ABSTRACT

Objective: To estimate plasma concentrations-time profiles of metronidazole commercial tablets through in vitro dissolution data using the Inverse Release Function approach and a convolution method.

Methods: Dissolution profiles of metronidazole reference tablets (500 mg) were obtained using USP Apparatus 1 at 100 rpm, USP Apparatus 4 at 16 ml/min, and 0.1 N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer as dissolution media. Additionally, three generic drug products were tested using USP Apparatus 1 and pH 4.5 acetate buffer. Drug was quantified at 278 nm until 60 min. Dissolution parameters such as mean dissolution time, area under the cumulative dissolution curve, and dissolution efficiency were calculated. Metronidazole plasma levels were predicted considering the in vitro release data and published information. Percent of prediction error (PE) for $C_{\text{max}}$ and AUC$_{0-\text{inf}}$ at each condition was calculated.

Results: When comparing dissolution profiles with common dissolution parameters (USP 1 vs. 4) significant differences were found ($P<0.05$). Values of PE for $C_{\text{max}}$ and AUC$_{0-\text{inf}}$ were within range ($\pm15\%$) only with USP Apparatus 1 and pH 4.5 acetate buffer. Using these conditions when comparing generic drug products vs. reference formulation, significant differences were found ($P<0.05$) and values of PE for AUC$_{0-\text{inf}}$ were out of the range.

Conclusion: The obtained information suggests using USP Apparatus 1 and pH 4.5 acetate buffer to predict the in vivo performance of metronidazole tablets. The impact of in vitro differences of all generic formulations was confirmed with differences in predicted in vivo performance.

Keywords: Convolution, Inverse release function, Metronidazole, Prediction error, Tablets

INTRODUCTION

An important objective of pharmaceutical product development is to gain a better understanding of the in vitro and in vivo drug performance. Through the successful development and application of an in vitro/in vivo correlation (IVIVC), in vivo drug performance can be predicted from its in vitro behavior [1]. Prediction of plasma concentration-time profiles can be established by a convolution approach. The convolution technique has some advantages: a) the procedure does not require an in vivo study for the test product to obtain pharmacokinetic parameters (bioavailability factor, $F$; volume of distribution, $V_d$; elimination rate constant, $k_e$) since values of these parameters are available in the literature and b) it is not necessary to purchase sophisticated computer software since a simple spreadsheet software (MS Excel) may be used [2].

For immediate-release dosage forms, the successful development of IVIVC models may be limited to Class 2 and Class 3 compounds classified under the Biopharmaceutics Classification System (BCS). According to Food and Drug Administration (FDA) guidelines, bioequivalences can also be requested for Class 1 compounds provided the drugs are solubilized in the gastric fluid sufficiently rapidly that gastric emptying does not become the rate-limiting step [1]. Gastric emptying time is 15-20 min under fasting conditions [3]. Metronidazole is a BCS Class 1, being a highly soluble and highly permeable drug [4]. The monograph states that a bioequivalent for metronidazole solid immediate-release formulations is justified provided, among other characteristics: the test product and its comparator are both rapidly dissolving [4]. According to FDA guidance, immediate-release formulations are considered rapidly dissolving products when a mean of 85% or more dissolves within 30 min using USP basket apparatus (USP Apparatus 1) and dissolution media of pH 1.2 as well as pH 4.5 and pH 6.8 buffers [5].

Pharmacopeial dissolution test for metronidazole tablets suggests USP Apparatus 1 at 100 rpm with 900 ml of 0.1 N HCl as dissolution medium and not less than 85% of the labelled amount should be dissolved in 60 min [6]. On the other hand, the flow-through cell method (USP Apparatus 4) is an alternative to conventional USP dissolution testing apparatuses [7]. The USP Apparatus 4 offers several advantages over the USP Apparatus 1 and paddle apparatus (USP Apparatus 2). These include the ability to maintain sink conditions due to the continuous introduction of dissolution medium to the dissolution cell in an open system and the ease with which the composition and pH of the dissolution medium can be changed over the course of a dissolution test [8].

The influence of dose and USP apparatus in the in vitro release performance of metronidazole reference tablets as well as the pharmaceutical equivalence of metronidazole generic drug products with media of physiological relevance has been previously reported [9, 10]. Significant differences were found between the therapeutic dose of 250 and 500 mg using USP Apparatus 1 and 4 ($P<0.05$). Some generic drug products showed different dissolution profiles than that observed with the reference formulation using 0.1 N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer as dissolution media. Benzoyl metronidazole suspensions have also been studied with the USP Apparatus 4 and simulated gastrointestinal fluids [11]. Significant differences in the rate and extent of drug dissolution of generic and reference drug products were found ($P<0.05$). Given the in vitro release conditions in which all formulations were tested, these differences could be of clinical implication.

The aim of this research is to predict the metronidazole plasma concentrations-time profiles through in vitro dissolution data using the Inverse Release Function approach [12] and to estimate the in vivo performance of metronidazole tablets with a convolution method [13]. Conditions included the use of the USP Apparatus 1 and 4 and dissolution media of physiological relevance (pH 1.2-2.0). A first set of experiments included the in vitro release of reference formulation and when obtaining the conditions that best reflect the in vivo performance, the metronidazole generic drug products for sale in the local market will be evaluated.
**MATERIALS AND METHODS**

**Reagents and chemicals**

Metronidazole reference tablets (coded as R formulation) (Flagyl 500 mg, Sanofi-Aventis de México S. A. de C. V. Mexico City, Mexico) and three generic formulations (coded as A, B, and C drug products) with the same dose were used. Mexican health authorities have established Flagyl drug product as the reference formulation for dissolution and bioequivalence studies [14]. HCl, sodium acetate, and phosphate monobasic and dibasic salts were acquired from J.T. Baker-Mexico (Xalostoc, Mexico). Metronidazole standard was acquired from Sigma-Aldrich Co. (St. Louis MO, USA).

**Uniformity of dosage units and assay**

Uniformity of dosage units and assay tests were performed with all formulations according to the procedures described in the USP [6].

**In vitro dissolution profiles**

In the first part of the in vitro release studies, dissolution profiles of R formulation were obtained using a USP Apparatus 1 at 100 rpm (Sotax AT7-Smart, Sotax AG, Switzerland) and 900 ml of dissolution medium. Additionally, the USP Apparatus 4 (Sotax C6, Sotax AG, Switzerland) at a laminar flow rate of 16 ml/min and 22.6 mm cells (ld) was used. In both dissolution apparatuses, 0.1 N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer at 37.0±0.5 °C were used as dissolution media. In all cases, dissolution samples were taken at 10-, 20-, 30-, 45-, and 60-min using fiberglass filters (n = 12). The amount of dissolved metronidazole was determined by UV measurement at 278 nm with the support of a standard calibration curve in each dissolution medium. In the second part of release studies, dissolution profiles of R formulation and all generic drug products were determined using the USP Apparatus 1 at 100 rpm and 900 ml of pH 4.5 acetate buffer at 37.0±0.5 °C. Samples of dissolution medium were withdrawn at same sampling times of the first part of dissolution studies and dissolved metronidazole was calculated with a standard calibration curve in pH 4.5 acetate buffer (n = 12).

**Data analysis**

Common dissolution parameters such as mean dissolution time (MDT), area under the cumulative dissolution curve (AUC), and dissolution efficiency (DE) were calculated and statistically compared. Dissolution parameters were determined with the Excel add-in DDSolver program [15]. After this, f2 similarity factor was also calculated with the aim of comparing dissolution profiles. Similar profiles were considered if f2 = 50-100 [16]. A first set of comparisons were considered with USP Apparatus 1 vs. 4 data (Student’s t-test) while the second set of comparisons were A-C generic drug products vs. R formulation (one-way ANOVA followed by a Dunnett’s multiple comparison test) with a model-independent approach [17]. Significant differences were considered if *P*<0.05.

**Prediction of metronidazole in vivo performance**

Metronidazole plasma levels were calculated by the support of the Inverse Release Function approach proposed by Cardot et al. [12]. This methodology allows and adjustment in the time scale of the in vitro release performance to facilitate the establishment of a meaningful IVIVC. Once the new time scale of the dissolution profile is calculated, predicted plasma concentrations-time profiles were determined with a simple numerical convolution method proposed by Qureshi [13]. The method used enables the calculation of metronidazole pharmacokinetic parameters such as bioavailability factor (F), elimination rate constant (k_e), and volume of distribution (V_d) [4]. From predicted plasma concentrations-time profiles, pharmacokinetic parameters such as peak concentration (C_max) and area under the concentration-time curve from zero to infinity (AUC_0-inf) were calculated by a compartmental method using the Excel add-in PK Solver program [19]. Reported data of a metronidazole bioequivalence study with R formulation (500 mg tablets) were used to estimate the predictability of the convolution method [19]. It has been established by the calculation of the percent of prediction error (%PE) for C_max and AUC_0-inf according to Eq. 1 (where %PE should not exceed 15%) [20-22].

\[
\%PE = \frac{\text{observed value} - \text{predicted value}}{\text{observed value}} \times 100 \quad \text{Eq. (1)}
\]

**RESULTS AND DISCUSSION**

**Uniformity of dosage units and assay**

Metronidazole tablets were within USP limits. The mean standard deviation (SD) of ten metronidazole reference tablets in uniformity of dosage units test was 104.36±0.30% (85-115% as USP limit); in assay test with three samples was 99.81±0.70% (90-110% as USP limit) [6]. All generic drug products also met both pharmacopeia tests.

**In vitro dissolution profiles**

Dissolution profiles of metronidazole R formulation using USP Apparatus 1 and 4 are shown in fig. 1 and fig. 2, respectively. Dissolved drug (mean±SD) at 30 min, using USP Apparatus 1 and 900 ml of 0.1 N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer, was 78.63±2.70%, 45.26±3.03%, and 41.7±4.89%, respectively. This formulation in no condition, was able to dissolve more than 85% of the drug at 30 min so R formulation cannot be considered as a rapidly dissolving product. The result differs from the reported solubility of metronidazole at 37 °C where at pH 1.0, pH 5.0 and pH 7.0 was 30.6, 12.8, and 11.6 mg/ml, respectively [4]. According to this information, the therapeutic dose used should have no problem to be completely dissolved from the oral dosage form; however, limited in vitro release was observed. Metronidazole reference tablets met the pharmacopoeia criteria as more than 85% of the dissolved drug was found at 60 min (98.78±2.96% with 0.1 N HCl). On the other hand, the use of USP Apparatus 4 significantly affected both rate and extent of released metronidazole, as the drug dissolved considerably slower. Dissolution parameters used to compare dissolution profiles and f2 similarity factor (USP 4 vs USP 1) are shown in table 1. In all cases, significant differences were found (*P*<0.05) and all f2 values were less than 50.

**Estimation of in vivo plasma levels**

To identify whether the conditions for the USP Apparatus 1 or USP Apparatus 4 reflected the in vivo performance of metronidazole in humans, it was necessary to predict the in vivo plasma concentration-time profiles from the in vitro dissolution data. An adjustment in the time scale of dissolution profiles, as well as the values of some metronidazole pharmacokinetic parameters, were used to estimate the in vivo performance of metronidazole commercial tablets. Plasma concentration-time data from USP Apparatus 1 and 4 dissolution data are shown in fig. 1 and fig. 2, respectively. Only using USP Apparatus 1 at 100 rpm and 900 ml of pH 4.5 acetate buffer as dissolution medium the values of PE for C_max and AUC_0-inf were<15%, indicating the validity of the convolution method [20] as well as the best in vitro conditions to predict the in vivo performance of metronidazole tablets.

**Comparison of generics and reference**

As the above result suggests the best conditions to test the in vitro release, three metronidazole generic drug products were studied, and dissolution profiles were compared with the dissolution profile of R formulation. Results are shown in fig. 3. At 30 min, all generic drug products released more than 85% of drug while R formulation dissolved 44.45±1.70%. At 60 min, all formulations dissolved more than 85% of metronidazole. Difference in in vitro release of all generic drug products and R formulation is evident even so, statistical comparison of MDT, AUC, and DE parameters were carried out. Significant differences in all parameters were found (*P*<0.05). Results are depicted in table 2. The calculation of the f2 similarity factor confirms the differences between dissolution profiles (f2<50). Predicted metronidazole plasma concentrations of all generic drug products and R formulations are shown in fig. 3. All PE values of AUC_0-inf from generic formulations were less than 15% (table 2). Therefore, it is considered that generic drug products would not have the same in vivo performance as the R formulation. On the other hand, PE values of C_max and AUC_0-inf of R formulation were in the range of±15% which maintains the prediction of the in vivo behavior of the R drug product used by us like that observed in the previously published bioequivalence study. It is known that metronidazole has a good bioavailability (90%) [23]; however, several complications have been documented such as absorption
problems [24], non-equivalence cases [25] and treatment ineffectiveness due to low plasma concentrations [26-28]. The results of the present in vitro release study could be related to previously reported problems. On the other hand, some efforts have been made to obtain better and more effective metronidazole formulations [29, 30].

Fig. 1: Dissolution profiles of metronidazole tablets using USP apparatus 1 (left) and predicted plasma levels (blue line at right). Observed data (full circles) were reported by Herrera [19]. Data is given as mean, n = 12

Fig. 2: Dissolution profiles of metronidazole tablets using USP apparatus 4 (left) and predicted plasma levels (blue line at right). Observed data (full circles) were reported by Herrera [19]. Data is given as mean, n = 12
The authors declare no conflict of interest.

CONFLICT OF INTERESTS

All authors have contributed equally.

AUTHORS CONTRIBUTIONS

Nil

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES


Table 1: Dissolution parameters and $f_2$ similarity factors calculated to compare dissolution profiles (USP 4 vs. USP 1)

<table>
<thead>
<tr>
<th>pH</th>
<th>USP Apparatus 1</th>
<th>USP Apparatus 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>19.3±0.29</td>
<td>30.47±0.23</td>
</tr>
<tr>
<td>4.5</td>
<td>29.07±0.28</td>
<td>4.5</td>
</tr>
<tr>
<td>6.8</td>
<td>27.84±0.28</td>
<td>31.59±0.47</td>
</tr>
</tbody>
</table>

Table 2: Dissolution parameters and $f_2$ similarity factors calculated to compare dissolution profiles (A-C vs. R formulations). Predicted errors for $C_{\text{max}}$ and $AUC_{0-\text{inf}}$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>R</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDT (min)</td>
<td>19.3±0.29</td>
<td>30.47±0.23</td>
<td>4.5</td>
<td>31.59±0.47</td>
</tr>
<tr>
<td>AUCC (%-min)</td>
<td>401.48±26.34</td>
<td>2440.96±32.97*</td>
<td>40.68±0.55 *</td>
<td>29.45</td>
</tr>
<tr>
<td>DE (%)</td>
<td>66.8±0.44</td>
<td>44.6±0.76</td>
<td>40.16±0.44</td>
<td>-</td>
</tr>
<tr>
<td>$f_2$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PE (%) for $C_{\text{max}}$</td>
<td>1.96</td>
<td>-7.32</td>
<td>-3.51</td>
<td>-7.17</td>
</tr>
<tr>
<td>PE (%) for $AUC_{0-\text{inf}}$</td>
<td>-11.62</td>
<td>-28.55</td>
<td>-17.47</td>
<td>-28.96</td>
</tr>
</tbody>
</table>

MDT: mean dissolution time, AUCC: area under the cumulative dissolution curve, DE: dissolution efficiency, PE: prediction error, R: reference, A-C: generic formulations, Data is given as mean±SD, n = 12, *P<0.05.


