Simultaneous determination of chlorogenic acid and baicalin in heat-clearing and detoxicating orally liquid by near infrared spectroscopy

Zhenyao Liu[a] and Jidong Yang[a,b,*]

[a]*College of Chemistry and Chemical Engineering, Southwest China University, Chongqing, China. E-mail: flyjd6400@sina.com*
[b]*College of Chemistry and Chemical Engineering, Yangtze Normal University, Chongqing, China*

Keywords: Near infrared spectroscopy; Partial least squares; heat-clearing and detoxicating oral liquid; chlorogenic acid; baicalin

Introduction

Heat-clearing and detoxicating oral liquid is very commonly used nowadays in China. It has the effects of, clearing lungs, moisturising dryness, and smoothing the throat to stop coughing. The main drugs of heat-clearing and detoxicating oral liquid are honeysuckle and scutellaria. The active components of honeysuckle and scutellaria are respectively chlorogenic acid and baicalin. The common method for determination of the main components of heat-clearing and detoxicating oral liquid quantitatively is high performance liquid chromatography.[1] High performance liquid chromatography requires tedious and complex processing. The method is time-consuming and destructive. The use of chemical reagents is also a factor if the economic benefit and safety are considered.[2] All of these factors have underlined the need for a reliable technique to quickly and nondestructively detect the quality of this important tonic.

In spectral analysis, the region between 780 nm and 2500 nm is called the near infrared (NIR) region. The chemical bonds of C-H, N-H, O-H and S-H have stretching vibrations in the NIR region, and can be determined by the near infrared method. In Figure 1 there are plenty of C-H bonds and O-H bonds in chlorogenic acid and baicalin, so chlorogenic acid and baicalin both have significant absorption in the NIR region.

Therefore, chlorogenic acid and baicalin in Heat-clearing and detoxicating oral liquid could be detected quickly and non-destructively by the NIR spectroscopy method.

NIR spectroscopy is a powerful analytical tool used in various industrial sectors, e.g. the agricultural, petrochemical, textile and pharmaceutical.[3] It is a fast, accurate, and non-destructive

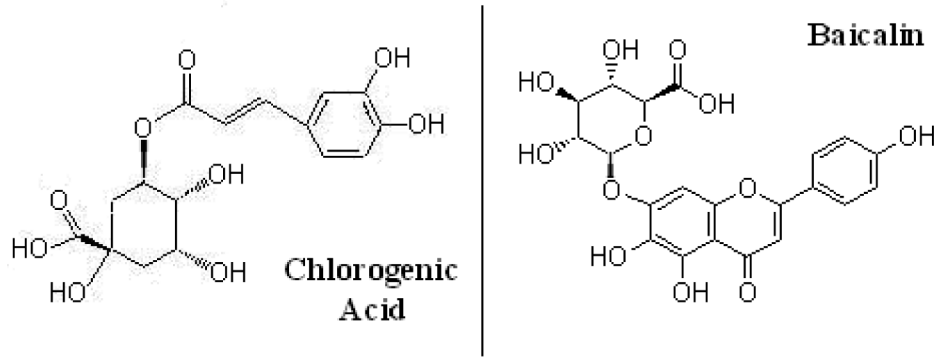

Figure 1. Structural formula of chlorogenic acid and baicalin.

analytical tool that can be considered as a replacement for traditional chemical analysis. The NIR method does not need sample pretreatment and does not destroy the sample. It is pollution-free, convenient and fast, and can be used on-line. It can simultaneously detect many components and its reproducibility is good. All of these advantages indicate that the NIR method is capable of detecting active components of traditional Chinese medicines. The NIR method has been used in analysis of Chinese medicine successfully in recent years.[4]

Materials and methods

Sample and reagents

In the experiment, 30 batches of heat-clearing and detoxicating oral liquid samples from six manufacturers (listed in Table 1) were investigated.

All the samples were placed in the same temperature-controlled room where the spectrometer was located before performing the analysis. The standard agent of chlorogenic acid (batch number: 1107532200413) and baicalin (batch number: 1107152200514) are approved by the National institute for the control of pharmaceutical and biological products.

Table 1. Ranges of contents of chlorogenic acid and baicalin from six manufacturers.

Manufacturer	Chlorogenic acid (mg mL^{-1})	Baicalin (mg mL^{-1})
Sichuan Taihuatang Pharmaceutical Co., Ltd	0.373–0.410	0.555–0.605
Chengdu Tianyin Pharmaceutical Co., Ltd	0.374–0.425	0.650–0.736
Sichuan Xuyang Pharmaceutical Co., Ltd	0.281–0.308	0.650–0.709
Zhengzhou Ruilong Pharmaceutical Co., Ltd	0.840–0.865	0.574–0.597
Jiangxi Nanchang Pharmaceutical Co., Ltd	0.380–0.416	0.238–0.255
Sichuan Good Doctor Pharmaceutical Co., Ltd	0.926–0.949	0.386–0.395

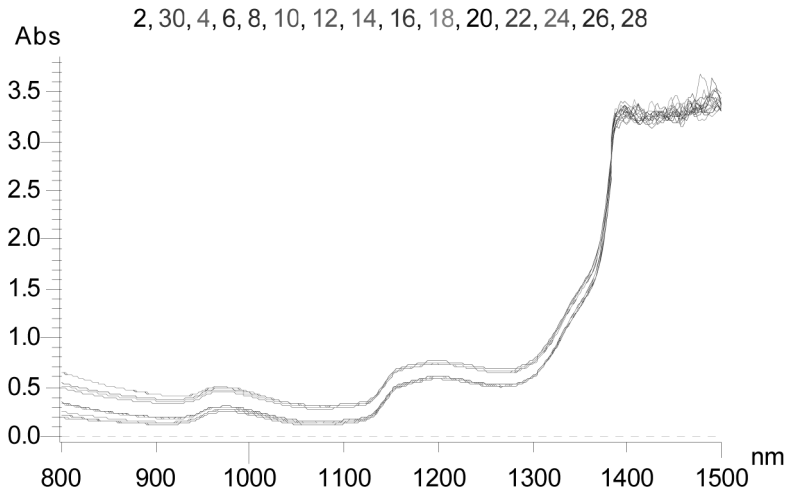

Figure 2. Near infrared spectra of heat-clearing and detoxicating oral liquid samples.

NIR Spectra collection

The NIR spectra were collected in transmission mode using a Near-infrared spectrophotometer (U-4100, Hitachi, Japan) with 1 cm quartz sample cell. Air was the reference, wavelength range was 800~1500 nm, wavelength spacing was 2 nm, slit width was 2 nm. Scanning speed was 1500 nm s^{-1}, every sample was scanned three times and averaged. The NIR Spectrum of wastewater is given in Figure 2.

High performance liquid chromatography analysis

Prior to the NIR spectral analysis, all samples were analysed by high performance liquid chromatography (LC-2010A, Shimadzu, Japan). Details are available on request.

Method (multivariate analysis of partial least square)

Multivariate analysis was used for quantitative and qualitative analysis. Partial least squares (PLS) algorithm was used in this experiment. The performance of the final PLS model was evaluated in terms of root mean square error of cross-validation (*RMSECV*) for cross-validation and root mean square error of prediction (*RMSEP*) during test validation, and the coefficient of determination (R^2).

Results and discussion

Choice of model algorithm

The basic idea of the model algorithm was to make use of the total spectrum information, in order to eliminate the effects of spectrum peak overlap. The absorption peaks of NIR spectra are broad

Table 2. Statistical results for calibration models for chlorogenic acid and baicalin.

	Chlorogenic acid			Baicalin		
	R^2	*RMSECV*	PCs	R^2	*RMSECV*	PCs
Smoothing processing	0.9646	1.379	8	0.9785	0.882	3
First derivative	0.9987	0.467	5	0.9847	0.480	4
Second derivative	0.9909	1.042	5	0.9832	0.908	2

and overlap, making single wavelength calibration impossible, due to the large amount of hidden information in all of the other spectral data. Multivariate calibration tools such as partial least-squares (PLS) regression are available. PLS regression extends and improves the potential application of spectroscopy technique in the pharmaceutical industry by extracting relevant features from the spectra. The calibration equations can be applied to unknown samples once the equation is established by PLS, from samples where the analyte has been determined by an approved reference method. PLS regression has no restriction in the number of wavelengths that can be selected for the calibration model, so the maximum information can be extracted from the spectra. The information can be condensed into latent variables, or factors, which can then be used in the calibration and prediction steps.

Choice of spectrum pretreatment method

The spectral signal processed by the detector contains noise, which can be corrected by smoothing of the signal. Processing the signal by a derivative protects against baseline drift, and assists in discriminating overlapping peaks, thereby increasing the resolution and sensitivity. But derivatives tend to increase the noise, and decrease the signal-to-noise ratio, so the pretreatment method should be optimised for different types of samples. The process of smoothing and derivative formation by first and second derivatives were compared in this experiment. Calibration statistics are summarised in Table 2.

From Table 2, the first derivative treatment was considered to be best for both chlorogenic acid and baicalin.

Optimisation of model and determination of PCs in model development

Evaluation of the calibration models was carried out to determine the optimal number of PCs for use in the regression model. Optimum number of PCs was determined by cross-validation. Outliers were detected using the Chauvenet test at the 95% confidence level.

External verification of model

As a prediction set, chlorogenic acid and baicalin contents were predicted in 15 heat-clearing and detoxicating oral liquid samples, using the models developed in this experiment. The *SEP* of chlorogenic acid and baicalin were respectively 0.356 and 0.370. The scatters of chlorogenic acid and baicalin between NIR predicted and measured are given in Figure 3 and Figure 4.

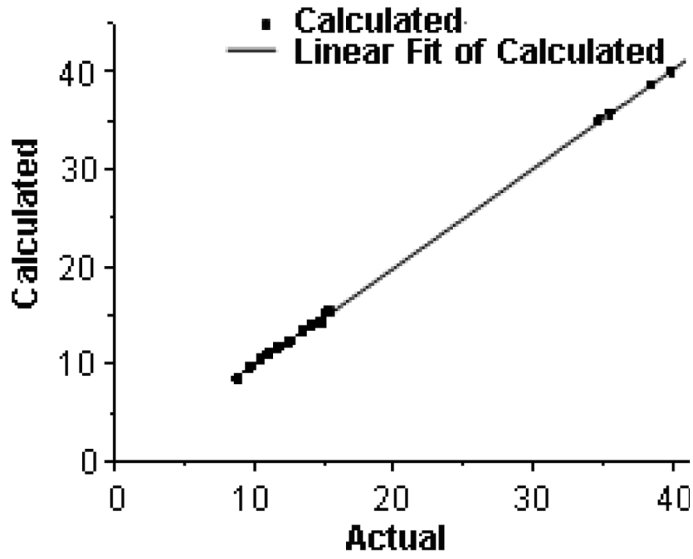

Figure 3. Correlation coefficient graph of chlorogenic acid between prediction result and actual value.

Application of model

Thirty heat-clearing and detoxicating oral liquid samples from six manufacturers were selected for the application experiment. The NIR spectra of the samples were collected, and predicted with the quantitative model. Ranges of contents of chlorogenic acid and baicalin from six manufacturers are given in Table 1.

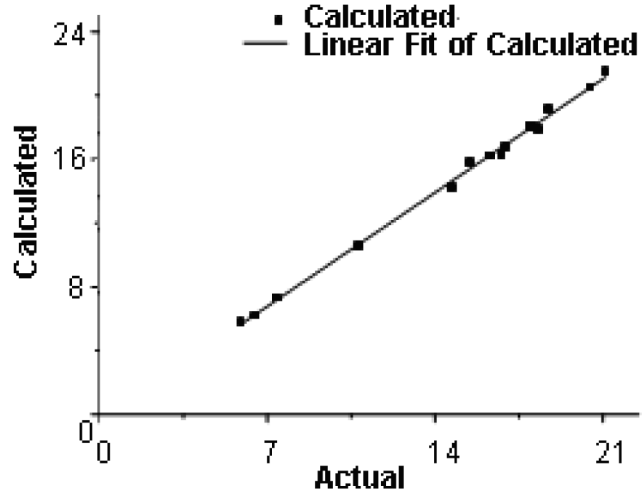

Figure 4. Correlation coefficient graph of baicalin between prediction result and actual value.

Conclusions

The results obtained in this research show the potential of NIR spectroscopy to detect chlorogenic acid and baicalin simultaneously in heat-clearing and detoxicating oral liquid by comparison with the HPLC reference method. The combination of NIR spectroscopy and PLS regression has been found to be a convenient, versatile method. It has the ability to dramatically reduce testing time and cost of monitoring, without using any chemical reagents, and was considered to be suitable for on-line monitoring n in pharmaceutical companies.

References

1. *Chinese pharmacopoeia*. Beijing Publications, Beijing, PR China (2005).
2. Q.S. Chen, J. Zhao, H. Lin, *Spectrochim. Acta Part A* **72,** 845 (2009).
3. P.M. Lucy, E.H . Stephen, I. David, V.V. Frank and L. Susan, *Agric. Food Chem.* **55,** 2791 (2007).
4. L. Ai, Q.L. Liang and G.A. Luo, *Application of NIR Spectrum in Asia-Pacific Traditional Medicine* **6,** 52 (2008).