Development and validation of the discriminating method of prasugrel dissolution in tablets using ultraviolet detection

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ABSTRACT. Current study develops and validates a dissolution test for Prasugrel hydrochloride 10 mg in coated tablets. After sink condition, filters and drug stability were evaluated, the discriminatory dissolution conditions were achieved with a USP apparatus 1 (basket) at 50 rpm stirring speed and 900 mL of 0.01 M HCl as dissolution medium. The UV spectrometric method at 220 nm was performed and validated for the determination of Prasugrel. The parameters specificity, linearity, accuracy, precision and robustness were evaluated according to international protocols. UV method and dissolution test proposed in current analysis may be applied for quality control of coated tablets containing Prasugrel since there is no official monograph for this drug.

Keywords: dissolution, ultraviolet spectrophotometry, validation studies, quality control.

Desenvolvimento e validação de método discriminativo de dissolução em comprimidos de Prasugrel utilizando detecção ultravioleta

RESUMO. O objetivo do presente estudo foi desenvolver e validar um teste de dissolução para cloridrato de Prasugrel 10 mg presente em comprimidos revestidos. Após avaliação da condição *sink*, filtros e estabilidade da droga, condições discriminatórias foram alcançadas utilizando equipamento USP 1 (cesta) em velocidade de 50 rpm e meio de dissolução composto por HCL 0,01 *M*. Foi desenvolvido e validado método de espectrofotometria na região do ultravioleta (UV) a 220 nm para a determinação de Prasugrel. Foram avaliados os parâmetros como especificidade, linearidade, exatidão, precisão e robustez de acordo com os guias internacionais. O método UV e o teste de dissolução propostos neste estudo podem ser aplicados para o controle de qualidade de comprimidos revestidos contendo Prasugrel, uma vez que não há monografia oficial para este fármaco.

Palavras-chave: dissolução, espectrofotometria ultravioleta, estudos de validação, controle de qualidade.

Introdution

The dissolution test is an important tool applied in the development of products, in the identification of critical manufacturing variables and in quality control processes, such as batch release or stability evaluation. It provides information on physicochemical properties and other factors influencing drug release in solid and semisolid pharmaceutical forms. It has been recently employed to select appropriate formulations for *in vivo* testing and to identify potential changes in bioavailability (DRESSMAN; KRÄMER, 2005). However, the method requires discriminatory capability, or rather, it must be able to detect differences of product performance in drug release derived from changes in the composition, manufacturing process or stability (AZARMI et al., 2007; USP 35, 2012).

Prasugrel (Figure 1), $C_{20}H_{20}FNO_3S$, is a third-generation thienopyridine compound approved by the Food and Drug Administration (FDA) in 2014.
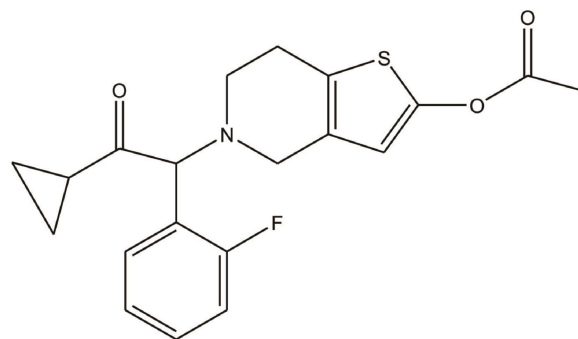

Figure 1. Chemical structure of Prasugrel.

It is an alternative for non-respondent patients to clopidogrel, constituting dual antiplatelet therapy recommended for the treatment of acute coronary

syndromes (ACS) and thrombotic complications in percutaneous coronary intervention (PCI) (GUERRA; TCHENG, 2009). Prasugrel is a prodrug and inhibits platelet aggregation by an irreversible blockade of ADP receptors. After oral administration, the drug has a fast action onset and the plasmatic levels of its metabolic activity may be detected in 15 minutes (VEVERKA; HAMMER, 2009).

According to the biopharmaceutical classification system (BCS), Prasugrel is listed in class II, or rather, a substance with low solubility and high permeability (EMEA, 2014). In literature, there are studies on the quantitative determination of Prasugrel in biological fluids (FARID et al., 2007; LUKRAM et al., 2011; WICKREMSINHE et al., 2007) employing liquid chromatography technique tandem mass spectrometry (LC-MS). In bulk and pharmaceutical dosage forms there are methods that make use of liquid chromatography (e. g. AHIRRAO et al., 2012; DESAI et al., 2012; SAHU et al., 2011) and spectrometry (KUMAR et al., 2011; JENA et al., 2011). At the moment, no monograph is described in any official compendium and few descriptions are available in the literature for dissolution test of Prasugrel. The FDA procedure for the drug's release consisted of apparatus 2 (paddle), rotational speed at 75 rpm, 900 mL of citrate-phosphate buffer at pH 4.0 and samples 10, 20, 30, and 45 minutes. However, no description for chromatographic or spectrophotometric quantification of the dissolved drug is extant (FDA, 2014). Moreover, the dissolution rate was a tool used during pre-formulation studies of Prasugrel tablets to evaluate the performance of the pharmaceutical substance. Artificial gastric juice without pepsin at pH 2.0 (900 mL), at 37°C, using USP apparatus 2 (paddle) was the condition described by the market (KUKEC et al., 2011). It should be underscored that no details are available on the stability of the drug in media; the rotational speed, the time points established for evaluation of the dissolution profile and the assay method have not yet been provided. In this study, Prasugrel solubility was evaluated in physiologic pH (1.2-6.8) and the best rates obtained in acid range were decisive for the choice of the medium.

Current study, therefore, develops and validates a dissolution test condition of Prasugrel in tablets to evaluate the drug's profile and contribute to quality control. Moreover, besides being a simple, fast and inexpensive technique, quantitative analysis by ultraviolet method is advantageous due to Prasugrel's instability in solutions.

Material and methods

Chemicals

Prasugrel reference substance was purchased from Ontario, Canada, where the tablets (Effient®) were commercially available. All the reagents and solvents used were of analytical grade and deionized water was used as dissolution medium and for analysis.

Apparatus

The development and validation of the dissolution test was performed with a Vankel VK 7000 dissolution, multi-bath (n = 8) and automated sampling manifold. Sample filtration was carried out with (10 μm) Vankel filters and 0.45 μm nylon membrane filter. UV-VIS spectrophotometer (Shimadzu), model UV 160 A, equipped with 1.0 cm quartz cells, was used for spectroscopic measurements. The software employed was UV Probe 2.33 (Shimadzu).

Determination of solubility and sink condition

The physical and chemical data of the drug, such as solubility, should be considered so that the medium could be determined. The dissolution process needs to ensure sink conditions, in other words, the volume of the medium must at least be three times greater than that required to form a saturated solution of a drug substance (USP 35, 2012). According to the literature, Prasugrel increases in solubility in proportion to decreases in pH (EMEA, 2014). Dissolution of Prasugrel was evaluated in a physiologic pH range determining the sink condition in different media: 0.01 M HCl; acetate buffer pH 4.5, 4.0, and 3.0; phosphate buffer pH 6.8; citrate buffer pH 3.0 and 2.0. Vessels containing media (300 mL) were heated at 37°C before adding Prasugrel (10 mg). Samples were rotated and the temperature maintained for 2 hours.

Stability determination

The solution's stability is an additional and important data in the choice of the dissolution medium. Standard solution stability was evaluated for 24 hours, at room temperature, and with the sample solutions; they were evaluated each 2 hours at 37 ± 0.5°C in 0.01 M HCl and for 24 hours at room temperature. Absorbance was verified at each time interval for comparison and the acceptable range for solution stability was fixed between 98.0 and 102.0%.

Dissolution test conditions

Different media, volume, type of apparatus and rotational speed were evaluated to obtain a discriminative dissolution test. Automatic sampling aliquots of 10 mL were withdrawn at 15, 30, 45, 60, 90, and 120 minutes. The adequate mathematic corrections were performed after optimization at 5, 10, 15, 30, 45, and 60 minutes, without medium reposition.

The standard solution used in the dissolution tests was prepared by transferring 11.11 mg of Prasugrel reference standard to 20 mL-flask containing 10 mL of methanol. The solution was kept in ultrasonic bath for 20 minutes and the volume was completed with the same solvent. An aliquot of 1.0 mL was transferred to 50 mL-flask and the volume was completed with the medium under analysis, obtaining 11.11 μg mL^{-1}.

After choosing the best conditions, the method was performed by employing a single point at 60 minutes to determinate the profile dissolution. Aliquots of 10 mL were withdrawn and filtered by a (10 μm) Vankel filter, submitted to another filtration through 0.45 μm nylon membrane filter and analyzed on a UV/VIS spectrometrometer at 220 nm. The UV method was selected due to the adequate determination of the analyte in the dissolution tests, easy automation and fast analysis time.

Assay validation method

The spectrometric method was employed for the quantification of the drug released. Specificity was carried out by mixing placebo with Prasugrel solution at 11.11 μg mL^{-1} and the absorption spectra changes were measured. Accuracy was evaluated by adding a known amount of Prasugrel reference standard to the final concentrations 12, 14, and 20 μg mL^{-1}. Each concentration was analyzed in triplicate. The precision, intermediate precision (interday) and repeatability (intra-run) with same concentration of accuracy test distributed in three days were carried out. On the last day, a different analyst carried out the analyses. Robustness was investigated by measuring changes in the absorption maxima at different wavelengths.

Validation of the dissolution method

Specificity

Specificity was evaluated by the analysis of the placebo. The placebo sample consisted of all the excipients of the commercial formulation in their usual concentration. It was transferred to vessels (n = 3) containing 900 mL of dissolution medium at 37 ± 0.5°C and was stirred for 1 hour at 150 rpm, using basket (USP apparatus 1). Aliquots of these solutions were filtered with a Vankel filter, submitted to another filtration by 0.45 μm nylon membrane filter and analyzed by UV spectrometric method.

Linearity

Appropriate aliquots of 500 ug mL^{-1} Prasugrel standard solution in methanol were transferred to 50 mL-flasks and diluted with the dissolution medium to a final concentration range between 2.0 and 20 ug mL^{-1}. The maxima of UV absorption of each solution were measured and the concentration determined. Three standard curves of Prasugrel were prepared on 3 different days and a linear regression analysis was applied to obtain the linearity parameters.

Precision

Precision was assessed by repeatability and intermediate precision. Repeatability was evaluated on the same day, in three vessels used for dissolution test, by relative standard deviation (RSD) from recovery data at 100% level. Intermediate precision was performed on two different days by a different analyst. The RSD was determined by comparing the assays performed according to dissolution test conditions previously described. Twenty tablets were triturated, accurately weighed for an equivalent amount of 10 mg of Prasugrel and submitted to a dissolution test, obtaining 11.11 μg mL^{-1}.

Accuracy

Accuracy was evaluated in two days. Amounts of a tablet pool at different levels were added. The pool was obtained by triturating twenty tablets with determined content assay. The accuracy was evaluated by adding the equivalent of 75, 100, and 125% of the total dose claimed in the tablet in dissolution vessels containing 900 mL of 0.01 M HCl and submitted to the dissolution conditions previously described. Final concentrations obtained in the vessels were 8.33, 11.11 and 13.88 μg mL^{-1} respectively. Further, 25% of placebo mixture were added in the first vessel. The recovery percentage was calculated for each sample by sample absorbance in 220 nm.

Robustness

During development, the dissolution medium deaeration influence on the release profile of Prasugrel in the tablets was evaluated. RSD percentage between dissolution data generated with

non-deaerated and deaerated medium was compared. The medium was deaerated at 48°C in an ultrasonic bath for 20 minutes and maintained at 37 ± 0.5°C.

Results and discussion

The selection of the dissolution medium was based on drug solubility, discriminatory ability of the method and stability of Prasugrel under test conditions. These characteristics were decisive in the choice of the medium, as described in USP. Dissolution characteristics were tested within the physiological pH range for the determination of sink condition. Prasugrel presented limited solubility in acetate buffer, phosphate buffer and citrate buffer, where large amounts of insoluble drug particles were observed after 2 hours in the solutions under the experimental conditions tested. However, solubility was higher in HCl (0.01 M) because Prasugrel bulk is soluble under low pH conditions. These results confirm the data provided by the manufacturer, where the solubility of Prasugrel base and its salts, such as hydrochloride, hydroiodide and hydrobromide, was established. The media in which the solubility was evaluated were artificial gastric juice without pepsin pH 1.2, acetate buffer pH 4.0 and phosphate buffer pH 6.8. Prasugrel base and all salts were best soluble in lower pH rates. The drug release (Figure 2) at six points of the pharmaceutical product was evaluated in different media by first using apparatus 2 (paddle) at the stirring speed of 50 rpm.
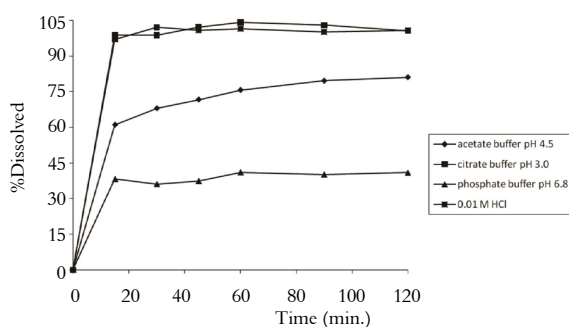

Figure 2. Dissolution profile of Prasugrel in different media (900 mL) at 15, 30, 45, 60, 90, and 120 minutes, with apparatus 2 (paddle), at the stirring speed of 50 rpm.

As observed, the solubility of Prasugrel was inversely proportionally to pH increase. The dissolution of Prasugrel achieved the plateau response within 60 minutes in all the media tested. Prasugrel presented fast dissolution rate in 0.01 M HCl, reaching 100% of the drug release in the first points. The above profile was expected since the

coating of the tablets was non-functional, especially in the presence of potent disintegrants in the product's formulation.

So that the discriminatory dissolution profile could be determined, experiment with apparatus 1 (baskets) at 50 rpm and 900 mL of 0.01 M HCl was carried out to distinguish the effects of flow turbulence and mesh of baskets compared to apparatus 2.

Apparatus 1 with baskets rotating at 50 rpm was selected as the dissolution apparatus and 900 mL of 0.01 M HCl was chosen as the dissolution method. These conditions were selected because the solubility of the drug in this medium and the characteristics of the apparatus, such as less turbulent flow conditions and mesh of apparatus 1, allow a discriminatory and satisfactory release profile of Prasugrel (Figure 3). Since the explanation for rate reduction is that USP apparatus 1 (basket) has specified mesh value, this uniformity retains large particles inside the basket and gradually exposes the drug to the medium. Moreover, the apparatus affects hydrodynamic conditions, influencing the dissolution profile. It should be underscored that, in current study, the 40-mesh basket was evaluated. Visual observations reveal uniform distribution of particles throughout the vessel and no coning formation. Moreover, no adhesion of particles inside the basket has been reported. Observations during the procedure contribute with the development of the dissolution test in search of a more discriminative method and with the optimization of the formulation. Dissolution time points were established taking into consideration the drug's stability in the medium. Moreover, the duration of the procedure for immediate release dosage forms is usually between 30 and 60 minutes.
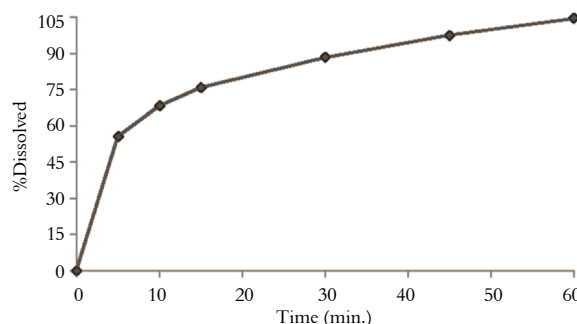

Figure 3. Prasugrel release in 900 mL of 0.01 M HCl with apparatus 1 (basket) at a 50 rpm rotational speed.

The results obtained for the solution's stability were within the acceptable range of 98 - 102% for standard and sample solution at 37 ± 0.5°C in 0.01 M HCl for 2 hours and at room temperature for 6

hours. Stability data contribute with the dissolution medium choice and provide important information to determine time testing and analysis that guarantee reliable results. It may be concluded that the best quantitative method for dissolution test of Prasugrel is UV spectrophotometry where the analyses were simpler and faster. Figure 4 shows the ultraviolet spectrum of Prasugrel standard obtained in 0.01 M HCl. The good absorbance observed at 220 nm justifies the choice of this wavelength for dissolution analysis.
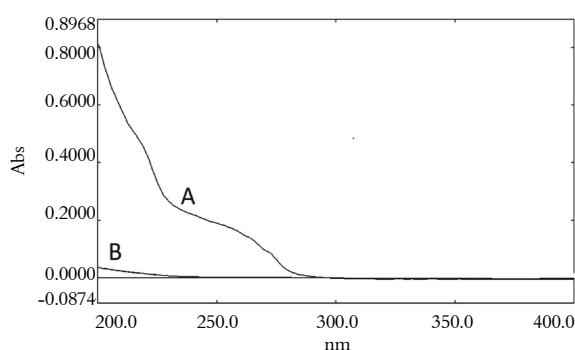

Figure 4. UV spectrum of standard reference substance of Prasugrel (A) and the placebo (B) solutions in 0.01 M HCl before filtration with Vankel filters and 0.45 μm nylon membrane in the final 12.0 μg mL^{-1} concentration.

Table 1 demonstrates the results of the UV method developed and validated in current study. Since no interference of the placebo (Figure 4) occurred when the samples were filtered through 0.45 nylon membrane filter (interference did not exceed 2% of the reference absorbance), the above detection method was consequently specific (ICH, 2005).

Good linearity, according to analysis of variance (ANOVA), ranging between 2.0 - 20.0 μg mL^{-1}, with a correlation coefficient of 0.9998, precision (RSD% in repeatability and intermediate precision results less than 2%), accuracy (100.50, 100.75, and 103.30%) and robustness (DPR%, less than 2%) were verified. Table 1 provided the results briefly.

The dissolution test validation results are demonstrated in Table 1. In the specify parameter, as observed in Figure 2, the analysis of the placebo solutions confirms no excipient interference using 0.45 μm nylon membrane filter. Filtration procedure is required to avoid undissolved drug and excipients particles from entering the analytical samples. Current analysis verified that the filter necessary to guarantee no interference of the formulation in Prasugrel absorbance is 0.45 μm nylon membrane filter.

Three standard curves for Prasugrel, plotting concentration (μg mL^{-1}) versus absorbance, were constructed to assess linearity. Within the concentration range studied (2.0 - 20.0 μg mL^{-1}), the method showed good linearity with a correlation coefficient of 0.9994 and the representative linear equation y = 0.0355x + 0.0103. The analysis of variance demonstrated regression (F calculated = 3345.51 > F critical; p = 0.05) and no deviation from linearity (p < 0.05). The precision was evaluated by repeatability and intermediate precision results. There was a 100.48% repeatability (intra-run) on the first day and 99.25% on the second day, with RSD of 1.08%. The intermediate precision (interday precision) presented an RSD of 0.87%. The RSD rates lower than 2% indicated the method's good precision.

Table 1. Results of the dissolution test and UV spectrophotometry method validation.

Parameters		Dissolution Test	UV spectrophotometry
Linearity		r = 0.9994	r = 0.9998
Precision	Repeatability (RSD%)	100.48 99.25	1.70 1.79 1.20
	Intermediate precision (RSD%)	0.87	0.86
Accuracy (%)		100.48 99.25	100.50 100.75 103.30
Robustness	Deaerated medium / Nondeaerated medium	98.78% / 100.97%	218 nm 220 nm 222 nm 99.6% 99.5% 98.7%
		RSD% 1.55	RSD% 0.49

Accuracy evaluation presented the measured recovery within the acceptable range (95 - 105%). The percentage of Prasugrel recovered from the solutions ranged between 97.17 and 98.93%. The assessment of the accuracy parameter presented in current paper is an alternative procedure in which the reference substance is unavailable or in insufficient quantities. Increasing levels of Prasugrel, investigated in the linearity curve, such as 75, 100, and 125%, were submitted to dissolution test according to the conditions described. Drug addition comes from a pool of samples with the content assay previously determined. Moreover, in the first vessel, the amount of placebo equivalent to 25% of the weight of the tables was also added to guarantee no interference of the excipients in the procedure. According to ICH guideline, the parameters specificity, linearity and precision had been previously established by inferring from accuracy results. The results of the experiments in the robustness test demonstrated that air bubbles in

the dissolution medium did not interfere in the drug's dissolution profile. The percentage of dissolved drug was 98.78% in deaerated medium and 100.97% in non-deaerated medium; however, the difference between the results was not significant (DPR = 1.55%), proving that deaeration of the medium for the dissolution test is not necessary. The presence of air bubbles in the dissolution medium may interfere in drug release but depends on the sensitiveness of the formulation for these conditions. The air dissolved may act as a barrier to dissolution when adhered in the dosage unit and basket mesh, or may facilitate the dissolution with increase of floatage of the particles (DRESSMAN; KRÄMER, 2005).

Conclusion

The developed and validated dissolution test with UV determination for Prasugrel in tablets presented discriminatory capability and solution stability throughout the analysis time. The method is reliable and may be an alternative method for routine analysis of Prasugrel tablets.

Acknowledgements

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