dx.doi.org/10.14227/DT310324P136

Life Cycle Application of AQbD for Formulation Development and Validation of a Dissolution Method for Nevirapine

Rayza A. D. de Almeida[1], Maria Luiza R. A. de Oliveira[1], Ivone de J. N. Lopes[1], Diogo D. Nascimento[1], Camila A. Oliveira[1], and Livia D. Prado[1*]

[1]Laboratório de Desenvolvimento e Validação Analítica, Instituto de Tecnologia em Fármacos (Farmanguinhos), Fundação Oswaldo Cruz, Rua Sizenando Nabuco, Rio de Janeiro, Brazil.

e-mail: liviaderis@gmail.com

ABSTRACT

Introduction: Dissolution plays a vital role as an in vitro test in the pharmaceutical product life cycle. For the evaluation of an appropriate dissolution test, analytical quality by design (AQbD) principles can provide increased confidence when deciding whether the product is of the expected quality. **Methods**: This study applied AQbD concepts for dissolution method development for nevirapine 200-mg tablets. Solubility tests were performed. The analytical target profile (ATP) was established for the dissolution and the quantification methods. Risk assessment was carried out through the construction of an Ishikawa diagram to identify the critical method parameters. Robustness was evaluated using a fractional factorial design and validation tests were conducted. **Results**: Nevirapine showed pH-dependent solubility and near-sink conditions were observed at pH 2.0. The ATP considered the targets for specificity, range, accuracy, and precision. The dissolution method was able to differentiate formulation attributes and changes in critical process parameters. The method showed robustness after 45 minutes, and pH control was the key element in ensuring analytical performance. Validation tests proved method specificity, linearity, accuracy and precision. **Conclusion**: This study demonstrated the application of AQbD to a dissolution method, making it possible to evaluate the discriminative power, robustness and to define the specification.

KEYWORDS: Nevirapine; dissolution; analytical target profile; design of experiments

INTRODUCTION

Nevirapine is a non-nucleoside reverse transcriptase inhibitor that is effective when used as part of combination therapy for the treatment of human immunodeficiency virus-1 infection (*1*, *2*). Nevirapine is a weak base whose conjugate acid has a pKa of 2.8 and the solubility depends strongly on the pH of the solution (*3*, *4*). Due to its low water solubility and high permeability, nevirapine is classified as a class II drug in the Biopharmaceutics Classification System (BCS). BCS class II compounds exhibit dissolution rate-limited bioavailability (*4*, *5*).

Dissolution is an important quality control test to evaluate the in vitro release performance of pharmaceutical dosage forms and represents a critical quality attribute of drug products (*6*–*8*). Dissolution tests frequently support the formulation development process to evaluate the stability of a drug product and ensure batch-to-batch consistency (*9*, *10*). The quality by design (QbD) approach strongly emphasizes the role of dissolution testing in evaluating critical process parameters that can affect dosage form performance (*11*). In this context, it is essential to have a dissolution methodology with sufficient discriminatory power to characterize potential differences, and such dissolution methodology should be a combination of justified parameters like media buffer pH, media volume, and mixing speed (*12*–*17*).

Regulatory guidelines have been published to present the steps for developing a dissolution method. If a method is described in a pharmacopeial monograph, its suitability

*Corresponding author

for the intended pharmaceutical product should be assessed (*18–20*). Therefore, the discriminative power can be determined prior to the analytical validation step. Also, international guidelines recommend performing a robustness assessment during method development (*21*). For dissolution, robustness is conventionally evaluated for the quantification method, varying parameters related to spectrophotometric or chromatographic methods (*22–24*). However, International Conference of Harmonization (ICH) Q2(R2) guideline addresses robustness as a performance characteristic associated with the reasoning for selecting dissolution parameters such as media pH and volume (*21*).

Analytical quality by design (AQbD) principles have been applied in the development of analytical methods, especially for chromatographic methods (*25–32*). AQbD strategy begins with the definition of an ATP and includes several steps to identify critical method attributes and parameters (CMAs and CMPs, respectively), develop or optimize experimental procedures (design of experiment [DoE] process), and determine method robustness. AQbD gained attention for enabling the development of robust and cost-effective procedures with regulatory flexibility and control strategies designed for life cycle monitoring. The significance of AQbD has been described in ICH Q14 guideline (*33*).

To our knowledge, few studies have been reported on the application of AQbD for dissolution methods to ensure adequate performance throughout the product life cycle. Furthermore, no study has presented ATP with the performance characteristics of dissolution and quantification methods, and a limited number of studies show the application of DoE for dissolution conditions (*34–36*). In this study, a dissolution method for immediate-release nevirapine 200-mg tablets was developed using AQbD elements to provide a robust and suitable method. Analytical validation was carried out to prove the suitability of the method.

METHODS

Chemical and Reagents

Materials used in experiments included: nevirapine reference standard (USP), nevirapine API (manufacturers A and B [manufacturer names were not disclosed due to confidentiality reasons), acetonitrile HPLC grade (J.T Baker), ethanol HPLC grade (Supelco), orthophosphoric acid (Merck), sodium phosphate monobasic monohydrate (Merck), hydrochloric acid (Êxodo), acetic acid (Biograde), and potassium phosphate monobasic (Êxodo).

Four nevirapine 200-mg tablet formulations were used, each one with variations in relation to the API manufacturer (A or B), hardness, or disintegrant amount. Sample N1 is the current formulation registered by the Brazilian regulatory agency (Anvisa), which was used as a reference product. The characteristics of the formulations were: N1 (API manufacturer A, 142 N hardness, 5% disintegrant), N2 (API manufacturer B, 154 N hardness, 5% disintegrant), N3 (API manufacturer B, 228 N hardness, 5% disintegrant), and N4 (API manufacturer B, 128 N hardness, 0% disintegrant).

Solubility Studies

Nevirapine suspensions in hydrochloric acid (HCl) 0.1 M pH 1.2, sodium phosphate buffer pH 2.0 (dissolution medium for nevirapine tablets described in the *United States Pharmacopeia* [USP]), acetate buffer pH 4.5 (preparation according to *USP*), and potassium phosphate buffer pH 6.8 (preparation according to USP) were maintained under agitation (100 rpm) at 37 °C using an IKA KS4000i (Germany) for determination of solubility by shake-flask method (*37*). Three independent experiments were carried out for each medium. Aliquots (10 mL) were taken at 2, 6, 12, and 24 h, filtered using a 0.22-µm PTFE syringe filter, and diluted for further quantification. The samples were quantified by high-performance liquid chromatography (HPLC) based on analytical curves in the range of 0.05–0.30 mg/mL. The HPLC method used was a previously developed and validated stability indicating method. A Metrohn (Switzerland) 780 potentiometer was used to determine the pH of all solutions.

Filter Suitability for Dissolution Test

Two PTFE syringe filters (Agilent UHMWPE 35 µm and BioNaky 0.22 µm) were evaluated. Leachability was tested by comparing chromatograms of the dissolution media before and after filtration. To evaluate if the dissolved API binds to the filter membrane, 10 mL of the standard solution was filtered, and the signal variation was calculated by comparing the peak area of the chromatogram with that of the unfiltered solution. Filtration efficiency was evaluated by taking a 20-mL aliquot from the dissolution vessel 5 minutes after adding formulation N2 to 900 mL of dissolution medium at 37 °C and 50 rpm. The sampled volume was divided into three parts. The first was immediately evaluated. The second and third parts were placed in an ultrasound bath for 5 and 10 minutes, respectively, after which the samples were evaluated by HPLC. The dissolution medium used was sodium phosphate buffer pH 2.0. The HPLC method used was the same as described for the quantification of dissolution test.

Analytical Target Profile (ATP) and Risk Assessment for Identification of Critical Method Parameters and Attributes (CMPs and CMAs)

The ATP was prepared based on the quality target product profile (QTPP) previously developed by the authors (unpublished data), in which dissolution was considered as a critical quality attribute (CQA). Risk assessment was conducted, with the elaboration of an Ishikawa diagram, to recognize the CMPs that can affect the final performance of the dissolution test, related to the CMAs (*38*).

Dissolution Test and Profile Comparison

Dissolution profiles were obtained for formulations N1, N2, N3, and N4 in a Varian (USA) VK7010 dissolution apparatus according to the method described in the *USP* (n = 6) (*37*). A USP apparatus 2 (paddle) at 50 rpm was used with 900 mL of 0.1-M sodium phosphate buffer pH 2.0 as dissolution medium. The bath temperature was set at 37 °C. Samples (10 mL) were drawn at 5, 10, 15, 30, 45, 60, and 90 minutes and filtered with a 35-µm Ultra High Molecular Weight Polyethylene (UHMWPE) filter. The percentage of drug dissolved was corrected in relation to the volume collected at each time point, and the absorbance was determined using a HPLC method. The HPLC system consisted of a Shimadzu (Japan) with an LC-20AT pump, CTO-20AC column oven, SIL-20A auto sampler, SPD-M20A PDA detector, and CBM-20A system controller. Chromatographic conditions included an X Terra C18 column (150 x 3.9 mm, 5 µm) at ambient temperature, mobile phase of water: acetonitrile (77:23 v/v), flow rate of 1.0 mL/min, detection at 214 nm, and injection volume of 20 µL.

Dissolution efficiency (DE) was obtained from the area under the curve (AUC) of the dissolution profile (*39*). The DE results were studied with analysis of variance (ANOVA) at 95% confidence level. In addition, two-way ANOVA was performed considering the percentage dissolved as the random variable and formulation and time as class variables (*40*).

Design of Experiment (DoE) for Robustness Evaluation

Robustness of the dissolution method was evaluated with batch N2 (n = 6) by carrying out a fractional factorial design (2^{4-1}). The DoE was created in Protimiza Experiment Design Software (http://experimental-design.protimiza.com.br). Four variables (X factors) were adopted: pH of the dissolution medium, volume of dissolution medium, degassing in the preparation of the dissolution medium, and sampling type (manual or automatic). Table 1 presents such variables and their respective levels. Percentage of API dissolved at each sampling time point (Y factor) was evaluated as the response. Effects were evaluated at 95% and 90% significance levels.

Table 1. Design of Experiment (DoE) Factors and Levels

Test no.	Coded Values				Real Values			
	X1	X2	X3	X4	X1	X2	X3	X4
1	-1	-1	-1	-1	1.9	800	No	Automatic
2	1	-1	-1	1	2.1	880	No	Manual
3	-1	1	-1	1	1.9	920	No	Manual
4	1	1	-1	-1	2.1	920	No	Automatic
5	-1	-1	1	1	1.9	880	Yes	Manual
6	1	-1	1	-1	2.1	880	Yes	Automatic
7	-1	1	1	-1	1.9	920	Yes	Automatic
8	1	1	1	1	2.1	920	Yes	Manual

X1: medium pH; X2: medium volume (mL); X3: degassing; X4: sampling type.

Validation of the Quantification Method

The quantification method used in the dissolution test was validated according to international guidelines. Specificity, linearity, precision, and accuracy were evaluated (*21*, *41*). Solution stability was also verified. Specificity was determined through injection of standard solutions (concentration = 0.0135 mg/mL) and placebo solutions obtained after a dissolution run and proper dilution. The placebo was composed of all constituents of the N2 formulation without nevirapine. Placebo interference was calculated (*37*). The linearity was evaluated through dilution of the nevirapine stock solution (concentration = 0.054 mg/mL) into dissolution medium at six concentrations levels (20%, 40%, 60%, 80%, 100%, and 120%) of the drug working concentration (0.0135 mg/mL). The determination of accuracy was accomplished by adding known amounts of nevirapine to the placebo solution to obtain the concentrations at 80%, 100%, and 120% levels. Each concentration was prepared in triplicate, and the percentage of recovery was calculated. The repeatability and the intermediate precision on consecutive days were established by performing the dissolution test with sample collection at 45 minutes. Relative standard deviations (RSD) were calculated.

The stability of nevirapine in 0.1-M phosphate buffer pH 2.0 was evaluated under storage condition at room

temperature. Samples were collected at 0 h, 6 h, 24 h, 48 h, and 72 h, filtering into the vial using a 0.22-µm PTFE syringe filter. Standard solution and sample solution were evaluated.

RESULTS AND DISCUSSION

Solubility Studies

Nevirapine exhibits pH-dependent solubility (Table 2), which has been reported in the literature (*42*, *43*). The dose/solubility ratio was found to be greater than 250 only in pH 1.2 medium. This result suggests that nevirapine has low solubility in the other media (pH 4.5 and 6.8). RSD was lower than 5%, indicating low variation between replicates and indicating reliability of the results. The sink condition was calculated, and it requires that drug solubility be greater than three times the total concentration of drug in the dissolution vessel (*37*). Sink condition was not achieved for buffer pH 4.5 and 6.8 because the dissolution should be performed in 900-mL vessels; near sink conditions were observed at pH 2.0. It is possible to perform the dissolution test in non-sink conditions; however, the method may have robustness problems (*44*). Therefore, it is necessary to assess whether small changes in dissolution conditions will have an impact on the amount of drug dissolved.

Table 2. Equilibrium Solubility (mg/mL) of Nevirapine in Different Media

Time (h)	pH 1.2	pH 2.0	pH 4.5	pH 6.8
0	2.14 (0.42)	0.56 (0.60)	0.12 (0.21)	0.11 (1.41)
2	2.18 (0.97)	0.57 (2.26)	0.12 (0.24)	0.11 (0.90)
6	2.19 (1.68)	0.57 (1.84)	0.12 (0.07)	0.11 (0.23)
12	2.13 (0.20)	0.56 (1.12)	0.12 (0.18)	0.11 (0.50)
24	2.17 (0.58)	0.56 (0.60)	0.12 (0.21)	0.11 (1.41)

Values are mean (relative SD).

Filter Suitability for Dissolution Test

The filtration step is fundamental in drug dissolution tests and should be evaluated during method development (*45*). In this study, tests were carried out to assess leaching, efficiency, and adsorption (*6*). No new peaks were observed in the chromatograms of the filtered medium, thus no leachability occurred for the 35-µm UHMWPE and 0.22-µm PTFE filters. Also, drug adsorption was not observed on the filter membranes: 0% variation in peak area between the filtered and unfiltered samples. In the filtration efficiency test, the samples kept in an ultrasound bath showed no significant increase in the nevirapine peak area (0% for the 35-µm UHMWPE filter and 1% for the 0.22-µm PTFE filter). The filter suitability tests showed that the 35-µm UHMWPE filter for sample collection and the 0.22-µm hydrophilic PTFE syringe filter used for sample preparation are suitable.

ATP and Risk Assessment for Identification of CMP and CMA

The established ATP for nevirapine tablet dissolution method must present the performance characteristics of the method with the intended target to guarantee the application throughout the life cycle (*21*, *33*, *37*, *46*). The scientific literature presents several papers with the application of AQbD and definition of ATP for chromatographic methods (*29–31*, *47*, *48*). However, there are no studies that define the ATP for dissolution methods, considering the performance characteristics. For the ATP established in this work (Table 3), we considered the performance characteristics for the dissolution and quantification methods as defined in the ICH Q2R2 (*21*).

The dissolution method must have adequate discriminative power for nevirapine 200-mg tablets. AQbD principles begin with elaboration of the ATP from an identified CQA. As dissolution is a CQA for the nevirapine 200-mg tablet, as previously established in the QTTP by the authors (unpublished data), the ATP link to the CQA was established. Understanding of the analytical procedure and link to the CQA allowed the definition of performance characteristics that ensure the quality of the measured dissolution result (Table 3).

A risk assessment was carried out through the construction of an Ishikawa diagram to define the CMP that may have a potential impact on the CMA and consequently on the performance of the dissolution method (Fig. 1). The Ishikawa diagram is the most adopted tool for the risk assessment of cause-effect phenomena (*49*, *50*). The percentage of API dissolved at each time point of the dissolution profile has been previously identified as a CMA. Factors related to people, equipment, measurement, and milieu are not considered CMAs, as they are controlled in the laboratory routine, such as training analysts in standard operating procedures, qualification of equipment, and control of the environmental conditions. As the method used is described in USP, some method parameters, such as apparatus, were not considered for the DoE study (*37*). Robustness was evaluated with the most critical factors, i.e., medium pH and volume, degassing, and type of sampling.

Dissolution Profiles

The dissolution profiles of the formulations are shown in Figure 2. Batches N1 and N2 represent the reference

Table 3. Analytical Target Profile for Dissolution Method for Nevirapine Tablets

Performance Parameter	Dissolution Test		Quantification Test	
	Target	Rationale	Target	Rationale
Selectivity and specificity	Statistically significant difference between batches (*21*)	Parameter assessed based on *USP* <1092> (*37*); discriminatory power demonstration.	No interference from excipients and dissolution medium (≤ 2%) (*37*)	Parameter assessed based on *USP* <1092> (*37*); API quantification shall not to be affected by the presence of other substances
Range	Not applicable (*21*)	Not applicable (*21*)	Interval between the upper and lower concentrations of the API observed in the dissolution profile (*37*)	Parameter assessed based on *USP* <1092> (*37*); stated range for intended use of the procedure
Accuracy	Not applicable (21)	Not applicable (*21*)	95–105% recovery (*37*)	Parameter assessed based on *USP* <1092> (*37*) and ICH Q2R2 (*21*) to ensure quality reportable results
Precision	RSD of ≤ 10% at time points with < 85% dissolved and ≤ 5% for time points > 85% (*37*)	Parameter assessed based on *USP* <1092> (*37*) to ensure quality reportable results	RSD ≤ 5% at specification time point (*37*)	Parameter assessed based on *USP* <1092> (*37*) and ICH Q2R2 (*21*) to ensure quality reportable results

CQA: critical quality attributes; RSD: relative standard deviation; USP: United States Pharmacopeia; API: active pharmaceutical ingredient; ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

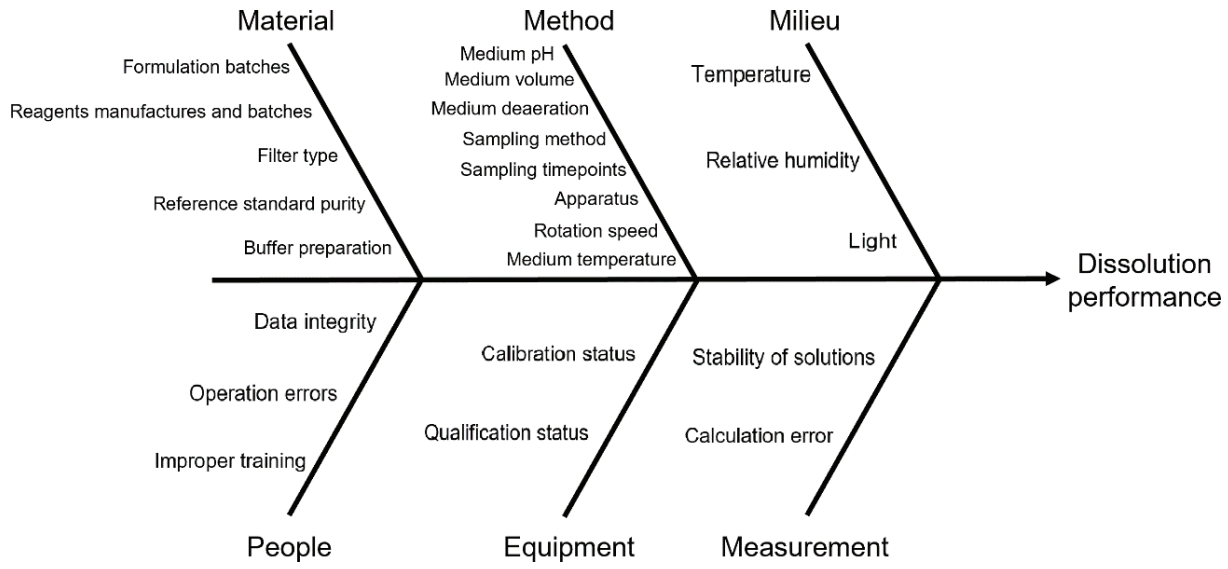

Figure 1. Ishikawa diagram used to identify critical method parameters for dissolution performance.

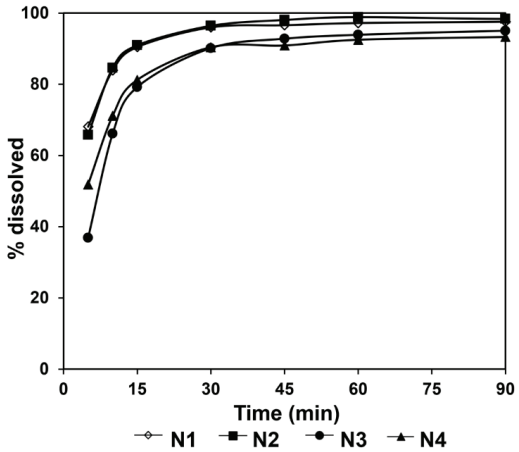

Figure 2. Dissolution profile of nevirapine batches N1, N2, N3, and N4.

product and the formulation with the API from manufacturer B, respectively. Batches N1 and N2 showed very fast dissolution (> 85% within 15 minutes), thus, the similarity of the profiles and the compliance with the ATP (i.e., link to CQA) was confirmed. Batches N3 and N4 showed fast dissolution (85% in 30 minutes), with the type of dissolution profile being different from batches N1 and N2, which proves the discriminative power of the method (selectivity for dissolution method described in the ATP). Because the calculation of the similarity factor (f_2) loses its discriminative power when very fast profiles are observed, and the difference between the dissolution of the formulations was proven by the difference in the profile types, f_2 was not calculated; however, the DE and ANOVA were used for comparison.

DE obtained for N1, N2, N3, and N4 were 88%, 89%, 78%, and 79%, respectively. By increasing hardness and reducing the amount of disintegrant in the formulation, the DE was lower. Comparison of DE values revealed a statistically significant difference ($p < 0.05$) between the formulations, thereby proving the discriminatory power of the method. DE is closely related to the performance of the formulations. It is then possible to evaluate the behavior of the formulations in comparison with each other and with the ideal 100% release.

Considering the DE results, it can be concluded that, this dissolution method is also relevant in the context of QbD, as it differentiates formulation attributes and changes in critical process parameters. A *p*-value < 0.05 indicated a statistical difference between the dissolution profiles. During drug development, several experimental formulations were produced to evaluate the discriminative power of the method and to evaluate the production process. In this case, it was found that the tablet disintegration in the vessel was important for the discriminative power of the dissolution method. Therefore, the previously described deliberate changes to the formulations were made.

Batch N2, the final test formulation with the API from manufacturer B, had acceptable RSD values for the dissolved amount of 5%, 3%, 2%, 2%, 2%, 3%, and 2% at times points of 5, 10, 15, 30, 60, and 90 minutes, respectively. This result is in accordance with precision of the dissolution method described in the ATP, as the RSD was ≤ 5%.

DoE for Robustness

Assessment for robustness of the dissolution method must involve evaluating the impact of small variations on the percentage dissolved. Robustness is traditionally determined by varying one factor at a time (*51*). In this study, DoE was performed with factors selected from the construction of the Ishikawa diagram. pH and volume can be critical due to possible analytical errors in the preparation of the medium, and the type of sampling and degassing are also essential for the application of the method in the quality control routine. Thus, these factors were selected.

The effects of variables (X factors) on responses (Y) were evaluated according to Table 4. Comparison of the pH was statistically significant ($p < 0.05$) for Y1 versus Y4. The pH factor was the CMP that had the most impact on the responses; the pH decrease caused an increase in the percentage dissolved of nevirapine between 5 and

Table 4. Main Effects and p-Values Obtained from Fractional Factorial Design

	Y1 Effect	p-value	Y2 Effect	p-value	Y3 Effect	p-value	Y4 Effect	p-value	Y5 Effect	p-value	Y6 Effect	p-value	Y7 Effect	p-value
Average	56.54	< 0.0001	76.35	< 0.0001	84.63	< 0.0001	91.0	< 0.0001	92.71	< 0.0001	93.5	< 0.0001	94.292	< 0.0001
X1	-15.17	< 0.0001	-13.79	< 0.0001	-12.25	< 0.0001	-3.8	0.0048	-2.00	0.1056	-1.67	0.1515	-1.250	0.2456
X2	0.75	0.6764	0.21	0.8958	-0.42	0.8341	-1.2	0.3705	-1.17	0.3403	-1.83	0.1155	-1.333	0.2160
X3	3.50	0.0563	2.13	0.1861	0.08	0.9666	0.7	0.6077	-1.08	0.3756	-1.58	0.1725	-0.333	0.7551
X4	-3.75	0.0415	-1.71	0.2861	-1.25	0.5306	1.2	0.3705	0.42	0.7322	0.08	0.9421	0.750	0.4838

X1: medium pH; X2: medium volume; X3: degassing; X4: sampling type; Y1: % dissolved at 5 min; Y2: % dissolved at 10 min; Y3: % dissolved at 15 min; Y4: % dissolved at 30 min; Y5: % dissolved at 45 min; Y6: % dissolved at 60 min; Y7: % dissolved at 90 min.

30 minutes. The use of degassed medium favored the dissolution of nevirapine during the first 5 minutes of the test ($p < 0.1$). The type of sampling also had an effect at 5 minutes ($p < 0.05$), with automatic sampling resulting in a higher percentage dissolved. The initial time points are expected to have greater variation and less robustness (*52*).

Establishing a control strategy is part of the AQbD approach and should be derived from data collected during method development phase (*33*). For the nevirapine dissolution method, pH control is the key element for proper method performance throughout the life cycle. Therefore, the medium must be carefully prepared with a pH of 2.0. In addition, for an adequate specification of the dissolution method, a time point of 45 minutes is recommended, as this time is the beginning of the plateau and presents robustness, demonstrated by the DoE (*19, 20*). The current specification described in *USP* is 60 minutes, but a 45-minute specification allows the reduction of testing time in quality control.

Validation of the Quantification Method

The specificity was demonstrated because no interference of excipients was observed. The quantification method showed good linearity at the concentration range of 20–120%. Correlation coefficient was $R^2 = 0.9999$ (*41*).

The accuracy of the method was considered adequate (between 95% and 105%) (*37*). Recovery results were 99% at 80% level (with replicate values of 98.9%, 99.2%, and 98.7%); 99% at 100% level (with replicate values of 99.4%, 98.8%, and 99.3%); and 99% at 120% level (with replicate values of 99.1%, 99.3%, and 99.2%). Repeatability and intermediate precision were evaluated, and RSD was ≤ 2% (with replicate values for analyst A of 92.5%, 89.3%, 93.5%, 97.2%, 98.6% ,and 95.7% and for analyst B of 97.1%, 97.0%, 97.8%, 93.4%, 100.5%, and 102.4%), demonstrating good precision (*37*). RSD obtained for repeatability was 3.6% and for intermediate precision was 3.8%.

The stability of nevirapine in 0.1-M sodium phosphate buffer pH 2.0 was evaluated up to 72 h. The RSD for the standard solution and sample solution was 1.2% (recovery of 101%) and 1.1% (recovery of 103%), respectively, being below the 2% acceptance limit (*37*). Thus, the solutions can be stored, prior to quantification, for up to 72 h at room temperature.

CONCLUSION

In this work, a systematic approach to development of the dissolution method for nevirapine tablets was demonstrated. The AQbD process was carried out including the definition of ATP and the use of experimental design as a multivariate approach for robustness. The suitability of the pharmacopoeia method with discriminative power for the product was demonstrated. The DoE allowed identifying the pH as the CMP to be controlled during the life cycle of the method. The quantification and dissolution methods can be used as a routine quality control test once the analytical validation has proven their performance. This study can be used as a reference for the development and evaluation of dissolution methods in the pharmaceutical industry, bringing scientific knowledge closer to regulatory requirements.

DISCLOSURES

This study was funded by Faperj, Brazil, grant no. E-26/010.002580/2019 and grant no. E-26/211262 /2019. The authors have no conflicts of interest.

REFERENCES

1. Balzarini, J. Current status of the non-nucleoside reverse transcriptase inhibitors of human immunodeficiency virus type 1. *Curr. Top. Med. Chem*. **2004**, *4* (9), 921–944. DOI: 10.2174/1568026043388420.
2. de Béthune, M. P. Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: a review of the last 20 years (1989–2009). *Antiviral Res*. **2010**, *85* (1), 75–90. DOI: 10.1016/j.antiviral.2009.09.008.
3. Garkal, A.; Avachat, A. Development and in-vitro in-vivo characterization of in-situ gelling sustained-release nevirapine suspension. *J. Drug Deli.v Sci Technol*. **2022**, *67*, 102938. DOI: 10.1016/j.jddst.2021.102938.
4. Cheeseman, S. H.; Hattox, S. E.; McLaughlin, M. M.; Koup, R. A.; Andrews, C.; Bova, C. A.; Pav, J. W.; Roy, T.; Sullivan, J. L.; Keirns, J. J. Pharmacokinetics of nevirapine: initial single-rising-dose study in humans. *Antimicrob Agents Chemother*. **1993**, *37* (2), 178–182. DOI: 10.1128/AAC.37.2.178.
5. Amidon, G. L.; Lennernäs, H.; Shah, V. P.; Crison, J. R. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res*. **1995**, *12* (3), 413–420. DOI: 10.1023/A:1016212804288.
6. Mann, J.; Hermans, A.; Contrella, N.; Nickerson, B.; Coutant, C. A.; Jede, C.; Kao, S.; Kou, D.; Scheubel, E.; Winge, F.; Milsmann, J. Dissolution method troubleshooting: an industry perspective. *Dissolut. Technol*. **2022**, *29* (4), 190–203. DOI: 10.14227/DT290422P190.
7. Medeiros, J. J. S.; Costa, T. M.; Carmo, M. P.; Nascimento, D. D.; Lauro, E. N. C.; Oliveira, C. A.; Duque, M. D.; Prado, L. D. Efficient drug development of oseltamivir capsules based on process control, bioequivalence and PBPK modeling. *Drug Dev. Ind. Pharm*. **2022**, *48* (4), 146–157. DOI: 10.1080/03639045.2022.2102647.

8. Alves, J. M.; Prado, L. D.; Rocha, H. V. Evaluation and correlation of the physicochemical properties of carvedilol. *Pharm. Dev. Technol.* **2016**, *21* (7), 856–866. DOI: 10.3109/10837450.2015.1073740.
9. Chang, R. K.; Raw, A.; Lionberger, R.; Yu, L. Generic development of topical dermatologic products: formulation development, process development, and testing of topical dermatologic products. *AAPS J*. **2013**, *15* (1), 41–52. DOI: 10.1208/s12248-012-9411-0.
10. Ansari, M.; Kazemipour, M.; Talebnia, J. The development and validation of a dissolution method for clomipramine solid dosage forms. *Dissolut. Technol*. **2004**, *11* (3), 16–24. DOI: 10.1208/s12248-008-9034-7.
11. Dickinson, P. A.; Lee, W. W.; Stott, P. W.; Townsend, A. I.; Smart, J. P.; Ghahramani, P.; Hammett, T.; Billett, L.; Behn, S.; Gibb, R. C.; Abrahamsson, B. Clinical relevance of dissolution testing in quality by design. *AAPS J*. **2008**, *10* (2), 380–390. DOI: 10.14227/DT150208P31.
12. Soni, T.; Nagda, C.; Gandhi, T.; Chotai, N. P. Development of discriminating method for dissolution of aceclofenac marketed formulations. *Dissolut. Technol*. **2008**, *15* (2), 31–35. DOI: 10.1208/s12249-018-1197-7.
13. Gray, V. A. Power of the dissolution test in distinguishing a change in dosage form critical quality attributes. *AAPS PharmSciTech* **2018**, *19* (8), 3328–3332. DOI: 10.1208/s12249-018-1197-7.
14. Khan, A. Development and validation of a discriminatory dissolution testing method for orally disintegrating tablets (ODTs) of domperidone. *Dissolut. Technol*. **2017**, *24* (2), 28–36. DOI: 10.14227/DT240217P28.
15. Khan, A.; Iqbal, Z.; Ullah, A.; Khadra, I.; Sherazi, M.; Zeb, N. Discriminatory dissolution testing for liquisolid compacts containing a poorly water-soluble drug (Hydrochlorothiazide). *Dissolut. Technol*. **2019**, *26* (1), 46–54. DOI: 10.14227/DT260119P46.
16. Sperandeo, N. R.; Kassuha, D. E. Development and validation of a dissolution test for 6 mg deflazacort tablets. *Sci. Pharm*. **2009**, *77* (4), 679–694. DOI: 10.3797/scipharm.090405.
17. Alves, J. M.; Prado, L. D.; Rocha, H. V. Dissolution Method Evaluation for Carvedilol Tablets. *Dissolut. Technol*. **2020**, *27* (1), 30–35. DOI: 10.14227/DT270120P30
18. Dissolution Testing of Immediate-Release Solid Oral Dosage Forms; Guidance for Industry. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, 1997.
19. Reflection paper on the dissolution specification for generic solid oral immediate release products with systemic action. EMA/CHMP/CVMP/QWP/336031/2017, Committee for Medicinal Products for Human Use, Committee for Medicinal Products for Veterinary use, Quality Working Party, European Medicines Agency, 2017.
20. Guia de Dissolução Aplicável a Medicamentos Genéricos, Novos e Similares. Guia no. 14/2018 – versão 2. Brazilian Health Regulatory Agency (Anvisa), 2022.
21. Q2(R2) Validation of Analytical Procedures: Step 5 Revision 1. ICH Harmonised Tripartite Guideline. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2023.
22. Kamalakkannan, V.; Puratchikody, A.; Ramanathan, L.; Jayapraba, S. Development and validation of a dissolution test with reversed-phase high performance liquid chromatographic analysis for Candesartan cilexetil in tablet dosage forms. *Arab. J. Chem*. **2016**, *9*, S867–S873. DOI: 10.1016/j.arabjc.2011.09.011.
23. Zaman, B.; Siddique, F.; Hassan, W. RP-HPLC method for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form and its application to in vitro dissolution studies. *Chromatographia* **2016**, *79* (23-24), 1605–1613. DOI: 10.1007/s10337-016-3179-9.
24. Pebriana, R. B.; Damayanti, O.; Agustin, Y. D.; Lukitaningsih, E.; Bestari, A. N. Validation of a high-performance liquid chromatographic method for the assay and dissolution of captopril in mucoadhesive tablet formulation. *J. Appl. Pharm. Sci*. **2021**, 11 (2), 66–74. DOI: 10.7324/JAPS.2021.110209.
25. Prajapati, P. B.; Bagul, N.; Kalyankar, G. Implementation of DoE and risk-based enhanced analytical quality by design approach to stability-indicating RP-HPLC method for stability study of bosutinib. *J. AOAC Int*. **2021**, *104* (6), 1742–1753. DOI: 10.1093/jaoacint/qsab078.
26. Moreira, C. S.; Lourenço, F. R. Development and optimization of a stability-indicating chromatographic method for verapamil hydrochloride and its impurities in tablets using an analytical quality by design (AQbD) approach. *Microchem. J*. **2020**, *154*, 104610. DOI: 10.1016/j.microc.2020.104610.
27. Prajapati, P.; Shah, H.; Shah, S. A. Implementation of QRM and DoE-based quality by design approach to VEER chromatography method for simultaneous estimation of multiple combined dosage forms of paracetamol. *J. Pharm. Innov*. **2022**, *17*, 2–18. DOI: 10.1007/s12247-020-09490-w.
28. Lopes, I. J. N.; Fujimori, S. K.; Mendes, T. C.; Almeida, R. A. D.; Sousa, F. F. M.; Oliveira, C. A.; Nascimento, D. D.; Lourenço, F. R.; Rodrigues, M. I.; Prado, L. D. Application of analytical quality by design (AQbD) for stability-indicating method development for quantification of nevirapine and its degradation products. *Microchem. J*. **2024**, *199*, 109939. DOI: 10.1016/j.microc.2024.109939.
29. Tome, T.; Časar, Z.; Obreza, A. Development of a unified reversed-phase HPLC method for efficient determination of EP and USP process-related impurities in celecoxib using analytical quality by design principles. *Molecules* **2020**, *25* (4), 809. DOI: 10.3390/molecules25040809.
30. Corredor, C. C.; Lozano, R.; Bu, X.; McCann, R.; Dougherty, J.; Stevens, T.; Both, D.; Shah, P. Analytical method quality by design for an on-line near-infrared method to monitor blend potency and uniformity. *J. Pharm. Innov*. **2015**, *10* (1), 47–55. DOI: 10.1007/s12247-014-9205-2.
31. Muchakayala, S. K.; Katari, N. K.; Saripella, K. K.; Schaaf,

H.; Marisetti, V. M.; Ettaboina, S. K.; Rekulapally, V. K. Implementation of analytical quality by design and green chemistry principles to develop an ultra-high performance liquid chromatography method for the determination of Fluocinolone Acetonide impurities from its drug substance and topical oil formulations. *J. Chromatogr. A* **2022**, *1679*, 463380. DOI: 10.1016/j.chroma.2022.463380.

32. Kaur, R.; Saini, S.; Sharma, T.; Katare, O. P.; Kaushik, A.; Singh, B. Implementation of Analytical Quality-By-Design for developing a robust HPLC method for quantitative estimation of voriconazole: application in drug formulations. *Anal. Chem. Lett*. **2021**, *11* (2), 168–186. DOI: 10.1080/22297928.2021.1888793.
33. Q14 on Analytical Procedure Development: Step 5. ICH Harmonised Tripartite Guideline. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2024.
34. Bloomfield, M. S.; Butler, W. C. Robustness testing, using experimental design, of a flow-through dissolution method for a product where the actives have markedly differing solubility properties. *Int. J. Pharm*. **2000**, *206* (1-2), 55–61. DOI: 10.1016/S0378-5173(00)00509-3.
35. Leon, R. M.; Issa, M. G.; Duque, M. D.; Daniel, J. S. P.; Ferraz, H. G. Development of a discriminative dissolution method, using in-silico tool for hydrochlorothiazide and valsartan tablets. *Pharmaceutics* **2023**, *15* (6), 1735. DOI: 10.3390/pharmaceutics15061735.
36. Fu, M.; Conroy, E.; Byers, M.; Pranatharthiharan, L.; Bilbault, T. Development and validation of a discriminatory dissolution model for an immediately release dosage form by DOE and statistical approaches. *AAPS PharmSciTech* **2021**, *22* (4), 140. DOI: 0.1208/s12249-021-02011-z.
37. <1092> The dissolution procedure: development and validation. In *USP–NF*. United States Pharmacopeia, 2023. DOI: 10.31003/USPNF_M643_05_01.
38. Liliana, L. A new model of Ishikawa diagram for quality assessment. *IOP Conf. Ser.: Mater. Sci. Eng*. **2016**, *161*, 012099. DOI: 10.1088/1757-899X/161/1/012099.
39. Khan, K. A.; Rhodes, C. T. Effect of compaction pressure on the dissolution efficiency of some direct compression systems. *Pharm. Acta Helv*. **1972**, *47* (10), 594–607.
40. Muselík, J.; Komersová, A.; Kubová, K.; Matzick, K.; Skalická, B. A critical overview of FDA and EMA statistical methods to compare in vitro drug dissolution profiles of pharmaceutical products. *Pharmaceutics* **2021**, *13* (10), 1703. DOI: 10.3390/pharmaceutics13101703.
41. Anvisa Resolução da Diretoria Colegiada - RDC no. 166. Brazilian Health Regulatory Agency (Anvisa), 2017.
42. Chadha, R.; Arora, P.; Garg, M.; Bhandari, S.; Jain, D. S. Thermoanalytical and spectroscopic studies on different crystal forms of nevirapine. *J. Therm. Anal. Calorim*. **2013**, *111* (3), 2133–2142. DOI: 10.1007/s10973-012-2666-y.
43. Stieger, N.; Caira, M. R.; Liebenberg, W.; Tiedt, L. R.; Wessels, J. C.; De Villiers, M. M. Influence of the composition of water/ethanol mixtures on the solubility and recrystallization of nevirapine. *Cryst. Growth Des*. **2010**, *10* (9), 3859–3868. DOI: 10.1021/cg901501d.
44. Simon, A.; de Almeida Borges, V. R.; Cabral, L. M.; de Sousa, V. P. Development and validation of a discriminative dissolution test for betamethasone sodium phosphate and betamethasone dipropionate intramuscular injectable suspension. *AAPS PharmSciTech* **2013**, *14* (1), 425–434. DOI: 10.1208/s12249-012-9920-2.
45. Smith, J.; George, J.; Nadler, T.; Joshi, V. Filters in dissolution testing: Evaluation and selection. *Dissolut. Technol*. **2020**, *27* (4), 6–13. DOI: 10.14227/DT270420P6.
46. Jackson, P.; Borman, P.; Campa, C.; Chatfield, M.; Godfrey, M.; Hamilton, P.; Hoyer, W.; Norelli, F.; Orr, R.; Schofield, T. Using the analytical target profile to drive the analytical method lifecycle. *Anal. Chem*. **2019**, *91* (4), 2577–2585. DOI: 10.1021/acs.analchem.8b04596.
47. Marie, A. A.; Hammad, S. F.; Salim, M. M.; Elkhodary, M. M.; Kamal, A. H. Deduction of the operable design space of RP-HPLC technique for the simultaneous estimation of metformin, pioglitazone, and glimepiride. *Sci. Rep*. **2023**, *13* (1), 4334. DOI: 10.1038/s41598-023-30051-x.
48. Teng, J.; Zhu, C.; Lyu, J.; Pan, L.; Zhang, M.; Zhang, F.; Wu, H. Analytical Lifecycle Management (ALM) and Analytical Quality by Design (AQbD) for analytical procedure development of related substances in tenofovir alafenamide fumarate tablets. *J. Pharm. Biomed. Anal*. **2022**, *207*, 114417. DOI: 10.1016/j.jpba.2021.114417.
49. Szoleczky, R.; Budai-Szűcs, M.; Csányi, E.; Berkó, S.; Tonka-Nagy, P.; Csóka, I.; Kovács, A. Analytical quality by design (AQbD) approach to the development of in vitro release test for topical hydrogel. *Pharmaceutics* **2022**, *14* (4), 707. DOI: 10.3390/pharmaceutics14040707.
50. Moure, R. B.; Sousa, F. F. M.; Nascimento, D. D.; Magalhães, J. L.; Oliveira, C. A.; Prado, L. D. Definições e escopo dos elementos-chave do Analytical Quality by Design (AQbD) para o desenvolvimento de métodos na indústria farmacêutica. *Vigil Sanit Debate* **2023**, *11*, e02162. DOI: 10.22239/2317-269x.02162
51. Gray, V.; Kelly, G.; Xia, M.; Butler, C.; Thomas, S.; Mayock, S. The science of USP 1 and 2 dissolution: present challenges and future relevance. *Pharm. Res*. **2009**, *26* (6), 1289–1302. DOI: 10.1007/s11095-008-9822-x.
52. Suarez-Sharp, S.; Abend, A.; Hoffelder, T.; Leblond, D.; Delvadia, P.; Kovacs, E.; Diaz, D. A. In vitro dissolution profiles similarity assessment in support of drug product quality: what, how, when—workshop summary report. *AAPS J*. **2020**, *22* (4), 74. DOI: 10.1208/s12248-020-00458-9.