Classification of Samples of the Traditional Remedy “Chinese Goldthread” by FT-IR Spectrometry and AssureID software

Introduction
Coptidis Rhizoma (the root of the Coptis chinensis plant), which is also known as goldthread, is one of the most commonly used traditional Chinese medicines (TCM) in China. It can clear heat and reduce fire and is usually used for diarrhoea, vomiting, jaundice, fever, toothache, conjunctivitis and some other conditions.1

In a separate note,2 we have shown that FT-IR spectrometry can be used to identify the presence of the marker compound (berberine) in Coptidis Rhizoma samples, and importantly, to distinguish it from other berberine-containing materials (Phellodendri Chinensis Cortex and Phellodendri Amurensis Cortex). Second-derivative processing and peak location methods were used for the identification.

TCM materials are normally processed before being used clinically, and different processing methods lead to different medicinal effects. The Chinese Pharmacopoeia1 lists three processing methods for Coptidis Rhizoma. Coptidis Rhizoma processed by rice wine is used for toothache and conjunctivitis, while that processed by ginger juice or the extract of Corni Fructus is used as an anti-emetic and to comfort the stomach. These different processing methods do not bring about an obvious difference in the appearance of the herb, so it is important to develop an analytical method that is capable of distinguishing these materials. In terms of chemical composition, the differences between the raw and the processed herbs may be not as significant as those between different kinds of herbs, so we can expect to require a more careful analysis of the spectra using chemometric tools.

In this note, we show that FT-IR spectrometry combined with AssureID™ software and the COMPARE™ and SIMCA algorithms can be used to distinguish the raw and processed Coptidis Rhizoma samples.
Experimental

Samples of raw Coptidis Rhizoma were obtained from the Institute of Chinese Materia Medica of the China Academy of Chinese Medical Sciences. Some raw Coptidis Rhizoma samples were processed according to the standard procedures of the Chinese Pharmacopoeia\(^1\) to obtain three types of processed Coptidis Rhizoma. Coptidis Rhizoma processed by rice wine was obtained by mixing 1 kg of raw Coptidis Rhizoma with 125 g of rice wine and stir-baking to dryness. Coptidis Rhizoma processed by ginger juice was obtained by mixing the juice from 125 g of raw ginger with 1 kg of raw Coptidis Rhizoma and stir-baking to dryness. Coptidis Rhizoma processed by Corni Fructus was obtained by mixing 1 kg of raw Coptidis Rhizoma with the liquid part from decocting 100 g of Corni Fructus with water and then stir-baking to dryness.

Spectra of the raw and the processed Coptidis Rhizoma samples were measured on a PerkinElmer® Spectrum™ 100 FT-IR spectrometer equipped with a single-bounce diamond attenuated total reflectance (ATR) sampling accessory (Figure 1). Spectra were acquired at 4 cm\(^{-1}\) resolution and for an accumulation time of 60 seconds. The COMPARE and SIMCA analyses were carried out using AssureID software.

Results and Discussion

As-measured and processed FT-IR spectra

Typical spectra of the raw and processed samples are shown in Figure 2. There are no obvious differences between the spectra of single samples of the raw and the processed Coptidis Rhizoma.

As the differences between materials become smaller, it becomes increasingly important to develop and validate classification models with large sets of data. If a small dataset is relied on too heavily, a classification may be developed based on differences that are due to chance, and not reproducible.

Spectra of 7 raw samples and 7 samples processed by each of rice wine, ginger juice and extract of Corni Fructus were used to develop the classification models. Separate sets of 3 samples of each material were measured in the same way and used to validate the performance of the classification models.

The spectra were pre-processed by AssureID software using second derivative and standard normal variate (SNV) transformations. SNV normalizes spectra to a common intensity, removing variations due to variable contact between the sample and the ATR crystal. The processed spectra are shown in Figure 3.

These processing steps greatly emphasize the differences between the sample spectra, and the presence of several samples for each material allows the comparison of within-group and between-group variations. The samples treated with ginger juice or extract of Corni Fructus cannot be distinguished from each other, but are clearly distinct from the raw and rice-wine-processed samples.
Discrimination using the SIMCA algorithm

While the COMPARE algorithm appears sufficient here, it cannot cope as well when there is variability within each class. For example, spectra of samples of the raw root taken from plants grown in different areas, or dried in different ways may show a greater degree of variation. In this case, a single mean spectrum is no longer representative of the set of spectra.

The SIMCA algorithm builds a principal components model of the spectra within each class, so that future spectra exhibiting variation from the mean that is consistent with the spectra used to calibrate the model can be correctly recognized as coming from the same material. With this approach, even materials exhibiting a large degree of within-class variability can be successfully distinguished, provided that the differences between classes are not the same as the differences within each class.

The classification model is developed by building individual principal components models for each class, and establishing thresholds on the distance from the center of each model. These calculations are performed automatically, and the Troubleshooting tool warns the user of any potential problems (such as materials that are poorly separated and cannot be distinguished).

Figure 5 shows a scatter plot of all the samples in the space defined by the first three principal components of the complete data set. Samples of each group are gathered in a region enveloped by an ellipsoid, and a good degree of separation is evident.

These spectra, using the wavenumber range 1250-1050 cm⁻¹, were used to develop classification models using the COMPARE and SIMCA algorithms in the AssureID software. Three materials were configured: one for raw Coptidis Rhizoma, one the rice wine treatment, and one for ginger juice or Corni Fructus (combined).

Discrimination using the COMPARE algorithm

COMPARE functions by measuring the correlation between each sample spectrum and the mean sample spectrum for each material. A threshold correlation coefficient is established for each material that allows the classification of future samples.

Figure 4 shows the correlation coefficients of all samples with the average spectrum for each group. If the correlation within each group is significantly higher than the correlations between the groups, a reliable classification model can be built – indicated by the dashed correlation thresholds. In Figure 4a, for example, we can see that the “raw” spectra all have a correlation of >0.99 with the mean raw spectrum, while the “rice wine” and “ginger juice/Corni Fructus” spectra have correlations with the mean “raw” spectrum of around 0.97 or lower. Similar observations can be made about the processed materials from Figures 4b and 4c. From all of this, and assuming that the samples are representative, we can conclude that the three materials can be distinguished.

This conclusion was supported by the correct classification of all of the independent validation samples.

Figure 4. Correlation coefficients of all samples with the average spectrum for each group. (a) Raw Rhizoma Coptidis (b) Rhizoma Coptidis processed by rice wine (c) Rhizoma Coptidis processed by either ginger juice or extract of Corni Fructus. The dashed line indicates the classification threshold.
As with the COMPARE model, 100% successful classification of both the calibration and validation samples was achieved.

**AssureID in routine use**

After a classification model is built and validated, it can be incorporated into a workflow that can be run using the dedicated Analyzer software module. The workflow automatically guides the analyst through the necessary sample preparation steps, configures the instrument, carries out the calculations and reports the result as a simple pass/fail for the selected material. All of the results are stored in a database and full traceability is maintained for compliance with electronic data-management regulations like 21 CFR Part 11.

**Summary**

In this note we have shown that the FT-IR spectrometry combined with AssureID software is a simple and powerful tool for quality control of TCMs. Samples of raw Coptidis Rhizoma and that processed by various means can readily be distinguished. It proved impossible to distinguish root processed by ginger juice from that processed by extract of Corni Fructus, but this is a less important distinction, as both are used to treat the same ailments.

The Spectrum 100 FT-IR equipped with a diamond ATR accessory and AssureID Analyzer software provides a very quick and simple way for analysts to identify samples.

**References**


2. Chen Jian-Bo, Zhou Qun, Sun Su-Qin, Ben Perston and Patrick Courtney, Rapid Quality Control of the Traditional Remedy “Chinese Goldthread” by FT-IR Spectrometry PerkinElmer Application Note 009318_01.