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OPTIMISING ANALYTICAL STRATEGIES FOR THE DEMONSTRATION OF BIOEQUIVALENCE IN A GENERIC NEBULISER

US FDA guidance for the *in vitro* demonstration of bioequivalence in a generic nebuliser directly references specific analytical strategies for the characterisation of aerosolisation performance. In this article, Déborah Le Pennec, Research Technician, University of Tours; Laurent Vecellio, R&D Engineer, University of Tours and Scientific Director, Aerodrug, DTF Medical; Paul Kippax, Team Leader, Advanced Materials, Malvern Instruments, and Stephane Rouquette, European Business Manager, Biosciences, also of Malvern Instruments, look at the optimal application of laser diffraction and automated imaging within this context.

The continuous drug delivery profile and ease of use of nebulisers makes them a popular choice for drug delivery, especially for high drug dosages and for paediatric or geriatric patients. Successful drug delivery relies on aerosolising a formulation to a particle size suitable for inhalation. Consequently, particle size measurements have an important role to play in supporting the *in vitro* demonstration of bioequivalence (BE) for a generic submission.

Draft product-specific US FDA guidance for nebulised Budesonide[1] highlights laser diffraction particle size measurement as one of the techniques that can be used to demonstrate BE in a nebuliser via *in vitro* testing alone – a valuable approach that minimises the need for more expensive studies. It also points to the need for agglomerate detection and measurement in suspension (in the ampoule) since this too can influence the effectiveness of drug delivery. In this article we examine the information provided by laser diffraction, within this context, and the optimisation of a test set-up for measurement,

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presenting data showing the impact of a range of experimental parameters. We also investigate the complementary use of automated imaging as an efficient technique for the detection of agglomerates within formulation suspensions.

DEFINING THE PERFORMANCE OF NEBULISERS

Rather than delivering a pre-metered dose, nebulisers are loaded with a reservoir of drug formulation and operate continuously once activated. The liquid drug formulation is aerosolised using a jet, ultrasonic or mesh nebuliser, through the application of compressed air, ultrasonics or vibration, respectively, to form a fine mist which is inhaled by the patient. When using a nebuliser, the patient breathes normally so the aerosol cloud is drawn into the lungs under regular tidal breathing conditions. Rapid and efficient delivery of the drug to the target area of the respiratory tract is dependent upon aerosolising the dose to a fine particle size, typically below 5 µm, with optimum particle deposition in the alveoli associated with even finer particle sizes. The fraction of a dose that will deposit in the lungs on the basis of size is called the respirable fraction or the fine particle fraction (FPF).

Within the clinical setting, nebulisers can be loaded with a range of alternative formulations for comparison. However, the effectiveness of drug delivery depends directly on interactions between the formulation and the nebuliser device, so testing the two together has become standard practice. Key performance metrics are: delivered droplet size, because of its influence on pulmonary deposition; and the rate of drug delivery.

DEMONSTRATING BIOEQUIVALENCE IN NEBULISERS

In the development of generics, the aim is to duplicate the performance of an established product precisely so that both innovator and generic can be used interchangeably to deliver an identical therapeutic effect. Identifying *in vitro* analytical strategies and instrumentation that provide the required information to demonstrate BE helps developers avoid the costs and time associated with *in vivo* testing and extensive clinical trials.

Steadily rising numbers of generic submissions have increased the regulatory burden of assessing and addressing concerns over the suitability of an *in vitro* testing strategy for any given product.

In response, the FDA has released a number of product-specific draft guidances detailing the tests required for popular targets. Nebulised budesonide, a steroid widely used for the treatment of asthma and chronic obstructive pulmonary disease (COPD), is one of the inhaled products for which product-specific draft guidance is already in place.[1]

This draft guidance for budesonide highlights seven discrete tests for the demonstration of bioequivalence by *in vitro* testing alone, including: "Comparative aqueous droplet size distribution of the nebulised aerosol by a laser diffraction method," and the need for "comparative drug particle and agglomerate particle size distribution in the suspension (in the ampoule)".

The following experimental studies show how laser diffraction and automated imaging can be optimally applied to meet these requirements.

STUDY 1: OPTIMISING LASER DIFFRACTION MEASUREMENTS

Laser diffraction particle size analysis allows the real-time measurement of droplet size in a nebuliser aerosol, making it complementary to the more time-consuming, but component-specific, sizing delivered by cascade impaction. Furthermore, laser diffraction measurements can be made at varying flow rates, enabling direct assessment of the impact of inhalation and exhalation on inhaled droplet size.

Researchers at the University of Tours (CEPR, INSERM U1100, Tours, France) use laser diffraction particle size analysis routinely to study the performance of standard jet, breath-enhanced jet and mesh nebulisers. To support this work, a series of experiments were carried out to optimise the analytical method used, by investigating the impact of certain

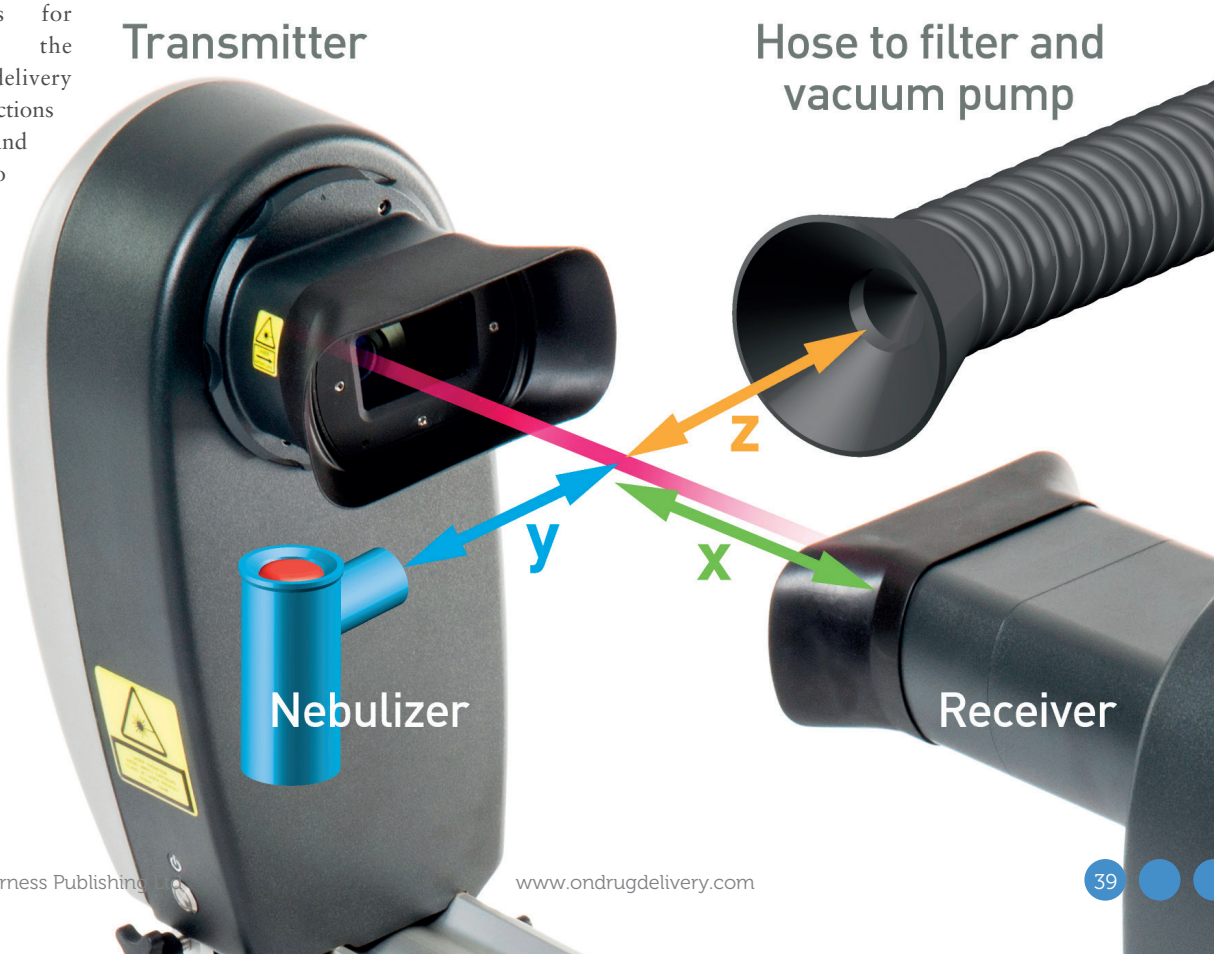

Figure 1: The test set-up for measuring particle size data for a nebuliser with Spraytec laser diffraction system, showing the variables that can be optimised.

experimental parameters on the measured data. All tests were carried out using a Spraytec laser diffraction analyser for particle size measurement (Malvern Instruments, UK) and a PARI LC PLUS nebuliser with a Pariboy compressor (PARI, Germany) as recommended in the FDA guideline for budesonide.[1] Aspiration of the sample was carried out at flow rates in the range 0 L/min to 100 L/min using a vacuum pump (Copley Scientific, UK). All measurements were made using 2 mL of saline solution over the course of 1 min.

Figure 1 shows the experimental set-up used and the method parameters that were varied, which included:

- X distance: the distance from the spray plume centre to the receiver lens along a central line between the detector and transmitter, across the range 1-32 cm
- Y distance: the distance of the nebuliser from the laser beam passing through the laser diffraction measurement zone, across the range 1-7 cm
- Z distance: the distance between the laser and the extraction system used to collect the aerosol created by the nebuliser, across the range 5-11 cm
- Extraction flow rate, across the range 0 L/min to 100 L/min.

"An optimised method was developed ... This provides valuable support for the use of laser diffraction to assess the performance of jet nebulisers robustly, either in BE studies or more generally."

The impact of these variables was quantified by recording:

- Dv10, Dv50 and Dv90: the measured diameters below which 10%, 50% and 90% of the particle population lies, on the basis of volume
- Span: defines the width of the particle size distribution and is equal to (Dv90 - Dv10)/Dv50
- % of the sample below 5 µm in size, which quantifies the sensitivity of the measured FPF.

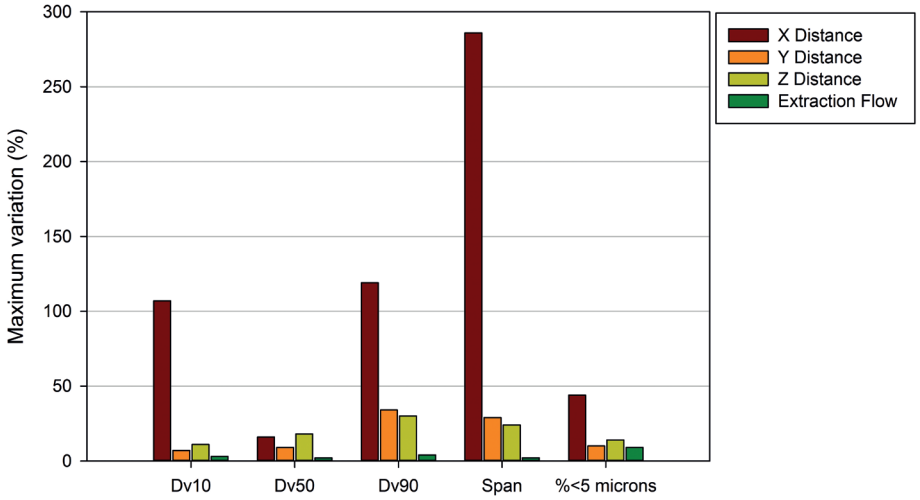

Figure 2: Maximum variability data for particle size distribution metrics measured using different laser diffraction / nebuliser set-ups. X distance (between the nebuliser spray plume and the receiver) is highlighted as a key parameter.

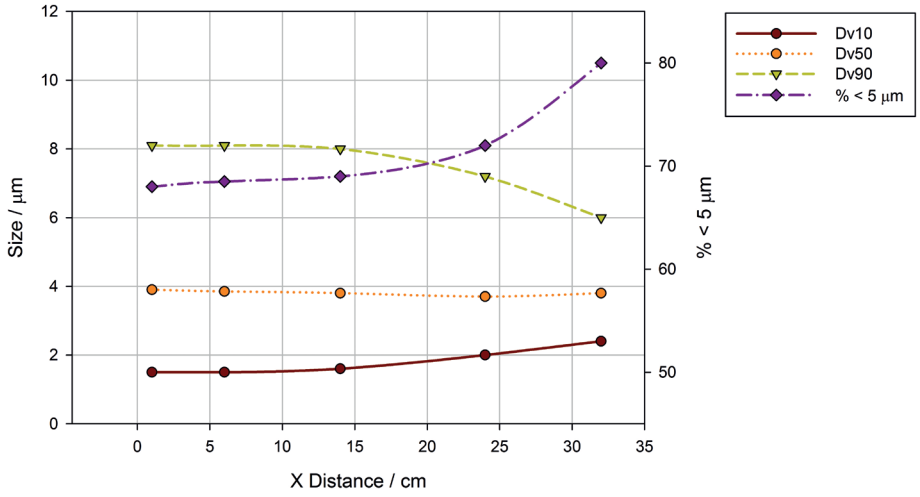

Figure 3: Dv10, Dv50, Dv90 and the % particles <5 µm, as a function of the distance between the nebuliser and the receiver lens. These data confirm that the measurement is robust to changes in distance in the 1-14 cm range.

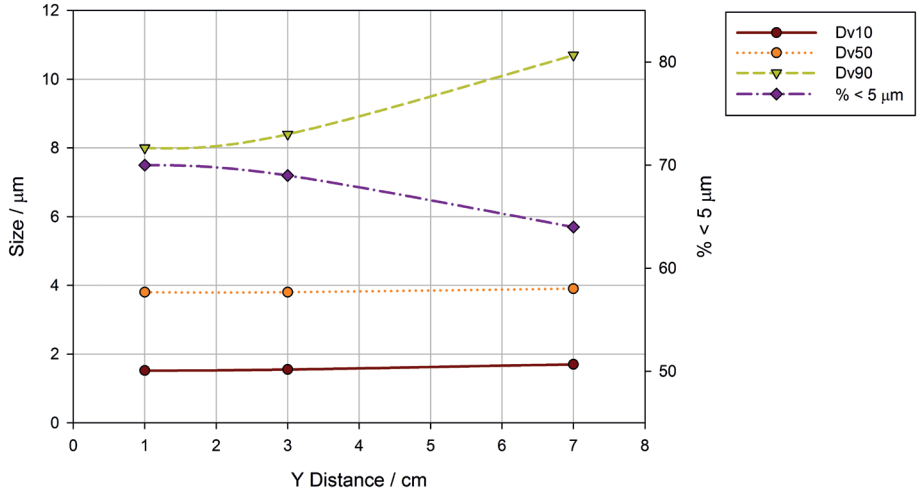

Figure 4: Dv10, Dv50, Dv90 and the % particles <5 µm, as a function of the distance between the nebuliser and the laser beam. These data show that moving the device too far away from the laser compromises sampling of the aerosol.

Figure 2 summarises the maximum variability associated with each parameter, calculated by considering the difference in the values obtained for the minimum and the maximum control value (e.g. 1 cm and 32 cm for the X distance). Generally speaking, measurements are robust with respect to the experimental set-up, especially the reported figures for the percentage of material below 5 µm. However, it is clear that the X distance, which describes the position of the nebuliser relative to the receiver lens, has the largest impact on the variability of the reported results. Y distance, the distance between the nebuliser and the laser, is the second most influential parameter.

The change in the reported size distribution parameters as the X distance is altered relates directly to the way the laser diffraction technique operates. Laser diffraction systems calculate droplet size distributions by measuring the light scattered from the spray droplets as they pass through the laser beam. As the nebuliser spray plume is moved further away from the receiver optics, there is a point at which light scattered at wide angles is no longer collected effectively (an effect called vignetting). This affects the ability of the system to detect smaller particles, causing changes in the reported values for the Dv10 and percentage of particles below 5 µm. In addition, there is an impact on the ability of the system to measure the width of the distribution, causing a variation in the reported Dv90 and Span. The influence of vignetting increases as the distance increases, as clearly shown in Figure 3 which confirms that the results obtained are robust in the range 1-14 cm but then change rapidly at larger distances. However, the fact that the nebuliser can be positioned up to 14 cm away from the receiver without a significant impact on the result is advantageous as it minimises the risk of spray deposition on the receiver optics.

An additional consideration is the sampling of the aerosol. Laser diffraction will only measure particles which pass through the measurement laser beam. Changing the Y distance leads to a change in the width of the spray plume at the point where it crosses the laser, which will in turn cause a change in the measurable percentage of the plume. Y must therefore be controlled to ensure that all droplets are sampled. The results gathered show that increasing Y distance causes the Dv90 to increase and the % particles <5 µm to decrease (Figure 4), suggesting that sampling of the spray plume is impacted by moving the device too far from the beam.

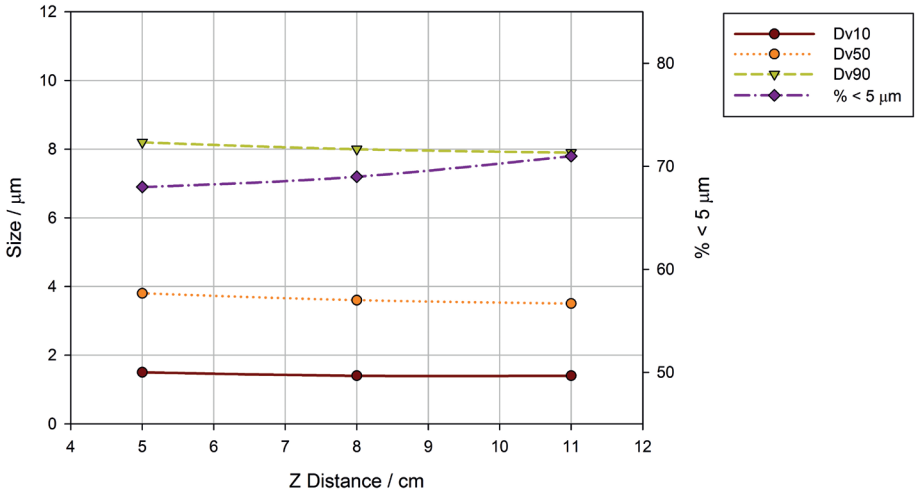

Figure 5: Dv10, Dv50, Dv90 and the % particles <5 µm, as a function of the distance between the laser beam and the extractor. This data shows that moving the extractor further away from the measurement zone results in a slight reduction in measured particle size.

Parameter	Setting	Comments
X-distance	1-6 cm	Ensure that optical contamination is minimised whilst also avoiding vignetting
Y-distance	1 cm	Measures close to the laser to ensure good sampling of the entire spray plume
Z-distance	5 cm	Ensures good extraction with minimal impact to the plume width
Extration Flow Rate	Use the lowest possible flow rate to avoid backflow or recirculation of the aerosol within the laser beam	

Table 1: Optimised method parameters for nebuliser characterisation by laser diffraction.

> "Speed of measurement, measurement reproducibility, and the number of particles that can be characterised in a single experiment make automated imaging a practical alternative to manual microscopy."

A possible rationale for the observed results is that the fines are recirculating before they reach the laser beam, causing them to be lost prior to measurement.

In this study, the impact of the distance between the extractor and measurement zone, Z distance, which is controlled to ensure that all droplets pass through the measurement zone without recirculation, was found to be minimal (see Figure 5). However, a slight decrease in particle size is observed at larger Z distances, with recirculation the most probable cause. Similarly, the extraction flow rate, which also influences recirculation, was also observed to have a minimal effect with this set-up (data not shown).

An optimised method was developed for subsequent studies, taking all of the measured data into account (see Table 1). This provides valuable support for the use of laser diffraction to assess the performance of jet nebulisers robustly, either in BE studies or more generally.

STUDY 2: AUTOMATED IMAGING TO COMPARE AGGLOMERATION

Automated imaging systems capture images of individual particles in a sample to build up statistically significant distributions of particle size and shape. Speed of measurement, measurement reproducibility,

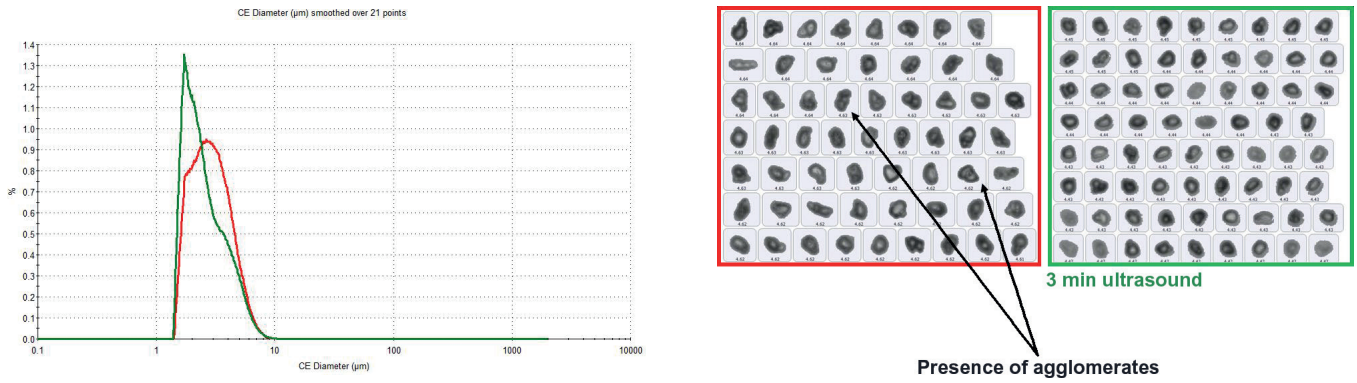

Figure 6: Particle size distribution data for the reference budesonide formulation indicates that there are agglomerates present which are dispersed by the application of ultrasound.

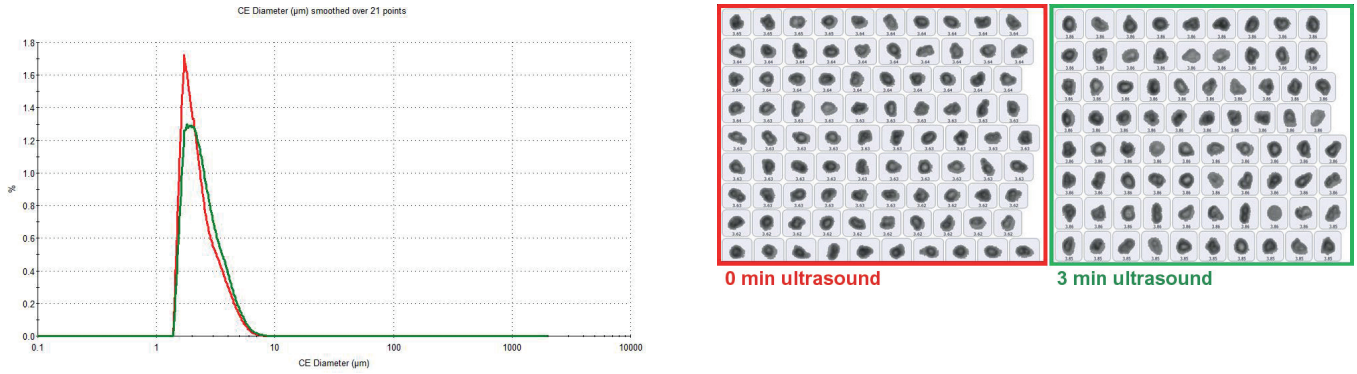

Figure 7: Particle size distribution data for the test budesonide formulation indicates that particle size is relatively unaffected by the application of ultrasound.

and the number of particles that can be characterised in a single experiment make automated imaging a practical alternative to manual microscopy. By combining particle size and particle shape data, automated imaging makes it easy to investigate and classify specific particle populations within a sample. For example, shape data can often be used successfully to differentiate agglomerates from primary particles. These capabilities make automated imaging a useful tool for nebuliser formulation development, and the demonstration of BE in accordance with FDA guidance.

In an experimental study, the particle size and state of agglomeration was studied in samples of innovator (reference) and generic (test) budesonide nebuliser formulations, before and after the application of ultrasound. Measurements were made after 0, 1, 2 and 3 minutes of ultrasound. For each measurement, 3 µL of sample was pipetted on to a glass microscope slide. A glass cover was placed over the sample and sealed in place, and the sample was then subjected to automated imaging using a Morphologi G3 automated imaging system (Malvern Instruments, UK). This measurement procedure was repeated four times for each sample, with the resulting data combined to form a single record for each sample. Particle size distribution data were presented in terms of circle equivalent (CE) diameter – the diameter of a circle with the same area as that of the particle.

The application of ultrasound reduced the overall particle size of the reference formulation (see Figure 6). Images of the largest particles in the sample clearly show agglomerated material in the original formulation that were not present in the samples after they were subjected to ultrasound. The particle size of the test formulation was, in contrast, relatively unchanged by the application of ultrasound and there was no evidence of agglomerates (see Figure 7).

In comparing these two formulations it is clear that they have somewhat different properties. The innovator is less stable than the generic, forming loose agglomerates which are relatively easily dispersed. However, the dispersed or primary particle size in both formulations is closely similar. Cascade impaction could be usefully applied as a follow-up analysis to determine whether any agglomerates present are dispersed during nebulisation, to support a claim of bioequivalence.

CONCLUSION

Draft product-specific guidance for the nebulised delivery of budesonide highlights laser diffraction particle sizing for the *in vitro* demonstration of bioequivalence. The data presented here illustrate the insight that laser diffraction measurements can provide in nebuliser studies and demonstrate how to optimise a test method for its beneficial application within this context.

Automated imaging is highly complementary to laser diffraction and efficiently enables particle size and agglomerate measurement in the formulation, as recommended in the regulatory guidance. The results presented here show how automated imaging can be used to detect agglomerated particles and assess the ease with which they are dispersed to support the demonstration of BE.

REFERENCE

1. *Draft US FDA Guidance for Budesonide (Suspension/Inhalation). Recommended Sep 2012. (http://www.fda.gov/downloads/Drugs/.../Guidances/UCM319977.pdf)*

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