Measurement of borneol based on infrared spectrometry

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The infrared spectrometry contains multiple information of the sample, and it is easy to be applied to on-line measurement. To Chinese medicine, this technology can improve the standard of quality control and accelerate the modernization course. In this paper, we investigate the spectral characteristics of borneol, an effective ingredient in many Chinese medicines. The following results are achieved. In middle infrared (MIR) region, utilizing the linear relationship between absorption and concentration, the concentration of borneol with relative error within 4.30% in the strongest absorption region ($2950-2970$ cm^{-1}) is measured; in near infrared (NIR) region, the predicted concentrations of borneol are calculated by using partial least squares (PLS) regression analysis, in which the wavelengths are selected by genetic algorithm (GA) from the absorption bands of borneol in NIR region. The predicted relative error of calibration model is less than 2%. This result shows that PLS regression analysis combining genetic algorithm is a good method to improve prediction and reduce data in NIR region.

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One of the key problems to the modernization of Chinese medicine is to enhance the quality control[1]. Chinese medicine will be unstable if the material qualities are irregular. So it is necessary to control the quality by using certain quality standards. Now, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) are the common analytical methods in quality control for Chinese medicine[2]. Random inspection is done by gas chromatography, e.g. we can detect the content of borneol after Suxiaojiuxinwan (a kind of cardiac Chinese medicine) is formed into pellet.

The purpose of this paper is to investigate the method of the component measurement in Chinese medicine based on infrared spectrometry. The optimal results show that this method can improve on-line measurement accuracy of components in Chinese medicine. And the subject studied in this paper is borneol, whose effective ingredient is camphol.

Suxiaojiuxinwan, a kind of compound pellet pharmaceutical dosage forms, is employed as disturbance in the experiments. Dissolving Suxiaojiuxinwan in organic solvent as background, then adding different quantity of borneol in a series of solutions, we obtained the samples . There are 27 samples for middle infrared (MIR) experiment and 32 samples for near infrared (NIR) experiment, the corresponding borneol concentration ranges are $1-32$ and $0.4-22.1$ mg/ml, respectively.

MIR spectra are collected from 400 to 4000 cm^{-1} by using a Fourier transform infrared (FTIR) spectrometer (GX, Perkin-Elmer) with a deuterated triglycine sulfate (DTGS) detector and KBr beam splitter. Samples are contained in a horizontal attenuated total reflection accessory. The path length is limited within 100 μm. NIR spectra are collected from 4000 to 10000 cm^{-1} with a tungsten-halogen lamp, a cryogenically cooled InSb detector, and SiO_2 beam splitter. Samples are contained in 3-mm path length transmission quartz cell.

In data processing, the absorption spectra obtained from the energy spectra of samples and corresponding backgrounds, which are measured by using this instrument and its accessories, are used in regression analysis. Absorbance is the negative logarithm of the transmittance spectrum (T), which provides the ratio of the transmitted against non-attenuated spectral radiant power. The absorbance values A are proportional to sample thickness and compound concentration ($A = \varepsilon cb$, where ε is the absorptivity, c is the concentration, and b is the sample path length of the absorbing substance), as stated by the Lambert-Beer Law.

In MIR region, as the characteristic absorption is obvious, the use of A values can provide a linear relationship between absorption and concentration. In NIR region, calibration and quantitative analysis are performed by using partial least squares (PLS) method, which has the ability to overcome problems common to spectroscopic data, such as collinearity, band overlaps and interactions[3,4]. In this paper, the method of 'leave-one-out' full cross-validation is applied to calibration model, and the prediction accuracy of the model is evaluated in terms of root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP). These values are calculated as

$$\mathrm{RMSEC} = \sqrt{\frac{\sum_{i=1}^{N_c} (c_i - \hat{c}_i)^2}{N_c - f - 1}},$$

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{N_p} (p_i - \hat{p}_i)^2}{N_p}}, \qquad (1)$$

where c_i is the true calibration concentration of the ith sample, $\hat{c}_i$ is the estimated calibration concentration, p_i is the true validation concentration of the ith sample, $\hat{p}_i$ is the estimated validation concentration, N_c is the number of calibration samples, N_p is the number of validation samples, and f is the number of PLS factors used in the model.

The characteristic absorption bonds of camphol (2956, 1479, 1390 cm^{-1})[5] are considered as observation object of borneol. Figure 1 shows the absorption spectrum of

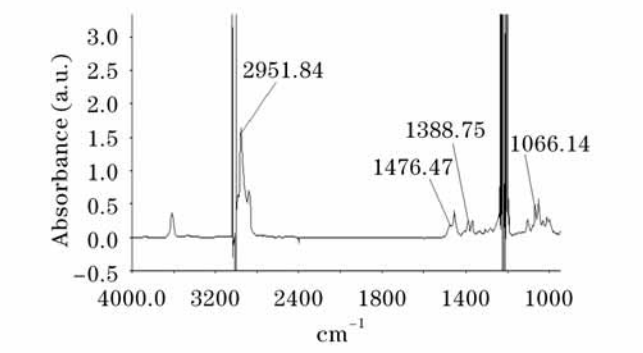

Fig. 1. Absorption spectrum of boeneol in MIR rgeion.

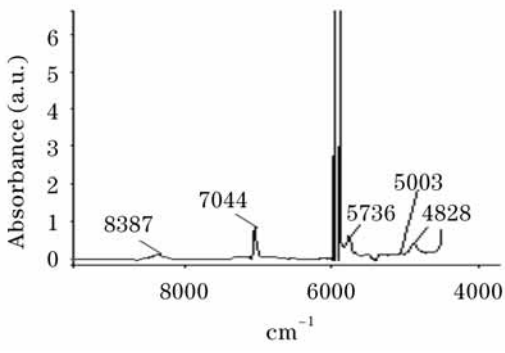

Fig. 2. Absorption spectrum of boeneol in NIR region.

Table 1. Results of the Linearity Regression in Different Bands

Bands (cm^{-1})	SEP (mg/ml)	Correlation
2950 − 2970	0.1587	0.983
1460 − 1490	0.2056	0.976
1380 − 1400	0.2133	0.973

SEP: square error of prediction.

Table 2. Relative Errors of Experiment Results

Sequence Number	Relative Error	Sequence Number	Relative Error
1	0.56%	16	−1.34%
2	0.39%	17	−1.45%
3	−2.50%	18	0.69%
4	−2.25%	19	3.38%
5	−2.56%	20	−3.19%
7	3.42%	21	−1.37%
8	1.26%	22	−0.18%
10	4.30%	23	−2.28%
12	0.69%	24	−0.34%
13	−1.96%	25	3.61%
14	0.60%	26	−2.70%
15	−0.70%	27	0.33%

borneol obtained through MIR experiment.

According to the linear relationship between absorption and concentration, Table 1 shows the results of linear regression in three characteristic absorption regions of borneol in MIR region. As the strongest absorption peak presents to the neighborhood of 2956 cm^{-1}, the best predictive result appears on the spectral range of 2950 − 2970 cm^{-1}. Table 2 shows the relative errors of the analysis results in the strongest absorption region. Thereinto, No. 6, 9, and 11 have been deleted because some mistakes are made in experiment. We can draw a conclusion from the results, i.e., the acceptability results can be obtained on the strong background interference as long as the background keeps invariable relatively.

Figure 2 shows the absorption spectrum of borneol in NIR region[6], which is obtained through the measurement in experiment. But only the spectral range of 5000 − 6000 cm^{-1} is used for PLS quantitative analysis, because of the presence of excessive noise, a result of low photonic throughput in the regions outside of the range. In Figs. 1 and 2, we can see some thick vertical lines around 1213, 3020, and 5988 cm^{-1} induced by the strong absorption of chloroform, which is a kind of organic solvent used in our experiment.

Before processing the PLS regression analysis, genetic algorithm (GA) is employed to select wavelengths, which establishes the calibration model. The parameters of GAs are determined according to our experiment demand. A population size of 50 is used, and the evolution is allowed to proceed for 500 generations. The fitness function to be minimized is $\frac{1}{1+\mathrm{RMSEP}}$, and the convergence criterion is 90% homogeneity of population[7].

The predictive results of 32 samples are obtained from Table 3, including before and after selecting wavelengths by GA. Table 4 shows the relative error between predictive and reference values. The values of relative errors reduce rapidly after selecting wavelengths by using GA, which are controlled within 2% except No. 5 and 6. There are even 17 samples whose relative errors are within 1%. It is noted that wavelength selection is effective when used with the NIR spectra of borneol solution containing multiple components in overlapping absorption bands. This result supports the use of wavelength selection not only as a means of improving prediction but also as a method for data reduction.

In this paper, the possibility of quality control and online monitoring of Chinese medicine is investigated based on infrared spectrometry. The maximal relative error is

Table 3. Calibration and Prediction Results Before and After Selecting Wavelengths by GA

Experiment	Number of Wavelengths	RMSEC ($\times 10^{-2}$ mg/ml)	RMSEP ($\times 10^{-2}$ mg/ml)
Before Selecting Wavelengths	250	15.74	17.72
After Selecting Wavelengths by the First GA	88	9.66	11.6
After Selecting Wavelengths by the Second GA	41	6.72	8.74

Table 4. Relative Errors Before and After Selecting Wavelengths by GA

No.	Before Selection	After Selection	No.	Before Selection	After Selection	No.	Before Selection	After Selection	No.	Before Selection	After Selection
1	1.076%	−0.986%	9	3.930%	1.721%	17	1.222%	1.102%	25	0.919%	0.880%
2	−2.279%	1.312%	10	0.365%	0.201%	18	−0.636%	−0.741%	26	−1.513%	−1.550%
3	−1.941%	1.023%	11	0.807%	0.565%	19	−1.567%	−1.665%	27	−0.286%	−0.286%
4	−2.018%	−1.658%	12	0.423%	0.204%	20	1.782%	1.680%	28	−0.826%	−0.826%
5	2.730%	2.218%	13	−0.644%	−0.842%	21	1.782%	1.675%	29	−0.177%	−0.212%
6	3.650%	2.120%	14	−3.342%	−1.329%	22	−1.190%	−1.281%	30	0.769%	0.684%
7	−0.391%	−0.755%	15	−2.100%	1.256%	23	0.784%	0.690%	31	−0.782%	−0.661%
8	3.259%	1.652%	16	−0.205%	−0.076%	24	0.317%	0.273%	32	0.680%	0.640%

Note: The results of after selection mean that the results are calculated by the second GA.

4.30% by using MIR spectrum, whereas the relative errors are controlled within 2% by using NIR spectrum. The criterion of off-line monitoring about the content of borneol in Suxiaojiuxinwan is 6 mg (±10%). The pills need to be redropped if it cannot reach the criterion. Apparently, the infrared spectrometry method can improve the controlling criterion of Chinese medicine.

The characteristic of MIR spectrum is the obvious absorption peaks which lead to the simple mathematic models. So, simple linear regression is enough to get good results. Whereas NIR spectrometry combined with chemometrics is complicated. The precision of analysis is related to the modelling quality and intelligent use of model. Wavelengths selection is an important step in modeling to simplify the design and minimize the cost.

If we design a kind of portable or on-line equipment, wavelength selection and optical design of the equipment may be limited because of MIR light source and other optical elements. Depending on its predominance of speediness and convenience, the NIR spectrometry has a wide application foreground. Especially, the management of good manufacturing practice (GMP) in pharmacy provides opportunity for NIR spectrometry to the one-time quality evaluation application. And we are utilizing several algorithms to optimize model in order to get a more robust and suitable model to meet the challenge of on-line monitoring in industry.

In short, infrared spectrometry can be used as an investigation method for on-line monitoring and quality controlling of effective principles in Chinese medicine. This method can improve the criterion of quality controlling and accelerate the modernization course of Chinese medicine.

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