



Research Article

Received: June 08, 2026
Revised: August 18, 2026
Accepted: August 19, 2026

DOI: 10.60101/past.2026.266075

Validated Analytical Approach for Particle Size Distribution Measurement in Acyclovir Oral Suspension Using Laser Diffraction

Nataraj Palaniyappan^{1*}, Vijayakumar Murugesan², Kannan Arumugam² and Ravisankar Mathesan³

¹Scientist, Novitium Pharma LLC, New Jersey, USA

²School of Pharmacy, JSS Academy of Higher Education and Research, Mauritius

³Srinivasan College of Pharmaceutical Sciences, Trichy, Tamil Nadu, India

*Email: palanatraj2020@gmail.com

Abstract

Particle size analysis is crucial for guaranteeing the quality and functionality of oral suspensions since it has a substantial impact on the dissolving profile and bioavailability of medicinal formulations. The current study used a laser diffraction particle size analyser to design and validate a straightforward, accurate, precise, and reliable Analytical technique for figuring out the particle size distribution in a 200 mg/5 mL oral solution of acyclovir. A Malvern Mastersizer 3000 fitted with a Hydro MV dispersion unit was used to examine the method, which was created utilising the wet dispersion approach using water as the dispersant. The developed technique was validated using the International Conference on Harmonisation (ICH) Q2(R1) standards for factors like method precision, intermediate precision, and robustness. Following the assessment of the Dv(10), Dv(50), and Dv(90) particle size distribution parameters, the obtained percentage of Relative Standard Deviation (%RSD) values were determined to be within acceptable bounds, indicating satisfactory repeatability and reproducibility of the approach. There was no discernible effect on the analytical performance in robustness tests conducted with different stirring speeds and obscuration settings. It was discovered that the established approach was robust, dependable, and appropriate for finding the acyclovir oral suspension's particle size distribution on a regular basis. Thus, quality control and formulation development studies of acyclovir oral suspension products can be successfully conducted using the established analytical approach.

Keywords: Acyclovir Oral Suspension, Particle Size Distribution, Laser Diffraction, Method Validation, Malvern Mastersizer 3000, ICH Q2(R1)

1. Introduction

Acyclovir is an antiviral medication that falls under the category of synthetic purine nucleoside analogues. Acyclovir is frequently used to treat viral infections, such as chickenpox, genital herpes, and herpes labialis, which are mostly triggered by the herpes simplex and varicella-zoster virus (1). Acyclovir works by selectively blocking viral DNA polymerase, which stops viruses from replicating and

spreading illness. (2). After oral administration, acyclovir is moderately absorbed and distributed throughout body fluids and tissues (3). Acyclovir is a crystalline powder that ranges in colour from white or almost white. Its molecular mass is 225.2 g/mol, and $C_8H_{11}N_5O_3$ is its empirical formula. It is nearly insoluble in organic solvents like ether and chloroform and only slightly soluble in water (4). 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one is the

chemical name for acyclovir, a synthetic guanine analogue intended to specifically target virus-infected cells (5). By increasing the surface area accessible for dissolution, particle size reduction significantly contributes to improving the dissolution rate and bioavailability of poorly soluble oral formulations (6). Particularly in paediatric dose forms like acyclovir oral suspension 200 mg/5 mL, the homogeneity, dissolving behaviour, stability, and bioavailability of oral suspension formulations are greatly impacted by the particle size distribution of acyclovir (7). The literature review states that a number of analytical techniques, such as RP-HPLC and UV spectrophotometric investigations, have been documented for acyclovir (8–11). Nevertheless, a survey of the literature showed that nothing is known about a proven particle size distribution technique for oral acyclovir solution. To ascertain the acyclovir oral suspension 200 mg/5 mL's particle size distribution, the current study was conducted to create an appropriate analytical method using a particle size analyser and to validate the established method in accordance with analytical requirements.

2. Methodology and Experimental Details

2.1 Materials

Acyclovir oral suspension 200mg/5 ml (Aurobindo Pharma Limited, Hyderabad–500032, India). As a dispersant, it uses ultra-pure water from Milli-Q.

2.2 Instrumentation and Equipment

A Malvern Mastersizer 3000 particle size analysis system equipped with both Aero S (dry dispersion) and Hydro MV (wet dispersion) accessories was employed for particle size measurements. Data acquisition, processing, and analysis were carried out using Mastersizer software with version of 3.88.

2.3 Method Optimization

Wet Dispersion Unit (Hydro MV)-equipped Malvern Mastersizer 3000. Water was recognised as the dispersion. The Sample RI was 1536 while the Dispersant RI was 1.330. Sample absorption of 0.1 and sample measurement time frame of 5 seconds were the next instrument parameters. Five seconds were spent measuring

the background. The obscuration range was 5–10%, the stirrer speed was set at 2200 rpm, and there were three measurement cycles. The obtained results of the analysis and obscuration readings of Dv(10), Dv(50), and Dv(90) were satisfactory.

2.4 Sample Preparation

Pour the dispersant solution into the sampling unit until it is completely filled. From the toolbar, select configure: Accessories. Manually enhance the stirrer/pump to 2200 RPM gradually. Replenish the dispersant solution in the sampling unit to the brim after a portion has been displaced. Confirm the instrument settings as per SOP for Malvern Mastersizer 3000, wet analysis mode using the parameters in Section 5.1. Ensure there are no air-bubbles in the Sampling unit (degas 3 to 4 times). From the toolbar, select Start SOP and select the SOP created using the Parameters in Section 5.1. Click Open to start the background measurement. If the laser intensity of the background measurement is <50%, drain the Sampling unit, repeat the cleaning procedure. Add sample directly into the sampling unit using a transfer pipette until obscuration attains in between 5%-10% (oscillating blue bar on green field). Record the results.

3. Results

3.1 Analytical Performance Characteristics

3.1.1 Precision study

The precision of the particle size approach has been assessed by placing six various sample preparations into the sampling instrument and documenting the particle size distribution. %RSDs for particle sizes at Dv (10), Dv (50), and Dv (90) should not exceed 15% and 10%, respectively. (Table 1 and Figure 1)

Table 1 Results of Method Precision study

Run No.	Dv(10)	Dv(50)	Dv(90)
1	15.524	50.652	110.955
2	15.685	50.845	110.456
3	15.843	51.652	110.852
4	15.956	50.456	110.483
5	15.745	50.845	110.856
6	15.935	51.054	110.965
Average	15.781	50.917	110.761
%RSD	1.0	0.8	0.2

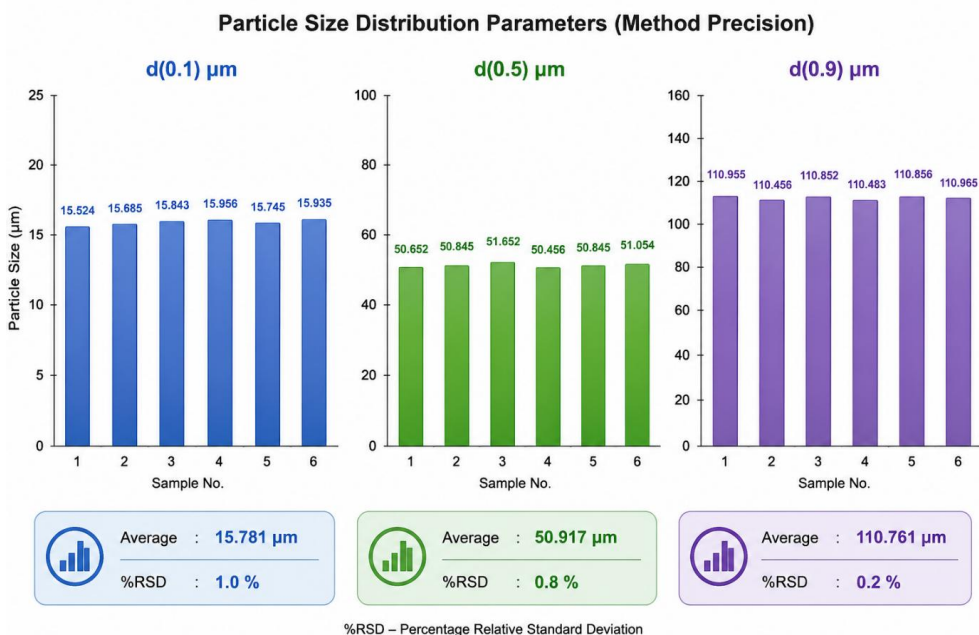


Figure 1 Graphical Representation of Method Precision Results for Acyclovir Oral Suspension

3.1.2 Robustness

By varying instrument parameters like obscuration (5%–10%) and stirring speed (± 200 RPM), one can evaluate the process's resilience. Following the determination of the particle size distribution (PSD) of the sample, the percentage relative standard deviation (%RSD) values for

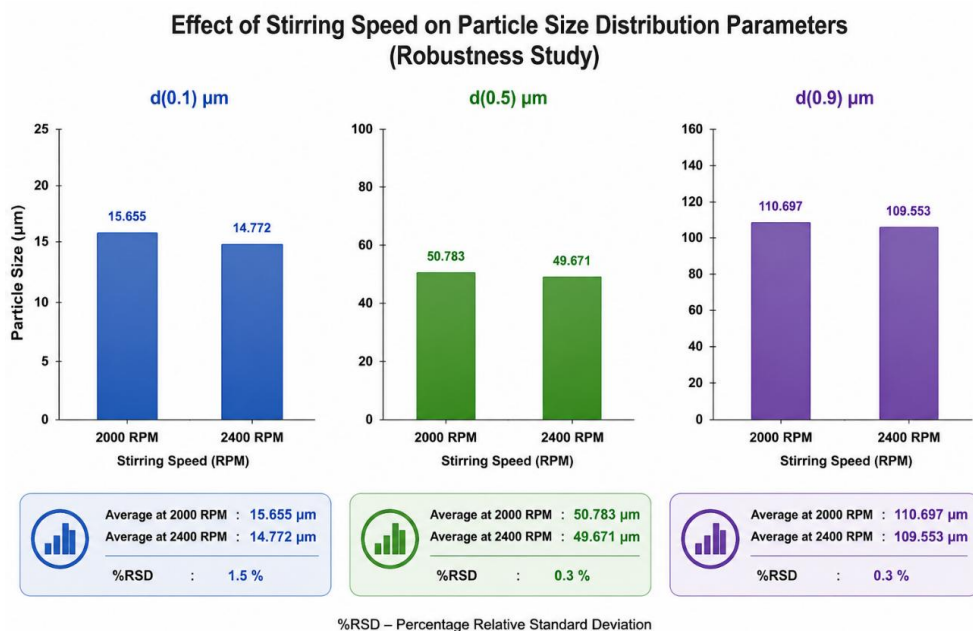
Dv(10), Dv(50), and Dv(90) were calculated. The acceptance criteria required the %RSD values at Dv(10) and Dv(90) to be not more than 15%, while the %RSD at Dv(50) was required to be not more than 10%. (Tables 2 and 3, Figure 2)

Table 2 Robustness Study Results Under Varying Agitation Rates

Run No.	Agitation (RPM)	Dv(10)	Dv(50)	Dv(90)
1	2000	15.625	50.652	110.963
2		15.856	50.745	110.842
3		15.485	50.952	110.285
Average		15.655	50.783	110.697
%RSD		1.2	0.3	0.3
1	2400	14.52	49.524	109.745
2		14.952	49.635	109.658
3		14.843	49.854	109.257
Average		14.772	49.671	109.553
%RSD		1.5	0.3	0.2

Table 3 Robustness Study Results Under Different Obscuration Levels

Run No.	Obscuration	Dv(10)	Dv(50)	Dv(90)
1	5%	15.452	50.856	110.524
2		16.152	50.254	110.652
3		15.685	50.686	110.325
Average		15.763	50.599	110.500
%RSD		2.3	0.6	0.1
1	10%	15.745	50.632	110.854
2		15.257	50.953	110.658
3		15.657	50.748	110.254
Average		15.553	50.778	110.589
%RSD		1.7	0.3	0.3

**Figure 2** Comparison of Particle Size Distribution Parameters at 2000 and 2400 RPM

3.1.3 Intermediate precision

Analyst-2 documented the particle size distribution after moving six distinct sample preparations into the sampling apparatus on a separate day. to evaluate the reliability of the particle size approach. Particle size percentage

RSD at d(0.1), d(0.9), and d(0.5) should not be greater than 15% for (0.1), d(0.9), and d(0.5), and 10% for d(0.5).(Table 4 and Figure 3)

Table 4 Comparison of Particle Size Distribution Parameters Obtained by Two Analysts

Run No.	User-1			User-2		
	Dv(10)	Dv(50)	Dv(90)	Dv(10)	Dv(50)	Dv(90)
1	15.524	50.652	110.955	14.854	50.652	110.527
2	15.685	50.845	110.456	14.956	49.658	109.568
3	15.843	51.652	110.852	15.124	50.124	110.538
4	15.956	50.456	110.483	15.352	49.965	110.956
5	15.745	50.845	110.856	14.958	50.352	110.475
6	15.935	51.054	110.965	15.541	50.245	110.325
Average	15.781	50.917	110.761	15.131	50.166	110.398
%RSD	1.0	0.8	0.2	1.8	0.7	0.4

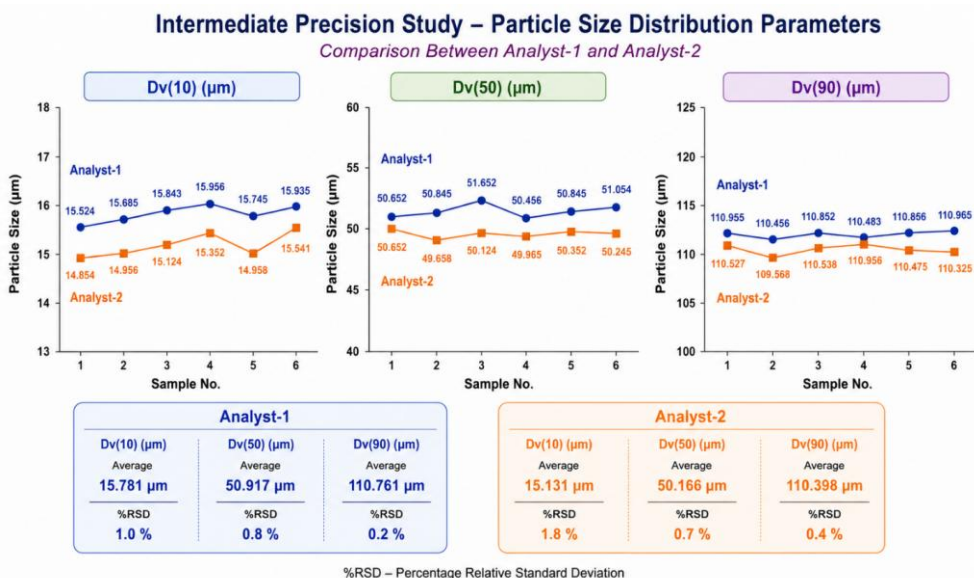


Figure 3 Comparative Assessment of Particle Size Distribution by Analyst-1 and Analyst-2

4. Discussion

The particle size distribution of pharmaceutical suspensions plays a significant role in product performance, stability, dissolution behaviour, and bioavailability. In the present study, a laser diffraction-based analytical method was successfully developed to characterize the particle size distribution of Acyclovir Suspension for oral administration 200 mg/5 mL. The method precision study demonstrated excellent repeatability, with very low %RSD values obtained for Dv(10), Dv(50), and Dv(90). These findings indicate that the developed analytical procedure can consistently generate reliable particle size measurements. The intermediate precision results confirmed the reproducibility of the method when performed by different analysts and on different days. The observed variability remained within the

established acceptance criteria, demonstrating the ruggedness of the analytical procedure. Robustness studies performed by varying stirring speed and obscuration levels showed no significant influence on particle size measurements. The low %RSD values obtained under all tested conditions indicate that minor operational variations do not affect the performance of the method. Overall, the validation results confirm that the developed laser diffraction method is precise, reproducible, and robust for routine particle size distribution analysis of Acyclovir oral suspension. The method can therefore be effectively applied in formulation development, process monitoring, stability studies, and quality control testing.

5. Conclusions

A robust laser diffraction-based analytical procedure was established and validated in order to characterization of distribution of particle sizes in Acyclovir oral suspension 200 mg/5 mL. Evaluation of validation parameters demonstrated consistent performance of the method, with low variability observed for the particle size descriptors Dv(10), Dv(50), and Dv(90). The results obtained during repeatability, intermediate precision, and robustness studies complied with the predefined acceptance criteria, indicating dependable analytical performance. Deliberate variations in operational parameters such as stirring speed and obscuration levels produced no meaningful changes in particle size measurements, further supporting the stability of the procedure. The findings confirm that the proposed method is suitable for routine application in pharmaceutical quality control and can serve as a valuable tool for formulation development, manufacturing process monitoring, and stability evaluation of Acyclovir oral suspension products.

Acknowledgements

The authors would like to thank Srinivasan College of Pharmaceutical Sciences-Trichy and JSS Academy of Higher Education and Research, Mauritius, for their assistance with this research project.

Declaration of Conflicting Interests

The authors affirm that they have no competing interests.

References

1. Sweetman SC, editor. Martindale: the complete drug reference. 38th ed. London: Pharmaceutical Press; 2014.
2. Brunton LL, Hilal-Dandan R, Knollmann BC, editors. Goodman & Gilman's the pharmacological basis of therapeutics. 13th ed. New York: McGraw-Hill Education; 2018.
3. de Miranda P, Blum MR. Pharmacokinetics of acyclovir after intravenous and oral administration. J Antimicrob Chemother. 1983;12(Suppl B):29-37.
4. Indian Pharmacopoeia Commission. Indian pharmacopoeia. Vol. 2. Ghaziabad: IPC; 2022.
5. O'Neil MJ, editor. The Merck index: an encyclopedia of chemicals, drugs, and biologicals. 15th ed. Cambridge: Royal Society of Chemistry; 2013.
6. Aulton ME, Taylor KMG, editors. Aulton's pharmaceuticals: the design and manufacture of medicines. 5th ed. London: Elsevier; 2018.
7. Bhalekar MR, Upadhyaya PG, Reddy S, Kshirsagar SJ, Madgulkar AR. Formulation and evaluation of acyclovir nanosuspension for enhancement of oral bioavailability. Asian J Pharm. 2014;8(2):97-104.
8. Al Quadeib BT. A simple RP-HPLC method for acyclovir determination in tablet and cream dosage forms. Drug Discov. 2019;13:107-14.
9. Weller DR, Balfour HH Jr, Vezina HE. Simultaneous determination of acyclovir, ganciclovir, and (R)-9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine in human plasma using high-performance liquid chromatography. Biomed Chromatogr. 2009;23(8):822-7.
10. Gupta A, Pathak S. Analytical methodologies for the estimation of acyclovir as key members of anti-viral agent: two decades in review. Curr Pharm Anal. 2023;19(8):1-18.
11. Yu L, Xiang B, Zhan Y. A simple high-performance liquid chromatographic method for the determination of acyclovir in human plasma and application to a pharmacokinetic study. Arzneimittelforschung. 2008;58(4):199-202.
12. Aboelezz A, Kharouba M, Mahmoud SH. A simple method for the determination of acyclovir concentrations in human plasma using high-performance liquid chromatography. Future J Pharm Sci. 2024;10:74.