Rapid monitoring the water extraction process of *Radix Paeoniae* Alba using near infrared spectroscopy

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Near infrared (NIR) spectroscopy has been developed into one of the most important process analytical techniques (PAT) in a wide field of applications. The feasibility of NIR spectroscopy with partial least square regression (PLSR) to monitor the concentration of paeoniflorin, albiflorin, gallic acid, and benzoyl paeoniflorin during the water extraction process of *Radix Paeoniae* Alba was demonstrated and verified in this work. NIR spectra were collected in transmission mode and pretreated with smoothing and/or derivative, and then quantitative models were built up using PLSR. Interval partial least squares (iPLS) method was used for the selection of spectral variables. Determination coefficients ($R^2_{\text{cal}}$ and $R^2_{\text{pred}}$), root mean squares error of prediction (RMSEP), root mean squares error of calibration (RMSEC), and residual predictive deviation (RPD) were applied to verify the performance of the models, and the corresponding values were 0.9873 and 0.9855, 0.0487 mg/mL, 0.0545 mg/mL and 8.4 for paeoniflorin; 0.9879, 0.9888, 0.0303 mg/mL, 0.0321 mg/mL and 9.1 for albiflorin; 0.9696, 0.9644, 0.0140 mg/mL, 0.0145 mg/mL and 5.1 for gallic acid; 0.9794, 0.9781, 0.00169 mg/mL, 0.00171 mg/mL and 6.9 for benzoyl paeoniflorin, respectively. The results turned out that this approach was very efficient and environmentally friendly for the quantitative monitoring of the water extraction process of *Radix Paeoniae* Alba.

**Keywords:** Near infrared spectroscopy; partial least squares regression; high performance liquid chromatography; *Radix Paeoniae* Alba.

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1. Introduction

*Radix Paeoniae* Alba (Baishao), the dried root of *Paeonia lactiflora* Pall, one of the most widely used herb medicines has been reported to exhibit pharmacological actions such as anti-inflammatory, anti-allergic, anti-thrombosis, immunoregulating, and kidney and liver protection properties.\(^1\)–\(^3\) Monoterpenepene glucosides are generally considered to be the main bioactive compounds responsible for most of the biological activities. Among them, paeoniflorin is the major active constituent and has been used as a phytochemical marker for the quality control of *Radix Paeoniae* Alba in Chinese Pharmacopoeia.\(^4\) Other bioactive components, such as albiflorin, benzoyl paeoniflorin, gallic acid, etc., have also been reported as the pharmacologically active ingredients of *Radix Paeoniae* Alba.\(^5\)–\(^7\)

As one of the most popular raw herb materials for the production of Chinese patent medicines, such as Shenzhiling oral solution, Si-Wu oral solution, Xiaoyaosan decoction, etc. Water extraction process is indispensable one of the key manufacturing units for most production of Chinese patent medicines containing *Radix Paeoniae* Alba. However, in large-scale traditional Chinese medicine (TCM) manufacturing process, the conventional extraction procedure is still the mainstream approach, and heat reflux extraction is the most widely used technique for the extraction of herbal materials.\(^8\) To improve the process efficiency and guarantee the final product quality, reliable process analytical technology (PAT) should be emphasized in the water extraction process of *Radix Paeoniae* Alba.

Near infrared (NIR) spectroscopy combined with chemometrics has found its useful applications in broad range of domains during the past decades, such as agricultural, food, pharmaceutical, and biomedical sectors.\(^9\)–\(^15\) NIR spectroscopy has also shown great power and gained wide acceptance in TCM manufacturing industry.\(^11\),\(^16\) The most well-known applications of NIR spectroscopy in the manufacturing processes of TCM include the separation monitoring,\(^17\) end point judgement of extraction process,\(^18\)–\(^20\) alcohol precipitation monitoring,\(^21\) etc. Obviously, NIR spectroscopy is a promise technology for the understanding of the TCM manufacturing processes.

The objective of this study is to investigate the feasibility and application of NIR spectroscopy in determination of paeoniflorin, albiflorin, gallic acid, and benzoyl paeoniflorin during the water extraction process of *Radix Paeoniae* Alba. NIR calibration models were built up by using partial least squares regression (PLSR) with NIR spectra and reference data of samples collected from the water extraction process of *Radix Paeoniae* Alba. The performances of NIR calibration models were evaluated by means of determination coefficients \((R^2_{\text{cal}}\) and \(R^2_{\text{pred}}\)), root mean squares error of prediction (RMSEP), root mean squares error of calibration (RMSEC), and residual predictive deviation (RPD). To our knowledge, this is the first report to demonstrate the feasibility of NIR spectroscopy with PLSR for rapid monitoring of the water extraction process of *Radix Paeoniae* Alba.

2. Materials and Methods

2.1. Chemicals and reagents

Authentic paeoniflorin, albiflorin, gallic acid, and benzoyl were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). High performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). *Radix Paeoniae* Alba was provided by Wohua Pharmaceutical Technology Co., Ltd, (Weifang, China). Distilled water was obtained from Milli-Q water purification system of Millipore (Bedford, MA, USA). Other reagents were obtained from VWR International (South Plainfield, NJ, USA).

2.2. *Radix Paeoniae* Alba water extraction and sampling

Extraction of *Radix Paeoniae* Alba was carried out under laboratory-scale simulating the actual procedure of *Radix Paeoniae* Alba water extract. Thirty gram *Radix Paeoniae* Alba was soaked in 300 mL tap water at room temperature (25°C) for 40 min, and then refluxed for 150 min at the first extraction stage, after filtration of the extract, the residues were extracted by another 180 mL tap water for 100 min at the second extraction stage. Sampling was from the same bottleneck of the 500 mL three-necked round-bottomed flask by glue dropper and each sampling was 3 mL. Samples were
collected at the beginning of boiling during each extraction, and then every 15 min during the first extraction and 20 min during the second extraction, four extraction batches were carried out and 68 samples were collected, but one sample was spilled, and then 67 samples were left.

2.3. Spectra acquisition
NIR spectra were collected using an Antaris II Fourier transform NIR spectrophotometer (Thermo Fisher, USA). Transmission spectra of samples were collected from 4000 cm$^{-1}$ to 10,000 cm$^{-1}$ at every 8 cm$^{-1}$ path interval. Each spectrum was obtained by averaging 32 scans with the gain value of 4 (B screen). To avoid error from the outer environment, all samples were equilibrated to room temperature (25°C) prior to NIR spectra collection. And the humidity was also kept at ambient level in the laboratory. Figure 1 shows the raw spectra of the extract samples.

2.4. Determination of reference values
The concentration of paeoniflorin, albiflorin, gallic acid, and benzoyl paeoniflorin in the extract samples were analyzed by HPLC coupled with UV detection (HPLC-UV). Chromatographic analysis was performed on an Agilent 1260 HPLC system (Agilent Corp., Santa Clara, CA, USA). An Agilent ZORBAX SB-C$_18$ column (4.6 $\times$ 250 mm, 5 $\mu$m) was employed for chromatographic separation with a flow rate of 0.8 mL/min. The mobile phase was consisted of acetonitrile (mobile phase A) and 0.1% phosphate acid in distilled water (mobile phase B). Gradient elution was carried with the following profile: 0–5 min, 5% A; 5–6 min, 5–15% A; 20–21 min, 15–25% A; 30–31 min, 25–35% A; 31–42 min, 35–50%; 42–45 min, 50–5% A; and 45–50 min, 5% A. The wavelength of UV detector was set at 230 nm and the injection volume was 10 $\mu$L.

2.5. Data analysis
All the computations, including division of the calibration and validation set, spectral pretreatment, and variable selection were carried out using MATLAB version 2010a (MathWorks Inc., Natick, USA) with the PLS tool-box (version 752) purchased from Eigenvector Research, Inc., (Wenatchee, WA, USA). The NIR spectra and HPLC data were modeled by PLSR to establish the quantitative models.

3. Results and Discussion
3.1. Reference values analysis
The HPLC-UV method was developed and validated for the determination of the four analytes in the 67 extract samples. Chromatogram of one sample is shown in Fig. 2.

The main methodology parameters and calibration curves of the validated HPLC-UV method are listed in Table 1. The concentration of the four analytes in the extract samples are listed in Table 2. The range of the concentrations of gallic acid, albiflorin, paeoniflorin, and benzoyl paeoniflorin in the extract samples were 0.077–0.331, 0.154–0.902, 0.240–1.386, and 0.011–0.044 mg/mL, respectively.

3.2. Division of calibration and validation set
Firstly, the samples with the highest and lowest concentrations of gallic acid, albiflorin, paeoniflorin,
and benzoyl paeoniflorin were divided into the calibration set, while the remaining samples were split into two sets, calibration set and validation set, by Kennard–Stone (KS) algorithm. In current study, 53 samples (80%) were placed in the calibration set, and the remaining 14 samples (20%) were signed to the validation set. The ranges of the concentration of gallic acid, albiflorin, paeoniflorin, and benzoyl paeoniflorin in calibration and validation sets are listed in Table 3 and score plot of principle components (PCs) for sample division is shown in Fig. 3, obviously, samples belonging to the validation set are evenly distributed throughout the calibration set.

### 3.3. Spectral pretreatments

NIR spectral analysis has been widely applied in virtue of the development of chemometrics, in which spectral pretreatment and variable selection methods play an important role in the development of the robust NIR models. In our current study, different spectral pretreatment methods such as first derivative (FD) conversion, Savitzky–Golay (SG) filter, standard normal variate (SNV), and the combinations of them were used and compared. For the optimization of the spectral pretreatment methods, all of the variables ($n = 1577$) were included for the PLSR modeling. For each analyte, the optimized spectral preprocessing method was

| Analytes           | Calibration curve | Linear range (µg/mL) | $r^2$ | Intra-day (RSD) | Inter-day (RSD) | Stability (RSD) | Repeatability (RSD) |
|--------------------|-------------------|----------------------|------|-----------------|-----------------|------------------|---------------------|
| Gallic acid        | $y = 0.0073x - 3.5131$ | 3.0–60.0             | 0.9995 | 0.3%            | 1.8%            | 0.0%             | 3.3%                |
| Albiflorin         | $y = 0.0132x - 0.2801$ | 5.6–112.0            | 0.9990 | 0.0%            | 1.1%            | 0.9%             | 2.9%                |
| Paeoniflorin       | $y = 0.0117x - 1.4902$ | 6.9–138.6            | 0.9994 | 0.0%            | 0.4%            | 0.2%             | 3.3%                |
| Benzoyl paeoniflorin | $y = 0.0060x - 0.4921$ | 0.7–6.5              | 0.9978 | 0.0%            | 3.8%            | 3.9%             | 3.4%                |

| Analytes           | Batch 1 | Batch 2 | Batch 3 | Batch 4 |
|--------------------|---------|---------|---------|---------|
| Gallic acid        | 0.105–0.331 | 0.108–0.327 | 0.077–0.310 | 0.070–0.303 |
| Albiflorin         | 0.189–0.875 | 0.236–0.902 | 0.154–0.841 | 0.142–0.797 |
| Paeoniflorin       | 0.292–1.349 | 0.311–1.386 | 0.254–1.326 | 0.240–1.336 |
| Benzoyl paeoniflorin | 0.014–0.040 | 0.015–0.041 | 0.013–0.041 | 0.011–0.044 |

### Table 3. The concentration values of the four target components in the calibration and validation sets.

| Analytes           | Sample set | Range (mg/mL) | Mean (mg/mL) |
|--------------------|------------|---------------|--------------|
| Paeoniflorin       | Calibration | 0.240–1.386   | 0.950        |
|                    | Validation | 0.311–1.374   | 0.801        |
| Albiflorin         | Calibration | 0.142–0.902   | 0.597        |
|                    | Validation | 0.203–0.888   | 0.504        |
| Gallic acid        | Calibration | 0.070–0.331   | 0.208        |
|                    | Validation | 0.106–0.305   | 0.184        |
| Benzoyl paeoniflorin | Calibration | 0.010–0.044   | 0.0303       |
|                    | Validation | 0.013–0.042   | 0.0266       |
selected based on the RMSEC, RMSEP, $R^2_{\text{cal}}$, and $R^2_{\text{pred}}$ values of the PLSR model. Take the PLSR model of albiflorin for example, as shown in Table 4, the RMSEC, RMSEP, $R^2_{\text{cal}}$, and $R^2_{\text{pred}}$ value of the PLSR model based on the SNV spectra were 0.0415 mg/mL, 0.0530 mg/mL, 0.9772, and 0.9709, respectively, which was the best among all the processing methods we tested with lower RMSEC and RMSEP, and higher $R^2_{\text{cal}}$, and $R^2_{\text{pred}}$ values. The optimization of the spectral pretreatment methods for the PLSR models of gallic acid, paoniflorin, and benzoyl paoniflorin were carried out following the same rule, while the details were omitted here.

Table 4. Detailed comparison of albiflorin models with different spectral pretreatments.

| Pretreatment     | Spectrum range (cm$^{-1}$) | RMSEC (mg/mL) | RMSEP (mg/mL) | $R^2_{\text{cal}}$ | $R^2_{\text{pred}}$ |
|------------------|----------------------------|---------------|---------------|-------------------|-------------------|
| Raw              | 4000–10,000                 | 0.0499        | 0.0565        | 0.9670            | 0.9655            |
| SG15+FD          | 4000–10,000                 | 0.0774        | 0.0749        | 0.9206            | 0.9377            |
| SNV              | 4000–10,000                 | 0.0415        | 0.0530        | 0.9772            | 0.9709            |
| SG15+FD, SNV     | 4000–10,000                 | 0.0623        | 0.0682        | 0.9486            | 0.9479            |

Fig. 3. PCA score plot of raw spectra for dividing sample sets.

Fig. 4. The variable regions selected by forward iPLS for each PLSR model of the four analytes: (a) Paoniflorin; (b) Benzoyl paoniflorin; (c) Gallic acid; (d) Albiflorin.
3.4. Variable selection

In this work, interval partial least square (iPLS) algorithm was first applied to split the full NIR spectral region (4000–10,000 cm\(^{-1}\)) into different intervals; then, the optimized interval(s) was (were) achieved with the lowest RMSECV. Take the variable selection for the PLSR model of albiotin as an example, the finally subinterval selected by iPLS is shown in Fig. 4(b) highlighted in green, corresponding to 5542–6310 cm\(^{-1}\). There were 200 variables selected for the modeling of albiotin. Using the same approach, the interval(s) selected for gallic acid, paeoniflorin, and benzoyl paeoniflorin are shown in Figs. 4(a), 4(c) and 4(d), and the corresponding spectral regions are listed in Table 5. The spectral range for paeoniflorin model contains 5542.4–6309.9 cm\(^{-1}\) related to the first overtones of \(-\text{CH}_3\), and \(-\text{CH}_2\) and 8627.9–9395.3 cm\(^{-1}\) related to the second \(-\text{OH}\) overtone. The spectral range for albiotin model is 5542.4–6309.9 cm\(^{-1}\) related to the first overtones of \(-\text{CH}_3\), and \(-\text{CH}_2\) and for gallic acid model is 7470.9–8624.0 cm\(^{-1}\) related to the second C–H overtone and stretching and deformation combination. For benzoyl paeoniflorin model, the range of 7856.6–9395.3 cm\(^{-1}\) is associated with the second \(-\text{OH}\) overtone was selected.

Each calibration model has an optimal LVs number. LVs lower or greater than the optimal one introduced in the model may cause the problem of ‘under-fitting’ or ‘over-fitting’, both of which will lead to the decrease in predictability. In this research, take the PLSR model of albiotin for example, the optimal number of LVs for this model was determined using a “Venetian blinds” cross validation protocol of seven data splits as shown in Fig. 5. The model result with number 5 of LVs corresponds to the lower values of RMSEP, RMSEC and root mean square error of cross-validation (RMSECV), and the smaller difference between RMSEP and RMSEC, thus the number 5 of LVs was chosen as the optimal latent variable number for the albiotin model. The same strategy was used to determine the most suitable LVs for the models of the other three analytes, and the results are listed in Table 5.

3.5. Evaluation of the calibration models

The performance of the models was evaluated in terms of RMSEC, RMSEP, \(R^2_{\text{cal}}\), and \(R^2_{\text{pred}}\). To further evaluate the predictability of each PLS model, the standard deviation of the validation set to standard error of prediction ratio (RPD) was also calculated. A cutoff point of three was recommended by Williams and Sobering,\(^{22}\) and a higher value of RPD would be considered to have better predictive capability. Generally, a good model should have higher \(R^2_{\text{cal}}\) \(R^2_{\text{pred}}\). RPD and lower RMSEC, RMSEP, as well as small differences between RMSECV and RMSEP. Table 5 shows the performance indexes of the established models. The results shown in Fig. 6 indicate that the established models give satisfactory fitting results and predictive ability, and the respective models can be applied to monitor the concentrations of the four

| Analytes            | Pretreatment | Spectrum range (cm\(^{-1}\)) | LVs | RMSEC (mg/mL) | RMSEP (mg/mL) | \(R^2_{\text{cal}}\) | \(R^2_{\text{pred}}\) | RPD  |
|---------------------|--------------|-------------------------------|-----|---------------|----------------|---------------------|---------------------|------|
| Paeoniflorin        | SG15+FD      | 5542.4–6309.9, 8627.9–9395.3  | 6   | 0.0487        | 0.0545         | 0.9873              | 0.9855              | 8.4  |
| Albiotin            | SNV          | 5542.4–6309.9                 | 5   | 0.0303        | 0.0321         | 0.9879              | 0.9888              | 9.1  |
| Gallic acid         | Raw          | 7470.9–8624.0                 | 6   | 0.0140        | 0.0145         | 0.9696              | 0.9644              | 5.1  |
| Benzoyl paeoniflorin| SNV          | 7856.6–9395.3                 | 6   | 0.00169       | 0.00171        | 0.9794              | 0.9781              | 6.9  |

Table 5. The performance indexes of each established model.
analytes in the water extract during the water extraction process of \textit{Radix Paeoniae Alba}.

4. Conclusion

In this study, a quantitative NIR spectroscopy method was explored and established for the simultaneous determination of paeoniflorin, albiflorin, gallic acid, and benzoyl paeoniflorin in the \textit{Radix Paeoniae Alba} extract during the water extraction process of \textit{Radix Paeoniae Alba}. By means of PLSR, quantitative NIR models were successfully built between the spectra and the corresponding reference values obtained by HPLC-UV. Compared with the HPLC-UV, the NIR spectroscopy method can significantly save manpower and time, and are potentially useful for monitoring the water extraction process of \textit{Radix Paeoniae Alba}.

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References

1. W. Li, S. Huang, R. Wang, "Advances in research on pharmacological actions and quality control of Paeoniae Radix Alba," \textit{Pharm. Care Res.} \textbf{2}, 016 (2012).
2. C. H. Wang, R. Wang, X. M. Cheng, Y. Q. He, Z. T. Wang, C. Wu, J. Cao, "Comparative pharmacokinetic study of paeoniflorin after oral administration of decoction of Radix Paeoniae Rubra and Radix Paeoniae Alba in rats," \textit{J. Ethnopharmacol.} \textbf{117}, 467–472 (2008).
3. H. Ohta, J. W. Ni, K. Matsumoto, H. Watanabe, M. Shimizu, "Peony and its major constituent, paeoniflorin, improved radial maze performance impaired by scopolamine in rats," \textit{Pharmacol. Biochem. Behav.} \textbf{45}, 719–723 (1993).
4. State Pharmacopoeia Committee of People’s Republic of China, Chinese Pharmacopoeia, Chemical Industry Press, Beijing (2015).
5. K. S. Suh, E. M. Choi, Y. S. Lee, Y. S. Kim, "Protective effect of albiflorin against oxidative-stress-mediated toxicity in osteoblast-like MC3T3-E1 cells," \textit{Fitoterapia} \textbf{89}, 33–41 (2013).
6. T. Okubo, F. Nagai, T. Seto, K. Satoh, K. Ushiyama, I. Kano, "The inhibition of phenylhydroquinone-induced oxidative DNA cleavage by..."
constituents of Moutan Cortex and Paeoniae Radix,” *Biol. Pharm. Bull.* 23, 199–203 (2000).

7. G. C. Yen, P. D. Duh, H. L. Tsai, “Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid,” *Food Chemistry* 79, 307–313 (2002).

8. J. P. Fan, J. Cao, X. H. Zhang, J. Z. Huang, T. Kong, S. Tong, Z. Y. Tian, Y. L. Xie, R. Xu, J. H. Zhu, “Optimization of ionic liquid based ultrasonic assisted extraction of puerarin from Radix Puerariae Lobatae by response surface methodology,” *Food Chemistry* 135, 2299–306 (2012).

9. Q. Dong, H. Zang, A. Liu, G. Yang, C. Sun, L. Sui, P. Wang, L. Li, “Determination of molecular weight of hyaluronic acid by near-infrared spectroscopy,” *J. Pharm. Biomed. Anal.* 53, 274–278 (2010).

10. H. Huang, H. Yu, H. Xu, Y. Ying, “Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: A review,” *J. Food Eng.* 87, 303–313 (2008).

11. P. Wang, Z. Yu, “Species authentication and geographical origin discrimination of herbal medicines by near infrared spectroscopy: A review,” *J. Pharm. Anal.* 5, 277–284 (2015).

12. E. W. Ciurczak, B. Igne, *Pharmaceutical and Medical Applications of Near-Infrared Spectroscopy*, CRC Press (2014).

13. A. Rohman, A. Nugroho, E. Lukitaningsih, Sudjadi, “Application of Vibrational Spectroscopy in Combination with Chemometrics Techniques for Authentication of Herbal Medicine,” *Appl. Spectrosc. Rev.* 49, 603–613 (2014).

14. F. Wang, W. Jiang, C. Li, H. Zhang, L. Nie, L. Li, P. Wang, H. Zang, “Application of near infrared spectroscopy in monitoring the moisture content in freeze-drying process of human coagulation factor VIII,” *J. Innov. Opt. Health Sci.* 8, 1550034 (2015).

15. P. Wang, J. Sun, T. Zhang, W. Liu, “Vibrational spectroscopic approaches for the quality evaluation and authentication of virgin olive oil,” *Appl. Spectrosc. Rev.* 51, 763–790 (2016).

16. X. L. Chu, Y. P. Xu, W. Z. Lu, “Research and Application Progress of Chemometrics Methods in Near Infrared Spectroscopic Analysis,” *Chinese J. Anal. Chem.* 5, 031 (2008).

17. H. Liu, X. Zhao, T. Qi, Y. P. Qi, G. R. Fan, “Establishment of the model for online monitoring of the column separation and purification process by near-infrared spectroscopy and determination of total ginsenosides in Folium Ginseng,” *Guang pu xue yu guang pu fen xi = Guang pu 33*, 3226–3230 (2013).

18. Y. Wu, Y. Jin, H. Ding, L. Luan, Y. Chen, X. Liu, “In-line monitoring of extraction process of scutellarein from Erigeron brevicapsus (vant.) Hand-Mazz based on qualitative and quantitative uses of near-infrared spectroscopy,” *Spectrochim. Acta A, Mol. Biomol. Spectrosc.* 79, 934–939 (2011).

19. Z. Wu, C. Sui, B. Xu, L. Ai, Q. Ma, X. Shi, Y. Qiao, “Multivariate detection limits of on-line NIR model for extraction process of chlorogenic acid from Lonicera japonica,” *J. Pharm. Biomed. Anal.* 77, 16–20 (2013).

20. P. Wang, H. Zhang, H. Yang, L. Nie, H. Zang, “Rapid determination of major bioactive isoflavonoid compounds during the extraction process of kudzu (Pueraria lobata) by near-infrared transmission spectroscopy,” *Spectrochim. Acta A, Mol. Biomol. Spectrosc.* 137, 1403–1408 (2015).

21. Z. Wu, B. Xu, M. Du, C. Sui, X. Shi, Y. Qiao, “Validation of a NIR quantification method for the determination of chlorogenic acid in Lonicera japonica solution in ethanol precipitation process,” *J. Pharm. Biomed. Anal.* 62, 1–6 (2012).

22. P. C. Williams, D. C. Sobering, How do we do it: A brief summary of the methods we use in developing near infrared calibrations, *Near Infrared Spectroscopy: The Future Waves*, 185–188 (1996).