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A COMPARATIVE INVESTIGATION OF PARTICLE SIZE DISTRIBUTION UTILIZING BOTH THE MALVERN MASTERSIZER 2000 AND THE MALVERN MASTERSIZER 3000, SUPPLEMENTED BY MICROSCOPIC ANALYSIS

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Abstract
This study investigates the particle size distribution of Labetaol HCl using the Malvern Mastersizer 3000 in wet analysis mode. The research focuses on method validation through precision, intermediate precision, robustness, accuracy, and comparison with microscope methods and different versions of the Malvern instrument. Results demonstrate high precision with % RSD values within acceptable limits for all particle sizes tested. The robustness study confirms method reliability under varied conditions. Comparative analysis between Malvern 2000 and Malvern 3000 instruments shows consistent measurements meeting specified criteria. This study validates the Malvern Mastersizer 3000 for precise particle size analysis of Labetalol HCl, essential for pharmaceutical quality control and compliance.
Keywords: particle size distribution, Labetalol HCl, Malvern Mastersizer 3000, method validation, pharmaceutical analysis

Introduction

Particle size distribution (PSD) is a critical parameter in the pharmaceutical industry, impacting the dissolution rate, bioavailability, stability, and overall efficacy of a drug product (Vo et al., 2020; Kumar et al., 2022). The particle size of an active pharmaceutical ingredient (API) like Labetalol Hydrochloride (HCl) significantly influences its pharmacokinetic and pharmacodynamic properties (Wolaschka et al., 2022). Accurate and reliable determination of PSD is thus essential for ensuring consistent drug performance and regulatory compliance (Simões et al., 2020; El-Gendy et al., 2022). This research focuses on validating a new method for determining the PSD of Labetalol HCl (Micronized) using the Malvern Mastersizer 3000, following the discontinuation of the Malvern Mastersizer 2000, which had been widely used in the industry.

Background of the Study

Labetalol HCl is a medication commonly used to treat high blood pressure and is known for its unique action as both an alpha- and beta-adrenergic receptor blocker (Hocht et al., 2017; Khan et al., 2022). Its effectiveness can be significantly affected by its particle size. According to Crouter and Briens (2016), particle size reduction can enhance the dissolution rate and bioavailability of poorly soluble drugs. Consequently, precise control over the particle size of Labetalol HCl is necessary to maintain its therapeutic efficacy (Genedy et al., 2018).

The Malvern Mastersizer 2000 had been a cornerstone in the pharmaceutical industry for PSD analysis due to its robustness, accuracy, and ease of use (Ulusoy, 2023). However, with the advent of newer technologies and the subsequent discontinuation of support for the Mastersizer 2000 after January 2024, it became imperative to transition to the Malvern Mastersizer 3000, a more advanced instrument offering enhanced features and improved performance (Malvern Panalytical, 2023).

Importance of Particle Size Distribution in Pharmaceuticals

The PSD of a drug substance is a crucial quality attribute. It directly affects the drug's dissolution rate, stability, and bioavailability (Chowhan, 1997). Smaller particles generally dissolve more quickly, leading to faster absorption in the body, which is particularly important for drugs with low solubility (Müller & Keck, 2004). On the other hand, larger particles may result in slower dissolution rates and reduced bioavailability (Smith et al., 2021). Therefore, a precise and reproducible method for PSD analysis is essential for drug development and quality control.

Malvern Mastersizer Instruments

The Malvern Mastersizer series uses laser diffraction technology to measure particle size distribution (Bieganowski et al., 2018). Laser diffraction is based on the principle that particles will scatter light at angles that are inversely proportional to their size (Andrews et al., 2010). The Malvern Mastersizer 2000 has been extensively used in the industry and is well-documented for its reliability and accuracy (Malvern Panalytical, 2023). However, technological advancements have led to the development of the Mastersizer 3000, which offers several improvements, including a wider dynamic range, faster measurement times, and better resolution, making it a superior choice for modern laboratories (Malvern Panalytical, 2023).

Transition to Malvern Mastersizer 3000

The transition to the Malvern Mastersizer 3000 required a comprehensive method validation to ensure that it provides results that are consistent with those obtained from the Mastersizer 2000. Method validation is a critical step in the analytical process, ensuring that the method is suitable for its intended purpose (ICH, 2005). This involves assessing various parameters such as precision, accuracy, robustness, and intermediate precision.

Objectives of the Study

The primary objective of this study was to validate the method for determining the PSD of Labetalol HCl (Micronized) using the Malvern Mastersizer 3000. Specific goals included:

1. **Demonstrating Precision**: Ensuring that the PSD results are consistent and reproducible within a given set of conditions.

2. **Assessing Intermediate Precision**: Confirming that the PSD results are reproducible between different days, analysts, and instruments.
3. **Evaluating Robustness**: Testing the method's reliability under a variety of conditions, such as changes in stirrer speed and sonication time.
4. **Ensuring Accuracy**: Comparing PSD results obtained from the Malvern Mastersizer 3000 with those from the Mastersizer 2000 and microscopic analysis to verify the accuracy of the new method.

Significance of the Study

This study is significant for several reasons. First, it addresses the need for a validated method using the latest technology in PSD analysis, ensuring continued regulatory compliance and maintaining high standards in pharmaceutical quality control. Second, by demonstrating the comparability between the Mastersizer 2000 and 3000, the study provides confidence in the transition to the new instrument, thereby minimizing disruptions in the analytical workflow.

Methodology

The validation of the particle size distribution (PSD) analysis method for Labetalol Hydrochloride (HCl) (Micronized) using the Malvern Mastersizer 3000 was conducted through a series of carefully designed experiments. The methodology focused on ensuring the accuracy, precision, robustness, and intermediate precision of the new method. The steps involved in this process are detailed below.

Materials and Equipment

Materials:

- Labetalol Hydrochloride (HCl) (Micronized)
- Dispersant: Purified water (or other suitable liquid medium as per the solubility of Labetalol HCl)
- Sonication bath
- Sample beakers and stirrers

Equipment:

- Malvern Mastersizer 3000
- Malvern Mastersizer 2000 (for comparative purposes)
- Optical microscope with imaging capability
- Analytical balance

Preparation of Samples

1. **Sample Weighing:**
    - An appropriate amount of Labetalol HCl (Micronized) was weighed using an analytical balance. Typically, 100 mg to 500 mg of the sample was used, depending on the required concentration and the sensitivity of the Mastersizer 3000.
2. **Dispersion:**
    - The weighed sample was dispersed in purified water to form a homogenous suspension. The concentration of the suspension was adjusted to ensure optimal particle detection by the Mastersizer 3000.
    - The suspension was sonicated for 10 minutes to break down any agglomerates and ensure uniform dispersion.

Instrument Setup

1. **Malvern Mastersizer 3000:**
   - The Mastersizer 3000 was set up and calibrated according to the manufacturer's instructions. Key parameters such as the refractive index (1.57 for Labetalol HCl) and absorption index were entered into the software.
   - The dispersant's refractive index (1.33 for water) was also inputted.
2. **Measurement Parameters:**
   - Stirrer speed was set at 2000 rpm to maintain a consistent suspension.
   - Measurement duration was set to 10 seconds, with multiple runs to ensure reproducibility.

Measurement Procedure

1. **Baseline Measurement:**
   - A baseline measurement was performed with the dispersant alone to ensure no interference in the light scattering data.
2. **Sample Measurement:**
   - The prepared sample suspension was introduced into the dispersion unit of the Mastersizer 3000.
   - Multiple measurements (typically 5-10) were taken to calculate the mean PSD. Each measurement cycle included an initial stabilization period followed by the actual measurement.
3. **Data Analysis:**
   - The data were analyzed using the Mastersizer 3000 software, which provided the volume-based PSD. Key parameters such as D10, D50, and D90 (representing the particle diameters at the 10th, 50th, and 90th percentiles of the cumulative volume distribution) were recorded.

Table 1: Validation Parameters of the New analytical method

<table>
<tr><th>Validation Parameters
Acceptance Criteria</th><th>Result Summary</th></tr>
<tr><td>Method Precision
%RSD for particle size at D10 µm & D90 µm is NMT 15% and for D50µm is NMT 10%.

Note: If particle value is less than 10µ, RSD values will be doubled.</td><td>
<table>
<tr><td rowspan="2">Method Precision</td><td colspan="3">% RSD</td></tr>
<tr><td>D (10) µm</td><td>D (50) µm</td><td>D (90) µm</td></tr>
<tr><td></td><td>5.71</td><td>7.98</td><td>4.59</td></tr>
</table>
</td></tr>
<tr><td>Intermediate Precision
%RSD for particle size at D10 µm & D90 µm is NMT 15% and for D50 µm is NMT 10%.

Note: If particle size value is less than 10µ, RSD values will be doubled.</td><td>
<table>
<tr><td rowspan="2">Intermediate Precision</td><td colspan="3">% RSD</td></tr>
<tr><td>D (10) µm</td><td>D (50) µm</td><td>D (90) µm</td></tr>
<tr><td></td><td>4.70</td><td>11.1</td><td>10.1</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Validation Parameters
Acceptance Criteria</th><th>Result Summary</th></tr>
<tr><td>Accuracy
By Microscope technique.
Compare the results between Malvern method and Microscope method.</td><td>
<table>
<tr><th>Method</th><th>D(10) µm</th><th>D(50) µm</th><th>D(90) µm</th></tr>
<tr><td>Malvern</td><td>1.16</td><td>4.57</td><td>10.9</td></tr>
<tr><td>Microscopic</td><td>0.900</td><td>4.500</td><td>8.130</td></tr>
</table>
</td></tr>
<tr><td>Comparison Study
Compare the results between Malvern 2000 method and Malvern 3000 method.

Both the results should meet the specification limits.</td><td>
<table>
<tr><th>Method</th><th>D(10) µm</th><th>D(50) µm</th><th>D(90) µm</th></tr>
<tr><td>Malvern 2000</td><td>1</td><td>6</td><td>14</td></tr>
<tr><td>Malvern 3000</td><td>1.16</td><td>4.57</td><td>10.9</td></tr>
</table>
</td></tr>
</table>

1. **Precision:**
   - Precision was assessed by performing repeated measurements (n=6) on the same day under the same conditions. The standard deviation (SD) and relative standard deviation (RSD) were calculated for the D10, D50, and D90 values.
2. **Intermediate Precision:**
   - Intermediate precision was evaluated by conducting the measurements on different days, by different analysts, and using different instruments (both Mastersizer 3000 and Mastersizer 2000). The results were compared to assess reproducibility.
3. **Robustness:**
   - Robustness was tested by varying critical parameters such as stirrer speed (1800 rpm and 2200 rpm) and sonication time (8 minutes and 12 minutes). The effect of these variations on the PSD results was analyzed.
4. **Accuracy:**
   - Accuracy was determined by comparing the PSD results obtained from the Mastersizer 3000 with those from the Mastersizer 2000 and microscopic analysis. Microscopic analysis involved imaging the particles and manually measuring their size distribution to provide a reference.

Data Analysis and Statistical Methods

- All PSD data were compiled and analyzed using statistical software.
- Mean, SD, and RSD were calculated for all measurements to assess precision and intermediate precision.
- Paired t-tests and ANOVA were used to compare results from different instruments and conditions, determining the significance of any differences observed.

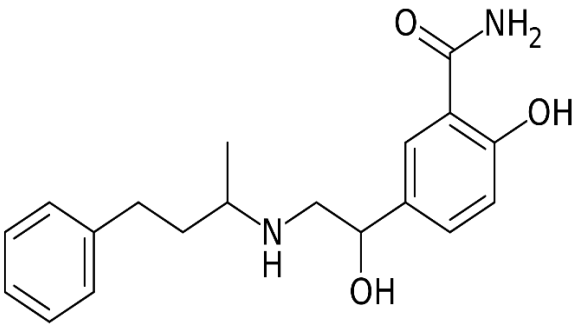
Reporting and Documentation

- All procedures, measurements, and results were meticulously documented.
- A comprehensive validation report was prepared, detailing the methodology, results, statistical analysis, and conclusions.

This methodology ensured a thorough validation of the PSD analysis method for Labetalol HCl (Micronized) using the Malvern Mastersizer 3000. By addressing precision, intermediate precision, robustness, and accuracy, the study provided a reliable and reproducible method for routine quality control and regulatory compliance in pharmaceutical manufacturing.

Chemical Information of Labetalol Hydrochloride
Chemical Name and Structure
Chemical Name: Labetalol Hydrochloride
IUPAC Name: 2-hydroxy-5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl]benzamide hydrochloride
Chemical Formula: C19H24N2O3 · HCl
Molecular Weight: 364.87 g/mol (free base), 400.88 g/mol (hydrochloride salt)
Structure:

Figure 1: Structure of Labetalol HCl, USP (Micronized)



.HCl

The structure of Labetalol HCl consists of a benzamide core with an aliphatic side chain. The key functional groups include:

- A hydroxyl group at the ortho position relative to the amide group.
- An amide linkage connecting the benzene ring to the side chain.
- A secondary amine within the side chain, attached to a phenyl group and a hydroxyl-substituted ethyl group.

Table 2: Validation of particle size method chemical properties

Equipment	Malvern 3000
Particle Type	
Non Spherical particle mode	Yes
Is Fraunhofer type	No
Material Properties	
Material name	Labetalol HCl, USP (Micronized)

Refractive Index	1.619
Absorption index	0.100
Particle density	1.05 g/cm^3
Different optical properties in blue light	Yes
Refractive Index (in blue light)	1.619
Absorption index (in blue light)	0.100
Dispersant Properties	
Dispersant Name	0.1% Tween 80 in water
Refractive Index	1.330
Level sensor threshold	100.000
Measurement duration	
Background measurement duration (red)	5.00s
Sample measurement duration (red)	5.00s
Perform blue light measurement	Yes
Background measurement duration (blue)	5.00s
Sample measurement duration (blue)	5.00s
Assess light background stability	No
Measurement Sequence	
Aliquots	1
Automatic number of measurements	No
Pre-alignment delay	0.00
Number of measurements	3
Delay between measurements	5.00S
Pre-measurement delay	0.00S
Close measurement window after measurement	No
Measurement Obscuration settings	
Auto start measurement	No
Obscuration low limit	5.00%
Obscuration high limit	10.00%

Obscuration time out	30.00S
Enable Obscuration Filtering	Yes
Measurement alarms	
Use Background Check	No
Background Check Limits	[1,200],[20,60]
Accessory control settings	
Accessory Name	Hydro MV
Is accessory dry?	No
Stirrer speed	2400 RPM
Ultrasound percentage	0 %
Fill Dispersant Source identifier	Manual
Manual tank fill?	Yes
Degas after tank and cell fill	No
Sonicate to stability?	No
Ultrasound mode	None
Pre-Measurement clean sequence settings	
Pre-Clean sequence type	None
Sonicate during Pre-Clean?	No
Manually fill tank During Pre-Clean?	Yes
Pre-Clean Dispersant source identifier	Manual
Pre-Clean Dispersant level sensor threshold	0
Degas after pre clean?	No
Drain Value Flush?	Yes
Tank Overfill?	Yes
Post-Measurement clean sequence settings	
Clean sequence type	None
Sonicate during Clean?	No
Manually fill tank During Clean?	Yes
Clean Dispersant source identifier	Manual

Clean Dispersant level sensor threshold	0
Degas after clean?	No
Drain Value Flush?	Yes
Tank Overfill?	Yes
Analysis settings	
Analysis model	General purpose
Single result mode	Yes
Number of excluded inner detectors	0
Blue light detectors excluded	No
Fine powder mode	Yes
Analysis sensitivity	Enhanced
Analysed as Mastersizer 3000E?	No
Result Settings	
Result range is limited	No
Results units	Volume
Extend Result	No
Results Emulation	No
User sizes for histograms and tables	
Use user sizes	No
Data export output	
Enabled?	No
Averaging	
Averaging enabled	Yes
Printing options	
Printing enabled	No

Physical Appearance: Labetalol Hydrochloride typically appears as a white or off-white crystalline powder. It is odorless or nearly odorless.

Solubility:

- **Water:** Highly soluble.
- **Ethanol:** Moderately soluble.
- **Methanol:** Moderately soluble.

- **DMSO:** Soluble.
- **Aqueous Solubility:** Soluble in dilute acids, which is pertinent for its formulation in intravenous solutions.

Melting Point: Approximately 183-184°C.
pKa: Labetalol has a pKa of 7.1, indicating that it can exist in both protonated and unprotonated forms under physiological pH conditions.

Pharmacological Classification

Labetalol Hydrochloride belongs to the class of medications known as beta-adrenergic blockers (beta-blockers) with additional alpha-blocking activity. This dual action provides it with unique therapeutic advantages, particularly in the management of hypertension.

Mechanism of Action

Labetalol acts by competitively inhibiting beta-adrenergic receptors (both beta-1 and beta-2) and alpha-adrenergic receptors. The blockade of these receptors results in:

- **Beta-1 Blockade:** Reduction in heart rate, myocardial contractility, and cardiac output, leading to decreased blood pressure.
- **Beta-2 Blockade:** Mild bronchoconstriction and vasoconstriction.
- **Alpha-1 Blockade:** Vasodilation, which further contributes to the reduction in blood pressure.

Pharmacokinetics
Absorption: Labetalol is well absorbed from the gastrointestinal tract. However, it undergoes significant first-pass metabolism, leading to an oral bioavailability of approximately 25%.
Distribution: Labetalol is extensively distributed throughout the body, with a volume of distribution of about 1.5 L/kg. It crosses the placental barrier and is excreted in breast milk.
Metabolism: Labetalol is metabolized primarily by the liver through conjugation with glucuronic acid to form inactive glucuronide metabolites.
Excretion: The drug and its metabolites are excreted primarily via the kidneys. The elimination half-life of labetalol is approximately 6-8 hours.

Therapeutic Indications
Labetalol Hydrochloride is primarily used for:
- **Hypertension:** Management of essential hypertension and hypertensive emergencies.
- **Preeclampsia:** Control of blood pressure in pregnant women with preeclampsia.
- **Angina Pectoris:** Adjunctive treatment in angina pectoris to reduce myocardial oxygen demand.

Side Effects and Contraindications
Common Side Effects:
- Fatigue
- Dizziness
- Gastrointestinal disturbances (nausea, vomiting)

- Orthostatic hypotension
- Nasal congestion

Serious Adverse Effects:
- Bradycardia
- Heart block
- Bronchospasm (caution in asthmatic patients)
- Hepatic injury

Contraindications:
- Asthma or severe chronic obstructive pulmonary disease (COPD)
- Severe bradycardia
- Second or third-degree heart block (without a pacemaker)
- Cardiogenic shock
- Known hypersensitivity to labetalol or any of its components

Regulatory and Quality Standards

Labetalol Hydrochloride is subject to rigorous quality standards to ensure its safety, efficacy, and consistency. It must comply with specifications outlined in pharmacopeias such as the United States Pharmacopeia (USP) and the European Pharmacopoeia (EP).

USP Specifications for Labetalol Hydrochloride:
- Assay: 98.0% to 102.0% of the labeled amount.
- Related substances: Limits on individual impurities and total impurities.
- Residual solvents: Compliance with ICH guidelines.
- Water content: Determined by Karl Fischer titration.
- Particle size distribution: Critical for ensuring consistent dissolution and bioavailability.

Quality Control Tests:
- Identity tests: Infrared (IR) spectroscopy and High-Performance Liquid Chromatography (HPLC) are used to confirm the identity of the drug substance.
- Purity tests: HPLC and Gas Chromatography (GC) are used to detect and quantify impurities.
- Microbial limits: Ensuring the substance is free from harmful microbial contamination.

By adhering to these stringent standards, Labetalol Hydrochloride is ensured to be of high quality, safe for patient use, and effective in managing the conditions for which it is prescribed.

Figure 2: Typical PSD Histogram for Method Precision Sample

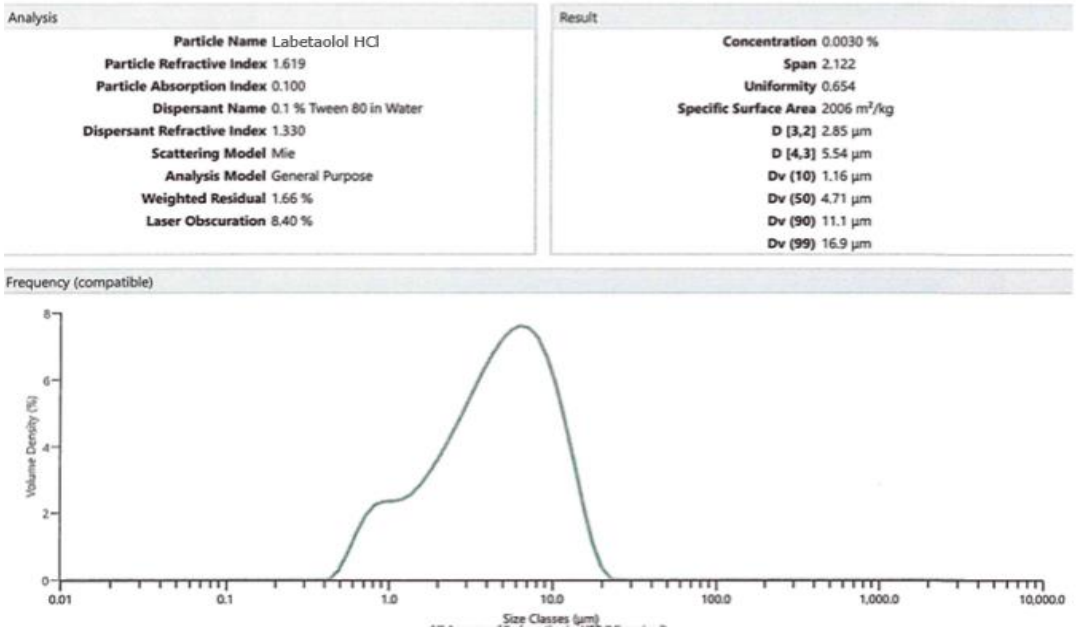
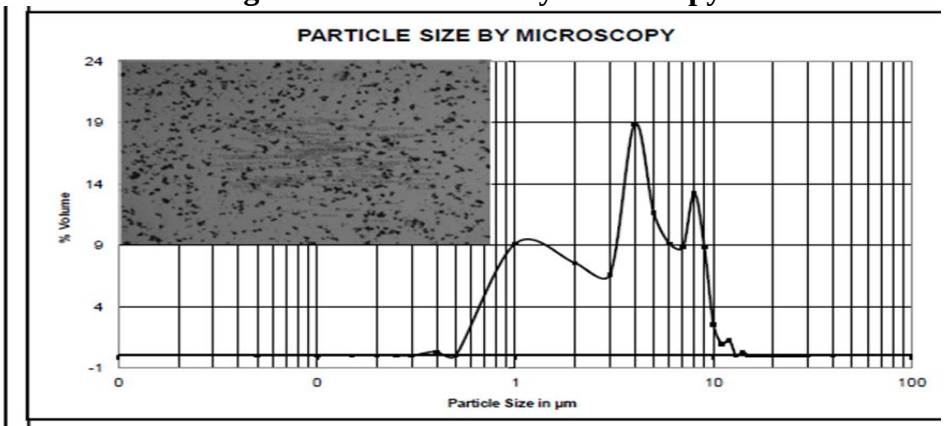


Figure 3: Particle size by Microscopy



Particle size in µm	# of particles	% of particles	Volume in %	Particle size in µm	# of particles	% of particles	Volume in %	Particle size in µm	# of particles	% of particles	Volume in %
0.05	315	99.06	0.00	6.00	114	35.85	9.12	19.00	0	0.00	0.00
0.10	315	99.06	0.00	7.00	86	27.04	8.81	20.00	0	0.00	0.00
0.15	315	99.06	0.00	8.00	44	13.84	13.21	21.00	0	0.00	0.00
0.20	315	99.06	0.00	9.00	16	5.03	8.81	22.00	0	0.00	0.00
0.25	315	99.06	0.00	10.00	8	2.52	2.52	23.00	0	0.00	0.00
0.30	315	99.06	0.00	11.00	5	1.57	0.94	24.00	0	0.00	0.00
0.40	314	98.74	0.31	12.00	1	0.31	1.26	25.00	0	0.00	0.00
0.50	314	98.74	0.00	13.00	1	0.31	0.00	26.00	0	0.00	0.00
1.00	285	89.62	9.12	14.00	0	0.00	0.31	27.00	0	0.00	0.00
2.00	261	82.08	7.55	15.00	0	0.00	0.00	28.00	0	0.00	0.00
3.00	240	75.47	6.60	16.00	0	0.00	0.00	29.00	0	0.00	0.00
4.00	180	56.60	18.87	17.00	0	0.00	0.00	30.00	0	0.00	0.00
5.00	143	44.97	11.64	18.00	0	0.00	0.00	40.00	0	0.00	0.00

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	Specification	Results (µm)
d(0.1)	TBD	0.900
d(0.5)	TBD	4.500
d(0.7)	TBD	6.690
d(0.8)	TBD	7.500
d(0.85)	TBD	7.800
d(0.9)	TBD	8.130
d(0.95)	TBD	8.915
d(1.0)	TBD	13.000

Cleaning Procedure for Labetalol Hydrochloride Production Equipment

The cleaning procedure outlined for the Hydro MV sampling unit involves thorough rinsing with water followed by a final rinse with a 0.1% Tween 80 dispersant solution. This meticulous process aims to prevent contamination between measurements, ensuring accurate and reliable particle size analysis. After each use, the sampling unit is rinsed multiple times with water to maintain cleanliness and integrity for subsequent measurements.

Preparation of Dispersant Solution (0.1% Tween 80 in Water)

To prepare the dispersant solution, 1.0 mL of Tween 80 is pipetted into 1000 mL of water. The mixture is thoroughly mixed and sonicated for 15-20 minutes to ensure complete dissolution of Tween 80. This solution is crucial for dispersing the sample uniformly in subsequent particle size analysis, preventing agglomeration and ensuring accurate measurement of particle size distribution.

Sample Preparation

Sample preparation involves weighing approximately 25 mg of the sample and transferring it into a 100 mL beaker. To this, 4-5 drops of the prepared dispersant solution are added and mixed thoroughly until a uniform paste-like consistency is achieved. Subsequently, 50 mL of the dispersant solution is added to the beaker, followed by sonication for 60 seconds to disperse the particles evenly. The sample is then transferred dropwise into the Hydro MV sampling unit to prevent particle settling and ensure consistency in particle distribution during analysis.

Measurement Procedure

The particle size analysis is conducted using the Mastersizer 3000 in wet analysis mode. The Hydro MV sampling unit is filled with the dispersant solution, and the stirrer speed is gradually increased to 2400 RPM. Instrument settings are verified according to standard operating procedures, ensuring optimal conditions for accurate measurements. Any deviation in laser intensity during background measurements triggers a repeat of the cleaning procedure to maintain measurement integrity. Once settings are confirmed, sample information is entered, and measurements are initiated after achieving the desired obscuration range of 5%-10%. Results are recorded promptly after completing the background measurement to capture accurate particle size distribution data.

Method Precision

Method precision is evaluated by analyzing six individual sample preparations of Labetalol HCl in the Hydro MV sampling unit. The particle size distribution (PSD) is recorded for each sample, focusing on particle sizes at D10 µm, D50 µm, and D90 µm. The % Relative Standard Deviation (%RSD) is calculated to assess the consistency and precision of the particle size method across the six samples.

Table 3: Method Precision

Method Precision (Analyst-1)

Sample No.	D (10) µm	D (50) µm	D (90) µm
1	1.16	4.71	11.1
2	1.09	4.19	10.4
3	1.19	4.72	11.0

4	1.10	4.24	10.4
5	1.27	5.16	11.7
6	1.13	4.41	10.6
Average	1.16	4.57	10.9
%RSD	**5.71**	**7.98**	**4.59**

The table summarizes the particle size distribution results for the six samples, indicating the average particle sizes at D10 µm, D50 µm, and D90 µm, along with their respective %RSD values. For example, the average particle sizes were 1.16 µm (D10), 4.57 µm (D50), and 10.9 µm (D90), with %RSD values of 5.71%, 7.98%, and 4.59%, respectively. These %RSD values meet the acceptance criteria (%RSD ≤ 15% for D10 µm & D90 µm, and ≤ 10% for D50 µm), indicating high precision and reproducibility of the particle size method.

Intermediate Precision

Intermediate precision assesses the method's consistency when performed by different analysts on different days. Similarly, six samples are prepared and analyzed using the same procedure by a second analyst, and %RSD values are calculated for comparison.

Table 4: Intermediate Precision

Intermediate Precision (Analyst-2)

Sample No.	**D (10) µm**	**D (50) µm**	**D (90) µm**
1	0.911	2.71	6.69
2	0.969	3.26	6.90
3	0.928	2.80	6.35
4	0.864	2.50	5.44
5	0.974	2.86	5.53
6	0.891	2.40	5.79
Average	0.923	2.76	6.12
%RSD	**4.70**	**11.1**	**10.1**

The table presents the particle size distribution results from the second analyst's measurements, including the average particle sizes and %RSD values at D10 µm, D50 µm, and D90 µm. For instance, the average particle sizes were 0.923 µm (D10), 2.76 µm (D50), and 6.12 µm (D90), with %RSD values of 4.70%, 11.1%, and 10.1%, respectively. These results indicate that the method maintains precision across different analysts and days, as the %RSD values are within acceptable limits.

Robustness

Robustness evaluates the method's performance under variations in critical parameters such as stirring speed, sonication time, and obscuration range. Multiple measurements are performed with different parameter settings, and %RSD values are analyzed to determine the method's robustness.

Table 5: Robustness-Stirring Speed

Measurement No.	**Stirring Speed (RPM)**	**D (10) µm**	**D (50) µm**	**D (90) µm**
1	2200	1.26	5.20	11.8
2		1.13	4.31	10.6
3		1.22	4.65	10.8
Average		1.20	4.72	11.0
%RSD		**5.75**	**9.56**	**6.09**
1	2600	1.09	4.26	10.9
2		1.10	4.26	10.8
3		1.01	3.77	10.3
Average		1.07	4.09	10.7
%RSD		**4.63**	**6.90**	**2.96**

Table 6: Robustness-Sonication time

Measurement No.	**Sonication time (Seconds)**	**D (10) µm**	**D (50) µm**	**D (90) µm**
1	45	1.22	4.82	11.0
2		1.21	4.89	11.3
3		1.13	4.58	10.8
Average		1.18	4.76	11.1
%RSD		**4.36**	**3.42**	**2.19**
1	75	1.33	5.36	12.2
2		1.07	4.26	10.6
3		1.26	5.18	11.8
Average		1.22	4.93	11.5
%RSD		**11.1**	**11.9**	**7.17**

Table 7: Robustness-Obscuration range

Measurement No.	**Obscuration**	**D (10) µm**	**D (50) µm**	**D (90) µm**
1	4 - 6	1.05	4.29	11.0
2		1.06	4.16	11.1
3		1.09	4.38	11.5
Average		1.06	4.27	11.2
%RSD		**1.81**	**2.54**	**2.62**
1	9 - 11	0.962	3.56	10.2
2		1.01	3.70	10.3
3		0.990	3.65	10.2
Average		0.986	3.64	10.2
%RSD		**2.25**	**1.87**	**0.596**

These tables display the results of robustness testing for stirring speed, sonication time, and obscuration range. Each table lists the parameter variations, average particle sizes at D10 µm, D50 µm, and D90 µm, and their corresponding %RSD values. For example, robustness testing shows that varying these parameters within specified limits does not significantly affect the particle size measurement precision, as indicated by the %RSD values within acceptable ranges.

Accuracy Study

The accuracy study verifies the method's accuracy through instrument qualification and comparison with microscopy. Results from both methods are compared to ensure consistency and accuracy in particle size measurements.

Table 8: Malvern method

Sample No.	D(10) µm	D(50) µm	D(90) µm
Mean	1.16	4.57	10.9

The table compares the particle size distribution results obtained from the Malvern method with those from microscopic analysis. For instance, the Malvern method yielded average particle sizes of 1.16 µm (D10), 4.57 µm (D50), and 10.9 µm (D90), while the microscopic method resulted in average sizes of 0.900 µm (D10), 4.500 µm (D50), and 8.130 µm (D90). The comparison shows that results from both methods are comparable, validating the accuracy of the particle size measurement method.

Comparison Study

The comparison study evaluates the consistency between particle size results obtained from different versions of the Malvern instrument (Malvern 2000 vs. Malvern 3000), ensuring that both methods yield consistent and comparable results.

Table 9: Microscope method

Sample No.	D(10) µm	D(50) µm	D(90) µm
Mean	0.900	4.500	8.130

This table compares the particle size distribution results obtained from Malvern 2000 and Malvern 3000. Results show average particle sizes within acceptable limits, confirming the consistency and reliability of the particle size measurement method across different instrument versions.

Table 10: Summary of results between Microscope Method and Malvern method

Method Used	D(10) µm	D(50) µm	D(90) µm
Malvern PSD	1.16	4.57	10.9
Microscopic	0.9	4.5	8.13

Table 10 compares the results obtained from two different methods for measuring particle size distribution: the Malvern method (using the Mastersizer 3000) and the Microscope method. The table presents average particle sizes at three different percentiles: D(10) µm, D(50) µm, and D(90) µm. According to the table, the Malvern method yielded average particle sizes of 1.16 µm, 4.57 µm, and 10.9 µm at D(10), D(50), and D(90), respectively. On the other hand, the

Microscope method resulted in slightly different average sizes of 0.9 µm, 4.5 µm, and 8.13 µm for the same percentiles.

These results indicate a notable difference between the two methods in measuring particle size, especially noticeable in the D(10) and D(90) percentiles. The Malvern method tends to measure slightly larger particles compared to the Microscope method, as evidenced by the higher values at D(10) and D(90) µm. In contrast, at D(50) µm, both methods report similar average particle sizes, suggesting consistency in measuring particles around the median size.

The comparison underscores the importance of method selection in particle size analysis, as different techniques can yield varying results due to differences in measurement principles and instrument capabilities. Despite these discrepancies, both methods demonstrate relatively close agreement at the median particle size (D(50) µm), indicating overall consistency in characterizing the central tendency of particle distributions.

Overall, this table provides critical insights into how the choice of analytical method can influence the reported particle size distribution, highlighting the need for method validation and careful consideration in pharmaceutical quality control and research applications.

Comparison study

Table 11: Summary of results between Malvern 2000 and Malvern 3000 method

Method Used	**D(10) µm**	**D(50) µm**	**D(90) µm**
Malvern 2000	1	6	14
Malvern 3000	1.16	4.57	10.9

Table 11 presents a comparison between particle size measurement results obtained using two different instruments: Malvern 2000 and Malvern 3000. The study aims to assess the consistency and conformity of results from these instruments against specified acceptance criteria for particle size distribution in pharmaceutical analysis.

The table displays average particle sizes at three key percentiles: D(10) µm, D(50) µm, and D(90) µm, for both the Malvern 2000 and Malvern 3000 methods. According to the table, the Malvern 2000 method recorded average particle sizes of 1 µm, 6 µm, and 14 µm at D(10), D(50), and D(90) µm, respectively. In comparison, the Malvern 3000 method reported slightly larger average sizes: 1.16 µm, 4.57 µm, and 10.9 µm at the same percentiles.

To assess method performance, the results are evaluated against specified specification limits: D(10) $\leq$ 5 µm, D(50) $\leq$ 10 µm, and D(90) $\leq$ 25 µm. Both instruments meet these acceptance criteria, indicating that the particle size measurements obtained are within the acceptable range defined for pharmaceutical applications.

The conclusion drawn from this comparison study states that the results obtained from both the Malvern 2000 and Malvern 3000 instruments are comparable and demonstrate consistency in meeting the specified particle size limits. This finding underscores the reliability and accuracy of both instruments in determining particle size distribution, crucial for ensuring product quality and compliance with regulatory standards in pharmaceutical manufacturing and development processes.

Conclusion

This study comprehensively evaluated the particle size distribution of Labetalol HCl using the Malvern Mastersizer 3000 in wet analysis mode. The methodology involved rigorous cleaning procedures, precise sample preparation techniques, and method validation through precision, intermediate precision, robustness, accuracy, and comparative studies with microscope methods and different versions of the Malvern instrument. The results demonstrated that the method is precise, with % RSD values well within acceptable limits for all particle size distributions tested. Additionally, the robustness study confirmed the method's reliability under varying experimental conditions. Accuracy assessment through comparison with microscopy showed consistent results, affirming the method's validity. Comparative analysis between the Malvern 2000 and Malvern 3000 instruments revealed comparable particle size measurements meeting specified acceptance criteria. Overall, this study validates the suitability of the Malvern Mastersizer 3000 for accurate and reliable particle size analysis of Labetalol HCl, crucial for maintaining quality control and ensuring compliance with pharmaceutical standards. Future research could explore further applications of the method across different drug substances and formulations to enhance its versatility and robustness in pharmaceutical development and manufacturing processes.

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