Rule of Proportion in Active Ingredient and Rapid Determination multi-components in Polygonum Perfoliatum L.

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Keywords: NIR；climate；polyphenol；tannins；flavone

Abstract. A set of rapid near infrared spectroscopy analysis methods were developed for the content determination of *Polygonum perfoliatum* L.. Investigate the relation between climate and proportion of the active ingredient content. Models were established with partial least squares (PLS) algorithm. The correlation coefficients between predicted value and measured value of the three calibration models are above 0.9 which indicated that there have no differences between NIR and UV in content determination. NIR has a broad prospect of application in the quality control of Chinese traditional medicine. Proportion of polyphenol and flavone is from 1.71 to 2.26. Combined with the local climate analysis shows: Tropical or subtropical humid monsoon climate conducive to the accumulation of polyphenol ingredient, large temperature difference between day and night conducive to the accumulation of flavone ingredient. The result can provide reference for the cropping of *Polygonum perfoliatum*L..

Introduction

Polygonum perfoliatum L. (PPL) is the aerial parts of the polygonaceae plant which was recorded in "all diseases rejuvenation" written by Tingxian Gong in Ming Dynasty(1587)[1]. Its nature and flavors are plain, sour and bitter. It can induce diuresis for removing edema, cleanup heat detoxifcation, relieve a cough. Modern pharmacological studies have shown that PPL have activities in anti-inflammatory and anti-tumor effect. Owing to its curative effects, it had been recorded in the 2010 and 2015 edition of the Chinese pharmacopoeia (ChP) as a new variety which is payed extensive attentionin in recent years [2].

PPL is widely distributed in China. Accordingly, the quality of the herbal raw materials fluctuates, owing to varying ecological environments [3-5]. For the efficacy of Traditional Chinese Medicine (TCM) are based on a certain ratio of multi-component, so study the ratio of active components and its relationship with climate are very important[6,7].

Near-infrared spectroscopy (NIR) technology combined with chemometric can directly analysis of multi-components in a few seconds no matter the opaque samples are granulated, paste or solid and needn't pretreated of the sample. AS a high-speed, non-destructive technique, it has showed vast prospect in TCM research [8-12]. This study aimed to establish a fast determination of multi-component method by NIR.

Materials and Methods

Equipment and Materials

NIR analyzer (Nicolet 6700, Thermo Scientific) with an InGaAs detector was used for NIR spectra collection and analysis; UV-visible spectrophotometer (UV 1200, Shanghai Precision & Scientific Instrument Co., Ltd.) was used to detect the content of PPL; gallic acid (110831-200302,

The National Institute for the Control of Pharmaceutical and Biological Products) and rutin (100080-200707, NICPBP) as reference substances. PPL samples (n = 94) from 12 provinces and 22 locations in China were collected. All samples were identified by Dr. Zhexiong Jin (Commercial University of Harbin).

Sample preparation

The samples were milled into powder with a grinder. The final powder samples were prepared by passing the ground powder through a 50-mesh sieve. To ensure that moisture was not an interfering factor, all samples were dried in an oven at 40°C for 12 h, placed in the dryer alternate.

NIR spectra collection

5g powders were put in quartz sample cell. The NIR diffuse reflection spectra were obtained from 10,000 to 4000 cm^{-1} at $4cm^{-1}$ intervals. Each sample was scanned 64 times, and the resolution was 8 cm^{-1}. The environmental temperature was controlled at 20-22°C and the relative humidity was 25-30%. In order to reduce operating error, each sample was scanned three times with air as reference.

Content detection

Samples (2 g) were reflex-extracted in a water bath with 50 ml of mixed solvent (70:30 [v/v] methanol and water) for 0.5 h. After filtration, samples were placed in a 250 ml brown volumetric flask and diluted with water to the mark. Folin-Ciocalteu colorimetry was used to determine total polyphenols at 760 nm by using gallic acid as a reference substance; Phosphomolybdic acid - casein colorimetric method was used to determine tannin by the same conditions as polyphenols detection; $AlCl_3$ colorimetry was used to determine total flavones at 413 nm by using rutin as a reference substance.

Quantitative calibration model

The 94 samples were randomly divided into calibration and validation sets, which were designed to examine the extrapolation performance. Calibration models were established in full spectrum band by using PLS. Multiplicative Signal Correction (MSC)、Standard Normal Variate（SNV）、first-derivative (FD)、second-derivative (SD)、Savitzky-Golay (SG)、Norris derivative (ND) were used in spectral preprocessing to reduce the effects of systematic noise, baseline variation, light scattering, and path length differences. Spectral pretreatment and chemometric analyses were implemented using the TQ analyst (V7.2, Thermo Scientific) and Matlab (V7.0, Math Works, Natick, MA, USA). Root Mean Square Error of Calibration (RMSEC) 、Root Mean Square Error of Cross-Validation (RMSECV)、correlation coefficient (R) and factor number were used to optimize the models. Root Mean Square Error of Prediction (RMSEP)、R and small differences between and performance index were used to examine the accuracy of the models.

Results and Discussion

Content analysis

Using the UV method described in Section 1.4, standard curve was drawnall and 94 samples were analyzed three times. The concentration of gallic acid had a good linear relationship between 0.001-0.006 mg/ml, the regression equation was C=0.009A-0.001, r=0.999; The concentration of rutin had a good linear relationship between 0.007-0.025 mg/ml, the regression equation was C=0.0302A-0.001, r=0.998. The content range of samples from different provinces are listed in Table 1. We found that samples from different provinces have obvious differences. For example, samples from Henan province had higher polyphenol, the average value was 44.12 mg/g, and however the average value of polyphenol was only 23.9 mg/g in Yunnan province. For the efficacy of TCM is based on a relatively fixed proportional relationship between the components, we can not find more discipline between the averages values of active components unless the ratios of them were calculate. Tannins is belong to macromolecular polyphenol, therefore, when calculating proportional relationship involves only polyphenol and flavone. The values are shown in Table 1.

Table 1 Proportion of Active Ingredient of PPL from different province

province	Content of polyphenol(mg/g)		Content of flavone (mg/g)		polyphenol:flavone
	range	average	range	average	
Guangxi	23.12-42.08	33.71	4.81-21.26	15.68	2.15
Guangdong	23.55-61.42	37.95	9.13-26.8	16.36	2.26
Henan	30.59-70.67	44.12	14.7-30.95	23.56	1.87
Fujian	20.28-77.81	35.48	6.47-26.37	16.28	2.18
Zhejiang	15.41-53.03	32.82	13.51-21.53	17.68	1.85
Sichuan	23.68-42.58	31.42	13.56-14.18	13.89	2.26
Hubei	20.24-46.18	38.21	15.89-27.04	21.46	1.76
Jiangxi	17.64-61.1	40.15	11.13-30.87	23.45	1.73
Hunan	18.44-41.94	30.19	13.21-22.14	17.69	1.71

The proportion ranges of polyphenol: flavone are between 2.15-2.26 in Guangdong, Guangxi, Fujian, Guizhou and Sichuan whose climate are belong to the tropical or subtropical humid monsoon climate. The climate character of these five provinces is having abundant annual rainfall compared with the others.

Yunnan's climate is plateau subtropical or tropical monsoon, the proportion only 1.71 can be classified as a class with Henan, Zhejiang, Hubei, Jiangxi and Hunan province. Compared to the above, these areas are almost having bigger temperature difference between day and night and less rainfall.

It can be concluded that abundant rainfall is conducive to polyphenols accumulation; large temperature difference is benefit for flavones generating.

Average near-infrared spectrum of PPL

Average near-infrared spectrums are shown in chart 1. It is showed that there have big absorbance values and rich information in 4000 - 7000 cm^{-1}; equipment noise are generated in 12000~10000cm^{-1} where the signal are larger; O-H band's combination frequency of stretching vibration and bending vibration is near 5000cm^{-1}, one grade frequency doubling appear in round 7000cm^{-1}, two grade frequency doubling arise about 10500cm^{-1}; C-H band's combination frequency of stretching vibration and bending vibration is near 4347cm^{-1}, one grade frequency doubling appear in round 5700cm^{-1}, two grade frequency doubling arise in about 8700 cm^{-1}. Chemometric analyses are needed because the spectrums are overlapped seriously.

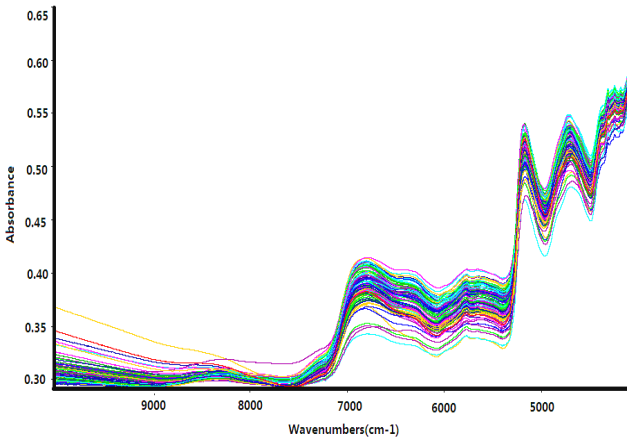

Figure 1 NIR Spectrum of PPL.

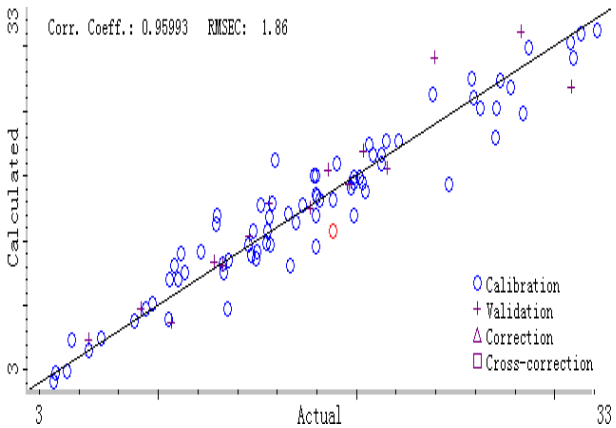

Figure 2 Correlation of predicted values with the reference values

Division of calibration and validation sets

The 94 samples were randomly divided into calibration and validation sets. The more sample number and wider coverage of content in Calibration, the stronger applicability of model are building. The statistical values for the excluded sample、calibration and validation sets are listed in Table 2.

T able 2 Distribution reference values of correct set, validation set, and excluded sample

Active ingredients	Calibration sets		Validation sets		Number of excluded samples
	Number of samples	Range of content (mg/g)	Number of samples	Range of content (mg/g)	
polyphenol	73	15.41-77.81	18	17.64～53.03	3
flavone	77	4.81～30.95	14	6.47～22.14	3
Tanins	75	5.21～54.29	14	11.6～34.36	5

Spectral pretreatment methods and wavelength range selection

Near infrared spectroscopy consists of frequency doubling and combination band of molecular vibration spectrum, but frequency doubling and combination band of molecular vibration spectrum in NIR zone is weak, band is complex and overlay. So spectral pretreatment must be used to reduce the effects of systematic noise, baseline variation, light scattering, and path length differences.

A good NIR calibration model should have a low RMSEC and RMSEP, high R, and small differences between RMSECV and RMSEP. In the spectral preprocessing, FD, SD, MSC, SNV, SG, and ND were compared to optimize the models. Optimized content prediction model parameters of tannins, polyphenols, and flavones are showned in table 3.

Table 3 Content prediction model parameters of tannins, polyphenols, flavones

Active ingredients	pretreatment methods	wavelength range (cm^{-1})	R^2	RMSEC	RMSEP	Factor	RMSECV	RMSECV /RMSEP
polyphenols	SNV+2ndDer +SG	4000-9500	0.95	4.49	4.87	5	7.23	1.48
Tanins	SNV+2ndDer +SG	4192-10000	0.94	2.94	3.06	5	5.60	1.83
flavones	1st Der+SG	4000-8000	0.96	1.86	2.15	11	3.81	1.77

Three models have better prediction accuracy, because R>0.94, RMSECV / RMSEP<1.83

Calibration model building

For the natural products used in TCM, which have a complex composition, PLS is a typically used qualitative analysis method. For example in flavones, Figure 2 shows correlation of predicted values with the reference values in the calibration sets have been interactive verified. The figure shows: calibration set samples are uniformly dispersed in both sides of the return line. Figure 3 is deviation of NIR predicted value with the reference value, from it we can see that deviation of NIR measuring method and UV method are between ± 6%. Figure 4 is correlation of RMSECV with principal components. Cumulative information of 11 main components can represented the 88.68% of the original spectral and RMSECV minimum, so the best factor is 11. Polyphenols and Tanins are unstable, so the deviations are bigger than flavones reached±10%， ±7%; Factors all are 5.

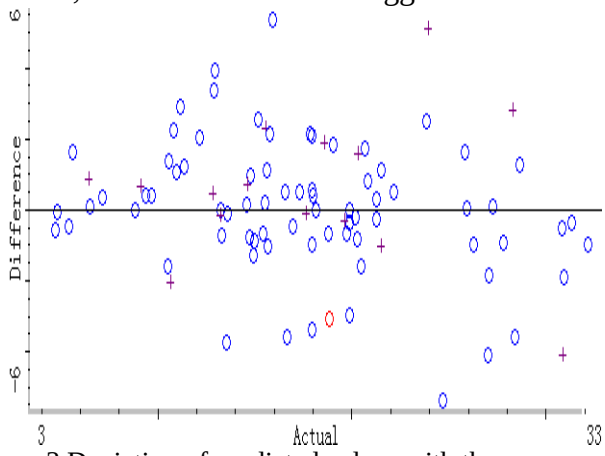

Figure 3 Deviation of predicted values with the reference values

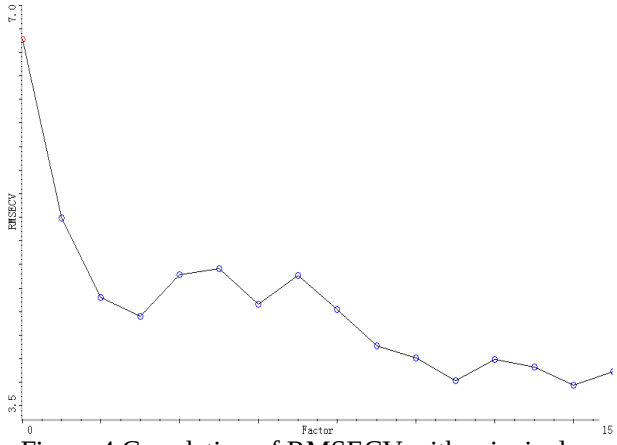

Figure 4 Correlation of RMSECV with principal components.

Verification of the calibration model

In order to validate the accuracy of the established calibration model, the sample content in the prediction set was determined with the NIR and UV method, and evaluated with the paired two sample *t*-test. In the 95% confidence interval, the polyphenols、tanins and flavones had statistical *t* values less than the two-tail critical value. The two-tail p-values were greater than 0.05, indicating that there was no significant difference between the NIR and UV method. The calibration model passed verification, can be used to detect the content of polyphenols、tanins and flavones in PPL.

Conclusions

Modernization of TCM needs detect fast and simple method of operation. In this study, the quantitative model of Near Infrared Spectrum were established which can detect the content of polyphenols, tannins and flavones within a few minutes. The results have broad application prospects.

There are big differences in the content of active components from different provinces, so the quality control of PPL is needed to pay more attentions. In the areas of samples collected， Henan and Jiangxi have higher content of polyphenols and flavones.

The proportions of polyphenols and flavones can be divided into two groups: One is represented by Guangdong and Guangxi in southern China, has a tropical or subtropical humid monsoon climate, hot and rainy in long-term, small temperature difference Year-round which are benefited for the accumulation of polyphenols; the other is represented by Henan, Hubei and Hunan in central China, has yare or temperate monsoon climate, mild climates, four distinctive seasons which are conducive to the accumulation of flavones. These results are instructive to the cultivation of PPL.

Acknowledgements

This work was financially supported by The National Research Foundation for the Doctoral Program of Higher Education of China (20132332110004), Science and Technology University of Henan (2014QN002, SY1213027).

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