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Development and validation of a dissolution test for rabeprazole sodium in coated tablets

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Abstract

The aim of this work is to develop and validate a dissolution test for rabeprazole sodium coated tablets using a reverse-phase liquid chromatographic method. After test sink conditions, dissolution medium and stability of the drug, the best conditions were: paddle at 75 rotations per minute (rpm) stirring speed, HCl 0.1 M and borate buffer pH 9.0 as dissolution medium for acidic and basic steps, respectively, volume of 900 ml for both. The quantitation method was also adapted and validated. Less than 10% of the label amount was released in the acid step, while more than 95% was achieved over 30 min in the basic one. The dissolution profile for tablets was considered satisfactory. The dissolution test developed was adequate for its purpose and could be applied for quality control of rabeprazole tablets, since there is no official monograph.

Keywords: Rabeprazole sodium; Dissolution test; HPLC; Method validation; Stability; Quality control

1. Introduction

Dissolution test has emerged in the pharmaceutical field as a very important tool to characterize drug product performance [1]. It provides measurements of the bioavailability of a drug as well as can demonstrate bioequivalence from batch-to-batch. Besides, dissolution is a requirement for regulatory approval for product marketing and is a vital component of the overall quality control program [2,3].

Rabeprazole (±)-sodium 2-[[4-(3-methoxypropoxy)-3-methylpyridine-2-yl]methylsulfinyl]-1*H*-benzimidazole (Fig. 1) is a proton pump inhibitor that covalently binds and inactivates the gastric parietal cell proton pump (H^+/K^+ ATPase). It has proven efficacy in healing, symptom relief and prevention of relapse of gastric ulcer, duodenal ulcer and gastro-oesophageal reflux disease [4]. Since it is an acid labile drug, it is commercialize as enteric coated tablets [5].

Although there is a crescent number of works describing the determination of rabeprazole in biological fluids [6–11] and pharmaceutical formulation [12–15] by several methods, this drug is not listed in any pharmacopoeia and there is no dissolution test for tablets reported in literature.

The aim of the present work is to develop and validate a dissolution test for rabeprazole sodium coated tablets using a high performance liquid chromatographic method (HPLC) adapted from the previously published method for drug determination in tablets [13].

2. Experimental

2.1. Chemicals

Rabeprazole sodium reference standard, 99.3% purity, was supplied by Janssen-Cilag (São Paulo, SP, Brazil) and was used as received. The coated tablets (Pariet®), containing 20 mg of rabeprazole sodium, were obtained commercially. The excipients of the pharmaceutical formulation were mannitol, hydroxypropyl cellulose, magnesium oxide, low-substituted hydroxypropyl cellulose, magnesium stearate, ethylcellulose, hydroxypropyl methylcellulose phthalate, diacetylated monoglycerides, talc, titanium dioxide, carnauba wax, and ferric oxide (yellow) as a coloring agent. All of them were obtained from different local distributors. Water was purified using Millipore®

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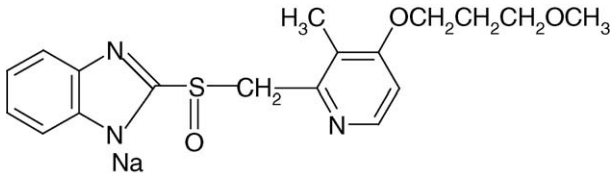

Fig. 1. Chemical structure of rabeprazole sodium.

system. All the other reagents were of analytical grade (Merck, Darmstadt, Germany). Buffer solutions were prepared according to USP 28 [16].

2.2. Instrumentation

The dissolution test was performed in a Sotax AT7 multi-bath ($n = 6$) dissolution test system (Basel, Switzerland), in accordance with the United States Pharmacopeia (USP) general methods [16].

A liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a model LC-10ADvp binary pump, SIL-10ADvp autosampler, CTO-10ACvp column oven, SPD-M10Avp PDA detector, SCL-10Avp system controller and CLASS-VP software was used to quantify the samples.

The Digimed potenciometer, model DM-20 (São Paulo, Brazil) was used to determine the pH of all solutions.

The ultrasonic bath used for deaeration was the model USC 2850 (Unique, São Paulo, Brazil) and the 0.45 μm nylon membranes were Millex (Millipore, São Paulo, Brazil).

2.3. Chromatographic conditions

The HPLC method was adapted from a previously published one [13] validated for rabeprazole sodium coated tablets analysis. The chromatographic conditions are listed in Table 1.

Standard preparation for content uniformity. An amount of powder equivalent to 10 mg of rabeprazole sodium was weighed and transferred to a 50 ml volumetric flask with 5 ml of water pH 10 (adjusted with ammonium hydroxide). The volume was completed with acetonitrile. An aliquot of 4 ml of this standard solution was transferred to 20 ml volumetric flask and diluted with acetonitrile obtaining the final concentration of 40.0 μg ml^{-1}. The solution was filtered in a 0.45 μm nylon membrane filter before the analysis. For the dissolution test, the solution was diluted to 11 μg ml^{-1} using the mixture of acetonitrile-borate buffer pH 9.0 (50:50, v/v) as solvent.

Table 1
Chromatographic conditions for rabeprazole sodium determination in tablets [13]

Equipment	Shimadzu SPD-M10A diode array detector
Column	Keystone Betabasic C8 (250 mm × 4.6 mm, 5 μm)
Mobile phase	Acetonitrile–water (35:65, v/v)
Flow rate	1.0 ml min^{-1}
Wavelength	282 nm
Injection volume	20.0 μl
Temperature	30 ± 1 °C

Sample preparation for content uniformity. One tablet was transferred to each 100 ml volumetric flask containing 10 ml of water pH 10 (adjusted with ammonium hydroxide). They were kept in the ultrasonic bath for 25 min, shaken for 15 min and the volume was completed with acetonitrile. Aliquots of 4 ml of the solutions were transferred to 20 ml volumetric flasks and diluted with the mixture of acetonitrile obtaining the final concentration of 40.0 μg ml^{-1}. The solutions were filtered in a 0.45 μm nylon membrane filter before the analysis. This procedure is according to the previous published method for tablets [13].

Sample preparation for acidic step. One tablet was transferred to each 100 ml volumetric flask containing 10 ml of water pH 10 (adjusted with ammonium hydroxide). They were kept in the ultrasonic bath for 25 min, shaken for 15 min and the volume was completed with acetonitrile. Aliquots of 1 ml of the solutions were transferred to 20 ml volumetric flasks and diluted with the mixture of acetonitrile-borate buffer (50:50, v/v) obtaining the final concentration of 10.0 μg ml^{-1}. The solutions were filtered in a 0.45 μm nylon membrane filter before the analysis.

2.4. Determination of sink conditions

Rabeprazole sodium sink conditions were determined in different solvents, such as: phosphate buffer pH 6.8, phosphate buffer pH 7.5 and borate buffer pH 9.0 (using an amount of drug equivalent a three times of the dose in the pharmaceutical formulation in 900 ml of medium).

2.5. Validation

In order to demonstrate the method was adequate for dissolution test purposes, it was validated through the analysis of stability, specificity, linearity, precision and accuracy parameters [17,18].

Stability. The rabeprazole stability were evaluated for at least 10 h in phosphate buffer pH 6.8, phosphate buffer pH 7.5 and borate buffer pH 9.0. The solutions were kept at 37 °C during the period of the test, verifying the chromatograms obtained by the HPLC method (peak area and degradation products formation).

Specificity. It was evaluated by preparing a placebo sample of commercial formulation of tablets in their usual concentration. This sample was transferred to a vessel with 900 ml of the dissolution medium and stirred for 2 h at 150 rpm using paddle (USP apparatus 2) and temperature of 37 °C. Aliquots of this solution were filtered and analyzed by HPLC and UV methods.

Linearity. Aliquots of a 100 μg ml^{-1} solution of rabeprazole sodium reference standard, prepared in a mixture of acetonitrile-borate buffer (50:50, v/v), were transferred to 25 ml volumetric flasks to obtain the final concentrations of 1.0, 2.0, 4.0, 5.0, 10.0 and 20.0 μg ml^{-1}. Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision. The evaluation of the intermediate precision of the dissolution tests was performed using a well-characterized lot of the drug product of tight content uniformity (according to USP 28) and compared with the results of the dissolution tests. The

repeatability was evaluated through the R.S.D. from the data of calibration curve.

Robustness. It was evaluated through deliberate changes in the chromatographic conditions, such as pH (7.3–8.6) and percentage of acetonitrile in the mobile phase (35–37% and 35–33%). The equipment change was also done (Shimadzu LC-10 A, C-R6A registrator, SPD 10 A UV–Vis detector and manual injection) in order to evaluate the system suitability.

Accuracy. It was inferred from precision, linearity and specificity data, according to ICH Q2B 1996 [19].

2.6. Dissolution test conditions

After preliminary studies, the dissolution test was conducted in two steps [20], both using paddle, at stirring speed of 75 rpm and temperature of 37 ± 0.5 °C.

2.6.1. Acidic step

The dissolution medium was 900 ml of 0.1 M hydrochloric acid, deaerated in ultrasonic bath for 15 min. After 2 h, the tablets were removed and the amount of rabeprazole determined by HPLC method (prepared as cited in Section 2.3).

2.6.2. Basic step

After 2 h in the acidic medium, the new set of tablets were transferred to the borate buffer pH 9.0 dissolution medium (900 ml), deaerated in ultrasonic bath for 15 min. Aliquots of 10 ml were withdrawn of each vessel at 5, 10, 15, 30, 45 and 60 min and equal volume of fresh medium was replaced to maintain a constant total volume. Samples were filtered using 0.45 μm nylon membrane (first 3 ml discarded), diluted with acetonitrile at 11 μg ml^{-1} and assayed by HPLC method. The dissolution profile was obtained.

3. Results and discussion

Drug solubility and solution stability are important properties to be considered when selecting the dissolution medium [21]. In this study, the first approach was compare pH 6.8 and 7.5 media, as commonly used for delayed-release solid dosage forms, and also a high pH medium (borate buffer pH 9.0), considering the higher stability of rabeprazole sodium under alkaline conditions.

The evaluation of sink conditions for rabeprazole sodium bulk demonstrated the drug is soluble in phosphate buffer pH 6.8, phosphate buffer pH 7.5 and borate buffer pH 9.0. However, the phosphate buffer pH 6.8 solution became yellow in few minutes, while the phosphate buffer pH 7.5 became green in almost 1 h, indicating poor stability of rabeprazole sodium in those media.

In order to evaluate the rabeprazole sodium stability in each medium, chromatograms were obtained. The representation of drug profile in phosphate buffer pH 6.8, phosphate buffer pH 7.5 and borate buffer pH 9.0, for 2 h at 37 °C, is shown in Fig. 2. It is possible to observe rabeprazole sodium rapid degradation at pH 6.8 and 7.5, even during the first hour (30% and 13.4%, respectively). On the other hand, it was more stable at pH 9.0 and degraded only 1.3% in the first hour, time set for the dissolution

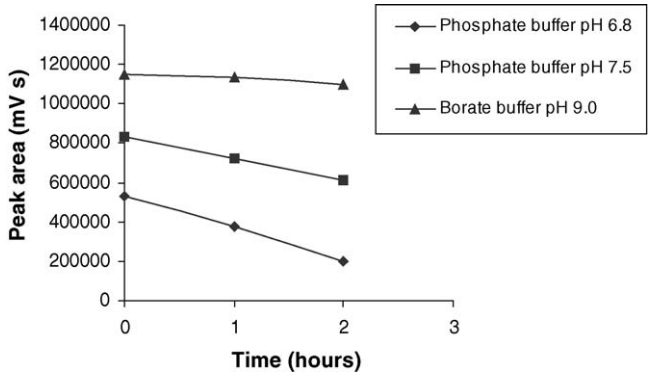

Fig. 2. Rabeprazole sodium profile in different media by HPLC: (♦) phosphate buffer pH 6.8; (■) phosphate buffer pH 7.5; (▲) borate buffer pH 9.0.

test. According to literature [18], the acceptable range for solution stability is 98–102%. The stability of the pH 9.0 solutions prepared for analysis (diluted in acetonitrile) was also evaluated for 11 h (considering the analysis time for routine quality control and dissolution profiles determination). The solutions remained stable for the period tested (Fig. 3). So, it was possible to guarantee the integrity of the drug during all the analysis time and observe that acetonitrile had an important whole in avoid drug degradation. Typical chromatograms of rabeprazole sodium reference standard and commercial sample are represented in Fig. 4.

The dissolution guidelines cite pH medium, in general, should not exceed 8.0 [22], and, although pH 9.0 is above physiologic conditions, in this case it was proved the drug required pH 9.0 to keep stable during the dissolution test. The same situation was reported by Farinha et al. [23], working with omeprazole in dissolution studies. The results obtained by Farinha et al. suggested that dissolution procedure for delayed-release solid oral dosage forms recommended by the general monograph of USP was not adequate for oral formulations containing omeprazole, which required pH 8.0 medium.

The specificity analysis revealed the HPLC method did not suffer interference by the formulation excipients, since there was not another peak in the retention time of rabeprazole sodium (about 7.5 min), only the buffer peak (Fig. 5). The chromatographic peak purity tool, applied for rabeprazole sodium peak, demonstrated that it was 100% pure. The same analysis was

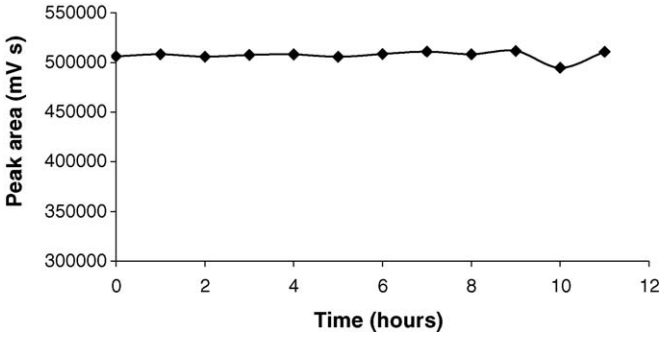

Fig. 3. Rabeprazole sodium profile in borate buffer pH 9.0 and acetonitrile (50:50, v/v) by HPLC.

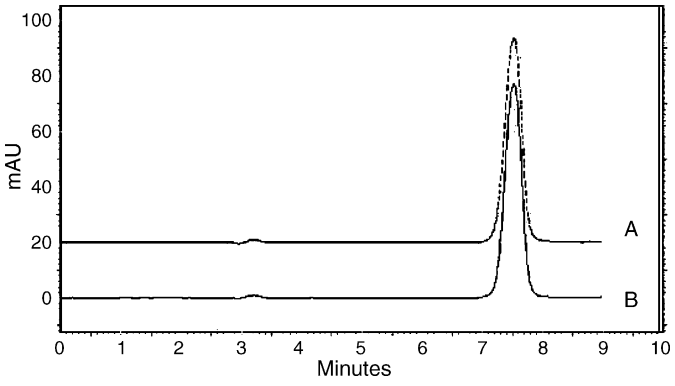

Fig. 4. Chromatograms of rabeprazole sodium (A) reference standard; (B) commercial sample (both at 40 μg ml^{-1}). Chromatographic conditions: Hypersil Keystone Betabasic C8 (250 mm × 4.6 mm; 5 μm); mobile phase acetonitrile–water (35:65, v/v); flow rate 1.0 ml min^{-1}; injection volume 20.0 μl; detection UV 282 nm and temperature of 30 ± 1 °C.

done using the UV method. The result obtained suggested the UV method could not be used for rabeprazole sodium coated tablets determination in dissolution tests, once the formulation excipients had significative absorbance at 282 nm.

The linearity was tested in the concentration range of 1.0–20.0 μg ml^{-1}. The method demonstrated to be linear, with a correlation coefficient of 0.9999. The slope obtained was 41702.6 and the intercept was −2789.07. The data were validated by means of analysis of variance (ANOVA), which showed significative linear regression and no-significative linearity deviation ($P < 0.05$) [20].

The stirring speed selection was done based on the range recommended (50–75 rpm) for apparatus 2 [21,22] and the usual value for tablets. Since the results obtained using 75 rpm in the preliminary studies were satisfactory, no other speed was tested. The filters were also evaluated and the 0.45 μm membrane was selected. It demonstrated not adsorb the drug during the process.

Once the rabeprazole sodium tablets are enteric coated, it is usual to carry out an initial acid step to evaluate the influence of acid pH on the integrity of the formulation. The amount of rabeprazole sodium eventually dissolved in the acidic medium

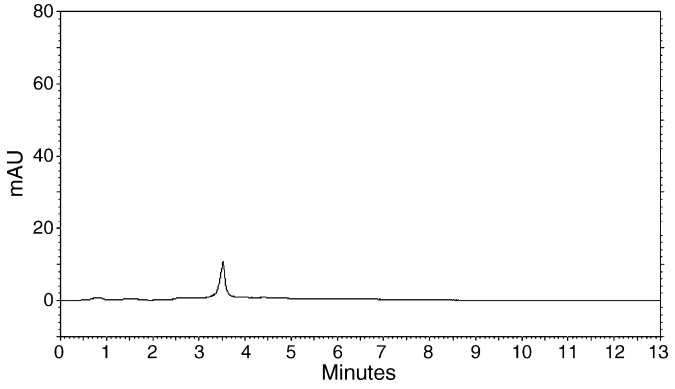

Fig. 5. Chromatogram of placebo sample in borate buffer pH 9.0. Chromatographic conditions: Hypersil Keystone Betabasic C8 (250 mm × 4.6 mm; 5 μm); mobile phase acetonitrile–water (35:65, v/v); flow rate 1.0 ml min^{-1}; injection volume 20.0 μl; detection UV 282 nm and temperature of 30 ± 1 °C.

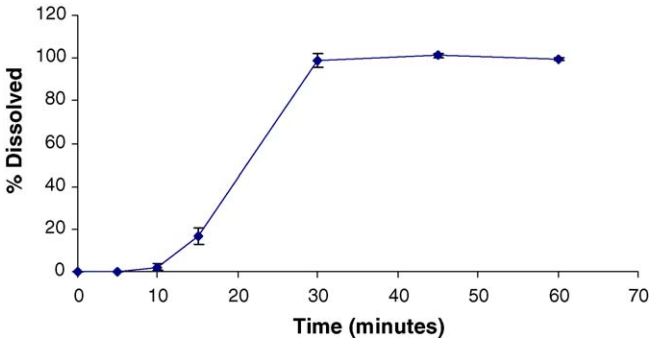

Fig. 6. Dissolution profile of rabeprazole sodium coated tablets ($n = 12$) using borate buffer pH 9.0 medium and 75 rpm stirring speed.

would become rapidly degraded. Thus, it was necessary to remove and analyze the tablets from the acidic step by HPLC method. The same procedure is applied for omeprazole pellets in USP 28 [16]. The mean percentage of rabeprazole sodium remaining in the tablets was 98.05% (R.S.D. = 1.18), indicating the resistance of the coating.

The drug release profile obtained in the dissolution test, at the conditions mentioned in Section 2.6, was considered satisfactory (Fig. 6). After 30 min, more than 95% of drug was dissolved in the medium. The values for percentage of rabeprazole sodium dissolved per time and the R.S.D. values are in Table 2. The results for R.S.D. were higher for times 10 and 15 min, since the concentration of rabeprazole sodium dissolved was small. Once there is only one rabeprazole sodium brand in the Brazilian market, it was not done the comparison of dissolution profiles between products.

The precision of the dissolution tests was evaluated through the comparison of the results of uniformity of content and the percentage drug release. The mean value found to uniformity of content to rabeprazole sodium tablets was 105.55%, with R.S.D. of 1.92. Considering that the remaining drug in acidic step was 98.05%, the loss of rabeprazole sodium was about 7.5% (less than 10%, as usually recommended). The amount reached in basic step was 101.26% (R.S.D. = 1.0). Adding the acidic and the basic steps mean amounts, the result is 108.7%. The small difference between the uniformity of content and percentage of drug release can be explained by the variation of the method applied for each step and for content uniformity. The repeatability evaluation demonstrated small R.S.D. for the results from each concentration in the calibration curve. The R.S.D. obtained

Table 2
Percentage of rabeprazole sodium dissolved in the dissolution test ($n = 12$), using borate buffer pH 9.0 medium and 75 rpm stirring speed

Time (min)	Drug dissolved (%)	R.S.D.
0	0	0
5	0	0
10	2.14	68.1
15	15.01	25.98
30	98.65	3.27
45	101.26	1.0
60	99.21	0.66

Table 3
Results of the robustness and system suitability evaluation of the chromatographic method for rabeprazole sodium

Chromatographic parameters	Modification			
	pH	Mobile phase acetonitrile:water (33:67, v/v)	Mobile phase acetonitrile:water (37:63, v/v)	Equipment[a]
Retention time (min)	6.5	8.9	6.4	7.6
Asymmetry	0.92	0.94	0.90	0.96

[a] Shimadzu LC-10 A, C-R6A registrator, SPD 10 A UV–Vis detector and manual injection.

were between 2.35 and 0.41. These results can demonstrate the good precision of the method for dissolution test.

The robustness of the method was demonstrated through the analysis of the chromatograms obtained under small variations in the chromatographic conditions. During these modifications, the retention time of rabeprazole sodium suffer small modifications, mainly with percentage of acetonitrile variation, but the symmetry of the peak was conserved, indicating there was no damage for the analysis (Table 3). The increase in the pH value of the mobile phase resulted in a smaller retention time. The theoretical plates kept around 3.49×10^3.

4. Conclusions

The dissolution test developed and validated for rabeprazole sodium coated tablets was considered satisfactory. It was carefully studied in order to guarantee the drug stability during all analysis time. The conditions that allowed the dissolution profile determination were borate buffer pH 9.0 medium, paddle (USP apparatus 2) and 75 rpm stirring speed. The method demonstrated to be adequate for quality control of rabeprazole sodium dosage form, since there is no official monograph.

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