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ARTIGO ORIGINAL / ORIGINAL RESEARCH

Efeito do meio de dissolução, aparato e velocidade de rotação na taxa de dissolução de cápsulas de atazanavir

Effects of dissolution media, apparatus and rotation speed in dissolution rate of atazanavir capsules

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RESUMO

O objetivo deste trabalho é avaliar os efeitos do meio de dissolução, aparato e velocidade de rotação na dissolução de cápsulas de atazanavir. Aliquotas de 6,0 mL foram amostradas após 5, 10, 15, 30, 45 e 60 minutos. Cestas (aparato 1 da USP) com velocidade de rotação de 75 rpm foram selecionadas como condições de dissolução e 900 ml de ácido clorídrico 0,1 N foi escolhido como meio de dissolução. A quantificação da dissolução de atazanavir foi determinada por espectrofotômetro ajustado a 246 nm, utilizando cubetas de 1,0 cm e meio de dissolução como branco. O método foi validado por meio da avaliação da estabilidade, especificidade, linearidade e precisão. De acordo com os resultados, a dissolução de atazanavir é altamente afetada pelo pH do meio de dissolução. A influência do aparato e rotação é menos significativa. A dissolução de atazanavir ocorre rápida e completamente em 30 minutos empregando HCl 0,1 N e aparato 1 com cestas em rotação de 75 rpm. Este método demonstrou-se adequado para o controle de qualidade de atazanavir em formulações farmacêuticas, uma vez que não há monografia oficial.

Palavras-chave: Atazanavir, teste de dissolução, validação de método

ABSTRACT

The aim of this paper is to evaluate the effects of media, apparatus and rotation speed in dissolution of atazanavir capsules. Manual sampling aliquots of 6.0 ml were withdrawn at 5, 10, 15, 30, 45 and 60 min. USP apparatus 1 with baskets rotating at 75 rpm was selected as the dissolution apparatus and 900 ml of hydrochloride acid 0.1 N was chosen as the dissolution medium. The quantification of atazanavir dissolution rate was performed using a spectrophotometer adjusted at 246 nm, using 1.0 cm cells and dissolution medium as blank. The method was validated through the analysis of stability, specificity, linearity and precision. According to the results, the dissolution of atazanavir is highly affected by pH of dissolution medium. The influence of aparatus and rotation in atazanavir dissolution is less significative. The dissolution of atazanavir was rapid and essentially complete within 30 min employing HCl 0.1 N and apparatus 1 with baskets rotating at 75 rpm. This method demonstrated to be adequate for quality control of atazanavir dosage form, since there is no official monograph.

Keywords: Atazanavir; dissolution test; method validation

INTRODUCTION

The dissolution tests for immediate release solid oral dosage forms, such as tablets, are used to assess lot-to-lot quality of a drug product; guide development of new formulations and ensure continuing product quality and performance after certain changes, such as changes in the formulation, and the manufacturing process (FDA, 1997). From a quality assurance point of view, a more discriminating dissolution method is preferred because the test will indicate possible changes in the quality of the product before in vivo performance is affected (Pharmacopeial Forum, 2004). The dissolution test is currently used as an in vitro bioequivalence (BE) test, generally for dissolution profile and profile comparison, establishing the similarity of pharmaceutical dosage forms (O'Hara et al., 1998; Shah, 2001).

Atazanavir (Figure 1), chemically methyl N-[(1S)-1-

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[[[(2S,3S)-2-hydroxy-3-[[(2S)-2 (methoxycarbonylamino)-3,3-dimethyl-butanoyl]amino]-4-phenyl-butyl]-[(4 pyridin-2-ylphenyl)methyl]amino]carbamoyl]-2,2-dimethylpropyl] carbamate (Merck, 2001), is an antiretroviral drug of the protease inhibitor class, used to treat infection of human immunodeficiency virus (HIV). It is distinguished from other protease inhibitors in that it can be given once-daily and has lesser effects on the patient's lipid profile. Like other protease inhibitors, it is used in combination with other HIV medications (Goodman & Gilman, 2006).

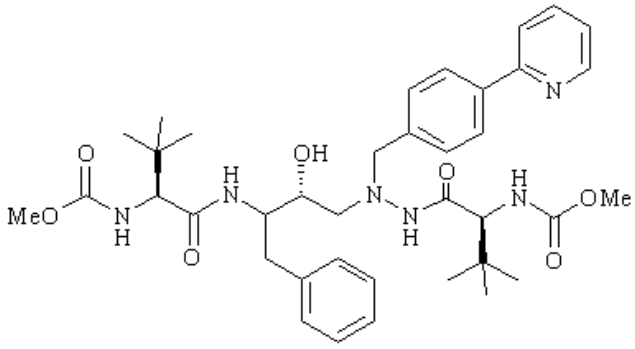

Figure 1. The chemical structure of atazanavir

The aim of this paper is to evaluate the effects of dissolution media, apparatus and rotation speed in dissolution rate of atazanavir capsules. This paper also describes the development of dissolution test and UV method validation for quantification of atazanavir in capsule dosage form.

MATERIALS AND METHODS

Chemicals

Atazanavir reference substance (88.0%) was obtained from Bristol Myers-Squibb (São Paulo, Brazil). Atazanavir capsules used for dissolution test were Reyataz 150 mg from Bristol Myers-Squibb (São Paulo, Brazil).

Instrumentation

The dissolution test was performed in a Vankel VL 7010 and Nova Ética Mod 299 multi-bath (n = 6) dissolution test system, in accordance with United States Pharmacopoeia (USP) general method (USP, 2011; FB, 2010). Both dissolutors were submitted to physical and chemical qualification.

A UV–VIS Spectrophotometer Nicolet Evolution 100 at 246 nm, using 1.0 cm cells was used to quantify the samples.

Dissolution test conditions

The filter evaluation is necessary to determine if it could be used in the dissolution test without adsorption of the drug and that it removes insoluble excipients that may otherwise cause high background or turbidity (Pharmacopeial Forum, 2004). A standard was prepared in different dissolution media proposed (purified water, hydrochloride acid 0.1 N, acetate buffer pH 4.5 and phosphate buffer pH 6.8) with a final concentration of 20.0 μg/ml. Aliquots of 20.0 ml were filtered with a quantitative paper filter (Whatman), a Millex 0.45 μm PTFE filter (Millipore) and a cannula polyethylene filter. The standard solutions were prepared in volumetric flasks and the final solution was analyzed without filtration and filtered with the same filters listed above. All the filtrates were analyzed by UV method. For a filter to be acceptable for use, the results of the filtered portions are to approach (95–105%) the original concentrations of the unfiltered standard solution and the centrifuged sample solution (Pharmacopeial Forum, 2004).

Dissolution testing was performed in compliance with USP 30 testing apparatus 1 and 2 rotating at 75 rpm and 50 rpm respectively, and 900 ml of the different dissolution media: purified water, hydrochloride acid 0.1 N, acetate buffer pH 4.5 and phosphate buffer pH 6.8. The medium, which was deaerated using an ultrasonic bath for 20 min, was maintained at 37 ± 0.5 ºC. The 900 ml glass dissolution vessels were covered to minimize evaporation. Manual sampling aliquots of 6.0 ml were withdrawn at 5, 10, 15, 30, 45 and 60 min.

The standard solution was prepared using an amount of powder equivalent to 20.0 mg of atazanavir that was transferred to a 100 ml volumetric flask, dissolved in 2 ml of methanol and diluted with purified water (200 μg/ml). Aliquot of 6.0, 5.0, 4.0, 2.0 and 1.0 ml of this standard solution was transferred to a 50 ml volumetric flasks and diluted with the dissolution media obtaining the final concentrations of 24.0, 20.0, 16.0, 8.0 and 4.0 μg/ml.

The dissolution efficiency (DE) was calculated from the area under the dissolution curve at time (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

Validation

In order to demonstrate whether the method was adequate for dissolution test purposes, it was validated through the analysis of stability, specificity, linearity and precision (FDA, 1997; Pharmacopeial Forum, 2004; Marques & Brown, 2002).

Stability

The standard diluted solution stability was evaluated for 3 days stored at 4 ºC and at room temperature in hydrochloride acid 0.1 N, verifying the absorbance obtained by the UV method.

Specificity

It was evaluated by preparing a standard, sample and placebo sample of the formulation of capsules. The placebo sample was transferred to vessels with 900 ml of purified water, hydrochloride acid 0.1 N, phosphate buffer pH 6.8 and acetate buffer pH 4.5. Aliquots of these solutions were analyzed by UV method.

Linearity

Aliquots of a 200 μg/ml solution of atazanavir reference standard, prepared with purified water, were transferred to volumetric flasks and diluted with the dissolution media to obtain the final concentrations of 24.0, 20.0, 16.0, 8.0 and 4.0 μg/ml. The linearity was evaluated by linear regression

analysis, which was calculated by the least square regression method and analysis of variance (ANOVA).

Precision

Repeatability and intermediate precision were used to assess the precision of the method. Repeatability was evaluated through relative standard deviation (RSD) from three measurements of the same sample and the intermediate precision through the RSD from three different samples. Aliquots of each sample were analyzed by UV method.

RESULTS AND DISCUSSION

The evaluation of the filters demonstrated that only the quantitative paper filter (Whatman) were within 98–102% of the initial values and could be used in the dissolution tests in the different dissolution medium.

To evaluate the atazanavir stability four dissolution media were used, which were over the physiologic pH range of 1.2 to 6.8. According to the literature, the acceptable range for solution stability is 98–102% of the initial value (Marques & Brown, 2002). The solutions remained stable in all dissolution media tested for the time period specified and no degradation products were observed. So, it was possible to guarantee the integrity of the drug during all the analysis time. The standard solution is therefore considered stable for at least 3 days at temperature room.

The specificity analysis revealed the UV method did not suffer interference by the formulation excipients. The results obtained suggested that the UV method could be used for atazanavir capsules quantification in dissolution tests, once the formulation excipients had not significative absorbance (interference not exceeds 2% of the reference absorbance) at 246 nm (Figure 2).

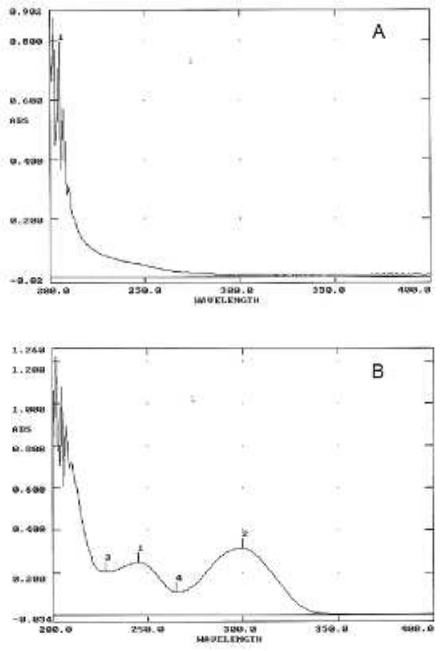

Figure 2. UV spectrum of atazanavir placebo (A) and reference standard (B).

The dissolution test conditions were selected based on a screening study with USP apparatus 1 (75 rpm baskets) and USP apparatus 2 (50 rpm paddles). The capsules were tested in 900 ml of purified water, hydrochloride acid 0.1 N, acetate buffer pH 4.5 and phosphate buffer pH 6.8 (Figures 3 and 4). The data for atazanavir are given in Table 1. The dissolution of atazanavir was rapid and essentially complete within 30 min employing HCl 0.1 N and apparatus 1 with baskets rotating at 75 rpm.

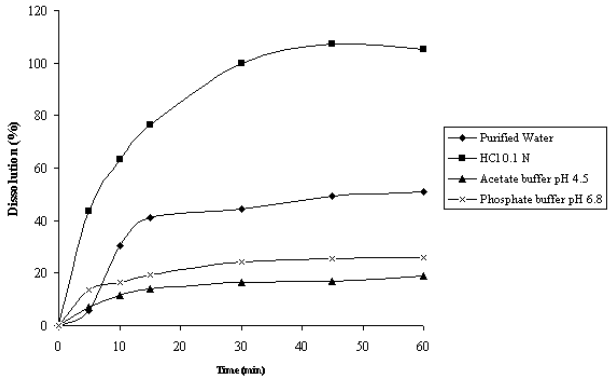

Figure 3. Comparison of atazanavir capsule's dissolution profiles using apparatus 1 rotating at 75 rpm.

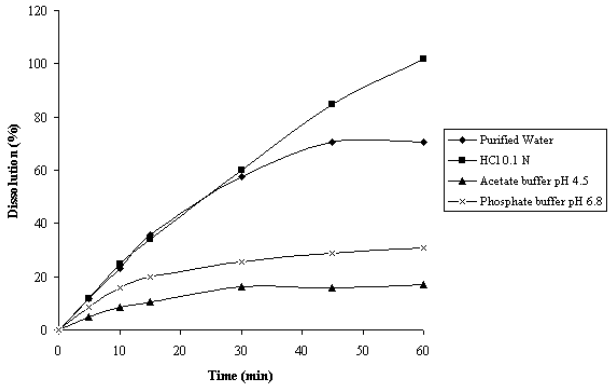

Figure 4. Comparison of atazanavir capsule's dissolution profiles using apparatus 2 rotating at 50 rpm.

Table 1. Screening study for atazanavir (% dissolved at 30 min)[a]

Dissolution media	USP Apparatus 1 / 75 rpm	USP Apparatus 2 / 50 rpm
Purified water	**44.2 (42.4–45.3)**	**57.6 (54.4–60.1)**
HCl 0.1 N	**100.0 (96.8–104.9)**	**60.1 (52.3–67.5)**
Acetate buffer pH 4.5	**24.3 (23.2–26.3)**	**25.6 (25.6–25.6)**
Phosphate buffer pH 6.8	**16.5 (16.0–16.9)**	**16.2 (13.7–20.0)**

[a] The average result is reported followed by the range in parentheses

To assess the linearity, a standard curve for atazanavir was constructed, plotting concentration (μg/ml) versus absolute area (absorbance) and showed good linearity in the range of 4.0–24.0 μg/ml range, with a correlation coefficient of 0.9943 (Figure 5). The slope obtained was 0.0133 and the intercept was 0.008. The analysis of variance (ANOVA) showed significative linear regression

and nosignificative linearity deviation ($P < 0.05$). These data indicate that the method is linear for atazanavir.

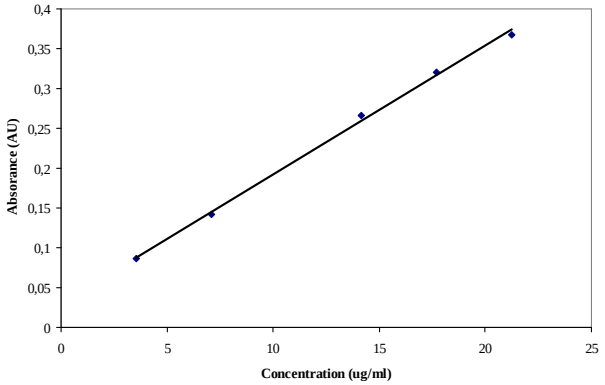

Figure 5. Linearity for atazanavir reference standard in hydrochloride acid 0.1 N

The precision of the dissolution tests was evaluated through repeatability and intermediate precision. The repeatability demonstrated small RSD for each day analyzed (0.001% in the first sample, 0.001% in second sample and 0.001% in the second day). The RSD for intermediate precision was 0.007% inter-samples. These results can demonstrate the good precision of the method for dissolution test.

The results of dissolution efficiency for each condition tested (Table 2) were compared in order to evaluate de effects of dissolution media, apparatus and rotation speed in dissolution rate of atazanavir capsules (Figure 6).

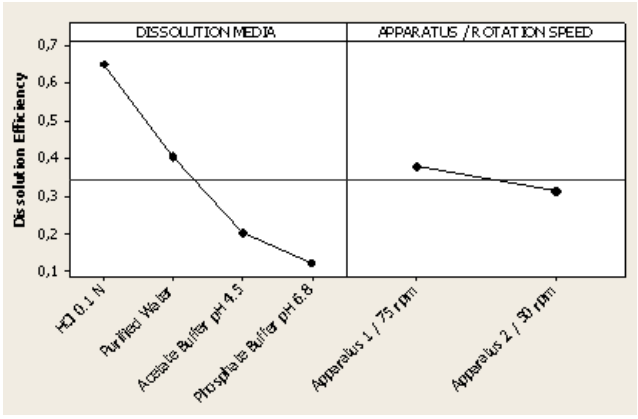

Figure 6. Effect of dissolution media (HCl 0.1 N, purified water, acetate buffer pH 4.5 and phosphate buffer pH 6.8), apparatus and rotation speed (Apparatus 1 rotating at 75 rpm and apparatus 2 rotating at 50 rpm) in dissolution efficiency of atazanavir capsules

According to the results, the dissolution of atazanavir is highly affected by pH of dissolution medium. The influence of aparatus and rotation in atazanavir dissolution is less significative. USP apparatus 1 with baskets rotating at 75 rpm was selected as the dissolution apparatus and 900 ml of hydrochloride acid 0.1 N was chosen as the dissolution medium. This dissolution test condition was selected because in general, mild conditions should be maintained during dissolution testing to allow maximum discriminating power and the drug release profile obtained in the developed dissolution test was considered satisfactory and discriminative.

Table 2. Results of dissolution efficiency for each condition tested

Dissolution media	Apparatus / Rotation speed	Dissolution Efficiency
Purified Water	**1 / 75 rpm**	**36,9%**
HCl	**1 / 75 rpm**	**81,7%**
Acetate Buffer pH 4.5	**1 / 75 rpm**	**19,9%**
Phosphate Buffer pH 6.8	**1 / 75 rpm**	**13,3%**
Purified Water	**2 / 50 rpm**	**44,4%**
HCl	**2 / 50 rpm**	**48,5%**
Acetate Buffer pH 4.5	**2 / 50 rpm**	**20,6%**
Phosphate Buffer pH 6.8	**2 / 50 rpm**	**11,6%**

The discriminatory power of the dissolution method depends on the method's ability to detect changes in the drug product. Drug solubility and solution stability are important properties to be considered when selecting the dissolution medium (Pharmacopeial Forum, 2004).

Dissolution tests for atazanavir capsules were performed using purified water, hydrochloride acid 0.1 N, acetate buffer pH 4.5 and phosphate buffer pH 6.8 to investigate the drug release in each medium. Dissolution rate decreases with increasing pH of dissolution media. The apparatus 1 rotating at 50 rpm shows higher dissolution rates than the apparatus 2 rotating at 75 rpm. This difference is greater for HCl 0.1 N medium.

In our study the effect of concentration of of hydrochloride acid in the dissolution rate of atazanavir was not evaluated. In spite of it, studies to evaluate the bioavailability enhancement of HIV proteases inhibitors showed that HCl 0.1 N was appropriated for dissolution of atazanavir (Fukushima et al., 2007) and ritonavir (Sinha et al., 2010). Rossi and collaborators (Rossi et al., 2011) indicate the use of apparatus 1 (basket) at a rotation speed of 75 rpm to be employed in a discriminating method of dissolution for fosamprenavir tablets.

Typical acceptance criteria for the amount of drug dissolved are in the range of 70–80% dissolved. These criteria including test times are usually established on the basis of an evaluation of the dissolution profile data (Pharmacopeial Forum, 2004; Marques & Brown, 2002). In this article, it was observed that a dissolution of 100.0% in 30 min takes place. So, this acceptance criterion was utilized.

CONCLUSION

The dissolution test developed and validated for atazanavir capsules was considered satisfactory. It was carefully studied in order to guarantee the drug stability during all the analysis time. The conditions that allowed the dissolution profile determination were 900 ml of hydrochloride acid 0.1 N at 37 ºC, USP apparatus 1 with baskets rotating at 75 rpm. This method demonstrated to be adequate for quality control of atazanavir dosage form, since there is no official monograph, collaborating to the

official codes.

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