Developing and Validating Commonly Employed Particle Sizing Methods to Support Bioequivalence and Product Quality

SBIA 2020: Advancing Innovative Science in Generic Drug Development Workshop

Session 1: Method Development / Validations for Non-traditional Analytical Methods
Topic 2: Advanced Analytical and Statistical Methods for Assessing Particle Size Distributions

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Learning Objectives

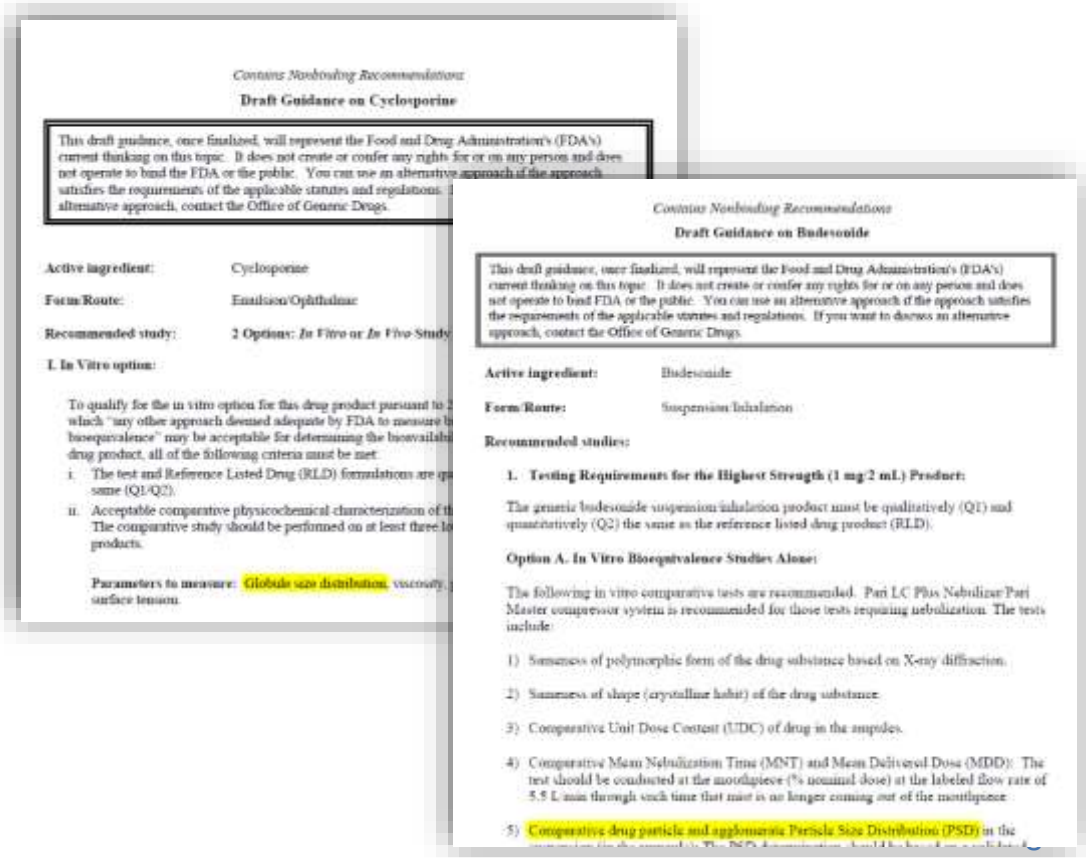
- Identify the importance of particle size distribution (PSD)
- Understand the challenges associated with PSD measurement
- Familiarize with parameters important for PSD method validation
- Recognize the common deficiencies in PSD assessment

Particle Sizing: the Why

- Particle size is an important product quality attribute for formulations in a dispersed state, e.g., emulsions, suspensions, liposomes, aerosols, colloidal irons

- Also a critical physicochemical property in supporting the bioequivalence (BE) determination (in vitro option), e.g., budesonide suspension, cyclosporine emulsion

- Concerns with products of a wide range of sizes (e.g., 10 nm to 100 µm) and with different distributions (e.g., unimodal or multi-modal)



Contains Nonbinding Recommendations

Draft Guidance on Cyclosporine

This draft guidance, once finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind the FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the Office of Generic Drugs.

Active ingredient: Cyclosporine

Form/Route: Emulsion/Ophthalmic

Recommended study: 2 Options: *In Vitro* or *In Vivo* Study

I. In Vitro option:

To qualify for the in vitro option for this drug product pursuant to 2
which "any other approach deemed adequate by FDA to measure b
bioequivalence" may be acceptable for determining the bioavailabi
drug product, all of the following criteria must be met:

i. The test and Reference Listed Drug (RLD) formulations are qu
same (Q1/Q2).

ii. Acceptable comparative physicochemical characterization of th
The comparative study should be performed on at least three lo
products.

Parameters to measure: Globule size distribution, viscosity,
surface tension.

Contains Nonbinding Recommendations

Draft Guidance on Budesonide

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Active ingredient: Budesonide

Form/Route: Suspension/Inhalation

Recommended studies:

1. Testing Requirements for the Highest Strength (1 mg/2 mL) Product:

The generic budesonide suspension/inhalation product must be qualitatively (Q1) and quantitatively (Q2) the same as the reference listed drug product (RLD).

Option A. In Vitro Bioequivalence Studies Alone:

The following in vitro comparative tests are recommended. Pari LC Plus Nebulizer/Pari Master compressor system is recommended for those tests requiring nebulization. The tests include:

1) Sameness of polymorphic form of the drug substance based on X-ray diffraction.

2) Sameness of shape (crystalline habit) of the drug substance.

3) Comparative Unit Dose Content (UDC) of drug in the ampules.

4) Comparative Mean Nebulization Time (MNT) and Mean Delivered Dose (MDD): The test should be conducted at the mouthpiece (% nominal dose) at the labeled flow rate of 5.5 L/min through each time that must is no longer coming out of the mouthpiece.

5) Comparative drug particle and agglomerate Particle Size Distribution (PSD) in the

But, what we are interested usually is not “size”

The analysis of the particle size is not an objective in itself, but is a means to an end.

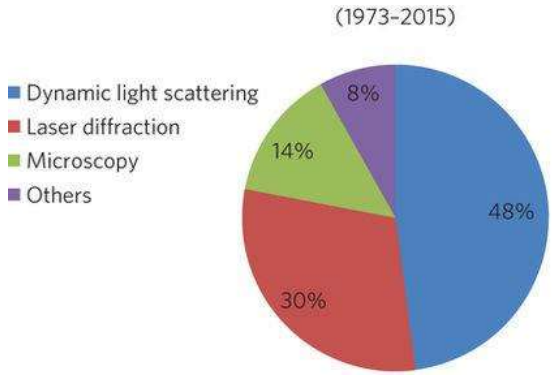
- Physical stability (e.g., dispersion sedimentation, agglomeration)
- Dissolution/drug release (e.g., due to surface area differences)
- Bioavailability
- Process capability (e.g., power flow, packing density)
- Bioequivalence (e.g., sameness or difference)

Depending on the purpose of the size measurement, the expectations on analysis outcome (i.e., numbers) may vary, e.g.,

Product A is 10 nm smaller than Product B ($p<0.05$). Are they “different”?

Techniques to Determine Particle Size Distribution

Technique	Size range	Principle
Dynamic light scattering (DLS)	1 nm to 1 µm	Brownian motion + light scattering
Laser diffraction (LD)	30 nm to 3000 µm	Static light scattering (Mie or Fraunhofer)
Electron microscopy (EM)	0.1 nm to a few micron	Electron density contrast
Image analysis	1 µm to a few hundred micron	Image analysis
Light obscuration	Subvisible particles (0.5 µm to 400 µm)	Single particle light blockage
Nanoparticle tracking analysis (NTA)	20 nm to 1 µm	Brownian motion + image analysis
Field flow fraction (FFF) + multi-angle light scattering (MALS) + DLS	1 nm to a few micron	Brownian motion + flow based separation + light scattering
Resonant mass measurement (RMM)	50 nm to 5 µm	Buoyant mass
Focused Beam Reflectance Measurement	1 µm to 1000 µm	Chord length



S. D'Mello, et al. *Nature Nanotechnology*, 2017, 12, p.523-529

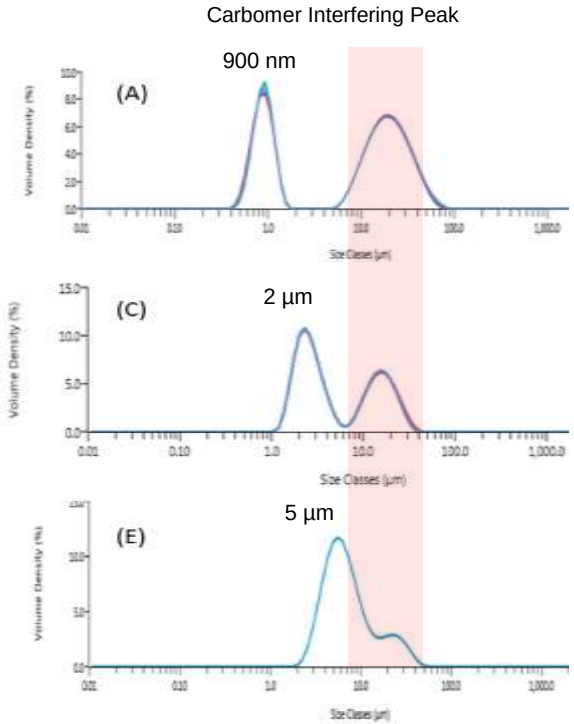
Particle Sizing: Development Considerations

(not an exhaustive list)

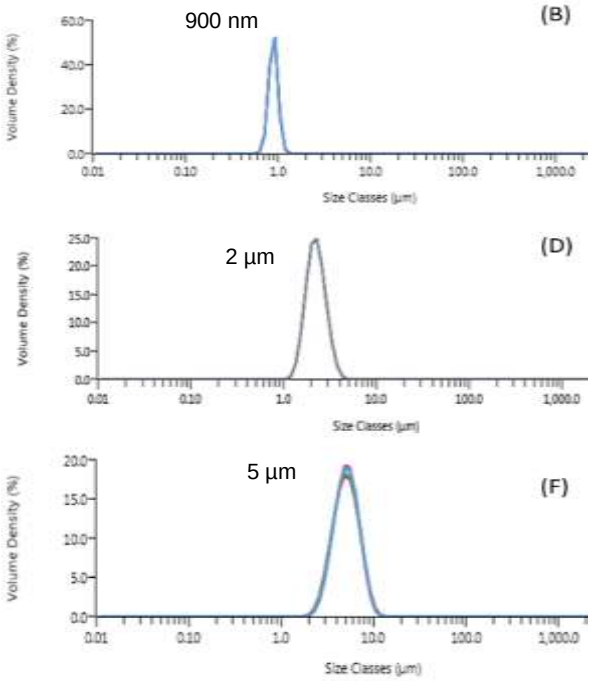
- ❑ What is the size range?
  - <100 nm: dynamic light scattering (DLS)
  - >1 µm: laser diffraction (LD)
- ❑ Interested in shape or if the shape is important: microscopy, e.g., Cryo-TEM
- ❑ Is the sample sensitive to dilution (e.g., suspension particles may dissolve)?
- ❑ Does sample disperse well or remain stable in the medium (e.g., in air or in water)?
- ❑ Is the sample colored (e.g., may absorb light and thus reduce the signal quality in DLS or LD)?
- ❑ Does sample preparation impact the stability of the particles (e.g., sonication, dilution, filtration)?
- ❑ Does the formulation component (e.g., polymeric excipient) interfere with the analysis?

Method Interference (Excipients)

Formulation excipients (e.g., polymers, surfactants) may interfere with the size analysis, leading to high variability and erroneous results. For example, in an ophthalmic suspension formulation, presence of carbomer (a viscosity enhancer) was found to interfere with the laser diffraction measurement (demonstrated below using NIST standards with known sizes). The size of the API particle was close to 5 µm which overlapped with the excipient interfering peak.



Interference eliminated by using the Placebo formulation as a blank



Internal data (not published)

Particle Sizing: Development Considerations

Cont.

- ❑ What is the appropriate measurement setting (instrument/software dependent)?
  - Laser power (DLS)
  - Measurement position (DLS)
  - Stirring speed (LD)
  - Sonication (LD)
  - Optical inputs (DLS and LD)
  - Analysis algorithms (e.g., cumulant vs. distribution, CONTI, Mie vs. Fraunhofer)
- ❑ What should be reported?
  - Mean or median (e.g., D[4,3], D[3,2], D[1,0], z-average, d50)
  - Distribution (e.g., Polydispersity index, SPAN)
  - Cumulant vs. distribution (for DLS)
  - Everything has to do with how the data is generated (e.g., settings, sample preparation)

Particle Sizing: Development Considerations

Cont.

- ❑ Sensitivity (e.g., formulation, process changes) and specificity (e.g., any interference?)
- ❑ Single technique (robustness) vs. complementary techniques (strength/limitation of each one, comparison)

PSD Method Validation

PSD method needs to be properly validated to demonstrate it is suitable for its intended purpose. However, validation of particle sizing methods is not the same as validation of other analytical methods described in ICH Q2 guideline.

Characteristics recommended in ICHQ2	Relevant for Size?	Comment
Specificity	No	Almost all PSD methods are non-specific to the particles being measured
Linearity	No	Most of the size measurement do not rely on calibration, therefore no need to establish linearity
Range	No	Range of the method is pre-defined by the choice of the technique, e.g., DLS or cryo-TEM, and not the method itself
Accuracy	Maybe	For system qualification, it is useful to use size standards to ensure the system operates correctly. But accuracy of the method cannot be determined using the size standards. It is more important to demonstrate that the method is reliable in measuring test and reference samples
Detection limit	No	Not relevant
Quantitation limit	No	Not relevant
Precision	Yes	Very important (3Rs, see next slide)
Robustness	Yes	Very important

Validation: Repeatability, Reproducibility, Robustness

Repeatability: closeness of agreement between multiple measurement results of a given property in the same dispersed sample aliquot, executed by the same operator in the same instrument under identical conditions within a short period of time (e.g., 6 measurements for the same sample).

-Machine, Testing method, Sample stability

Reproducibility: closeness of agreement between multiple measurement results of a given property in different aliquots of a sample, prepared and executed by same or different operators in similar instruments according to the same method (e.g., 6 samples prepared by the same operator).

-Sampling procedure, dispersion, machine

Robustness: reliability of an analysis with respect to deliberate variations in method parameters, i.e., it should be both sensitive (able to detect significant changes in the underlying measured parameter) and precise (repeatable with a high signal to noise ratio). For example, change in sonication power, sonication duration, flow rate, particle concentration (i.e., obscuration%), temperature, analysis algorithm.

Example Deficiencies: 1

We acknowledge that you have set acceptance limit for PSD based on distribution analysis in 3.2.P.5.3. Please clarify if the results (i.e., d10, d50, and d90) were “intensity-“ or “volume-“ weighted and if the mode of the analysis was “General purpose” or “Multi narrow”. Based on your response, please update the method in section 3.2.P.5 accordingly.

Related to:

1) How data are reported (i.e. cumulant or distribution for DLS)
2) What is the analysis algorithm

Example Deficiencies: 2

We acknowledge that you have performed method validation for particle size. However, the robustness of the method against dilutions has not been determined. You have diluted the sample 1000 times prior to the size measurement. The impact of serial dilutions on size results should be determined. Accordingly, please validate the method robustness in terms of serial dilutions (e.g., 10, 100, 1000 times using water). Additionally, please include polydispersity index (PdI) in the validation result (precision and robustness).

Related topic:

1) What should (not) be validated
2) 3Rs (repeatability, reproducibility, and robustness)

Example Deficiencies: 3

a. We acknowledge that …. Furthermore, the increase in the acceptance limit from NMT150 nm (product release) to NMT170 nm (stability test) is not justified based on the 9-months stability testing results. Please revise the acceptance limit for particle size to a range, and use the same acceptance limit for both product release and stability test.

b. We acknowledge that .... However, the variability in the provided results appears much narrower than the proposed range of 75-200 nm. Please tighten the acceptance limit for particle size, either based on the variation of the reference listed drug (e.g., ± 3σ) or a range that you can justify. In addition, please establish appropriate acceptance limit for PdI, and provide justification accordingly.

Related topic:

1) How to set the specification and acceptance limit
2) Which parameter to set the specification

Other Common Deficiencies

- Incorrect choice of the instrument or technique, e.g., choosing laser diffraction (LD) for measuring particle size of colloidal iron product.
- Incorrect use of material/dispersant refractive index (RI), especially if reported based on distribution analysis (DLS).
- Not clear on which analysis was used, e.g., cumulant vs. distribution (DLS).
- Intensity-weighted distribution is always recommended; use volume-weighted distribution only if it is adequately justified; and avoid the use of number-weighted distribution (DLS).
- Validation performed incorrectly using only the reference standard (e.g., NIST standard); should use actual samples (reference list drug (RLD) samples are also ok)
- Lack of method details, such as measurement position, attenuator settings, cuvettes (DLS).
- Sample preparation missing critical details or lack of justifications, e.g., if the dispersion medium has been saturated with the drug before measuring using LD, lack of justification for use of sonication.

Summary

- Particle size is one of the critical quality attributes that also affects the BE
- Every particle sizing technique has its strengths and limitations (welcome new techniques which provide better understandings)
- It is important to ensure the method is properly developed and adequately validated
- Correct interpretation of the result relies on full and complete information of the method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Challenge Question (Single choice)

What parameters are needed to be critically examined as part of the method validation for PSD:

A. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

B. Accuracy and Precision

C. Precision and Robustness

D. Specificity and Accuracy