Lifecycle-Oriented AQbD Development and Six Sigma Validation of a Green Dry Laser Diffraction Method for Particle Size Distribution Analysis of Metronidazole API

Pradip Patil[1], Pankajkumar Nariya[2], Patil Aditi Rajesh[3], Mahesh Savant[4*]
[1] Department of Chemical Sciences, Faculty of Science, Atmiya University, Rajkot, Gujarat, India.
Email Id : pradippatil99@gmail.com ORCID ID - 0009-0008-4549-5947
[2]Department of Chemical Sciences, Faculty of Science, Atmiya University, Rajkot, Gujarat, India.
Email Id: Pankaj.nariya@atmiyauni.ac.in
[3] Department of Chemistry Faculty of Science, P.D.V.P Collage Tasgaon , Maharashtra , India.
Email Id : patiladiti361@gmail.com
[4]Department of Chemical Sciences, Faculty of Science, Atmiya University, Rajkot, Gujarat, India. Email Id : mahesh.savant@atmiyauni.ac.in ORCID ID - 0000-0001-7106-4253
[*]***Correspondence : Mahesh Savant***
Department of Chemical Sciences, Faculty of Science, Atmiya University, Rajkot, Gujarat, India.
Email Id: mahesh.savant@atmiyauni.ac.in ORCID ID - 0009-0008-4549-5947

Abstract
The accurate description of the particle size distribution (PSD) is a key quality attribute in pharmaceutical industrial management, which affects the final performance of active pharmaceutical ingredients (APIs). In this work, an eco-friendly, robust laser diffraction method for the determination of the PSD of metronidazole is described, developed, optimized and validated with stringent statistical validation. The study was performed using a Malvern Mastersizer 3000 with an Aero S dry powder dispersion unit using a Quality-by-Design (QbD) approach combined with an advance process capability analytics. The framework was implemented in full compliance with ICH Q14 and ICH Q2(R2) regulatory requirements for industrial quality control. Computational method was used to optimize critical process parameters such as air pressure, feed rate, refractive index and measurement time. The dry dispersion protocol was statistically validated with impressive precision with %RSD metrics of 0.94%, 0.53% and 1.30% for d(0.1), d(0.5), and d(0.9) percentiles respectively. Moreover, the method showed intermediate precision RSDs of 2.39%, 1.12% and 0.41%. The protocol avoided any solvent-induced analytical biases completely by using a dry dispersion technique, minimized operator dependence and followed the green analytical chemistry paradigms by eliminating hazardous organic solvents. Robustness studies and Six Sigma process capability analysis (Cp, Cpk) confirmed long term stability and reproducibility of the method to intentional variations of parameters . In the end, this computational approach provides a rapid, low-cost, non-destructive industrialmanagement tool for life-cycle management, ongoing process verification, and routine pharmaceutical manufacturing control.
Industrial Quality Management, Statistical Process Control, Malvern 3000, Metronidazole API, Quality-by-Design, Laser Diffraction, Green Analytical Chemistry.

Keywords: Analytical Quality by Design (AQbD); Laser diffraction; Particle size distribution (PSD); Metronidazole; Active pharmaceutical ingredient (API); Dry powder dispersion; Method validation; Analytical method development; Aero S dry dispersion unit; Pharmaceutical quality control., computer validation with 21CFR.
How to cite this article: Pradip Patil, Pankajkumar Nariya, Patil Aditi Rajesh, Mahesh Savant. Lifecycle-Oriented AQbD Development and Six Sigma Validation of a Green Dry Laser Diffraction Method for Particle Size Distribution Analysis of Metronidazole API. Int J Drug Deliv Technol. 2026;16(88s):822-829. DOI: 10.25258/ijddt.16.88s.84.

Introduction

The pharmaceutical industry has experienced a paradigm shift from traditional quality-by-testing approaches to science- and risk-based product development strategies emphasizing process understanding, lifecycle management, and continuous quality improvement. The implementation of Quality by Design (QbD) principles recommended by the International Council for Harmonisation (ICH) through Q8(R2), Q9, Q10, and the recently introduced Q14 guidelines is driving this transformation. These regulatory frameworks support systematic identification and control of Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs) to assure that pharmaceutical products consistently meet their predetermined quality, safety and efficacy throughout the product lifecycle [1–4,11,12]. This implies that analytical methods used for quality assessment must also be developed through a scientific, risk-based, and lifecycle-oriented approach, often termed Analytical Quality by Design (AQbD).

Particle size distribution (PSD) is considered one of the most important critical quality
attributes among the different physicochemical properties of pharmaceutical raw materials due to its significant influence on the manufacturing performance and product quality.

Particle size controls many basic properties of

pharmaceutical powders, such as flowability, bulk and tapped density, compressibility, blend homogeneity, segregation tendency, electrostatic behavior, and content uniformity. More importantly, PSD has a significant impact on dissolution rate, drug release kinetics, wettability, suspension stability, bioavailability and therapeutic efficacy, especially for poorly water-soluble active pharmaceutical ingredients (APIs) [5-10]. Variability in particle size may therefore lead to inconsistent manufacturing performance, reduced process capability, and undesirable batch-to-batch variations. Accurate particle characterization is therefore an indispensable component of pharmaceutical development, process optimization, technology transfer, and routine quality control.

Reliable particle size determination requires not only the choice of an appropriate analytical technique but also the development of a robust analytical procedure that can generate precise, accurate, and reproducible measurements under routine operating conditions. Analytical method development has evolved from empirical trial and error approaches to scientifically designed methodologies supported by risk assessment, statistical optimization, and lifecycle management as regulatory expectations have changed with the times. In this respect, the ICH Q14 offers a comprehensive framework for analytical procedure development, while ICH Q2(R2) defines internationally accepted validation requirements to demonstrate that an analytical procedure is fit for its intended purpose [11,12]. Moreover, the US Food and Drug Administration (US FDA) strongly recommends the use of validated analytical procedures and Process Analytical Technology (PAT) to enhance process understanding, enable continuous process verification, and have robust pharmaceutical quality systems [13,14].

Various analytical techniques have been applied for particle size characterization, such as

sieve analysis, sedimentation techniques, optical microscopy, Coulter counter analysis, dynamic light scattering (DLS), and laser diffraction [15–18]. Each of the analytical techniques has its own advantages. However, laser diffraction (LD) is the preferred technique in industry because of its rapid analysis, wide measuring range, excellent repeatability, the least sample preparation, non-destructive operation and suitability for routine quality control of pharmaceutical powders [19–22]. For these reasons, laser diffraction has become the internationally accepted standard method for particle size analysis and is formally recognized by ISO 13320:2019 and USP General Chapter <429>, making it the most widely used technique in pharmaceutical, chemical, food, pigment, cosmetic, and advanced materials industries [19,22].

The basic principle of laser diffraction is that the particle size is correlated to the angular distribution of the scattered laser light. Big particles mostly scatter light at small angles, but little particles scatter light at large angles. The resulting scattering profile is mathematically converted into a particle size distribution by using appropriate optical models. For transparent and submicron particles, Mie scattering theory is usually used because it considers the refractive index and the absorption properties of the material. On the other hand, the Fraunhofer approximation gives an acceptable estimation for relatively large opaque particles where optical properties have a negligible influence on scattering behavior [25–27].

However, the reliability of laser diffraction measurements is heavily dependent on the proper optimization of sample preparation, dispersion conditions, obscuration level, optical parameters and instrumental operating variables. Insufficient dispersion could lead to particle agglomeration, while too much dispersion energy can cause particle fracture, both of which affect measurement accuracy and reproducibility [17,20–24]. Thus, systematic optimization of analytical parameters is crucial to develop a robust and reliable PSD analytical method.

Sample dispersion is one of the most critical stages in laser diffraction analysis. Particle dispersion can be carried out either by wet or dry techniques, depending on the physicochemical properties of the material. Wet dispersion usually uses an appropriate dispersing medium, often with the help of ultrasonication to break agglomerates, while dry dispersion uses compressed air to separate free-flowing powder particles with controlled dispersion pressure [23,24]. Compared to wet dispersion, dry dispersion avoids the use of organic solvents and dispersing liquids, minimizes sample preparation time, reduces analytical waste and offers a more environmentally sustainable analytical alternative for routine industrial quality control. Therefore, dry laser diffraction has gained growing interest as a green analytical technology that enables the sustainable manufacture of pharmaceuticals without sacrificing analytical robustness and regulatory compliance.

Metronidazole is a synthetic 5-nitroimidazole antimicrobial agent extensively prescribed for the treatment of anaerobic bacterial infections and protozoal diseases including amoebiasis, giardiasis, trichomoniasis, and various gastrointestinal infections. Because of its broad antimicrobial spectrum, good clinical efficacy and well-established safety profile, metronidazole is still included in the World Health Organization (WHO) Model List of Essential Medicines and is one of the most widely produced antimicrobial agents in the world [28–32]. The physicochemical properties of metronidazole, especially the particle size distribution, are decisive factors for dissolution behaviour, suspension stability, tablet compression properties, granulation efficiency and overall formulation performance.

Therefore, precise control of PSD is critical for consistent product quality and regulatory compliance during

commercial manufacturing.

In recent years, advances in pharmaceutical development have encouraged the use of Analytical Quality by Design (AQbD) principles in the development of analytical methods. In this context, PSD is considered a Critical Quality Attribute, while instrumental settings, dispersion conditions, optical parameters, feed rate, air pressure, and measurement duration are Critical Method Parameters (CMPs) that must be systematically evaluated and optimized. The integration of quality risk management with Design of Experiments (DoE) enables the identification of significant analytical variables, a quantitative evaluation of factor interactions, the establishment of an analytical design space, and the development of robust operating conditions able to minimize analytical variability due to instrumentation, operators, materials, and environmental influences [1–4,11,33]. Such a systematic strategy significantly improves method robustness, enhances measurement reliability, minimizes analytical variability, allows for regulatory flexibility, and supports the lifecycle management of analytical procedures.

Although several studies have been conducted to investigate particle engineering and formulation optimization of metronidazole, relatively little attention has been paid to the systematic development of robust analytical methodologies for routine determination of metronidazole API particle size. Most of the published studies are mainly on nanoparticle formulation, microsphere development, or chromatographic assay methods. Comprehensive analytical procedure development and validation for dry laser diffraction based on AQbD are still rare. Moreover, no validated analytical workflow combining ICH Q14, ICH Q2(R2), quality risk management, and statistical optimization has been reported for routine particle size characterization of metronidazole active pharmaceutical ingredient.

Hence, the present study was undertaken to develop and validate a robust, environmentally sustainable, and an industrially applicable dry laser diffraction analytical method for particle size characterization of metronidazole API using the principles of Analytical Quality by Design (AQbD). The analytical procedure was systematically developed through quality risk assessment and Design of Experiments to establish a scientifically justified analytical design space and was subsequently validated according to ICH Q2(R2) recommendations. The developed method is anticipated to offer a reliable, reproducible, regulatory-compliant and eco-friendly analytical platform for routine quality control, lifecycle management and continuous quality assurance of metronidazole active pharmaceutical ingredient in pharmaceutical manufacturing.

1. Literature Review and Operational Context

Previous industrial and computational modelling studies have demonstrated the intrinsic link between particle size and formulation success, as summarized in the context of our current operational study (Table 1).

Table 1: Computational and Formulation History of Metronidazole PSD Studies

Sr No	Reference	Objective	Key Industrial Outcomes
1	Sabbagh et al. [34]	Nanocomposite development	Optimized particle size via regressior
2	Sreeharsha et al. [35]	QbD nanoparticle engineering	Identified Critical Process Parameters (CPPs).
3	Roy et al. [36]	Microsphere formulation	Mapping polymer ratios to particle dimensions.
4	Kumar et al. [37]	Colon-targeted particles	Size correlation with release kinetics
5	Thomas et al. [38]	MDRS API analysis	Reduced analytical time (90 to 8 hrs).
6	Bhosale et al. [39]	HPLC method development	Established baseline quantification
7	Current study	Green QbD - based dry laser diffraction with 21 CFR validated computerised Malvern Mastersizer 3000	Developed highly precise, solvent-free, robust PSD solvent-free, robust PSD method , optimized for industrial Lifecycle management and reduced analytical time 8 Hrs to 15 min. Eco friendly and human safety

Scientific Rational: For this Research is Superior

1. Optimization of Mie Theory for Non-Spherical API Crystals

Unlike the Fraunhofer approximation, which assumes all particles are opaque and spherical, this study utilizes Mie Theory [18, 20]. Since Metronidazole API crystals are often irregular and non-spherical, Mie theory is

scientifically superior as it accounts for the complex refractive index and absorption characteristics of the material. This ensures that the light scattering pattern is not just "measured" but "interpreted" with physical accuracy, providing a more reliable d(10), d(50), and d(90) result than historical studies using simplified optical models.

Dry Solvent-Free Dispersion: The Green Analytical Paradigm

Historical studies have frequently relied on wet dispersion (Table 1, items 1–4). However, wet dispersion introduces solubility-induced analytical bias—where the API begins to dissolve in the suspending liquid (e.g., water or IPA) during measurement, leading to erroneous "finer" results [15]. By utilizing a high-energy Aero S dry powder dispersion unit, this research: Eliminates Solvent Bias: The particle size measured represents the true, dry bulk state.

Enhances Operational Safety: It removes the necessity for hazardous organic solvents, strictly adhering to Green Analytical Chemistry (GAC) principles and improving occupational health for laboratory personnel. Increases Throughput: As noted, reducing analytical time from 8 hours (using MDRS [38]) to just 15 minutes is a massive industrial efficiency gain, facilitating real-time release testing and rapid batch-to-batch monitoring.

2.2 CFR Part 11 Compliance and Data Integrity

Modern industrial management, as mandated by the US FDA, requires that analytical data be secure, attributable, and immutable. This study is superior because it leverages the 21 CFR Part 11-compliant architecture of the Malvern Mastersizer 3000 software. Historical studies (Table 1, items 3–6) often lacked the computerized audit trails and electronic signature security necessary for modern pharmaceutical lifecycle management. This integration ensures that the particle size data is not only scientifically accurate but also legally defensible during regulatory inspections.

2.3 AQbD and Lifecycle Robustness

This study does not simply "test" the particle size; it establishes a Design Space [30]. Through the systematic use of DoE, we have identified the Critical Method Parameters (CMPs)—such as air pressure and feed rate—that prevent "Out-of-Specification" (OOS) results. While previous studies (items 1–4) focused on formulation optimization, this research provides the analytical bedrock upon which those formulations must be built.

2. Materials and Methodological Framework

2.1 Materials and Reagents

To conduct the optimization trials, ultra-pure water was generated via a Milli-Q purification system (Millipore, USA). Analytical grade Tween 80 (Sigma Aldrich), 2-Propanol/Isopropyl Alcohol (Merck), and high-purity liquid paraffin (Thermofisher) were utilized for dispersion testing. Metronidazole API (research grade) was gifted by C. Pharmaceuticals Pvt. Ltd.

2.2 Instrumentation and Software Architecture

Measurements were captured utilizing a Malvern Mastersizer 3000, outfitted with both Aero S (dry) and Hydro MV (wet) automated dispersion units. The system was governed by Mastersizer software (Version 1.70), configured to strictly comply with 21 CFR Part 11 requirements for industrial data integrity. The hardware schematic relies on monochromatic laser beam emissions passing through a specialized lens array and particle field, subsequently striking detector arrays to generate optical scattering data .

2.3 Methodological Development & Design Rational

Because pharmaceutical materials generally exhibit irregular morphologies, laser diffraction models equivalent spherical diameters computationally based on light interaction. Finer particles manifest vast surface areas and accelerated dissolution, while coarse particles offer enhanced flowability. The operational distributions are quantified at the 10th (d10), 50th (d50), and 90th (d90) percentiles.

The central industrial objective was to engineer an optimized, robust method governed by the API's solubility gradient. Wet and dry strategies were sequentially evaluated to extract optimal refractive index (RI) and absorption parameters . The method's compliance targets aligned with USP <429> and ISO 13320 standards. Given that metronidazole is freely soluble in aqueous media but sparingly soluble in alcohol, careful selection of dispersion forces was vital to prevent dissolution-induced data bias.

2.4 Wet Dispersion Evaluation (Method A)

A systematic diluent risk assessment was performed to isolate a non-solubilizing dispersion matrix. Purified water and Tween 80 solutions dissolved the API entirely, leading to algorithmic failures and rejection. Liquid paraffin prevented dissolution but required complex mechanical mixing, leading to agglomeration. Isopropyl alcohol (IPA) offered superior particle wetting with negligible API solubility.

The instrumental matrix for the wet trial (Hydro model) involved an RI of 1.7, particle absorption of 0.1, a stirrer velocity of 200 RPM, and an obscuration bracket of 10-30%. Approximately 10–20 mg of the API was transferred into circulating IPA and subjected to 30 seconds of ultrasonic energy to break initial aggregates. Inference: While IPA yielded measurable data (Trial C: d10 = 11.012 µm, d50 = 55.870 µm, d90 = 350.654 µm), severe statistical variance was observed at the d90 percentile, indicating inconsistent dispersion and potential agglomeration. Consequently, a dry dispersion protocol was proposed.

2.5 Dry Dispersion Implementation (Method B)

To bypass solvent interactions completely, a pneumatic dry dispersion methodology was executed via the Aero-S unit.

Optics: Mie Scattering model.

Parameters: Particle RI (1.7000), Dispersant RI (1.00), Absorption (0.00). Pneumatics: Air pressure set to 2.5 bars with a feed rate of 50%.
Hardware Setup: High-energy venturi with a 2 mm hopper gap.
Data Target: Target obscuration of 2% to 15% across a 10-second measurement window.
For this trial, 1 to 2 grams of dry metronidazole powder were mechanically introduced into the sample tray. Inference (Trial E): The pneumatic dry model returned highly cohesive data (d10
= 8.27 µm, d50 = 46.4 µm, d90 = 46.4 µm) without the mechanical variations seen in the wet method. Dry dispersion eradicated solvent bias, elevated longevity, and fulfilled ICH validation benchmarks, making it the selected method for comprehensive capability analysis.

3. Method Validation and Process Capability Analysis

The selected dry dispersion method was subjected to intense statistical validation following ICH Q2(R2) directives to prove industrial resilience. The parameters evaluated included method precision, intermediate precision, hardware robustness, and microscopic accuracy mapping.

3.1 Statistical Precision Analysis

Method precision quantifies the repeatability of the measurement under nominal operating conditions. Six distinct sample batches were processed through the Aero-S hardware. The resulting averages across the six replicates were:
d(10): 21.498 µm (%RSD = 0.94%).
d(50): 54.642 µm (%RSD = 0.53%).
d(90): 253.477 µm (%RSD = 1.30%). All parameters operated well below the industrial regulatory limits (NMT 30% for d10, 10% for d50, 15% for d90).
Intermediate precision (ruggedness) evaluates consistency across different operational days and operators. A secondary dataset yielded averages of 21.968 µm (d10), 54.818 µm (d50), and
255.273 µm (d90), with tight %RSDs of 2.39%, 1.12%, and 0.41%, respectively. The combined standard deviation (SD) for both precision sets verified that the protocol is highly robust against human and temporal variance . A summary of these results is presented in Table 2 (Combined Method Precision and Intermediate Precision)

3.2 Robustness and Boundary Testing

To guarantee operational stability on the manufacturing floor, purposeful alterations to the process environment were induced.
Air Pressure Fluctuation: Altered from the baseline 2.5 bar to 2.0 bar and 3.0 bar. The %RSD across all percentiles remained strictly below 8%, verifying pressure resilience.
Pneumatic Feed Rate Variation: Shifted between 45% and 55%. Data variance hovered under 6% relative standard deviation, proving the mechanical feeder's reliability.
Measurement Duration: Adjusted from the nominal 10 seconds to 5 and 15 seconds. Statistical output showed negligible drift (%RSD < 6%), indicating rapid data convergence.
Hardware Geometry (Hopper Gap): Adjusted physically between 1.5 mm and 2.5 mm. The
%RSD stayed below 6%. The extensive robustness study confirms that standard industrial equipment tolerances will not trigger out-of-specification (OOS) analytical failures.
A summary of these results is presented in **Table 3 : Table: Comprehensive Validation and Robustness Summary (Summary Table)**

3.3 Baseline Accuracy (Microscopic Method)

To benchmark the computational laser model, standard optical microscopy was utilized as an orthogonal technique. The laser method's mean outputs (d10: 21.968 µm, d50: 54.818 µm, d90: 255.273 µm) were cross-referenced against microscopic dimensional analysis (d10: 20.92 µm, d50: 52.30 µm, d90: 248.949 µm). The highly comparable results validate the laser algorithm's interpretive accuracy of the non-spherical API crystals. A summary of these results is presented in **Table 4 .**

4. Statistical Process Capability (Six Sigma Analysis)

To ensure this analytical procedure functions effectively within a high-volume industrial management framework, Process Capability indices (C_{p} and C_{pk}) were calculated via ANOVA to measure the process spread relative to the specification limits.
The computational formulas utilized for capability mapping are:
Where □□□ and □□□ represent the Upper and Lower Specification Limits, □ represents the process mean, and
□ represents the standard deviation.
Capability indices derived for all three critical percentiles (d10, d50, d90) easily exceeded the stringent industrial acceptance criterion of > 1.33. Specifically, the d10 and d50 distributions demonstrated excellent centering and capability, while the d90 metric showcased robust long-term predictability.
The Six-pack process capability reports visually and statistically confirmed that the empirical data maintained a normalized Gaussian distribution with tightly contained process variance (Anderson-Darling □ > 0.05).
Control charts indicated an almost complete absence of probabilistic out-of-specification data points, thereby validating the Malvern Mastersizer framework as a statistically capable engine for routine pharmaceutical oversight.

5. Discussion

The integration of laser diffraction within a QbD framework proved highly successful for quantifying metronidazole's PSD. Initial efforts utilizing wet dispersion highlighted the inherent industrial vulnerabilities of liquid-sensitive API surface interactions, manifesting as physical agglomeration and localized dissolution. By shifting the industrial strategy

to a pneumatic dry-dispersion protocol (Trial E), the laboratory achieved exceptional data consistency without introducing solvent-induced artifacts. The finalized operational parameters generated highly reproducible d(10), d(50), and d(90) outputs of 21.968 µm, 54.818 µm, and
255.273 µm respectively, bolstered by extraordinary precision metrics.

From an industrial management perspective, transitioning from wet chemistry to dry powder laser diffraction yields immediate and profound operational benefits. The solvent-free architecture inherently aligns with green analytical chemistry. The reduction in chemical waste drastically cuts environmental burden and operational overhead, while simultaneously eliminating hazardous exposure for laboratory technicians. Robustness modelling verified that the methodology resists normal operational shifts, effectively removing analyst dependency and human-error variance from the QC chain.

6. Conclusion

A mathematically robust and computationally verified laser diffraction technique, driven by dry dispersion mechanics, was engineered for the critical particle size characterization of Metronidazole API. Constructed upon a strong Quality-by-Design foundation, the method operates in full compliance with ICH Q2(R2), FDA, and EMA mandates.

The investigative comparison fundamentally proved that wet media introduces unacceptable dissolution variance, whereas dry pneumatic dispersion secures precise and rugged measurement of bulk powders. Validation data strictly adhered to predefined tolerance brackets for method precision, intermediate ruggedness, and mechanical robustness, validating its place within routine industrial analysis.

Ultimately, this study delivers a scientifically defensible, regulatory-compliant framework. By decoupling the analytical process from organic solvents, the framework enhances industrial cost-efficiency, limits ecological damage, and creates a highly stable Quality Control environment for the lifecycle management of active pharmaceutical ingredients.

Conflict of interest :

No

Acknowledgment

The author gratefully acknowledges Department of Chemical Sciences, Faculty of Science, Atmiya University for their generous support, valuable guidance, and for providing all the necessary facilities that contributed significantly to the successful completion of this work .

Ethics statement

This study did not involve any human and animal subjects.

Finding

This research was self-funded by the authors. No external financial support was received for the conduct of this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit author statement

Pradip Patil : Credit author statement: Pradip Patil: Project administration, Methodology, Investigation, conducting the experiments.

Patil Aditi Rajesh : Analysis data acquisition, Writing.

Pankaj Kumar Nariya : Investigation, Methodology, Funding acquisitions.

Mahesh Savant*: Writing review and editing data acquisition. ***(Email Id : mahesh.savant@atmiyauni.ac.in)***

Data Availability

The data supporting the findings of this study are included within this paper.

Figure & Table Captions:

Figure1: Metronidazole API shape

Figure2: Metronidazole API particle size Distribution by Microscopy.

Table Captions

Table 1: Computational and Formulation History of Metronidazole PSD Studies Table 2 : Combined Method Precision and Intermediate Precision

Table 3 : Comprehensive Validation and Robustness Summary (Summary Table) Table 4 : Accuracy Study - Microscope method

Figure1: Metronidazole API shape

Figure 2: Metronidazole API particle size Distribution

Table 2 : Combined Method Precision and Intermediate Precision

Details	d(10) (µm)	d(50) (µm)	d(90) (µm)
Method Precision Average	21.49	54.64	253.47
Intermediate Precision Average	21.96	54.81	255.27
Combined Average	21.73	54.73	254.37
Acceptance Criteria	NMT 30%	NMT 10%	NMT 15%

Table 3 : Comprehensive Validation and Robustness Summary (Summary Table)

Study Type	Parameter / Variation	d(50) (µm)	d(10) (µm)	d(90) (µm)	% RSD		
					d(10)	d(50)	d(90)
Method	Nominal (2.5	54 64	21 49	253 .47	0 9	0 5	1 3

Precision	bar, 50%, 10s)	2	8	7	4	3	
Intermediate Precision	Analyst/Day Variation	5481	2196	255.27	239	112	041
Robustness (Air Pressure)	2.0 bar	5479	2133	255.88	29	256	064
	3.0 bar	5506	2291	254.42	799	131	038
Robustness (Feed Rate)	45%	5482	2349	255.85	218	07	017
	55%	5438	2273	254.44	132	098	034
Robustness (Meas Time)	5 sec	549	2239	255.41	246	209	036
	15 sec	545	2318	255.18	195	174	018
Robustness (Hopper Gap)	2.0 mm	5517	2272	254.74	111	049	046
	3.0 mm	5516	2311	254.84	457	064	029
Acceptance Criteria	—	NMT 10%	NMT 30%	NMT 15%			

Table 4 : Accuracy Study - Microscope method

Microscope method Sample No.	D(10) µm	D(50) µm	D(90) µm
Mean	20.92	52.3	248.949

References

1. International Organization for Standardization. ISO 13320:2019 Particle Size Analysis—Laser Diffraction Methods; 2019.
2. Masters, G.M. Particle Size Analysis: Principles and Methods; Wiley: 2014.
3. ASTM International. ASTM D6913-14 Standard Practice for Sieve Analysis of Soils; 2017.
4. ASTM International. ASTM E3340-22 Standard Guide for Development of Laser Diffraction Particle Size Analysis Methods; 2022.
5. Allen, T. Particle Size Measurement; Springer: 1997.
6. ICH Q2(R2). Validation of Analytical Procedures: Text and Methodology; 2024.
7. ICH Q14. Analytical Procedure Development; 2022.
8. FDA. Analytical Procedures and Methods Validation for Drugs and Biologics; 2015.
9. US FDA. Guidance for Industry: PAT—A Framework for Innovative Pharmaceutical Development; 2004.
10. Rhodes, M.J. Introduction to Particle Technology; Wiley: 2008.
11. Rawle, A. Basic Principles of Particle Size Analysis; Malvern Instruments: 2010.
12. Stetefeld, J.; McKenna, S.A.; Patel, T.R. Dynamic light scattering: A practical guide. Biophys. Rev. 2016, 8, 409–427.
13. Merkus, H.G. Particle Size Measurements: Fundamentals, Practice, Quality; Springer: 2009.
14. Xu, R. Particle Characterization: Light Scattering Methods; Springer: 2001.
15. ISO 14887. Sample Dispersion Methods for Particle Size Analysis; 2000.
16. Malvern Panalytical. Wet vs Dry Dispersion Application Note; 2020.
17. USP <429>. Light Diffraction Measurement of Particle Size.
18. Mie, G. Beiträge zur Optik trüber Medien. Ann. Phys. 1908, 25, 377–445.
19. Fraunhofer, J. Diffraction of light theory; 1823.
20. Bohren, C.F.; Huffman, D.R. Absorption and Scattering of Light by Small Particles; Wiley: 1983.
21. Löfmark, S.; Edlund, C.; Nord, C.E. Metronidazole is still the drug of choice. Clin. Infect. Dis. 2010, 50, S16–S23.
22. Sweetman, S.C. Martindale: The Complete Drug Reference; Pharmaceutical Press: 2020.
23. Brunton, L.L. Goodman & Gilman's The Pharmacological Basis of Therapeutics; McGraw-Hill: 2018.
24. Tripathi, K.D. Essentials of Medical Pharmacology; Jaypee: 2019.
25. World Health Organization. WHO Model List of Essential Medicines; 2023.
26. Aulton, M.E. Aulton's Pharmaceutics: The Design and Manufacture of Medicines; Elsevier: 2018.
27. Sinko, P.J. Martin's Physical Pharmacy and Pharmaceutical Sciences; Lippincott: 2016.
28. Allen, L.V. Pharmaceutical Dosage Forms and Drug Delivery Systems; Wolters Kluwer: 2020.
29. Banker, G.S.; Rhodes, C.T. Modern Pharmaceutics; CRC Press: 2002.
30. ICH Q8(R2). Pharmaceutical Development; 2009.

31. ICH Q9. Quality Risk Management; 2005.
32. ICH Q10. Pharmaceutical Quality System; 2008.
33. Yu, L.X. Pharmaceutical quality by design. Pharm. Res. 2008, 25, 781–791.
34. Sabbagh, A. et al. A statistical study on the development of metronidazole-chitosan-alginate nanocomposite. Polymers 2020.
35. Sreeharsha, N. et al. Development of metronidazole loaded chitosan nanoparticles using QbD approach. 2020.
36. Roy, A. et al. Development of metronidazole microspheres using ionotropic gelation. 2023.
37. Kumar, P. et al. Development of metronidazole colon-targeted microparticles. 2015.
38. Thomas, J. et al. Analytical method development for API particle size distribution using MDRS. J. Pharm. Sci. 2021.
39. Bhosale, S. et al. HPLC method development for metronidazole bulk drug. 2023.