

# Development and Validation of a Discriminating Dissolution Method for Atorvastatin Delayed-Release Nanoparticles Using a Flow-Through Cell: A Comparative Study Using USP Apparatus 4 and 1

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## ABSTRACT

Atorvastatin, an HMG CoA reductase inhibitor, is widely used for the treatment of dyslipidemia and prevention of cardiovascular disease. It belongs to Class 2 of the Biopharmaceutics Classification System owing to its low solubility and high permeability. In vitro dissolution testing is an essential tool for the design of a dosage form. Appropriate selection of dissolution test conditions is essential since it will help establish an appropriate in vitro–in vivo correlation (IVIVC), which is imperative for a waiver of costly bioequivalence studies. Compounds belonging to BCS Class 2 are suitable for establishing a significant IVIVC. USP Apparatus 4 (flow-through cell) is an attractive alternative to conventional dissolution methods like Apparatus 1 and 2. The ability of the apparatus to maintain sink conditions over a longer period of time has proved useful in the development of dissolution methods for poorly soluble drugs, making the development of IVIVCs easier for such drugs. Nanoparticles of atorvastatin were formulated by an emulsion–solvent evaporation method to overcome its bioavailability problems. Development of a discriminatory dissolution method for the same was attempted using a flow-through cell (Apparatus 4) because dissolution performed using Apparatus 1 did not provide complete release of the drug within 60 min. Phosphate buffer pH 6.8 with 1.5% SLS was selected as the dissolution medium based on the results of the solubility studies. Various parameters such as flow rate and sample-loading method were optimized. The dissolution method was validated with respect to various parameters as per ICH guidelines.

**KEYWORDS:** Flow-through cell; in vitro–in vivo correlation; BCS classification; dissolution method validation; ICH guidelines.

## INTRODUCTION

Atorvastatin is an HMG CoA reductase inhibitor or statin, which reduces levels of low density lipoproteins and triglycerides in the blood while increasing levels of high density lipoprotein. The primary uses of atorvastatin are for the treatment of dyslipidemia and the prevention of cardiovascular diseases. Atorvastatin belongs to Class 2 of the Biopharmaceutics Classification System (BCS), which implies that the drug has very low solubility and high permeability. The absolute bioavailability of atorvastatin is 12% after a 40-mg oral dose due to its poor aqueous solubility and poor absorption (1). Formulation of the drug into colloidal carriers may help resolve such issues. Therefore, polymeric nanoparticles of atorvastatin were formulated using an emulsification–solvent evaporation method to improve the solubility and bioavailability of the drug.

Dissolution testing has become an important tool in solid dosage form development and quality control.

Though in vitro dissolution testing cannot replace in vivo bioavailability assessment, it provides valuable biopharmaceutical information in dosage form design (2) that is suitable for establishing a significant in vitro–in vivo correlation (IVIVC) for a BCS Class 2 compound. Appropriate selection of the dissolution study conditions is essential to establish a method that discriminates between products with potential problems of bioavailability. The establishment of a significant IVIVC provides the basis for predicting in vivo performance and waives costly bioequivalence studies (3).

Apparatus 4 has several advantages over conventional dissolution systems such as Apparatus 1 and 2. This apparatus can be operated as either an open configuration; wherein fresh solvent from the reservoir continuously passes through the cell containing the dosage form, or as a closed system, which recycles a fixed volume of the medium. A distinct advantage of the flow-through cell is that medium and flow rate changes can be performed

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easily in the same run. Intraluminal hydrodynamics are more efficiently simulated in this system than in other in vitro systems (4). It is possible to sustain sink conditions in the open flow-through apparatus for longer periods. This application is especially important for poorly soluble drugs, making the development of in vitro–in vivo correlations easier for such drugs. The flow-through system has proved useful in the development of a more discriminating dissolution method than the official one in the *USP* for the poorly soluble compound albendazole and in the establishment of an IVIVC for paracetamol suppositories, a Class 3 drug. In addition, dosage forms such as extended-release tablets, drug-eluting stents, microspheres, nanosuspensions, and implants have shown appropriate results when evaluated using Apparatus 4 (5).

This research focused on the development and validation of a dissolution method for atorvastatin nanoparticles using Apparatus 4 and compared it with the innovator dissolution method employing Apparatus 1.

## EXPERIMENTAL SECTION

### Materials

Atorvastatin calcium (AC) was obtained as a gift sample from Cadila Pharmaceuticals Ltd. (Ahmedabad, India). Eudragit L100-55 was purchased from Evonik Industries (Mumbai, India). Surfactants were procured from S. D. Fine Chemicals (Mumbai, India). Potassium dihydrogen phosphate and hydrochloric acid were purchased from S. D. Fine Chemicals (Mumbai, India). Milli-Q water (Millipore, Bedford, MA, USA) was used for the preparation of buffer media.

### Instrumentation

Dissolution method development in a flow-through cell was performed using a Sotax CE7 Apparatus 4 equipped with 22.6-mm diameter cells, while an Electrolab tablet dissolution tester was used to perform dissolution using baskets.

### AC Solubility

The solubility of AC was determined in phosphate buffers of four different pH values (5.5, 6.0, 6.8, and 7.4) by the shake–flask method (6). Nanoparticles were prepared using Eudragit L 100–55, a polymer that dissolves above pH 5.5, justifying the selection of buffers. Sodium lauryl sulfate (SLS) in several concentrations (0.5%, 1%, and 1.5% w/v) was added to pH 6.8 and pH 7.4 buffers, and the resulting solutions were used for solubility assessment. Solubility determination was carried out by adding an excess weighed quantity of AC to 1 mL of each buffer solution in plastic Eppendorf tubes. The tubes were mixed

for 2 min on a cyclomixer and placed in a reciprocating water bath shaker (Boekel Scientific, Feasterville, PA, USA) at 37 °C for 24 h. The tubes were centrifuged (Etek TC 4100D, Elektrocraft, Mumbai, India) at 10,000 rpm for 15 min and subsequently filtered through 0.1- $\mu$ m PVDF membrane syringe-driven filters (Millex VV, Millipore Corporation, Bedford, MA, USA). The filtrates were assayed for drug content by a validated in-house HPLC method. All experiments were performed in triplicate.

### Preparation of Atorvastatin Calcium Delayed-Release Nanoparticles

Atorvastatin calcium delayed-release nanoparticles (ACDR NPs) were prepared by a nanoprecipitation method employing Eudragit L 100-55 as an encapsulating polymer. Polymer and drug were dissolved in an organic solvent that was then slowly added to the surfactant containing the aqueous phase under constant stirring. The organic solvent was then allowed to evaporate. The resulting nanoparticulate dispersion was dried using a spray drier (JISL Lab Spray Dryer, Mumbai, India).

### Content Uniformity and Assay

Content uniformity and assay tests were performed for ACDR NPs according to the validated in-house HPLC method. The RP–HPLC system for determination of AC consisted of Agilent 1100 modules (G1310A isocratic pump with solvent container, G1314A VW detector with standard flow cell, G1328A manual injector). The output signal was monitored and processed using an Agilent single G2220AA 2D-Value Solution ChemStation. Chromatographic separation was achieved on a 5- $\mu$ m Thermo Scientific RP C<sub>18</sub> ODS column (4.6 mm  $\times$  150 mm). Mobile phase solvent A was ammonium acetate adjusted to pH 4 with 10% acetic acid, and solvent B was acetonitrile/tetrahydrofuran (92.5:7.5). Prior to use, water was filtered through a 0.45- $\mu$ m filter membrane. Mobile phase was pumped through the column at a flow rate of 1.0 mL/min. The injection volume was 20  $\mu$ L, and the detector wavelength was 252 nm.

### Dissolution Profile Using Basket Method (Apparatus 1)

The dissolution profiles of ACDR NPs using the basket method were determined using an automated dissolution Apparatus 1 (Electrolab TDT, India). The prepared ACDR NPs equivalent to 10 mg AC USP were enclosed in a dialysis bag and suspended in a basket containing 900 mL of pH 6.8 phosphate buffer with 1.5% SLS at 37.0  $\pm$  0.5 °C. The rotational speed was set at 75, 100, and 125 rpm. Sampling using filter probes was carried out over 60 min at 5-min intervals using 12 replicates. The amount of AC dissolved was determined with a known concentration of the standard solution using the validated RP–HPLC method developed in-house.

## **Dissolution Profile Using Flow-Through Cell System (Apparatus 4)**

Dissolution profiles of ACDR NPs were established on an automated flow-through cell system, Apparatus 4 (Sotax TM CE7, Sotax AG, Switzerland) with 22.6-mm i.d. cells and a piston pump (Sotax CY7–50, Sotax AG, Switzerland). In all experiments, laminar flow with a bed of glass beads (6 g) was used. The degassed dissolution medium, pH 6.8 phosphate buffer maintained at  $37.0 \pm 0.5$  °C, was pumped at flow rates of 8 mL/min and 16 mL/min. A closed-loop system was utilized by recycling the dissolution medium. A ruby bead (5-mm diameter) was placed at the base of the 22.6-mm sample cell, and 4 g of 1-mm glass beads was added to fill the bottom conical part of the sample cell. ACDR NPs were added to the dialysis bag and placed in the Apparatus 4 sample cell for release studies. Phosphate buffer (900 mL) with varying concentrations of SLS maintained at  $37 \pm 0.5$  °C was used as the release medium in these studies. Sequential sampling using 0.45- $\mu$ m nitrocellulose membranes (Millipore) was carried out over 60 min. At each time point, 10-mL samples were withdrawn from the medium reservoir containers of the apparatus. The samples were replenished with fresh medium. Sink conditions were maintained throughout the experiment. The content of AC in the aliquots was analyzed using the validated in-house HPLC method. The results are reported as mean  $\pm$  SD ( $n = 3$ ). The amount of dissolved AC was determined with a known concentration of the standard solution using an RP–HPLC system consisting of Agilent 1100 modules (G1310A isocratic pump with solvent container, G1314A VW detector with standard flow cell, G1328A manual injector).

## **Optimization of Dissolution Method Using Apparatus 4**

Various sample-loading patterns have been reported for Apparatus 4. These include mixing the drug substance homogeneously with 1-mm glass beads, sandwiching the drug between the 1-mm glass beads, layering the sample on the bottom of the cylindrical portion below the bed of 1-mm glass beads, and packing the glass beads in the lower cone over the layer of the drug. Among these methods, homogeneously mixing the sample with glass beads was inferred (7) to be the best method of drug-powder loading in terms of achieving maximum dissolution with minimum variability of results. Mixing was carried out very gently with the help of a spatula.

The effect of flow rate on drug release from ACDRNPs was evaluated by varying the flow rates, 8 mL/min and 16 mL/min. Dissolution release studies from Apparatus 4 conducted at a flow rate of 16 mL/min were used for

comparison with the Apparatus 1 dialysis bag method. AC content from the dissolution aliquots was analyzed using the same RP–HPLC method used for content uniformity and assay tests.

## **Dissolution Method Validation**

To demonstrate that the developed RP–HPLC method was suitable for analysis of drug content from the dissolution aliquots, it was validated on the parameters of stability, specificity, linearity, accuracy, precision, and robustness according to *USP 32* (8) and ICH guidelines (9).

### *Standard and Sample Solution Stability*

The stability of AC in the dissolution medium was evaluated using reference standard and aliquot samples. A standard solution of AC was prepared in the dissolution medium of pH 6.8 phosphate buffer containing 1.5% SLS (w/v) and maintained at ambient temperature ( $23 \pm 2$  °C) for 0, 15, and 24 h and at refrigerated temperature ( $8 \pm 2$  °C). Aliquots withdrawn during dissolution testing of the nanoparticles were also subjected to similar conditions. The solutions were protected from light during this study. The assay was performed in triplicate.

### *Specificity*

Specificity was evaluated by preparing a placebo of nanoparticles at their usual concentration. An amount equivalent to that contained in one tablet (i.e., 100 mg) was transferred to vessels with 900 mL of medium at  $37 \pm 0.5$  °C and stirred for 1 h at 150 rpm using Apparatus 1. Aliquots of this solution were withdrawn, filtered, and analyzed.

### *Linearity*

Aliquots of a stock solution containing 100  $\mu$ g/mL of AC reference substance prepared in methanol were transferred to 25-mL volumetric flasks and diluted with medium to final concentrations of 5, 10, 15, 20, 25, and 30  $\mu$ g/mL for HPLC analysis. The solutions were analyzed in triplicate every day for three consecutive days. The linearity was evaluated by linear regression analysis, which was calculated by the least-squares regression method and analysis of variance (ANOVA).

### *Accuracy and Precision*

The accuracy of the method was evaluated through the recovery of known amounts of AC reference substance added to the placebo. A stock solution containing 1 mg/mL of AC was prepared in methanol. Aliquots of 10, 15, and 20 mL of this solution were added to vessels containing dissolution medium for a final volume of 900 mL kept at  $37 \pm 0.5$  °C (final concentrations of 10, 15, and 20  $\mu$ g/mL). Samples were stirred at 150 rpm for 1 h. Aliquots from

each vessel were then collected and analyzed. These studies were performed in triplicate on three different days. The solutions used in the accuracy test were also analyzed to ensure the precision of the method. Intraday and interday precision was established based on relative standard deviation (RSD) of the results.

#### Robustness

Robustness of the method was evaluated during development by making small but deliberate changes to the protocol parameters. The release of AC in different pH values of the dissolution medium (6.4 and 6.8) was also evaluated for the same.

#### Evaluation of Release Kinetics

Zero-order, first-order, Higuchi, and Hixon–Crowell mathematical models were applied to evaluate the kinetics of drug release. The equations are as follows:

Zero-order kinetics	$Q_t = Q_0 + K_0 t$
First-order kinetics	$\log Q_t = \log Q_0 + (K_1 t)/2.303$
Higuchi model	$f_t = K_H t^{1/2}$
Hixon–Crowell model	$W_0^{1/3} - W_t^{1/3} = k_s t$

where  $Q_t$  is the amount of drug dissolved in time  $t$ ;  $Q_0$  is the initial amount of drug in the solution;  $K_0$  and  $K_1$  are zero-order and first-order release constants, respectively;  $f_t$  is the amount of drug released in time  $t$  by surface unity;  $K_H$  is the Higuchi dissolution constant;  $W_0$  is the initial amount of drug in the pharmaceutical dosage form;  $W_t$  is the remaining amount of drug in the pharmaceutical dosage form at time  $t$ ; and  $K_s$  is a constant incorporating the surface–volume relation. The curves were constructed applying these models, considering only one point above 80% of the drug released. The mathematical model that best expressed the dissolution profile of ACDR NPs was selected based on the coefficient of determination,  $R^2$  (10).

## RESULTS AND DISCUSSIONS

### AC Solubility in Various Buffers

Solubility studies show the minimum solubility of AC in pH 5.5 phosphate buffer (19.6  $\mu\text{g}/\text{mL}$ ) and the highest solubility in pH 6.8 phosphate buffer with 1.5% (w/v) SLS (218.9  $\mu\text{g}/\text{mL}$ ). The addition of SLS increased the solubility of AC from 98.1 to 218.9  $\mu\text{g}/\text{mL}$  in pH 6.8 phosphate buffer, and the same phenomenon was observed in pH 7.4 phosphate buffer. The solubility of AC was highest in pH 6.8 buffer compared with other buffers; hence, further dissolution testing of ACDR NPs was performed using pH 6.8 phosphate buffer with the addition of SLS in concentrations of 0.5%, 1%, and 1.5% (w/v).

### Content Uniformity and Assay

All products met in-house product specifications for content uniformity and assay. The AC results for the content uniformity test were in the range of 100.20–101.34%, while assay results were between 101.42% and 105.11%.

### Dissolution Profiles Using Basket Method

When a dissolution test is not defined in the dosage form monograph, or if the monograph is not available, a comparison of drug dissolution profiles is recommended in three different dissolution media in the pH range of 1–7.5. The selection of a dissolution medium may be based on the solubility data and dosage range of the drug product (11). Based on the data obtained from the solubility studies, pH 6.8 phosphate buffer with various concentrations of SLS showed the highest solubility for AC; hence, the same medium was used for further dissolution studies of ACDR NPs.

The dissolution study using the dialysis bag method was performed employing different rotation speeds, but there was no significant difference observed in terms of release of AC. The dialysis bag rather served as a hindrance to the release of the drug from the nanoparticles, and this was reflected in the release profile of the drug since at the end of 60 min; not more than 68% of the drug was released (Figure 1). Hence, it was decided to use a flow-through cell as an alternative to ensure complete release of the drug.

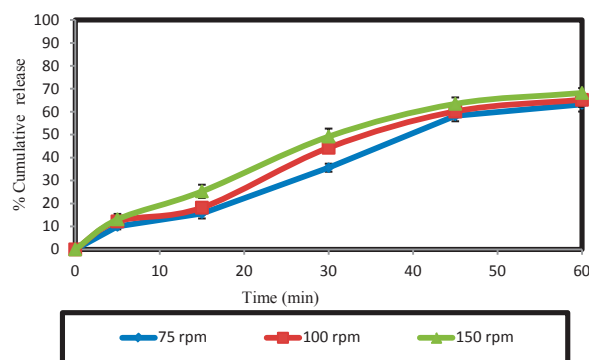


Figure 1. Dissolution release profiles in pH 6.8 phosphate buffer with 1.5% SLS using Apparatus 1 dialysis bag method.

### Dissolution Profile Using Flow-Through Cell System

The protocol for dissolution studies with Apparatus 4 was established as explained earlier using the dialysis bag method, and the aliquots were analyzed according to the validated RP–HPLC method. It was observed that the rate of drug release increased with increasing SLS

concentration. However, though the addition of SLS increased the rate of drug release, it still could not afford complete release of the drug within 1 h. Dissolution media containing 1.5% SLS also demonstrated only 65% drug release at the end of 60 min (Figure 2). Hence, further optimization and modifications were required in the dissolution methodology to ensure complete release of this BCS Class 2 drug.

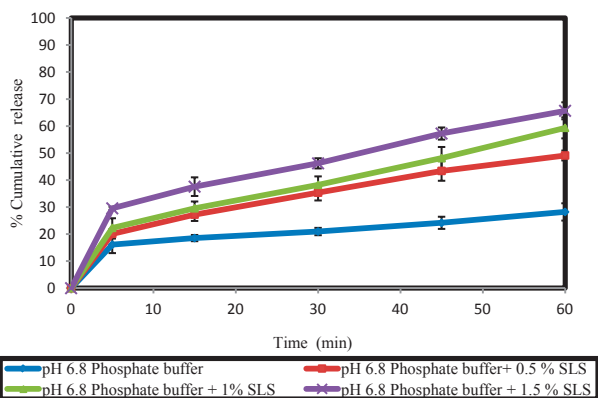


Figure 2. Dissolution of ACDR NPs using dialysis bag in Apparatus 4 system.

### Optimization Using Apparatus 4

The release profile of ACDR NPs by dialysis did not show complete release of drug at the end of 60 min. The sample-loading method was modified by mixing the nanoparticles with 1 mm glass beads before loading in the sample cell. This method has been reported to provide complete release of drug in Apparatus 4 (7). These findings were confirmed in the current study. Use of the glass-bead mixing method provided complete release of the drug from ACDR NPs in the dissolution media of pH 6.8 buffer with 1% and 1.5% SLS.

Variation in flow rate did not show any effect on the rate of drug release (Figures 3 and 4). Hence it can be concluded

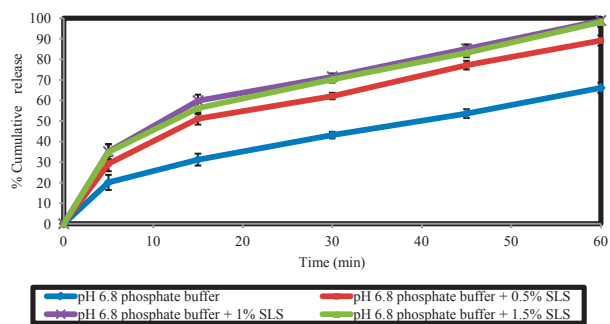


Figure 3. Dissolution in pH 6.8 phosphate buffer at a flow rate of 8 mL/min using glass-bead mixing method.

that the flow rate does not have a significant impact on the release profile of the drug from nanoparticles.

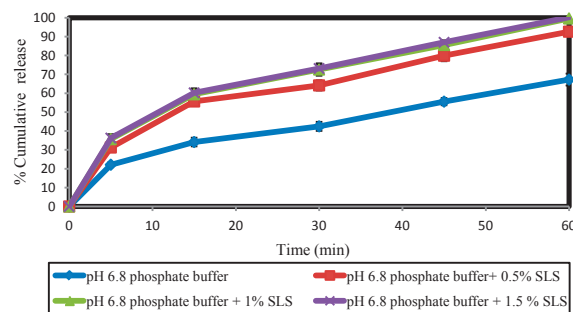


Figure 4. Dissolution in pH 6.8 phosphate buffer at a flow rate of 16 mL/min using glass-bead mixing method.

### Comparison of USP Apparatus 1 and 4 Release Profiles

ACDR NPs dissolution profiles obtained with the Apparatus 1 method and the flow-through cell system are shown in Figure 5. Dissolution profiles obtained using the flow-through cell system are more precise and complete as compared with that of Apparatus 1. When the dialysis bag was used in Apparatus 1, 65% AC was released from the formulation. Similarly, when used in Apparatus 4, the dialysis bag method does not show release greater than 67%. However, the modifications made in the sample loading method (blending of nanoparticles with glass beads) demonstrate 100% release in 60 min. This observation confirms earlier findings wherein the use of a similar method ensured complete release of the drug.

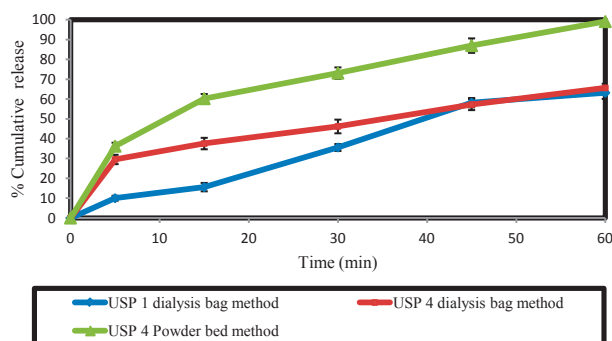


Figure 5. Comparison of the release profiles obtained from USP Apparatus 1 and 4 in pH 6.8 phosphate buffer with 1.5% SLS.

### Dissolution Method Validation

The HPLC method used for the analysis of the aliquots was validated for various parameters to ensure suitability for estimating the release profile of the drug from ACDR NPs (12).

### Reference Substance and Sample Solution Stability

The stability of the reference and sample solutions was evaluated at room temperature for 24 h to estimate the stability of AC in the solutions over the period of dissolution profile determination and further analysis. The drug content of the reference and sample solutions stored at room temperature and under refrigeration ( $8 \pm 2$  °C) for 24 h was within 99–103% of the initial value. Moreover, no degradation products were observed at these conditions.

### Specificity

The specificity of the dissolution test by the RP–HPLC method demonstrated no interference of excipients. Overlaid HPLC chromatograms of a formulation and its placebo in the dissolution medium are shown in Figure 6. The chromatogram for the lowest dose strength did not show any interference from excipients at the peak of interest, confirming the specificity of the method

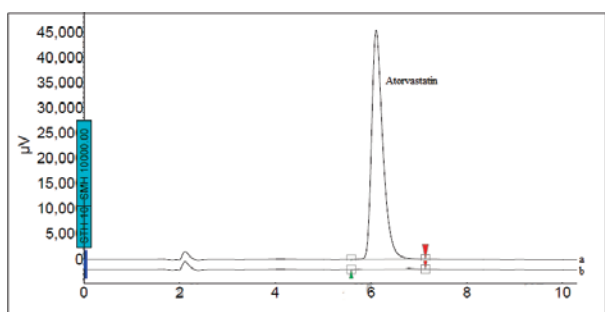


Figure 6. Overlaid chromatograms of (a) 10 mg AC DR NPs in dissolution medium and (b) placebo formulation in dissolution medium using the dissolution HPLC method.

### Linearity

Linearity of the method was evaluated with a six-point calibration curve in the concentration range of 5–30  $\mu\text{g}/\text{mL}$  of drug substance. The correlation coefficient value of 0.9999 indicates excellent linearity of the analysis method over the concentration range of 5–30  $\mu\text{g}/\text{mL}$ .

### Accuracy and Precision

The accuracy of the method was demonstrated by the recovery of a known amount of AC added to the dissolution vessels. Recoveries from 95.0% to 105.0% of the added amounts are recommended in the dissolution tests (10). The mean percentage recovery for three different days ranged from 97.80% to 100.6% (Table 1), corroborating the accuracy of the method. The intraday precision was evaluated at three different concentration levels. The intermediate precision was evaluated for the same solutions on different days. Values presented in Table 1 show good precision of the method with RSD values less than 2%.

Table 1. Recovery and Precision of AC

Concentration of drug added ( $\mu\text{g}/\text{mL}$ )	Concentration of drug recovered ( $\mu\text{g}/\text{mL}$ )	% drug recovered	%RSD
10	10.06	100.6	1.23
15	15.11	100.7	0.78
20	19.56	97.80	1.10
Intraday precision			
10	9.99	99.80	0.87
15	15.02	100.13	0.28
20	20.04	100.13	0.74
Interday Precision			
10	10.97	99.40	0.47
15	15.01	100.06	0.97
20	20.01	100.03	0.24

### Robustness

Robustness indicates the ability of the method to withstand changes in the method protocol. The analytical method was evaluated by varying the chromatographic conditions of wavelength, flow rate, pH, and mobile phase composition. Changes in the mobile phase flow rate, column temperature, and  $\lambda_{\text{max}}$  of the method did not result in any significant change in the retention time of the drug as shown in Table 2. Hence method robustness was confirmed.

Table 2. Robustness Studies

Sr. No.	Parameter	Level	RT (min)	per USP
1	Flow rate (mL/min)	0.8	7.15	0.91
		1.0	6.10	0.94
		1.2	4.90	0.92
2	Column temperature (°C)	25	6.10	0.93
		30	6.10	0.78
		35	6.10	0.95
3	Wavelength $\lambda_{\text{max}}$ (nm)	257	6.10	0.97
		252	6.10	0.97
		247	6.10	0.98

### Evaluation of Release Kinetics

The dissolution profile was used to evaluate the kinetics of drug release (13). According to the obtained  $R^2$  values, the dissolution profile is best described by the Higuchi model ( $R^2 = 0.9933$ ). When the drug release is governed by this model, it is assumed that the release rate is limited by diffusion through the polymeric matrix.

## CONCLUSION

A discriminating dissolution method is an important in vitro test for evaluating change in a drug product formulation or process. Since there is no dissolution method specified for ACDR NPs in the literature, an attempt was made to develop a discriminating dissolution method.

The dissolution medium was pH 6.8 phosphate buffer with 1.5% SLS to enhance dissolution. Use of the dialysis bag method did not demonstrate complete release of the drug from either Apparatus 1 or 4. Sample loading in Apparatus 4 by a glass-bead mixing method ensured complete release of the drug from the nanoparticles. Flow rate of the dissolution medium did not have an impact on the release profile of the drug. Thus the Apparatus 4 dissolution method was found to be discriminatory.

The dissolution method was validated for various parameters according to ICH guidelines. The method is specific, linear, accurate, precise, and robust. The reference and sample solutions were stable over 24 h at room temperature. The drug release follows the Higuchi model. Hence, a discriminatory dissolution method for ACDR NPs was developed and validated.

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## CONFLICT OF INTEREST

No conflict of interest has been declared by the authors.

## REFERENCES

1. Lau, Y. Y.; Okochi, H.; Huang, Y.; Benet, L. Z. Pharmacokinetics of Atorvastatin and Its Hydroxy Metabolites in Rats and the Effects of Concomitant Rifampicin Single Doses: Relevance of First-Pass Effect From Hepatic Uptake Transporters, and Intestinal and Hepatic Metabolism. *Drug Metab. Dispos.* **2006**, *34* (7), 1175–1181. DOI: 10.1124/dmd.105.009076.
2. Anand, O.; Yu, L. X.; Conner, D. P.; Davit, B. M. Dissolution Testing for Generic Drugs: An FDA Perspective. *AAPS J.* **2011**, *13* (3), 328–335. DOI: 10.1208/s12248-011-9272-y.
3. Yu, L. X.; Amidon, G. L.; Polli, J. E.; Zhao, H.; Mehta, M. U.; Conner, D. P.; Shah, V. P.; Lesko, L. J.; Chen, M.-L.; Lee, V. H. L.; Hussain, A. S. Biopharmaceutics

Classification System: The Scientific Basis for Biowaiver Extensions. *Pharm. Res.* **2002**, *19* (7), 921–925. DOI: 10.1023/A:1016473601633.

4. Nicolaides, E.; Symillides, M.; Dressman, J. B.; Reppas, C. Biorelevant Dissolution Testing to Predict the Plasma Profile of Lipophilic Drugs After Oral Administration. *Pharm. Res.* **2001**, *18* (3), 380–388. DOI: 10.1023/A:1011071401306.
5. Medina, J. R.; Salazar, D. K.; Hurtado, M.; Cortés, A. R.; Domínguez-Ramírez, A. M. Comparative in vitro dissolution study of carbamazepine immediate-release products using the USP paddles method and the flow-through cell system. *Saudi Pharm. J.* **2014**, *22* (2), 141–147. DOI: 10.1016/j.jsps.2013.02.001.
6. Baka, E.; Comer, J. E. A.; Takács-Novák, K. Study of equilibrium solubility measurement by saturation shake-flask method using hydrochlorothiazide as model compound. *J. Pharm. Biomed. Anal.* **2008**, *46* (2), 335–341. DOI: 10.1016/j.jpba.2007.10.030.
7. Bhattachar, S. N.; Wesley, J. A.; Fioritto, A.; Martin, P. J.; Babu, S. R. Dissolution testing of a poorly soluble compound using the flow-through cell dissolution apparatus. *Int. J. Pharm.* **2002**, *236* (1–2), 135–143. DOI: 10.1016/S0378-5173(02)00027-3.
8. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. *Stability testing of New Drug Substances and Products, Q1A(R2)*. ICH Harmonised Tripartite Guideline: Geneva, Switzerland, 2003.
9. <1225> Validation of Compendial Procedures. In *The United States Pharmacopeia and National Formulary USP 32–NF 27*; The United States Pharmacopeial Convention, Inc.: Rockville, MD, 2009.
10. <1092> The Dissolution Procedure: Development and Validation. In *The United States Pharmacopeia and National Formulary USP 32–NF 27*; The United States Pharmacopeial Convention, Inc.: Rockville, MD, 2009.
11. Brown, C. K.; Chokshi, H. P.; Nickerson, B.; Reed, R. A.; Rohrs, B. R.; Shah, P. A. Acceptable Analytical Practices for Dissolution Testing of Poorly Soluble Compounds. *Pharm. Technol.* **2004**, *28* (12), 56–65.
12. Gite, S.; Patravale, V. Validation of RP–HPLC Method and Stress Degradation for the Combination of Metformin HCl, Atorvastatin Calcium and Glimperide: Application to Nanoparticles. *J. Chromatogr. Sci.* **2015**, *53* (10), 1654–1662. DOI: 10.1093/chromsci/bmv068.
13. Costa, P.; Sousa Lobo, J. M. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.* **2001**, *13* (2), 123–133. DOI: 10.1016/S0928-0987(01)00095-1.