Application note

Visualizing content distribution in tablets with Raman imaging

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Thermo Scientific DXR3xi Raman Imaging Microscope.

Introduction

Pharmaceutical formulations are complex, multicomponent mixtures. There is a need to identify and verify components, and also to evaluate the distribution of these components. The distribution of components within a product can affect the stability and functionality of the final product, which is why blend and content uniformity are important for pharmaceutical formulations.

With the wide variety of active pharmaceutical ingredients (API) that can be combined with numerous excipients, it is important to have flexible analytical tools that can quickly provide accurate data on a variety of formulations. Raman spectroscopy is a proven analytical method for the analysis of pharmaceutical formulations. It not only can be used for identifying materials but also can provide detailed chemical and structural information. When Raman spectroscopy is used for imaging a sample, it provides information on the spatial distribution of components and variations in chemical structure throughout the sample. Images based on the Raman spectroscopic data are powerful visual tools for quickly evaluating samples. With single-point Raman spectroscopy, information about a specific location on the sample is provided. With Raman imaging, an expanded view of the sample is provided, allowing for a more thorough analysis of the homogeneity and spatial distribution of components. There are ways with single-point analysis to expand the analysis area to gain a better representation of the whole sample, but this is not the same as Raman imaging. When single-point analysis is expanded, the contributions from the various locations are combined into a single spectrum and the information from individual locations is lost. Raman imaging provides rapid access to vast amounts of spectroscopic data. This ability to rapidly collect more data over a larger area represents a more statistically relevant analysis of the samples and a vast improvement in the analysis of pharmaceutical products.

There are many types of pharmaceutical formulations and each has its own set of analytical requirements. Raman spectroscopy is flexible enough to be used to solve a great number of these analytical problems. This application note will focus on one of these problems, the rapid evaluation of whole tablets using Raman imaging. The goal is to identify and evaluate the spatial distribution of the tablet components in a fast and efficient manner.

The Raman data from the samples in this application note were collected using a Thermo Scientific™ DXRxi Raman Imaging Microscope and the accompanying Thermo Scientific™ OMNIC™xi Raman imaging software. This product represents an evolution in the DXR Raman product line, making it possible to collect Raman spectral data at astoundingly fast rates. This increase in acquisition speed means that collection of large area Raman images is now not only practical but routine. The product retains the best qualities of the original Thermo Scientific DXR Raman microscope such as autoalignment and calibration, and user changeable laser, filters and grating, but with a new state-of-the-art high-speed microscope stage synchronized with a sensitive EM CCD detector. These imaging components accurately and reliably collect a very large amount of data in a very small amount of time. The OMNICxi software also represents an evolution in software specifically designed for imaging; providing a convenient and easy-to-use graphic interface for harnessing all additional data. New data collection options allow for quick surveys to locate important areas of interest, easy optimization of collection parameters, and single, multiple or auto region collects. The software also contains powerful data analysis options for processing the data into informative Raman images.

API distribution

The sample used to demonstrate the advantages of Raman images in this application note is a common over-the-counter pain relief product specifically formulated for treating migraines. In this product, multiple APIs make up a relatively large portion of the total tablet. The tablet is reported to contain 250 mg of acetaminophen, 250 mg of aspirin, and 65 mg of caffeine. The exact amounts of the inactive ingredients were not provided. The average weight of a tablet was 676 mg, so the approximate percentages of the active components are 37, 37 and 9.6% respectively. The remaining 16.4% represents a variety of inactive excipients. The tablet has a diameter of approximately 11 mm and the outer coating of the tablet was removed before analysis to provide better access to the internal structure. Residual outer coating was observed in the analysis of the whole tablet (see Figure 1).

A quick Raman image of the whole tablet (see Figure 1) can be collected to show the API distribution. It took approximately eight minutes at an acquisition rate of 550 Hz (1.8 ms per spectrum) to collect the 226,000 spectra that make up the Raman image. The spacing between the spectra was set at 25 microns. A 10× objective was used to focus the beam from a 532 nm laser onto the sample. The image is the result of a multivariate curve resolution (MCR) analysis of the Raman data. The different colors indicate the presence of different components distinguished by the MCR routine. The components were identified by an automatic searching routine against integrated commercial libraries. This single-step analysis using the OMNICxi software takes the initial data and provides a final identified image in one efficient step. In this image, the blue particles represent aspirin, the green particles represent acetaminophen and the yellow particles represent caffeine. Specific colors used to create the images can be selected by the user. The red layer on the outside of the tablet represents titanium dioxide in the outer coating of the tablet. This coating was removed from the top surface of the tablet, but still remains on the sides.

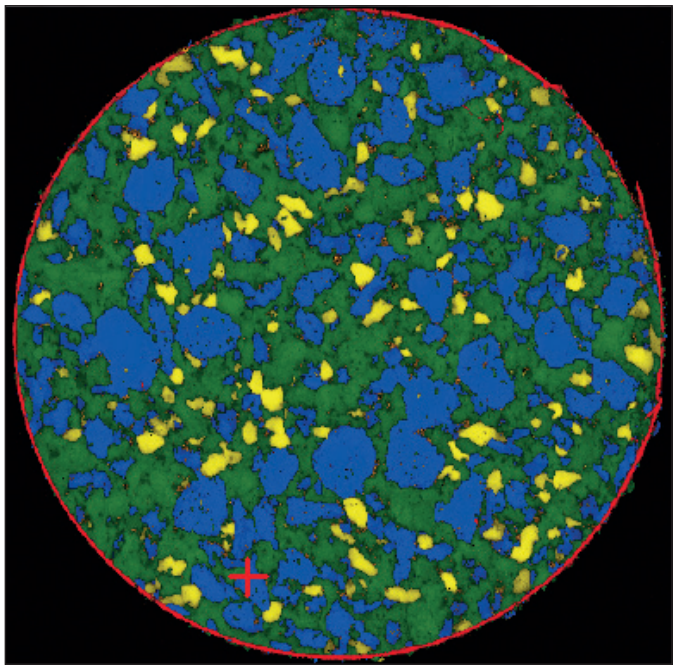

Figure 1. Raman image of the whole tablet. MCR image: Blue – Aspirin, Green – Acetaminophen, Yellow – Caffeine, and Red – titanium dioxide.

In this sample, particle size of the active ingredients is relatively large, resulting in experimental parameters sufficient for obtaining a relatively good representation of the spatial distribution of the components. However a more detailed image with greater spatial resolution may be required. The initial image can be used as a guide to select an area or areas of interest on the sample where higher resolution images could provide additional information. The software supports the automated collection of multiple regions if more than one area of interest is identified.

Additional excipient identified

Figure 2 shows a Raman image based on an MCR analysis of an area on the sample of approximately 1.6 × 1.7 mm. The Raman image consists of 116,000 spectra with a spacing of 5 microns. Image collection time was approximately 55 minutes. A higher magnification objective (50×) was used to support the higher spatial resolution measurements and a longer exposure time was used (200 Hz, 5 ms per point) because the excipients tend to have lower Raman scattering coefficients compared to the APIs. In this case, along with the 3 APIs, the analysis now indicates the presence of an excipient (starch). This excipient is present in low concentrations with small particles, and it has a relatively lower Raman scattering coefficient compared to the APIs – all factors contributing to it going unnoticed during the first analysis.

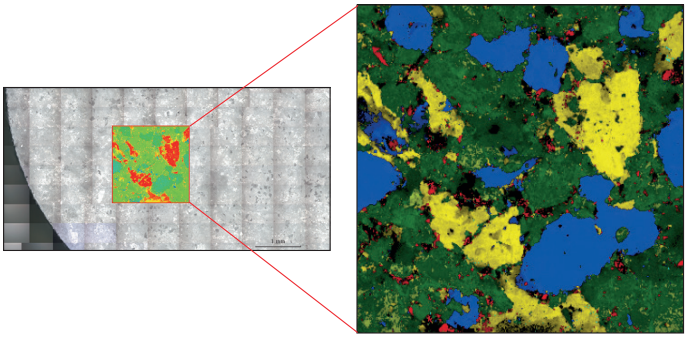

Figure 2. Higher spatial resolution Raman image of an area on the tablet. MCR image: Blue – Caffeine, Green – Acetaminophen, Yellow – Aspirin, and Red – starch.

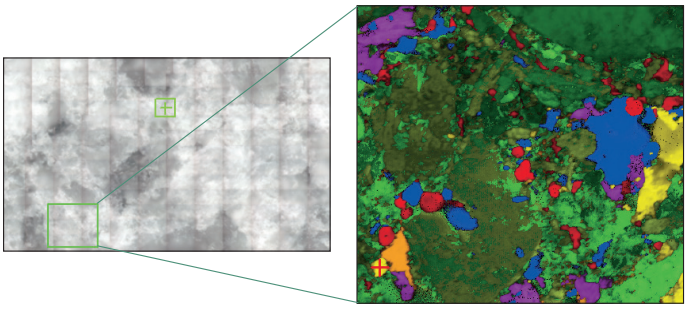

Figure 3. Higher spatial resolution Raman image of an area on the tablet. MCR image: Blue – Aspirin, Green – Acetaminophen, Yellow – Caffeine, Red – starch, Fuchsia – microcrystalline cellulose, and Orange – sodium lauryl sulfate.

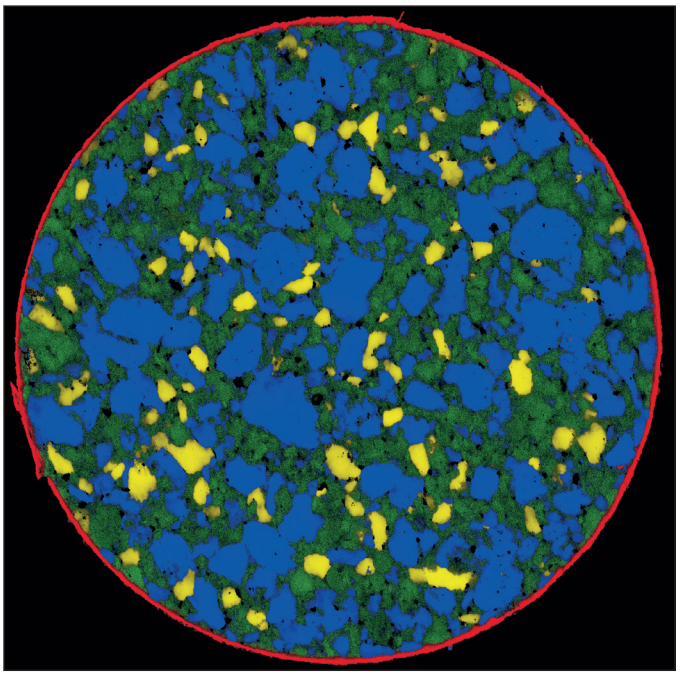

Figure 4. Raman (5.4 million spectra) image of the whole tablet. 5 micron steps. MCR image: Blue – Aspirin, Green – Acetaminophen, Yellow – Caffeine, and Red – titanium dioxide.

It is possible to acquire an even more detailed view of the sample by extending this approach to a higher spatial resolution image. Figure 3 shows a Raman image based on the MCR analysis of data collected from an area of approximately 225 × 250 microns. This image is derived from 229,000 spectra with a spacing of 0.5 microns. A 100× objective was used to provide access to higher spatial resolution data. Using an acquisition rate of 100 Hz (10 ms per point), the image collection time was approximately three hours, but provided significantly better detail at a much better spatial resolution.

Using this level of detail, it was possible to identify additional excipients. In addition to starch, it was now possible to locate and identify microcrystalline cellulose and sodium lauryl sulfate – demonstrating how the details of the image can change with spatial resolution. Conversely, and if appropriate, the process of quickly evaluating the whole tablet to select specific areas of interest for more detailed investigations saves time compared to collecting higher spatial resolution images of the entire sample, and in most cases will be equally effective.

5.4 million spectra

The amount of data that can be collected is only limited by the ability of the computer to process and store the required quantity of data and the time the user wishes to invest in collecting such large images. An example of this is shown in Figure 4.

This Raman image consists of 5.4 million spectra with a spacing of 5 microns. The spectra were collected at a rate of 550 Hz (1.8 ms per spectrum) and even at that rate it took over three hours to collect the whole image. The image was produced from an MCR analysis of the Raman data. The components identified by the MCR routine are assigned different colors and shown in the image. The aspirin is blue, the acetaminophen is green, the caffeine is yellow and the titanium dioxide in the outer coating is red. The MCR analysis not only provides a visual representation of the spatial distribution of the components and an automatic searching routine to identify the components, but also includes a particle size analysis for each of the components. The relative areas occupied by the various components are calculated as part of this analysis routine. While this is not strictly a quantitative analysis, the relative areas occupied by the various components can be used to give an approximate measure of the relative amounts of the components on the exposed surface. See Table 1 for percentages obtained. This certainly has some assumptions built into it and will not replace actual quantitative analysis methods, but it does provide some additional information.

The spectral quality was sufficient to observe the spatial distributions of the APIs and the outer coating. Longer exposure times will be required to observe much weaker Raman scatters, such as the excipients. This does give a more

detailed view of the spatial distribution than the first image of the whole tablet (Figure 1), but it does not provide the fine detail seen in Figure 3. With this particular sample, locating significant amounts of excipients required the higher spatial resolution and exposure times used in Figure 3. However, collecting the whole tablet at that resolution is not practical. In this case, it was more effective to collect a quick image of the whole tablet and then focus on specific targeted areas for the higher spatial resolution images. While the ability to collect larger higher spatial resolution images is available, the time and effort put into collecting and handling such large amounts of data needs to be weighed against the benefits.

Component	Percentage obtained	Reported actual weight percentage
Acetaminophen	35.4	37
Aspirin	38.6	37
Caffeine	7.7	9.6

Table 1. Approximate percentages of components based on areas occupied in the image.

Conclusions

This application note has shown how Raman imaging can be used for the analysis of whole tablets and detailed imaging of specific areas of interest. Raman imaging provides a fast way of identifying a variety of components and evaluating the spatial distribution of those components in the tablet. It provides a much more detailed method for assessing content uniformity by providing the actual distribution of components within the tablet and not just a single composite spectrum representing the whole tablet. Whether the goal of the analysis is the quick imaging of a whole tablet or a detailed investigation of a specific area at high spatial resolution, the DXR3xi Raman Imaging Microscope and the OMNICxi software provides a fast, flexible, and convenient way of collecting and analyzing Raman images.

The data were collected using an older model instrument DXRxi Raman Imaging Microscope. Currently, Thermo Fisher Scientific offers an improved model, the DXR3xi Raman Imaging Microscope, which offers superior speed and performance over its predecessor.