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Original Article

PHARMACEUTICAL EQUIVALENCE OF METRONIDAZOLE TABLETS USING THE FLOW-THROUGH CELL (USP APPARATUS 4) AND MEDIA OF PHYSIOLOGICAL pH RANGE

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ABSTRACT

Objective: The aim of this work was to evaluate the pharmaceutical equivalence of metronidazole tablets through the study of the hydrodynamics of the flow-through cell (USP Apparatus 4) on the dissolution performance of four commercial formulations (500 mg). The results were compared with those found using the USP basket apparatus.

Methods: Experiments were performed with 0.1 N hydrochloric acid (pH 1.2), acetate buffer pH 4.5 and phosphate buffer pH 6.8. A USP Apparatus 4 was used with laminar flow at 16 ml/min and 22.6-mm cells. USP basket apparatus was used with 900 ml of each dissolution medium. The dissolution profiles were compared in terms of the mean dissolution time and dissolution efficiency.

Results: Significant differences in MDT and DE values of generic formulations *vs*. reference with both USP apparatuses were found (*P<0.05); hence, dissolution profiles of generic metronidazole formulations cannot be considered similar to the dissolution profile of the reference. After using some equations to explain the release performance of metronidazole, dissolution data were well adjusted to Peppas-Sahlin and logistic models when the flow-through cell was used.

Conclusion: The main problem found with the studied formulations was that generic drug products showed different dissolution performances than the reference, and they did not meet the biowaiver criteria for either class I or class III drugs; therefore, they cannot be considered therapeutic equivalents.

Keywords: Flow-through cell, Generic drug products, Metronidazole tablets, USP Apparatus 4

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INTRODUCTION

Metronidazole is used with other antibiotics for the treatment of peptic ulcer disease caused by *Helicobacter pylori*. It is also used in the treatment of trichomoniasis, vaginitis, and urethritis caused by *Gardnerella vaginalis*, giardiasis, and amoebiasis. Metronidazole is indicated for intraabdominal infections, skin structure infections, bacterial septicaemia, bone and joint infections, central nervous system infections, lower respiratory tract infections, and endocarditis [1]. The chemical structure of metronidazole is shown in fig. 1.

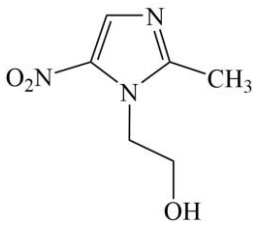

Fig. 1: Chemical structure of metronidazole

Metronidazole is widely used as a generic drug product, and it is available as tablets, suspensions, and ovules. Mexican health authorities have established dissolution and bioavailability studies to consider the interchangeability between test products and the reference formulation [2]. Due to available information on its chemical and biological characteristics, a biowaiver monograph for metronidazole solid dosage forms has been published [1]. This monograph suggests that *in vitro* dissolution studies should be carried out under certain conditions to avoid *in vivo* studies.

The classification of metronidazole according to the Biopharmaceutics Classification System has been controversial. Rediguieri *et al.* [1] classified metronidazole as a class I drug (high solubility/high permeability), Kasim *et al.* [3] classified it as a class III drug (high solubility/low permeability), and Celebioglu and Uyar [4] classified metronidazole as a poorly water-soluble hydrophobic drug. To suggest a biowaiver for class I drugs, several conditions should be fulfilled, particularly two of them: the test and reference formulations are rapidly dissolving, and the test formulation does not contain any excipients that will affect the rate or extent of absorption [5].

To suggest a biowaiver for class III drugs, some conditions related to the previous ones are requested: the drug product (test and reference) is very rapidly dissolving, and the test product is qualitatively the same and quantitatively very similar to the reference product [5]. According to FDA guidance, immediate-release formulations are considered very rapidly dissolving when a mean of 85% or more of the labelled amount of the drug dissolves within 15 min and a rapidly dissolving product when a mean of 85% or more dissolves within 30 min using, among other alternatives, the USP basket apparatus (USP Apparatus 1) at 100 rpm and dissolution media of 0.1 N hydrochloric acid (pH 1.2) as well as buffer solutions of pH 4.5 and 6.8 [5].

Pharmacopeial conditions to test metronidazole tablets indicate the use of the USP basket apparatus at 100 rpm with 900 ml of 0.1 N hydrochloric acid as the dissolution medium and not less than 85% of the labelled amount should be dissolved in 60 min (Q=85%) [6]. An alternative to evaluate the dissolution behaviour of solid dosage forms is the flow-through cell (USP Apparatus 4). This dissolution equipment has the advantage of generating a hydrodynamic environment similar to that found inside the gastrointestinal tract [7]. Several authors have reported a significant *in vitro*/*in vivo* correlation (IVIVC) between data obtained with the flow-through cell and human behaviour [8, 9], which makes it a suitable option to estimate the release of the drug in the human body. On the other hand, *in vitro* dissolution studies with new metronidazole formulations have been reported, including release data with USP Apparatus 4 [10-12].

The aim of this work was to test four metronidazole commercial formulations, three generic drug products and the reference under the hydrodynamic environment of the flow-through cell (USP Apparatus 4) and dissolution media of physiological relevance (pH 1.2, 4.5, and 6.8) to document whether these formulations meet standard criteria to waiver *in vivo* studies and consider them pharmaceutical equivalents. Data were compared with results found using the USP basket apparatus and the same dissolution media. The results may support the design of better metronidazole generic formulations.

MATERIALS AND METHODS

Materials and quality control tests

The metronidazole standard was purchased from Sigma–Aldrich Co. (St. Louis MO, USA). Hydrochloric acid, acetic acid, and phosphate salts were purchased from J. T. Baker-Mexico (Xalostoc, Mexico). Metronidazole tablets (500 mg) of the reference product Flagyl® (Sanofi-Aventis de Mexico S. A. de C. V., Mexico City, Mexico) and three generic formulations (randomly coded as A, B, and C) were used in this study. Mexican health authorities have established the Flagyl® brand as a reference formulation for dissolution and bioequivalence studies [13]. Assay and uniformity of dosage unit tests were carried out with all commercial formulations according to the techniques described in the United States Pharmacopeia [6].

Dissolution studies

Flow-through cell (USP apparatus 4)

Dissolution profiles were determined with flow-through cell equipment (Sotax Model CE6, Sotax AG, Switzerland). Laminar flow (generated with a bed of 6 g of glass beads) at 16 ml/min and 22.6-mm cells (i.d.) were used. All tablets were tested with 0.1 N hydrochloric acid (pH 1.2), acetate buffer pH 4.5 and phosphate buffer pH 6.8. The dissolved metronidazole was determined with a UV/Vis spectrophotometer (Perkin Elmer Model Lambda 10, USA) using 1-mm cells. Automatic samples were taken every 5 min for 60 min (n=12). Dissolved metronidazole was calculated with a standard calibration curve (4 to 32 µg/ml) in each dissolution medium at 278 nm.

USP basket apparatus

Dissolution curves of metronidazole tablets were determined using the official dissolution test described in the USP [6]. A USP basket apparatus (Sotax AT7-Smart, Sweden) at 100 rpm was used. In each vessel, 900 ml of 0.1 N hydrochloric acid (pH 1.2) at 37.0±0.5 °C was used (Q>85% at 60 min). Additionally, 900 ml of acetate buffer pH 4.5 and phosphate buffer 6.8 were used. Automatic samples were taken every 5 min for 60 min (n=12). Dissolved metronidazole was quantified with standard calibration curves in each dissolution medium.

Data analysis

To compare the dissolution profiles of metronidazole formulations, the model-independent parameters mean dissolution time (MDT) and dissolution efficiency (DE) were calculated. MDT is the time at which 63.2% of the dose dissolves and is calculated with the statistical moment's theory [14]. MDT can be calculated by Eq. 1 [15].

$$\mathrm{MDT} = \frac{\sum_{j=1}^{n} \hat{t}_j \Delta M_j}{\sum_{j=1}^{n} \Delta M_j} \ldots\ldots (1)$$

Where *j* is the sample number, *n* is the number of dissolution sample times, $\hat{t}_j$ is the time at the midpoint between t_j and t_{j-1} and ΔM_j is the additional amount of the drug dissolved between t_j and t_{j-1}.

DE is defined as the area under the dissolution curve up to a certain time, *t*, expressed as a percentage of the area of the rectangle described by 100% dissolution at the same time. It can be calculated by Eq. 2 [15]:

$$\mathrm{DE} = \frac{\int_0^t y \times \mathrm{dt}}{y_{100} \times t} \times 100\%. \ldots\ldots (2)$$

Where *y* is the drug percent dissolved at time *t*.

For comparison of MDT and DE data, a one-way analysis of variance (ANOVA) following a Dunnett multiple comparisons test was carried out with the support of SigmaPlot software (Version 11). Significant differences were considered if *P<0.05. To compare dissolution profiles with a model-dependent approach, metronidazole dissolution data were adjusted to some mathematical equations commonly used in dissolution studies. Zero-order, Peppas-Sahlin, Weibull, and logistic models were considered. The best-fit model exhibited a higher adjusted correlation coefficient ($R^2_{adjusted}$) and lower Akaike Information Criterion (AIC) [15]. All data were adjusted with the add-in DDSolver program [16].

RESULTS AND DISCUSSION

Quality control tests

All metronidazole formulations met the requirements of assay and uniformity of dosage unit tests stipulated in the USP. The results are shown in table 1.

Table 1: Results of the pharmacopeial test of the metronidazole reference (R) and generic formulations (A–C)

Key	Assay (%)*	Uniformity of dosage units (%min–%max)†
R	94.52	92.20–94.07
A	94.76	93.82–95.61
B	95.90	95.24–96.39
C	103.47	101.57–104.47

Mean, *n=3. †n=10

Dissolution profiles

The dissolution profiles of all metronidazole drug products under the hydrodynamics of the flow-through cell (USP Apparatus 4) and USP basket apparatus are shown in fig. 2. Differences in their *in vitro* release performance were observed among commercial formulations and between both USP apparatuses.

Metronidazole formulations revealed a slower dissolution rate with the flow-through cell than the dissolution rate found with the USP basket apparatus, especially reference and generic product B, while with the USP basket apparatus, generic products A and C maintained a similar behaviour using the three-dissolution media. In the flow-through cell, the hydrodynamics are less drastic, and the drugs that present rapid dissolution show a more efficient release by the formulation. The slower dissolution rate can be explained by differences in the hydrodynamic environment of USP Apparatus 4. This apparatus utilizes no stirring mechanisms, so the tablets are continuously exposed to homogeneous, non-turbulent laminar flow, causing a slow dissolution rate [17]. Under pharmacopeial conditions, the reference and generic formulations A and C met the pharmacopeial Q criterion (>85% dissolved at 60 min). Generic product B showed that almost 76% of the drug dissolved at the same time. To identify whether metronidazole tablets are very rapidly or rapidly dissolving formulations, fig. 2 shows dashed lines at 15 and 30 min at 85% dissolved.

It is important to emphasize that under no dissolution conditions used, the reference product dissolved more than 85% at 30 min, while generic product A showed a rapid dissolution performance only with the USP basket apparatus at pH 1.2, 4.5 and 6.8 as well as with the USP Apparatus 4 at pH 4.5 and pH 6.8. According to BCS-

based biowaivers for class I drugs, the following criteria should be demonstrated: 1. the drug substance is highly soluble, 2. the drug substance is highly permeable, 3. the test and reference products are rapidly dissolving, and 4. the product does not contain any excipients that will affect the rate or extent of absorption of the drug. These characteristics must be observed with the use of the USP basket apparatus at 100 rpm and the following dissolution media: 0.1 N hydrochloric acid (pH 1.2) and buffers pH 4.5 and 6.8 [5].

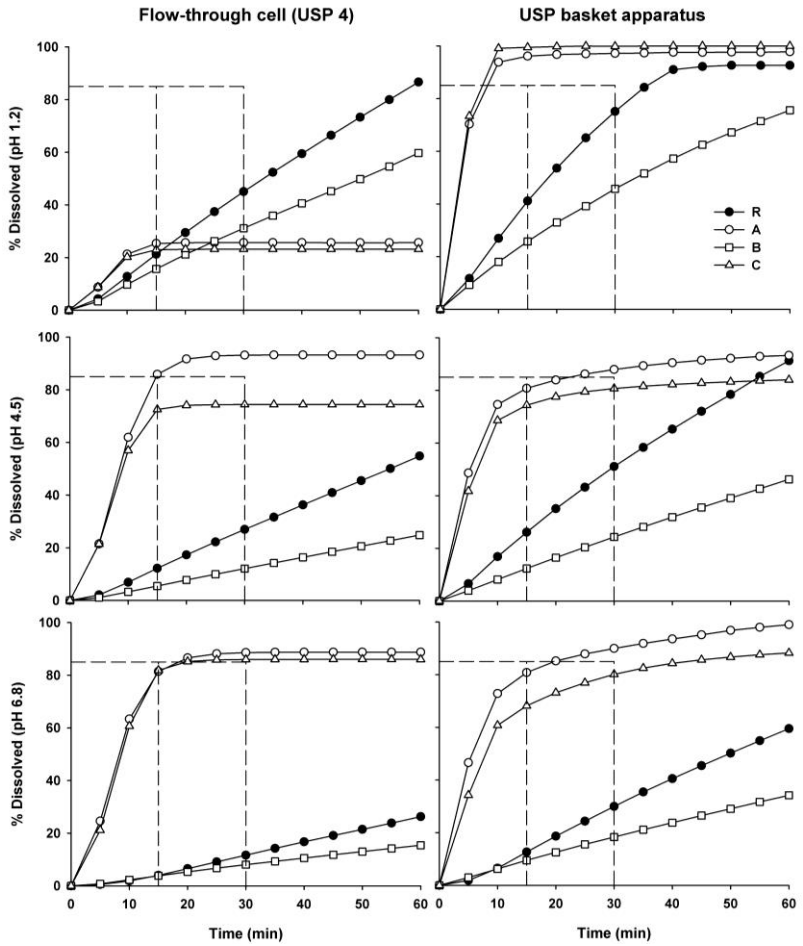

Fig. 2: Dissolution profiles of metronidazole tablets (500 mg) of reference (R) and generic formulations (A−C). Mean, n=12. Dashed lines show the very rapidly or rapidly dissolving conditions. For better clarity, error bars have been omitted

Metronidazole solubilities at 37 °C and pH 1, 5, and 7 were reported as 30.6, 12.8, and 11.6 mg/ml, respectively [2]. Apparently, the dose, type, and volume of the dissolution medium should not be a problem for the complete dissolution of the drug; however, in some cases, limited dissolution was observed with the studied formulations. By the obtained results, only generic product A appeared to not have release problems in any dissolution media, and a biowaiver can be requested for this product if the other requirements are met. Regarding the type and proportion of excipients, there is no information available, as all drug products used were commercial formulations, and we assumed that they met adequate manufacturing criteria.

A simple visual inspection of dissolution profiles (fig. 2) indicates different *in vitro* release performances of metronidazole, regardless of the used condition. The reference product is very sensitive to changes in the pH and hydrodynamic environment of the USP apparatus, while generic product A is the opposite. As some formulations showed coefficients of variation greater than 20% at early sampling times and greater than 10% at other sampling times, no f_2 similarity factors were calculated. For a better comparison of dissolution data, model-independent and model-dependent approaches were used. To compare dissolution profiles by a model-independent method, MDT and DE were calculated and statistically compared. These parameters provide reliable information about the *in vitro* performance of pharmaceutical dosage forms. The results are shown in table 2.

In almost all comparisons, significant differences were found (*P<0.05). The dissolution profiles of generic metronidazole formulations cannot be considered similar to the dissolution profile of the reference. Several authors have considered MDT and DE as adequate parameters to compare dissolution profiles [18]. Additionally, these parameters have been used to establish significant IVIVC levels B and C, respectively. IVIVC level B is defined as the relationship of MDT and mean residence time (mean time that a molecule stays in the body). These two parameters are calculated by statistical moment theory. IVIVC level C is defined as the association of a dissolution time parameter ($t_{50\%}$, $t_{80\%}$, or another time value) and a pharmacokinetic parameter, e. g., area under the curve, C_{max} or T_{max} [19, 20].

To compare dissolution profiles by a model-dependent method, metronidazole dissolution data were adjusted to four common dissolution models. This action was carried out without any physiological significance and only with the aim of finding a mathematical equation that explains the *in vitro* release performance of metronidazole formulations under used conditions. The aim of using mathematical equations to fit dissolution data is that they facilitate the analysis and interpretation of results describing dissolution curves as a function of a few parameters that can be statistically compared [21]. The adjustment of dissolution data to common mathematical models is shown in table 3.

Table 2: Mean dissolution time (MDT) and dissolution efficiency (DE) of the metronidazole reference (R) and generic formulations (A–C)

pH	Key	Flow-through cell (USP 4)		USP basket apparatus	
		MDT (min)	DE (%)	MDT (min)	DE (%)
1.2	R	29.67±0.22	43.75±0.92	18.29±0.20	64.36±0.78
	A	6.66±0.20*	22.77±0.73*	4.39±0.21*	90.63±0.74*
	B	29.59±0.11	30.25±0.52*	25.71±0.15*	43.21±0.52*
	C	6.27±0.11*	20.75±0.69*	4.12±0.10*	93.52±0.60*
4.5	R	30.89±0.21	26.60±0.84	28.00±0.20	48.65±0.51
	A	8.52±0.18*	79.96±3.02*	8.28±0.14*	80.42±0.49*
	B	30.92±0.24	12.03±0.30*	29.06±0.12*	23.84±0.22*
	C	7.36±0.08*	65.23±1.41*	7.70±0.35*	73.17±0.77*
6.8	R	32.99±0.31	11.83±0.40	30.62±0.23	29.17±0.90
	A	8.14±0.13*	76.71±2.27*	10.11±0.17*	82.32±1.30*
	B	29.69±0.22*	7.79±0.20	28.66±0.09*	17.85±0.30*
	C	8.04±0.07*	74.47±1.13*	11.01±0.20*	72.01±1.16*

Mean value±SEM, n=12. *P<0.05

Table 3: Adjusted coefficients of determination and AIC values to choose the best-fit model of metronidazole reference (R) and generic formulations (A–C)

pH	Key	Flow-through cell (USP 4)				USP basket apparatus			
		Zero-order	Peppas-sahlin	Weibull	Logistic	Zero-order	Peppas-sahlin	Weibull	Logistic
$R^2_{adjusted}$									
1.2	R	0.9970	0.9979	0.9859	0.9711	0.8564	0.9005	0.9854	0.9689
	A	0.2671	0.6839	0.4148	0.4513	0.2584	0.5796	0.7916	0.6355
	B	0.9971	0.9982	0.9959	0.9934	0.9869	0.9949	0.9877	0.9837
	C	0.2524	0.6926	0.4155	0.4442	0.2524	0.5408	0.6456	†
4.5	R	0.9986	0.9987	0.9966	0.9955	0.9927	0.9961	0.9829	0.9625
	A	0.3768	0.6917	0.7540	0.8022	0.5940	0.9187	0.9479	0.9618
	B	0.9990	0.9992	0.9986	0.9971	0.9987	0.9997	0.9929	0.9953
	C	0.3074	0.6808	0.6313	0.6946	0.4935	0.8887	0.8604	0.8603
6.8	R	0.9969	0.9979	0.9900	0.9843	0.9968	0.9969	0.9972	0.9970
	A	0.3802	0.7248	0.8031	0.8444	0.6840	0.9358	0.9721	0.9596
	B	0.9965	0.9975	0.9948	0.9899	0.9978	0.9993	0.9961	0.9986
	C	0.3450	0.6807	0.7488	0.8078	0.6975	0.9333	0.9651	0.9720
AIC									
1.2	R	37.55	33.89	57.24	66.11	88.78	85.09	61.59	68.64
	A	65.76	56.41	63.80	62.28	75.75	72.36	60.54	66.22
	B	29.83	24.35	33.58	39.68	52.84	41.02	52.92	55.63
	C	62.07	52.14	59.86	58.51	62.07	66.56	68.41	†
4.5	R	16.75	16.71	29.66	32.16	50.82	43.50	62.64	71.45
	A	99.46	91.74	87.73	83.60	81.25	62.09	55.41	50.52
	B	-4.71	−6.80	−3.05	5.00	11.30	−3.54	34.97	29.76
	C	93.04	84.48	86.12	83.11	82.46	63.91	65.35	63.50
6.8	R	12.22	6.99	24.65	30.41	30.04	30.23	27.87	30.36
	A	96.49	87.48	83.00	79.36	81.98	62.36	52.28	56.29
	B	-2.02	−5.32	1.93	10.57	12.61	−2.17	19.73	4.42
	C	97.56	89.67	86.61	82.50	82.87	64.06	54.14	50.67

Mean value, n=12. †Data not adjusted

When the flow-through cell apparatus with a dissolution medium of pH 1.2 was used, all formulations were adjusted to the Peppas-Sahlin equation, while at pH 4.5 and pH 6.8, some formulations were adjusted to Peppas-Sahlin and others to the logistic model. However, with information on the USP basket apparatus at all dissolution media, some data were adjusted to the Peppas-Sahlin equation, Weibull model, and only one formulation was adjusted to the logistic equation (pH 6.8, product C). As dissolution data of all formulations tested with USP Apparatus 4 and pH 1.2 were adjusted to the Peppas-Sahlin model, dissolution profiles of generic drug products and the reference were compared with the k_1 and k_2 parameters. The Peppas-Sahlin model is shown in Eq. (3). The results are shown in table 4.

$$F = k_1 \cdot t^m + k_2 \cdot t^{2m} \ldots. (3)$$

Table 4: Parameters of the Peppas-Sahlin model used to compare dissolution profiles of the metronidazole reference (R) and generic formulations (A–C). Mean value±SEM, n = 12. *P<0.05

Key	k_1	k_2	m
R	-2.38±0.22	2.58±0.07	0.45
A	9.46±0.24*	-0.85±0.01*	0.45
B	-1.10±0.10*	1.67±0.03*	0.45
C	8.80±0.25*	-0.81±0.01*	0.45

Significant differences in the dissolution profiles of all generic drug products were found (*P<0.05). Due to variability in the adjustments at all other dissolution conditions used, the comparison of dissolution profiles could not be carried out by a model-dependent approach; however, both comparison methods (model-independent and model-dependent) revealed high variability in the release

performance of all formulations and the need for *in vivo* studies to ensure the interchangeability of metronidazole 500 mg tablets.

Although basket and paddle apparatuses are currently the most popular methods to study the *in vitro* release of many drug products, both methods are operated under closed finite *sink* conditions and cannot mimic the conditions observed in the digestive system [22]. Some authors have shown that the gastrointestinal tract acts as a natural *sink*: the drug is absorbed as soon as it dissolves; thus, *in vivo,* there is no concentration build-up at the absorption site; the drug concentration in the surrounding fluids remains very low, and dissolution occurs under *sink* conditions. The advantage of the flow-through cell is its ability to permit a suitable continuous flow through an open circuit [23]. For such features, the flow-through cell can be used to assess the intraluminal disintegration of dosage forms [24].

Metronidazole generic formulations are widely available worldwide. Manufacturing of these products follows well-defined quality standards, but it is important to maintain an evaluation of commercial drug products for the following reasons: metronidazole has good oral absorption (bioavailability ≥ 90%) [25], but nonbioequivalence and treatment ineffectiveness due to low plasma levels in patients have been reported [26-28]. Moreover, significant differences in dissolution behaviour from commercial formulations, even between batches of the same brand, have been reported [29].

The importance of a postmarketing evaluation of commercial metronidazole formulations has been pointed out by several authors. Medina *et al.* [30] studied the *in vitro* release performance of metronidazole tablets with two therapeutic doses (250 and 500 mg). The flow-through cell and USP basket apparatus with 0.1 N hydrochloric acid as the dissolution medium was used. With the flow-through cell, formulations of 250 mg were considered rapidly dissolving products, and tablets of 500 mg were not considered as rapidly dissolving products, while with the USP basket apparatus, formulations of 250 mg were classified as very rapidly dissolving products, and tablets of 500 mg were classified as rapidly dissolving products.

Additionally, Medina *et al.* [31] studied the dissolution behaviour of benzoyl metronidazole suspensions under the hydrodynamics of the flow-through cell and simulated gastrointestinal fluids. Significant differences between a generic formulation and the reference were found in the following dissolution media: simulated gastric fluid with/without pepsin, simulated intestinal fluid without pancreatin and fasted state simulated intestinal fluid. As a recommendation of both research works, bioequivalence studies should be carried out to classify metronidazole generic drug products as interchangeable with the reference.

CONCLUSION

It is important to perform postmarketing monitoring of metronidazole tablets to ensure safety and efficacy, as the four commercial metronidazole formulations tested with the flow-through cell and dissolution media of physiological relevance showed significant differences. With the USP basket apparatus, only one drug product met the criterion of a rapidly dissolving product within dissolution media of pH 1.2 to 6.8. Considering this result, generic formulation A could be a candidate to waiver *in vivo* studies, but different results were obtained with the flow-through cell. Considering USP Apparatus 4 as equipment that better simulates the hydrodynamic environment of the gastrointestinal tract, these differences are likely to be reflected in the clinic. The main problem found with the studied formulations is that the reference showed limited *in vitro* release, while generics showed different dissolution performance than the reference; therefore, they cannot be considered therapeutic equivalents. Bioequivalence studies are suggested for metronidazole 500 mg generic tablets. More research with metronidazole oral drug products is also necessary.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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