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Short communication

Dissolution test for lamivudine tablets: Optimization and statistical analysis

Christian Fernandes [a,*], Roberto Gonçalves Junqueira [b], Ligia Maria Moreira Campos [a], Gerson Antônio Pianetti [a]

[a] *Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos, 6627 Pampulha, CEP 31270-901, Belo Horizonte, MG, Brazil*

[b] *Departamento de Alimentos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos, 6627 Pampulha, CEP 31270-901, Belo Horizonte, MG, Brazil*

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Abstract

A comparison of different methods for dissolution test used by five different manufacturer laboratories of lamivudine tablets is made, evaluated, and discussed. Dissolution medium (water and hydrochloric acid pH 1.2), apparatus (paddles and baskets) and time (30 and 60 min) were analyzed. The determination was accomplished by spectrophotometry at 270 nm. Analysis of variance (ANOVA) factorial design $5 \times 2 \times 2 \times 2$ with six repetitions, with post hoc multiple comparisons between means conducted by Duncan test at 0.05 significance level was used. After the comparative analysis of the results, optimal dissolution conditions were determined as follows: water as dissolution medium, paddles at the stirring speed of 50 rpm as apparatus and time of 30 min. The method was applied to the dissolution test of samples from eleven batches of tablets, produced by five different laboratories.

Keywords: Lamivudine; Dissolution test; Generic drug; Duncan test; Anti-HIV drugs

1. Introduction

Lamivudine belongs to a class of drugs named nucleoside analogues. It is a potent and selective inhibitor of type 1 and 2 human immunodeficiency virus (HIV) [1–3].

It exhibits polymorphism and can be obtained either as acicular crystals or as bipyramidal crystals. However, only bipyramidal crystals are appropriate to be used in the manufacture of tablets because they have adequate fluidity and are stable [4]. Lamivudine has a pK_a of 4.3 and exists primarily in the un-ionized form when dissolved in distilled water. It is very stable to light and temperature in both the solid state and in aqueous solution. Moreover, it is soluble in water and it is considered class 1 in the biopharmaceutics classification system, which means that it has high permeability and high solubility [4,5].

It has been widely used in internationally recognized Brazilian governmental programs to treat patients with acquired immunodeficiency syndrome (AIDS) [6,7].

Solid dosage forms for oral administration are widely prescribed in clinical practice because they are practical, stable, economical, and usually safe [8]. On the other hand, they pose bioavailability problems related to the absorption process [9]. Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract [10]. For that reason, the importance of dissolution tests and dissolution profile for the establishment of pharmaceutical equivalence as well as the importance in further bioequivalence studies must be highlighted. These tests are also essential to evaluate batch-to-batch quality, to guide the development of new dosage forms and to guarantee quality and performance after any changes in the dosage form, the production process or the scale of the manufacturing process [10–12]. In addition, dissolution is a requirement for regulatory approval for product marketing [13].

* Corresponding author. Tel.: +55 16 33739966; fax: +55 16 33739983.
E-mail address: cfernandes@iqsc.usp.br (C. Fernandes).

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This study compares three different methods used to evaluate the dissolution of lamivudine tablets. Due to the lack of methodological standardization in official pharmacopoeias, the methods were obtained from five different laboratories.

2. Experimental

2.1. Chemical and solvents

All reagents were of analytical grade. Hydrochloric acid (37%) and potassium chloride were from Merck (Darmstad, Germany) and Vetec (Rio de Janeiro, Brazil), respectively. Distilled water purified with a Milli-Q Ultra-Pure Water System (Millipore, Bedford, USA) was used. Standard lamivudine was supplied by Glaxo Wellcome (England) with 99.9% of purity. Lamivudine tablets were supplied by Glaxo Wellcome (containing microcrystalline cellulose, sodium starch glycolate, magnesium stearate, hydroxypropylmethylcellulose, titanium dioxide, polyethyleneglycol, and polysorbate 80 as excipients) and Brazilian governmental pharmaceutical laboratories A (containing microcrystalline cellulose, starch, polyvinylpyrrolidone, sodium starch glycolate, colloidal silicon dioxide, magnesium stearate, hydroxypropylmethylcellulose, polyethyleneglycol, and titanium dioxide as excipients), B (containing lactose monohydrate, maize starch, microcrystalline cellulose, polyvinylpyrrolidone, sodium starch glycolate, colloidal silicon dioxide, magnesium stearate, Opadry White, ethylcellulose, and ethanol as excipients), C (containing lactose, cellulose, magnesium stearate, sodium starch glycolate, maize starch, polyvinylpyrrolidone, ethanol, talc, Eudragit E 100, acetone, polyethyleneglycol, titanium dioxide, and propyl alcohol as excipients), and D (containing microcrystalline cellulose, magnesium stearate, sodium starch glycolate, colloidal silicon dioxide, and Opadry White as excipients). The tablets supplied by Glaxo Wellcome were used as reference [14]. Three batches from each laboratory were used, with the exception of laboratory D, with only one batch (Table 1). All tablets were coated and labeled as containing 150 mg of lamivudine.

Table 1
Batches used to evaluate dissolution test for lamivudine tablets

Laboratory	Batches
Glaxo Wellcome	W1158KA (G1) W1509AC (G2) BO18603 (G3)
A	00080226 (A1) 00080227 (A2) 00080228 (A3)
B	00090878 (B1) 00090879 (B2) 00090880 (B3)
C	000808 (C1) 000809 (C2) 000810 (C3)
D	520 (D1)

2.2. Instrumentation and analytical conditions

All dissolution tests were performed in a 72RL multi-bath ($n = 6$) dissolution test system (Hanson Research, CA, USA), in accordance with The United States Pharmacopoeia (USP) general method [15]. The drug release percent (DR%) was assayed by ultraviolet spectrophotometry at the wavelength of 270 nm, using a UV 160A spectrophotometer (Shimadzu, Kyoto, Japan).

2.3. Optimization of ultraviolet spectrophotometry conditions

Spectra of lamivudine standard were built in the range from 200 to 400 nm using quartz cuvettes with 1 cm of path length and water as blank. Solutions of lamivudine standard at 15 $\mu g\,mL^{-1}$ prepared either in water or hydrochloric acid pH 1.2 were used in this analysis. Spectra of the tablets in the range from 200 to 400 nm were also built and compared. One batch of each laboratory was employed in this analysis (G3, A1, B1, C1, D1). Twenty tablets were weighed and powdered. The equivalent of 150 mg of lamivudine was weighed and transferred into a 100 mL volumetric flask with water. This solution was filtered and 1.0 mL was transferred into a 100 mL volumetric flask using either water or hydrochloric acid pH 1.2, obtaining a solution at 15 $\mu g\,mL^{-1}$. A calibration curve with five points, in the range from 5 to 25 $\mu g\,mL^{-1}$, was built at the wavelength of 270 nm. Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. Samples were prepared in triplicate in two different days at 5, 10, 15, 20, and 25 $\mu g\,mL^{-1}$ in order to test the precision. The repeatability was evaluated by calculating the relative standard deviation (R.S.D.) [16].

2.4. Comparison of methods

Three different dissolution test conditions were used by the five laboratories: (1) water as dissolution medium, basket as apparatus, and time of 60 min; (2) hydrochloric acid pH 1.2 as dissolution medium, basket as apparatus, and time of 60 min; (3) water as dissolution medium, paddle as apparatus and time of 30 min. All laboratories employed tolerance of 80%, wavelength of 270 nm, and stirring speed of 50 rpm. One batch from each laboratory was chosen (G1, A2, B1, C2, and D1) in order to perform the comparative analysis.

A factorial design $5 \times 2 \times 2 \times 2$ with six repetitions was used together with a statistical method based on the analysis of variance (ANOVA) in order to evaluate the significance of the main factor effects as well as their interactions. Later, post hoc multiple comparisons between means were performed to compare the drug release percent by Duncan test at 0.05 significance level in order to make a detailed statistical analysis of the data [17].

The following parameters were compared: water (pH 6.2) × hydrochloric acid pH 1.2; paddle × basket; and 30 min × 60 min. The analysis was divided into four steps, as follows: (1) water, paddle, 30 and 60 min; (2) water, basket, 30 and 60 min; (3) hydrochloric acid pH 1.2, paddle, 30 and 60 min; (4) hydrochloric acid pH 1.2, basket, 30 and 60 min.

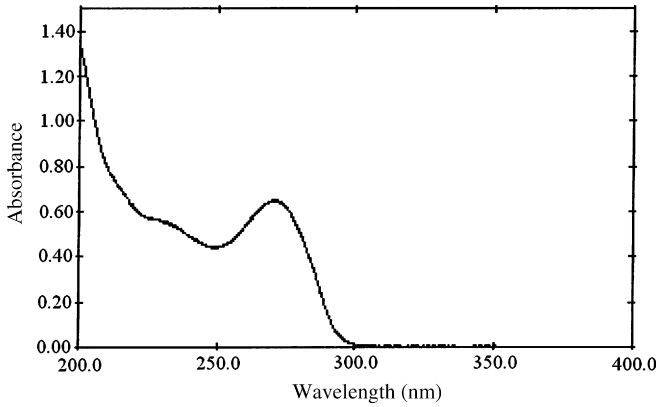

Fig. 1. Ultraviolet spectrum of lamivudine standard at 15 μg mL^{-1} in water.

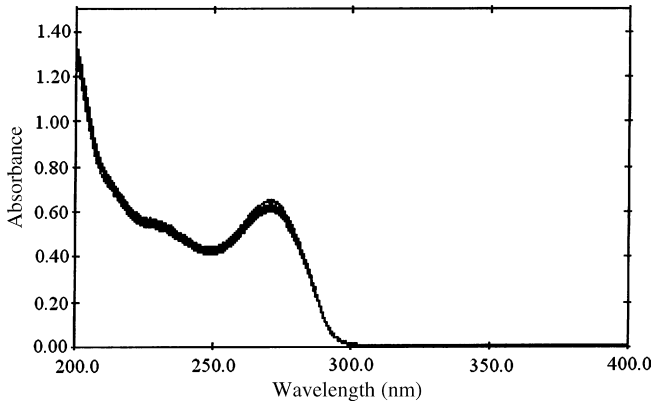

Fig. 2. Ultraviolet spectra of lamivudine standard and tablets (batches G3, A1, B1, C1, and D1) at 15 μg mL^{-1} in water.

2.5. Solutions

A solution of hydrochloric acid pH 1.2 was prepared by adding 4.0 g of potassium chloride and 140.0 mL of hydrochloric acid in 2 L of water. The pH value was checked with a calibrated pHmeter. A standard solution used to evaluate the DR% was prepared in water (16 μg mL^{-1}). Sample solutions were prepared by placing one tablet in each vessel containing the dissolution medium at the temperature of 37 ± 0.5 °C. Samples were collected using a syringe at the end of the specified time and filtered in a 0.45 μm nylon membrane filter. Sample solutions of 5 mL were transferred into a 50 mL volumetric flask, later completed with water.

2.6. Conditions of dissolution test

After comparison of the results, the dissolution test was performed in all batches under the following conditions: 900 mL of water (pH 6.2), paddle, 30 min, 50 rpm, 37.0 °C, tolerance of 80%, and determination accomplished by spectrophotometry at 270 nm.

3. Results and discussion

3.1. Optimization of ultraviolet spectrophotometry conditions

The ultraviolet spectrum for lamivudine standard is shown in Fig. 1. Spectra obtained in water and in hydrochloric acid pH 1.2 were similar. A maximum absorbance close to 270 nm, a minimum at 250 nm and a shoulder at 230 nm can be observed in the obtained spectrum. Therefore, 270 nm was chosen as wavelength in the dissolution test analysis.

The overlaid spectra of lamivudine standard and tablets (batches G3, A1, B1, C1, and D1) are shown in Fig. 2. All spectra were similar and had the same profile, with maximum absorbance close to 270 nm. Spectra obtained in water and in hydrochloric acid pH 1.2 were similar. Moreover, no interference was observed in the tablet dosage forms.

The linearity was tested in the concentration range of 5.0–25.0 μg mL^{-1}. The method demonstrated to be linear, with a correlation coefficient of 0.9999. The linear regression equation was $Y = -0.03447 + 0.04151X$. Table 2 reports the absorbance values obtained in the evaluation of precision. The R.S.D. obtained in all concentration were lower than 1.5% demonstrating that this method has an adequate precision.

Table 2
Absorbance values obtained by ultraviolet spectrophotometry in the evaluation of precision

Concentration (μg mL^{-1})	5	10	15	20	25
Absorbance (average, $n = 3$)	0.174	0.380	0.587	0.797	1.004
R.S.D. (%)	1.48	1.11	1.10	0.49	0.68

3.2. Comparison of methods

The values of DR% obtained in the comparison of the dissolution test methods are shown in Fig. 3. Table 3 presents the data obtained by ANOVA, where the drug release percent results were statistically compared. The results obtained by ANOVA show that there are significant effects for the main factors as well as some interactions. The Duncan test was employed to analyze comparatively the obtained means in full detail.

The analysis interacting the four parameters (batch × time × medium × apparatus) showed that, for batches G1, D1, and B1 the results were similar regardless of medium, apparatus or time. For batch A2, hydrochloric acid pH 1.2 × paddle × 30 min differed significantly from the other tested conditions. For batch C2 the analysis performed with hydrochloric acid pH 1.2 presented DR% higher than that using water, independently of the time or apparatus.

Considering the results, water can be employed as dissolution medium because water and hydrochloric acid pH 1.2 were not significantly different except for batches C2 and A2 (Fig. 3). The small DR% presented by batch A2 using hydrochloric acid pH 1.2, paddle, and 30 min was possibly a consequence of individual variations of the tablets, considering the high standard deviation obtained (11.5%). On the other hand, the small DR% presented by batch C2 in water was most likely caused by the coating, which prevents the penetration of water and hinders disintegration and dissolution. It must be considered that lamivudine is

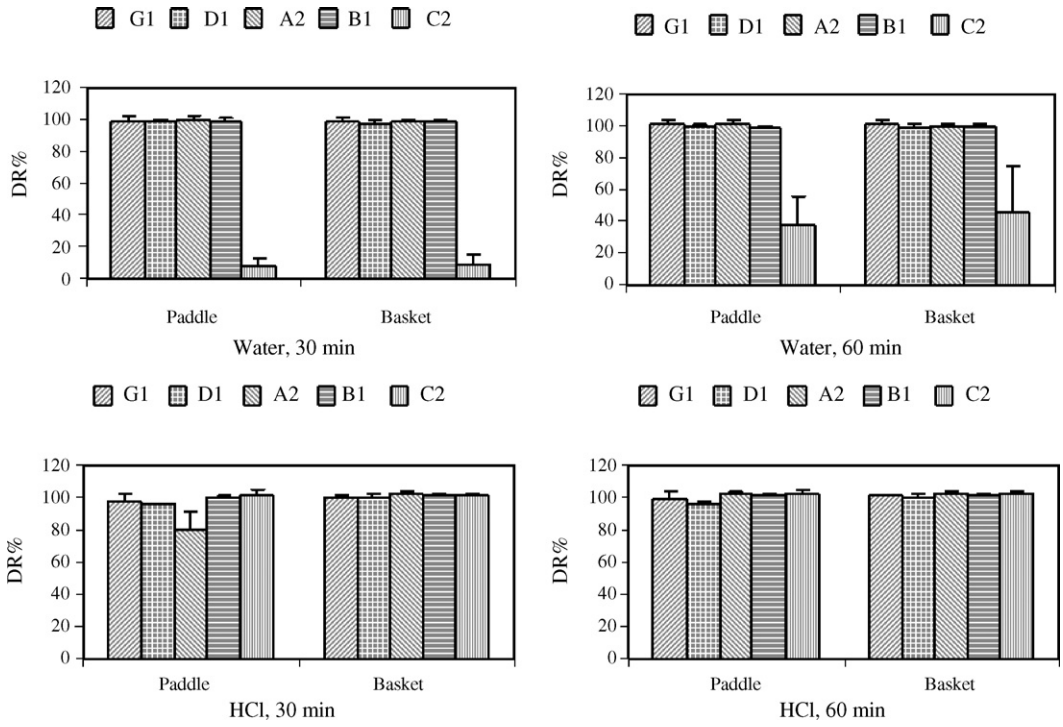

Fig. 3. Values of DR% and standard deviation obtained for batches G1, D1, A2, B1, and C2 in different conditions of medium, apparatus, and time.

highly soluble in water, and that water is the choice of preference according to USP 26 [15]. pH of water remained the same during the analysis.

Paddle apparatus is generally recommended by USP 26 for dissolution test of tablets, while basket is preferred for the analysis of capsules. The result for batch A2 using hydrochloric acid pH 1.2, 30 min, and paddles was lower than that using hydrochloric acid pH 1.2, 30 min, and basket (80.1% and 102.3%, respectively), possibly due to individual variations of the tablets when paddle was used, as previously discussed. For batch C2 the result obtained with water, 60 min, and basket was higher (45.6%) than that employing water, 60 min, and paddle (37.6%). Both values were lower than the recommended tolerance limit (85%). Therefore, paddle was considered adequate and was chosen as stirring apparatus.

Lamivudine tablets are dosage forms for immediate release. Thus, the drug should be rapidly available for absorption. Only batches A2 and C2 presented significantly different results

Table 3
ANOVA data for comparative analysis of different dissolution tests

Factors	D. F.	S. S.	M. S.	*F*	*p*	Sign (*p*)
Laboratory	4	48601.77	12150.44	314.8896	4.19E − 85	<0.001
Medium	1	13709.31	13709.31	355.2889	3.15E − 46	<0.001
Time	1	1652.175	1652.175	42.8176	4.93E − 10	<0.001
Apparatus	1	295.926	295.926	7.669188	6.15E − 03	<0.01
L × M	4	57515.39	14378.85	372.6407	1.78E − 91	<0.001
L × A	4	138.8137	34.70344	0.899371	4.65E − 01	n.s.
L × T	4	2317.178	579.2944	15.01293	9.53E − 11	<0.001
M × A	1	136.957	136.957	3.549364	6.10E − 02	n.s.
M × T	1	351.142	351.142	9.10016	2.89E − 03	<0.01
A × T	1	29.75104	29.75104	0.771025	3.81E − 01	n.s.
L × M × A	4	484.7111	121.1778	3.14043	1.56E − 02	<0.05
L × T × M	4	3185.973	796.4932	20.64183	2.96E − 14	<0.001
L × T × A	4	349.6621	87.41552	2.265451	6.35E − 02	n.s.
T × M × A	1	135.751	135.751	3.51811	6.22E − 02	n.s.
L × T × M × A	4	293.3688	73.34219	1.900728	1.12E − 01	n.s.
Treatment	39	129197.9				
Residue	200	7717.272	38.58636			
Total	239	136915.2				

Table 4
Values of DR% obtained in the established conditions in dissolution test for lamivudine tablets using water as dissolution medium, paddles as apparatus and time of 30 min

Batches	DR%						Average	R.S.D.%
G1	90.2	96.6	99.9	100.9	99.9	100.0	97.9	4.14
G2	94.7	103.4	101.3	105.7	102.1	103.4	101.8	3.70
G3	96.6	101.7	102.4	99.9	99.7	95.9	99.4	2.65
A1	103.3	104.3	102.2	103.6	103.2	104.8	103.6	0.88
A2	98.9	98.2	98.7	101.6	99.0	105.1	100.3	2.65
A3	96.8	102.9	102.6	97.6	102.0	101.6	100.6	2.66
B1	96.1	98.2	101.2	99.0	99.3	99.0	98.8	1.68
B2	100.3	100.9	100.0	101.4	101.7	99.6	100.7	0.82
B3	97.4	102.9	102.6	100.9	103.2	100.4	101.2	2.17
C1	68.7	87.7	100.0	30.7	93.0	63.4	73.9	34.45
C2	7.1	4.4	5.9	3.7	17.8	4.2	7.2	74.30
C3	25.4	6.3	14.8	5.2	14.1	4.3	11.7	69.35
D1	95.4	98.4	101.3	97.8	98.9	95.6	97.9	2.25

when samples were collected after 30 or 60 min. For batch A2 the result employing paddle, hydrochloric acid pH 1.2, and 30 min was lower than that using paddle, hydrochloric acid pH 1.2, and 60 min, possibly as a consequence of tablets individual variations. For batch C2 the difference between 30 and 60 min was significant only when water was used. Therefore, the time of 30 min was chosen considering the analysis speed.

The established conditions after statistical analysis employing ANOVA and Duncan test were: 900 mL of water at 37 °C as dissolution medium, paddle as apparatus at the stirring speed of 50 rpm and collected in 30 min. A method for dissolution test in these established conditions was proposed to be included in a monograph for lamivudine tablets developed by the authors for Brazil Pharmacopoeia [18,19].

The values of DR% and R.S.D. for all batches in the established conditions are shown in Table 4. The three batches from laboratory C (C1, C2, and C3) presented small DR%, lower than the 85% limit ($Q+5\%$). In all other evaluated batches the tablets presented high DR%, showing good capacity to release the drug in the established conditions for the dissolution test.

4. Conclusions

This study presented an investigational approach to develop dissolution test conditions for lamivudine tablets and evaluated the results employing a factorial design. The comparison of different dissolution methods allowed us to define the test conditions as follows: 900 mL of water at 37 °C as dissolution medium, paddle as apparatus at the stirring speed of 50 rpm, collected in 30 min, and tolerance of 80%. Using the methods and conditions established by the manufacturer laboratories, all batches presented equivalent results to the reference product. However, when the method and criteria proposed by this work were used, smaller DR% values were observed for batches C1, C2, and C3 when compared to those of reference, and therefore could not be considered equivalent. This study illustrates the importance of an official method for dissolution test in order to standardize the analysis performed by manufacturer laboratories.

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