

Method validation for laser diffraction measurements



Introduction

When undertaking particle size measurements it is important to validate the analysis method to ascertain both its robustness and integrity. This allows the key variables associated with variability in the results to be determined and then controlled as part of the measurement procedure. The implementation of a validation study is an absolute requirement in the pharmaceutical industry and of growing significance throughout the manufacturing sector where the need for result consistency is important to ensure efficient production and quality control.

Validation is described by the US Food and Drug Administration (FDA) as “establishing documentary evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specification and quality attributes” [1]. The FDA Guidance to Industry document goes on to state that for particle sizing methods (including laser diffraction) that the intermediate precision and robustness of the method should be studied.

Users of laser diffraction instruments for particle characterization applications have a wealth of information on the theory behind the technology as well as guidance on both sampling and dispersion [2,3,4]. In 1999 the Pharmaceutical Analytical Sciences Group (PASG) [5] laid down some guidelines as to the process which should then be followed during method validation. The work of the PASG group is built upon here with practical examples of how validation can be carried out. Lerke and Adams [6] have also published their ideas on

the subject in a useful paper. Their paper covers both method development and method validation, whereas this note is mainly concerned with the latter.

Note that following the procedures outlined here cannot guarantee that an auditing body will always approve a given method. Rather the minimum amount of work expected to be performed is outlined. Any additional studies will only strengthen the case for the candidate method.

Method Validation

The development of a validated method should be carried out using an instrument that has validated software and is regularly tested to confirm its performance. Validated software has lifecycle documentation detailing its development and maintenance and should be numerically validated using a peer package such as Microsoft Excel. The instrument installation should conform to the manufacturers Installation Qualification (IQ). The recommended manufacturer’s Operational Qualification (OQ) should also be carried out at least yearly, with the instrument performance being tested using secondary standards on a routine basis between each OQ visit. In the current regulatory environment, it is also essential that the software is compliant to 21 CFR part 11, the FDA’s rule regarding the use of electronic records and signatures.

When validating a laser diffraction method for particle characterization, the following main variables must be considered: sampling; sample preparation; instrument range; appropriateness of the technique; robustness of the analytical method;

the amount of light scattered by the sample; and the reproducibility and precision of the measurement.

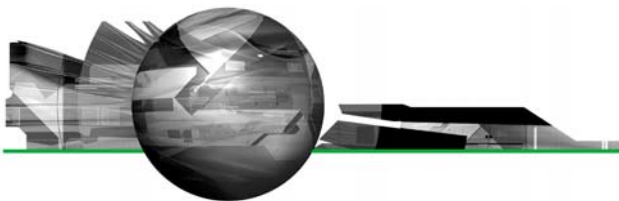
Sampling

One of the first considerations in particle size analysis is whether the sample selected for analysis is representative of the bulk material. If the sample is not representative the results obtained may be atypical and the experiment is a pointless exercise. During transit of the sample, settling can occur - in powders large particle tend to settle at the top of the sample container, whereas suspensions show the reverse within large particles undergoing sedimentation. In each case the material must be sampled in such a way as to remove the bias caused by these processes.

Research has shown that use of a spinning riffler is the most reproducible method of obtaining a representative sample for powder samples when compared with other methods [7] (table 1). Riffling works best for free-flowing particles but can take a great deal of time if a large amount of powder is to be handled. If the powder is not free-flowing then particle segregation is minimized and techniques such as scoop sampling

Table 1: Variability Associated with different sampling methods [7].

Method	Relative Standard Deviation (%)
Cone & Quartering	6.81
Scoop Sampling	5.14
Table Sampling	2.09
Chute Riffling	1.01
Spinning Riffling	0.13



may yield a representative sample.

For slurries it is important to overcome sedimentation by re-suspending the sample. This can often be achieved by simple stirring. However, use of certain stirrers, such as magnetic fleas, can lead to the large particles being thrown to the outside of the container (in a similar way to hydrocyclone separation) where they are not sampled.

Sample Preparation

The PASG group defined sample preparation as “the pre-treatment and the presentation of the sample to the measuring technique in a meaningful manner” [5]. Selection of the correct method will therefore depend on the interests of the user. Where, for example, the primary particle size is important, correct dispersion of the sample will be important. This may be the case when control of the solubility of the powder is important in defining its functionality. If the natural agglomerated state is of interest, as is the case in some granulation

processes, sample preparation should take this into account in order to avoid the break-up of agglomerated particles. In either case, the dispersion medium – whether air or a liquid – should not cause irreversible changes to the particle size through processes such as dissolution, milling or aggregation.

For both dry and wet measurements it will be important to understand how the dispersion energy affects the reported particle size. For wet measurements the addition of surfactants and additives and the use of sonication for dispersion must be investigated. Comparison of the state of the powder before and after dispersion using microscopy can then be used to assess if irreversible particle break-up has occurred. In the case of dry powder measurements the effect of dispersion pressure must be ascertained, with the correct pressure being selected by comparing the results against wet dispersion.

Dispersion parameters are normally assessed as part of method

development rather than as part of the method validation process. Details of the method development process are given elsewhere [8,9].

Detection Limit and Range

Assessment of the detection limit of the laser diffraction technique is not required as part of method validation. However, it is important that the dynamic range of the particle-sizing instrument covers the size range of the sample being tested. This normally does not present a problem for most pharmaceutical samples as modern laser diffraction instrumentation can cover a size range from 20nm to 2000µm in a single measurement. Older instrumentation, however, may have to use many lenses in order to cover the same dynamic range. Here the lens that covers the largest proportion of the particle size distribution should be used. Alternatively the result from two lenses can be blended together, although this is not recommended since the result depends on the mathematical efficacy of the blending routines.

Specificity

Specificity, in terms of whether or not the technique is appropriate to the material under analysis, should be addressed as part of method development and does not have to be revisited for method validation. It is, of course, true that different sizing techniques display different sensitivities and will therefore provide different results for the same sample. Selection of an appropriate technique depends on what is of interest – for instance is the detection of a small amount of over-sized material required or does the technique need to differentiate between different particle types? Laser diffraction provides a good method for assessing small changes in the size distribution in this regard. However, it is difficult to differentiate between the different

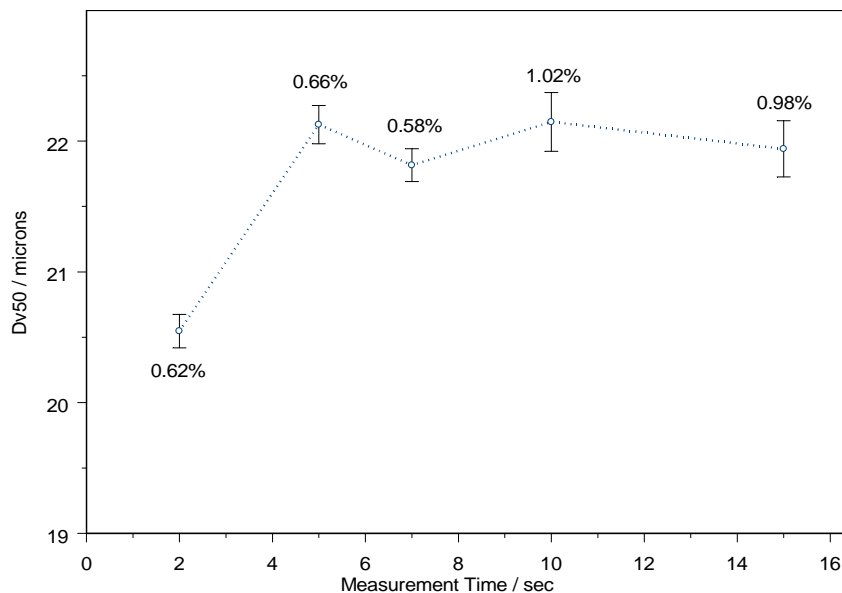


Figure 1: Variation in the Dv50 as a function of measurement duration. The error bars represent the COV over ten measurements.

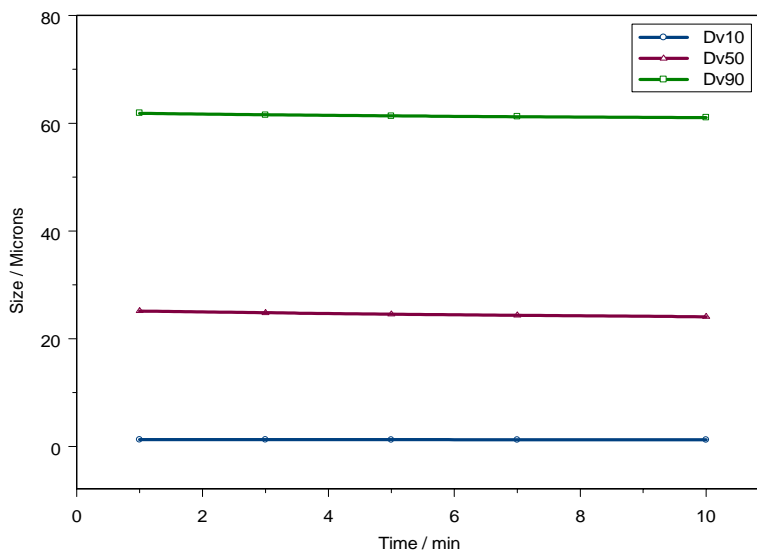
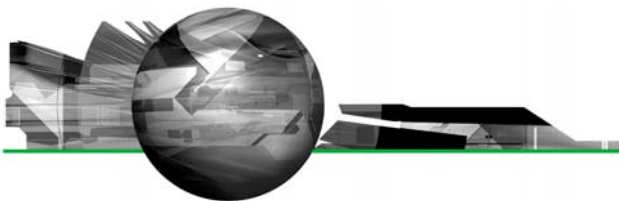


Figure 2: Lactose sample stability measurement summary.

components within pharmaceutical dosage forms using the technique.

Robustness

The robustness of an analytical method is an indication of its ability to remain unaffected by small variations in the test parameters and so provides assurance of its reliability during routine use. The method robustness should be considered before repeatability, reproducibility and intermediate precision are assessed.

Measurement duration and measurement stability are the two main variables to be considered as part of a robustness study. Others, such as air pressure (dry measurements) and pump/stir rates (wet measurement) are normally considered as part of method development, but are briefly described here.

Measurement Duration

The duration of each laser diffraction measurement should be set so as to ensure that representative sampling has been achieved. For narrow distributions or fine particles relatively

short measurements time can be used. If polydisperse or coarse particle size distributions are analyzed then longer measurement times may be required in order to ensure that a representative volume of larger particles has been sampled.

The suggested procedure for assessing the correct measurement duration is to carry out ten repeat measurements using measurement times of 2, 5, 7, 10 and 15 seconds. The individual and mean readings for each measurement time can be over-plotted and any shift in particle size distribution noted. The appropriate duration period can be selected by looking at the coefficient of variation (COV) for the median particle size (Dv50), Dv10 and Dv90.

The COV should be within limits of acceptability laid down in ISO13320 [2]. This states that for samples with a median size of greater than 10 μm , the COV should be less than 3% for cut-off values close to the centre of the distribution (e.g. Dv50) and less than 5% for cut-off values towards the limits of the distribution (e.g. Dv10 and Dv90). For samples with a median size of less than 10 μm , the

COV limits are doubled. The increased variability allowed within ISO13320 for fine particles reflects the fact that dispersion is important within this size range. Note that typically COV values of less than 3% are achievable across the measurement range if both dispersion and sampling are controlled.

Figure 1 shows an example of how the Dv50 reported for a lactose excipient varies with measurement duration. In this case measurement duration of 7 sec was chosen. Although the COV is low for the 2 sec measurement, the Dv50 is significantly smaller than for the other measurements, suggesting that the large particles were not correctly sampled using such a short measurement time.

Measurement Stability

In order to determine whether samples are stable over the period of analysis and not subject to agglomeration, de-agglomeration or dissolution, it is necessary to monitor the particle size distribution at known time points.

A sample should be prepared in accordance with the method under investigation. It is recommended that at least five repeat measurements at the previously established duration be taken at varying times after sample dispersion (1, 3, 5, 7 and 10 minutes). The mean and COV for the Dv10, Dv50 and Dv90 values should then be determined and should be shown to be within the limits of ISO13320 [2].

Typical measurement stability results obtained for a lactose sample are shown in figure 2. From these results it is clear that the lactose sample is stable whilst in suspension. It was decided from these results that measurements should be taken after 1 minute in order to give the sample time to equilibrate.

An example of how the dispersion conditions can yield poor



measurement stability is shown in figure 3. Here, the particle size of an emulsion product dispersed in tap water and deionised water is shown as a function of measurement number

(and therefore time). The results obtained for single measurements are similar. Only by assessing the measurement stability over time can the instability of the dispersion in tap

water be observed. Obviously the precision of measurements carried out in tap water will be poor in this case and deionised water should be used.

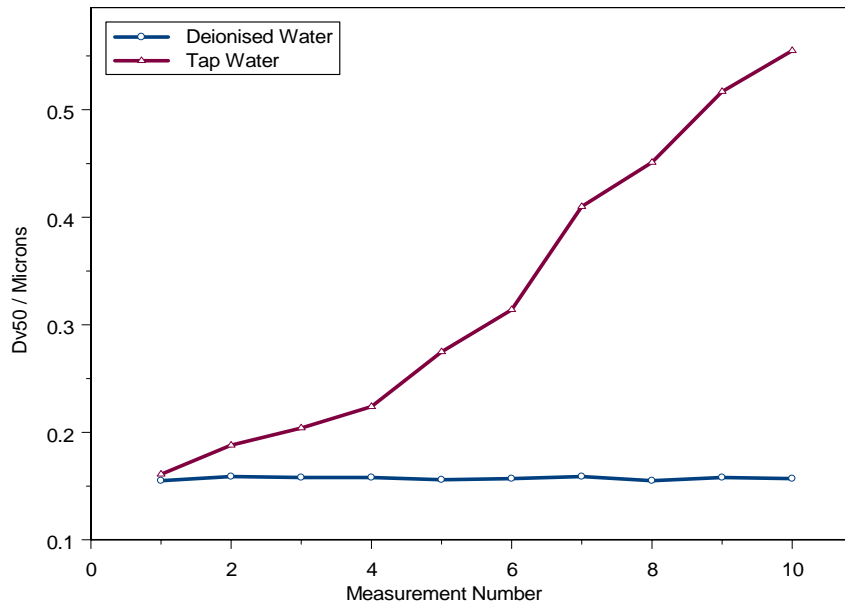


Figure 3: Emulsions sample stability measurements.

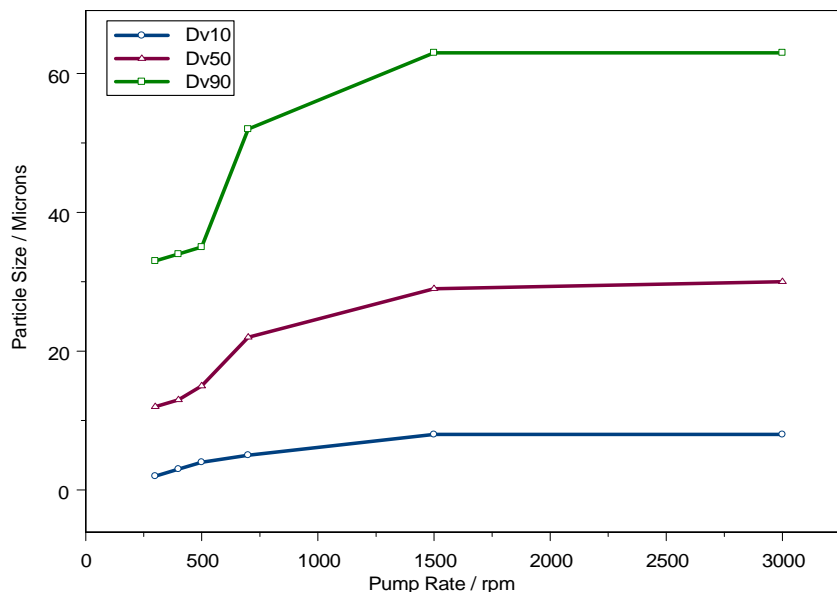


Figure 4: Effect of varying the stirrer rate on the result obtained for a typical lactose.

Sample Dispersion

As part of method development the process of sample dispersion should have been explored and understood.

In the case of dry measurements users are required to understand how the measured particle size varies with the air pressure selected for dispersion [2]. A suitable pressure is one at which dispersion is achieved without milling of the particles. In the case of pharmaceutical materials, the sample under test can be friable and may be milled to a finer particle size if the dispersion air pressure is set too high. Often both dispersion and milling occur simultaneously (which leads to a broadening of the distribution) [10]. The best way of proving that no attrition is occurring is to achieve near-identical results for both wet and dry dispersion [2]. Full details of how dry method development may be carried out are given elsewhere [8].

For measurements made using wet dispersion the role of ultrasound in assisting dispersion must be understood [2]. Using high-energy ultrasound can fracture some crystalline materials, although this is extremely unusual. As part of method development the affect of varying the duration and power of ultrasound on the particle size distribution should be examined. Ideally measurements should be taken before, during and after ultrasound to examine what effect sonication has on the robustness of the measurement. Micrographs should also be obtained to ensure that particle break-up has not occurred during dispersion.

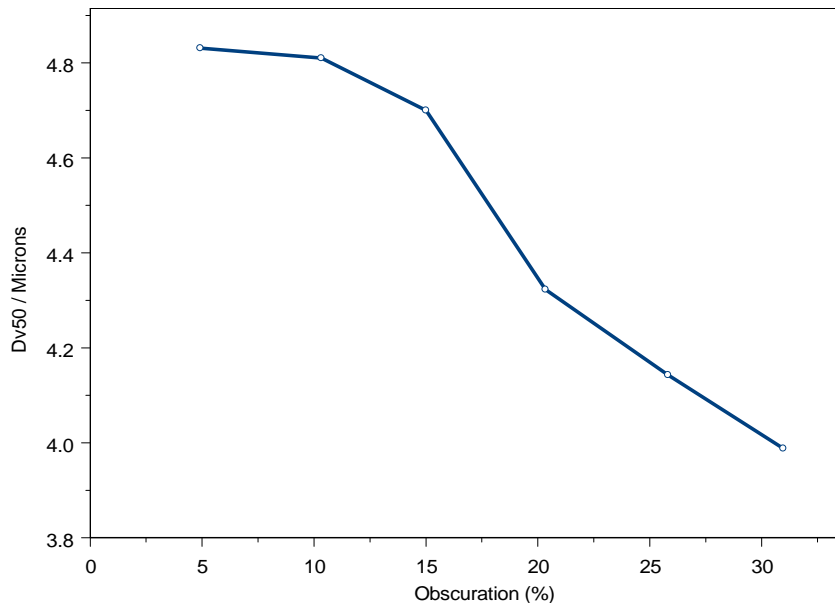
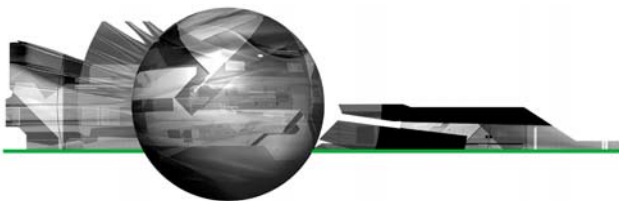


Figure 5: Change in Dv50 as a function of obscuration for a pharmaceutical powder.

It should also be noted that the application of ultrasound can cause agglomeration in some cases. If this occurs then it may be necessary to look at the use of different dispersing agents to bring about stability. Full details of how wet method development may be carried out are given elsewhere [9].

Pump and Stir rates

The pump and stir speeds used during a wet measurement should be examined as part of method development. The chosen conditions should be capable of suspending all the material without causing air bubble formation (a particular problem if surfactants are being used).

Figure 4 shows how the result obtained for a lactose sample varies according to the stirrer settings. As can be seen, the result reaches a plateau above 2000rpm. It is at this point that all of the material is correctly suspended and dispersed. Sample sedimentation causes the results to be smaller than expected at stirrer rates below 2000rpm.

Confirmation of refractive index

As part of the method development work, the choice of refractive index should be examined. Index matching fluids can be used to provide experimental evidence of the real refractive index. The Fraunhofer approximation should not be used if there are particles smaller than 40 times the wavelength of light used for the measurement (25 microns for a system using a HeNe red laser light source) present in the distribution¹ as it may erroneously report the presence of fine material. ISO13320-1 provides guidance as to when the optical properties will be critical in configuring the laser diffraction analysis [2].

Linearity and Obscuration

Assessing the linearity of a sizing technique is not considered to be part of the method validation as sizing techniques seldom display a linear response as a function of particle size. However, it is important to consider how the sample obscuration affects the measurement. Obscuration

is a measure of the amount of light scattered by the sample and correlates with the concentration of material present within the measurement zone. For most particle size distributions the reported particle size should be independent of the measurement obscuration for a wide obscuration range. At extremely low obscurations results with large COVs may be obtained (owing to the low signal to noise ratio) whilst at extremely high concentrations the results obtained may be smaller than expected due to the effects multiple scattering. It is suggested that obscurations of 5, 10, 15, 20 and 25% are investigated in the same way that the measurement duration test is performed, with the acceptable COVs being similarly specified.

An example of how multiple scattering affects the results obtained is given in figure 5 for a pharmaceutical powder. At low obscurations the result is constant. However, beyond 10% obscuration multiple scattering causes the reported size to decrease. In this case a measurement obscuration of around 7.5% would provide a robust method where small changes in the obscuration from sample to sample would not overly affect the results.

Reproducibility

Bell et al defined reproducibility as an indicator of precision between laboratories [5]. It can indeed show this, but experience at Malvern Instruments has shown that it is far more likely to provide a measure of the effectiveness of the chosen sampling method. It can also be used to flag differences between different instruments (be it of the same or different models). Another consideration will be environment conditions experienced in different laboratories, especially where saturated solutions are used as dispersants, as small temperature differences can cause result changes due to either particle dissolution or recrystallization.

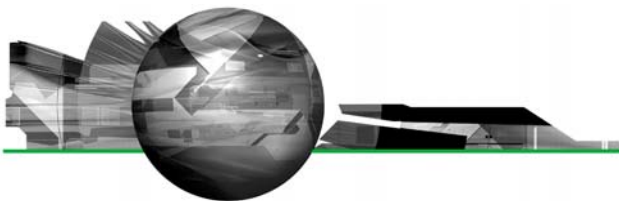


Table 2: Variation in the results obtained for seven separate scooped-sampled lactose samples.

Sample Number	D(v,0.1) μm	D(v,0.5) μm	D(v,0.9) μm
1	1.22	23.68	63.23
2	1.17	23.77	60.02
3	1.09	22.79	56.59
4	1.16	23.63	62.55
5	1.11	22.26	59.68
6	1.18	22.78	65.36
7	1.12	23.41	61.47
Mean	1.15	23.19	61.27
%RSD	3.95	2.50	4.63

To assess reproducibility, a number of samples (at least five) should be taken from the same batch and tested in accordance with the method under investigation. For each sample at least five repeat measurements should be taken and the individual and average results should be obtained. The sample-to-sample COV for should then be determined and should be within the limits stated in ISO13320 [2], or in certain cases within the limits of USP<429> [4].

An example of the results obtained using scoop sampling for a lactose excipient is shown in Table 2. In this case the COV obtained was within the limits expected on the basis of the sampling statistics shown in Table 1.

Intermediate Precision

Assessing the Intermediate Precision requires users to determine the method variability when it is followed by a second analyst or using a second instrument (or both). This is essentially a repeat of the reproducibility test, thus similar COV limits should be applied. Both sets of results should then be combined to

give a pooled mean and a pooled RSD (which should be <3%).

The reproducibility data obtained for a second analyst analyzing the lactose sample mentioned above is shown in Table 3. From this a pooled mean and COV can be determined across both operators and is shown in Table 4. As can be seen, the variation in the Dv50 is only just within the ISO13320 limits. This is related to the method of sampling used within this study. If the powder had been sampled using a spinning riffler this would have improved the overall precision. The broader acceptance limits employed in USP<429> could also be applied in this case if scoop sampling is the only viable method for obtaining a sample.

Other Considerations

Validation terms such as quantification limit do not apply to laser diffraction methods and are therefore outside the scope of this application note. Assessment of the measurement accuracy is also not a requirement as, when measuring the particle size of non-spherical particles, it is difficult to define exactly what accuracy actually means. This is because all particle sizing techniques use approximations to derive a characteristic particle diameter. In the case of laser diffraction there is also no calibration procedure involved in deriving a particle size distribution that would need to be assessed as part of an accuracy study. However, it is important that the system's performance is verified following the manufacturers recommended OQ procedure. The requirements for verification are also outlined in ISO13320 [2].

Conclusions

The FDA's guidance regarding the validation of particle size analysis methods states that the validation concepts associated with other analytical methodologies, such as HPLC, do not transfer to techniques

Table 3: Lactose results obtained for a second analyst.

Sample Number	D(v,0.1) μm	D(v,0.5) μm	D(v,0.9) μm
1	1.06	22.92	61.01
2	1.08	22.08	56.54
3	1.04	21.66	62.17
4	0.97	22.55	60.23
5	1.04	22.74	57.98
6	0.99	23.58	59.86
7	0.95	22.11	62.78
Mean	1.02	22.52	60.08
% RSD	4.79	2.83	3.69

Table 4: Pooled mean and COV values.

Mean Dv50 / microns	22.85
Standard Deviation	0.68
COV (%)	2.98

such as laser diffraction. Instead, analysts are only required to assess the intermediate precision and robustness in order to show that the selected method provides a reproducible method of controlling product quality [1]. This application note has outlined some of the important variables that should be assessed when considering the precision of laser diffraction measurements. However, the validation protocol applied to measurements carried out on a given material will ultimately depend of an



assessment of the risk associated with any measurement errors and is therefore the responsibility of the analyst involved.

laser diffraction" Paper 0208 14th International Congress of Chemical and Process Engineering "CHISA'2000", 27-31 August, Praha, Czech Republic

References

- [1] FDA Draft CMC Guidance for Industry; Analytical Procedures and Methods Validation; Section XI; Part F (Aug 2000).
- [2] ISO 13320-1 Particle Size Analysis – Laser Diffraction Methods Part 1: General Principles (1999)
- [3] Jillavenkatesa, A, Dapkunas, S. J. and Lum, L. S., (2001) Particle Size Characterization Practice Guide, N.I.S.T 960-1
- [4] USP General Chapter <429> "Light Diffraction Measurement of Particle Size", Pharmacopoeial Forum, 28(4), 1293-1298 (2002).
- [5] Bell, R., Dennis, A., Hendriksen, B., North, N. and Sherwood, J., (1999) "Position paper on Particle Sizing: Sample Preparation, Method Validation and Data Presentation, Pharmaceutical Technology Europe, November 1999
- [6] Lerke, S.A. and Adams, S.A, (2002) "Development and Validation of a Particle Size Distribution Method for Analysis of Drug Substance", American Pharmaceutical Review, Fall 2002
- [7] Allen, T. Particle Size Measurement, (1999) 5th edition, Volume 1, p 38, Chapman and Hall, London
- [8] Developing a Method for Dry Powder Analysis, Malvern Application Note MRK524.
- [9] Wet Method Development for Laser Diffraction Measurements, Malvern Application Note MRK561.
- [10] Rawle, A. F., (2000) "Attrition, dispersion and sampling effects in dry and wet particle size analysis using

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