application note

Automation of Manual QC analysis of Nasal Spray on the Morphologi® G3

Introduction

A nasal spray drug product contains an active ingredient and often other excipients dissolved or suspended in a formulation which is delivered to the patient by nasal inhalation. Delivery by such a method is common for drugs to alleviate, for example, sinus congestion or allergic rhinitis or to deliver vaccines, proteins and peptides. Typically the formulation is administered via a metered spray pump activated by the patient.

When producing such sprays pharmaceutical manufactures have to ensure many Quality Control (QC) procedures are followed. Guidance for such procedures are outlined by the US Food and Drug Administration's (FDAs), Centre for Drug Evaluation and Research (CDER) "Guidance for Industry" documents entitled *Bioavailability (BA) and Bioequivalence (BE) Studies for Nasal Aerosols and Nasal Sprays for Local Action* [1] and Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products — Chemistry, Manufacturing, and Controls Documentation [2].

One important parameter that is controlled is the droplet size delivered by the spray pump. This is often measured by laser diffraction methods as described in another Malvern application note [3]. Additionally for some nasal spray formulations QC procedures must also be carried out to characterize the active pharmaceutical ingredient. Such QC procedures may specify the proportion of large active particles that the sample may contain along with the proportion of smaller active particles thus the method involves identifying and counting the particles meeting specified criteria. Often such procedures are carried out by manual microscopy analysis which can be time consuming, requires a skilled analyst to identify particles that meet the criteria and may be subject to human bias or fatigue.

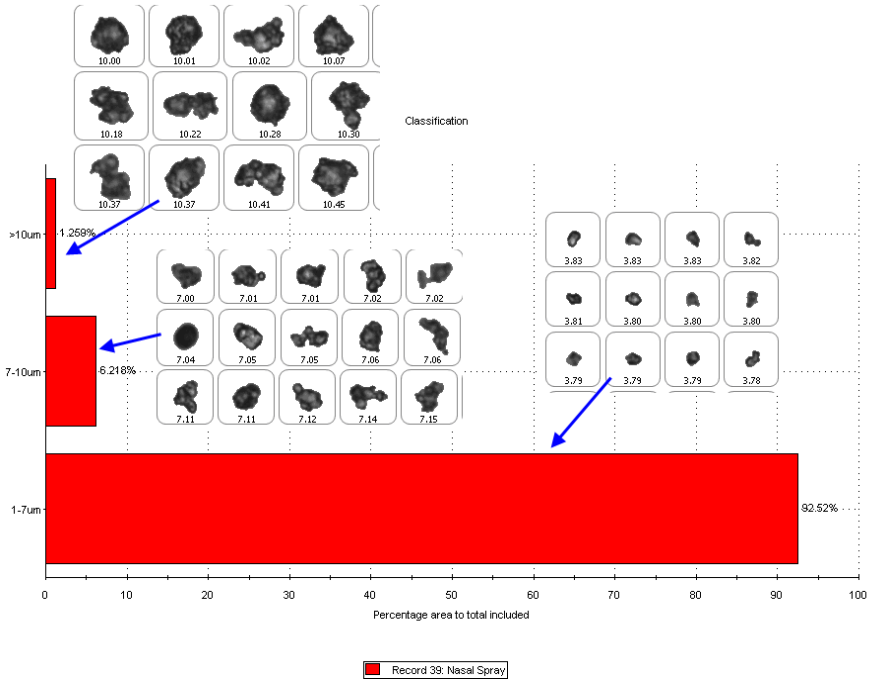

Figure 1: Classification chart showing the proportion of active particles found in each size class along with example particle images

Automation of such procedures with the Morphologi® G3 not only reduces the analysis time but also frees up the analyst for other tasks and reduces subjectivity.

Example

A manual microscopy analysis currently employed for particle size determination of an active ingredient in a nasal spray involves the analyst identifying 500 active particles in a sample whilst ensuring they are not touching any excipient particles. Digital images of the active particles are taken and independent image analysis software is used to size the particles and provide statistics. The QC procedure specifies that no more than 2 % of the active particles can be larger then 10 µm in terms of Circular Equivalent Diameter (CE Diameter). Additionally, 70 – 95 % of the particles have to be between 1 and 7 µm in

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terms of CE diameter. For each batch tested two samples have to be measured. The time to complete the analysis is around 2 hours for an experienced analyst. Automating such a procedure on the Morphologi G3 not only reduced the analysis time to around 20 minutes, but it also allowed the identification of over 3800 active particles; significantly improving the statistical significance of the analysis.

Methodology

The samples were dispersed in the same way as detailed in the specification for the manual analysis, thus a sample of the nasal spray was spayed onto a glass slide and a coverslip was placed on top. The sample was measured on the Morphologi G3 according to a standard operating procedure (SOP) which defined all the hardware and software variables such as the sample area to be measured. Classifications were set up to identify active particles in the required size classes so the proportion in each could be established. Post analysis, any images of active particles identified that were touching excipient particles were excluded from the final result. Each measurement took around 20 minutes and identified over 3800 active particles. Increasing the sample area measured would increase the number of particles identified even further. Figure 1 shows the result of an analysis in terms of the classification chart showing the proportion of active particles found in each size class along with example particle images.

Developing the classification using the scattergram

When developing the method for the automated analysis, the scattergram function of the Morphologi software was employed to help define the particle classifications that enabled the particles of interest to be detected. The particles falling into the each of the size groups defined in the specification were selected in turn and scattergrams of size VS the other measured parameters were viewed.

Figure 2 shows a screen shot of the scattergram feature. The top left distribution allows interactive filtering; the example shows that all particle images containing less than 100 pixels are excluded from the result since at least 100 pixels per image are required for meaningful shape information. The top right and bottom left distributions show size or shape parameters as selected by the user. In this example the top right distribution shows CE diameter and the bottom left distribution shows the mean intensity. The scattergram in the bottom right graph then shows the relationship between these two morphological parameters.

In this example the scattergram shows the relationship of size in terms of CE diameter VS intensity mean. It was found that for particles larger than 10 µm the intensity mean parameter separated the dark active particles from the light excipient particles well as shown in Figure 2. On selecting a region of the scattergram only particles images contained within that region are displayed. The region can then be resized as required to include only particle images of interest. The parameters selected are automatically detailed in the selection section and can be defined as a class if required. Once classes are established they can then be added to the SOP so the result in terms of the classification is presented directly at the end of the measurement. The classification applied in the SOP for the complete analysis is detailed in Table.1

Once the classification has been defined in the SOP it is applied to subsequent measurements so results can be obtained directly on completion of the measurement. Results of measuring different samples can then be easily compared by overlaying results in a classification chart or by presentation of results in a classification table.

Table 1: Description of the classification applied to the SOP

Size class	Class description.
>10µm	CE Diameter (µm) > 10.00 Intensity Mean < 90.0
7-10µm	CE Diameter (µm) ≥ 7.00 and < 10.00 Intensity Mean <= 90.0
1-7µm	CE Diameter (µm) ≥ 1.00 and < 7.00 Intensity Mean <= 90.0 Aspect Ratio > 0.500

Summary

Performing quality control procedures that require the identification of certain types of particles by manual microscopy, for example on suspended drug formulations such as nasal sprays, require highly skilled analysts and can be time consuming and open to subjectivity or bias. Automating such procedures on the Morphologi G3 not only removes the subjectivity it also frees up the analyst for other tasks and allows methods to be easily transferred between sites with out the need for intensive training.

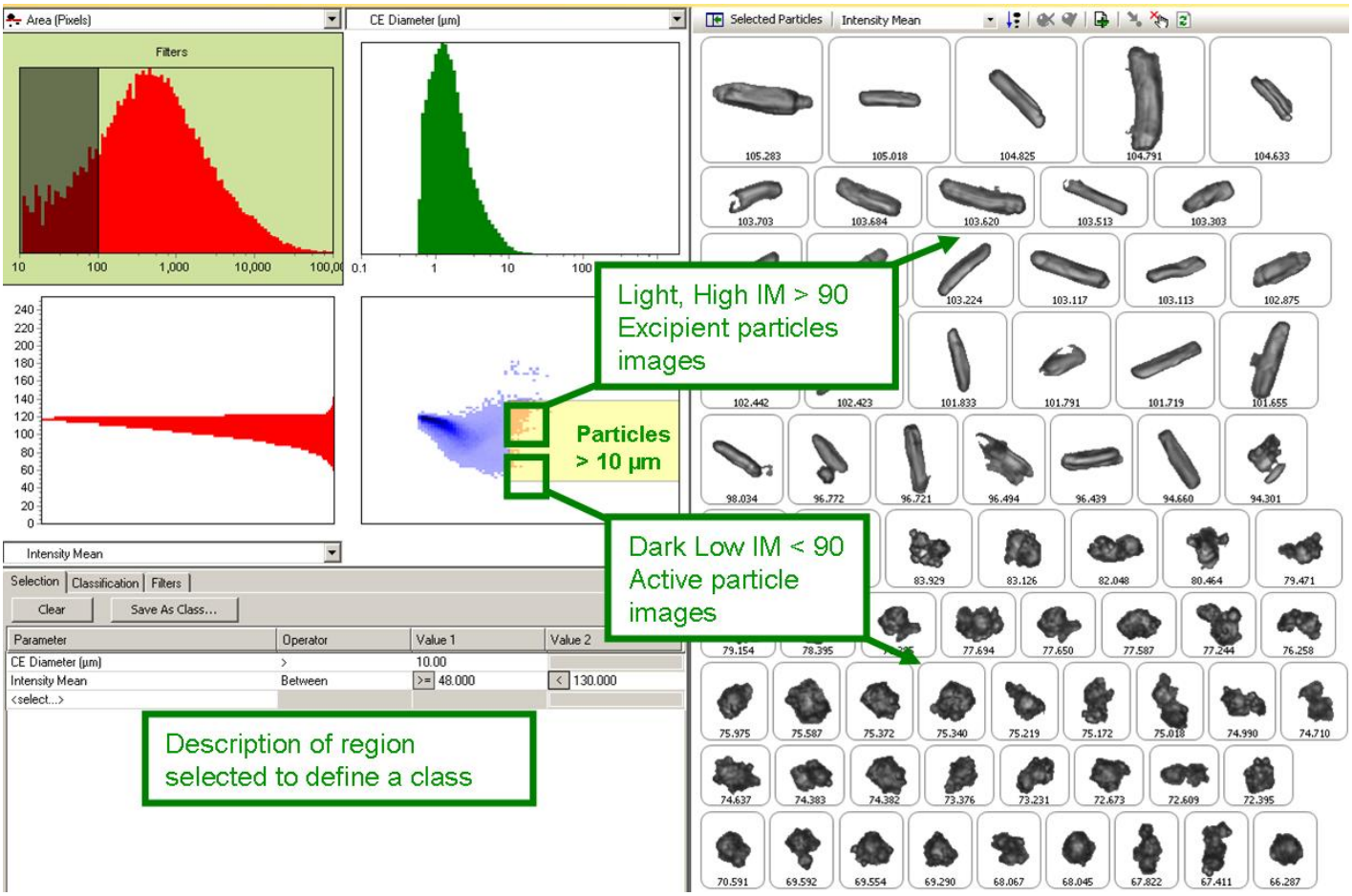

Figure 2: Screen shot of the Morphologi G3's scattergram feature and selected particle images sorted in terms of Intensity mean

1 This can be downloaded from www.fda.gov/cder/guidance/5383DFT.pdf

2 This can be downloaded from http://www.fda.gov/cder/guidance/4234fnl.htm

3 http://www.malvern.com/malvern/kbase.nsf/allbyno/KB001006/$file/MRK753-01.pdf

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