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Research Article

Influence of dose and the USP basket and flow-through cell dissolution apparatuses in the release kinetics of metronidazole immediate-release products

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ABSTRACT

Metronidazole has been classified as a drug with high solubility/high permeability and for this compound a bio-waiver monograph has been published however, no significant *in vitro-in vivo* correlation was found using the current pharmacopeial conditions. On the other hand, ineffectiveness due to low drug plasma levels in patients as well as differences in dissolution performance from commercial products even between batches of the same product, have been found. The objective of this study was to evaluate the dissolution performance of two metronidazole generic products and the reference product Flagyl® at two doses (250- and 500-mg) under the hydrodynamic environment generated by the flow-through cell (USP Apparatus 4) and to compare it with the results obtained with the USP official basket method. USP Apparatus 4 with laminar flow at 24 ml/min and 0.1 N hydrochloric acid solution as dissolution medium was used. In USP Apparatus 1, 250-mg products were classified as very rapid dissolution products (> 85% dissolved in 15 min), whereas the 500-mg products were classified as rapid dissolution (> 85% in 30 min). With the USP Apparatus 4, 250-mg products were considered rapid dissolution products (> 85% dissolved in 30 min) and 500-mg products were not considered rapid dissolution products due to not reaching 85% dissolution in 30 min. Under this system, that best simulates the gastrointestinal flow, only the 500-mg reference product achieved 100% release up to 52 min. Dissolution profiles were compared using model-dependent and -independent methods and with both approaches significant differences were found ($p < 0.05$). Metronidazole is considered an ideal drug for waiver of bioequivalence studies but their dissolution equivalence is not as obvious when doses or system are changed, which impacts on request biowaiver.

Keywords: Dissolution kinetics; Dose; Generic products; Metronidazole; USP Apparatus 4

INTRODUCTION

Metronidazole [1-(2-hydroxymethyl)-2-methyl-5-nitroimidazol] is an antimicrobial drug indicated primarily for treating trichomoniasis, intestinal amebiasis, giardiasis and in the prophylaxis and treatment of anaerobic infections (Lamp et al., 1999). Metronidazole is prepared in different dosage forms but the tablets are the most commonly used dosage form mainly for the advantages of patient management and the intake of a solid pharmaceutical form as well as the location of the infection to be treated.

In general, oral absorption of metronidazole is good with a bioavailability $\geq 90\%$ (Freeman et al., 1997); however, absorption variations (Mc Gilveray et al., 1978a), cases of bioinequivalence (Boix and Barrera, 1996), and treatment ineffectiveness due to low drug levels have been found in patients (Idkaidek and Najib,

2000; Itiola and Pilpel, 1986; Kane et al., 1961; McGilveray et al., 1978b). Furthermore, *in vitro* dissolution data offer the best method to predict *in vivo* performance formulation. In this regard, there have been documented differences in release characteristics of metronidazole from commercial products and even between batches of the same product (Gadalla et al., 1984). Cases have also been reported of bioequivalence predictability of only some products based on their *in vitro* performance (Ibezim et al., 2008), which may account for some *in vivo* differences as reported by other authors.

Guidelines for industry-based FDA Biopharmaceutics Classification System (BCS) indicate the criteria by which bioequivalence studies can be replaced by *in vitro* dissolution studies (FDA, 2000). This waiver is based mainly on the fulfillment of the similarity factor criterion (f_2) of the dissolution profiles of test and reference products in different dissolution media with pH values of physiological relevance and compliance with other criteria relating to the excipients used in the formulation (Lennernäs and Abrahamsson, 2005). Considering the criteria of BCS and the information reported in the literature, Rediguieri et al. (2011) classified metronidazole as a Class I drug (high solubili-

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ty/high permeability) and proposed guidelines to justify the bioequivalence studies waiver for immediate-release solid dosage forms. For Class I compounds, dissolving the active pharmaceutical ingredient in 0.1 N hydrochloric acid of not less than 85% of the dose within the first 15 min of testing ensures similarity of dissolution profiles of test and reference products (Alt et al., 2004). However, it is important to mention that unlike Rediguieri et al. (2011), Kasim et al. (2004) classified metronidazole as a Class III drug (high solubility/low permeability). Recently, a private company specializing in drug delivery studies (Dahan et al., 2009; TSRL, 2012) has classified metronidazole as a Class IV drug (low solubility/low permeability), leading to confusion when considering the biowaiver products containing metronidazole.

Beginning in 2005, Mexican health authorities established the requirement to demonstrate bioequivalence of metronidazole immediate-release solid dosage forms with regard to the reference product to consider test products eligible to join the "Catalog of Interchangeable Generic Medicines" (Diario Oficial de la Federación, 2005). Additionally, the dissolution profiles between products must comply with the f_2 similarity factor. To carry out the evaluation of the dissolution profiles of metronidazole tablets, the USP 30 (2007) specifies the use of USP Apparatus 1 at 100 rpm with 900 ml of 0.1 N hydrochloric acid as dissolution medium and not less than 85% (Q) of metronidazole is dissolved in 60 min. However, to date there is no information confirming adequate *in vitro* correlation between *in vivo* results obtained under these conditions.

An alternative to evaluate drug dissolution is the flow-through cell system (USP Apparatus 4). Their advantages over conventional basket and paddle apparatuses (USP Apparatus 1 and 2, respectively) are widely demonstrated, especially in the dissolution of poorly soluble drugs (Bhattachar et al., 2002; Langenbucher et al., 1989; Medina et al., 2013) and in the dissolution of drugs contained in modified-release dosage forms (Chevalier et al., 2009). The USP Apparatus 4 best simulates the hydrodynamic conditions that are found in the gastrointestinal tract. Therefore, it is important to investigate the applicability of the flow-through cell system in the assessment of the metronidazole release for the purpose of developing methods for ensuring proper evaluation of the dissolution process of products sold nationally.

The aim of this study was to investigate the release of metronidazole from two generic products and the reference products (at 250- and 500-mg doses) to have information on the dissolution kinetics of metronidazole under the hydrodynamic environment generated by the flow-through cell system. Results were compared using the conventional vessel system, USP Apparatus 1.

MATERIALS AND METHODS

Products

Six commercial metronidazole products purchased directly from public pharmacies were used: three at doses of 250 mg and the remaining at a 500-mg dose. Test products (designated by the letters A and B) were compared with Flagyl® (designated by the letter R) as reference product. Standard metronidazole (Sigma-Aldrich, St. Louis MO, USA) and hydrochloric acid 37%, AR grade (J.T.Baker-México) were used.

Content uniformity and assay

Content uniformity and assay tests were performed with all products according to the procedures described in the United States Pharmacopeia (USP 30, 2007).

Analytical method validation

Prior to dissolution study, validation of the analytical method for quantifying metronidazole in 0.1 N hydrochloric acid was carried out. The analytical method was validated following Mexican regulations (Norma Oficial Mexicana, 2008).

To demonstrate the linearity of the spectrophotometric system, three calibration curves with five different metronidazole concentrations (4 – 32 µg/ml) prepared in 0.1 N hydrochloric acid were analyzed at 278 nm. Data obtained were fitted by least squares to a linear function and the coefficients of regression, regression analysis of variance (ANOVA) and 95% confidence interval ($CI_{95\%}$) for the intercept value were calculated. The system precision was demonstrated by calculating the percentage coefficient of variation (CV%) of the response factor of the data obtained for linearity. To evaluate the filter influence, a 25 µg/ml solution of metronidazole in 0.1 N hydrochloric acid was prepared. The solution was filtered through 0.45-µm nylon filters and 0.45-µm nitrocellulose filters. Prior to and after filtration, samples were analyzed at 278 nm using 1-cm quartz cell.

Method linearity (drug with excipients) was determined by dissolving in 900 ml of 0.1 N hydrochloric acid grinding quantities of the tablets equivalent to 10, 20, 40, 80, 100 and 120% of the dose. The USP Apparatus 1 at 100 rpm was used. At 60 min the amounts of metronidazole dissolved in each sample was calculated with reference to a calibration curve prepared on the day of the experiment. This procedure was performed in duplicate with all study products. Data (dissolved vs added amounts) were fitted by least squares and calculated regression coefficients. Regression ANOVA was performed and $CI_{95\%}$ for the values of the slopes and intercepts were calculated. The accuracy was assessed by calculating the $CI_{95\%}$ of the average percentage of metronidazole recovered from the known added amount of the drug. The precision was determined by calculating the CV for the percentage of the drug dis-

solved (repeatability) and by duplicate analysis of a homogeneous sample of milled tablets equivalent to 100% of the dose by two analysts carried out on two different days (reproducibility). Results obtained were analyzed by two-way ANOVA; a $p < 0.05$ was considered statistically significant.

Release kinetics in the baskets equipment (USP Apparatus 1)

Dissolution profiles of metronidazole tablets were determined according to the guidelines described in the United States Pharmacopeia (USP 30, 2007) in an automated dissolution USP Apparatus 1 (Vankel VK 7000, Erweka, Germany) coupled to a multi-channel peristaltic pump (Vankel VK 810, England) and an UV/Vis spectrophotometer (Varian Cary 50 Tablet, USA). Metronidazole tablets were placed in 900 ml of 0.1 N hydrochloric acid at 37.0 ± 0.5 °C as dissolution medium. The baskets were rotated at 100 rpm. In all experiments, 0.1 N hydrochloric acid was degassed with vacuum and the computer was programmed to take samples of the dissolution medium every 5 min for a total of 60 min. The dissolved amount of metronidazole was determined spectrophotometrically at 278 nm with 12 units of each product comparing the absorbances obtained with a metronidazole standard solution of known concentration.

Release kinetics in the flow-through cell (USP Apparatus 4)

With the same products, dissolution profiles of metronidazole in an automated flow-through cell system (Sotax CE6, Sotax AG, Switzerland) connected to a piston pump (Sotax CY-7, Sotax AG, Switzerland) and an UV/Vis spectrophotometer (Perkin Elmer, Lambda 10, USA) were determined. The tablets were placed in 22.6 mm cells (i.d.), and laminar flow (6 g glass beads) at the rate of 24 ml/min was used. An open system without recirculating the dissolution medium was also used. In all experiments, the 0.1 N hydrochloric acid solution was degassed with vacuum and the computer was programmed to take samples of the dissolution medium every 5 min for 60 min through nitrocellulose filters. The dissolved amount of metronidazole was determined at 278 nm in 12 units of each product comparing the absorbance obtained with a metronidazole standard calibration curve prepared on the same day of analysis.

Data analysis

With the dissolution data of all study products, model-independent parameters mean dissolution time and dissolution efficiency were calculated. The obtained values for the test products were compared with the values of the reference products using an univariate ANOVA followed by Dunnett's or Dunnett's T3 multiple comparison test as appropriate. Differences were considered significant if $p < 0.05$.

Finally, in order to assess the metronidazole release kinetics under the hydrodynamic environments generated by the vessel and the flow-through cell systems, the dissolution data were fitted by nonlinear regression to the kinetic models: 1st order, Hixson-Crowell, Higuchi, Logistic and Weibull. The best fit model was selected considering the highest coefficient of determination (R^2_{ajustado}) and the lowest value of Akaike information criterion (Yuksel et al., 2000). Some parameter values derived from the selected kinetic model were compared with a univariate ANOVA followed by Dunnett's or Dunnett's T3 multiple comparison test. The data fit with the Excel add-in DDSolver program (Zhang et al. 2010) was performed and statistical comparisons with SPSS program (version 17.0) were used; a $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Content uniformity and assay

All products met the content uniformity and assay tests described in the United States Pharmacopeia. The percentage of metronidazole on the content uniformity test ranged from 85 to 115% and the assay test was between 90 and 110% (Table 1).

Analytical method validation

Validation results showed that the analytical method for measuring metronidazole in 0.1 N hydrochloric acid is linear in the range of 4 – 32 µg/ml with an $R^2 > 0.999$ and a relative error due to regression $< 2\%$. The regression equation calculated from the averaged data was $y = 0.0383x + 0.0049$ ($p < 0.05$). The estimated $CI_{95\%}$ of the intercept was -0.0014 to 0.0111 . The precision provided a CV response factor of 1.4%. Evaluation of the filter influence identified nitrocellulose filters as the most suitable for the dissolution study. Absolute difference in the average percentage of drug that adhered to this type of filter was 1.1%, whereas for the nylon filter it was 4.0%.

The validation results with the used products showed good linearity with R values > 0.99 ($p < 0.05$) and relative errors due to regression $< 2\%$. The one and zero values were included within the $CI_{95\%}$ for the slopes and intercepts values, respectively. The calculation of quantified percentage averages were between 97 and 103%, which showed adequate accuracy. CV values calculated to evaluate the repeatability and reproducibility of the analytical method in all cases were $< 2\%$ and two-way ANOVA showed no significant differences in drug dissolved between days and analysts ($p > 0.05$). The method was considered selective because it met the criteria of linearity, accuracy and precision.

Dissolution studies

In the biowaiver monograph of metronidazole, immediate-release dosage forms information is collected to justify the waiver from bioequivalence studies for this drug if the test product met four requirements that

reflect both the rate and extent of dissolution and the manufacturing process of the products under study. The requirements are as follows: 1) test and reference products should be rapidly dissolving products, 2) dissolution profiles should be similar in dissolution media of physiological relevance (pH 1.2, 4.5 and 6.8), 3) the test product should contain only certain excipients in amounts not exceeding those normally used for the preparation of immediate-release products and 4) whether the test product contains sorbitol, sodium lauryl sulfate or propyleneglycol, these excipients must be presented in a qualitative composition to that contained in the reference product (Rediguieri et al., 2011). The same monograph refers to metronidazole as a highly soluble drug as well as having a low risk for finding bioequivalence, even if the products differ in their dissolution profiles.

According to the first condition that includes the dissolution rate is important to analyze the following, Figure 1 shows the dissolution profiles of all products obtained with USP Apparatus 1 and 4. All products, and with the two USP Apparatuses, met the pharmacopoeial criterion ($Q = 85\%$, 60 min) with the exception of the 250-mg reference product in USP Apparatus 4. In the USP Apparatus 1, all 250-mg products reached 85% dissolution before 15 min and the 500-mg products after 15 min and before 30 min; therefore, these products can be classified as very rapidly dissolving products and rapidly dissolving products, respectively (Kortejärvi et al., 2010; Shokhin et al., 2011). Furthermore, the flow-through cell system shows differences in the rate and extent of dissolution of all the products used. In the USP Apparatus 4, A and B 250-mg generic products exceeded 85% after 15 min and before 30 min, whereas the reference product did not reach 85% dissolution at 30 min. Under the flow-through cell system, all 500-mg products reached 85% dissolution after 30 min. With the USP Apparatus 4, only the 500-mg reference product achieved complete release of the dose (100%) at 52 min.

In a previous study with two metronidazole products, Emami et al. (2006) classified the test product as very rapid dissolution ($\geq 85\%$ dissolved in 15 min) and the reference product as rapid dissolution ($\geq 85\%$ in 30 min). In addition to evaluating five products, Rodrigues et al. (2008) reported the dissolution of the reference product as very rapid dissolution ($\geq 85\%$ in 15 min), a generic and a patent product as rapid dissolution ($\geq 85\%$ in 30 min) and the two other products showed a lower dissolution rate and release of $< 85\%$ at 30 min. Both studies were conducted with 250-mg tablets under pharmacopoeial conditions. Their results are consistent with our findings in the sense of detecting differences in the dissolution rate in the first two 15-min intervals of the test.

To date, most of the dissolution data were derived using USP Apparatus 1 and 2. Results obtained in the present work shows that products containing metroni-

dazole even now change their classification, using different doses and the USP Apparatus 4 and whose results can be affected when considering the biowaiver. USP Apparatus 1 is recommended by the United States Pharmacopoeia for evaluating the extent of dissolution of metronidazole immediate-release solid forms but Levy et al. (1967) concluded that the *in vitro* dissolution rate correlated with the *in vivo* absorption rate only at a low speed of agitation (55 rpm). Hamlin et al. (1962) also demonstrated that products with *in vivo* differences may not differ whether *in vitro* dissolution test was performed with a high stirring rate and concluded that *in vitro* tests with relatively low stirring rates better correlated with the *in vivo* performance of the formulation. Therefore, the high stirring rate (100 rpm) presents a low probability of correlation with *in vivo* data. The flow generated by the USP Apparatus 4 is slower and it can be explained by the hydrodynamic conditions that characterize the flow-through cell system where no continuous stirring mechanisms and the dosage form is exposed continuously to a uniform laminar flow, causing a different dissolution pattern generated by the conventional vessel system (Langenbucher et al., 1989).

The second condition that considers the comparison of the dissolution profiles is noteworthy in this study was carried out only in acidic dissolution medium (0.1 N hydrochloric acid), considering the prevailing *in vivo* environment around the formulation within the first minutes after ingestion. As the solubility of metronidazole in an acid medium (pH 1.2) at room temperature is 64.8 mg/ml (Rediguieri et al., 2011), the 500-mg dose should be dissolved in 900 ml (USP Apparatus 1) or 1440 ml (USP Apparatus 4) of 0.1 N hydrochloric acid at $37.0 \pm 0.5^\circ\text{C}$ without difficulty and in a reasonable time interval. However, this does not occur, which suggests that the nature of the excipients and/or the manufacturing process have an important effect on dissolving the active pharmaceutical ingredient from products used. In addition, Galia et al. (1998) suggest the simulated gastric fluid as a suitable dissolution medium for the prediction of *in vivo* performance of class I drugs, which places them at a disadvantage to the 0.1 N hydrochloric acid solution used as current pharmacopoeial conditions. With all 250-mg products, dissolution of metronidazole using the pharmacopoeial method was rapid and complete ($100\% \leq 15$ min) and, in this case, calculation of the f_2 similarity factor was not required. With this result and according to the FDA criterion (1997), the dissolution profiles of 250-mg generic products were considered similar to the dissolution profile of the reference product (Alt et al., 2004). The results obtained with the other products and under remaining conditions did not meet the criteria required to perform the comparison of the dissolution profiles using the f_2 similarity factor. In some cases, high variability was found; however, other parameters with model-independent and -dependent methods

were calculated in order to compare the dissolution profiles.

Table 2 presents the average values of the mean dissolution time and dissolution efficiency of all studied products under the conditions described in the Methods section. This Table shows the values of the parameters of generic products that present significant difference ($p < 0.05$) compared to the values of the parameters of the reference products.

When comparing the dissolution profiles with model-independent methods, all mean dissolution time values of generic drugs used in both types of equipment showed a significant difference ($p < 0.05$) compared to the reference (Flagyl® 250- and 500-mg) values except the mean dissolution time of the 500-mg product B in USP Apparatus 1. Additionally, some dissolution efficiency values also had significant differences compared to the values of the reference products ($p < 0.05$). The results of this study show significant differences in 12/16 comparisons, although it should be noted that significant differences can be found in either parameter or either USP apparatus, e.g., 250-mg product B evaluated in the USP Apparatus 4 shows no significant difference in the value of the dissolution efficiency but has differences in the value of the mean dissolution time using the same equipment. Model-independent parameters: mean dissolution time and dissolution efficiency are essential for the establishment of *in vitro-in vivo* correlation levels B and C, respectively (Cutler et al., 1997). Level B is based mainly on the comparison of parameters calculated with the "statistical moment theory" as the mean dissolution time is that representing the time at which 63.2% of the drug is dissolved. Level C requires the calculation of some *in vitro* parameter expressing overall drug dissolution. Dissolution efficiency relates the area under the curve of the dissolution profile compared to the total area of the rectangle formed by the 100% theoretical dissolution of the dose and the time interval of the dissolution test.

Table 3 presents the individual data of the products adjusted to the previously mentioned kinetic models. Some experimental units were adjusted to more than one equation and none product was adjusted to Higuchi's kinetics. With the hydrodynamic environment generated by the flow-through cell system, especially for 500-mg products, there was more variability in adjusting the dissolution data to the kinetic models used.

All 250-mg products tested with the USP Apparatus 1 were adjusted to Weibull's kinetics whose expression is as follows: (Zhang et al., 2010)

$$F = F_{max} \left[e^{-\frac{(t - T_i)^\beta}{\alpha}} \right]$$

where F is the percent of drug dissolved vs t time, F_{max} is the maximum percentage of drug dissolved at infi-

nite time, α is the scale factor of the process, β is the form factor of the process and T_i is a location parameter time in which the drug begins to dissolve.

Because data of the 250-mg products evaluated in USP Apparatus 1 were adjusted to the same kinetic model, the dissolution profiles of generic products were compared with respect to the reference product with the calculation and statistical comparison of T_d values derived from fitting the dissolution data to the Weibull's function. The T_d value represents the time at which 63.2% of the drug is dissolved and is equivalent to the mean dissolution time calculated with statistical moments. Table 4 shows the average values of α , β , T_i , F_{max} and T_d derivatives of the adjustment of dissolution data to the Weibull's model.

There was a significant difference in the T_d values of generic products compared to the T_d values of the reference product ($p < 0.05$). With the dissolution data of 250-mg products in the USP Apparatus 4 and 500-mg products in both apparatuses, this type of comparison for the diversity to which the products were fitted to the kinetic models was unable to be performed.

Considering results in dissolution media with different acidity degrees (pH 1.2, 5.0 and 7.2), a previous report was carried out with five commercial products (coated formulations) in healthy volunteers and volunteers who showed high gastric acidity. Three of the five products showed very low dissolution at pH 5.0. These products showed low values of $C_{p_{max}}$ and area under the curve of plasma concentration vs time profile. However, using the area under the curve as *in vivo* parameter only an adequate *in vitro-in vivo* correlation with the USP Apparatus 2 in pH 5.0 buffer was found. These results were obtained with patients who had high gastric acidity (Ogata et al., 1985).

Rediguieri et al. (2011) mentioned that for Class I drugs manufactured in immediate-release dosage forms, the risk of bioinequivalence is minimal when the products fulfill the established criteria for comparing dissolution profiles. However, in the case of metronidazole, different sources classified the drug as belonging to different BCS classes, which may prevent this statement from being applied without difficulty to metronidazole products. On the contrary, according to reported sources, the β -blocker metoprolol is catalogued as a Class I drug. Rekhi et al. (1997), after carrying out a bioequivalence study, reported bioequivalence in regard to the immediate-release form of metoprolol salt, even when differences were shown in their dissolution profiles.

Regarding the last two requirements for applying for bioequivalence waiver studies of products containing metronidazole, in the present study we evaluated commercial products sold in public pharmacies and we are not aware of what type of excipients were used as well as whether they are presented in qualitative-quantitative values regarding the contents in the refer-

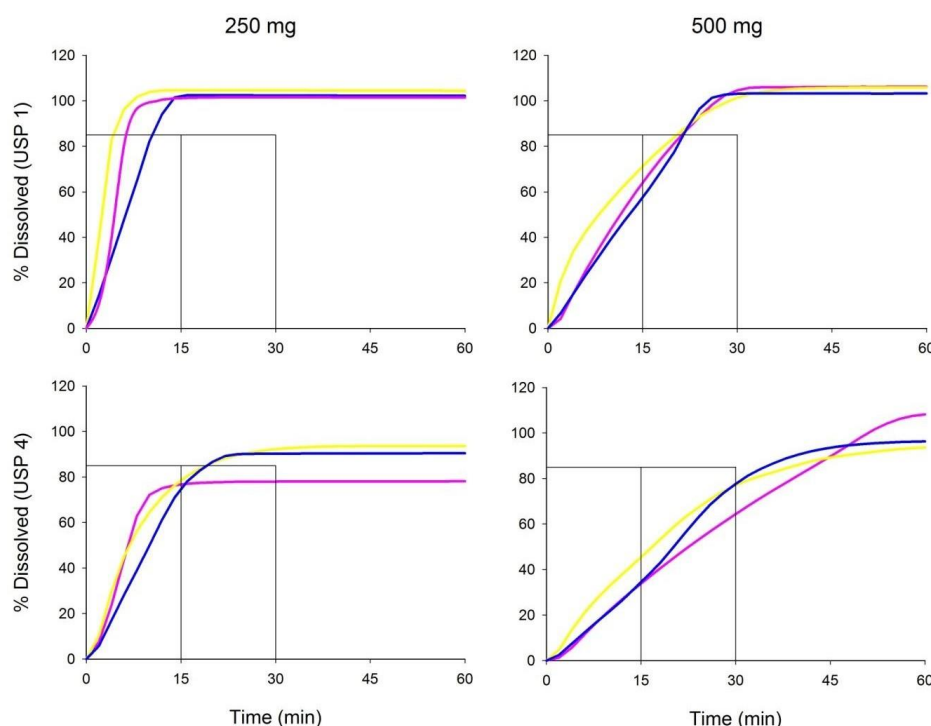


Figure 1: Dissolution profiles of metronidazole. Reference (pink), A (yellow) and B (blue) products. Mean, n = 12. Error bars were omitted for clarity

Table 1: Content uniformity and assay results

Dose (mg)	Product	Content uniformity (min – max)	Assay (%)
250	R	100.8 (94.9 – 105.2)	95.5
	A	94.5 (92.3 – 96.5)	99.2
	B	99.0 (91.2 – 101.3)	100.2
500	R	99.7 (96.1 – 101.0)	103.8
	A	97.7 (95.6 – 100.0)	99.6
	B	96.0 (93.3 – 100.0)	100.1

Mean, n = 10

ence product. Excipients such as sorbitol, sodium lauryl sulfate and propyleneglycol have a potential effect on bioequivalence. Sorbitol is frequently used in oral solutions and, when present in high amounts, accelerates gastrointestinal transit. Therefore, Rediguieri et al. (2011) mentioned that when any of these three excipients is presented in a test product, there is a high risk of establish bioinequivalence. For highly soluble drugs is important to consider the gastric emptying time which, for this kind of drugs, is the limiting factor of the absorption (FDA, 2000; Lennernäs and Abrahamsson, 2005). This value is 15 to 20 min under fasting conditions (Alt et al., 2004).

Since its introduction, the flow-through cell system (USP Apparatus 4) showed greater advantages to assess the rate and extent of the dissolution process compared to other widely used types of conventional equipment (Chevalier et al., 2009; Langenbucher et al., 1989; Medina et al., 2013; Nicklasson et al., 1991). The hydrodynamic environment generated by USP Apparatus 4 is similar to that presented *in vivo* and several reports confirm correlation of *in vitro* data generated

in this equipment with *in vivo* results (Emara et al., 2000; Štefanič et al., 2012; Sunesen et al., 2005). Currently, research is ongoing on the hydrodynamic conditions generated by the flow-through cell system (D'Arcy et al., 2011; Shiko et al., 2010) as well as finding dissolution conditions more similar to the natural environment of the gastrointestinal tract. These include the use of biorelevant dissolution media even from the developmental stage of the formulation (Fang et al., 2010; Sunesen et al., 2005). It is important to investigate the type of equipment and conditions under which it must carry out the evaluation of the dissolution of metronidazole tablets, especially to explain the difference in the dissolution process observed between doses. Pharmacopoeial conditions appear not to be the most appropriate to show such differences. This is irrelevant if these differences do not have any meaning regarding the *in vivo* performance of the formulation; however, previous reports reveal problems in the absorption of metronidazole. The objective is to design an *in vitro* test to warn of such a possibility, particularly with high-dose products. The test used the flow-

Table 2: Model-independent parameters

USP Apparatus	Product	Dose (mg)	
		250	500
Mean dissolution time (min)			
1	R	4.5±0.06	13.2±0.2
	A	2.7±0.1 ^a	11.3±0.5 ^a
	B	6.4±0.4 ^a	13.2±0.2
4	R	5.8±0.1	26.2±0.3
	A	8.4±0.3 ^a	17.8±1.0 ^a
	B	9.1±0.9 ^a	19.9±1.4 ^a
Dissolution efficiency (%)			
1	R	93.7±0.2	82.7±0.8
	A	99.5±0.4 ^a	85.8±0.8 ^a
	B	91.2±0.7 ^a	80.4±0.6
4	R	70.5±0.6	60.8±0.4
	A	80.4±1.0 ^a	65.6±1.6 ^a
	B	75.9±2.3	63.9±1.7

Mean ± SEM, $n = 12$; ^a Significant difference ($p < 0.05$).

Table 3: Individual adjustment to kinetic models

USP Apparatus	Dose (mg)	Product	1 st order	Hixson-Crowell	Logistic	Weibull
1	250	R				12
		A				12
		B				12
	500	R		4		8
		A		1		11
		B		2		10
4	250	R				12
		A	1		7	6
		B			4	11
	500	R		2		10
		A	2	2	3	6
		B			7	12

Note: Some experimental units adjusted to more than one kinetic model.

Table 4: Weibull's parameters and T_d values of 250-mg products

Product	α	β	T_i	F_{max}	$T_d (\pm SEM)$
R	1.6 _E ³	1.7	1.6	101.4	4.9±0.1
A	2.0	1.0	1.1	104.5	2.9±0.1 ^a
B	4.0 _E ⁵	4.5	-8.5	102.5	7.9±0.5 ^a

Mean, $n = 12$; ^a Significant difference ($p < 0.05$).

through cell as an alternative system that better reflects the prevailing hydrodynamic environment of the gastrointestinal tract. *In vitro* results generated in USP Apparatus 4 have a greater capacity to discriminate between products, offering a higher probability of finding a significant *in vitro-in vivo* correlation.

In México, the bioequivalence study of metronidazole tablets is a prerequisite to renew governmental health registration and to consider these as generic products, although metronidazole absorption is a function of gastric emptying and theoretically there are no drug dissolution problems. According to the results obtained in the present study, this requirement is necessary and appropriate. Furthermore, it is also important to explore other systems and dissolution conditions and to

try to establish any *in vitro-in vivo* FDA-accepted correlation level before suggesting that a drug does not require *in vivo* studies and designing conditions that better reflect the release characteristics of the drugs. In the absence of a significant *in vitro-in vivo* correlation, *in vivo* studies are the only method to ensure a performance equivalent to that presented by the reference product. For the characteristics shown, metronidazole is considered an ideal drug for biowaiver, but the dissolution equivalence is not as obvious when doses or systems are changed. Significant *in vivo* effects may not occur with the products used in this study; however, it is important to note that the *in vitro* behavior of the drug does not meet expectations and other reports have documented bioequivalence problems. Pending biopharmaceutical investigations remain

to be performed with metronidazole and that require attention and analysis to ensure its effectiveness.

CONCLUSION

For metronidazole immediate-release products it is important to discuss the effect of using only the pharmacopoeial quality *Q* criterion for comparison purposes using conditions that show little discriminating ability. The study reveals that products that meet the *Q* value show differences in the dissolution rate even when the official method is used. These differences are more evident with the use of the flow-through cell system. It is necessary to investigate whether the dissolution conditions evaluated in USP Apparatus 4 overestimate the differences between doses or if there is a correlation between the *in vitro* decreased high-dose performances with bioavailability.

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