Nota Técnica

DEVELOPMENT OF DISSOLUTION METHOD FOR BENZNIDAZOLE TABLETS

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The aim of this work was the development of a dissolution method for benznidazole (BNZ) tablets. Three different types of dissolution media, two stirring speeds and apparatus 2 (paddle) were used. The accomplishment of the drug dissolution profiles was compared through the dissolution efficiency. The assay was performed by spectrophotometry at 324 nm. The better conditions were: sodium chloride\ hydrochloride acid buffer pH 1.2 with stirring speed of 75 rpm, volume of 900 mL and paddle as apparatus. Ahead of the results it can be concluded that the method developed consists in an efficient alternative for assays of dissolution for benznidazole tablets.

Keywords: dissolution; Chagas disease; benznidazole.

INTRODUCTION

Although not the ideal drug due to its low solubility (biopharmaceutical class II) and high toxicity Benznidazole (N-benzyl-2-nitro-1-imidazole acetamide) (Figure 1) is currently the choice for Chagas disease (CD) treatment.[1] The drug is an imidazole group agent used in CD treatment since 1973, though CD has had its relevance as a disease still neglected nowadays.[2] CD, also called American trypanosomiasis, is a tissue and hematologic parasatism caused by *Trypanosoma cruzi.*[3]

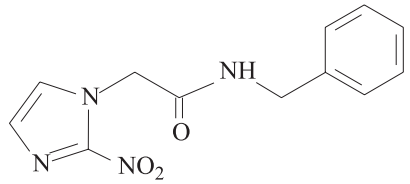

Figure 1. *Benznidazole chemical structure*

According to the World Health Organization (WHO), there are about 120 million people living at risk of contracting parasitosis and 16-18 million people already infected by the parasite.[4] The battle against the disease has succeeded with the interruption of vectorial and transfusional transmission in most of endemic countries. The infected people have no access to an adequate drug treatment, however.[5]

Adequate analytical methods as the assay and dissolution tests are important for research and rational development of medicines in attendance to the regulating agencies needs. Then, it is necessary to develop and validate these methods for posterior application in pharmaceutical products R & D.[6]

Dissolution can be defined as the process which one the drug is freed from its pharmaceutical form for a certain time into solution in standard conditions so that the organism becomes able to absorb it.[7,8] Dissolution tests are requirements currently used to demonstrate the performance of all solid oral dosage forms[8] and are an important quality control tool in different stages of the medication cycle of life.[9]

These methods are useful during the first steps of pharmaco-technical development to identify critical variants in the production, to give a possibility of choice among different formulations, optimize them and make risk factor assessments such as in cases of controlled release form development.[10] There are three kinds of dissolution tests for immediate release drugs: one time, and two time points and a dissolution profile (several time points). These last ones allow assessing and comparing dissolution kinetic and efficiency of products.[11]

For drugs with no official monograph neither method it has been necessary to develop and validate dissolution tests which are capable to predict the same *in vivo* behavior of the drugs. For dissolution profile assessment it is recommended the use of three different media in physiological pH band as a support for the dissolution method development and optimization as well as in the establishment of *in vitro - in vivo* correlations.[12-15]

The biopharmaceutical classification of the drug is an important aspect to be taken into consideration in the choice of the dissolution medium. Class I drugs will easily dissolve in any aqueous medium, since there is not any disintegration problem. For class II drugs it is necessary to take care in choosing the medium. Some proposed media are clearly non-physiological, what can be acceptable for quality control but not when the goal is making some inference about the *in vivo* behavior of formulations. The use of surfactants[14] is recommended for low water soluble drugs products in the aim of reaching the Sink condition.[7] Then, an adequate analytical method must be chosen for quantifying the drug into solution. The method must be accurate enough to determine the exact amount of the substance in the sample.[16]

The choice of the apparatus must be based on how capable of presenting consistent results and admitting some automation degree they are.[17] The basket apparatus is usually used for non-disintegrating forms with 50-100 rpm agitation speed, whereas the apparatus 2 (paddle) can be used both to disintegrating and to nondisintegrating forms in 50 or 75 rpm.[14]

The aim of the present work is the development and validation of an appropriate dissolution method for benznidazole tablets (100 mg) due to the inexistence of any published material in official compendium or scientific journal about the above cited one.

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EXPERIMENTAL

Instrumentation

The tests were performed in a Varian VK 7010, 8000 multi-bath (n=8) dissolution test system in accordance to the United States Pharmacopeia (USP) general method.[18]

Analytical balance (Bioprecisa FA 2104N), ultrasonic bath (Limp Sonic), durometer (Varian VK 200), friabilometer (Press Test), disintegrator (Nova Ética 301-AC) and UV-Visible spectrophotometer (Micronal B 582) were used in physicochemical tests.

Materials and reagents

Benznidazole reference substance (99.9%) supplied by Roche® was used in the product standard control test. Purified water obtained through water purification system with a reverse osmose (Gehaka 20) ht accomplished with a Gehaka deionizator; benznidazole raw material; ethanol purchased from Vetec, Rio de Janeiro, Brazil; all standard aqueous buffer solutions (sodium acetate pH 4.5, sodium phosphate pH 7.2, HCl 0.1 N + NaCl pH 1.2), benznidazole tablets (100 mg) were developed at the Laboratório de Tecnologia dos Medicamentos (LTM) and Rochagan® reference tablets supplied by Roche®.

Validation of the assay method by the UV-Visible spectrophotometer

The method used to accomplish the assay of BNZ dissolved percentile in the dissolution tests was adapted from Soares-Sobrinho *et al*.[6] to be used in our experiment. The method validation was accomplished according to ICH[18] where the specificity, linearity, precision and accuracy parameters were evaluated.

Specificity

Absorption specters were obtained in the UV-Visible region to the placebo solutions, standard and samples in order to verify the formularizations interference in the BNZ spectrophotometric determinations.

Linearity

This assay was carried through a linear regression analysis with the use of the minimum-square method of three authentic curves average points with six points in the concentrations of 5, 10, 15, 20, 30 and 50 µg/mL of benznidazol working standard. The correlation coefficient was obtained through the average of the three curves.

Accuracy

Accuracy was accomplished with the use of concentrations of 20, 80 and 120%. The values were based on the percentages which might be found during the dissolution profile.

Precision

Solutions of the standard substance in the concentrations of 50 and 100% were obtained and assessed through the samples coefficient of variation.

Physical tests and assay/tablets content uniformity

The hardness, friability, disintegration, average weight, content uniformity tests and assay of the products used in the study (Benznidazol-LTM and Rochagan) were carried according to USP30.[19]

Assay

Preparation of standard solution

25 mg of benznidazole working standard were precisely weighed and quantitatively moved to a 50 mL balloon. The volume was completed with ethanol and placed in the ultra sonic per 10 min. 2 mL of the solution were transferred to a 50 mL volumetric balloon and completed with water. The reading proceeded in an UV-Visible spectrophotometer with a wavelengt of 324 nm with water as the blank. The reading theoretical concentration was 20 µg/mL.

Samples preparation

Raw material

The preparation of this solution proceeded the same way the standard solution preparation, with the use of 25 mg of benznidazole raw material.

Tablets

The preparation of this solution also obeyed the same procedure above-cited with the use of 25 mg of benznidazole. Before the first step, however, the solution must be filtered for proceeding the technique. The 10 tablets were weighed, triturated in a mortar and the assay was carried through according to the method described by Soares-Sobrinho *et al*..[6]

Development of dissolution method

For dissolution method development they were used benznidazol-LTM tablets 100 mg and considered the conditions which were tested in accordance to Table 1, three dissolution media, obeying the physiological pH band, Apparatus 2 (paddle), Rotational speed of 50 and 75 rpm.[18] They were collected 2 mL from the cubes with immediate replacement of the media. The dissolution media were warmed at 40 ºC and soniced. 900 mL volume was used in cubes (n=8). The temperature stabilization in test was 37.0 ± 0.5 °C and the aliquots were collected in 15, 30, 45, 60, 90 and 120 min time points and the samples were assayed in UV-Visible at 324 nm. The stability of the drug in the dissolution medium was analyzed within a 24 h period and the influence of the sample filtration was tested at the same time.

Table 1. Conditions used in dissolution tests

Condition	Apparatus	Medium (900 mL)	Speed (rpm)
I	Paddle	Sodium chloride\hydrochloric acid pH 1.2 buffer	50
II		Acetate pH 4.5 buffer	
III		Phosphate pH 7.2 buffer	
IV		Sodium chloride\hydrochloric acid pH 1.2 buffer	75
V		Acetate pH 4.5 buffer	
VI		Phosphate pH 7.2 buffer	

Samples preparation

The collected sample was filtered in membrane with 0.45 µm porosity and diluted in extreme-pure water in order to reach a 22.2 µg/mL final concentration of BNZ.

The assessment of influence in sample filtration was checked by the preparation of raw material BNZ solutions and Benznidazol-LTM in sodium chloride\hydrochloric acid pH 1.2 buffer. The samples were analyzed in UV-Visible spectrophotometer before and after the filtration process with 0.45 µm acetate membrane. For the BNZ stabil-

ity study in solution (medium used in dissolution), it was assessed through BNZ and Benznidazol-LTM solutions which were kept in ambient temperature for a 24 h period at 37.5 ºC.

Comparative study between products and dissolution profiles

Tests of average[9] weight, hardness, friability, disintegration, assay and content uniformity USP 30[19] were made for the comparative study between the local attainment products (benznidazol-LTM) and the product produced by Roche (Rochagan®) as well as a comparative analysis of the dissolution profiles with the use of the dissolution method developed and dissolution efficiency (DE)[24] (Equation 1), as we might previously observe in Martinello and Serra.[20]

$$DE(\%) = \frac{AUC\ (0\text{-}120)}{ATR} \times 100\% \quad (1)$$

DE- dissolution efficiency, AUC- Area under the curve, ATR- Total rectangular area

This comparative tool was used to demonstrate the influence of factors as nature of the dissolution medium and variations in stirring speed between the conditions which were tested during the dissolution test development.[21]

RESULTS AND DISCUSSION

UV method validation

The method for BNZ quantification demonstrated to be specific because there was no placebo interference in BNZ maximum absorption peak at 324 nm. It was obtained $r^2 = 0.999$ and the linear equation $y = 0.0301x + 0.001$ for linearity tests with no lack of adjustment to the line obtained beyond the residues random distribution.

A 99.3 to 100.8% variation, and a recovery average within 99.4 to 100.4% were verified to accuracy parameter. Variation coefficients for solutions which were tested 50 and 100% of 0.7 and 0.6%, respectively, were obtained in the verification of precision. The results assure reliability for this method use in development of dissolution test.[17]

Physical tests and assay/products uniformity

The physical controls, besides the analyzed products assay and content uniformity allow a more complete analysis. Important data to clarify any doubt during the comparison between the dissolution profiles of medicines in study are represented in Table 2, which contains the results of all parameters above- cited.

Development of dissolution method

Actually there is neither pharmacopeia monograph nor described method in literature although it has been a long time since benznidazole tablets are available on the market. That is why the dissolution test for this product was developed as well as verified for other medicines available in the world-wide market.[22]

Apparatus 2 (paddle) was used for pharmaceutical form tablet and 50 or 75 rpm as stirring speed, following the guideline for dissolution tests.[14] The influence of stirring speed can be observed in Figure 2. Differences between stirring speed in Figure 2 are notorious in the dissolution profile with buffer pH 7.2 (Conditions III and VI).

It was observed a great influence of the stirring speed parameter for pH 4.5 buffer (conditions II and V) (Figure 2). The tests were done in a pH 1.2 medium (conditions I and IV) and showed little differences between the two speeds as observed in Figure 2. To define the most adequate dissolution medium (pH 7.2, 4.5 or 1.2) in the use of the method,[14] three media with different pH buffer (basic, neutral and acid) were suggested. Dissolution profiles were compared in the two speeds which were established according to Brier *et al*.,[23] as observed in Figure 2. An accentuated difference present in Figure 2 was observed in dissolution profiles using 50 rpm speed although the percentages of dissolved drug in the end of test (120 min), except for the test which medium pH 1.2 was used, presenting values lower than 80%. The tests carried with stirring speed of 75 rpm presented differences between the media tested. The difference was observed as less intense compared to the one verified in 50 rpm. The selective function of dissolution test with a 75 rpm speed was reduced, however a higher percentage of drug was dissolved to the end of the test (120 min), as observed for the pH 1.2 and 4.5 medium with approximate values of 90% and for pH 7.2 medium with approximate values of 80%. Due to affinities between the drug and acid pH the percentile which was obtained in pH 1.2 buffer was more effective for solubilization.

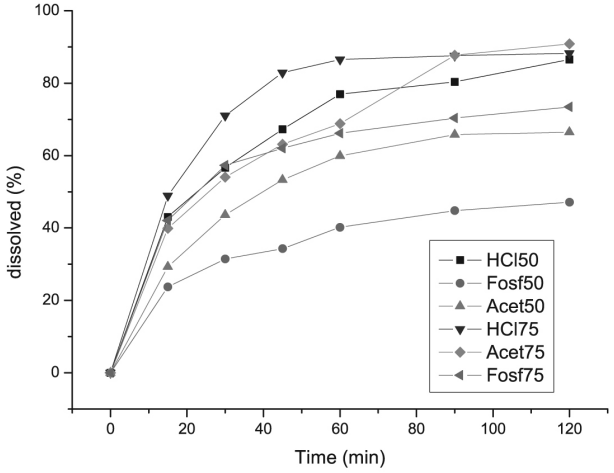

Figure 2. *Dissolution profiles of BNZ tablets in each medium with a comparison between two stirring speeds. Phosphate buffer pH 7.2; Acetate buffer pH 4.5; NaCl/HCl buffer pH 1.2. Comparative dissolution profile of BNZ tablets among the three dissolution media 50 and 75 rpm*

A way also used for comparing dissolution is the dissolution efficiency (DE),[22] according to Table 3 differences between the tested conditions can be verified, showing condition IV as the one with

Table 2. Physical quality control of tablets: Benznidazole-LTM and Rochagan

	Hardness Kgf	Friability %	Average weight RSD	Desintegration Seconds	Assay % (RSD)	Content uniformity % (RSD)
Benznidazole-LTM	6.30	0.01	0.35	>30	99.80 (0.5)	99.70 (0.25)
Rochagan	5.10	0.01	0.45	>30	100.10 (0.3)	100.20 (0.3)

the higher dissolution efficiency. Significant changes with reliable interval of p> 0.05 (ANOVA) were not verified for results which were obtained before and after the filtration. The results for BNZ solution stability and BNZ tablets were between 99 and 101%, demonstrating the maintenance of the sample chemical integrity during the assay.

Table 3. Dissolution efficiency values in the time 120 min

Test Conditions	DE% (RSD)
I	43.27 (1.8)
II	33.25 (2.7)
III	17.14 (2.4)
IV	**44.12 (0.5)**
V	41.00 (0.9)
VI	36.73 (2.6)

Comparative study between products

The comparative study developed between Benznidazole-LTM and Rochagan® through quality control analysis to tablet pharmaceutical dosage form, using general methods from the fourth edition of Farmacopéia Brasileira (Brazilian Pharmacopeia),[25] besides the dissolution profile after development of method for dissolution test demonstrated that although the physicochemistry tests (Table 2) were similar, the opposite was verified on dissolution profile, through the comparison of tested products profiles (Figure 3). The difference can be confirmed through dissolution efficience in 45, 60, 90 and 120 min time points (Table 4).

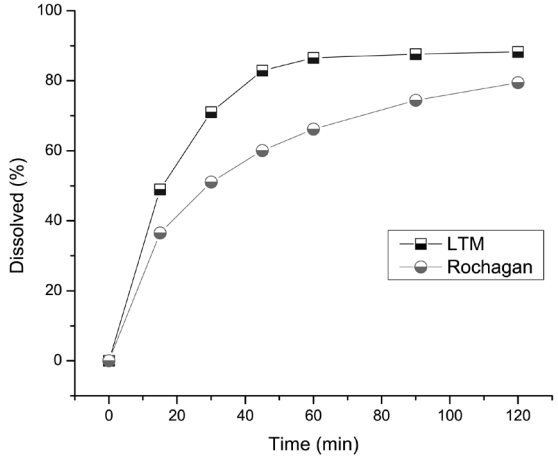

Figure 3. *Comparative dissolution profile between benznidazole-LTM (LTM) and Rochagan.products*

Table 4. Dissolution efficience of benznidazole-LTM X Rochagan in the times points 45, 60, 90 and 120 min

Time (min)	*Benznidazole-LTM % (RSD)*	*Rochagan% (RSD)*
45	41.46 (3.5)	30.10 (4.1)
60	43.26 (1.8)	33.10 (3.4)
90	43.80 (0.8)	37.22 (2.1)
120	44.12 (0.7)	39.70 (0.5)

CONCLUSION

The development of tests for *in vitro* dissolution assays of medicines is very important. The inexistence of specific method for BNZ tablet (100 mg) in official summaries and scientific literature highlights the importance of the above-cited tests to the definition of specific method for such purpose constitutes an appropriate method to use. With apparatus 2 (paddle), stirring speed of 75 rpm, medium volume 900 mL, dissolution medium buffer Sodium chloride\hydrochloric acid pH 1.2, experiment duration of 120 min, with two time points to quality control assays lot to lot with specifications of >Q of 70 and 80% for the 60 and 120 min time points, respectively, according to the dissolution tests guide (FDA, 1997) for biopharmaceutical class II substances (low solubility), besides the accomplishment of dissolution profiles to the BNZ formulations development and optimization.

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