality control and market management of *Poria*.

2. Materials and Methods

2.1. Materials and Reagents. A total of 108 batches of *Poria* samples were collected in small cube shapes from the market in 2019. The collected regions covered the main production provinces and the Dabie Mountains of China, including Hunan, Anhui, Yunnan, Hubei, Sichuan, Guizhou, Chongqing, Fujian, Guangdong, Henan, Jiangxi, Zhejiang, and Guangxi Provinces.

2.2. Main Instruments and Reagents. Spot Light 400 NIR Spectrometer (PerkinElmer Inc., USA), Multiskan MK3 Microplate (Thermo Electron Corp., USA), and LC-20AT high performance liquid chromatograph (Shimadzu Corp., Japan) were used in this research.

During this research, acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Longxi Science Co., Ltd., acetic acid (HPLC grade) was purchased from Tianjin Fuyu Fine Chemical Co., Ltd., purified water were from Hangzhou Wahaha Baili Food Co., Ltd., and pachymic acid (Batch No. 18062101) was purchased from Chengdu Pufei Biotechnology Co., Ltd.

2.3. Method for the Determination of Pachymic Acid Content

2.3.1. Preparation of the Reference Solution. Pachymic acid was accurately weighed and dissolved in methanol to prepare a 0.49 mg/mL reference substance solution, which was filtered through a 0.22 μm microporous membrane and reserved for testing.

2.3.2. Preparation of the Test Solution. Each sample was crushed into powder of 80 mesh fineness and placed in a 10 mL conical flask. The powder was dissolved with methanol to make a 0.125 g/mL solution and was disposed of by ultrasonic (1200 W) for 40 min. The supernatant was taken and reserved for testing after filtering through a 0.22 μm microporous filter membrane.

Table 1: LC time program.

t/min	Acetonitrile (%)	0.04% acetic acid solution (%)
0	50	50
5	55	45
12	60	40
15	65	35
19	70	30
25	75	25
30	75	25
35	100	0

2.3.3. The HPLC Conditions. The pachymic acid content of the reference solution and test solution was determined by HPLC with the chromatographic column being Thermo BDS Hypersil C18 (250 × 4.6 mm, 5 μm). The LC time program is shown in Table 1. The flow rate was 1.4 mL/min, the column temperature was 30°C, the detection wavelength was 210 nm, and the sample size was 20 μL. Based on this condition, the corresponding chromatogram is shown in Figure 1. The peak with the retention time of 26 min was the pachymic acid.

2.3.4. Evaluation of HPLC Methods. Linear Relation Investigation. In this part, the chromatograph and corresponding peak areas of 5 injections of reference solutions with different concentrations (0.0245, 0.049, 0.49, 0.98, and 1.47 mg/mL) were recorded. The linear regression was established with the content of reference solutions (x) and corresponding chromatographic peak areas (y) and shown as the equation $y = 3 \times 10^8\ x + 4162.2$. The linear regression coefficient of this equation was 0.9999, indicating that the peak area of pachymic acid had a good linear correlation with the content in the range of 0.0245~1.4700 mg/mL.

Reproducibility. A total of 6 testing solutions prepared from the same sample were tested separately. The RSD of the test was 0.48%, indicating that this method had a good repeatability.

Precision. In this part, one reference solution was injected 6 times continuously for the precision testing of this method. The RSD was 0.31%, which indicated that the instrument and method could determine the content of pachymic acid accurately.

Stability. Samples prepared according to 2.3.2 were injected at 0, 4, 8, 16, 20, and 24 h, respectively, for testing the stability of the testing solution. The results showed that the RSD of this method was 0.28%, indicating that pachymic acid in the test solution was basically stable within 24 h.

Recovery. In this part, 6 samples with a known content of pachymic acid were added with a certain amount of standard pachymic acid, respectively. The average recovery rate of this

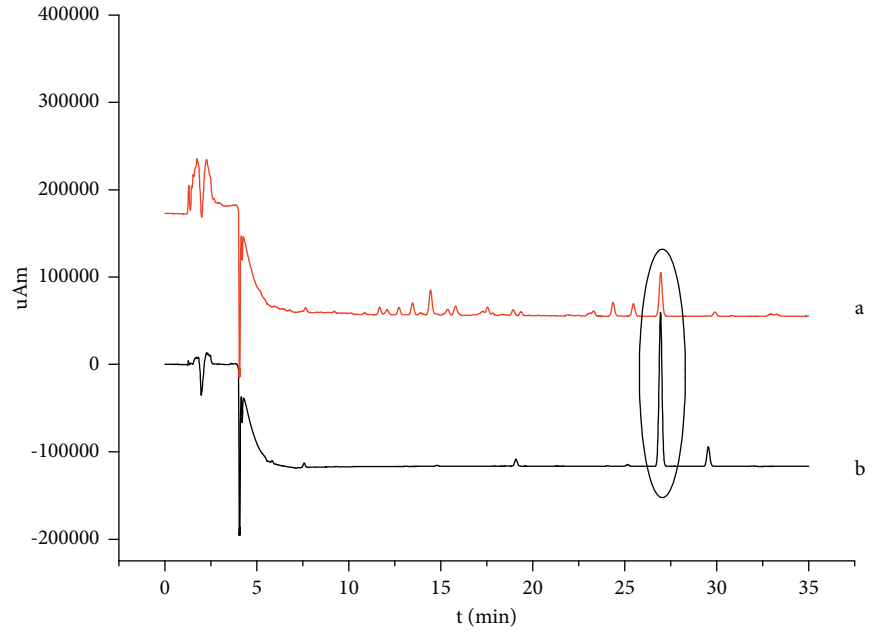

Figure 1: Chromatogram of (a) *Poria* sample and (b) reference solution.

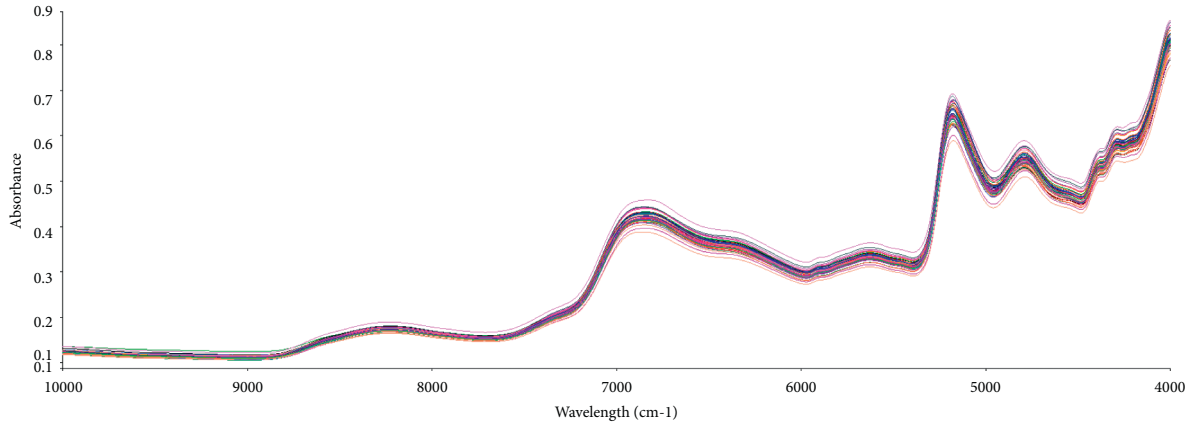

Figure 2: NIR spectrum of *Poria*.

method for the pachymic acid was 101.55% and 2.46%, and the RSD value was 2.36%, which was eligible.

2.4. Procedure of NIR Spectroscopy Analysis

2.4.1. NIR Spectrum Acquisition and Analysis. The *Poria* samples were crushed into powder, sieved through 80 mesh, and evenly spread on the bottom of the test dish (its diameter is 5 cm). The resolution was set to 8 cm^{-1}, scanning was 64 times per average spectrum within the spectral range of 4000–10000 cm^{-1}, the scanning type was interleaved mode, and the internal reference was automatically used as the background. Based on the determination parameters, the spectrum of each sample could be obtained (Figure 2).

2.4.2. Establishment of the NIR Model. PLS, artificial neural networks (ANN), support vector machines (SVM), and other nonlinear correction methods are used to establish the model with their own advantages and disadvantages [22], and in this research, PLS was adopted to build the NIR analysis model with the software Spectrum Quant. In addition, 4 parameters including r_c^2, RMSEC, RMSEP, and r_v^2 were used to evaluate the established model. r_c^2 is used to evaluate the degree of linearity between the predicted and measured values of the samples. The closer r_c^2 is to 1, the better the regression or prediction result is. RMSEC is mainly used to evaluate the feasibility and prediction ability of modeling methods. RMSEP is mainly used to evaluate the prediction ability of the model for external samples. The smaller the value is, the better the prediction ability of the model for external samples is. r_v^2 is used to evaluate the linearity between the predicted value and the measured value of the model for the unknown sample during the external validation of the model [21]. Its value is approximately close to 1, indicating that the model predicts better results. Spectrum Quant was used for modeling in this study.

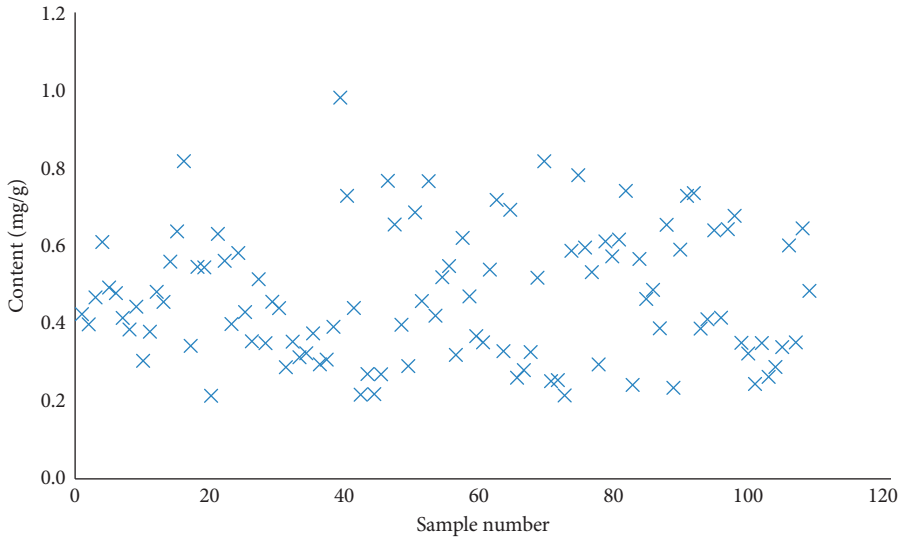

Figure 3: Scatter plot of pachymic acid content in *Poria* samples.

Table 2: Pachymic acid content range in *Poria* samples from the calibration set and validation set.

	Size	Maximum value (mg/g)	Minimum value (mg/g)	Mean ± SD (mg/g)
Calibration set	91	0.981	0.214	0.472 ± 0.166
Validation set	17	0.735	0.242	0.429 ± 0.144

Table 3: Test results for the ranges of NIR spectroscopy.

Spectral range (cm^{-1})	r_c^2	RMSEC (mg/g)	RMSEP (mg/g)
4200–5390	0.504	0.124	0.131
5390–6070	0.524	0.121	0.127
6070–7500	0.832	0.072	0.079
4000–10000	0.915	0.051	0.054

3. Results and Analysis

3.1. The Content Value of Pachymic Acid. Figure 3 is a scatter plot about the pachymic acid content of 108 batches of *Poria*.

A total of 91 *Poria* samples were randomly selected from 108 samples according to the sequence of each sample by using the random function in Excel for composing the calibration set, and the remaining samples were included in the validation set. At the same time, the maximum and minimum values of pachymic acid content from the calibration set should cover the validation set. The information characteristics of the samples from the two sets are shown in Table 2.

3.2. Selection of Spectral Interval of NIR Analysis Model. The absorption effect of substances varies in different NIR spectra, and it is necessary to determine the proper spectral range for the PA determination [23]. In this study, 4 potential spectral intervals were adopted, and the evaluation results of the corresponding analysis model are shown in Table 3.

According to the results, the spectral range is 4000–10000 cm^{-1}, the full spectral range with the largest r_c^2, the smallest RMSEC and RMSEP, as well as the proper ratio of latter two indices were selected for modeling.

Table 4: The results for the data preprocessing methods.

Methods	r_c^2	RMSEC (mg/g)	RMSEP (mg/g)
MSC	0.209	0.156	0.163
SNV	0.208	0.156	0.164
FD	0.504	0.124	0.129
SD	0.922	0.049	0.052
MSC + SD	0.915	0.051	0.054
SNV + SD	0.915	0.051	0.054
MSC + FD	0.582	0.113	0.120
SNV + FD	0.590	0.112	0.118

3.3. Selection of Spectral Pretreatment Methods for NIR Analysis Models. During the data analysis of NIR spectra, some random factors, such as sample particle size and light interference, could cause the baseline drift, affecting the reliability and stability of model building [24–26]. Single and combination data preprocessing methods were used for the modeling and the results are listed in Table 4.

MSC mainly eliminates the scattering effect caused by uneven sample size and particle size. SNV is mainly used to eliminate the influence of particle size, surface scattering light, and optical path change on the near-infrared spectrum. Derivatives can effectively eliminate baseline and other background interference [21]. It can be seen from Table 4 that SD, SNV + SD, and MSC + SD are the better preprocessing methods for the spectral information of the NIR analysis model. The r_c^2 of the three pretreatment methods is

Table 5: The test results for the number of principal components.

No.	Method, principal factor number	r_c^2	RMSEC (mg/g)	RMSEP (mg/g)
1	SNV + SD, 10	0.949	0.040	0.042
2	SNV + SD, 9	0.915	0.051	0.054
3	SNV + SD, 8	0.873	0.062	0.065
4	MSC + SD, 10	0.949	0.040	0.042
5	MSC + SD, 9	0.915	0.051	0.054
6	MSC + SD, 8	0.873	0.062	0.065
7	SD, 10	0.941	0.043	0.046
8	SD, 9	0.922	0.049	0.052
9	SD, 8	0.853	0.067	0.069

Table 6: The parameters of the optimal NIR detection model.

Project		Parameters
Model parameters	Spectral pretreatment method	SNV + SD
	Principal factor number of PLS	9
	Spectral range	4000–10000 cm^{-1}
	r_c^2	0.915
The evaluation results	RMSEC (mg/g)	0.051
	RMSEP (mg/g)	0.054
	r_v^2	0.917

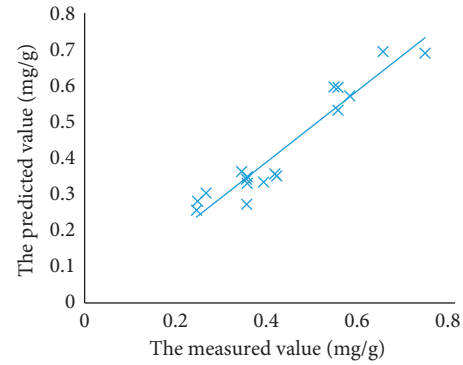

Figure 4: Comparison between predicted values and experimental values.

greater than 0.9, and RMSEC and RMSEP meet the basic requirements of model building. Among them, the values of the three indexes (r_c^2, RMSEC, and RMSEP) of SNV + SD and MSC + SD pretreatment methods have a slight difference, so these three pretreatment methods are taken into consideration.

3.4. Determination of PLS Principal Factor Number in the NIR Analysis Model. When PLS was used to establish the NIR analysis model, the number of PLS principal factors was also a key factor affecting the quality of the model. Either too many or too less principal factors would cause underfitting or overfitting problems, respectively, increasing the prediction error [27]. In this part, the corresponding results of the models with specific data preprocessing method and principle factors are listed in Table 5.

It can be seen from Table 5 that all alternative data preprocessing methods with more principal factors could cause higher r_c^2 and smaller RMSEC and RMSEP, and the models with over 9 principal factors would have a suitable r_c^2 higher than 0.90. So 9 principal factors were the basic requirements for the modeling.

4. Validation and Evaluation of the Model

On the basis of the former parts of this research, 6 aforementioned models (No. 1, 2, 4, 5, 7, and 8 in Table 5) with r_c^2 over 0.90 were further verified by using validation set samples in this part, and it was found that the maximum r_v^2 of model 2 is 0.917. From this, the optimal NIR analysis model (No. 2 model in Table 5) for predicting the content of pachymic acid of *Poria* was obtained, and its parameters are shown in Table 6.

The distribution of the predicted and experimental values was scattered intuitively in a line graph (Figure 4) [28]. At the same time, SPSS statistical software (SPSS Inc., Chicago, USA) was used to analyze the difference between the two values by comparing the ratio of them with 1, and there was no significant difference found between them ($P = 0.512$). This result indicated that the NIR analysis model had good prediction accuracy for pachymic acid of *Poria*.

5. Conclusion

There are already many national standards by using NIR for qualitative and quantitative detection now [29, 30]. NIR has also been widely recognized in the field of traditional Chinese medicine. It is widely used in the production process of traditional Chinese medicines, including qualitative identification of the types and authenticity of traditional Chinese medicines [31], quantitative determination of effective components, and monitoring of the drug production process [32, 33]. In this study, different NIR analysis models for pachymic acid were evaluated with different parameters by r_c^2, RMSEC, RMSEP, and r_v^2 values. The results showed that the NIR analysis model with pretreatment method of standard normal variate (SNV) + second derivative (SD), the spectral interval of 4000–10000 cm^{-1}, and 9 PLS major factors could predict the pachymic acid content in *Poria* exactly. Besides, there was no significant difference

between the predicted value and the measured value of the prediction set ($P = 0.512$).

According to Williams et al.'s results, this NIR model is more precise than the standard for most applications and almost reaches the standard for quality assurance ($r^2 \geq 0.92$) [34]. Hence, many improvements still need to be made to this model in further study, in order to promote its applicability and practicability.

The pachymic acid content of *Poria* was successively determined by NIR combined with stoichiometry, expanding the applicability of NIR. Compared with other analytical methods, the NIR method has the advantages of simple pretreatment, fast analytical speed, high multisample processing capacity, and low cost, which is beneficial to the development promotion of the *Poria* industry [35].

Data Availability

The data used to support the findings of this study are included within the article.

Ethical Approval

Not applicable.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Jie Lu and Changqin Li contributed equally to this work.

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