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Short Communication

IN VITRO RELEASE AND PREDICTED *IN VIVO* BEHAVIOR OF METRONIDAZOLE VAGINAL FORMULATIONS

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ABSTRACT

Objective: To document the *in vitro* release and to predict the *in vivo* behavior of metronidazole ovules (reference and generic formulations) using USP Apparatus 1 and 4. Prediction of metronidazole plasma concentrations was proposed with the Inverse Release Function approach. The information generated can be considered for the development of new metronidazole vaginal drug products.

Methods: Dissolution profiles were obtained using USP Apparatus 1 at 100 rpm and 900 ml of pH 4.5 acetate buffer. Additionally, USP Apparatus 4 at 16 ml/min was used. Drug was quantified at 278 nm every 10 min until 60 min. Mean dissolution time (MDT) and dissolution efficiency (DE) were calculated. Mathematical models such as Korsmeyer-Peppas, Makoid-Banakar, Peppas-Sahlin, Logistic and Weibull were used to fit *in vitro* data. Percent of prediction error (%PE) for C_{max} and $AUC_{0\text{-}inf}$ were calculated.

Results: Metronidazole ovules of reference formulation released<2% at 60 min in both dissolution methods. Generic formulation released>85%. Values of DE and MDT using USP Apparatus 1 and 4 were 40.40%, 31.94 min, 70.91% and 15.44 min, respectively. *In vitro* release of generic drug product was better described by Weibull function. %PE for C_{max} and $AUC_{0\text{-}inf}$ were <15%.

Conclusion: Due to limited drug release of reference formulation it was not possible to know the *in vitro* behavior of this drug product. Generic formulation showed a better *in vitro* performance by being able to characterize the main dissolution parameters DE and MDT and a release kinetics well defined by a mathematical equation.

Keywords: Convolution, Inverse release function, Metronidazole, Ovules, USP apparatus 4

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Bacterial vaginosis is a common vaginal condition marked by the presence of a thin white or gray vaginal discharge and a fishy odor [1]. This condition is characterized by a loss of the normal hydrogen peroxide (H_2O_2)-producing vaginal lactobacilli and an increase in the presence of anaerobic bacteria [2]. Bacterial vaginosis is a prevalent women's health issue that affects millions of women worldwide [3]. Treatment of bacterial vaginosis is mostly applied with oral and vaginal formulations of antibiotic drugs such as metronidazole or clindamycin [4, 5] however, oral administration of metronidazole would experience first-pass metabolism and can cause gastrointestinal adverse effects [5].

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) is a synthetic oral nitroimidazole antibiotic drug which is used for the treatment of infections caused by anaerobic bacteria and protozoa [6]. Metronidazole has a good bioavailability (≥ 90%) [7] . However, several oral absorption difficulties [8], lack of *in vivo* equivalence [9] and treatment ineffectiveness due to low plasma concentrations have been reported [10-12].

Metronidazole is a Biopharmaceutic Classification System Class I, being highly soluble and highly permeable drug [13]. Pharmacopeial dissolution test for metronidazole tablets suggests USP Apparatus 1 (basket method) at 100 rpm with 900 ml of 0.1 N HCl and not less than 85% of the labelled amount should be dissolved in 60 min [14]. To date, no dissolution test for metronidazole ovules or vaginal suppositories has been suggested by the USP [14].

Intravaginal administration of drugs, especially for treatment of *Trichomonas* and *Candida* infections, may lead to a systemic effect due to remarkable absorption of some drugs from the vaginal wall [15]. Some investigations have focused on the study of the release and absorption of metronidazole vaginal formulations [15-18]. Gel, vaginal mucoadhesive tablet, ovule, mucoadhesive film, tablet and suspensions have been used for the vaginal administration of metronidazole [19]. The authors reported that a study comparing vaginal gel and oral formulations containing metronidazole for the treatment of bacterial vaginosis it has been found to be nearly close treatment rate.

The aim of this research is to document the *in vitro* release of metronidazole ovules and to predict the drug plasma concentrations to estimate the *in vivo* behavior of commercial drug products. Conditions include USP Apparatus 1 and USP Apparatus 4 (flow-through cell method) and dissolution media with vaginal pH. The vaginal pH of a healthy woman of reproductive age is acidic (pH = 4- 5) [15]. The information generated can be considered for the development of new metronidazole vaginal formulations.

Metronidazole ovules of reference formulation (Flagyl V 500 mg, Sanofi-Aventis de México S. A. de C. V. Mexico City, Mexico) and a generic drug product with the same dose were used. Mexican health authorities have established this commercial brand as the reference formulation for dissolution and bioequivalence studies [20]. HCl, HPLC acetonitrile, HPLC methanol, acetic acid and sodium acetate were acquired from J. T. Baker-Mexico (Xalostoc, Mexico). Metronidazole standard was acquired from Sigma-Aldrich Co. (St. Louis MO, USA). Metronidazole content in commercial ovules was determined by a previously validated HPLC method.

Dissolution profiles of metronidazole ovules were obtained using USP Apparatus 1 at 100 rpm (Sotax AT7-Smart, Sotax AG, Switzerland) with 900 ml of pH 4.5 acetate buffer at 37.0±0.5 °C (n = 12). Additionally, metronidazole ovules were tested with USP Apparatus 4 (Sotax CE6, Sotax AG, Switzerland) at a flow rate of 16 ml/min using 22.6 mm cells (i.d.). Turbulent flow was used. The amount of dissolved metronidazole was determined at 278 nm every 10 min during 60 min with the support of a calibration curve.

Common dissolution parameters such as dissolution efficiency (DE) and mean dissolution time (MDT) were calculated. Metronidazole dissolution data were fitted with different mathematical equations: Korsmeyer-Peppas, Makoid-Banakar, Peppas-Sahlin, Logistic and Weibull. The model with the highest adjusted determination

coefficient ($_{Adjusted}$ R^2) and the lowest Akaike Information Criterion (AIC) was chosen as the best-fit model [21]. Data analysis was carried out using the Excel add-in DDSolver program [22].

Metronidazole plasma concentrations were predicted through the Inverse Release Function approach proposed by Cardot *et al.* [23]. This procedure allows an adjustment in the time scale of the dissolution process to facilitate the establishment of a significant *in vitro*/*in vivo* correlation. With the new time scale of dissolution profile predicted plasma concentrations were calculated with a simple numerical convolution method created by Qureshi [24] using an MS Excel spreadsheet. The method uses reported pharmacokinetic parameters such as bioavailability factor (F), elimination rate constant (k_e), and volume of distribution (V_d) to construct metronidazole plasma concentration-time profiles [13]. Using this, pharmacokinetic parameters such as peak concentration (C_{max}) and area under the concentration-time curve from zero to infinity ($AUC_{0\text{-}inf}$) were calculated by a compartmental method using the Excel add-in PKSolver program [25]. Reported data about the metronidazole plasma concentrations-time profile [26] of the reference drug product Flagyl (tablets, 500 mg) were used to estimate the predictability of the convolution method, which was established by the calculation of the mean absolute percent of prediction error (%PE) for C_{max} and $AUC_{0\text{-}inf}$ according to Eq. 1 (where %PE should not exceed 15%) [27-29].

$$\%PE = \frac{(\text{observed value} - \text{predicted value})}{\text{observed value}} \times 100 \ldots\ldots \text{Eq. (1)}$$

The mean drug content±SD of ten metronidazole ovules was 103.27±2.57% for reference formulation and 102.40±3.77% for generic drug product. As can be seen, the drug content corresponds to what is declared on the label.

Dissolution profiles of metronidazole ovules from reference formulation using USP Apparatus 1 and 4 are shown in fig. 1. As a limited *in vitro* release behavior was observed at 60 min with both dissolution methods (<2%) no MDT values and data adjustment were carried out. An image of both dissolution apparatuses with the release experiment of this formulation at 60 min is shown in fig. 2. The image shows that at the last sampling time, the ovules only slightly modified their original shape, which justifies the poor release from this formulation. For this reason, the prediction of plasma concentrations-time profile was also not carried out. In this regard, some authors point out several factors associated with the treatment of bacterial vaginosis by standard administration of metronidazole. The data suggest that treatment failure might not be caused by drug resistance [30]. Drug release from vaginal suppositories is influenced by some factors such as drug-vehicle interactions, solubility, the particle size of the drug and drug concentration in the vehicle [15].

On the other hand, dissolution profiles of metronidazole ovules from generic drug product using USP Apparatus 1 and 4 are shown in fig. 1. A better *in vitro* release behavior was observed. Dissolution parameters such as percent of the dissolved drug at 60 min (Q_{60}), DE and MDT are shown in table 1. As no values of reference formulation were calculated no dissolution profiles comparison, using these dissolution parameters or f_2 similarity factor, was possible. The adjustment of dissolution data to several mathematical equations are shown in table 2. The *in vitro* release of metronidazole from generic drug product using both dissolution apparatuses was well described by the Weibull function. The fit of dissolution data to this function emphasizes the S-shaped or sigmoidal dissolution profiles [31].

Dissolution of metronidazole from vaginal suppositories (500 mg) have been studied by some authors [15]. Formulations of metronidazole were tested with the USP Apparatus 1 at 100 rpm and 900 ml of pH 4.5 lactate buffer as dissolution medium. The complete dose was released at 90 min. In other report, several authors have studied the *in vitro* release of metronidazole from vaginal suppository formulations. The USP Apparatus 1 at 100 rpm and 900 ml of pH 4.5 lactate buffer was used. Five kinds of 500 mg metronidazole suppositories released the drug at different rates; however, all of them released the complete dose at 60 min [32].

Some authors have tested the USP Apparatus 2 (paddle method) and USP Apparatus 4 to study vaginal formulations [33]. The use of pH 5.2 acetate buffer containing 1% sodium dodecyl sulphate with both methods gave different dissolution profiles. Results showed that with USP Apparatus 2 almost 90% of the drug was released at 20 min while with USP Apparatus 4 at 16 ml/min more than 80% of the drug was dissolved after 30 min. As conclusion the slower rate of dissolution obtained in the USP Apparatus 4 would help to distinguish between different formulations.

New metronidazole vaginal formulations have been recently proposed as nanofibers where they were tested using USP Apparatus 1 at 100 rpm and pH 4.5 phosphate buffer as dissolution medium [19], while comparative bioavailability of metronidazole vaginal film-coated tablets has also been reported [16].

The predicted plasma concentrations-time profiles of metronidazole from generic drug products are shown in fig. 1. *In vivo* data from a bioequivalence study of metronidazole reference tablets (500 mg) reported by Herrera [26] were used as comparison data. Several authors have reported that the effect of intravaginal administration of metronidazole was as effective as oral administration but with less gastrointestinal issues [5]. The %PE between the observed pharmacokinetic data and those calculated by the convolution method, using USP Apparatus 1 and 4 did not exceed 15%; this indicated the validity of the convolution method [27]. A previous report state that the effectiveness of metronidazole vaginal and oral regimens is similar [19]. Another work details that the efficacy of oral metronidazole for the treatment of bacterial vaginosis has been reported to range from 78-92% when evaluated four weeks after treatment, compared to 61-94% for vaginal metronidazole [2].

Several authors have recommended that physiological parameters should be considered during the design and preclinical evaluation of vaginal formulations. Drug release tests should be performed to prove the efficacy of vaginal formulations developed in order to obtain systemic or local effect. The pH and low liquid volume of the medium must be taken into consideration in order to form biocompatible media during vaginal drug release tests [19].

Due to poor drug release from the reference formulation, it was not possible to characterize the *in vitro* performance of metronidazole. The generic drug product showed a better dissolution behavior by being able to characterize the main dissolution parameters and a clearly defined *in vitro* release kinetics through a mathematical equation. It is important to discuss the characteristics that the reference formulations must show to be considered as comparison formulations. It is necessary to carry out bioavailability studies with these vaginal drug products to establish the absorption rate and relate it to the observed *in vitro* release behavior.

Table 1: Dissolution parameters of metronidazole ovules mean±SEM, n = 12

Code	Q_{60} (%)	DE (%)	MDT (min)
USP Apparatus 1			
R	1.64±1.09	1.00±0.07	-
G	85.83±2.57	40.40±2.10	31.94±0.76
USP Apparatus 4			
R	1.35±0.09	0.70±0.05	-
G	95.50±0.60	70.91±0.40	15.44±0.22

R: reference, G: generic, Q_{60}: drug released at 60 min, DE: dissolution efficiency, MDT: mean dissolution time

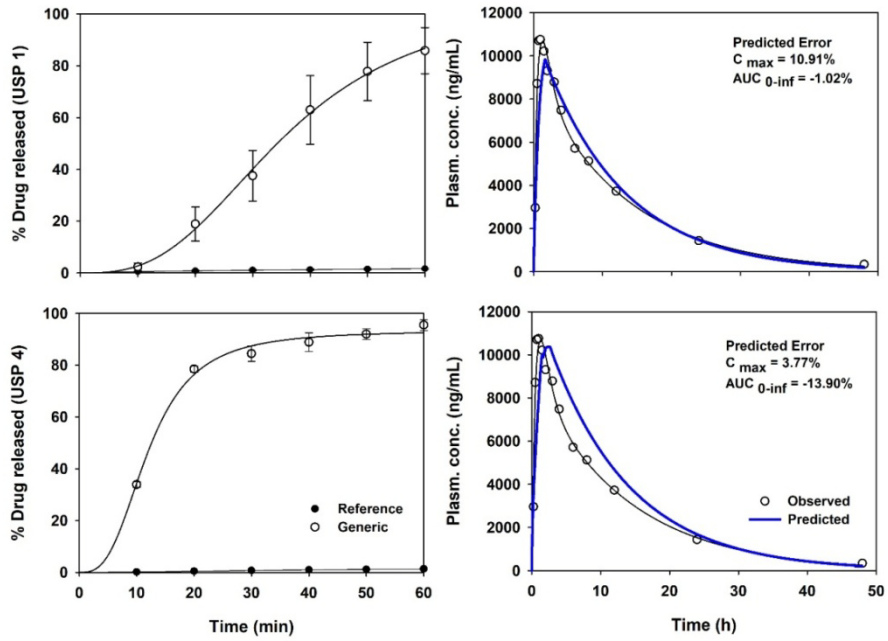

Fig. 1: Dissolution profiles of metronidazole ovules using USP basket apparatus (USP 1) and flow-through cell method (USP 4) and predicted plasma concentrations of generic formulation. Observed data were reported by Herrera [26]. mean±SD, n = 12

Fig. 2: Image of the dissolution of the reference formulation after 60 min using the USP Apparatus 1 (A) and 4 (B). The ovules only slightly modified their original shape

Table 2: Used criteria to choose the best-fit model of *in vitro* release data. Mean, n = 12

Parameter	Code	Korsmeyer-Peppas	Makoid-Banakar	Peppas-Sahlin	Logistic	Weibull
USP apparatus 1						
$_{Adjusted}R^2$	R	-	-	-	-	-
	G	0.9519	0.9958	0.9745	0.9943	**0.9961**
AIC	R	-	-	-	-	-
	G	35.36	20.19	30.96	21.83	**19.10**
USP Apparatus 4						
$_{Adjusted}R^2$	R	-	-	-	-	-
	G	0.7432	0.8815	0.8480	0.9571	**0.9877**
AIC	R	-	-	-	-	-
	G	41.69	36.98	38.64	29.59	**20.08**

R: reference, G: generic, AIC: Akaike Information Criterion, the best-fit results are in bold

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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