Sensitivity analysis of laser diffraction for measuring particle size distributions of inhalation actives

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Summary
A validated laser diffraction (LD) wet dispersion method developed for the Mastersizer2000 was copied onto the Masterszer3000 instrument from Malvern Instruments Ltd, keeping as many parameters as possible the same. These two methods were tested using a series of powder blends from a range of inhalation powders to demonstrate the sensitivity of the methods to detect small changes in particle size distribution (PSD).

Blending experiments demonstrate these methods on these instruments are capable of measuring a shift in the d(0.1), d(0.5) and d(0.9) PSD for this powder of 0.04 micron, 0.05 micron and 0.3 micron respectively, with statistical confidence. This sensitivity analysis of an analytical method is important when considering the suitability of product specifications. It is also important to recognise that there may be a bias when comparing data from one instrument to another, even when manufactured by the same manufacturer, and this needs to be taken into consideration when transferring methods from one instrument to another.

Introduction
LD is the most commonly used analytical method for measuring the PSD of inhalation powders within the pharmaceutical industry. The powders can be de-agglomerated in an air stream by high sheer mixing, or by placing the powder in a dispersion and de-agglomerating with ultrasonic power [1,2]. This publication is based on the later approach. The pharmacopeia guidance is based on the ASTM and ISO standards for measurement of PSD [3,4].

3M has developed a Quality by Design (QbD) approach for the optimisation and validation of methods [5,6] for inhalation powders and shown that a well designed method can produce data to a repeatability much closer than specified in the regulatory guidance. This publication describes the use of blending experiments to give an indication of the method sensitivity ie the ability of the method to see small changes in PSD.

Methodology
An analytical method was developed and validated on the Mstersizer2000 instrument for powder samples of Active A using a Quality by Design (QbD) approach [5,6] and this methodology was transferred to the Mastersizer3000 instrument using the same parameters wherever possible, as described in Table 1.

Instrument	Mastersizer2000 and Mastersizer3000
Accessory	Hydro 2000SM
Ultrasonic treatment	30W with a Sonics Vibracell VC 130
obscuration	10 %
Stirrer speed	3000 rpm
Number of records	10
Dispersant	Iso-octane and 0.05 %w/v lecithin
Dispersant refractive index	1.392
Blue LED	On
Active Refractive Index	1.750
Active Absorption Coefficient	0.050
Analysis Model	Mie theory - General Purpose Model – irregular shaped particles

Table 1 - Description of equipment and settings for a LD PSD method based on the Mastersizer2000 and Mastersizer3000 instruments.

Results and discussion
A range of samples of an inhalation grade API (Active A) was selected and these were analysed simultaneously on the Mastersizer2000 and Mastersizer3000 instruments with an n=5 in order to form a baseline of information. Typical examples are shown in Figure 1 and a summary of PSD data is provided in Table 2.

It is interesting that the two instruments showed a significant bias in results, which is demonstrated in Figure 2 by the shift away from the line of identity. The bias is particularly significant when considering the volume% of particles less than 1 micron [p=0.000 for t-test between d(0.1) values for both instruments]. The Mastersizer3000 instrument interpreted the scattering data as having a much higher level of fines than the Mastersizer2000 instrument, as noted previously [7]. This is attributed to improvements in the instrument design of the Mastersizer3000 instrument, which includes an increased power blue LED, folded optics and an increased number of detectors. Otherwise, the 6 samples show similar profiles and the trending is very consistent across the two instruments, including a shoulder in the PSD above 10 micron for Sample 6. The shoulder in this sample had a significant effect on the d(0.9) values.

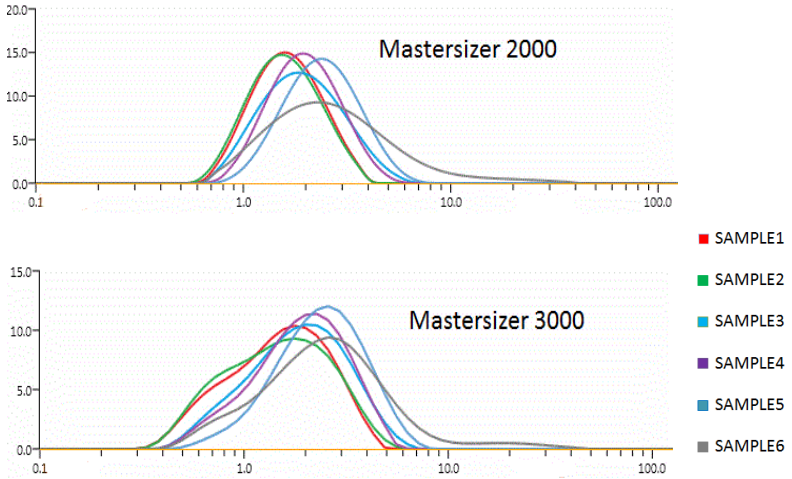


Figure 1 - PSD of Active A Samples 1 to 6 measured on the Mastersizer2000 and Mastersizer3000 instruments.

Sample		M2000			M3000	
	d(0.1)	d(0.5)	d(0.9)	d(0.1)	d(0.5)	d(0.9)
1	0.99	1.60	2.64	0.66	1.51	2.88
2	0.94	1.56	2.67	0.65	1.48	3.02
3	1.06	1.91	3.64	0.83	1.87	3.63
4	1.15	1.96	3.37	0.86	1.93	3.57
5	1.37	2.35	4.04	1.18	2.37	4.30
6	1.15	2.47	6.39	0.95	2.43	5.56

Table2 - PSD values for the 6 Samples in micron

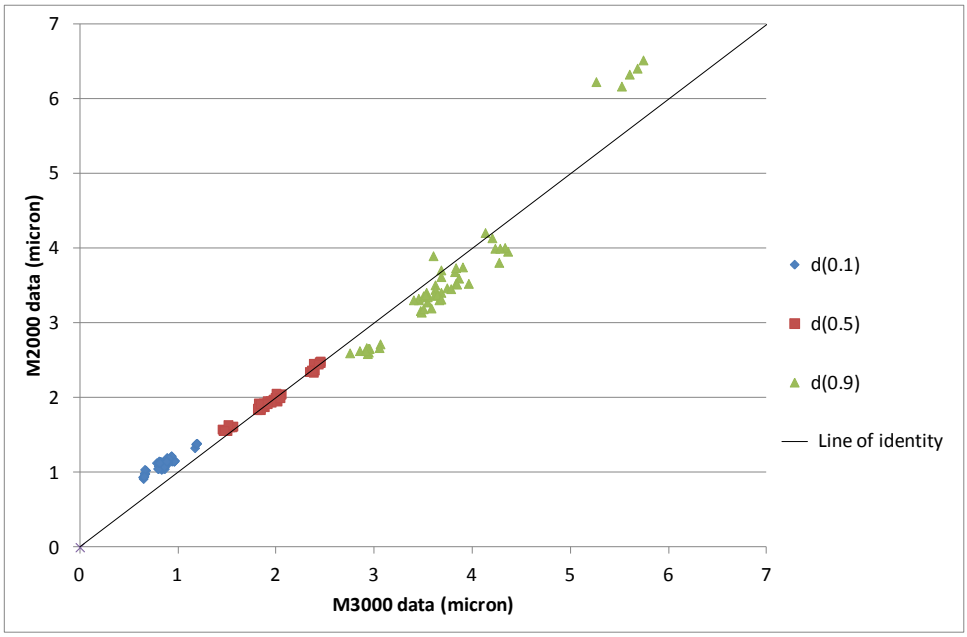
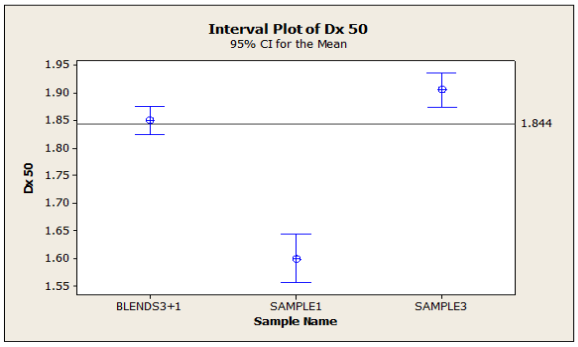


Figure 2 - Comparison of PSD data for Active A measured on the Mastersizer2000 and Mastersizer3000 instruments.

Blending experiments

A series of 8 blends were produced with a d(0.5) of just less than or just greater than 2 micron. Sample 3 and 4 had a d(0.5) of approximately 2 micron and these were used as base materials for the blends, representing 80% of each blend prepared. Sample 1 and 2 had a lower d(0.5) and these were used with Samples 3 and 4 to produce blends with a smaller d(0.5). Samples 5 and 6 had a higher d(0.5) and these were used with Samples 3 and 4 to produce blends with a higher d(0.5). These 8 blends were tested (n=3) using the Mastersizer2000 and Mastersizer3000 methods by placing the samples on the two instruments simultaneously.

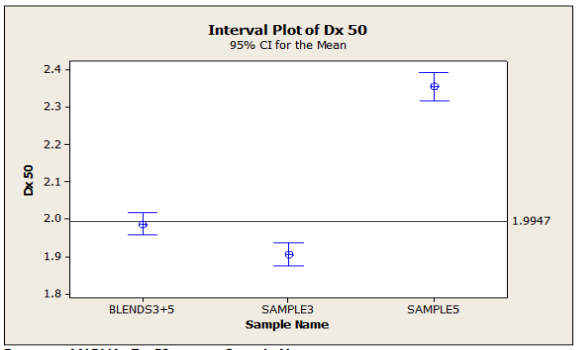
The predicted value of d(0.5) for each blend was calculated using a weighted average of the d(0.5) values of the individual samples and these will be referred to as theoretical results. The actual d(0.5) of each blend was measured experimentally (n=3) and the difference between theoretical and measured d(0.5) gives an indication of the ability of the method to track small changes to the PSD. The data was entered into Minitab and statistics were performed on the Sample data relative to the Blend data, and the output from 2 blends is presented in Figure 3a and 3b. The ANOVA (Analysis of Variance) showed that the blends of Blend 3+1 and Blend 3+5 respectively had statistically different d(0.5) values from Sample 3. This was confirmed with all 8 blends on the Mastersizer2000 and the data demonstrates the method is able to determine a shift in d(0.5) of about 0.05 micron with a good degree of confidence. On the Mastesizer3000 instrument there were 2x Blends (Blend 3+1 and Blend 4+2) where the method was not able to distinguish a shift in d(0.5) of 0.05 and 0.07 micron respectively. The limit of resolution, with statistical confidence, for a shift in the d(0.5) for this method on both instruments is approximately 0.05 micron.



One-way ANOVA: Dx 50 versus Sample Name

Grouping Information Using Tukey Method and 95.0% Confidence

Sample Name	N	Mean	Grouping
SAMPLE3	4	1.905	A
BLENDS3+1	3	1.850	B
SAMPLE1	3	1.600	C



One-way ANOVA: Dx 50 versus Sample Name

Grouping Information Using Tukey Method and 95.0% Confidence

Sample Name	N	Mean	Grouping
SAMPLE5	3	2.353	A
BLENDS3+5	3	1.987	B
SAMPLE3	4	1.905	C

Figure 3a and 3b - Theoretical and experimental d(0.5) values for blends of Sample 3 + Sample 1 and Sample 3 + Sample 5 respectively for the Mastersizer2000 instrument

Figure 4a shows the shift in PSD data for all 8 blends on the Mastersizer2000 and Mastersizer3000 instruments. The red bar denotes the theoretical shift in the d(0.5) for each of the blends on each instrument whilst the blue bar represents the shift measured by the analytical method. It is clear that the analytical methods were able to track the small shifts in d(0.5) associated with preparing and testing the blends of powders. Another way of presenting this data is to show the difference between theoretical and measured d(0.5) (See Figure 4b) and this gives an indication of the "accuracy" of the measurements. It is clear that the measured values were typically within 0.04 micron of the theoretical values. The 2 results with a variance greater than 0.04 micron were blends containing Sample 6, which had a shoulder in the PSD, and this behaviour might not be considered typical for all the samples measured.

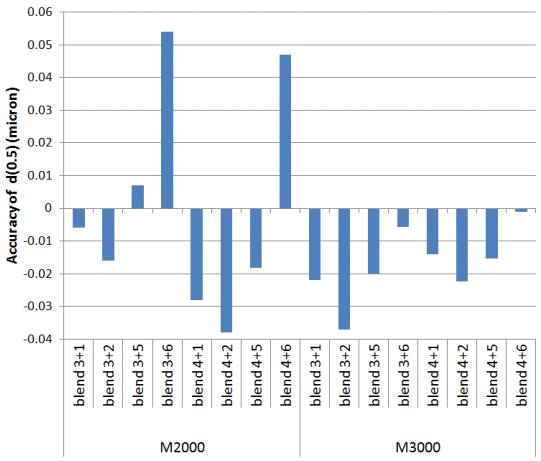
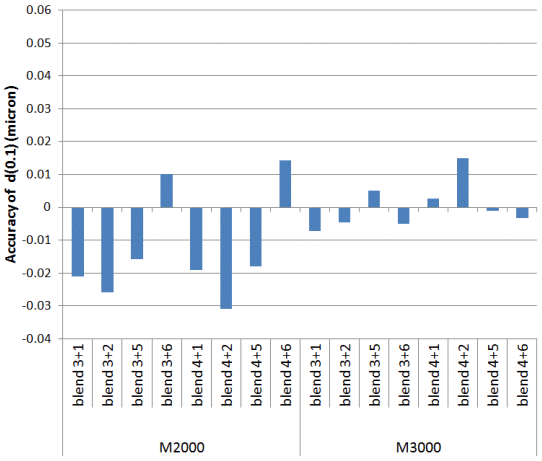


Figure 4a - Theoretical d(0.5) shift (red bars) and the measured d(0.5) shift (blue bars) for a range of blends of Active A on both instruments

Figure 4b - Accuracy of d(0.5) for a range of blends of Active A on both instruments

There was not a large shift in the d(0.1) data between the samples and the blends, as shown in Figure 5a. The Mastersizer2000 and Mastersizer3000 methods were not able statistically to determine these small shifts in the blends relative to the base powders, however both instruments were able to see shifts in the d(0.1) above about 0.04 micron.

In comparison the d(0.9) data showed much more variability for both instruments and although the PSD shift between samples and blends was quite high, typically between 0.1 and 0.4 micron, the methods were often not able to measure these shifts with statistical confidence. This is related to the higher RSD% associated with the d(0.9) data produced by these methods. Both instruments were able to see shifts in the d(0.9) above about 0.3 micron.



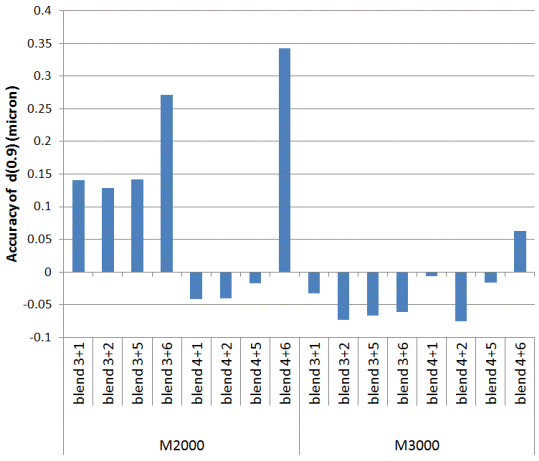


Figure 5a and 5b - Accuracy of d(0.1) and d(0.9) for a range of blends of Active A on both instruments

Conclusions

It is an important factor when developing a laser diffraction method to recognise its capability of measuring small changes in PSD and confirming this is aligned to product specifications. This is the first step in setting up a method based on Quality by Design. The sensitivity of a method could be considered the smallest shift in d(0.5) which is consistently statistically different to a base material. Another metric could be to measure the difference between the theoretical d(0.5) value and the measured d(0.5) value for a range of blends. This could be considered a measure of the accuracy of the method. In this case we have shown, with blending experiments, that the Mastersizer2000 and Mastersizer3000 instruments are both capable of measuring a shift in the d(0.1), d(0.5) and d(0.9) PSD of 0.04 micron, 0.05 micron and 0.3 micron respectively, with statistical confidence.

It is also important to recognise that different laser diffraction instrument designs, even when purchased from the same manufacturer, can have a significant effect on the PSD data. In this case there is a significant bias in the results whereby the Mastersizer3000 instrument shows a much higher proportion of sub-micron particles relative to the Mastersizer2000 instrument. This must be taken into consideration when crossing over methods from one instrument type to another.

References

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