

Dissolution Profile of Apixaban Tablets: Method Development and Validation Using HPLC Analysis

Natália Olegário dos Santos*, Nathalie Ribeiro Wingert, and Martin Steppe

Laboratory of Pharmaceutical Quality Control, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil.

e-mail: nati.olegario@hotmail.com

ABSTRACT

Apixaban is an anticoagulant agent that inhibits factor Xa, commercially available as coated tablets at the dosages of 2.5 and 5.0 mg. There is no official monograph of the formulation in the current international pharmacopoeias. From research and development to finished product quality control, the dissolution test is an important tool to evaluate the quality of pharmaceutical formulations. This study aimed to develop and validate an in vitro method to assess the dissolution profile of apixaban immediate-release (5 mg) coated tablets. Several conditions were tested in this study until the most suitable one was reached. The selected method included the following parameters: 0.01 M hydrochloric acid (pH 2.3, 500 mL), USP paddle apparatus, 75 rpm, 37 °C, with seven sampling points and a total test time of 90 minutes. Quantitative analysis was performed by high performance liquid chromatography using a previously validated method. The dissolution method was validated following the official guidelines, demonstrating specificity, linearity, precision, accuracy, and robustness. This study enabled the development of an adequate, effective, and reliable method, which contributes to the evaluation of apixaban release in new products and the quality control of formulations containing this drug.

KEYWORDS: Apixaban, dissolution, validation, HPLC, quality control, analytical method

INTRODUCTION

Apixaban (APX) is a direct, highly selective, and reversible inhibitor of the activated coagulation factor X (FXa), whose effect does not depend on antithrombin for antithrombotic action. The drug acts by inhibiting the FXa (free and bound) to the clot and also the activity of prothrombinase, thereby preventing the generation of thrombin and thrombus formation (1). APX is indicated for the prevention of venous thromboembolism after elective hip or knee arthroplasty, reduction of the risk of systemic embolism and stroke in patients with non-valvular atrial fibrillation, treatment of deep venous thrombosis, and to prevent the recurrence of venous thromboembolism after the occurrence of a thromboembolic event (2). APX is currently sold under the trade name of Eliquis (Bristol-Myers and Pfizer, USA) as coated tablets at dosages of 2.5 and 5 mg. APX has an acidic pKa of 13.12 and a basic of -1.60, therefore it does not ionize at physiological pH and remains in its neutral form (3). According to the Biopharmaceutical Classification System, APX is a class III molecule with high solubility and low permeability (4). It has an oral bioavailability around

50% and aqueous solubility of about 0.04 mg/mL in the physiological pH range (pH 1.2–6.8) (5).

The dissolution test aims to study the drug release process into a solvent over time (6). The test aims to reproduce as closely as possible what will happen with the pharmaceutical form when administered. Therefore, it is important to assess several factors that may influence the process in vivo by selecting appropriate parameters and methodology for the in vitro dissolution test. During the event in which a solid drug is released into a solvent, a liquid film called a “diffusion layer” is formed, which influences the speed of dissolution. According to the Noyes-Whitney equation, the larger the thickness of this layer, the slower the dissolution rate (7). Factors such as temperature and agitation speed are also important because the in vivo temperature is 37 °C and agitation influences the thickness of the diffusion layer in addition to representing the peristaltic movements of the gastrointestinal tract (8). Another factor to consider is the presence of air and gases in the dissolution medium, which can cause changes in the movement of particles, decrease the contact

* Corresponding author.

between solid particles and the liquid medium (i.e., small bubbles may form on the surface of the pharmaceutical form, acting as a barrier), and cause particles to stick to the apparatus or vessel, thereby hindering dissolution and impacting the final test result (9, 10). Also, to perform the tests, the concept of sink condition (i.e., three times the saturation volume within a range of 500 to 1000 mL) should be considered (11).

The dissolution test is a substantial resource both for development and monitoring of formulations and for quality control and characterization of in vitro-in vivo correlations in bioequivalence studies (12). To obtain adequate and reliable results, the method employed must be well characterized and undergo a validation process by measuring the parameters to ensure that the proposed analytical method is appropriate for its purpose (10, 13).

So far, no studies have been found in the literature related to the dissolution process of pharmaceutical products containing APX and the development of analytical methods applied for this purpose. Also, the APX monograph is not reported in official guidelines. Therefore, the present work aimed at proposing an adequate dissolution method to assess this important quality parameter in formulations containing APX.

MATERIALS AND METHODS

Chemicals

The APX chemical standard (> 99%) was obtained from Carbosynth (Berkshire, UK). APX immediate-release (5 mg) coated tablets (Pfizer, USA) were purchased in local drugstores in Porto Alegre, Brazil. All chemicals and reagents used for samples preparation and dissolution media were analytical grade. The mobile phase solvents were HPLC grade.

Stock Solutions Preparation

The Previously weighed APX tablets were crushed into a homogeneous powder. A sample solution from this powder was prepared in methanol. The dispersion was sonicated for 15 minutes, filtered, and stored in an amber flask, resulting in a concentration of 500 µg/mL of APX.

The same process, apart from filtration, was performed for preparation of the standard solution to obtain a final solution, also at the concentration of 500 µg/mL. APX sample and standard stock solutions were diluted in dissolution media up to 10 µg/mL for high performance liquid chromatography (HPLC) analysis.

Solubility Test

To determine the solubility of the APX, a sufficient amount of sample was added to 5 mL of 0.01 M HCl or pure water

up to media saturation. The suspension was stirred for 24 h in a water bath at 37 °C. After this period, the samples were filtered and analyzed in HPLC to determine drug solubility.

Dissolution Medium

Preliminary tests were carried out to choose the dissolution medium. Initially, traditional dissolution media were selected including 0.025 M sodium phosphate (pH 6.8), 0.025 M ammonium acetate (pH 6.4), 0.025 M sodium phosphate + 0.01% SDS (pH 6.7), 0.01 M HCl (pH 2.3), and ultrapure water (pH 6.9). Likewise, 50 mM sodium citrate was tested and sodium dodecyl sulphate (SDS) was added at concentrations of 0.01% and 0.5%.

Dissolution Test

The dissolution test was carried out in a multi-bath dissolution test station (VK 7010, Varian, USA) with automated sampling and filters of 35 µm porosity. The conditions selected were as follows: USP paddle apparatus, 75 rpm, with 500 mL of 0.01 M HCl at 37 ± 0.5 °C. The total test time was 90 min, and samples (5 mL) were collected at the following times: 5, 10, 15, 30, 45, 60, and 90 min.

Analytical Methodology

After the dissolution test, quantitative analysis of the samples was performed in a Shimadzu Prominence (LC-20A) HPLC with diode array detection (HPLC-DAD) with an Inertsil CN-3 column (150 x 4.6 mm; 5 µm) according to a previously validated method (14). The mobile phase used was a mixture of methanol and water (50:50, v/v) with a flow rate of 1.0 mL/min, temperature of 30 °C, and injection volume of 20 µL. UV detection was carried out at 220 nm, and the analysis time was 5 min.

Dissolution Test Validation

The method was validated according to the specifications contained in the official guides and current legislation covering the following analytical parameters: specificity, linearity, accuracy, precision and robustness (10, 15–17).

RESULTS AND DISCUSSION

Average Weight and Drug Content

Average drug content in the tablets was determined to be 103.33% (relative standard deviation [RSD]: 1.01%). This result was considered adequate because it is within the range usually stipulated by official compendia (90–110%).

Dissolution Method Development

The solubility study aimed to determine the drug saturation concentration in aqueous media (0.01 M HCl and ultrapure water) and if the sink condition could be established. In water, APX had a solubility of 49.96 µg/mL,

which is consistent with the literature (i.e., approximately 40.00 µg/mL) (18). In 0.01 M HCl, APX solubility was 40.66 µg/mL. According to the classification of descriptive solubility terms, APX would be considered as a practically insoluble substance; however, this classification refers to the physical chemical solubility of the substance, not taking into account the solubility in equilibrium (i.e., physiological solubility of the drug). The BCS classifies APX as a class III molecule, meaning high solubility and low permeability. In the BCS a drug is considered highly soluble when its maximum oral dose is completely solubilized in 250 mL of solvent. The results found for APX show the high physiological solubility of the drug in pure water and 0.01 M HCl media according to the BCS (4).

During the dissolution test, the maximum concentration obtained in the vessels was 10 µg/mL, because a volume of 500 mL was used for the dissolution of a drug with a dose of 5 mg. Therefore, we can conclude that drug saturation in the dissolution medium is about four times greater than the maximum concentration reached during the tests, which according to the literature indicates to be in sink condition as desired (11).

Several alternatives available in the literature were tested to choose the dissolution medium. Initially, not only the behavior of the drug in the medium was evaluated but also whether the medium was suitable for the analytical method to be used without causing interference that would impair APX quantification. Then, dissolution tests were carried out with the medium defined according to the results of the first stage. Figure 1 presents the dissolution profile of the five media tested.

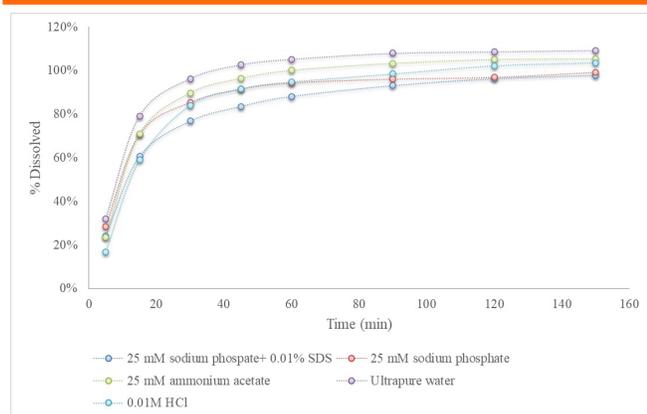


Figure 1. Apixaban dissolution profiles in the tested media.

Tests with phosphate buffer and acetate demonstrated a higher variation between vessels than those tested with other media. In the case of phosphate buffer with SDS, the reason for adding the surfactant was to increase the solubility of the drug in the medium for faster dissolution.

However, it did not show a significant difference in dissolution time compared to other media, including phosphate without the surfactant. Table 1 shows the average percentage of dissolution obtained in each medium and their respective RSD after 90 min. Among the five media tested, two presented results considered satisfactory: 0.01 M HCl and ultrapure water. Both showed more uniform dissolution rates between the vessels, with the highest drug release and lowest RSD. The dissolution medium chosen was 0.01 M HCl because ultrapure water has a greater pH variation that does not allow adequate control over this parameter. Another major factor in choosing 0.01 M HCl as the dissolution medium is related to its greater reproduction and similarity to the in vivo conditions to which the drug is exposed, because APX is for immediate release with disintegration and dissolution in the stomach. In addition, 0.01 M HCl is one of the suggested media for high solubility drugs, as in the case of APX (16).

Table 1. Comparison of Tested Dissolution Media

Dissolution Medium	% Dissolved at 90 min, Mean (RSD)
Ultrapure water	102.59 (0.77)
0.01 M HCl	101.06 (1.46)
25 mM Ammonium acetate	100.07 (3.75)
25 mM Sodium phosphate	99.20 (3.39)
25 mM Sodium phosphate + 0.01% SDS	97.81 (1.05)

RSD = relative standard deviation; HCl = hydrochloric acid; SDS = sodium dodecyl sulphate.

USP apparatus 1 (basket) and 2 (paddle) were available for the dissolution study. Apparatus 2 was chosen because it is the apparatus most commonly used for the evaluation of tablets. Filters with a pore size of 35 µm were used. The rotation speed typically applied to the paddle ranges from 25 to 75 rpm. Agitation at 50 rpm was first tested but the experiment did not generate a complete drug dissolution within 90 min. The use of 75 rpm resulted in a dissolution percentage close to 100% in the total time interval of the test as well as the maintenance of a gradual increase in the drug release percentage. Dissolution test results at 50 and 75 rpm are shown in Figure 2.

Dissolution Method Validation Specificity

The specificity was intended to assess if any compounds present in the formulation or the dissolution medium showed any sign or significant interference that would impair APX quantification. Samples containing only the mixture of excipients were subjected to the dissolution test. The results proved that the excipients did not present any analytical interference.

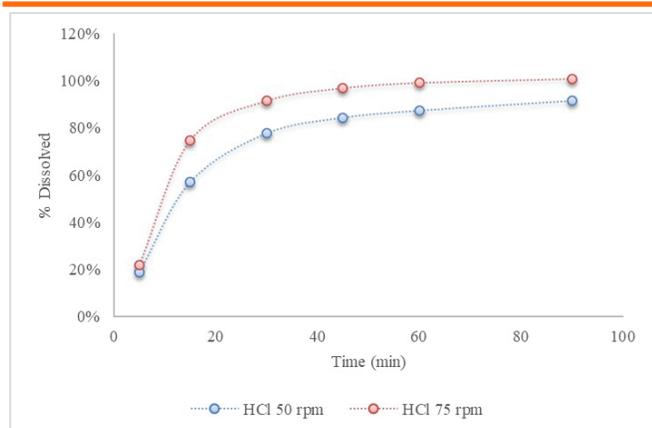


Figure 2. Apixaban dissolution profiles at speeds of 50 and 75 rpm.

Linearity

Linearity was evaluated by generating three standard curves to represent seven concentration levels over the range of 1.0 to 15 µg/mL. The resulting curves obtained were plotted, and the following linear equation was obtained using the least squares method: $y = 67518x - 9982$. The correlation coefficient (R^2) was 0.99995, proving compliance with national and international guidelines (15, 19). Linear regression was also evaluated by one-way analysis of variance (ANOVA). The F value was 50,034.83 between the curves, also confirming that the calculated values represent a linear regression ($F_{\text{calc}} > F_{\text{tab}}$, $p < 0.05$). The residues were also analyzed, and randomness was perceived with no bias, demonstrating homoscedasticity of the results.

Precision

To assess precision, three dissolution tests with six vessels each were performed for three different days, and the repeatability of the dissolution profile was evaluated at all sampling points. Repeatability (intraday) and intermediate precision (interday) were assessed using the RSD values. For high solubility drugs, the percentage of dissolution accepted is at least 80% in 30 min and the RSD values at the sampling points of 5 and 10 min must be below 20%,

and for the other times it must be less than 10% (16, 17). As shown in Table 2, all values were within the stipulated range, demonstrating the precision of the method. Figure 3 shows the dissolution profiles obtained from inter-day precision assessments.

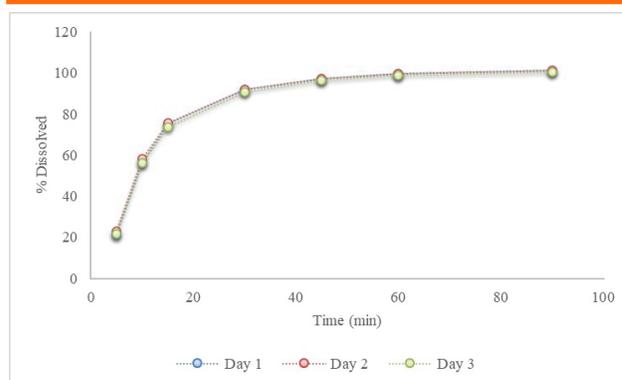


Figure 3. Apixaban dissolution profiles in interday precision.

Accuracy

Three concentration levels were analyzed, low (8 µg/mL), medium (10 µg/mL), and high (12 µg/mL). In the recovery evaluation, it is expected that the values found will be in the range of 95–105% (10). Recovery values for each level studied at the 15-min sampling point were 97.50%, 98.71%, and 90.39% for the low, medium, and high level, respectively. At the end point (90 min), recovery values and concentrations found for the low, medium, and high levels were 99.46% (7.97 µg/mL), 100.87% (10.11 µg/mL), and 98.17 (11.80 µg/mL), respectively, thus confirming method accuracy.

Robustness

Robustness was studied through small changes such as increased filter porosity (70 µm) and medium without deaeration. Two vessels were used for each change, and it was expected that none of the design changes made would significantly affect the results. At 90 min, the results for the modified conditions, i.e., without deaeration (102.79%;

Table 2. Results of Intra- and Interday Precision Tests

	5 min	10 min	15 min	30 min	45 min	60 min	90 min
Day 1	21.45 (12.83)	56.34 (7.76)	75.30 (2.12)	92.02 (0.64)	97.24 (0.96)	99.43 (1.03)	101.13 (1.12)
Day 2	23.05 (14.27)	58.36 (6.43)	75.77 (2.09)	92.12 (1.01)	97.35 (1.12)	99.62 (1.17)	101.20 (1.14)
Day 3	21.70 (9.82)	56.32 (5.25)	73.74 (2.09)	90.87 (0.63)	96.50 (0.72)	98.86 (0.79)	100.51 (0.83)
Interday	22.06 (12.22)	57.01 (6.40)	74.94 (2.30)	91.67 (0.97)	97.03 (0.98)	99.30 (1.01)	100.95 (1.03)

Values are mean (RSD) % dissolved.
RSD = relative standard deviation.

RSD 1.33%) and increased filter porosity (100.06%; RSD 1.10%), were similar to the nominal condition (100.95%; 1.03%), indicating that the changes did not affect the dissolution process. Also, the percentage of drug release at the sampling points remained virtually the same, i.e., approximately 20% in 5 min and 92% in 30 min. Figure 4 corroborates these results through the total overlap of the dissolution profiles of APX in the different conditions.



Figure 4. Apixaban dissolution profiles in robustness test.

CONCLUSION

The present study aimed to develop and validate a dissolution method for APX coated tablets (5 mg). Several conditions were evaluated until reaching the one that proved to be most suitable for this purpose. The preferred method was established under the following conditions: USP paddle apparatus, 75 rpm, 0.01 M HCl (pH 2.3, 500 mL), 37 °C, filters with 35 µm porosity, total experimental time of 90 min, and quantification by HPLC.

The validation was carried out as recommended in the official guidelines and covered the parameters of specificity, linearity, precision, accuracy, and robustness, with all results found in accordance with the recommended validation parameters.

All the objectives presented in this study were achieved as the proposed method demonstrated to be adequate, feasible, and reliable, presenting a profile of gradual dissolution that would favor the detection of potential production process problems in the dissolution performance of the product. This study contributes to the future monograph of APX immediate-release coated tablets as well as the development of new products containing this drug.

CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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