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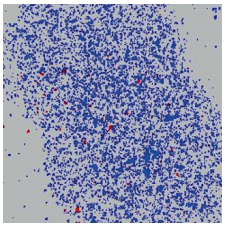
Raman Spectroscopy

Morphological and chemical characterization of pharmaceutical formulations



Application Note

Pharmaceutical RA73

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Abstract : In order to control formulations of pharmaceutical products, characterizing their active compounds is critical, especially in terms of morphological and chemical characterization of particles. In this application note, two formulations from a generic and an innovator nasal spray have been analyzed to compare their compounds size and shape, and chemically characterized to compare the two formulations based on the ParticleFinder™ app for LabSpec 6. A focus is done on the active compound.

Keywords: Raman microscopy, ParticleFinder™, Particle Analysis, Pharmaceutical Products, API Characterization

Introduction

One of the major challenges in the pharmaceutical development of a new product consists in its formulation, highly impacting the release and the efficiency of the active molecules in the body. During this step, actives and excipients are mixed together to produce the drug in its final form. The size and shape of the particles, the chemical form of the active, as well as its distribution, are multiple parameters that could have a huge effect on the drug efficiency.

In this paper, we present how Raman microscopy can be used as a powerful tool for pharmaceutical compounds characterization, both morphologically and chemically. Raman microscopy is the combination of optical microscopy and Raman spectroscopy. Optical microscopy provides information about the 2D morphology of particles down to a few hundred of nanometer-size, while Raman spectroscopy gives access to chemical composition of the dispersed particles. This technique not only distinguishes the different chemicals in a mixture but also identifies polymorphs.

We focus on the analysis of generic and innovator nasal sprays, by investigating their differences in terms of particles' size and shape distribution, as well as chemical fraction homogeneity.

Materials and methods:

The LabRAM Soleil Raman microscope from HORIBA has been used for the morphological and chemical characterizations of the pharmaceutical product. This system incorporates unique and powerful functions in a reliable, high performance system, ideally suited to research and analytical labs. It is fully confocal, providing high image quality and spatial or depth resolution.

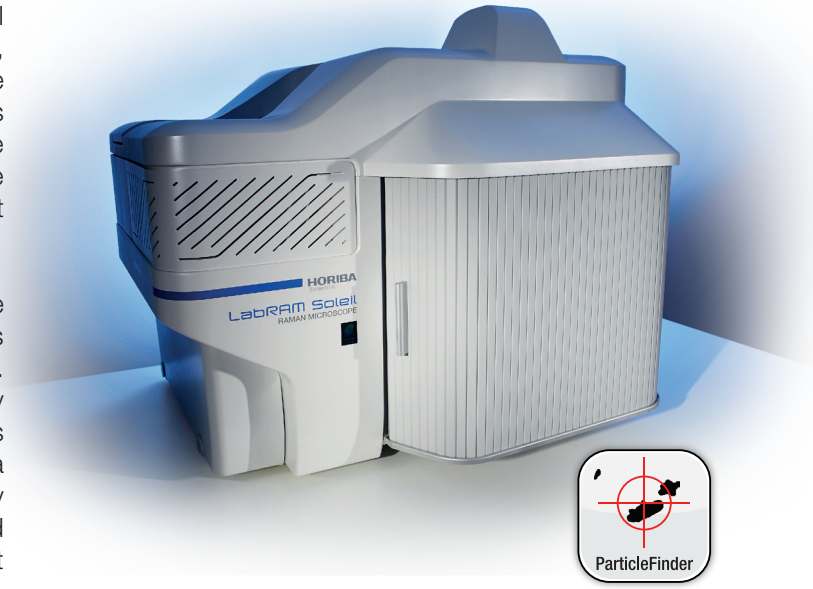

Figure 1: LabRAM Soleil Raman microscope.

A specific software tool has been used to achieve these analyses: the software application (or App) ParticleFinder™ proposed in the LabSpec 6 Spectroscopy Suite that operates the LabRAM Soleil. ParticleFinder™ automates static image analysis and Raman identification via an intuitive user interface. It identifies, locates and counts all the particles present, then characterizes their size and shape before performing the Raman analysis of each one individually. It offers the possibility to record a spectrum at the center of the particle, an average of multiple spectra from the particle, or a complete map covering the particle.

For the experiments, the samples have been dispersed by spraying one shot of the product onto a clean glass slide from a distance of approximately 40 cm. This operation has been repeated twice per nasal spray, on different slides.

Morphological characterization

Based on the image contrast, particles are automatically identified by the software. Statistical distributions for various morphological parameters, such as size and shape, can then be obtained. Here, we have focused on the equivalent circular diameter, i.e. the diameter of a circle having the same area than the particle considered, and the circularity. Figure 2 shows a optical image of the particle spatial distribution considered for Innovator sample 1 (a), the equivalent circular diameter distribution (b) and the circularity distribution (c) of 8,584 particles.

As this operation has been applied on the different nasal spray dispersions, the statistical distributions have been easily obtained for innovator and generic products. These results are summarized in Table 1.

Based on this table, one can easily conclude that the circularity is highly similar between generic and innovator products. However, the generic particles are slightly smaller. Static image analysis is also useful in that case to monitor the size and shape distributions between a generic and an Innovator nasal spray.

Sample	# of particles	Equivalent circular Diameter (µm)			Circularity		
		D10	D50	D90	D10	D50	D90
Innovator 1	8584	0.50	0.93	2.43	0.42	0.61	0.82
Innovator 2	14843	0.67	1.12	2.85	0.42	0.61	0.82
Generic 1	4894	0.40	0.76	2.08	0.40	0.59	0.77
Generic 2	6444	0.50	0.90	2.38	0.40	0.59	0.82

Table 1: Morphological statistics for the different nasal spray dispersions.

Chemical characterization

Based on particle locations obtained with ParticleFinder™, the Raman spectral analysis of every particle has been realized for one of each product (Innovator 1 and Generic 1). The total measurement times were 2:20 and 4:07 hours for Generic (4,894 particles) and Innovator (8,584 particles), respectively. A statistical clustering (K-means Clustering Analysis) has then been applied on each spectra assembly to separate Active Principal Ingredient (API) from excipient particles. Reference spectra have also been obtained based on these classifications.

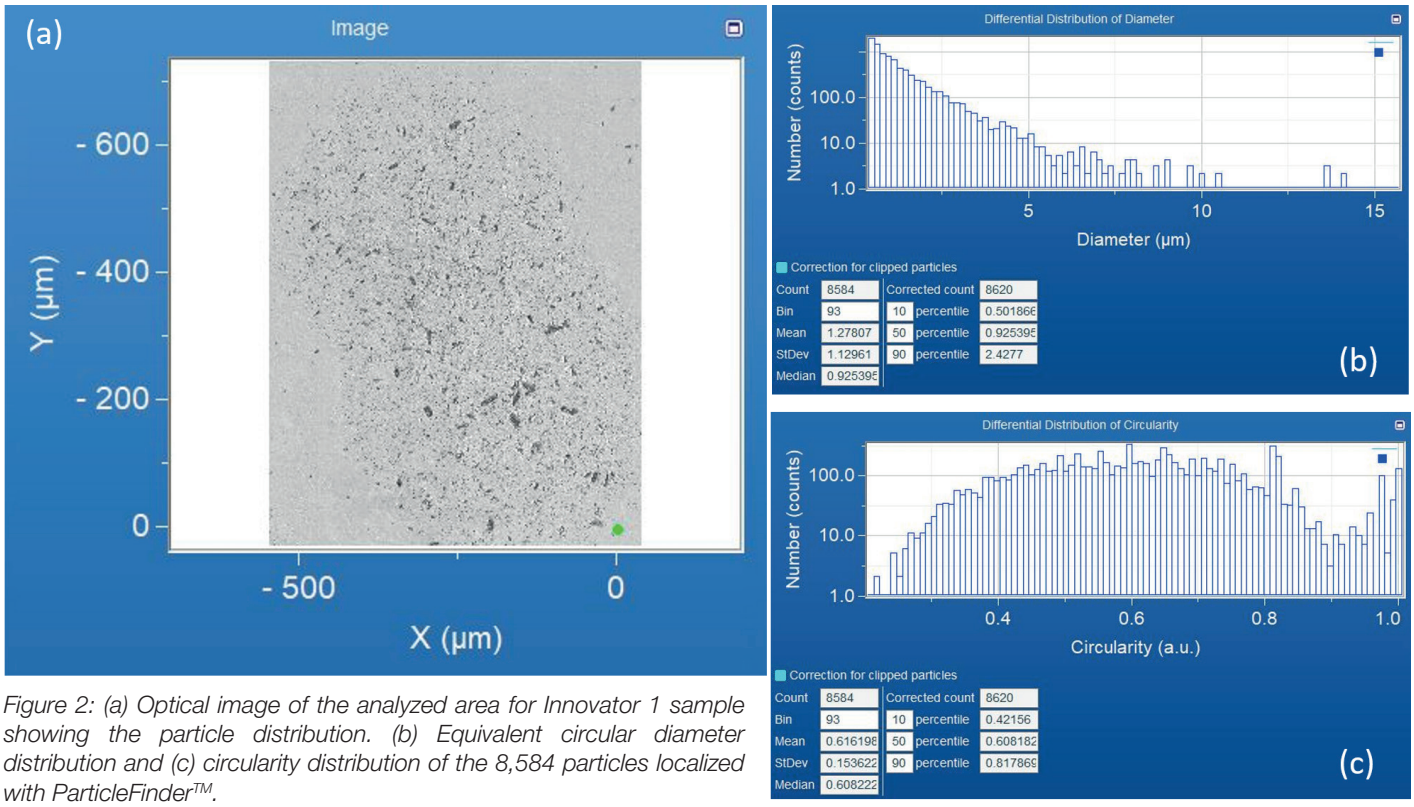

Figure 2: (a) Optical image of the analyzed area for Innovator 1 sample showing the particle distribution. (b) Equivalent circular diameter distribution and (c) circularity distribution of the 8,584 particles localized with ParticleFinder™.

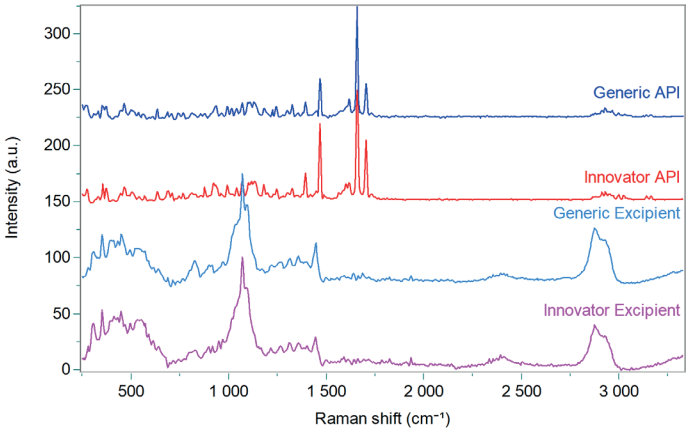

Figure 3: Average Raman spectra of generic and innovator particles obtained from K-means clustering analysis

As shown in Figure 3, the reference spectra of generic and innovator products are similar, confirming the formulation similarities between the two products. The spatial distributions of the different chemicals have also been obtained to inform about the homogeneity of dispersion of the actives within the product. Each particle has been so tagged with a specific color on the optical images, as shown on Figure 4a-b (red for API and blue for excipient particles).

It has been also easy to obtain the diameter and circularity distributions of the API in these two products to compare the morphological distribution of the API only between generic and innovator formulations (Figure 4c-f). Table 2 summarizes the size parameters and the estimated fraction of API in the two forms. The results indicate that API in the generic and innovator sprays have similar forms and that API D-estimates are in a similar range.

Sample	API estimated fraction	Equivalent circular Diameter of API particles (µm)		
		D10	D50	D90
Innovator 1	1.13 %	1.0	2.5	4.5
Generic 1	1.31 %	0.5	2.5	5.0

Table 2 : API particle size statistics.

Conclusion

To summarize, the ParticleFinder™ application for LabSpec 6 Spectroscopy Suite enables a quick and accurate analysis of nasal spray products. Morphology of particles, identification of API and estimated fraction of API have been determined. The LabRAM Soleil high-performance Raman microscope enables fast chemical identification of more than 5,000 particles. The static image analysis with ParticleFinder™ application is a perfect solution to morphologically and chemically compare generic and innovator nasal sprays, and more generally to characterize pharmaceutical formulations.

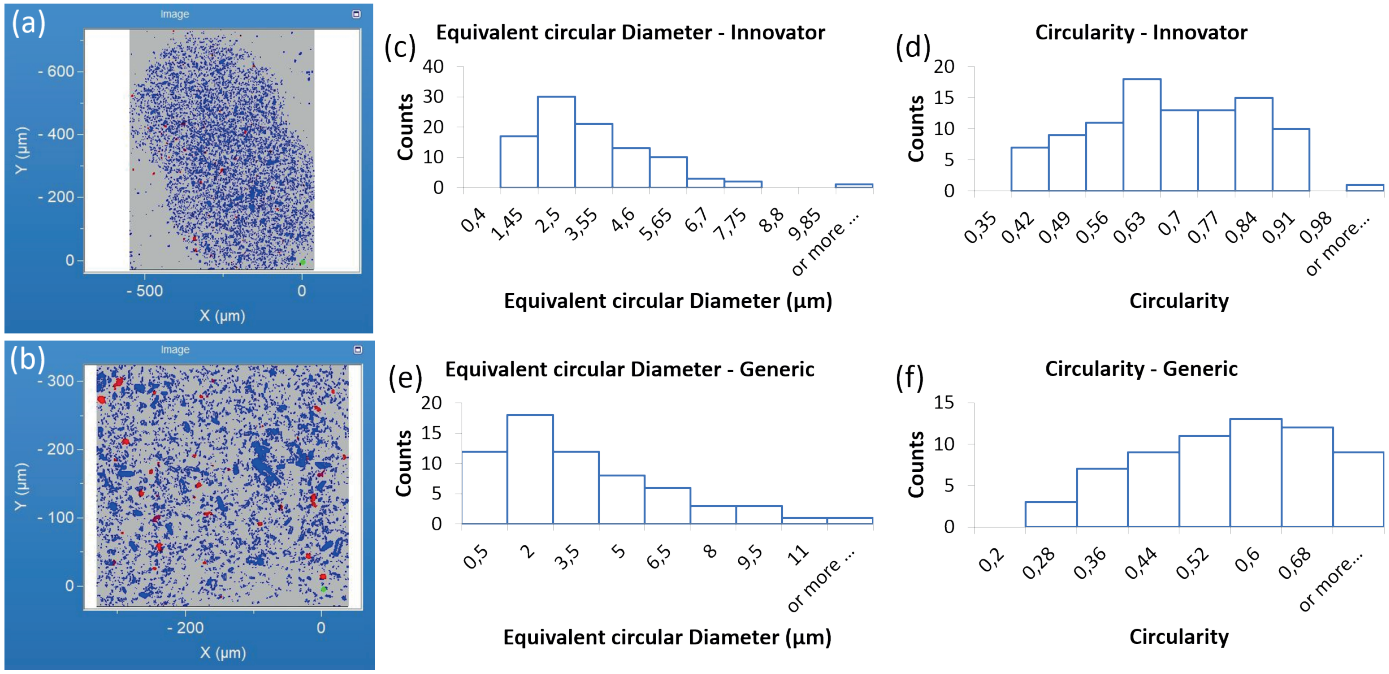

Figure 4: API distributions. (a) Spatial distribution of API (red) and excipient (blue) in the innovator spray area. (b) Spatial distribution of API (red) and excipient (blue) in the generic spray area. (c-f) Equivalent circular diameter and circularity distributions of API only in the innovator and generic sprays.

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