

Particle Size Distribution (PSD) Method Validation Using Malvern Mastersizer 3000 for Drug Substance and Drug Product: A Risk-Based Regulatory Lifecycle Approach

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Abstract

Particle Size Distribution (PSD) is a critical quality attribute (CQA) for a wide range of pharmaceutical drug substances and drug products, directly influencing dissolution, bioavailability, blend and content uniformity, stability, and manufacturability. Laser diffraction has become the compendial and industrial standard for PSD analysis, with the Malvern Mastersizer 3000 widely used in pharmaceutical development and quality control laboratories. However, regulatory inspections continue to identify deficiencies related to dispersion optimization, method validation, sampling representativeness, and lifecycle management of PSD methods. This study presents a comprehensive evaluation of PSD method validation using both dry and wet dispersion accessories of the Malvern Mastersizer 3000, specifically the Aero S (dry dispersion) and Hydro MV (wet dispersion) systems. Experimental datasets generated during method development and validation were used to evaluate measurement performance for two representative pharmaceutical materials: Drug substance and Drug product. Key PSD parameters, including D10, D50, and D90, were assessed under optimized dispersion conditions. Method validation parameters such as repeatability, intermediate precision, and robustness were evaluated using risk-based analytical validation principles aligned with ICH Q2 (R2). The study is further contextualized within the integrated pharmaceutical quality framework described in ICH Q7-Q11, linking PSD measurement to drug substance characterization, process understanding, and lifecycle man-

agement. The findings demonstrate that appropriate dispersion selection and statistically justified validation are essential for ensuring reliable PSD control. Overall, PSD measurement should be viewed not merely as an analytical test but as a critical tool for process understanding and quality control within modern pharmaceutical manufacturing systems. This article reviews the scientific and regulatory basis of laser-diffraction PSD testing and presents a case study validating dry and wet Mastersizer 3000 methods for a drug substance powder and a cream drug product. The study applies dispersion optimization, sampling controls, and precision, intermediate precision, and robustness assessments within an ICH Q2 (R2)-aligned framework. The main conclusion is that material-specific dispersion selection and lifecycle-based validation are necessary for reliable PSD control.

Keywords

Particle Size Distribution, Laser Diffraction, Malvern Mastersizer 3000, Dry Dispersion, Wet Dispersion, ICH Q7, ICH Q8, ICH Q9, ICH Q10, ICH Q11, Method Validation, FDA 483, PAT, Lifecycle Management, Quality Risk Management

1. Introduction

Particle size is one of the most influential physical characteristics of a pharmaceutical material. Although often viewed simply as a numerical distribution D10, D50, or D90 particle size significantly influences how a drug behaves during manufacturing, Dissolution/testing behavior, and bioavailability. From early-stage development through commercial production, particle size distribution (PSD) serves as a bridge between material science and clinical performance, and it impacts process performance as well (flow, blending, milling etc.). A clear scientific connection between particle size and product performance can be found in the dissolution behavior as suggested by the Noyes Whitney equation: The dissolution rate of a solid is directly proportional to its surface area. As the particle size decreases, total surface area increases dramatically, often leading to a faster dissolution [1]-[3]. For poorly soluble drug substances particularly those classified as Biopharmaceutics Classification System (BCS) Class II or IV, this relationship becomes critical. In such cases, dissolution is frequently the rate-limiting step for absorption. Even modest changes in particle size can significantly alter bioavailability, systemic exposure, and potentially clinical outcomes. Consequently, PSD control is not merely a physical measurement; it is a clinically relevant parameter. Beyond dissolution, particle size influences nearly every aspect of pharmaceutical processing. During blending, differences in particle size and density can lead to segregation, resulting in poor blend uniformity and potential content uniformity failures. Fine particles may adhere to equipment surfaces or larger particles, while coarse fractions may settle preferentially. In solid oral dosage manufacturing, ac-

tive pharmaceutical ingredient (API) particle size affects powder flowability, compressibility, and tablet hardness [4]. Overly fine materials may exhibit poor flow and electrostatic behavior, whereas excessively coarse particles may lead to weak compacts or capping. Thus, PSD becomes an essential process variable, closely tied to manufacturing robustness and batch-to-batch consistency. Given its widespread impact, accurate and reliable PSD measurement is paramount. Among available analytical techniques, laser diffraction has become the industry standard due to its practicality, speed, and broad applicability. Modern instruments such as the Malvern Mastersizer 3000 can measure particle sizes over a wide dynamic range from submicron particles to millimeter-scale materials within seconds. This broad measurement capability makes laser diffraction suitable for a diverse range of pharmaceutical materials, including APIs, excipients, granules, and finished dosage forms. Laser diffraction offers several practical advantages such as rapid analysis and enabling high sample throughput both in development and quality control environments. It demonstrates high reproducibility when methods are properly optimized. Importantly, it reports volume-based distributions, which align well with manufacturing considerations where bulk material behavior is volume-dependent. However, despite its technological maturity and widespread adoption, PSD measurement by laser diffraction is not a “plug-and-play” test. The technique is extremely sensitive to dispersion conditions and sample preparation. In dry dispersion, excessive air pressure can fracture fragile particles, artificially shifting the distribution toward smaller sizes. Inadequate pressure, on the other hand, may fail to break apart agglomerates, resulting in false measurements. Similarly, in wet dispersion systems, insufficient sonication can leave agglomerates intact, while excessive sonication may induce particle breakage or alter surface characteristics. Sampling presents an equally significant challenge. Powders are inherently heterogeneous, and segregation can occur during storage, transport, or handling. If sampling procedures are not carefully designed and validated, the measured PSD may not represent the true bulk distribution. Even minor inconsistencies in sampling technique, sample mass, or feed rate can introduce variability that rivals or exceeds true process variation. These realities underscore a critical point: PSD analysis is not solely an instrumental measurement but rather a system-dependent analytical process. Structured method development, dispersion optimization studies, risk assessment, and lifecycle monitoring are necessary to ensure reliability. Without such controls, PSD results may appear precise but lack scientific robustness. In today’s regulatory environment, where lifecycle management and risk-based approaches are emphasized, PSD methods must be validated and continuously verified within an integrated quality framework. Particle size is not merely a release specification; it is a material attribute that connects formulation design, process engineering, analytical science, and patient outcomes. Recognizing this interconnected role transforms PSD measurement from a routine laboratory test into a strategic component of pharmaceutical quality assurance [5]. The Product Quality Research Institute (PQRI) recommendations provide guid-

ance for measuring and reporting particle size distributions using validated techniques such as laser diffraction, sieving, and dynamic light scattering [6].

2. Scientific Principles of Laser Diffraction

Laser diffraction is an optical measurement technique used to determine particle size distribution (PSD) by analyzing how a population of particles scatters light. When a coherent light beam passes through a dispersed particulate system, each particle interacts with the incident light, producing a characteristic scattering pattern. The angular distribution of this scattered light contains quantitative information about particle size. Larger particles predominantly scatter light at smaller angles relative to the incident beam, while smaller particles generate scattering at wider angles. By collecting and mathematically interpreting this angular intensity profile, the instrument reconstructs the PSD of the sample. The measurement is inherently indirect. The instrument does not “see” particles individually; instead, it records the collective scattering signature of millions of particles simultaneously. An array of detectors positioned at multiple angular locations measures the intensity of scattered light. The resulting dataset represents scattering intensity as a function of angle. Mathematical inversion algorithms are then applied to determine the particle size distribution that best explains the measured scattering pattern. Because this reconstruction depends on theoretical light-scattering models, the final PSD output is model dependent. Two principal optical theories are used in laser diffraction calculations: the Fraunhofer approximation and Mie theory. The Fraunhofer approximation is based on diffraction theory and assumes that particles are completely opaque and significantly larger than the wavelength of the incident light [7] [8]. Under these assumptions, scattering is dominated by edge diffraction effects, and internal optical properties of the particle are not explicitly considered. This model is computationally straightforward and can be adequate for coarse, non-transparent materials. However, when particles are small, translucent, or partially absorbing, as is frequently encountered in pharmaceutical materials, the Fraunhofer approximation may oversimplify scattering behavior and introduce systematic errors. Mie theory provides a more comprehensive electromagnetic solution to light scattering by spherical particles [7] [8]. Unlike the Fraunhofer approach, Mie calculations require specification of both the real and imaginary components of the particle refractive index, as well as the refractive index of the surrounding medium in wet dispersion measurements. The real refractive index describes how light propagates through the particle relative to the surrounding environment, while the imaginary component accounts for absorption or attenuation of light within the material. Because scattering intensity at higher angles is particularly sensitive to optical contrast, accurate refractive index inputs are essential for reliable characterization of fine particle fractions. Even modest inaccuracies in refractive index values can shift calculated distributions, especially below approximately 10 μm . Modern instrumentation enhances the robustness of these theoretical calculations. Systems such as the Malvern Mastersizer

3000 employ a dual-wavelength configuration that combines a red laser with a blue LED light source. The shorter wavelength of the blue LED increases sensitivity to smaller particles by improving detection of wide-angle scattering. In addition, wide-angle detector arrays capture scattered light across an extensive angular range, enabling simultaneous characterization of broad or multimodal size distributions. Advanced inversion algorithms iteratively refine the calculated PSD by minimizing residual differences between measured and predicted scattering intensities. These computational procedures improve the stability of the solution, particularly when dealing with complex or highly polydisperse samples (**Figure 1**, **Figure 2**).

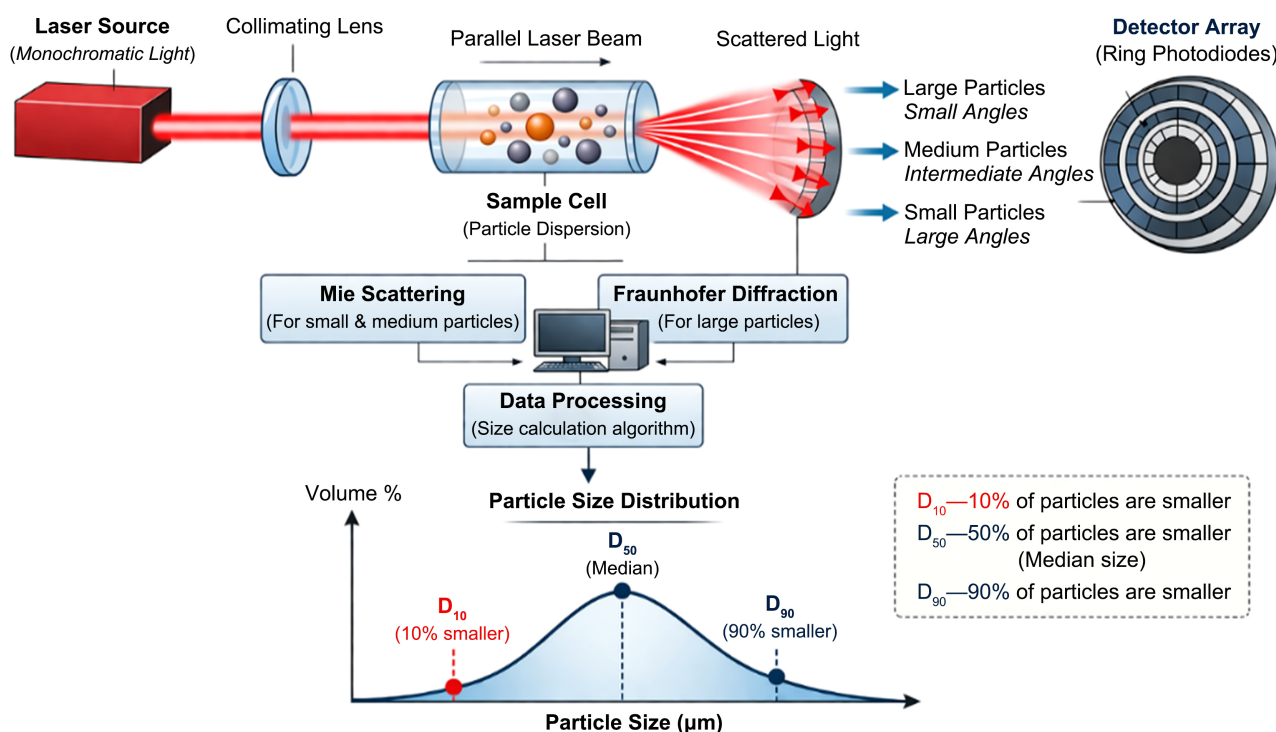


Figure 1. Laser diffraction principle optical system diagram.

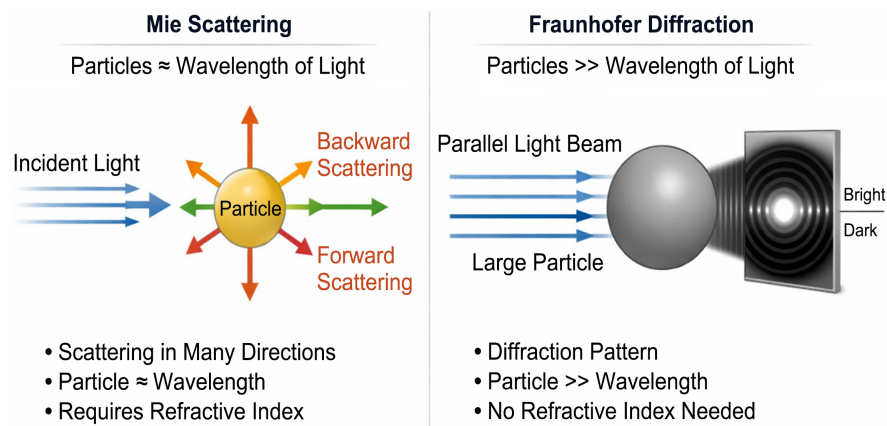


Figure 2. Mie scattering and fraunhofer diffraction pattern difference.

The results of laser diffraction analysis are typically expressed as volume-based particle size distributions. Volume weighting reflects the proportional contribution of particles to total sample volume, which aligns well with bulk material behavior in manufacturing contexts. Commonly reported statistical descriptors include D10, D50, and D90. The D50 value represents the volume median diameter at which half of the total particle volume consists of smaller particles and half consists of larger particles. The D10 and D90 values indicate the lower and upper bounds of the central distribution, providing insight into the fine and coarse tails, respectively (*i.e.*, D10 - 10% of particles smaller than this size, D50 - median particle size, and D90 - 90% of particles below this size) (**Figure 3**). To quantify distribution breadth, the span parameter is frequently calculated using the expression $(D_{90} - D_{10})/D_{50}$. Span provides a normalized measure of distribution width relative to the median size. Narrow span values indicate relatively uniform particle populations, whereas broader spans suggest heterogeneity, potential agglomeration, or multimodal behavior. In process monitoring applications, shifts in span may signal changes in milling efficiency, granulation conditions, or aggregation phenomena.

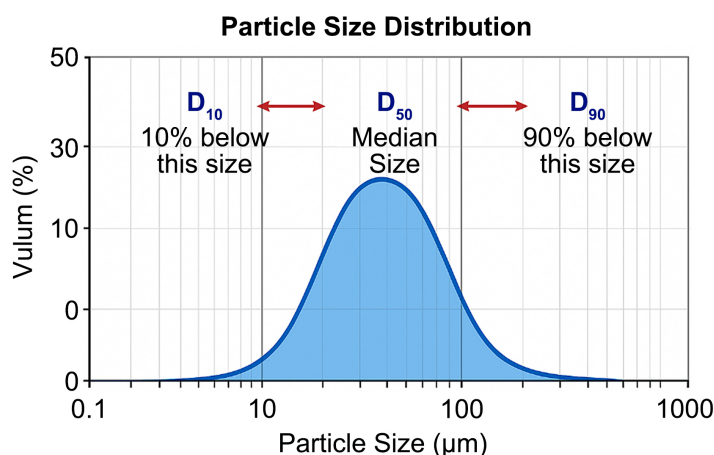


Figure 3. Particle scattering pattern vs size.

A critical aspect of laser diffraction analysis is the recognition that the reported PSD is influenced by model assumptions and user-defined optical inputs. Incorrect refractive index values, inappropriate selection of scattering theory, or failure to account for absorption effects can introduce systematic bias. Such bias may not be immediately apparent from precision data alone, as repeat measurements can remain highly reproducible while consistently inaccurate. Therefore, scientific justification of optical parameters, supported by literature values, experimental verification, or sensitivity analysis, is essential during method development. In summary, laser diffraction integrates optical physics, detector technology, and mathematical modeling to generate rapid and comprehensive particle size distributions. Its strength lies in its ability to characterize wide size ranges efficiently and reproducibly. However, because the technique relies on theoretical reconstruction

of scattering data, careful selection of optical models and parameters is fundamental to ensuring scientifically defensible and regulatory-compliant results.

3. Regulatory Framework: ICH Q7-Q11 Integration

3.1. ICH Q7: GMP Foundation

A scientifically sound particle size method must rest on a strong Good Manufacturing Practice (GMP) foundation. Under ICH Q7, analytical procedures used for release and stability testing are required to be appropriately validated and documented. This expectation applies fully to particle size distribution (PSD) methods, even though they are physical rather than chemical tests. The data generated is often used for batch disposition decisions, making regulatory reliability essential. Method validation alone, however, is not sufficient. ICH Q7 also requires that analytical equipment be properly qualified through Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ). For laser diffraction systems, this means demonstrating that the instrument is installed correctly, operates within defined parameters, and consistently produces reliable results over time. Routine performance verification with appropriate standards further strengthens confidence in ongoing system suitability. Equally important are well-controlled Standard Operating Procedures (SOPs), particularly for dispersion conditions. In particle size analysis, parameters such as dry dispersion pressure or wet sonication time directly influence results. These are not minor settings; they are critical method variables. If such parameters are selected arbitrarily or lack documented scientific justification, the integrity of release data may be questioned. From a regulatory perspective, failure to justify dispersion energy settings represents a GMP risk, as it undermines the assurance that reported particle size results truly reflect the material being tested [9].

3.2. ICH Q8: Pharmaceutical Development

ICH Q8 introduces the concept of Quality by Design (QbD), shifting pharmaceutical development from empirical trial-and-error approaches toward science- and risk-based understanding. Within this framework, particle size distribution (PSD) must be formally evaluated to determine whether it constitutes a critical quality attribute (CQA). When particle size directly influences dissolution, bioavailability, blend uniformity, or downstream manufacturability, it should be clearly designated as a CQA and controlled accordingly. For drug substances manufactured through size-reduction processes, such as milling or micronization, ICH Q8 expects the establishment of a defined design space. This design space should describe the relationship between process parameters such as mill speed, feed rate, classifier settings, or energy input and the resulting PSD. Rather than relying on fixed operating points, development studies should demonstrate a scientifically justified operating range within which consistent particle size outcomes are achieved. Such understanding supports process robustness and facilitates regulatory flexibility. A particularly important expectation is the demonstration of a mechanistic

link between PSD and dissolution performance. Experimental data should show how changes in particle size influence dissolution rate and, where relevant, bioavailability. Establishing this relationship strengthens the clinical relevance of PSD specifications. Additionally, development reports should include dispersion plateau studies conducted during analytical method development. These studies confirm that measurement conditions do not artificially alter particle size through agglomeration or fracture, ensuring that reported PSD values accurately reflect the true material characteristics [10].

3.3. ICH Q9: Quality Risk Management

ICH Q9 formalizes the application of Quality Risk Management (QRM) principles throughout the pharmaceutical lifecycle. In the context of particle size distribution (PSD) analysis, risk management is not theoretical; it directly influences the reliability of analytical results and the robustness of process control. Structured tools such as Failure Mode and Effects Analysis (FMEA) and risk-ranking matrices should be applied during method development and validation to systematically identify and mitigate potential failure points. Several key risk areas are particularly relevant to laser diffraction methods. Over-dispersion can generate particle fracture, artificially shifting the distribution toward smaller sizes. Conversely, under-dispersion may allow agglomerates to persist, producing falsely coarse results. Sampling bias represents another significant risk, especially for heterogeneous powders prone to segregation. In addition, optical model error arising from incorrect refractive index selection or inappropriate theoretical assumptions can introduce a systematic measurement bias. Through formal risk assessment, each potential failure mode can be evaluated in terms of severity, occurrence, and detectability. The resulting risk prioritization enables targeted mitigation strategies, such as dispersion optimization studies, standardized sampling procedures, and refractive index sensitivity analysis. Importantly, dispersion pressure or sonication time should not be arbitrarily selected; instead, statistically justified dispersion windows must be defined based on experimental data. A risk-based framework ensures that PSD methods are not only precise but scientifically defensible and aligned with regulatory expectations [11].

3.4. ICH Q10: Pharmaceutical Quality System

ICH Q10 extends quality principles beyond development and validation into the commercial lifecycle. Within a Pharmaceutical Quality System (PQS), PSD control should not end at method approval; rather, it must be continuously monitored to ensure sustained process performance. Lifecycle oversight transforms PSD from a static specification parameter into a dynamic indicator of process stability. Routine trending of PSD parameters is a central expectation. Control charts for D50 values can reveal subtle shifts in median particle size that may indicate changes in milling efficiency, equipment wear, or raw material variability. Monitoring of the span parameter provides additional insight into distribution breadth

and the potential emergence of fines or coarse fractions. These statistical tools allow early detection of drift before specification limits are breached. When trends indicate atypical behavior, the PQS requires structured investigation and corrective and preventive action (CAPA). For example, repeated upward shifts in D90 may prompt evaluation of milling parameters or classifier performance. Integration of PSD data into the broader quality system ensures alignment between analytical monitoring, process control, and continuous improvement initiatives. Through systematic trending and CAPA integration, ICH Q10 promotes sustained assurance that particle size remains within the validated design space across the product lifecycle [12].

3.5. ICH Q11: Drug Substance Development

ICH Q11 emphasizes a science-based approach to drug substance development, particularly for active pharmaceutical ingredients (APIs). When milling or micronization is used to achieve target particle size, it must be recognized as a critical process step. The parameters governing size reduction such as mill type, energy input, feed rate, and classifier settings directly determine the final PSD and therefore require thorough process understanding. Under this framework, PSD specifications should not be arbitrarily selected. Instead, they must be clinically and biopharmaceuticals justified. Development studies should demonstrate how variations in particle size influence dissolution behavior and, when applicable, systemic exposure. Establishing this linkage ensures that PSD limits are meaningful and connected to product performance rather than solely manufacturing convenience. Scale-up represents another key consideration. Changes in equipment scale, throughput, or energy transfer can alter particle-breakage dynamics, potentially altering the PSD. ICH Q11 therefore expects evaluation of scale-up impact to confirm that commercial manufacturing reproduces the particle size profile established during development. By integrating process understanding, clinical relevance, and scalability assessment, ICH Q11 reinforces the central role of PSD in ensuring consistent and reliable drug substance quality [13].

4. Risk-Based Framework for PSD Method Development

A scientifically robust particle size distribution (PSD) method cannot rely solely on instrument capability; it must be built upon a structured, risk-based development strategy. Because laser diffraction measurements are highly sensitive to dispersion and sampling variables, method development should systematically identify and control sources of variability. Applying a risk-based framework ensures that method parameters are selected based on experimental evidence rather than operator convenience or vendor defaults. The goal is to define operating conditions that consistently reflect the true particle characteristics of the material, without inducing artificial changes such as agglomeration or fracture [14]. The Ishikawa diagram depicting cause-and-effect relationships on the potential critical analytical attributes (CAA) of laser light diffraction (**Figure 4**) [15]–[19].

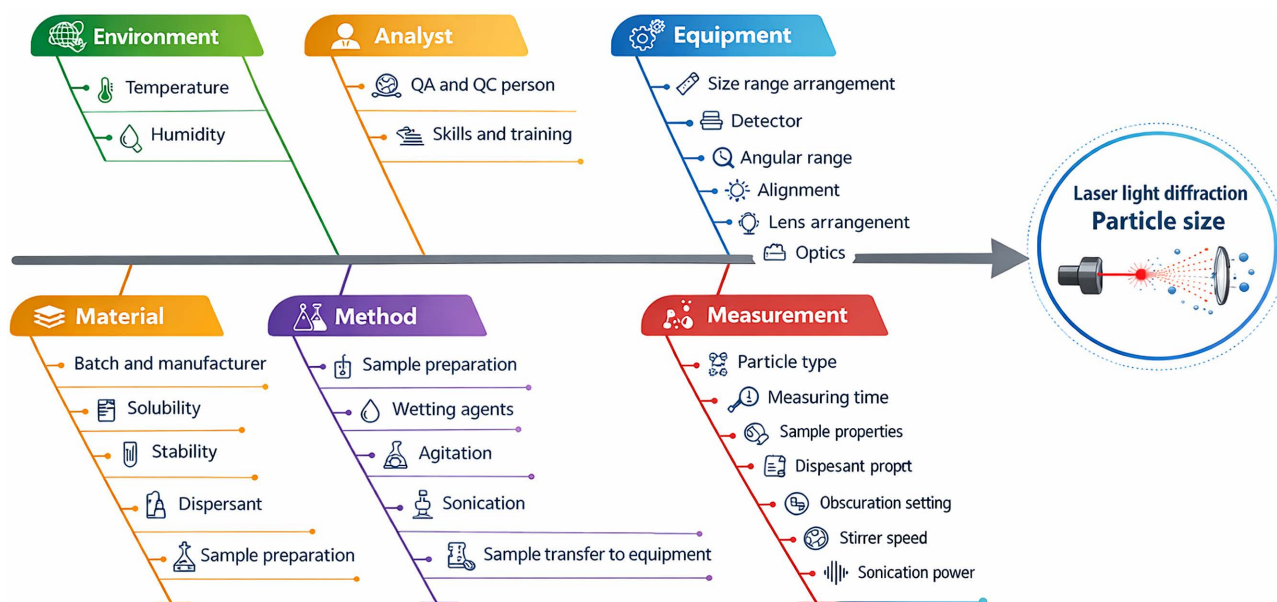


Figure 4. Ishikawa diagram depicting relationship on the potential CAA of laser light diffraction.

4.1. Dispersion Energy Optimization

Dispersion is the most critical variable in laser diffraction analysis. The objective is to separate individual particles without altering their intrinsic size. Both insufficient and excessive energy dispersion can compromise data integrity, making optimization studies essential.

In dry dispersion systems, pressure titration studies are commonly performed across a defined range, typically between 0.5 and 4.0 bar. At low pressures, cohesive forces may prevent complete deagglomeration, resulting in artificially coarse distributions. As pressure increases, agglomerates disperse and particle size values decrease until a stable plateau is reached. This plateau region represents the optimal dispersion window where further pressure increases no longer significantly reduced D50 or D90 values. Pressures beyond this plateau may cause mechanical fracture of fragile particles, leading to artificially fine distributions. Identifying and justifying this plateau region through documented experimentation is fundamental to establishing a scientifically defensible method.

Dispersion energy optimization is a central component of developing a reliable wet-dispersion method for particle size distribution (PSD) analysis. In liquid-based systems, dispersion energy refers to the combined mechanical and acoustic forces applied to a particle suspension to separate loosely bound agglomerates and ensure that the measurement reflects the true primary particle size. Because each material responds differently to applied energy, the process must be approached deliberately and experimentally to balance effective deagglomeration with preservation of particle integrity. A cornerstone of dispersion energy control is the optimization of sonication. Sonication introduces acoustic cavitation energy into the medium, producing micro-turbulence and shear forces that efficiently break down agglomerates. However, this energy must be carefully titrated: insufficient soni-

cation allows agglomerates to persist, while excessive energy can fracture fragile particles, leading to artificially reduced particle sizes. To establish the ideal sonication conditions, analysts typically perform incremental sonication studies, exposing the sample to increasing durations or intensities of ultrasound and monitoring the PSD profile after each step. A plateau in the distribution where further sonication does not change D10, D50, or D90 values indicates that agglomerates have been effectively dispersed without damaging the native particle structure. Beyond sonication, mechanical mixing energy is also essential. Wet suspensions must remain homogeneous during measurement to avoid bias from sedimentation or concentration gradients. Stirring or circulation flow rates should be optimized to maintain stable suspension conditions without introducing new artifacts such as air bubbles, excessive shear, or swirling effects that can distort scattering patterns. Validation of stirring speed is often performed by evaluating PSD consistency at multiple mixing intensities; a method is considered robust when the PSD remains stable across a suitable range of operating speeds. Another critical aspect of dispersion energy optimization is the interaction between the sample and the dispersing medium. Even with appropriate sonication and stirring, incompatibility with the liquid phase can confound results. Certain materials may dissolve partially in aqueous media, swell upon hydration, or undergo surface modifications in the presence of specific surfactants. These chemical or physical changes can mimic dispersion-energy effects and obscure the true PSD. Therefore, dispersant compatibility studies including solubility checks, chemical stability assessments, and pH or ionic-strength screening are integrated into the optimization process to confirm that energy input, not chemical alteration, is responsible for observed dispersion behavior. Together, these optimization steps incremental sonication tuning, controlled mechanical mixing, and confirmation of medium compatibility define a controlled dispersion environment tailored to the material under study. By carefully balancing dispersion energy against particle fragility and medium effects, the method achieves the dual goals of complete deagglomeration and preservation of native particle characteristics, ultimately improving the accuracy and reproducibility of PSD measurements in wet dispersion systems.

4.2. Sampling Risk

While dispersion is often emphasized, sampling frequently represents the largest contributor to PSD variability. Powders are inherently heterogeneous and prone to segregation based on particle size, density, and shape. During storage or transport, finer particles may migrate downward or adhere to container walls, while coarser particles may accumulate in localized regions. Without a validated sampling strategy, analytical results may not accurately represent the bulk material. Risk-based method development must therefore include evaluation of sampling procedures. Factors such as sampling location, depth, sample mass, and container handling should be standardized and documented. The use of appropriate sampling tools, such as thief samplers designed for uniform withdrawal, can minimize bias. Rep-

licate sampling studies are recommended to assess variability introduced at this stage. Ultimately, even the most optimized dispersion protocol cannot compensate for non-representative sampling. By integrating validated sampling procedures into the PSD method, analytical variability can be reduced, ensuring that measured distributions genuinely reflect the true particle population.

5. Critical Parameters Influencing Particle Size Distribution Using the Malvern Mastersizer 3000

Particle size distribution (PSD) is a critical quality attribute in pharmaceutical, chemical, and materials science applications, as it directly influences dissolution rate, bioavailability, stability, flow properties, and processability. Laser diffraction has become one of the most widely employed techniques for PSD determination due to its broad dynamic range, reproducibility, and rapid analysis. The Malvern Mastersizer 3000 operates based on the principle that particles scatter light at angles inversely proportional to their size, allowing calculation of particle size distribution through appropriate optical models (Mie or Fraunhofer theory). Although the instrument provides highly robust measurements, the accuracy of PSD data is strongly dependent on sample dispersion conditions. Improper dispersion can lead to measurement of agglomerates, fractured particles, or sedimented fractions, thereby misrepresenting the true primary particle size. Therefore, understanding and controlling dispersion parameters in both dry and wet systems is essential for method development, validation, and routine quality control.

5.1. Critical Parameters in Dry Dispersion (Dry Powder Dispersed Using Compressed Air-Typically Aero S Module)

Dry dispersion involves aerosolization of powder using compressed air to separate particles prior to laser measurement. This approach is particularly suitable for free-flowing powders and materials sensitive to liquid media. Critical Parameters Affecting Dry Particle Size Distribution (PSD) Measurement presented in **Table 1**.

5.1.1. Air Pressure

Air pressure is the primary dispersive force in dry measurement. Increasing air pressure enhances deagglomeration efficiency by applying mechanical energy to particle clusters. Adequate pressure results in reduced median particle size (D50) and narrower distributions due to effective separation of loosely bound agglomerates. However, excessive pressure may induce particle fracture, leading to artificially smaller size measurements and distorted distribution profiles. Conversely, insufficient pressure results in incomplete dispersion and overestimation of particle size. Method optimization therefore requires determination of a pressure setting that achieves stable particle size values without evidence of particle attrition.

5.1.2. Vibration Feed Rate

The vibration feed rate controls the rate at which powder enters the measurement zone. A higher feed rate may cause temporary overloading of the optical path,

increasing obscuration beyond optimal limits and promoting multiple scattering phenomena, which can artificially increase measured particle size. In contrast, an excessively low feed rate results in weak signal intensity and poor reproducibility. A steady and controlled feed that maintains obscuration within the recommended range (typically 1% - 5% for dry systems) is essential for accurate analysis.

5.1.3. Sample Weight

Sample weight is closely related to feed rate and obscuration. Excessive sample loading increases light attenuation and multiple scattering effects, thereby compromising measurement accuracy. Insufficient sample quantities produce weak scattering signals and increased variability. During method development, sample weight must be optimized to ensure consistent obscuration and repeatable measurements.

5.1.4. Obscuration

Obscuration represents the percentage of laser light attenuated by particles during measurement and serves as an important system-suitability indicator. For dry dispersion, an obscuration range of approximately 1% - 10% is generally recommended. High obscuration levels may result in multiple scattering and overestimation of particle size, while very low obscuration may reduce signal-to-noise ratio and data reliability. Maintaining appropriate obscuration ensures measurement precision and robustness.

Table 1. Critical parameters affecting dry particle size distribution (PSD) measurement: definition, impact, and optimization.

Parameter	Definition/Role	Impact on PSD Measurement	Optimization/Considerations
Physical Air Pressure	Compressed air used to disperse powder in the measurement chamber (dry dispersion).	Too low → incomplete de-agglomeration, larger apparent particle sizes; too high → particle breakage or excessive fines.	Optimize pressure for material type; follow instrument manufacturer guidelines; validate reproducibility.
Process Vibration Feed Rate	Rate at which powder is fed into the measurement zone via a vibratory feeder.	Too slow → insufficient sample in measurement zone, longer measurement times; too fast → particle collisions, aggregation, or measurement errors.	Adjust feed rate to achieve stable, reproducible sample delivery; verify histogram stability.
Physical Sample Weight	Amount of powder used per measurement.	Too little → poor statistical representation, noisy PSD data; too much → overcrowding, multiple scattering, or instrument saturation.	Use recommended sample amount per instrument and method SOP; ensure uniform loading.
Optical Obscuration	Percentage of laser light blocked by particles (in laser diffraction) or fraction of particles in measurement zone.	Too low → low signal, high noise, inaccurate PSD; too high → multiple scattering, distorted histogram, overestimation of large particles.	Target instrument-specified obscuration range (e.g., 1% - 10%); adjust feed or dispersion parameters to maintain within range.

5.2. Critical Parameters in Wet Dispersion (Sample Dispersed in Liquid Medium Using Hydro MV-Medium Volume)

Wet dispersion involves suspending particles in a suitable liquid medium, typically using a recirculating dispersion unit. This approach is advantageous for cohesive powders or materials prone to strong agglomeration.

Critical Parameters Affecting Wet Particle Size Distribution (PSD) Measurement presented in **Table 2**.

Table 2. Critical parameters affecting wet particle size distribution (PSD) measurement: definition, impact, and optimization.

Parameter	Definition/Role	Impact on PSD Measurement	Optimization/Considerations
Physical Dispersion Media Selection	The liquid or solvent in which particles are suspended for wet measurement.	Incompatible media can cause aggregation, swelling, dissolution, or altered refractive properties, leading to inaccurate PSD.	Choose media compatible with the material; maintain constant temperature and ionic strength if relevant.
Chemical Physicochemical Compatibility	Interaction of the dispersion medium with the sample (chemical, pH, ionic strength, etc.).	Incompatible media can cause particle breakdown, aggregation, or chemical reaction, distorting PSD.	Evaluate chemical stability, avoid reactive solvents, adjust pH or ionic strength.
Optical Refractive Index (RI)	Optical property of particle and medium used in laser diffraction or imaging.	Incorrect RI leads to erroneous particle size calculations.	Measure or use literature values for sample and medium; adjust in software settings.
Physical Viscosity & Sedimentation Effect	Resistance of the dispersion medium to flow; tendency of particles to settle under gravity.	High viscosity slows particle settling but may dampen Brownian motion. Settling causes apparent increase in particle size or loss of fine fraction.	Use appropriate viscosity for instrument; may dilute high-viscosity samples. Use stirring, sonication, or stabilizers; measure quickly after dispersion.
Chemical Surface Tension & Wetting Behavior	Ability of the medium to wet particle surfaces.	Poor wetting can leave agglomerates, underrepresent fines, and bias PSD.	Use surfactants or dispersants; ensure complete wetting.
Process Sonication & Medium Stability	Use of ultrasonic energy to break aggregates in the chosen medium.	Insufficient sonication leaves agglomerates; excessive may break particles.	Optimize power, duration, and medium type; verify particle integrity.
Optical Obscuration	Stability of suspension and optical density (obscuration) during measurement.	High obscuration or unstable suspension reduces measurement accuracy, increases noise.	Target recommended obscuration of 5% - 20% (% of laser blocked) and verify dispersion uniformity.

Continued

Process Stirring Speed	Rate of mechanical stirring of the suspension during measurement or preparation.	Too slow → uneven dispersion or sedimentation; too fast → foaming, air bubbles, or particle breakage, leading to PSD errors.	Optimize speed to maintain uniform suspension without inducing damage; verify visually or via stability check.
Process Sonication Time	Duration of ultrasonic treatment applied.	Too short → incomplete dispersion; too long → particle breakage or heating.	Determine empirically for each material.
Process Vortex Mixing Time	Duration and intensity of mechanical mixing.	Insufficient mixing → sedimentation or aggregation; excessive → foaming or particle breakage.	Optimize to achieve uniform suspension without damaging particles.
Physical Sample Dispersion Stability	Ability of the sample to remain uniformly dispersed during measurement.	Instability leads to biased PSD, higher variability, and irreproducibility.	Use dispersants, control time between preparation and measurement, verify visually or via stability checks.
Chemical Thermodynamic Equilibration	Time for the system to reach a stable particle distribution with balanced interactions.	Measuring before equilibrium creates artifact peaks, over- or underestimation of particle size fractions.	Allow sufficient stabilization, monitor histogram for artifacts, ensure reproducibility.

5.2.1. Dispersion Medium Selection

In wet laser diffraction analysis, the selection of an appropriate dispersion medium is a critical determinant of accurate particle size measurement. In systems such as the Malvern Mastersizer 3000, particles are suspended in a liquid prior to laser exposure, and the integrity of the measurement depends on the stability and optical compatibility of that suspension. An improperly selected medium can result in agglomeration, dissolution, swelling, sedimentation, or optical artifacts, all of which may compromise the reliability of the particle size distribution (PSD) data.

5.2.2. Physicochemical Compatibility

The dispersion medium must be chemically inert with respect to the analyte. Dissolution of partially soluble materials can lead to an apparent reduction in particle size, while swelling (e.g., in polymeric or hydrophilic materials) may produce artificially increased size values. Therefore, solubility screening is a fundamental step in method development. Ideally, the material should remain physically stable and chemically unchanged throughout the measurement period. Additionally, the medium should not induce chemical degradation or polymorphic transformation. In pharmaceutical systems, for example, exposure to aqueous media may alter crystalline structure or surface properties, thereby affecting particle size results and reproducibility.

5.2.3. Refractive Index Considerations

Accurate PSD determination using Mie theory requires knowledge of both the

particle refractive index and the refractive index of the dispersion medium. The contrast between these two values directly influences scattering intensity and size calculation accuracy. A mismatch or incorrect refractive index input can distort the calculated distribution, particularly for fine particles ($<10\ \mu\text{m}$). Therefore, the optical properties of the selected medium must be well characterized and properly entered into the instrument software. Typical dispersion media employed in wet laser diffraction analysis include purified water, aqueous surfactant solutions such as 0.1% Triton X-100, 0.1% or 1.0% Tween 80 in water, as well as organic solvents including ethanol, isopropyl alcohol, and n-hexane. The selection of an appropriate dispersion medium depends primarily on the physicochemical properties of the analyte, including solubility, surface characteristics, density, and chemical stability. Purified water is the most commonly used dispersion medium due to its availability, low cost, and compatibility with a wide range of pharmaceutical and inorganic materials. However, its relatively high surface tension may limit effective wetting of hydrophobic particles, potentially resulting in agglomeration or floating particles. To overcome this limitation, nonionic surfactants such as Triton X-100 or Tween 80 are frequently incorporated at low concentrations (e.g., 0.1% w/v). These surfactants reduce surface tension, enhance particle wetting, and promote deagglomeration without significantly altering the refractive index or viscosity of the medium. In certain cases, increasing the surfactant concentration to 1.0% may be necessary for highly hydrophobic materials, although excessive surfactant levels should be avoided to minimize foaming and potential micelle-related interference. Ethanol is often selected for materials that exhibit limited aqueous solubility or poor wettability in water. Its lower surface tension compared to water improves dispersion of hydrophobic particles. Additionally, ethanol evaporates readily, which may be advantageous during post-analysis cleaning; however, volatility must be considered during sonication to prevent changes in concentration or temperature. Nonpolar solvents such as n-hexane are suitable for highly hydrophobic or moisture-sensitive materials. These solvents provide improved compatibility for lipid-based or water-reactive substances. Nevertheless, safety considerations, solvent volatility, and compatibility with instrument components must be carefully evaluated prior to routine use. Overall, the choice of dispersion medium should ensure chemical inertness, minimal solubility of the analyte, adequate wetting, stable suspension behavior, and compatibility with optical model requirements. A systematic screening approach during method development is recommended to identify the medium that provides reproducible and physically meaningful PSD data.

5.2.4. Viscosity and Sedimentation Effects

The viscosity of the dispersion medium plays a significant role in suspension stability. According to Stokes' law, sedimentation velocity is inversely proportional to the medium's viscosity [20]. Low-viscosity media may allow rapid settling of larger particles, resulting in biased D90 values and poor reproducibility. Increasing viscosity can reduce sedimentation but may introduce challenges in pumping,

circulation, and background stability. Therefore, viscosity should be optimized to balance suspension stability and instrument performance.

5.2.5. Surface Tension and Wetting Behavior

Effective wetting of particle surfaces is a critical factor in wet PSD measurements, as inadequate wetting can lead to aggregation, floating clusters, or trapped air bubbles that distort particle size analysis. Surface tension governs the ability of the liquid medium to spread across and penetrate particle surfaces. High surface tension media, such as pure water, often fail to adequately wet hydrophobic or poorly water-soluble particles, resulting in floating aggregates, broad PSD distributions, and overestimation of particle sizes. To improve wetting, surfactants or dispersing agents (e.g., polysorbates, sodium dodecyl sulfate, or other nonionic/anionic surfactants) are commonly added. These reduce the liquid's surface tension, allowing the medium to fully penetrate interstitial spaces and wet particle surfaces. Improved wetting enhances dispersion stability, minimizes air entrapment, and ensures more accurate PSD measurements. However, surfactant use requires careful control: excessive concentrations can cause foam formation, micelle-related artifacts, or altered particle behavior, introducing secondary errors in PSD analysis. Optimization involves selecting surfactant type, concentration, and mixing protocol to achieve stable, reproducible dispersions without introducing measurement artifacts. A schematic figure can clearly illustrate the effect of surface tension and wetting: High surface tension (poor wetting): Particles float, form aggregates, and trap air; PSD histogram shows broader peaks and artifact tails. Reduced surface tension (good wetting with surfactant): Particles are uniformly dispersed, air bubbles are minimized, and PSD histogram accurately represents the true particle size distribution (**Figure 5**).

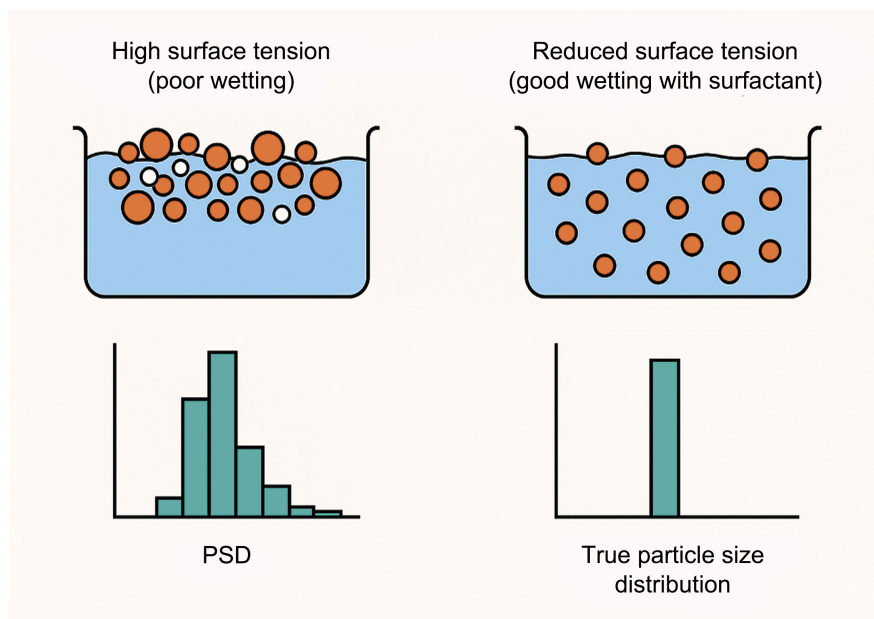


Figure 5. Surface tension and wetting effect on the PSD.

5.2.6. Sonication and Medium Stability

The dispersion medium must also remain stable under sonication if ultrasonic energy is applied to break agglomerates. Some solvents may undergo temperature rise or evaporation during prolonged sonication, altering viscosity and refractive index. Consequently, temperature monitoring and control are important during method development.

5.2.7. Obscuration

In wet systems, the acceptable obscuration range is typically higher (approximately 5% - 20%) due to improved dispersion uniformity and reduced multiple scattering compared to dry systems. Gradual addition of sample to reach the target obscuration is recommended to prevent sudden overloading and ensure consistent measurement conditions.

5.2.8. Stirring Speed

Stirring maintains a homogeneous suspension and prevents sedimentation during analysis. Insufficient stirring allows larger particles to settle, resulting in artificially increased D90 values and poor reproducibility. Excessively high stirring speeds, however, may induce mechanical attrition of fragile particles, leading to reduced particle size measurements. Also, higher stir speed generates air bubbles. Therefore, stirring speed must balance suspension uniformity and mechanical stability of the sample.

5.2.9. Sonication Time

Sonication is frequently employed to disrupt soft agglomerates through ultrasonic energy. Progressive increases in sonication time typically reduce measured particle size until a plateau is reached, indicating effective deagglomeration. Beyond this point, extended sonication may cause particle breakage or alter material properties. A sonication-time study during method development is essential to identify optimal conditions that achieve reproducible results without inducing structural changes.

5.2.10. Vortex Mixing Time

Vortex mixing prior to measurement ensures uniform sample distribution in the dispersion medium. Inadequate mixing may result in localized concentration gradients and measurement variability. Controlled vortexing improves reproducibility by promoting consistent dispersion prior to laser analysis.

5.2.11. Sample Dispersion Stability

Sample dispersion stability is a critical parameter in wet PSD measurement methods because the accuracy and reproducibility of particle size data depend on maintaining a uniform suspension throughout the analysis. If the sample is not properly dispersed or settles/agglomerates during measurement, larger clusters or agglomerates may form, leading to apparent particle sizes that are artificially high. Conversely, inadequate wetting of particles can prevent fine particles from being

measured, resulting in an underestimation of small particle fractions. This introduces bias, increased variability, and poor method precision, potentially causing out-of-specification (OOS) results or incorrect process control decisions in continuous manufacturing. Ensuring stable dispersion typically requires optimization of dispersant type, concentration, sonication, stirring, and measurement timing, with verification of stability over the measurement period. Incorporating dispersion stability evaluation into method development and validation is essential for robust, reproducible PSD data that accurately reflects the true particle distribution of the product.

5.2.12. Thermodynamic Equilibrium

Thermodynamic equilibrium is an important consideration in wet PSD measurement because particles in suspension may undergo aggregation, agglomeration, or settling until a stable equilibrium state is reached. If measurements are taken before the system reaches equilibrium, the PSD data can show artifact peaks in the histogram, representing transient or unstable particle clusters rather than the true distribution. These artifacts can lead to overestimation of large particle fractions or misinterpretation of particle population dynamics. Ensuring that the sample reaches thermodynamic equilibrium through adequate dispersion, mixing, and stabilization time helps obtain reproducible and accurate PSD measurements. Monitoring artifact peaks in the histogram is a practical way to verify that equilibrium has been achieved, ensuring that the PSD reflects the true particle size distribution of the material rather than transient measurement artifacts.

6. PSD Validation Considerations (A Case Study Validating Dry and Wet Dispersion Methods for Drug Substance and Drug Product Respectively)

The analytical validation of PSD methods was conducted in accordance with the principles described in the International Council for Harmonization guideline ICH Q2(R2) [21]-[23]. This guideline emphasizes scientific justification, statistical evaluation, and a lifecycle-based approach to analytical methods. Because PSD results (e.g., D10, D50, D90) are highly sensitive to dispersion conditions, these parameters must be systematically optimized and justified during method development. Robustness studies should evaluate the impact of small variations in air pressure, stirring speed, and sonication time. Repeatability and intermediate precision assessments ensure measurement reliability. Furthermore, confirmation that selected dispersion conditions do not induce particle fracture is essential to maintain scientific validity. This article presents a full experimental validation paper documenting the development and validation of particle size distribution methods for drug substance powder and drug product topical cream formulations.

6.1. Materials, Equipment and Methods

Malvern Mastersizer 3000 equipped with the Aero S and Hydro MV accessories

system was used for particle size method validation, with Software version 5.61 used for data processing and evaluation. Drug Substance and drug Product, and purified water. A single batch/lot of sample (drug substance powder and drug product cream) was used for each dry, wet, precision, intermediate precision, and robustness study.

6.2. Sample Preparation and Instrument Parameters for Dry Dispersion

A structured sampling approach was implemented for dry-dispersion analysis to ensure representative characterization of the drug substance powder. During method development and feasibility studies, three production lots from different manufacturing campaigns were evaluated to capture potential lot-to-lot variability. For each lot, samples were collected from multiple bulk container locations including top, middle, and bottom sections to address potential segregation during storage and handling. Subsampling was performed using a standardized riffling procedure, where 50 - 100 g of bulk powder was collected and progressively divided to obtain homogeneous subsamples. A sample mass of approximately 3000 mg was selected for Aero S hopper was based on instrument recommendations and preliminary studies to ensure adequate powder flow and representative particle loading. For dry-dispersion measurements, refractive index of 1.50 was obtained from the Merck Index, consistent with the transparent nature of the drug substance under dry conditions with negligible absorption characteristics, supporting the selected absorption index of 0.010. Sensitivity analysis confirmed that $\pm 5\%$ variation in these optical parameters resulted in $<5\%$ change in D50 values, demonstrating robustness of the optical model. Dispersion optimization was systematically evaluated through pressure titration studies. Feed pressure varied from 0.5 to 4.0 bar while monitoring particle size distribution parameters. Results demonstrated that D50 values decreased from 6 μm at 0.5 bar to 4 μm at 1.5 bar, then plateaued at $3.9 \pm 0.2 \mu\text{m}$ between 1.5 - 3.0 bar, indicating complete deagglomeration without particle breakage. Pressure increases beyond 3.0 bar to 4.0 bar showed artificial size reduction ($\text{D50} = 2.5 \mu\text{m}$) with increased fine particle fraction, suggesting mechanical fracture. The optimal pressure of 1.5 bar was selected based on plateau achievement within the safe operating range and measurement reproducibility. Feed rate optimization confirmed that 60% provided consistent powder flow and stable particle size measurements across the pressure range.

For dry-dispersion measurements were performed using compressed air at 1.5 bar and a 60% feed rate to ensure stable powder flow. The instrument was operated in General-Purpose Fine Powder Mode, which is recommended for materials with a nominal particle size below 10 μm . The Standard Venturi disperser and general-purpose tray were utilized with a hopper gap of 1.25 mm to promote consistent powder entrainment. The optimized dry-dispersion conditions applied for routine analysis are summarized in **Table 3**.

Table 3. Instrument parameters for dry dispersion accessories.

Sample Weight	3000 mg
Sampler	AERO S
Material Name	Drug Substance
Particle Type	Non-Spherical
Refractive Index	1.50
Absorption Index	0.010
Density	1.00 (g/cm ³)
Refractive Index	1.33
Background Measurement Duration (Red)	5.00 s
Sample Measurement Duration (Red)	5.00 s
Number of Measurements	3
Obscuration Lower Limit	2.00%
Obscuration High Limit	8.00%
Vibration Feed Rate	60%
Air Pressure	1.5 Bar
Venturi Type	Standard Venturi Disperser
Tray Type	General Purpose tray with hopper
Hopper Gap	1.25 mm
Analysis Model	General Purpose Fine power mode was selected for particles less than 10-micron size

6.3. Sample Preparation and Instrument Parameters for Wet Dispersion

The tested materials comprised 0.5% topical cream formulations in an oil-in-water emulsion base containing carbomer, glycerin, octyl hydroxy stearate, polyethylene glycol 400, polysorbate 80, propylene glycol, sorbitan monooleate, stearic acid, water, pH adjusters, and preservatives. The expected particle size distribution ranged from 1 - 15 μm for primary micronized drug substance, 15 - 75 μm for cream matrix-associated aggregates, and 75 - 300 μm for storage-induced agglomerates within the semi-solid matrix. Given the drug substance's high aqueous solubility (12.2 mg/mL at 25°C), the API exhibits moderate wettability challenges when dispersed in the lipophilic cream vehicle. The API crystals exhibit moderate fragility requiring controlled dispersion to prevent artificial size reduction, while the cream matrix components form deformable, dispersible agglomerates. Based on these characteristics, wet dispersion was selected to effectively break down ma-

trix-associated agglomerates while minimizing drug dissolution through rapid analysis enabling accurate particle size characterization.

A comprehensive sampling plan was implemented to ensure representative analysis across the study materials. Approximately 10 - 15 g of cream was collected using a cut-and-open tube method, mixed thoroughly, and subsampled to obtain representative 100 mg aliquots. Sample mass selection was optimized to achieve 10% - 20% obscuration while maintaining adequate particle loading for statistical reliability. Purified water was selected as an appropriate dispersant for the cream matrix through comprehensive compatibility studies. Dissolution assessment demonstrated that less than 2% of the total drug dissolved during the 10-minute analysis window, confirming minimal impact on particle size measurements. Matrix swelling was negligible (<5%), when exposed to the aqueous medium, with no observable changes in particle morphology or size distribution over the analysis period. Placebo contribution studies revealed minimal background interference (<3% of the total volume distribution) confirming negligible impact on active formulation measurements. Short-term suspension stability was confirmed through time-course studies in which particle size parameters (D10, D50, D90) remained stable within $\pm 5\%$ over 15 minutes post-dispersion, with no evidence of particle settling, re-agglomeration, or continued matrix breakdown. Additionally, microscopic evaluation of dispersed samples confirmed the maintenance of individual particle identity without morphological changes. No significant chemical interactions were observed between the cream components and the aqueous dispersant as indicated by the stable pH measurements (6.8 ± 0.2). These comprehensive validation studies support the suitability of purified water for effective matrix dispersion while preserving the particle integrity of drug substance particles.

For wet-dispersion measurements in an aqueous medium, the effective refractive index was set to 1.49 to account for the drug-water interface and potential hydration effects on particle surfaces, as confirmed by immersion microscopy observations. An absorption index of 1.00 was selected through preliminary obscuration studies to account for complex light scattering contributions from the cream matrix components, drug-water interfaces, and suspended particulates. The dispersant refractive index was set to 1.330 for purified water at 25°C based on merck index.

Comprehensive dispersion studies evaluated multiple parameters to achieve optimal matrix breakdown. Sonication was systematically evaluated during wet-method development but was excluded from the finalized routine method. Optimization studies revealed that particle size D50 reached a plateau at 22 μm within 2 - 5 minutes of sonication, with no further size reduction upon extended treatment. Since sonication had no significant impact on achieving the final particle size distribution and the same results were obtained through stirring alone, sonication was deemed non-critical and excluded from the routine protocol to simplify the method. Consequently, sonication parameters were not included in the robustness evaluation, focusing validation efforts on the critical stirring parame-

ters that directly influence dispersion quality. Stirring speed evaluation (1000 - 2000 RPM) demonstrated optimal dispersion at 1500 RPM, where D90 values stabilized. Vortex studies (30-second intervals up to 4 minutes) confirmed that brief vortexing prior to analysis improved initial dispersion homogeneity without affecting the final particle size distribution. The optimized protocol (120-second vortexing followed by 1500 RPM stirring) provided reproducible dispersion for key percentiles while preserving drug substance particle integrity.

Table 4. Instrument parameters for wet dispersion accessories.

Sampler	Hydro MV
Material Name	Drug Product (Cream)
Refractive Index	1.49
Absorption Index	1.00
Dispersant Name	Water
Refractive Index	1.33
Level Sense of Threshold	100.00
Background Measurement Duration (Red)	15.00 s
Sample Measurement Duration (Red)	15.00 s
Aliquots	1
Automatic Number of Measurements	No
Pre-Alignment Delay	10.00
Number of Measurements	3
Delay between Measurements	10.00
Premeasurement Delay	60.00
Obscuration Lower Limit	10.00%
Obscuration High Limit	20.00%
Stir Speed	1500 rpm
Analysis Model	General Purpose
Single Result Mode	No
Analysis Sensitivity	Normal

For wet-dispersion measurements, the drug product (0.5% cream) was transferred to a clean beaker and gently mixed to ensure uniformity. Approximately 100 mg of sample was weighed into a 50 mL centrifuge tube, followed by the addition of 25 mL purified water as the dispersant. The tube was capped, inverted several times, and vortex-mixed for approximately 120 seconds to disperse the

semi-solid matrix and produce a homogeneous suspension. The preparation was then introduced into the Hydro MV dispersion unit and the system was operated at 1500 rpm, with obscuration maintained between 10% - 20% to achieve suitable particle loading while minimizing multiple-scattering effects. Background and sample acquisition times were 15 seconds, and three replicate measurements were collected. The instrument was operated under the General-Purpose Analysis Model and the single-result mode was disabled to allow averaging across replicates. The finalized wet-dispersion method parameters and instrument settings are summarized in **Table 4**.

7. Result and Discussion

Table 5. ICH Q2 (R2) applicability matrix.

Validation Characteristic	Applicable	Rationale
Accuracy	Yes	Assessed using reference materials; absolute accuracy challenging for PSD
Precision (Repeatability)	Yes	Critical for quality control and batch release
Precision (Intermediate)	Yes	Required for multiple analyst/instrument scenarios
Robustness	Yes	Essential for routine laboratory operations
Specificity	Yes	Method must distinguish drug particles from matrix components
Range	Yes	Method must cover expected particle size variations
Detection Limit	No	Not applicable - method measures physical particle distribution, not chemical concentration
Quantitation Limit	No	Not applicable - particle counting method, not quantitative chemical analysis
Linearity	No	Not applicable - instrument response is particle-count based, not concentration-dependent

The objective of validation was to demonstrate that the PSD method is reliable, reproducible, and suitable for its intended application in pharmaceutical quality control [24]-[26]. Key validation parameters evaluated in this study included precision, intermediate precision, and robustness [27]-[30]. As a part of accuracy, the instrument performance verification was conducted using manufacturer-supplied reference standards (QAS4002_AeroS/M for Dry Accessories and QAS4001_Hy-

droSM for Wet Accessories) to confirm instrument functionality before sample analysis. Method validation for the drug substance and drug product included precision, robustness, and range characteristics. These parameters collectively assess the method's ability to produce consistent and accurate particle size measurements under routine laboratory conditions and potential operational variations [31]–[33]. The PSD ICH Q2(R2) applicability matrix was presented in **Table 5**. The validation claims are narrowed to exclude specificity, and range, as these parameters were not experimentally evaluated in this study. The revised applicability matrix focuses solely on precision (demonstrated through repeatability and intermediate precision studies) and robustness (confirmed through systematic parameter variation).

7.1. Precision

Method precision, also referred to as repeatability, evaluates the closeness of agreement among a series of measurements obtained under identical experimental conditions. For PSD analysis, repeatability was assessed by preparing six independent sample preparations from the same homogeneous batch. Each sample preparation was measured in triplicate, and the average result for each preparation was calculated. Precision performance was evaluated using the percent relative standard deviation (%RSD) of the average particle sizes. Acceptance criteria were defined based on common industry practices, such that the %RSD for the six average values of D50 was required to be not more than (NMT) 10%, while the %RSD values for D10 and D90 were required to be NMT 15% individually. For materials with particle sizes below 10 μm , the acceptance criteria were adjusted to account for increased analytical variability, and the %RSD limits were doubled to reflect this higher inherent variance. The doubled limits for sub-10 μm materials reflect the increased measurement uncertainty at smaller particle sizes due to Brownian motion effects and increased sensitivity to optical parameter variations, consistent with established analytical principles. The results demonstrated acceptable repeatability, indicating that the PSD method produces consistent particle size measurements under the defined laboratory conditions (**Table 6**, **Table 7**) (**Figure 6**). Weighted residuals of less than 1% in the Mastersizer 3000 analysis indicate an excellent fit between the measured and calculated data, confirming that the results are reliable and of high quality as per instrument guidelines.

7.2. Intermediate Precision

Intermediate precision evaluates the reproducibility of the analytical method under normal laboratory variations, such as different analysts, different days, and potentially different instruments of the same model. This parameter provides an indication of the method's reliability during routine quality control operations. For this study, six additional sample preparations were prepared using the same batch employed in the repeatability study. The analysis was conducted by a different analyst on a different day, thereby introducing realistic operational variability.

Each preparation was analyzed in triplicate, and the average result for each sample was calculated. The same acceptance criteria used for repeatability were applied to the intermediate precision study. The results confirmed that the PSD method maintained consistent performance across different analysts and days, demonstrating adequate reproducibility under typical laboratory conditions (Table 6, Table 7).

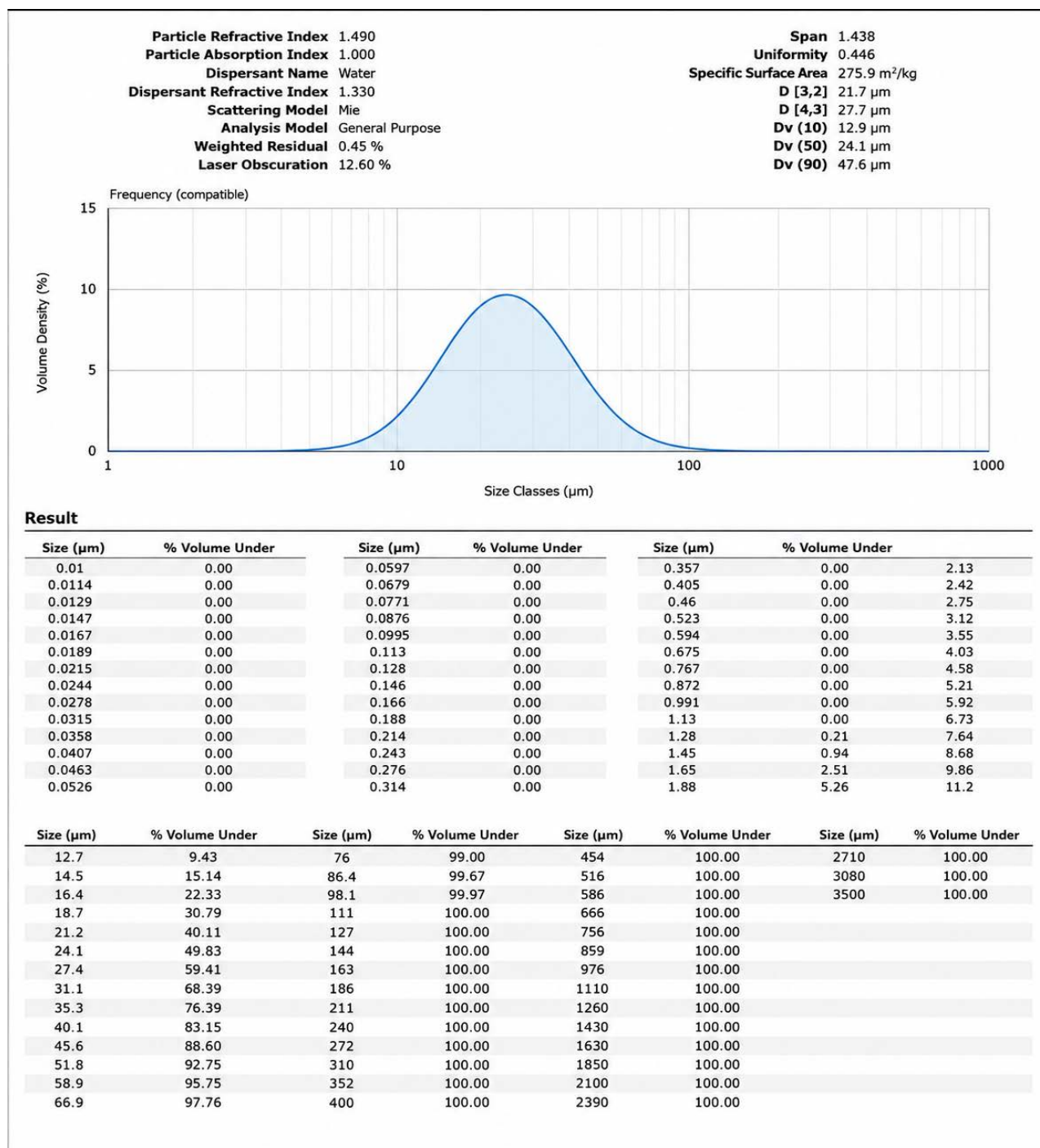


Figure 6. PSD precision histogram for drug product (Cream).

Table 6. Precision results for dry accessories.

Average Results in μm					
Samples		D(10)	D(50)	D(90)	% Obscuration
Method Precision	1	1.54	4.09	8.27	5.21
	2	1.51	4.00	8.07	4.93
	3	1.59	4.02	7.93	4.73
	4	1.50	4.01	8.08	4.52
	5	1.47	3.93	7.89	4.53
	6	1.46	3.98	8.13	4.82
	Average (n = 6)	1.51	4.01	8.06	4.79
%RSD (n = 6)		3	1	2	NA
Intermediate Precision	1	1.33	3.78	7.53	4.28
	2	1.34	3.77	7.54	4.94
	3	1.32	3.79	7.60	4.26
	4	1.32	3.78	7.59	5.36
	5	1.35	3.84	7.95	4.44
	6	1.36	3.85	8.01	4.26
	Average (n = 6)	1.34	3.80	7.70	4.59
%RSD (n = 6)		1	1	3	NA

Table 7. Precision Results for Wet Accessories.

Average Results in μm							
Samples		Sample Weight (mg)	D(10)	D(50)	D(90)	% Obscuration	% Weighted Residual
Method Precision	1	103.90	12.9	24.1	47.6	12.60	0.45
	2	99.50	12.9	24.2	47.9	13.09	0.47
	3	100.60	12.9	24.1	47.2	12.40	0.46
	4	102.00	12.8	24.1	47.6	12.26	0.46
	5	99.70	12.9	24.1	47.5	12.77	0.45
	6	99.60	12.9	24.1	47.6	12.71	0.48
	Average (n = 6)		12.9	24.1	47.6	12.64	0.46
%RSD (n = 6)			0	0	1		NA

Continued

Intermediate Precision	1	98.88	12.8	23.8	45.7	14.47	0.46
	2	94.45	12.9	24.0	46.3	14.82	0.44
	3	102.22	12.9	23.9	46.2	13.52	0.44
	4	103.98	12.9	24.0	46.3	13.63	0.44
	5	102.05	12.8	24.1	47.6	14.29	0.45
	6	99.60	12.9	24.0	46.5	14.38	0.45
Average (n = 6)			12.9	24.0	46.4	14.19	0.45
%RSD (n = 6)			0	0	1	NA	

7.3. Robustness

Robustness studies were performed to evaluate the method's ability to remain reliable when subjected to small, deliberate variations in analytical parameters. Such studies help ensure that the method is resilient to minor operational fluctuations that may occur during routine use. Robustness was assessed by introducing controlled variations to selected critical analytical parameters. For each variation, three independent sample preparations were analyzed, and each preparation was measured in triplicate. The specific parameter variations evaluated in this study are summarized below in **Table 8**.

Table 8. Robustness evaluation parameters for dry and wet dispersion methods.

Condition	Nominal	Modified Conditions
Dry Accessories		
Vibration Feed Rate	60%	54%
		66%
Air Pressure	1.5 bar	1.3 bar
		1.7 bar
Wet Accessories		
Obscuration	10% - 20%	9%
		21%
Stir Rate	1500 RPM	1350 RPM
		1650 RPM
Vortex Time	120 seconds	90 seconds
		150 seconds

These variations were selected based on their potential impact on particle size measurements. The robustness results were evaluated using the same acceptance criteria applied in precision and intermediate precision studies. Overall, the robustness assessment demonstrated that the method remained stable and produced consistent PSD results despite small variations in analytical conditions, confirming its suitability for routine pharmaceutical analysis (**Table 9**, **Table 10**).

Table 9. Robustness results for dry accessories.

Parameter	Sample	Average Results in μm			
		D(10)	D(50)	D(90)	% Obscuration
Vibration Feed Rate 54%	1	1.42	3.91	8.01	4.59
	2	1.45	3.97	8.09	4.92
	3	1.42	3.97	8.12	4.45
	Average (n = 3)	1.43	3.95	8.07	4.66
	%RSD (n = 3)	1	1	1	NA
Vibration Feed Rate 66%	1	1.45	3.98	8.13	4.89
	2	1.44	3.94	8.13	5.27
	3	1.49	3.90	7.66	3.99
	Average (n = 3)	1.46	3.94	7.98	4.72
	%RSD (n = 3)	2	1	3	NA
Air Pressure 1.3 bar	1	1.42	4.04	8.90	5.69
	2	1.41	4.03	8.75	4.18
	3	1.40	4.04	8.87	4.73
	Average (n = 3)	1.41	4.04	8.84	4.87
	%RSD (n = 3)	1	0	1	NA
Air Pressure 1.7 bar	1	1.39	3.98	8.97	5.03
	2	1.33	3.83	7.90	4.48
	3	1.31	3.79	7.57	4.90
	Average (n = 3)	1.34	3.87	8.15	4.80
	%RSD (n = 3)	3	3	9	NA

Table 10. Robustness Results for Wet Accessories.

Parameter	Sample	Sample Weight (mg)	Average Results in μm			% Obscuration	% Weighted Residual
			D(10)	D(50)	D(90)		
Obscuration 9%	1	100.77	12.9	23.9	46.1	9.53	0.52
	2	101.83	13.0	24.2	47.3	9.45	0.51
	3	100.44	12.8	24.1	47.4	9.39	0.50
	Average (n = 3)		12.9	24.1	47.0	9.46	0.51
	%RSD (n = 3)		1	1	2	NA	
Obscuration 21%	1	100.76	12.8	24.0	47.3	20.70	0.48
	2	99.56	12.8	24.2	48.0	20.62	0.48
	3	100.56	12.8	24.2	48.1	20.72	0.48
	Average (n = 3)		12.8	24.1	47.8	20.68	0.48
	%RSD (n = 3)		0	0	1	NA	
Stir Rate 1350 RPM	1	99.06	12.9	24.2	48.1	11.84	0.49
	2	100.02	12.8	24.3	48.7	12.91	0.50
	3	101.08	12.8	24.3	48.6	11.37	0.52
	Average (n = 3)		12.8	24.3	48.5	12.04	0.50
	%RSD (n = 3)		0	0	1	NA	
Stir Rate 1650 RPM	1	101.40	12.9	24.6	50.7	11.41	0.51
	2	100.24	12.8	24.3	48.3	11.95	0.51
	3	99.42	12.8	24.3	48.8	11.95	0.53
	Average (n = 3)		12.8	24.4	49.3	11.77	0.52
	%RSD (n = 3)		0	0	3	NA	
Vortex Time 90 Seconds	1	98.15	12.8	23.8	45.4	13.87	0.44
	2	100.31	12.9	23.9	46.0	15.50	0.44
	3	97.05	12.8	23.9	46.5	15.07	0.44
	Average (n = 3)		12.9	23.9	46.0	14.82	0.44
	%RSD (n = 3)		0	0	1	NA	
Vortex Time 150 Seconds	1	97.10	12.9	23.9	46.4	14.18	0.43
	2	95.63	12.8	24.1	48.0	15.51	0.46
	3	98.10	12.9	23.9	46.4	15.69	0.43
	Average (n = 3)		12.8	24.0	46.9	15.13	0.44
	%RSD (n = 3)		0	1	2	NA	

Air pressure comparison between nominal (1.3 bar) and increased pressure (1.7 bar) conditions revealed minimal impact on particle size distribution. At 1.7 bar, the D50 and D10 showed a small reduction from 4.04 μm to 3.87 μm (4.2% decrease) and 1.41 μm to 1.34 μm (5.0% decrease), respectively. The D90 demonstrated a more pronounced but acceptable shift from 8.84 μm to 8.15 μm (7.8% decrease). These shifts indicate slightly improved deagglomeration at higher pressure without causing significant particle breakage, as evidenced by the maintained particle size distribution profile. Based on these results, an operating range of 1.3 - 1.7 bar was established, within which particle size variation remained within $\pm 8\%$ of nominal values.

Stir rate evaluation between 1350 RPM and 1650 RPM demonstrated excellent method robustness. At the higher stir rate (1650 RPM), D50 and D10 remained essentially unchanged (24.3 μm vs 24.4 μm , 0.4% increase) and (12.9 μm vs 12.8 μm , 0.8% decrease). The D90 exhibited minimal variability, increasing from 48.5 μm to 49.3 μm (1.6% increase). The consistent results across both stir rates indicate that mixing intensity within this range does not significantly affect cream matrix dispersion or cause particle breakdown. An operating of 1350-1650 RPM was therefore established, providing operational flexibility while maintaining measurement precision within $\pm 2\%$ for all key percentiles.

The same acceptance criteria used for repeatability were applied to the robustness study. Robustness conclusions were drawn based on the criterion that parameter variations should not exceed 15% deviation from nominal results, ensuring method reliability within normal operational ranges while maintaining fitness-for-purpose performance. These limits consider the multi-parameter nature of PSD analysis (D10, D50, D90) and provide appropriate discrimination for quality control applications.

This validation scope was limited to characteristics relevant to physical particle size measurement, while excluding concentration-dependent parameters not applicable to PSD methodology, thereby ensuring a scientifically justified and regulatory-compliant validation approach. This case study validation is specific to one drug substance with defined physicochemical properties and drug product topical cream formulation matrix. The optimized dispersion conditions (1.5 bar dry pressure, and 1500 RPM wet stirring) are material-specific and may require re-optimization for different APIs or formulation matrices with varying solubility, particle morphology, or cream base compositions. The findings should not be generalized to other drug substances or topical formulations without appropriate method development studies to account for different material characteristics and matrix interactions. Future validation efforts should consider API-specific and matrix-specific optimization to ensure appropriate method performance across diverse topical formulation types.

8. FDA 483 Observations and Systemic Failures

Inspection findings frequently reveal systemic weaknesses in particle size distri-

bution (PSD) method validation, and testing. While FDA Form 483s and warning letters cite regulatory sections rather than specific analytical techniques, the deficiencies documented under laboratory controls directly apply to PSD methods. Among the most frequently cited observations in FY 2024, 21 CFR 211.160(b) was invoked for laboratory controls that did not include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures. FDA guidelines For PSD methods, this encompasses the absence of dispersion optimization studies, lack of intermediate precision evaluation, and unjustified selection of optical parameters deficiencies that often stem from over-reliance on vendor-recommended default settings without experimental verification. A major root cause is the assumption that instrument qualification alone ensures method suitability. However, regulatory expectations under 21 CFR 211.160(b) require product-specific justification of method conditions. Warning letters issued under 21 CFR 211.160 specifically cite the use of unsuitable or non-validated test methods without scientifically supported justification, as well as the absence of test specifications for incoming and final inspections, as recurring deficiencies. GMP Trends Analysis of FDA warning letters under 21 CFR 211.160 has identified a primary failure pattern: absence of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures including for topical drug products, where failures to establish identity test procedures and written calibration programs have been cited. Inadequate OOS investigation of PSD results is an additional systemic risk. Under 21 CFR 211.192, FDA has cited firms for preparing new test preparations of original samples for products with OOS results without first adequately investigating the initial failing result, with the agency finding no scientific justification for this practice. GMP-journal Warning letter analysis covering fiscal years 2017-2021 found that laboratory records frequently lacked complete data, including OOS results, root cause analyses of OOS results, data from failed tests, and re-test data each of which is essential to a defensible PSD investigation [34]-[38]. Systemic improvement requires integration of scientific method development, robust validation design, and continuous performance monitoring. By addressing these root causes, organizations can reduce compliance risk and enhance data reliability.

9. Future Trends

Advances in pharmaceutical manufacturing are steadily transforming particle size distribution (PSD) analysis from a traditional, laboratory-based quality control test into a dynamic, process-integrated decision-making tool. Historically, PSD testing was performed after production, often serving as a confirmatory release parameter. Today, however, evolving regulatory expectations and technological capabilities are driving a paradigm shift. Modern pharmaceutical quality systems emphasize real-time monitoring, predictive control, and lifecycle management. As a result, PSD measurement is increasingly positioned not merely as an analytical endpoint, but as a critical enabler of process understanding and control. This

transformation is strongly aligned with regulatory initiatives promoting scientific and risk-based manufacturing. Agencies such as the U.S. Food and Drug Administration have actively encouraged innovation through frameworks that support Quality by Design (QbD), Process Analytical Technology (PAT), and continuous manufacturing [39]-[41]. Within this evolving landscape, PSD analysis plays a pivotal role because particle size directly influences dissolution rate, blend uniformity, content homogeneity, and overall product performance. Future trends therefore focus on integrating PSD measurement into manufacturing workflows, enhancing real-time responsiveness, and leveraging advanced analytics. Technologies that once operated independently are now interconnected, creating intelligent systems capable of adaptive process control. The shift from reactive testing to proactive quality assurance practices represents one of the most significant advancements in pharmaceutical analytical science and supports more reliable, robust manufacturing outcomes.

9.1. Process Analytical Technology (PAT)

Process Analytical Technology (PAT) represents a foundational pillar in modern pharmaceutical manufacturing. Encouraged by the U.S. Food and Drug Administration, PAT promotes real-time measurement and control of critical quality attributes during production rather than relying solely on end-product testing. In the context of PSD, PAT enables inline or at-line particle size monitoring directly within unit operations such as milling, granulation, or spray drying. Real-time PSD measurement provides immediate insight into process performance. For example, during milling operations, particle size can be continuously tracked, allowing operators or automated systems to adjust milling speed, feed rate, or classifier settings as soon as deviations occur. This immediate feedback significantly reduces batch-to-batch variability and minimizes the risk of producing out-of-specification material. Instead of discovering deviations after completion of a batch, corrective actions can be implemented dynamically, preserving product quality and reducing waste. Beyond operational efficiency, PAT strengthens process understanding. Continuous data collection generates comprehensive datasets that reveal process trends, variability patterns, and potential failure modes. This knowledge supports robust design space development and enhances regulatory confidence in manufacturing controls. Moreover, continuous process verification, an essential component of lifecycle management, becomes feasible when PSD is monitored in real time. Ultimately, PAT transforms PSD from a static laboratory measurement into a strategic control parameter. By embedding PSD analysis within the manufacturing process, pharmaceutical companies move closer to predictive, science-driven quality assurance rather than solely retrospective quality testing [42][43].

9.2. Continuous Manufacturing

Continuous manufacturing (CM) represents a major evolution from traditional

batch production, offering improved efficiency, flexibility, and product consistency. In this model, materials flow through interconnected unit operations without interruption, requiring tight coordination and immediate response to variability. Within such systems, PSD becomes a critical control variable, particularly in processes involving milling, blending, and downstream formulation. Unlike batch manufacturing, where PSD may be evaluated at discrete time points, continuous manufacturing requires constant monitoring. Real-time PSD feedback must be integrated into automated control loops that adjust process parameters instantly. Real-time PSD feedback must be integrated into automated control loops that adjust process parameters instantly. Laser diffraction is widely used in-line or at-line to measure particle sizes across a broad range, providing rapid and quantitative PSD data. Focused Beam Reflectance Measurement (FBRM) enables real-time monitoring of particle growth and breakage by measuring chord lengths in suspensions or powders, making it particularly useful in wet or dry granulation processes. Imaging techniques, such as Particle Vision and Measurement (PVM), capture in-line images to evaluate both particle size and morphology, allowing detection of fines, agglomerates, and shape variations. For submicron particles, dynamic light scattering (DLS) is employed at-line to assess nanoparticles or dissolved APIs. Traditional sieving methods are primarily used off-line for validation or cross-checking purposes. Overall, these PSD measurement techniques in continuous manufacturing enable integration into process control loops, supporting real-time quality assurance and alignment with Quality by Design (QbD) principles. For instance, if particle size begins to drift toward the upper specification limit, the control system can automatically modify milling intensity or adjust feed rates to restore equilibrium. This closed-loop control strategy significantly reduces process variability and enhances overall system stability. Advanced statistical modeling plays a central role in this environment. Multivariate analysis and predictive algorithms help interpret complex datasets generated by inline sensors. These tools allow early detection of subtle process shifts before they result in specification failures. Rather than reacting to deviations, manufacturers can anticipate and prevent them. The integration of PSD into continuous systems also supports regulatory expectations for enhanced process control and real-time release testing. By demonstrating sustained control over particle size, companies can justify reduced reliance on end-product testing. Continuous manufacturing, supported by robust PSD monitoring, therefore represents a future-ready model that combines operational efficiency with scientifically sound quality assurance [44].

9.3. Advanced Analytical Techniques

As pharmaceutical processes become more sophisticated, analytical technologies are evolving to provide deeper and more multidimensional insight into particle characteristics. Traditional laser diffraction remains a powerful tool; however, emerging techniques are expanding the scope of PSD analysis beyond simple size metrics. Dynamic image analysis, for example, provides detailed morphological

information such as particle shape, aspect ratio, and surface texture. This additional characterization is particularly valuable for materials where shape influences flowability, compaction, or aerodynamic behavior. By complementing size distribution data with morphological parameters, scientists gain a more comprehensive understanding of material performance. Integration of Raman spectroscopy within PAT frameworks further enhances analytical capability. While PSD measurements describe physical characteristics, Raman spectroscopy provides chemical identity and polymorphic information. The combination of these techniques enables simultaneous physical and chemical monitoring, supporting a holistic approach to process control. Artificial intelligence (AI) and machine learning are also emerging as transformative tools in PSD analysis. AI-driven distribution modeling can identify subtle trends, predict process outcomes, and automate anomaly detection with high sensitivity. By leveraging historical datasets, machine learning algorithms can optimize dispersion parameters, predict milling behavior, and enhance decision-making accuracy. Together, these advanced analytical approaches signal a shift toward intelligent, data-driven pharmaceutical manufacturing. PSD analysis is no longer confined to size measurement alone it is evolving into a multidimensional, predictive, and integrative component of modern quality systems.

10. Conclusion

Particle Size Distribution measurement by laser diffraction remains a fundamental analytical tool for controlling critical quality attributes in pharmaceutical development and manufacturing. The validation studies presented for both dry and wet dispersion accessories of the Malvern Mastersizer 3000 demonstrate that reliable PSD characterization requires systematic dispersion optimization, scientifically justified method parameters, and statistically supported validation. Evaluation of representative materials, including a drug substance and drug product, confirmed that dispersion technique selection significantly influences measured PSD parameters such as D10, D50, and D90, highlighting the importance of material-specific method development. The results show that robust PSD methods can be achieved through a risk-based validation strategy incorporating repeatability, intermediate precision, and robustness assessments. When aligned with modern regulatory expectations, particularly those described in ICH Q2(R2) and the integrated quality framework of ICH Q7-Q11, PSD measurement becomes more than a routine analytical test; it serves as an essential component of process understanding, quality control, and lifecycle management. Ultimately, effective PSD validation requires integration of analytical science, regulatory compliance, and operational quality systems. Adoption of structured, risk-based methodologies supported by appropriate dispersion selection and validated performance parameters can significantly reduce regulatory risk while improving analytical reliability. Such an approach ensures that PSD measurements remain scientifically defensible, reproducible, and fully aligned with contemporary pharmaceutical quality

standards.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Chu, K.R., Lee, E., Jeong, S.H. and Park, E. (2012) Effect of Particle Size on the Dissolution Behaviors of Poorly Water-Soluble Drugs. *Archives of Pharmacal Research*, **35**, 1187-1195. <https://doi.org/10.1007/s12272-012-0709-3>
- [2] Zheng, K., Lin, Z., Capece, M., Kunnath, K., Chen, L. and Davé, R.N. (2019) Effect of Particle Size and Polymer Loading on Dissolution Behavior of Amorphous Griseofulvin Powder. *Journal of Pharmaceutical Sciences*, **108**, 234-242. <https://doi.org/10.1016/j.xphs.2018.11.025>
- [3] Wilson, D., Crampton, A., Sullivan, R. and Liljeblad, A. (2022) An Analysis of the Technical and Economic Influences of Particle Size Control of an Active Pharmaceutical Ingredient. *Journal of Pharmaceutical Innovation*, **17**, 501-509. <https://doi.org/10.1007/s12247-020-09526-1>
- [4] Liu, L.X., Marziano, I., Bentham, A.C., Litster, J.D., White, E.T. and Howes, T. (2008) Effect of Particle Properties on the Flowability of Ibuprofen Powders. *International Journal of Pharmaceutics*, **362**, 109-117. <https://doi.org/10.1016/j.ijpharm.2008.06.023>
- [5] Sun, Z., Ya, N., Adams, R.C. and Fang, F.S. (2010) Particle Size Specifications for Solid Oral Dosage Forms: A Regulatory Perspective. *American Pharmaceutical Review*, **13**, 68-73. <https://www.americanpharmaceuticalreview.com/Featured-Articles/36779-Particle-Size-Specifications-for-Solid-Oral-Dosage-Forms-A-Regulatory-Perspective/>
- [6] Snorek, S.M., Bauer, J.F., Chidambaram, N., Doub, W.H., Duffy, E.P., Etzler, F.M., *et al.* (2007) PQRI Recommendations on Particle-Size Analysis of Drug Substances Used in Oral Dosage Forms. *Journal of Pharmaceutical Sciences*, **96**, 1451-1467.
- [7] United States Pharmacopeia (USP) (2025) USP <429> Light Diffraction Measurement of Particle Size. United States Pharmacopeial Convention.
- [8] Shekunov, B.Y., Chattopadhyay, P., Tong, H.H.Y. and Chow, A.H.L. (2007) Particle Size Analysis in Pharmaceutics: Principles, Methods and Applications. *Pharmaceutical Research*, **24**, 203-227. <https://doi.org/10.1007/s11095-006-9146-7>
- [9] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (2000) ICH Q7: Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients. ICH.

- [10] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (2009) ICH Q8 (R2): Pharmaceutical Development. ICH.
- [11] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (2023) ICH Q9 (R1): Quality Risk Management. ICH.
- [12] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (2008) ICH Q10: Pharmaceutical Quality System. ICH.
- [13] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (2012) ICH Q11: Development and Manufacture of Drug Substances (Chemical and Biotechnological/Biological Entities). ICH.
- [14] US Food and Drug Administration, Guidance for Industry (2022) Physicochemical and Structural (Q3) Characterization of Topical Drug Products Submitted in ANDAs, Guidance for Industry. <https://www.fda.gov/media/162471/download>
- [15] Chiarentin, L., Moura, V., Major, F., Catita, J., Miranda, M. and Vitorino, C. (2025) Particle Size by Design: Standardizing Measurements for Complex Topical Drug Product Assessment. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **705**, Article ID: 135679. <https://doi.org/10.1016/j.colsurfa.2024.135679>
- [16] Barber, D., Keuter, J. and Kravig, K. (1998) A Logical Stepwise Approach to Laser Diffraction Particle Size Distribution Analysis Methods Development and Validation. *Pharmaceutical Development and Technology*, **3**, 153-161. <https://doi.org/10.3109/10837459809028491>
- [17] Dubey, J., Rajyaguru, B., Vankawala, K., Vaidya, F., Mistri, J. and Surati, K. (2025) Development and Validation of New Analytical Method for the Determination of Particle Size Distribution in Empagliflozin Using Laser-Based Particle Size Analyzer. *Journal of Advances in Medical and Pharmaceutical Sciences*, **27**, 65-74. <https://doi.org/10.9734/jamps/2025/v27i11828>
- [18] Medendorp, J., Bric, J., Connelly, G., Tolton, K. and Warman, M. (2015) Development and Beyond: Strategy for Long-Term Maintenance of an Online Laser Diffraction Particle Size Method in a Spray Drying Manufacturing Process. *Journal of Pharmaceutical and Biomedical Analysis*, **112**, 79-84. <https://doi.org/10.1016/j.jpba.2015.04.019>
- [19] Nataraj, P., Ravisankar, M., Vijayakumar, M., Sivagamy, M. and Senthilkumar, G.P. (2025) Analytical Method Development and Validation to Assess the Particle Size Distribution in Azithromycin Oral Suspension 200 mg/5 ml. *Saudi Journal of Medical and Pharmaceutical Sciences*, **11**, 949-952. <https://doi.org/10.36348/sjamps.2025.v11i10.004>
- [20] Pearson, J., Nguyen, T.L., Cölfen, H. and Mulvaney, P. (2018) Sedimentation of C₆₀ and C₇₀: Testing the Limits of Stokes' Law. *The Journal of Physical Chemistry Letters*, **9**, 6345-6349. <https://doi.org/10.1021/acs.jpclett.8b02703>
- [21] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (2023) ICH Q2 (R2): Validation of Analytical Procedures. ICH. (Revised Guideline Adopted November 2023)
- [22] Gosar, A., Jadhav, S., Lalge, A. and Patil, M. (2020) Development and Validation of Novel Analytical Method for the Determination of Particle Size Distribution in Lumefantrine Using Laser-Based Particle Size Analyzer. *Indo American Journal of Pharmaceutical Research*, **11**, 1457-1465.
- [23] Gosar, A., Parange, C., Narvankar, S., Jadhav, S. and Patil, V. (2022) Development and Validation of Novel Analytical Method for the Determination of Particle Size Distribution in Ciprofloxacin Hydrochloride Using Laser-Based Particle Size Analyzer. *Journal of Advances in Medical and Pharmaceutical Sciences*, **24**, 40-47.

- <https://doi.org/10.9734/jamps/2022/v24i730316>
- [24] Xie, L., Su, L., Xiao, Y., Zhang, R.-Q. and Zheng, P. (2024) Determination of Particle Size Distribution of Budesonide Suspension for Inhalation by Laser Diffraction Method. *Chinese Journal of Pharmaceutical Analysis*, **44**, 2004-2010. (In Chinese)
 - [25] Guo, B., Hao, G., Qian, X., Pan, X.-W. and Chen, R. (2021) Establishment and Validation of Particle Size Distribution Determination Method of Rivaroxaban by Laser Light Scattering. *Chinese Journal of Pharmaceutical Analysis*, **41**, 1649-1654. (In Chinese)
 - [26] Wang, Y., Wu, S., Xue, D., Zhang, K., Shao, H., Xu, W., Zhang, D., Zhang, S. and Yang, Q.-Y. (2021) Methodological Study of Particle Size Distribution of Bromhexine Hydrochloride by Laser Diffraction Method. *Chinese Journal of Pharmaceutical Analysis*, **41**, 1448-1453. (In Chinese)
 - [27] Cheng, D., Xue, M., Yin, J., Qiu, X., Liu, M. and Lu, Y. (2025) Study on the Influence of Ultrasound Parameters on the Determination of Particle Size and Particle Size Distribution of Budesonide Suspension for Inhalation by Laser Diffraction Method. *Herald of Medicine*, **44**, 1784-1788. (In Chinese)
 - [28] Tinke, A.P., Vanhoutte, K., Vanhoutte, F., De Smet, M. and De Winter, H. (2005) Laser Diffraction and Image Analysis as a Supportive Analytical Tool in the Pharmaceutical Development of Immediate Release Direct Compression Formulations. *International Journal of Pharmaceutics*, **297**, 80-88.
<https://doi.org/10.1016/j.ijpharm.2005.03.009>
 - [29] Silva, A.F.T., Burggraef, A., Denon, Q., Van der Meeren, P., Sandler, N., Van Den Kerkhof, T., *et al.* (2013) Particle Sizing Measurements in Pharmaceutical Applications: Comparison of In-Process Methods versus Off-Line Methods. *European Journal of Pharmaceutics and Biopharmaceutics*, **85**, 1006-1018.
<https://doi.org/10.1016/j.ejpb.2013.03.032>
 - [30] Vo, A., Feng, X., Smith, W.C., Zhu, D., Patel, M., Kozak, D., *et al.* (2021) Analyzing Ophthalmic Suspension Particle Size Distributions Using Laser Diffraction: Placebo Background Subtraction Method. *International Journal of Pharmaceutics*, **598**, Article ID: 120401. <https://doi.org/10.1016/j.ijpharm.2021.120401>
 - [31] Marriott, C., MacRitchie, H.B., Zeng, X.M. and Martin, G.P. (2006) Development of a Laser Diffraction Method for the Determination of the Particle Size of Aerosolised Powder Formulations: Comparison with Inertial Impaction. *International Journal of Pharmaceutics*, **326**, 39-49. <https://doi.org/10.1016/j.ijpharm.2006.07.021>
 - [32] Ransmark, E., Åkerfeldt, E., Liu, X., Sørensen, H., Gómez Galindo, F. and Håkansson, A. (2025) Evaluation of Laser Diffraction as a Fast Method for Particle Size Distribution Measurement of Plant Cell Structures in Whole Oat and Soy Beverages. *Food Research International*, **217**, Article ID: 116781.
<https://doi.org/10.1016/j.foodres.2025.116781>
 - [33] Pei, Y., Hinchliffe, B.A. and Minelli, C. (2021) Measurement of the Size Distribution of Multimodal Colloidal Systems by Laser Diffraction. *ACS Omega*, **6**, 14049-14058.
<https://doi.org/10.1021/acsomega.1c00411>
 - [34] U.S. Food and Drug Administration (2025) Inspection Observations (Form FDA 483). FDA.
<https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-references/inspection-observations>
 - [35] U.S. Food and Drug Administration (2026) Warning Letters. FDA.
<https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/compliance-actions-and-activities/warning-letters>

- [36] U.S. Food and Drug Administration (2017) Code of Federal Regulations, Title 21, Part 211.160: Current Good Manufacturing Practice for Finished Pharmaceuticals—General Requirements for Laboratory Controls. U.S. Government Publishing Office. <https://www.ecfr.gov/current/title-21/chapter-I/subchapter-C/part-211/subpart-I/section-211.160>
- [37] U.S. Food and Drug Administration (2017) Code of Federal Regulations, Title 21, Part 211.192: Current Good Manufacturing Practice for Finished Pharmaceuticals—Production Record Review. U.S. Government Publishing Office. <https://www.ecfr.gov/current/title-21/chapter-I/subchapter-C/part-211>
- [38] U.S. Food and Drug Administration (2022) Guidance for Industry: Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production (Revision 1). CDER. <https://www.fda.gov/media/71001/download>
- [39] Dubey, J., Jariwala, K.J., Srivastava, B.K., Koundinya, J., Rajyaguru, B. and Vaidya, F. (2026) A Quality by Design Approach to Development and Validation of a Laser-Based Analytical Method for Particle Size Distribution of Dapagliflozin Propanediol. *Journal of Advances in Medical and Pharmaceutical Sciences*, **28**, 15-38. <https://doi.org/10.9734/jamps/2026/v28i1839>
- [40] Yang, S., Li, J., Zhang, Y. and Wang, X. (2025) Aspects and Implementation of Pharmaceutical Quality by Design from Conceptual Frameworks to Industrial Applications. *Journal of Pharmaceutical Innovation*, 20(2), 145-160.
- [41] Pharma Excipients (2020) De-Risking Excipient Particle Size Distribution Variability with Automated Robust Mixing: Integrating Quality by Design and Process Analytical Technology. *European Journal of Pharmaceutics and Biopharmaceutics*, **154**, 123-134.
- [42] U.S. Food and Drug Administration (2004) PAT—A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance. U.S. FDA Guidance. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/pat-framework-innovative-pharmaceutical-development-manufacturing-and-quality-assurance>
- [43] Dahlgren, G., *et al.* (2020) Quality & Regulatory Solutions for PAT in Continuous Manufacturing. *Pharmaceutical Engineering*, **40**, 30-38. <https://ispe.org/pharmaceutical-engineering/september-october-2020/quality-regulatory-solutions-pat-continuous>
- [44] U.S. Food and Drug Administration (2023) ICH Q13 Continuous Manufacturing of Drug Substances and Drug Products. U.S. FDA Guidance. <https://www.fda.gov/media/165775/download>