Rapid analysis of Fructus forsythiae by near-infrared spectroscopy

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***Abstract*—In this paper, Near-infrared spectroscopy (NIRS) was introduced into the field of Chinese herbal medicines and a rapid analytical method which can not only differentiate the two species of *Fructus forsythiae*, QingQiao (QQ) and LaoQiao (LQ), but also determine the contents of Phillyrin, Forsythoside A and Moisture in *Fructus forsythia* was established. The total content of volatile oil in Fructus forsythia was used as an important parameter for discrimination of QQ and LQ. Two lignans components , Phillyrin and Forsythoside A were analyzed successively by high-performance liquid chromatography (HPLC). Scattering effect and baseline shift in the NIR spectra were corrected by several pre-processing methods. By using discriminant analysis, a model which can be used to identify the species of *Fructus forsythiae* was established and samples were separated successfully into two different clusters corresponding to QQ and LQ. Finally, partial least squares (PLS) regression was used to build the correlation model. The results showed that the correlation coefficients of the calibration models are R = 0.959 for the Moisture, R = 0.957 for Phillyrin and R = 0.960 for Forsythoside A. The outcome showed that NIRS can provide a simple and accurate way in the quality control of Chinese herbal medicine (CHM).**

Keywords- Fructus forsythiae, discriminant analysis, Phillyrin, Forsythoside A, Near-infrared spectroscopy

I. INTRODUCTION

Fructus forsythiae, a Chinese herbal medicine known as 'Lianqiao', refers to the dry fruit of the plant Forsythia suspensa（Thunb.）Vahl.[1] It's used commonly in Chinese medicine treatment and has the pharmacological efficacy in antibacterial, cardiotonic, diuretic, and antiemetic, ect.[2–5] According to different plucking time, *Fructus forsythiae* could be divided into two species—Qingqiao and Laoqiao. [1] In autumn, when the fruit is still green and begins to be ripe, it will be picked, removed the impurities, braised and dried up, then the fruit is called "Qingqiao" (QQ); In winter, when the fruit is ripe and the seeds fall off, it will be picked, removed the impurities, then the fruit is called "Laoqiao" (LQ).

The main chemical compositions of *Fructus forsythiae* are Volatile Constituents、Lignans compounds, such as phillyrin, Forsythoside A etc and Triterpene Acids, such as Oleanolic Acid, Ursolic Acid etc. Volatile Constituents are the major components which have the pharmacological efficacy in Antibacterial and antiviral.[6] Phillyrin and Forsythoside A are officially defined to be the target components to control the Quality of *Fructus forsythiae*. To determine the chemical compositions contents of *Fructus forsythiae*, conventional methods such as colorimetry, thin layer chromatography, and high performance liquid chromatography (HPLC) are often used. [7-9] But these methods are not only time-consuming, where complicated pretreatment and long analysis time was required, but also the limited by depending on one or several target components for the quality of *Fructus forsythiae*. Given the herbal medicines, especially the compound prescription, which is a complicated system of mixture,[10] the existing methods can't reflect the overall and internal quality information of *Fructus forsythiae*.

Near infrared spectroscopy (NIRS) has several advantages as an analytical method, including convenience of operation, rapid detecting speed, higher efficiency, lower cost, less pollution, and no complicated preparation procedures.[11-12] In recent years, NIR has been widely applied in the fields of agriculture, food, petroleum, and biochemistry.[13] Also, it has been applied successfully in the qualification analysis in herbal medicine.[14–16] In this paper, NIR method was used as an analysis tool to distinguish the species of *Fructus forsythiae* and determine the contents of Moisture, Phillyrin and Forsythoside A.

II. MATERIALS AND METHODS

A. Samples and sample pre-treatment

Ninety-eight QQ and seventy-six LQ were collected from Fu-niu Mountains in China. All of the samples were identified by professor S.-Q. Chen (Department of Pharmacognosy, Henan College of Traditional Chinese Medicine, Zhengzhou, China). After being dried up, all the samples were crushed into powders that can pass through a 40-mesh (0.45mm) sieve. Then, the powers were filled into plastic bags separately and stored in the dry container for analysis.

B. Chemical analysis

The content of moisture and total volatile oil were determined by the methods indexed by Chinese Pharmacopoeia (2010 edition)1.

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Phillyrin and Forsythoside A were purchased from The National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Their purities were determined as 98%, 97% respectively by quantitative H-NMR. HPLC grade acetonitrile and methanol were purchased from Tedia (USA). Water was purified by a Milli-Q academic water purification system (Milford, MA, USA). Other reagents were analytical grade.

C. Near-infrared spectroscopic analysis

All samples were scanned and recorded by Nicolet 6700 FT-NIR Systems (Thermo Fisher, USA) equipped with an InGaAs detector. The spectra were collected in the diffuse reflectance mode with a gold foil reference standard over the spectral region of 12000-4000 cm^{-1} with a resolution of $8cm^{-1}$. The spectra were determined with a circular sample cup. Each spectrum is the average of 64 scans and the measurement was repeated three times.

A discriminant model which can differentiate QQ and LQ was established by using discriminant analysis (DA) combining with Principal component analysis (PCA). And a multiple quantitative regression model was established using Partial Least Squares(PLS). 65% of the samples were used for calibration set while 20% and 15% of the samples were used for validation sets, respectively. PLS regression was used to establish the correction model and the number of principal components factors used in the PLS regression is determined by minimizing predicted residual error sum square (PRESS) value. A VISION (8.0.1.24), TQ Nir systems, MATLAB (version 7.0) were used for the data analysis.

III. Results and discussion

A. Chemical analysis of Fructus forsythiae

Fig. 1 shows typical chromatograms of the standard solution of Phillyrin, Forsythoside A and the extracts of *Fructus forsythiae*. The presence of Phillyrin and Forsythoside A was confirmed by comparing the retention time and UV spectrum of the corresponding peak with those of the standards.

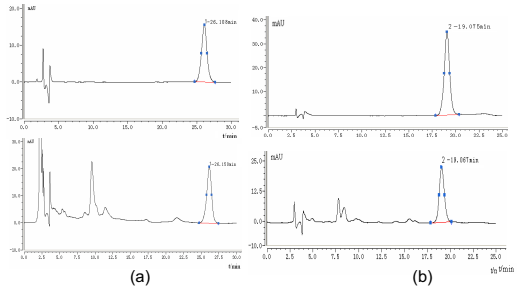

Fig. 1 HPLC chromatograms of *Fructus forsythiae* (1- Phillyrin; 2- Forsythoside A)

(a) HPLC chromatograms of Phillyrin and the extracts of *Fructus forsythiae* in 277 nm

(b) HPLC chromatograms of Forsythoside A and the extracts of *Fructus forsythiae* in 332 nm

The results of chemical analysis and method validation were tabulated in Table 1. The calibration curves of the two standards exhibited good linearity ($r^2 > 0.9999$) within the test range. Also, the analytical method had good accuracy with recovery 97.44 for Phillyrin and 97.8 for Forsythoside A. The variabilities of Phillyrin and Forsythoside A contents in *Fructus forsythiae* were less than 3%.

TABLE 1 Results of the contents of Phillyrin, Forsythoside A, moisture, total volatile oil in QQ and LQ and HPLC method validations

Phillyrin and Forsythoside A in all the samples of *Fructus forsythiae* were analyzed by the developed HPLC method. The results showed that the contents of Phillyrin and Forsythoside A in QQ were nearly twice than that in LQ, the

Components	Content range of QQ	Content range of LQ	Mean content of QQ	Mean content of LQ	r2	RSD% of Precision (n = 5)	Recovery (%) (n = 5)
Phillyrin	0.103-0.407%	0.069-0.278 %	0.267%	0.143%	0.9999	1.61	97.44±2.23
Forsythoside A	1.146-5.121%	0.558-1.914 %	2.408%	1.094%	0.9999	1.95	97.87±1.89
moisture	3.81-9.57%	6.1-11.4%	6.35%	9.19%			
total volatile oil	1.38-3.31ml/mg	0.20-0.61%	2.09%	0.38%			

contents of total volatile oil in QQ was about 2% but little in LQ.

B. Discriminant analysis of Fructus forsythiae by NIRS

Fig. 2a shows the raw NIR spectra of QQ and LQ and visual inspection suggested these two species are so similar that can't be identified by their raw spectra. The most intensive band is contributed by combination bands, such as C–H stretching and deformation vibration in CH_3 (4400 cm^{-1}), C–H deformation vibration in CH_2 (4310 cm^{-1}), and O–H stretching and deformation vibration in CH_3 (4820 cm^{-1}). These vibrational modes are present in Phillyrin and Forsythoside A as well as other constituents such as moisture and total volatile oil.

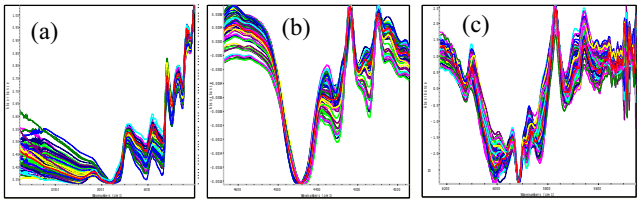

Fig. 2 NIR spectra of *Fructus forsythiae*
(a) Raw spectrum in the range of 4000-12000cm^{-1}
(b) Second derivative spectra in the range of 4200–4600cm^{-1}
(c) Second derivative spectra in the range of 5500–6200cm^{-1}

Fig. 2 shows the NIR spectrum of QQ and LQ, the second derivative spectrum in 4200–4600 cm^{-1} and 5500–6200 cm^{-1} where the differences between QQ and LQ are most significant. Discriminant analysis models using all the spectra and the given regions (4200–4600, 5500–6200cm^{-1}) were established and the results are shown in Table 2. The accuracy for the prediction of the model using original spectrum is obviously less than that using second derivative. This proved

that the pre-treatment is crucial in establishing a practical models.

TABLE 2 Results of the discrimination of QQ and LQ using different pre-processing methods

pre-processing method	Factor	Successful rate (calibration)	Successful rate (validation)
4100–10000 cm^{-1}	1	75.7%	79.5%
4100–10000 $cm^{-1}$with 2nd derivative	2	83.1%	86.4%
5500–6200 cm^{-1} with 2nd derivative	2	85.7%	88.6%
4200–4600cm^{-1} with 2nd derivative	2	86.4%	87.3%
4200–4600, 5500–6200cm^{-1} with 2nd derivative	2	88.9%	89.6%
4200–4600, 5500–6200cm^{-1} with 2nd derivative	**3**	**94.8%**	**93.1%**

The models established shows a clear classification between QQ and LQ. The successful identification may be contained in the total volatile oil content from QQ and LQ.

C. Quantification of moisture, Phillyrin and Forsythoside A content with PLS algorithm

To build a reliable PLS models, all averaged sample spectra including two *Fructus forsythiae* species were divided into calibration and validation sets. One hundred and thirty samples were used in the calibration set and the remaining forty-four samples were divided into validation.

Quantitation models for moisture, Phillyrin and Forsythoside A contents were established by combining NIR spectram and PLS method. The spectral pre-treatment and the number of PLS factors are all important parameters. The number of PLS factors used in the regression is determined by minimizing predicted residual error sum square (PRESS) value. Using more PLS factors in the model may fit the calibration set better, but rupture the predictions of other samples. This is the phenomenon of 'overfitting' and it can result in the poorer prediction for the other samples that in the test set. [17]

Table 3 lists the RMSECV, RMSEC, RMSEP and R for quantitation models of Phillyrin, Forsythoside A and moisture established by different data pre-treatment. For Phillyrin, the MSC and first derivative of the NIR spectra (4500–9750 cm^{-1}) was used. For Forsythoside A, the first derivative of the NIR spectra(4500–9500 cm^{-1}) was used. For moisture, the model using SNV, first derivative of the whole NIR spectra(5000–8000 cm^{-1}) gives the lowest RMSECV value.

Fig. 3 shows that the correlation of the predicted values obtained by NIR methods and the stand values determined by chemical analysis methods. The circle and cross represent the calibration and validation data points. RMSECV (root-mean-square error of cross-validation) of the model for Phillyrin, Forsythoside A and Moisture content are 0.371, 0.306 and 0.541. The correlation coefficients of the calibration model for Phillyrin, Forsythoside A and Moisture content are 0.957, 0.960 and 0.959. RMSEP of the models for Phillyrin, Forsythoside A and Moisture content are 0.290, 0.223 and 0.511. The R values of the validation sets of the Phillyrin, Forsythoside A and Moisture content models are 0.951, 0.958 and 0.947, respectively. It shows that the NIR predicted values have no significant difference with the values determined by chemical analysis methods.

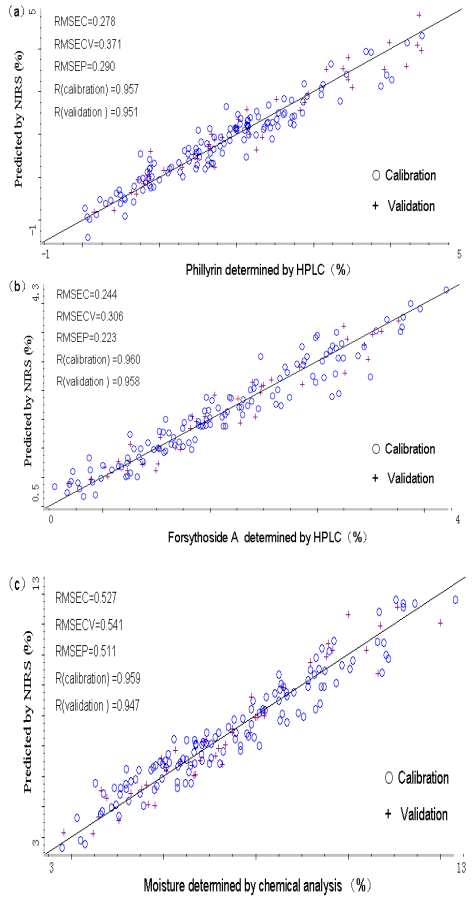

Figure 3. NIRS predicted values vs. chemical analysis measurement for the content of (a) Phillyrin, (b) Forsythoside A and (c) moisture in *Fructus forsythia*

TABLE 3 Results of models for (a) Phillyrin, (b) Forsythoside A and (c) moisture content

Pre-treatments	Factor	RMSEC	RMSECV	RMSEP	R(calibration)	R(validation)
(a) Model for Phillyrin content						
No treatment	7	0.402	0.470	0.426	0.907	0.893
MSC	7	0.385	0.465	0.403	0.920	0.913
SNV	8	0.405	0.474	0.467	0.910	0.897
SNV, First Derivative	8	0.308	0.416	0.350	0.947	0.935
MSC, First Derivative	**7**	**0.278**	**0.371**	**0.290**	**0.957**	**0.951**
(b) Model for Forsythoside A content						
No treatment	8	0.336	0.391	0.323	0.923	0.908
MSC	10	0.318	0.404	0.308	0.931	0.923
First Derivative	**7**	**0.244**	**0.306**	**0.223**	**0.960**	**0.958**
SNV, First Derivative	7	0.281	0.374	0.297	0.947	0.932
MSC, First Derivative	7	0.282	0.398	0.278	0.946	0.938
(c) Model for moisture content						
No treatment	6	0.597	0.640	0.601	0.947	0.941
MSC	6	0.536	0.571	0.566	0.958	0.943
First Derivative	7	0.539	0.614	0.585	0.944	0.938
SNV, First Derivative	**5**	**0.527**	**0.541**	**0.511**	**0.959**	**0.947**
MSC, First Derivative	5	0.657	0.694	0.612	0.936	0.925

D. Conclusion

The results confirms that it's feasible to apply NIRS into the quality control of *Fructus forsythia*. It can be used not only in the identification the species of QQ and LQ, but also in the determination of the contents of Moisture, Phillyrin and Forsythoside A. Compared with the conventional chemical analysis methods, NIRS is simple, fast, no-pollution, no-destructive and can be used in the on-line monitor of the production process of CHM.

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