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Simultaneous Determination of Ginsenosides, Ginsenosides, Notoginsenosides and Panaxtrol Saponin of *Panax notoginseng* by Near Infrared Spectroscopy

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The object of this research was to develop a method for rapid and simultaneous determination of the ginsenosides (Rg_1), ginsenosides (Re), notoginsenosides (R_1) and panaxtrol saponin of *Panax notoginseng* by near infrared spectroscopy. Calibration models are generated by performing partial least-squares regression and optimized individually by considering spectral range, spectral pretreatment methods and number of model factors. Calibration models were developed for Rg_1, Re, R_1 and panaxtrol saponin with root mean square errors of cross-validation (RMSECV) of 0.157, 0.022, 0.0546 and 0.213, and the correlation coefficient (R^2) of 89.33, 91.10, 96.12 and 91.64 %, respectively. The established model was validated and showed it was fast, non-destructive and accurate. This method provides a new efficient approach for determining the active components in the complex system of *Panax notoginseng*.

Keywords: Near infrared, *Panax notoginseng*, Rapid and simultaneous determination.

INTRODUCTION

Panax notoginseng, also called Sanchi, is an important medicinal plant, which has been cultivated in Yunnan and Guangxi provinces in the southwest of China for its remarkable and valuable hemostatic effect. It has been used as a health tonic and a healing drug in the east countries of Asia such as China, Korea and Japan for hundreds of years. The herb possesses anti-hypertensive, anti-thrombotic, anti-atherosclerotic, hepatoprotective, and neuroprotective activities[1], and is a major component of common household medicines, such as the Yunnan Paiyao powder and the compound danshen dripping pill. The principal active components of *Panax notoginseng* are ginsenosides and notoginsenosides, including panaxadiol, panaxatriol and oleanolic acid type saponins, present either as aglycones or in glycosylated forms[2,3]. Now, most quality controls about *Panax notoginseng* are limited to the identification and determination of the following six major ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1[2,3]. Traditional analytical methods include HPLC, TLC, and colourimetry. However, most of the methods are based on complex processing of samples, use of expensive chemicals, besides involving a considerable amount of manual work. In addition, these methods are destructive. Therefore, there is a need for fast, non-destructive techniques for the assessment of medicinal herbs internal quality.

As defined by the American Society of Testing and Materials, the near infrared region of the electromagnetic spectrum spans wavelengths ranging from 780 to 2526 nm. The most prominent absorption bands of near infrared are related to the overtones and combinations of fundamental vibrations exhibited by -CH, -NH, -OH and -SH functional groups[4,5]. Near infrared is a fast, non-destructive, environment friendly technique widely used in the pharmaceutical industry. And it requires little or no sample preparation and is both flexible and versatile, *i.e.*, it is applicable to multiproduct and multicomponent analysis. Near infrared spectra also allows testing of raw material and end products, and simultaneous measurement of several analytical parameters as well. Furthermore, near infrared spectra generates no waste, is less expensive to run than conventional methods, since a single instrument can be used for a wide range of medicinal herbs species and parameters, and can be built into the processing line, enabling large-scale individual analysis and real-time decision making[6,7].

In this paper, we use near infrared to establish prediction models of ginsenosides (Rg_1), ginsenosides (Re), notoginsenosides (R_1) and panaxtrol saponin in *Panax notoginseng*. High performance liquid chromatographic (HPLC) methods were developed for the determination of 126 types of *Panax notoginseng* and were used as reference methods for near-infrared (NIR) spectroscopy. On the above foundation, calibration models were generated by performing partial least-

squares (PLS) regression and optimized individually by considering spectral range, spectral pretreatment methods and number of model factors. The established model was validated and showed that it was fast, non-destructive and accurate. This method provides a new efficient approach for determining the active components in the complex system of *Panax notoginseng*.

EXPERIMENTAL

Samples and reagents: One hundred and twenty six types of *Panax notoginseng* were collected from Wenshan of Yunan Province. All samples were first milled into powder and then passed through a 100-mesh sieve. To ensure that moisture was not an interfering factor, all samples were dried for at least 7 h in a silica gel desiccator at room temperature until the weight loss was less than 0.0003 g. Rg_1, Re and R_1 standards were from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). HPLC-grade acetonitrile was obtained from Tianjin Kermel Chemical Reagent Company (Tianjing, PR China). Water was purified by an ultrapure water instrument. All other reagents were of analytical grade.

Data collection: Near infrared spectra of *Panax notoginseng* powder samples were recorded using a Bruker Matrix-I FT-NIR spectrometer (Bruker Optik, Ettlingen, Germany) equipped with a PbS detector, sample cup and rotary tables. The system was operated by OPUS spectral acquisition and processing software (Bruker Optik, Ettlingen, Germany). The spectra were obtained at a resolution of 8 cm^{-1} over a wavelength range of 12000-4000 cm^{-1} with 64 scans per spectrum, and air absorbance was recorded as the reference standard.

High performance liquid chromatography: About 0.3 g sample of the *Panax notoginseng* powder was extracted by ultrasonic extraction for 0.5 h in 15 mL mobile phase of HPLC, and then was diluted to 25 mL. A 1260 HPLC system (Agilent Technologies Inc., USA) consisting of UV-visible detector was used to separate and analyze 10 µL sample injections at a wavelength of 210 nm over a Phenomen C18 column (250 × 4.6 mm, 5 µm). The mobile phase was composed of acetonitrile-water (20:80). Chromatographic peaks were identified by comparing their retention time against the known standards. The concentration of every sample was shown in Fig. 1.

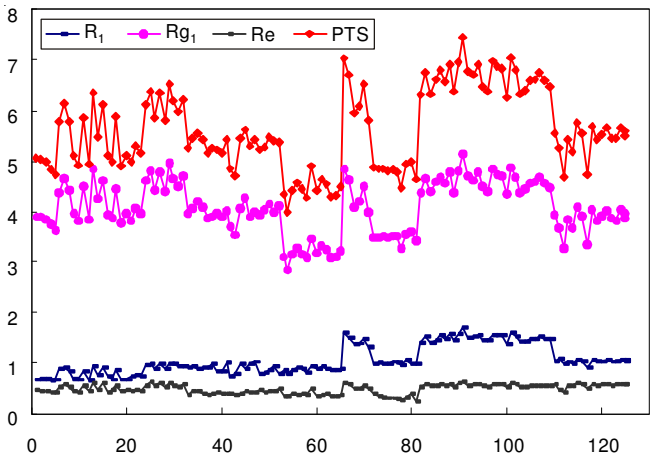

Fig. 1. Concentration of 126 samples

Data processing: Intensity of the measurements at different wavenumbers can be correlated to the concentrations of the relevant components in the sample through a series of mathematical procedures. These processes involve multivariate statistical calculations such as multiple linear regression (MLR), principal component regression (PCR), the partial least squares (PLS), the artificial neural networks (ANN) and so on. partial least-squares was the most frequently used in these methods. Therefore the near infrared spectroscopic calibration models were constructed respectively by using partial least-squares with the OPUS software[4,8].

RESULTS AND DISCUSSION

Spectral features and sample set selection: Fig. 2 shows the original near infrared spectra of 126 samples of *Panax notoginseng*. Among these samples, 20 samples were selected randomly for the validation set and the remaining 106 samples were for the calibration set.

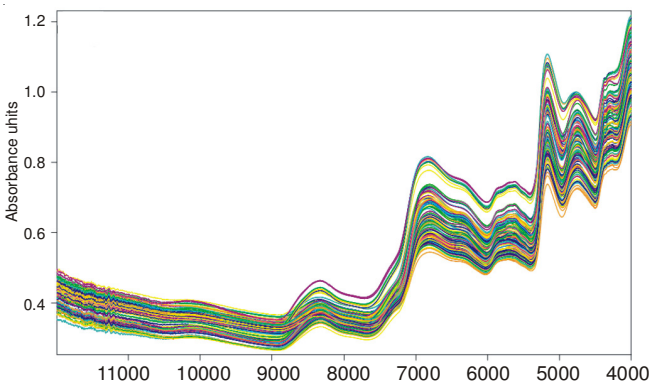

Fig. 2. NIR spectrum of the 126 samples of *Panax notoginseng*

Spectral pretreatment methods: To develop a robust model, different spectral pretreatment methods are often utilized to eliminate noise, baseline shift and matrix background interference and enhance the spectral features to extract the relevant information before partial least-squares modeling. The OPUS software provides eleven kinds of important spectral pretreatment methods, including: Untreated, Constant offset elimination (COE), Straight line subtraction (SLS), Vector normalization (VN), Min max normalization (MMN), Multiplicative scatter correction (MSC), First derivative, Second derivative, First derivative + SLS, First derivative + VN and First derivative + MSC.

In the spectral pretreatment process, the partial least-squares model is validated by cross-validation to assess the average predictive ability. The cross validation method is a resampling technique based on 'leaving one sample out' (LOO) procedure. This means that the calibration is performed N times (N is the number of samples in the calibration data set), each time leaving one sample out and testing the calibration equation on this single sample. The partial least-squares methods must be computed for each number of components and the optimum number of factors for calibration was selected basing on the correlation coefficients of the calibration set (R_{cal}) and the root mean square errors of cross-validation (RMSECV). The model with the best prediction ability is usually selected by computing

TABLE-1
OPTIMIZED PARAMETERS USED BY THE PLS MODEL AND CALIBRATION AND VALIDATION RESULTS FOR ESTIMATION BY NIR SPECTROSCOPY

	Spectral pretreatment method	Factor	Spectrum region (cm^{-1})	R_{cal} (%)	RMSECV	RMSEP	R_{val} (%)
Ginsenosides (Rg_1)	Min max normalization	7	6101.9-4597.6	89.33	0.157	0.302	86.32
Ginsenosides (Re)	First derivative + MSC	4	6101.9-5446.2 4601.5-4246.7	91.10	0.022	0.032	91.11
Notoginsenosides (R_1)	Vector normalization	11	6101.9-4597.6	96.12	0.055	0.074	97.22
Panaxtrol saponin (PTS)	First derivative + VN	8	6101.9-4246.7	91.64	0.213	0.321	93.79

the root mean square error of validation (RMSEP) and the correlation coefficients of the validation set (R_{val})[4,6,9].

The OPUS could give the value of the R, RMSECV and RMSEP, and then the best models were chosen according to these evaluation parameters, anyhow, the calibration models with highest R (both in calibration and validation) as well as lowest RMSECV and RMSEP were considered optimal.

According to the above criteria, the best calibration models were generated based on Min max normalization (MMN) for Rg_1, First Derivative + MSC for Re, Vector Normalization (VN) for R_1, and First Derivative + VN for panaxtrol saponin as shown in Table-1.

Spectral region and factors: In the complex system, it was difficult to use a classical univariate calibration method for quantitative analysis. In addition, one or several wavelength (s) related to obtain content could not be found because of the interferences of the other components on the near infrared, so the spectral region rich in chemical information was used usually to establish the calibration model. In the same spectral region and spectral pretreatment method, the number of partial least-squares factors (F) would directly affect R, RMSECV and RMSEP. Not enough information would be obtained from the spectrum when F is too small, and the actual information is misrepresented or "over fitted" when F is too big.

Partial least squares (PLS) method of the OPUS is a powerful multivariate calibration method in recommending the appropriate spectral region and the ideal F according to the criteria of the best calibration. Table-1 showed the appropriate spectral region for the near infrared models, respectively.

With the partial least-squares (PLS) regression, the calibration models were optimized individually by considering spectral range, spectral pretreatment methods and number of model factors. The values of the R, RMSECV and RMSEP for the best calibration models were shown in Table-1.

Compared to the reference values determined by HPLC, the T and F tests showed the predicted result of the validation set was satisfactory with a significant level of 0.05 by SPSS 12.0.

Conclusion

In this study, the near infrared spectroscopy provided robust, accurate, repeatable and rapid analysis for Rg_1, Re, R_1 and panaxtrol saponin in *Panax notoginseng*. Compared to the reference method, the analysis time of simultaneous determination for Rg_1, Re, R_1 and panaxtrol saponin of every sample was reduced to less than 1 min by near infrared from 180 min by HPLC, and near infrared method is an environment friendly technique and requires little or no sample preparation. The near infrared method provides a new efficient approach for determining the active components in the complex system of *Panax notoginseng*.

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