Application of near infrared (NIR) spectroscopy as a tool for quality control in traditional Chinese medicine (TCM)

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Introduction

Due to the increasing consumption of traditional Chinese medicine (TCM) products, there is a permanent and challenging demand for new and high-throughput analytical methods to ensure efficacy, safety and quality. Authentication of plant material and origin, identification of parts of the plant, and qualitative and quantitative analysis of primary and secondary metabolites are the key challenges to efficiently ensure quality. Standardisation, stability and quality control based on chromatographic methods including thin layer chromatography (TLC), liquid chromatography (LC), mass spectrometry (MS) and biochemical methods are relatively time-consuming and high cost, the equipmentis very expensive and can only be operated by trained staff. Traditionally applied identification as well as physical and chemical descriptions follow distinct and complex Chinese experience, rules and protocols,[1] which in many cases do not enable an objective determination. Often only a (dried) part of the plant (or even animal) is present and then identification is based on personal and subjective operator decisions.[2]

As an alternative, near-infrared (NIR) spectroscopy can carry out objective, fast, non-invasive, high-throughput measurements. Combining the measurement of spectral "fingerprints" and physico-chemical parameters with chemometric methods allows for novel insights and strategies in TCM quality control.

Materials and Methods

Samples and sample preparation

To obtain representative samples, 27 cultivated and 22 wild samples of skullcap root (*Scutellariae* sp.; "radix scutellariae") were collected from nine different regions in China from August to September. Each sample was a mixture of 5 plants and all of the samples were morphologically identified. Plants were first cut into small pieces with scissors and were then milled using a grinder. The final powders were sieved using a 100-mesh (150 µm) sieve and stored in air-tight containers before analysis.

Reagents

Baicalein (B), baicalin (baicalein-7-glucuronide, BG) and wogonin (W) (≥95%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol, formic acid, and ethanol (all analytical grade) were purchased from the Beijing Chemical Reagent Company (Beijing, China). Acetonitrile (high-performance liquid chromatography grade) was obtained from Fisher-Scientific (Fisher Scientific, Hampton, USA). Double deionised water and distilled water were prepared using a Milli-Q water-purification system (Millipore, Molsheim, France).

High-performance liquid chromatography (HPLC) as a reference method

0.1 g of radix scutellariae was dissolved in 20 ml ethanol (70 vol. %) and ultrasonicated for 60 minutes. The total volume of the extract was adjusted to 20 ml with ethanol (70 vol. %). The mixture was then filtered through quantitative filter paper and finally through a 0.45 µm syringe filter (Agela, Newark, USA), prior to HPLC analysis.

Chromatographic analysis was carried out on a Diamonsil octadecylsilane (ODS) column (Dikma, Richmond Hill, USA) (250 mm × 4.6 mm internal diameter, 5 µm particle size) at 30°C using a Waters Alliance HPLC system (Waters, Milford, USA), equipped with a quaternary solvent delivery system, an auto-sampler and a diode-array detection system. The detection wavelength was 280 nm. The gradient elution of the mobile phase consisting of (a) acetonitrile, water and formic acid (21:78:1, v/v/v) and (b) acetonitrile, water and formic acid (80:20:1, v/v/v) was as follows: 100 % (a) at 0–10 min, from 100 % to 90 % (a) at 10–35 min, from 90 % to 65 % (a) at 35~70 min, from 65 % to 5 % (a) at 70–90 min, and 5 % (a) at 90~100 min. 20 min re-equilibrium was allowed before the next injection. The flow rate was 1.0 $ml.min^{-1}$.

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NIR spectroscopy

NIR spectra of samples were recorded with a Nirvis FT-NIR spectrometer (Büchi, Flawil, Switzerland) at a wavenumber range from 4008 to 9996 cm^{-1} with a resolution of 4 cm^{-1} in reflectance mode by fibre optics. Each spectrum was the average of 60–70 scans. Each sample measurement was repeated three times, agitating the powder before each measurement. The corresponding spectra were averaged. The temperature was kept constant at 20°C. Spectra were divided into a calibration set (67%, c-set), and a validation set (33%, v-set). All pre-treatments and calculations were carried out using The Unscrambler (version X 10.0.1; Camo, Oslo, Norway) and the selection of the best quantitative regression model was based on the following quality parameters:

1.) Bias, the average deviation between the predicted values (y_n) and the actual values (x_n) in the c-set, should be close to zero.

$$\text{BIAS}=\frac{1}{N}\sum(x_n - y_n)$$

2.) Standard error of estimation (SEE) is the standard deviation of the differences between reference values and NIR-results in the c-set.

$$\text{SEE}=\sqrt{\frac{1}{N}\sum(x_n - y_n - \text{BIAS})^2}$$

2.) Standard error of prediction (SEP) is the counterpart for the v-set samples. SEE and SEP should be as small as possible.

$$\text{SEP}=\sqrt{\frac{1}{N}\sum(x_n - y_n - \text{BIAS})^2}$$

4.) Correlation coefficients (R^2) should approach 1.

Results and Discussion

NIR spectroscopy for the analysis of medicinal plants (“phytomics”) was introduced in Austria at the Institute of Analytical Chemistry and Radiochemistry, Universtiy of Innsbruck, in 1999. At approximately the same time, NIR spectroscopy was established as a novel tool for quality control of TCM in China. Now, NIR spectroscopy is used not only for authentication, identification and quantification of raw material but also for process quality control and/or extraction monitoring. Both raw materials and complex formulae are current subjects of investigation. TCM includes medicinal plants as well as medicine prepared from animals, fungi and minerals; NIR spectroscopy is used not only for the monitoring of secondary metabolites but also for the determination of additional parameters (e.g., fibre, moisture). Due to short measurement times of only a few seconds, the use of a fibre-optic probe makes NIR spectroscopy attractive for on-line monitoring. In Figure 1 the main application fields of NIR spectroscopy in TCM include the control of raw materials, production and extraction processes, and preparation of medicinal formulations. Here we describe the applicability of NIR spectroscopy for the qualitative and quantitative analysis of radix scutellariae.

Spectral data pre-processing

In this study, four data pre-processing methods were compared. Data were pretreated with multiplicative scatter correction (MSC), standard normal variate (SNV), 1st derivative, or 2nd derivative. MSC is an important procedure for the correction of scattered light in powders. It is used to modify the additive and multiplicative effects in the spectra resulting from different particle sizes. SNV is another mathematical transformation method used to remove slope variation and correct scattering effects in spectra. 1st and 2nd derivatives focus on eliminating baseline drifts and enhancing small spectral differences. Smoothing (Savitzky–Golay) was used in combination with first and second derivatives to reduce noise. The segment size of smoothing was 3. Figure 2 shows the mean spectra for all original NIR data of the wild and cultivated varieties. Spectral differences between wild and cultivated plants were enhanced after 2nd derivative pre-processing. The most intense band in each spectrum could be assigned to the second overtone of the carbonyl group (5352 cm^{-1}), followed by the CH stretch and CH deformation vibration (7212 m^{-1}), the OH vibration (4440 cm^{-1}), the CH_2 (5742 cm^{-1}) and the CH_3 overtone (5808 cm^{-1}).

Cluster analysis

Principal component analysis (PCA) is a multivariate data reduction method in which the original variables are transformed into new orthogonal ones referred to as principal components (PCs). In this study, PCA was

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used to develop a cluster model for qualitative analysis of wild and cultivated radix scutellariae. All recorded spectra were transformed by applying a 2nd derivative before calculating the cluster model. Figure 3 shows the 2-dimensional cluster plot represented by the first (PC1) and second (PC2) principal components. The wavenumber range used for calculation was 4200–7716 cm^{-1}. The total variance explained by the first principal component was 81%. Wild and cultivated varieties of radix scutellariae could be distinguished by examining PC1 and PC2 score values (Figure 3).

Quantitative analysis

Table 1 compares the different pre-treatments and flavonoid calibrations. In this study, the best results were obtained when models were based on the 4200 to 7716 cm^{-1} spectral region. 2nd derivative is obviously superior to other pre-treatments as demonstrated by the similarity of the results for the standard error of calibration SEC and SEP.

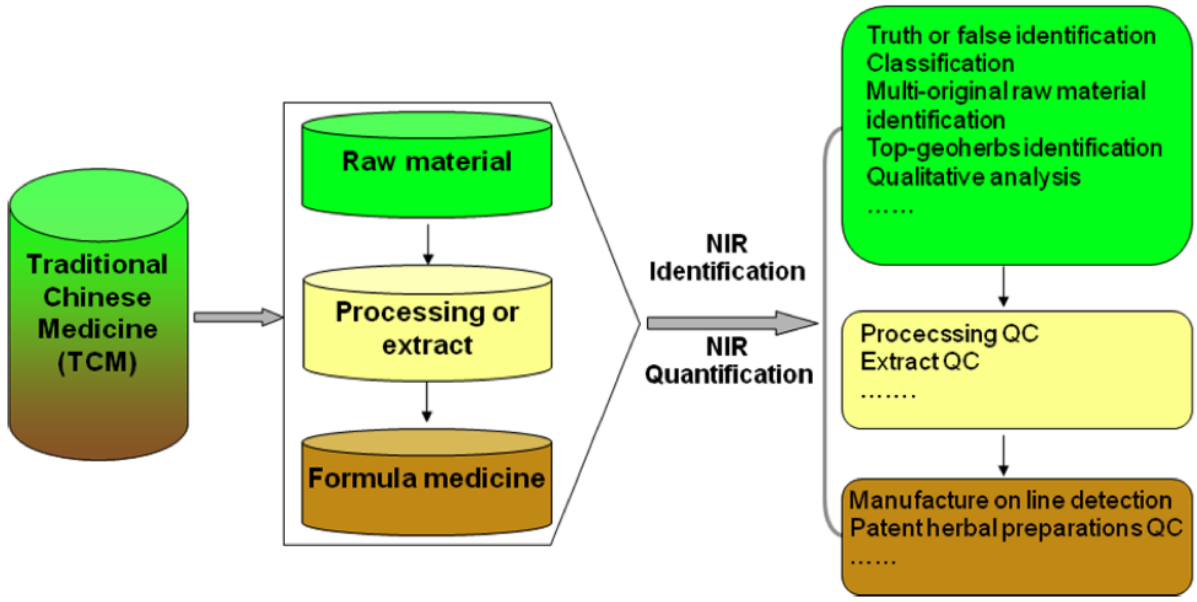

Figure 1. Flow diagram of NIR spectrosocpy application fields in TCM.

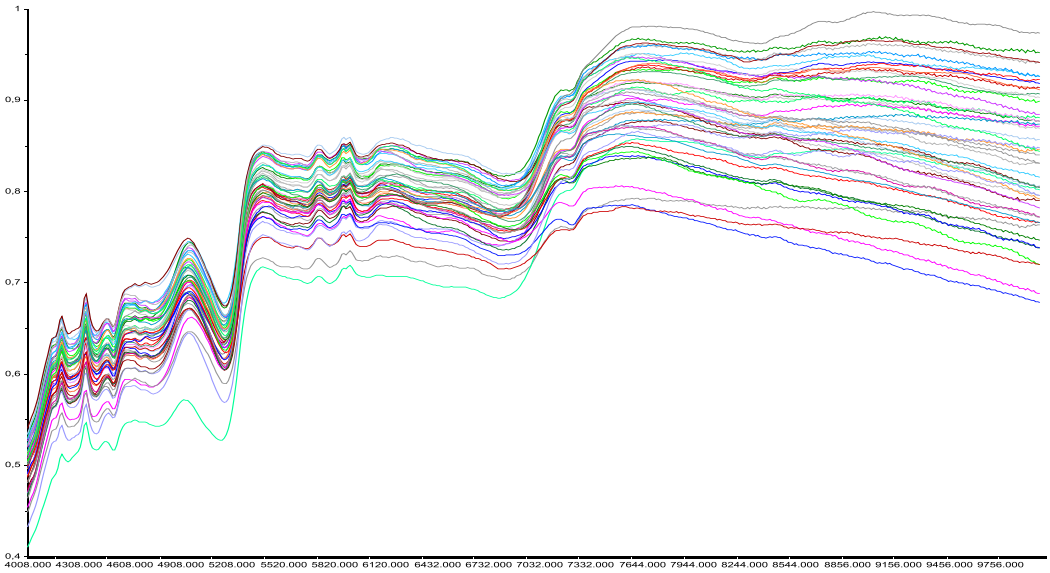

Figure 2. Original NIR spectra recorded from 49 radix scutellariae samples.

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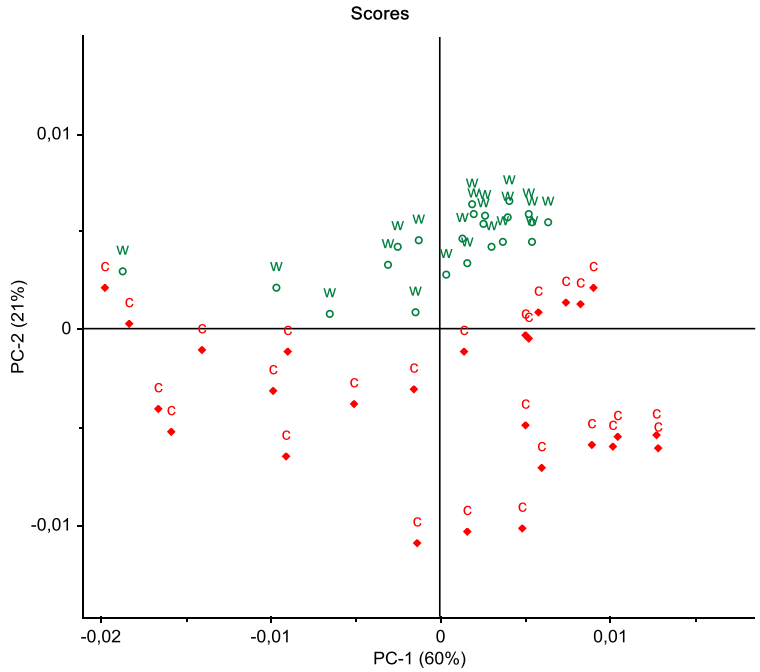

Figure 3. Principal component score values of the wild and cultivated radix scutellariae samples.

Table 1. Performance parameters of the models of B via different spectra pre-processing methods.

Pre-treatment	wavenumber [cm^{-1}]	PLS factors	SEC [%]	SEP [%]	Bias	R^2 [c-set]	R^2 [v-set]
none	4200-7716	14	0.148	0.142	-0.0411	0.890	0.932
MSC	4200-7716	14	0.180	0.182	-0.0383	0.837	0.893
SNV	4200-7716	14	0.156	0.138	0.0048	0.878	0.942
1st derivative	4200-7716	9	0.0768	0.0599	-0.00719	0.970	0.989
2nd derivative	4200-7716	9	0.0484	0.0522	-0.0205	0.988	0.990

Conclusion

It can be concluded that NIR spectroscopy in combination with multivariate data analysis can distinguish wild from cultivated varieties of radix scutellariae and simultaneously determine the concentration of three flavonoids. A simple, rapid and reliable overall characterisation of radix scutellariae quality can be obtained at low cost. The results indicate that the proposed method can be readily utilised as a quality control method in the phytopharmaceutical industry.

Acknowledgements

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