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# Development and validation of a dissolution method for a BCS class IV drug – tadalafil

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## Abstract

The present study describes the development and validation of a dissolution method for tadalafil, a Biopharmaceutical Classification System class II drug. 0.1N hydrochloric acid (HCl)+0.5% sodium lauryl sulphate (SLS), pH 4.5-acetate buffer+0.5% SLS and pH 6.8–phosphate buffer+0.5% SLS were tested as dissolution medium, and influences of apparatus, and rotation speed were evaluated. Samples were analyzed by UV spectrophotometric method at 225 nm. The result also shows a better dissolution profile using pH 6.8–phosphate buffer + 0.5% SLS as medium and paddle as apparatus is a speed of 100 rpm. The conditions that allowed dissolution determination were USP type II apparatus at 100 rpm, containing 900 mL of pH 6.8–phosphate buffer+0.5% SLS as dissolution medium, with analysis at wavelength of 225 nm. Samples were analyzed by UV spectrophotometric method and validated as per ICH guidelines, showing specificity, linearity, precision and accuracy.

**Keywords:** Dissolution, BCS Class IV drug, Validation.

## 1. Introduction

Development of dissolution tests for immediate release oral dosage forms such as tablets and coated tablets has become an important tool for pharmaceutical companies in order to assess lot-to-lot quality of a drug product; to guide development of new drug formulations and ensure consistent product quality and performance after certain changes such as alterations in formulation and the manufacturing process [1–2]. Besides, when is possible an *in vitro*/*in vivo* correlation, dissolution can be used as a test in order to predict the drug bioavailability, thus, being able to determinate drug bioequivalence [3–5].

The development of an analytical method involves an evaluation process to estimate their efficiency on laboratory routine. As long the method are developed, in order to scientifically prove their efficiency, it is essential to carry out the validation of the developed method. Validation must guarantee, by experimental studies, that the method meets the requirements of the analytical applications, ensuring results reliability. Thus, validation is intended to demonstrate that the method is suitable for the intended purpose. This is done by demonstrating parameters such as specificity, linearity, accuracy and precision suitable for analysis [6–13].

Developing an appropriate *in vitro* dissolution test for drug products with lower water solubility has been a challenge for pharmacists. Those drugs, classified into classes II or IV by the Biopharmaceutical Classification System (BCS), depending on their apparent permeability. Drug release is usually the rate-limiting step for oral absorption of these substances[14].

Tadalafil, a drug used on erectile dysfunction treatment, is commercially available on Brazil by the brand name Cialis® as coated tablets with a labeled amount of 20 mg. Tadalafil, a water insoluble drug, is classified as BCS Class II drug and shows a LogP: 1.42 and a pKa: 0.85 [10,14–17]. Low water solubility drugs do not show a good dissolution on water, even at the pH physiological range, and may require the use of surfactants [18]. Surfactants act by reducing the interfacial tension between the drug and the

solvent, increasing drug dissolution. One of the action mechanisms is related to the particle wettability, where the surfactant favors the contact between the drug particle and the surrounding liquid.

As a dissolution methodology for tadalafil tablets is described at USP 39 – NF 34 [10], that method use HPLC in order to quantify the drug. As UV spectrophotometry is a simpler method, and also faster than HPLC,this paper aims to describe the development and validation of a dissolution test for tadalafil coated tablets by an ultraviolet method.

## 2. Materials & Methods

### 2.1 Materials and equipment

All chemicals and reagents were of analytical reagent grade. Pharmaceutical company Prati–Donaduzzi (Toledo, Brazil), kindly donated tadalafil reference substance (99.83 %). The reference drug product (Cialis®), labelled as containing 20 mg of tadalafil and the following excipients (croscarmelose sodium, hydroxypropyl cellulose, hypromellose, magnesium stearate, microcrystalline cellulose, monohydrate lactose, sodium laurylsulphate, titanium dioxide, triacetin and yellow iron oxide) were obtained commercially.

Equipment and instruments used in the present study were analytical scale (Gehaka, AG–200 model), dissolution test apparatus (Nova Ética, 301-6 AUT model), ultrasonic bath (Químis, Q355D model) and UV spectrophotometer (UV-1600 Pró–Análise).

### 2.2 Methodology

#### 2.2.1 Maximum wavelength absorption determination

It was prepared three different standard solutionswith 22.22 µg/mL of tadalafil in the following medium: 0.1N hydrochloric acid (HCl) + 0.5 % sodium lauryl sulphate (SLS), pH 4.5-acetate buffer + 0.5 % SLS and pH 6.8–phosphate buffer + 0.5 % SLS. The samples were submitted to an UV scan spectrum between 800 to 200 nm in order to determinate tadalafil maximum wavelength absorption.

#### 2.2.2 Dissolution test conditions

Dissolution testing of tablets was performed with the reference drug product (Cialis® 20 mg) in accordance with the USP to define the dissolution test conditions. Initially, this was performed using paddles (USP apparatus II) at a stirring speed of 50 rpm and 900 mL of the following dissolution media, pre-heated to 37 °C ± 0.5°C: 0.1N HCl + 0.5 % SLS; pH 4.5–acetate buffer + 0.5 % SLS and pH 6.8–phosphate buffer + 0.5 % SLS. Manual sampling aliquots of 20.0 mL were withdrawn at 5, 10, 15, 30 and 45 minutes, filtered in a Millex® filter and analyzed on a UV/VIS Spectrophotometer (225 nm). There was no medium replacement after the sampling. The tadalafil standard solution was prepared in order to obtain a final concentration of 0.0222 mg/mL.

After these preliminary studies, tests to determinate the apparatus (paddles and baskets) and rotation speed (50 and 100 rpm for paddles; 75 and 100 rpm for baskets) were performed using as dissolution medium pH 6.8–phosphate buffer + 0.5 % SLS maintained at 37 ± 0.5ºC.Also, sampling aliquots of 20.0 mL were withdrawn manually at 5, 10, 15, 30 and 45 minutes, filtered(Millex® filter) and analyzed on a UV/VIS Spectrophotometer (225 nm), together with a standard solution of tadalafil in 22.22 µg/mL final concentration.

#### 2.2.3 Validation

In order to demonstrate the method's suitability for use as a dissolution test, it was validated based on specificity, linearity, precision and accuracy parameters [6,19].

##### 2.2.3.1 Specificity

Placebo samples of Cialis® (a mix of the excipients from tadalafil-coated tablets) were prepared empirically in their usual compositions, according to literature [20–21]. The placebo samples were transferred to different vessels (n=6) with 900 mL of pH 6.8–phosphate buffer + 0.5 % SLS as dissolution medium at 37 °C ± 0.5 °C and stirred for 1 h at 100 rpm using a paddle (USP apparatus II). Aliquots of these solutions were filtered through a Millex® filter and analyzed by the UV method (225 nm).

##### 2.2.3.2 Linearity

A stock solution was prepared containing 222.22 µg/mL of tadalafil using pH 6.8–phosphate buffer + 0.5 % SLS as solvent. Aliquots of this solution were transferred to volumetric flasks to obtain final concentrations of 4.44; 8.89; 13.33; 17.78; 22.22; and 26.67 µg/mL. Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least squares regression method and analysis of variance (ANOVA).

##### 2.2.3.3 Precision

The evaluation of intermediate precision (inter-day precision) of the dissolution test was performed on three different days. The repeatability was evaluated on the same day for intra-day precision in six vessels used for the dissolution test. The relative standard deviation (RSD) from the results was calculated.

##### 2.2.3.4 Accuracy

Accuracy was determinate by the recovery percentage of a known amount of tadalafil reference substance added to a placebo solution. A recovery study was conducted by adding known amounts of the tadalafil stock solution to the dissolution vessels containing the placebo solution (50, 100 and 150 %) of the nominal assay

(20 mg). Each concentration was prepared in triplicate and analyzed by the UV method at 225 nm.

## 3.Results and Discussion

### 3.1. Dissolution method development

As tadalafil is classified as a Class II drug (low solubility and high permeability) in Biopharmaceutical Classification System (BCS), it is justified the use of surfactants in order to improve drug solubility. Sodium lauryl sulphate (SLS), which increased drug dissolution by decreasing the interfacial tension between drug and solvent, was selected as surfactant to be added in an amount of 0.50 % in all the dissolution mediums tested [22].

The first test was to determine in which wavelength tadalafil showed UV absorption. The results of the UV scan spectrum between 800 – 200 nm showed demonstrated that tadalafil showed a maximum absorption at 225 nm (Figure 1).

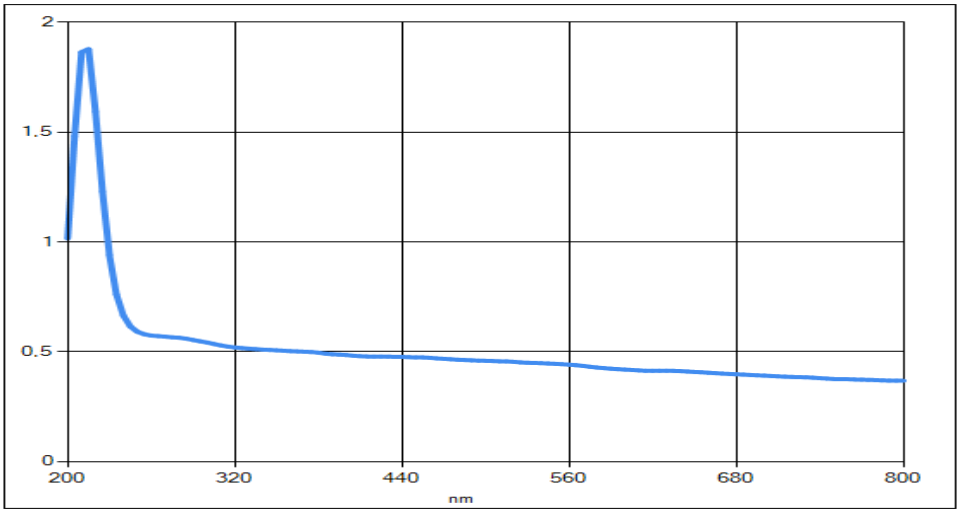


**Figure 1: Tadalafil standard solution UV scan spectrum**

The dissolution profiles in media at physiological pH range (1.2 to 6.8) need to be evaluated for immediate release drugs. Thus, dissolution tests on 0.1 N HCl, pH 4.5-acetate buffer and pH 6.8–phosphate buffer as dissolution medium, all added with 0.50 % SLS allowed evaluate which medium showed better discriminative dissolution profile. The results (Figure 2) showed that there was no total tadalafil dissolution on 0.1 N HCl + 0.5 % SLS, where the final drug dissolution was 74.77 %. Similar results were obtained when pH 4.5-acetate buffer + 0.5 % SLS, when the final drug dissolution was close to 90 % (89.51 %).

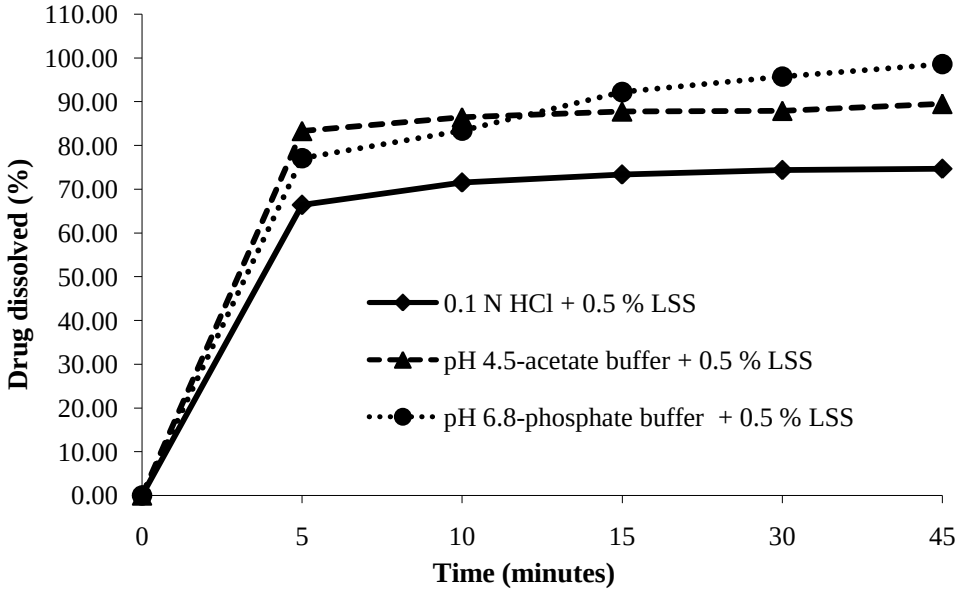


**Figure 2: Dissolution profile of tadalafil tablets in 0.1 N HCl + 0.50 % SLS, pH 4.5-acetate buffer + 0.50 % SLS and pH 6.8-phosphate buffer + 0.50 % SLS.**

The dissolution medium which showed complete tadalafil dissolution was pH 6.8–phosphate buffer + 0.5 % SLS, where the final drug release was 98.59 %. Also, the dissolution profile at five points (5, 10, 15, 30 and 45 minutes) showed that tadalafil dissolved more than 85% in 15 minutes (Figure 2). As tadalafil must be ready to act in the men body around 30 minutes after the drug intake [14], a rapidly drug dissolution is required for immediate-release dosage forms [10,21].

Therefore, the choice of pH 6.8-phosphate buffer + 0.5 % SLS as dissolution medium was due to its good solubility, accessibility, low cost and the fact that it is a typical dissolution medium [23–24].

After the dissolution medium was determinate, apparatus type and speed were tested. The most common dissolution conditions are basket (USP apparatus I) at 50 or 100 rpm as rotation speed and paddles (USP apparatus II) in a rotation of 75 or 100 rpm [13]. The tadalafil dissolution (Figure 3) showed a better result when paddles at 50 rpm are used, when a drug release of 99.93 % were obtained. The other apparatus conditions, paddles at 100 rpm was not able to provide a more discriminated dissolution profile, since shows more than one point with more than 90 % of drug dissolution, and baskets at 75 rpm and at 100 rpm didn't provide total drug dissolution, showing drug final dissolution of 92.30 % and 94.71 %, respectively.

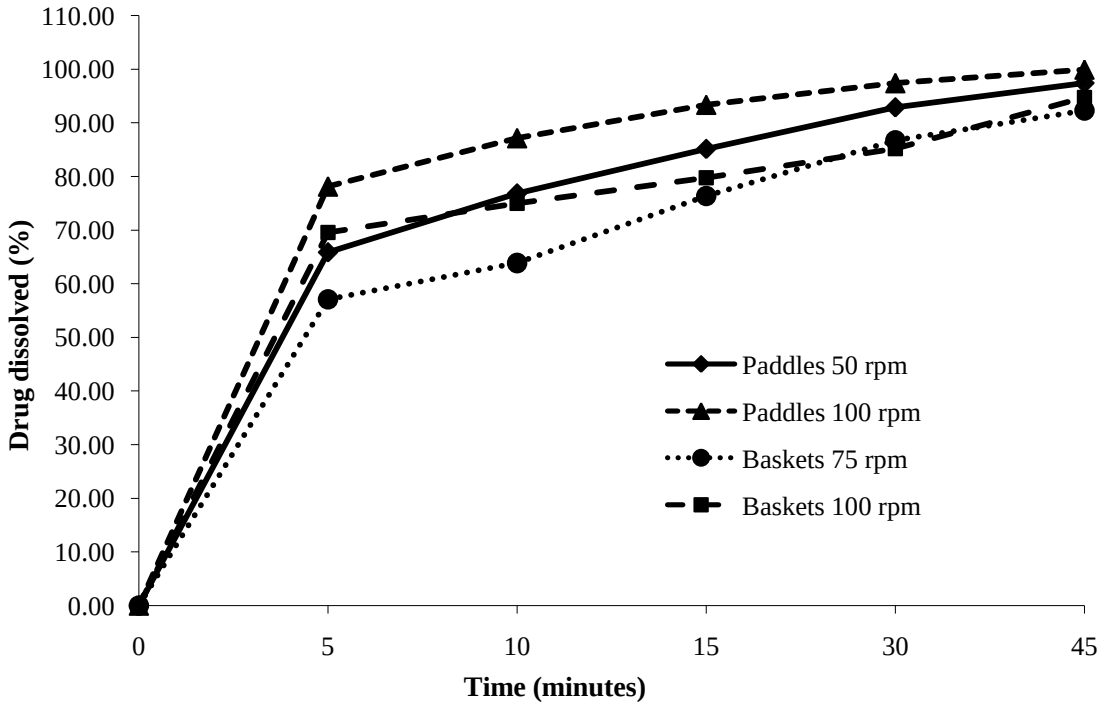


**Figure 3: Dissolution profile of tadalafil tablets in paddles (50 and 100 rpm) and baskets (75 and 100 rpm) using pH 6.8–phosphate buffer + 0.50 % SLS as dissolution medium.**

Based on the results, is possible to determinate that a good dissolution method for tadalafil-coated tablets is using 900 mL pH 6.8–phosphate buffer + 0.5 % SLS as dissolution medium with paddles at 50 rpm, samples withdraw at 5, 10, 15, 30 and 45 minutes, filtered and analyzed at spectrophotometer at 225 nm.

### 3.2. Dissolution method validation

Validation of an analytical method is the processes that establish that performance characteristics of the procedure meet the requirements for the intended analytical applications [10]. In order for the developed dissolution method meets the requirements of the analytical applications, ensuring results reliability, it should be validated.

The determinate the specificity, which is the ability to assess unequivocally the substance in the presence of components that may be expected to be present [26], analysis of a tadalafil placebo solutions showed that the UV method suffer no interference from the formulation of the tablet evaluated, demonstrating to be specific (Figure 1). Since there was no interference from the excipients with the selected wavelength (225 nm), UV can be used in order to quantify tadalafil. Generally, UV is usually used in quality control of pharmaceuticals because most of drugs absorbed energy in UV region, and is a method, which does not require a complex or expensive equipment, and there is no need for toxic solvents [27]. In addition, by using UV method, results can be obtained faster, analysis is simpler

and fewer solvents are used, making this valuable in routine analysis [28–29].

In order to assess linearity, three calibration curves of tadalafil were constructing and plotted graphically as concentration (µg/mL) versus absorbance. The results (Figure 4) showed a good correlation coefficient ($R^2$: 0.9993) in the studied concentration range (4.44 – 26.67 µg/mL$^{-1}$). The representative linear equation was y = 0.0354x + 0.1331 and the data were validated by means of the analysis of variance (ANOVA), which demonstrated significant linear regression and no significant linearity deviation ($p < 0.01$) [10,25]. According to the results, linearity was proved because an appropriate linear correlation was found since the obtained correlation coefficients showed values higher that 0.99 [6–7].

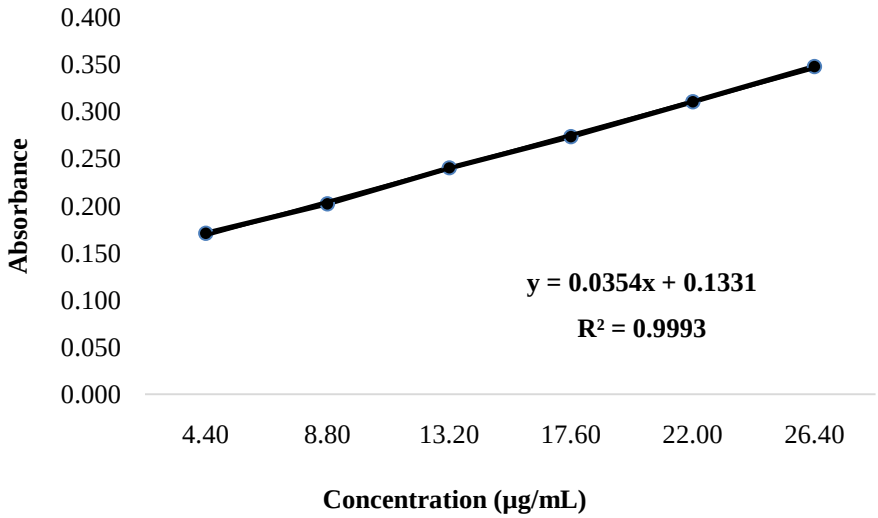


**Figure 4: Linearity curve of tadalafil standard solution in pH 6.8–phosphate buffer + 0.50 % SLS**

Precision of the method was determined by measuring the repeatability and intermediate precision. The results showed a low relative standard deviation (RSD) ranging from 0.49 to 0.45 % for intra-day precision and 0.94 % for inter-day precision. As RSD values were lower than 2%, the results indicated the good precision of this method [6–7].

The accuracy of the analytical procedure, which is the accordance between the accepted value and the value found, was demonstrated by the recovery of known amounts of tadalafil in the dissolution vessels. In the present study, three concentrations were evaluated (11.11, 22.22 and 33.33µg/mL) and each concentration was measured three times. The recovery percentage found ranged from 99.25 to 100.70 %, indicating method's accuracy. As the measured recovery is typically 95–105%, the results indicated good accuracy of the method. The recovery percentage was calculated in triplicate and the mean value was considered.

## 4. Conclusions

A discriminative dissolution method to evaluate tadalafil-coated tablets by ultraviolet spectrophotometry was success developed and demonstrated to be an easy, fast and simple method. The conditions allowing dissolution determination were 900 mL of pH 6.8–phosphate buffer with 0.50 % of SLS as dissolution medium, using USP type II apparatus (paddles) at 50 rpm and analysis by spectrophotometric detection in a wavelength of 225 nm. The spectrophotometric method was validated and showed to be specific, linear, precise and accurate.

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