Application Note: 51254

Classification of Nutraceutical Herbal Powders by FT-IR Using ATR and Discriminant Analysis

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Key Words

- OMNIC
- Smart Orbit
- TQ Analyst
- ATR (attenuated total reflectance)
- Discriminant Analysis
- Nutraceutical

Introduction

Most classical pharmaceutical agents have a specific chemical structure, and target a specific molecular receptor. Holistic and naturopathic medicines are generally less specific, and use multi-component, naturally occurring materials, such as herbs or plant extracts. These "nutraceuticals" are widely used in holistic treatments for a variety of therapies, such as boosting the immune system, or regulating organ and bodily functions. Consumers are often motivated by frustration with the expense of drugs, or out of a desire for general wellness enhancement rather than specific disease treatment. Most nutraceuticals are available without prescriptions.

While not as strictly regulated as the pharmaceutical industry, nutraceutical suppliers still must monitor variations in materials (both incoming raw materials and final product) to ensure quality. As the claims for efficacy become more aggressive and use becomes more pervasive, the FDA may further regulate the industry, so QA/QC procedures embedded now will head off problems in the future. Fourier transform infrared (FT-IR) can serve as an excellent analytical tool for the analysis of nutraceuticals, with advantages in sensitivity, selectivity, speed and regulatory compliance through validation protocols. Also, our OMNIC™ software provides powerful tools for automation, so non-specialists can achieve satisfactory results with minimal training.

The analytical problem is that, unlike classical drugs with their specific chemical composition, a nutraceutical is a mixture, which can experience substantial variations due to lot-to-lot disparities in source materials. Essentially, no matter how similar two nutraceuticals may appear, the same FT-IR spectrum will not be observed. Also, the biological origin means that many herbal powders contain similar components, like cellulose, proteins and sugars, which can make the FT-IR spectra similar as shown in Figure 1. Further, some spectral differences may be caused by sample handling or varying environmental conditions, such as humidity. Monitoring sample-specific variations is extremely important in guaranteeing quality.

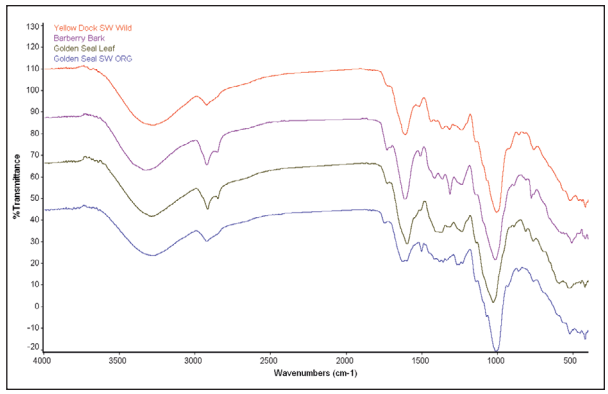

Figure 1: Spectra from the nutraceutical powders Golden Seal, Barberry Bark, Yellow Dock, and Golden Seal Leaf

As noted, many nutraceutical powders exhibit similar spectral features due to their inherent cellulose, protein or sugar content. This makes them difficult to classify uniquely using standard library search routines. It is crucial to classify herbal powders, but it may also be important to identify the source of a given lot of material. In the worst case, library searches result in matches to a majority component (like cellulose). Even in the best cases, library searching may not discriminate sufficiently for this analysis – a sequence of candidates with similar match values (a search result "cluster") may emerge. This clustering may not provide sufficient information to classify the material.

Our TQ Analyst™ software includes powerful tools that can be utilized for QC of nutraceutical materials. Discriminant Analysis (DA) coupled with the fingerprinting capability of the FT-IR provides an excellent solution for the nutraceutical evaluation process. DA works well as a classification technique both to determine the major classes of known materials and to determine if an unknown substance falls within a certain class. This allows FT-IR to be used to screen incoming materials to determine if they belong to any of the major classifications.

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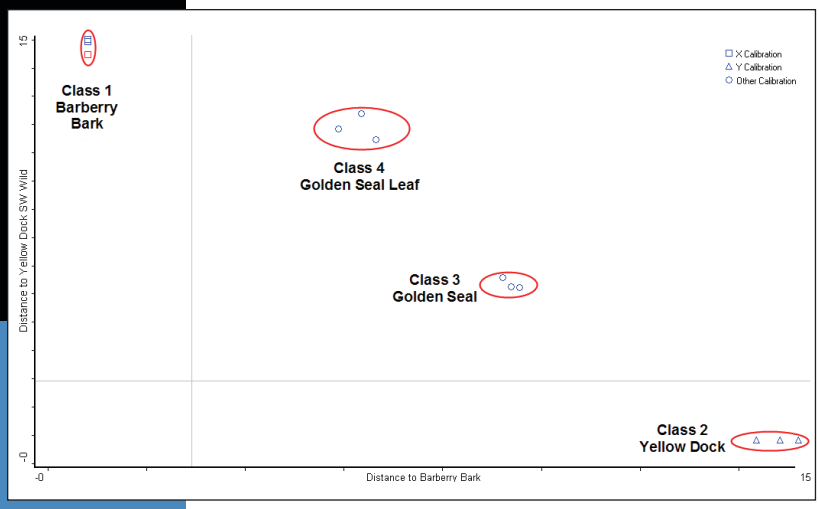

Figure 2: TQ Analyst plot showing the clustering of the four classes, as described in the text. Each point is linked to a spectrum in the table of standards shown in Figure 3.

In a quantitative calibration, spectra from a series of standards of known concentrations are processed to provide a relationship which can be used to predict unknowns. The classification process similarly begins by collecting spectra from many different batches of material with known compositions. These standards are fed into the DA algorithm, which forms clusters based on the spectral features and given identity. An example involving herbal powders is shown in Figure 2. An unknown sample is then processed by computing the "distance" between the cluster centers and that of the sample spectrum. This distance from each class center to the unknown material is known as the Mahalanobis distance, and is calculated as follows:

$$D_M = \sqrt{(X - X_{avg})^T S^{-1} (X - X_{avg})}$$

With a spectrum containing n data points, D_M equals the Mahalanobis distance (a scalar), X is the spectrum (represented as an n x 1 vector), X_{avg} is the mean spectrum (n x 1 data vector), S^{-1} is the inverse of the covariance matrix (an n x n matrix), and the T denotes the vector transpose.

Any region of the spectrum can be selected to build the calibration model. Once the calibration is constructed, the method uses this region to calculate D_M relative to all the classes of standards provided. D_M, if small, indicates inclusion in a class (and large D_M suggests exclusion). If no small D_M for any class is found, then the material may represent a completely unknown substance. The threshold value for inclusion can be set by the end user.

Another critical consideration is sample presentation. We offer robust sample handling through the Smart Orbit™ diamond attenuated total reflectance (ATR) accessory. The Smart Orbit provides enhanced sampling speed and ease of use combined with the longevity of a diamond accessory. Briefly, in ATR, the IR light is focused into an IR-transmittant crystal (diamond, germanium, ZnSe or Si). The choice of crystal and accessory (single bounce versus multi-bounce, for instance) is optimized depending upon the physical and optical properties of the sample. Using the Smart Orbit, samples are run simply by placing a small amount of material onto the diamond, and then ensuring intimate contact using a pressure device. No mixing or grinding is necessary, and data can be collected in seconds.

Experimental

Our Nicolet™ 380 FT-IR spectrometer and Smart Orbit ATR accessory, with a diamond crystal, were used. The diamond, brazed into a stainless steel puck, is an ideal material for analysis due to its hardness, spectral range (25000 to below 200 cm^{-1} due to the reflective optics of the Smart Orbit), and chemical inertness. The spectrometer was equipped with a DTGS detector and a KBr beamsplitter. Nitrogen was used for purging the spectrometer and the accessory. Several nutraceutical herbal powders were provided by Western Botanicals for this test. The samples included Barberry Bark, Golden Seal Leaf, Golden Seal, and Yellow Dock. The data was collected in 12 seconds (16 scans at 8 cm^{-1} resolution) over the full mid-infrared range (4000 to 400 cm^{-1}). The spectra were used for the DA without further processing.

Results

Calibration spectra were obtained from multiple examples of four types of herbal nutraceutical powders. Figure 2 shows the groupings that resulted from the method calibration and validation. Note each point represents a spectrum, not just one value. The closer a point (spectrum) is to a cluster, the more likely that the sample belongs to that class. TQ Analyst allows different classes to be selected for visualization. Here, the distance to Yellow Dock is shown on the Y (vertical) axis with distance to Barberry Bark on the X (horizontal) axis. The calibration results for some of the standards seen in Figure 2 are shown in Figure 3. The class of an unknown is determined by calculating the D_M between the unknown and all classes; the software then selects the class with lowest D_M. An analysis report, as seen in Figure 4, cites the Mahalanobis distance between the unknown sample and the nearest class.

Index	Spectrum Title	Actual Class	Calculated Class	Distance	Next Class	Next Distance	Distance to Yellow Dock SW Wild	Distance to Barberry Bark
1	Barberry Bark	Barberry Bark	Barberry Bark	0.8158	Golden Seal Leaf	6.0355	14.4735	0.8158
2	Golden Seal SW ORG	Golden Seal SW ORG	Golden Seal SW ORG	0.8175	Yellow Dock SW Wild	6.2351	6.2351	9.5635
3	Yellow Dock SW Wild	Yellow Dock SW Wild	Yellow Dock SW Wild	0.8163	Golden Seal SW ORG	6.3185	0.8163	14.8369
4	Barberry Bark	Barberry Bark	Barberry Bark	0.8154	Golden Seal Leaf	6.4292	14.9331	0.8154
5	Golden Seal Leaf	Golden Seal Leaf	Golden Seal Leaf	0.8163	Barberry Bark	5.8941	11.8448	5.8941
6	Golden Seal Leaf	Golden Seal Leaf	Golden Seal Leaf	0.8165	Barberry Bark	6.6479	11.4731	6.6479
7	Golden Seal Leaf	Golden Seal Leaf	Golden Seal Leaf	0.8166	Barberry Bark	6.3551	12.3818	6.3551
8	Yellow Dock SW Wild	Yellow Dock SW Wild	Yellow Dock SW Wild	0.8161	Golden Seal SW ORG	5.9025	0.8161	14.3553
9	Yellow Dock SW Wild	Yellow Dock SW Wild	Yellow Dock SW Wild	0.8168	Golden Seal SW ORG	6.8221	0.8168	15.2121
10	Golden Seal SW ORG	Golden Seal SW ORG	Golden Seal SW ORG	0.8171	Yellow Dock SW Wild	6.2598	6.2598	9.3888
11	Golden Seal SW ORG	Golden Seal SW ORG	Golden Seal SW ORG	0.8165	Yellow Dock SW Wild	6.5762	6.5762	9.2105
12	Barberry Bark	Barberry Bark	Barberry Bark	0.8171	Golden Seal Leaf	6.4466	15.0045	0.8171

Figure 3: Partial list of standards used for the DA calibration. The "Actual" class of the material (entered by the user) is compared with the calculated class to reassure its proper classification. The D_M values indicate the distance to the center of that class. The smaller the distance, the better the classification match.

Method title:	Discriminate Analysis for Classification of Holistic Herbs
Method file name:	C:\My Documents\Omnic\Quant\quant for herbs\Herbals Application Note.qnt
Revision:	3
Saved on:	Thu Jun 08 10:27:57 2006 (GMT-07:00)
Spectrum title:	Barberry Bark 2
Spectrum file name:	C:\My Documents\Omnic\Spectra\Application Western Botanical\quant test BB 2.SPA
Spectrum date:	Thu Mar 16 10:33:51 2006 (GMT-08:00)
Full spectrum fit:	97.7
Measurement region fit:	97.7
Index:	1
Class name:	Barberry Bark
Distance to class:	0.82
Pass/Fail:	Pass

Figure 4: Example report for an unknown material. The report includes the class name, its distance to the mean point of that class, and if it passes or fails based on the classification.

Ideally, in a DA calibration, the classes will be well separated (no overlap). In Figure 2, data shown in the upper left region of the plot is strongly correlated with Barberry Bark (note the x-axis value is small) and weakly with Yellow Dock (the y-axis value is large). Data in the lower right similarly correlates strongly with Yellow Dock but poorly with Barberry Bark (small y, large x). The data clusters near the diagonal, due to the Golden Seal and Golden Seal Leaf, have poor correlations with the materials along the axes (both x and y are large). TQ Analyst allows the user to page through all possible correlations, generating a series of pair-wise correlation plots.

The calibration table in Figure 3 shows multiple pieces of information. The title of each calibration spectrum, the class to which the sample is assigned by the user, the class determined (lowest D_M) by the DA calibration, the relevant D_M value, and the name and D_M for the next nearest class are all reported. Every spectrum in this calibration table is linked to a point in the Mahalanobis distance plot. If a standard was not properly grouped (assigned to A while D_M indicates it should be B), an error message will pop up. A warning will also indicate if the spectral fit and distance is beyond a given threshold value.

The analysis of an unknown using the calibrated method is shown in Figure 4. In this case, the user told the software into which class the powder should be assigned. The customizable report specifies how well the unknown spectrum fits within that closest group, and gives the Mahalanobis distance with respect to the average of that group. The report also provides a clear pass/fail indication, where fail indicates the material is classed by the DA into another group, or no group was found.

A simple SOP (standard operating procedure) can now be developed. The sample is run on the Smart Orbit, and the expected identification is entered. The data is processed, and TQ Analyst can provide instructions for what to do: if pass, then allow material to be used, if fail, then quarantine the material for further analysis. The user need not have extensive knowledge of spectroscopy to use this method.

Conclusion

The Discriminant Analysis procedures within TQ Analyst, combined with the Nicolet 380 FT-IR Spectrometer and the Smart Orbit diamond ATR Accessory, provide a powerful analytical characterization tool for nutraceutical herbal powders. Quick, error free, classification of these materials provides both screening and quality assurance tools. The low cost, high throughput and simple operation of our hardware and software make this an ideal solution for the rapidly growing nutraceutical industry.

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