Application note

The analysis of low dose tablets and polymorphs using Raman imaging

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Keywords

DXR3xi Raman Imaging Microscope, active pharmaceutical ingredient, pharmaceuticals, polymorphs, Raman imaging, tibolone

Goals

Using Raman imaging for evaluating the spatial distribution of active pharmaceutical ingredients in lower concentration tablets including the differentiation of polymorphs.

Introduction

The pharmaceutical industry must be able to fully characterize the active pharmaceutical ingredient (API) in product formulations. Some APIs adapt different structural forms that affect the functional behavior of the drug. Solvates, polymorphs, degree of crystallization, and salt formation all represent some of the possible structural variation of APIs. These variations can affect how the drug is delivered and its therapeutic effectiveness. Homogeneous distribution of components is often an important factor in the development of formulation and processing procedures. Various manufacturing processes have the potential to alter the nature and distribution of APIs, so it is often necessary to check the product after these processing steps and not just the final product.

Raman spectroscopy can be used to identify and verify components, as well as providing detailed information on molecular structure and chemical environments. It can be used to distinguish very similar materials. For instance, Raman spectroscopy can distinguish between polymorphs, which are the same chemical compounds occurring in different crystalline forms. The particular polymorph present will depend on the crystallization conditions and the stability of the various forms. Some polymorphs can convert to other forms during processing steps, which is important to note because polymorphs can vary in therapeutic effectiveness. Additionally, issues with intellectual property may arise if one polymorph is covered in a patent and another is not. Raman imaging takes this powerful spectroscopic analysis and extends it across a sample to create images of the sample – proving it to be a very powerful imaging and analysis tool.

Raman micro-spectroscopy can be used to analyze very small samples. In the case of pharmaceutical formulations, the sample itself is not necessarily small, but the API (or other components) may be present in small quantities. Single point Raman spectroscopy is excellent for providing information about a specific location on the sample, but it is not always easy to locate which areas of the sample are important or representative of the whole sample. Larger spot sizes can be used to cover more of the sample, but then the spectrum becomes a convolution of many components. While it still might be possible to decipher the spectral contributions of the various components, any spatial information is lost.

When using Raman imaging, a view of the whole sample is provided, while preserving spatial information – proving particularly important when evaluating homogeneity and spatial distribution of components, as well as for locating lower concentration components. Raman imaging provides this vast amount of spectroscopic data at a rapid speed, allowing for improved analysis efficiencies a more statistically relevant analysis of the samples.

DXR3xi Raman Imaging Microscope.

Instrumentation

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 Imaging 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.

Raman imaging results

This application note will illustrate how Raman imaging can be used with tablets where the API is present in relatively low concentrations. This is a common occurrence with many pharmaceutical formulations and the requirements for the analysis of these types of products are somewhat different from those where the majority of the tablet is the API. The sample used for this analysis was a tablet of Tibolone. Tibolone is a synthetic steroid used in hormone replacement therapy in postmenopausal women. Tibolone is also known to exist in different polymorphic forms. It has been reported to crystallize in both a monoclinic and in a triclinic form. The concentration of Tibolone in the tablets was small (about 3% by weight). The goal of the analysis was to identify the Tibolone in the tablet, to show the spatial distribution of the Tibolone and to see if there was any evidence for the existence of different polymorphs in the tablet. This particular example was chosen to illustrate the way that Raman imaging can be utilized for these types of samples and this approach certainly can be extrapolated to other types of samples.

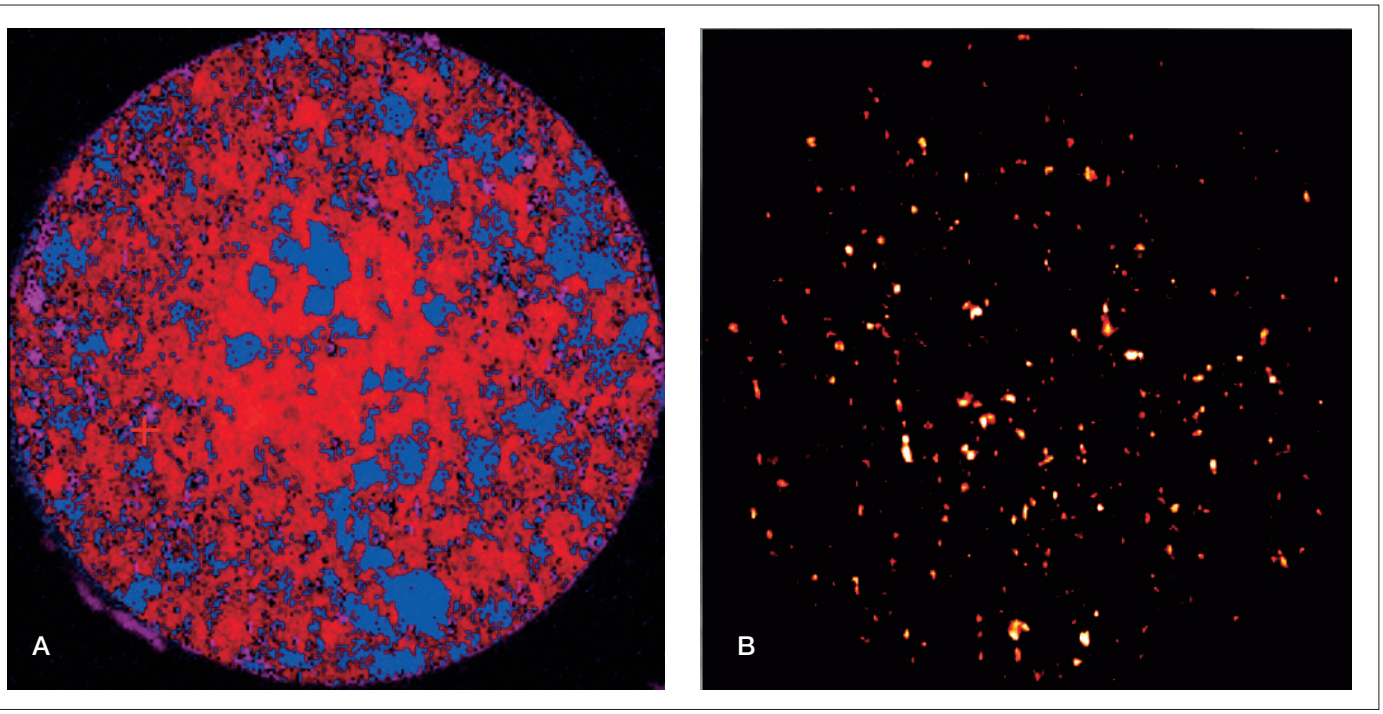


Figure 1. (A) MCR derived Raman image of the whole Tibolone tablet. Red: Starch and Blue: Lactose. The Fuchsia particles are highly fluorescent. (B) A Raman image based on the peak height at 2102 cm^{-1} from the spectrum of Tibolone and showing the distribution of Tibolone in the tablet.

The first step in the analysis was to generate a Raman image from the whole tablet (Figure 1A).

This image is generated from an MCR routine from 52,000 spectra with a spacing of 25 microns. A relatively slow acquisition rate (50 Hz, 20 ms per spectrum) and 10 scans were used because the excipients that make up this tablet have a lower Raman scattering coefficient and the API is present in lower concentrations. It took about three hours to collect an image of the tablet. A 532 nm laser was used along with a 10× objective. In this case, the majority of the tablet is defined by two excipients. The red particles represent starch and the blue particles represent lactose. The presence of the Tibolone in the tablet was most evident when the Raman intensity (peak height) at 2102 cm^{-1} was used to generate a Raman image. Image B in Figure 1 shows the spatial distribution of the Tibolone in the whole tablet, and while it was possible to visualize the distribution of the API this way, the data was not sufficient to clearly differential polymorphs.

In an attempt to improve the analysis for the existence of polymorphs, it was thought that a higher spatial resolution image would be more effective. Figure 2 shows a Raman image of an area approximately 1.1 × 1.6 mm on the sample.

This image consists of 75,000 spectra with a spacing of five microns. A rate of 50 Hz and 25 scans were used and it took about 10 hours to collect this image. The size of the analysis area should be sufficient to give a statistically relevant example of the whole tablet. The greater spatial resolution of this image allows for a more definitive evaluation of the distribution of Tibolone. It was even possible to detect evidence of two polymorphs of Tibolone. Figure 3 shows the spatial distribution of the two polymorphs. The spectral differences observed in the Tibolone spectra were confirmed as belonging to the different polymorphs by comparison to the Raman spectra of the isolated polymorphs.

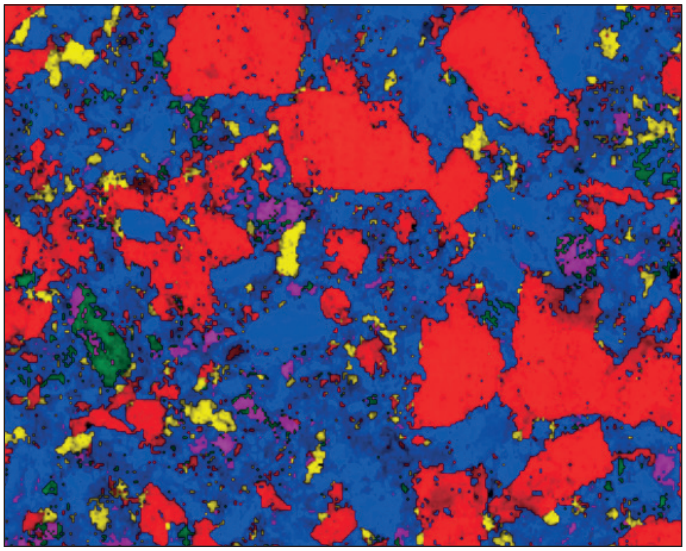
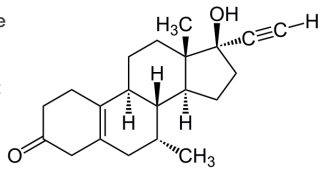


Figure 2. A MCR derived Raman image from an area on the Tibolone tablet. The Blue and Green particles are Lactose and the Red particles are Starch. The Fuchsia particles are highly fluorescent and the Yellow particles are Tibolone.



Chemical structure of Tibolone

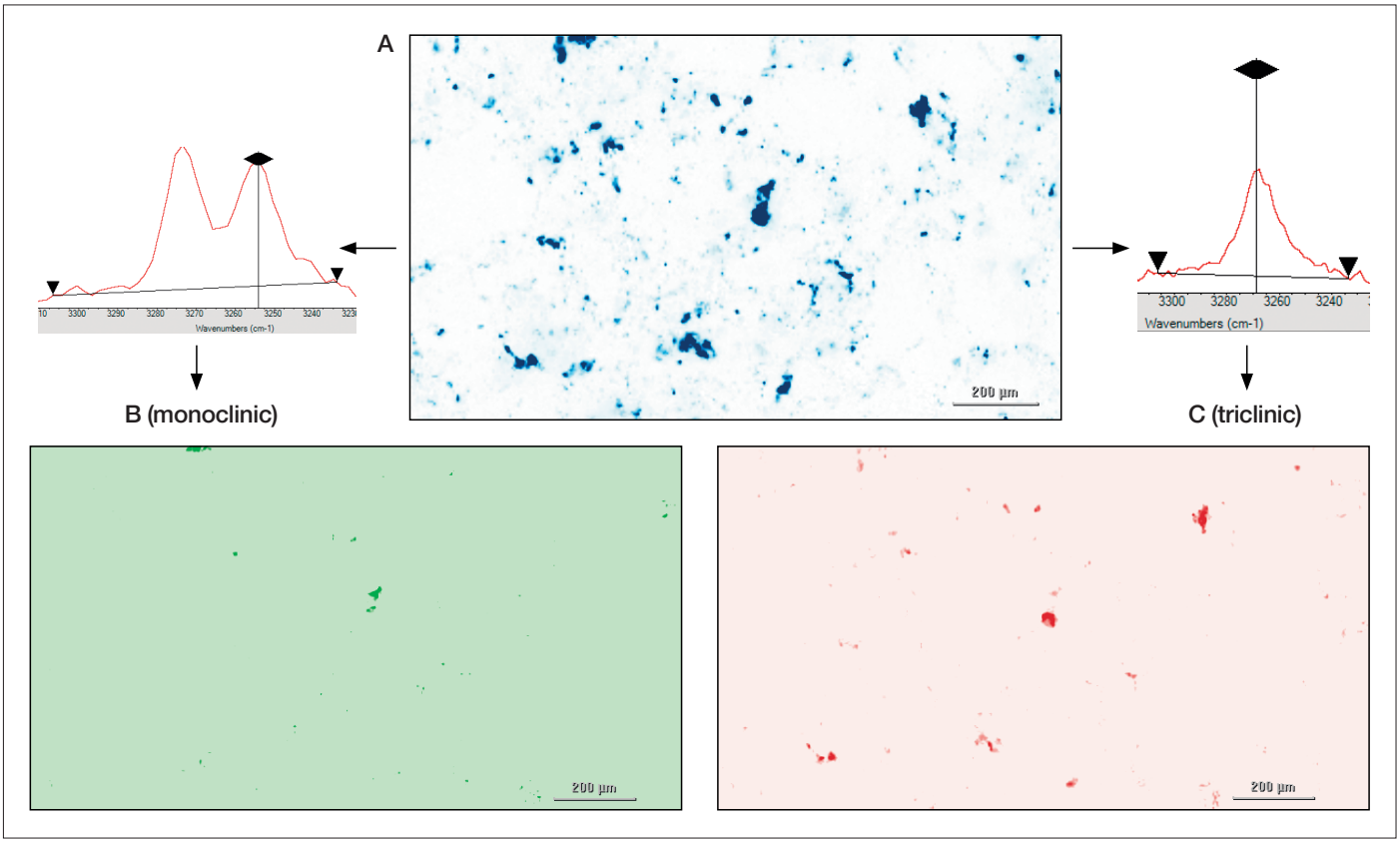


Figure 3. Distribution of polymorphs of Tibolone. Raman images derived from peak heights indicating the distribution of different polymorphs of Tibolone. (A) All Tibolone, (B) Form I (monoclinic), (C) Form II (triclinic)

Peak height profiles were more effective than the MCR routine for distinguishing the two polymorphs, which is likely due to the subtle differences between the spectra of the polymorphs and the small concentration of API. The spectral variation between the polymorphs is a very small contribution to the overall spectral variation observed in the data set as a whole and, thus, is not readily picked up with MCR routine. However, since the software provides access to different profile choices, it is possible to select the profile that works the best for a particular data set. If the goal of the analysis was just to evaluate the spatial distribution of the API in the tablet, the initial image would have been sufficient; but, if the analysis requires more detail, such as the identification of polymorphs, this can be accommodated by acquiring higher spatial resolution images with good spectral quality.

Conclusions

This application note illustrated how Raman imaging can be used for the analysis of pharmaceutical products where the API is present in fairly low concentrations – a common situation for many pharmaceutical products. Raman imaging can readily locate and identify API, as well as the excipients. The speed at which a whole image of the tablet can be collected represents a vast improvement over simple Raman mapping, making this kind of analysis practical. Not only can Raman imaging be used for identifying and displaying the spatial distribution of API, but it also can be used for more detailed spectral analysis, such as differentiation of polymorphs. TheDXR3xi Raman Imaging Microscope provides the power of Raman imaging to solve analysis challenges and the OMNICxi software provides a flexible and convenient interface for collecting and processing 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 model.