Technical Note: 51768

NIR and Raman: Complementary Techniques for Raw Material Identification

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Key Words

- Antaris
- DXR
- Raman
- NIR
- Raw Material
- RMID

Raw Material Identification (RMID) is one of the most important quality control aspects in pharmaceuticals, food, and chemical manufacturing. Being able to quickly and reliably identify materials before they are used in a process will help ensure the final product meets specifications and reduces costs associated with wasted materials and time. Unfortunately, traditional chromatographic or wet chemistry techniques used to identify materials requires considerable time, technical training and expense. Additionally, these techniques destroy the sample in the process, which adds to the cost and prevents archiving the physical specimens. Ideally, a method of decisive raw material identification would be rapid, easy to perform and non-destructive. Such a method would enable testing and identification at multiple stages of a process (from loading dock to process line) which is a critical part of Process Analytical Technology (PAT) initiatives.

Fortunately, spectroscopic techniques can meet these requirements. Since spectroscopy involves the interactions of light with materials, the samples are not necessarily damaged or destroyed. Spectroscopy is much more rapid than chromatographic or wet chemical methods and can provide proper identification in less than two minutes. Fourier transform spectroscopic instruments can provide the information even faster, often in less than 15 seconds. The Thermo Scientific Antaris II Fourier transform near-infrared (FT-NIR) and the Thermo Scientific DXR SmartRaman spectrometers are ideally suited for even the most challenging RMID applications. These techniques are complementary to each other which increase their value and empower their more widespread use within a facility. These techniques can be used to identify a wide variety of raw materials and can even distinguish between closely related ones that have similar chemical structures. Figure 1 shows both the Antaris™ II Method Development Sampling (MDS) NIR analyzer and the DXR SmartRaman spectrometer.

Figure 1: DXR SmartRaman (left) and Antaris II MDS NIR (right) analyzers used for the raw material analysis. Both systems are designed to be easily used by process personnel.

Near-infrared spectroscopy is a technique that uses light between 10,000 and 4000 cm^{-1} to excite certain molecular vibrations. The molecular bonds that are most sensitive to excitation in this region of the spectrum tend to be polar with the vibrations changing the bond's dipole moment. Absorptions in the near-infrared region are less intense than in the mid-infrared region which allows for deeper penetration of the source light into the material. This has two advantages: a larger amount of material is exposed, which reduces sampling error; and glass vials and plastic films used as containers produce less spectroscopic interference. These advantages allow for spectroscopic sampling to be done without removing the material from the original container or exposing the operator to potentially hazardous conditions. Finally, the Antaris NIR operating software, Thermo Scientific RESULT, is designed to be easy to operate, such that non-technically trained individuals can definitively identify materials as they arrive in the facility on the loading dock, and throughout the process.

Raman spectroscopy is also a vibrational technique but differs substantially from other spectroscopic techniques. Raman spectroscopy relies strictly on the non-elastic scattering of photons from a laser source. As the photons from the laser interact with the material, a few are absorbed. The energy from these photons is partially absorbed and the remaining energy is re-emitted as scattered light at a different frequency. The scattered light is shifted from the original laser frequency by an amount that depends on the energy absorbed by the molecular bonds. In contrast to NIR, the molecular bonds associated with Raman scattering are non-polar. Therefore, it is common for Raman to provide information about the carbon-carbon bonds along the backbone of organic raw materials. The spectrum generated by a Raman instrument generally has sharper and better resolved peaks than NIR which can provide more chemical information of unknown samples. While Raman spectroscopy requires more technical expertise than NIR, the DXR SmartRaman incorporates features such as autoexposure, Smart backgrounds and automated alignment and calibration, making it easily used with minimal training. The Raman excitation laser can penetrate optically clear materials such as glass or plastic which also limits the spectroscopic interference from the sample containers.

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The two methods are technically different in that they excite different vibrational states. Therefore, molecular bonds that are not active with one method are often active with the other. Furthermore, the information that is generated from each method is often treated differently. NIR spectroscopy usually relies on chemometrics to interpret the resulting spectra. Chemometrics involves statistical methods to classify raw materials into groups based on a standard training set of materials. Developing the standard training set is application specific and uses the materials likely to be encountered at the specific facility. Once the standards spectra have been loaded in the software, NIR provides results very easily and rapidly. Raman spectroscopy, by contrast, will provide more chemical data and is commonly used to learn information about a material that little is known about. Large library databases that are often commercially available are easily searchable with Raman because the sharper, well-defined peaks are conducive to this methodology. Because of the contrasting features of the two complementary technologies, the two in tandem are ideally suited for comprehensive RMID for even the most challenging applications.

Raw materials identification is one of the most common types of process analysis in a variety of industries. For this study, common pharmaceutical materials including excipients, active ingredients, lubricants and buffer salts were analyzed. The materials were subjected to both NIR and Raman analysis to determine the most efficient means of rapidly and decisively identifying all of the materials in a typical process environment. Several samples were collected and placed in polyethylene bags for the analysis. While the samples were analyzed through the polyethylene bags in this study, other plastic or glass containers can also be used, or, if required, the materials can be analyzed directly.

Table 1 lists the 27 materials collected and analyzed for this study. Included in this list are various sugars and polysaccharides that have similar chemical structures, hydrated and anhydrous inorganic salts used as buffers, common pharmaceutical ingredients and other buffers. These materials are commonly found throughout the pharmaceutical industry and were selected as representative of the wide variety of chemicals capable of being detected with spectroscopy. Based on the strengths of both NIR and Raman, it was quickly determined that initial and rapid analysis of the materials could usually be done with NIR; while more difficult or challenging samples were identified by Raman as a follow on technique.

Index	Class Name	Index	Class Name
1	acetaminophen	15	mannitol
2	acetylsalicylic acid	16	$MgSO_4$ $7H_2O$
3	aspartame	17	$MgSO_4$ anhyd
4	$CaCl_2$ $2H_2O$	18	micro cellulose
5	$CaCO_3$	19	$MnSO_4$
6	$CaHPO_4$ anhyd	20	$NaHPO_4$ anhyd
7	calcium stearate	21	pearlitol
8	crosspovidone	22	polyethylene
9	CuO	23	salicylic acid
10	ethyl paraben	24	sodium citrate
11	folic acid	25	stearic acid
12	lactose monohydrate	26	sucrose
13	magnesium stearate	27	talc
14	maltose		

Table 1: List of 27 items used in the raw material analysis. Items were selected as examples of the wide variety of materials found in pharmaceutical process plants.

The Antaris II FT-NIR system was used to produce spectra from the test raw materials. Five samples of each material were scanned by placing the bags containing the samples on top of the integrating sphere. Figure 2 demonstrates the positioning of the samples on the instrument. The samples were analyzed using 16 scans with four wavenumber resolution between 10,000 and 4000 cm^{-1}. The resulting standard scans were used to create a chemometric method using Thermo Scientific TQ Analyst software. A Discriminant Analysis algorithm was chosen using multiplicative signal correction (MSC) for the pathlength. To limit the influence of baseline offsets that might occur when using reflection measurements, the raw spectra were first treated to a first derivative filter. Additionally, the first derivative spectra were smoothed using a Norris smoothing algorithm (segment 9; gap 7). Representative raw spectra of some of the raw materials are shown in Figure 3.

Figure 2: Typical sample scanned on the Integrating Sphere of the Antaris II FT-NIR system. Samples were easily scanned through the plastic bag in about 15 seconds.

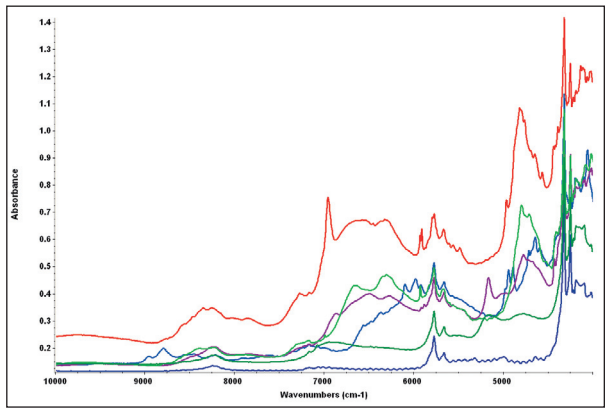

Figure 3: Representative NIR raw spectra from some of the standards in the training set. The spectra were analyzed using the first derivative with Norris smoothing (not shown).

A diagnostic to determine the quality of the chemometric method is found by comparing Mahalanobis distances. Materials that belong to the same class will have similar spectral characteristics. These similar spectra will group together around a central typical spectrum, and the distance an individual spectrum falls from that typical spectrum is quantified by assigning it a Mahalanobis distance. If a given spectrum is close to this central spectrum it will be assigned a lower Mahalanobis distance. Conversely, if a spectrum is very different, it will be assigned a higher Mahalanobis distance. Mahalanobis distances are similar to standard deviations in that they provide a quantitative measurement of how much a given spectrum deviates from the central typical spectrum. Figure 4 is an example plot comparing the Mahalanobis distances of the salicylic acid spectra and the acetaminophen spectra. By comparing the distance a particular spectrum lies from the origin of the axis in the plot the identity of the material can be deduced. The plot in figure 4 shows the Mahalanobis distances for a selected spectrum. It falls 21.9 units from the center typical spectrum of salicylic acid, but only 0.89 units from the typical acetaminophen spectrum. With such a low Mahalanobis distance this spectrum is correctly identified as being obtained from an acetaminophen sample.

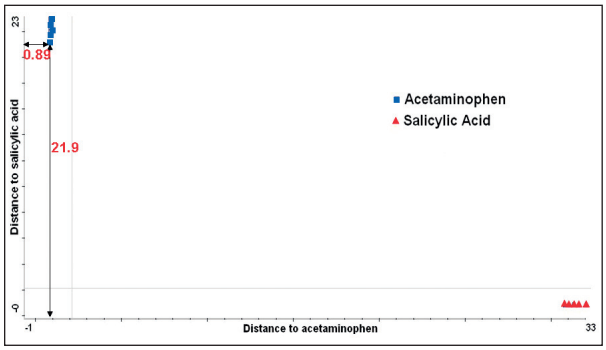

Figure 4: Example Mahalanobis distance plot of salicylic acid (red triangles) and acetaminophen (blue squares). The indicated spectrum in the upper left is 0.89 Mahalanobis units from the typical acetaminophen spectrum while it is 21.9 units from the typical salicylic acid spectrum. It is therefore classified as acetaminophen.

Most of the samples were correctly and decisively identified in their correct classes. Table 2 indicates how close samples are to the nearest incorrect class. The average Mahalanobis distance to the nearest incorrect class will indicate how likely the material will be incorrectly classified. Most of the numbers are relatively high, indicating there is wide separation from the next closest class and low chance of being incorrectly identified. However, there were four cases where the spectra were similar enough to an incorrect class that misidentification may occur. These four materials had Mahalanobis distances to the nearest incorrect class of less than three. Calcium carbonate was particularly problematic in that it was incorrectly classified completely. The chemometric method showed that the calcium carbonate samples provided very little spectroscopic information and the information that was there was due to the plastic bag used to contain the material. Figure 5 shows the NIR spectra of the calcium carbonate and the polyethylene bag; note the spectra are virtually identical as the calcium carbonate has no absorbance in the NIR region.

Index	Class Name	Distance to Next Class
1	acetaminophen	7.95
2	acetylsalicylic acid	9.82
3	aspartame	7.26
4	$CaCl_2$ $2H_20$	4.52
5	$CaCO_3$	0.40
6	$CaHPO_4$ anhyd	3.89
7	calcium stearate	5.34
8	crosspovidone	7.57
9	CuO	4.27
10	ethyl paraben	8.14
11	folic acid	6.50
12	lactose monohydrate	5.33
13	magnesium stearate	2.57
14	maltose	6.71
15	mannitol	6.87
16	$MgSO_4$ $7H_2O$	10.25
17	$MgSO_4$ anhyd	1.36
18	micro cellulose	6.79
19	$MnSO_4$	2.57
20	$NaHPO_4$ anhyd	5.59
21	pearlitol	6.33
22	polyethylene	6.12
23	salicylic acid	9.57
24	sodium citrate	8.98
25	stearic acid	6.36
26	sucrose	7.75
27	talc	3.41

Table 2: Mahalanobis distances to the nearest incorrect class for the samples. Larger numbers indicate clear separation and little chance of misidentification. Two samples (yellow) were less than three Mahalanobis distance units from an incorrect class and two others (blue) were less than two units from an incorrect class indicating higher chances of misidentification.

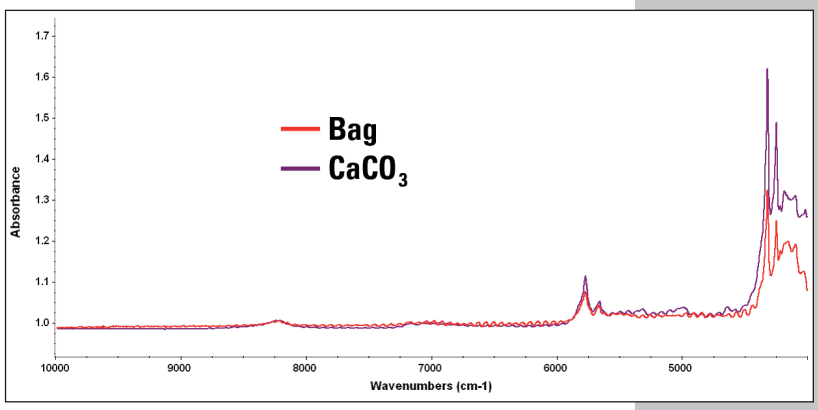

Figure 5: NIR spectra of calcium carbonate compared to the polyethylene bag. Calcium carbonate does not have significant spectral information in the NIR region and is therefore not easily identified with NIR.

While NIR analysis performed well for most of the samples, some samples were shown to be challenging. Anhydrous polyatomic ionic salts such as calcium carbonate or magnesium sulfate often will not have enough absorbance in the NIR region to produce useable spectra. These materials may be confused with each other and incorrectly classified. Alternatively, materials that are chemically similar to the container may also be misclassified. Magnesium stearate, for example, has a similar spectral signature as the polyethylene bag and may be classified incorrectly.

Raman spectroscopy was selected to determine its ability to properly identify the four samples that were challenging for NIR. Raman analysis is usually performed in a laboratory setting where the measurement parameters can be more easily controlled by the operator. The nature of Raman spectroscopy dictates more control over measurement parameters and therefore more skilled operators then NIR. The result, however, is that more chemical information can be obtained and challenging samples may be identified.

The four samples that were not definitively identified with NIR were analyzed with the DXR SmartRaman instrument. The samples were placed on the Thermo Scientific Universal Platform Sampling accessory and analyzed using a 780 nm laser. An autoexposure feature optimized data collection parameters to achieve a signal to noise ratio of at least 100. Data collection using this feature could take up to two minutes of acquisition time. Whereas the NIR sampling used Discriminant Analysis the Raman sampling was more conducive to large database library searches. Figure 6 demonstrates the success of this method with the calcium carbonate sample. Note the spectral peaks of the sample compared to that found in the library. Peaks located at 2850 and 1400 cm^{-1} shift are residual from the bag. In all four cases the samples were correctly identified by the library search with the Raman spectra.

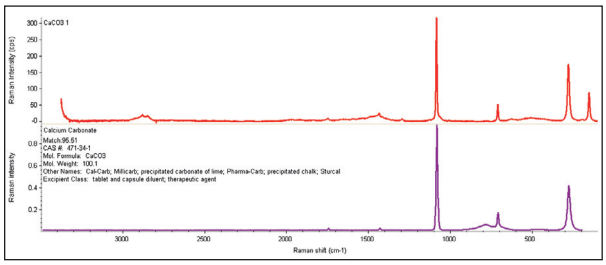

Figure 6: Raman spectra of calcium carbonate sample (top) and standard calcium carbonate from the library database (bottom). The spectra are virtually identical with a spectral match value of 95.5.

A library search is advantageous because it is a simple, fast way to identify unknown materials. How close the sample is to a given standard in the library can be quantified with a spectral match score, where a perfect match has a score of 100. The spectral match scores for the four samples were: calcium carbonate 95.5; magnesium stearate 88.2; magnesium sulfate 96.3; manganese sulfate 99.4. Large library databases are also commercially available or are easily built from a set of standards. As long as the libraries are comprehensive, they are usually very successful. However, if the library is not comprehensive it is possible to incorrectly identify an unknown.

By using a combination of NIR and Raman spectroscopy even the most challenging raw materials can be identified. The two spectroscopic techniques are complementary with some materials being better suited for NIR, while others are better suited for Raman. NIR has better sample penetration and samples a larger area, whereas Raman is generally limited to the width of the laser. Therefore, large grained heterogeneous samples are more conducive to analysis with NIR. In aqueous samples where the water bands might be interfering, Raman is possibly a better choice than NIR. Figure 7 shows a chart of various sample types and how they might be best analyzed by the two techniques. Samples that fall to the left are better suited for NIR analysis while those on the right are better suited for Raman. The method described in the application above initially uses NIR for rapid and easy analysis of the raw materials. The more challenging samples were then re-analyzed with the Raman spectrometer. In this way a wide variety of raw materials can be most efficiently identified very rapidly.

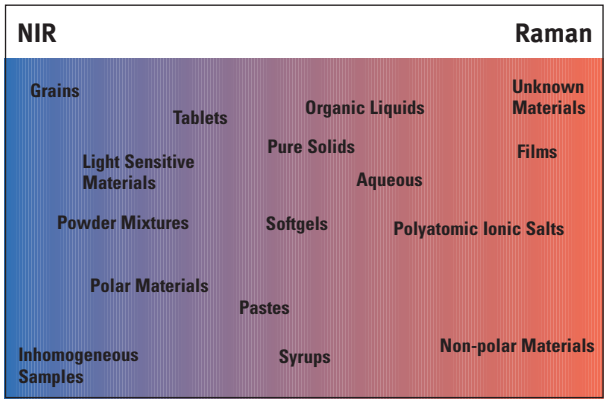

Figure 7: Chart of sample types and how they are best suited for analysis. Samples to the left tend to be better suited for NIR, those to the right tend to be better suited for Raman spectroscopy.

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TN51768_E 04/09M