Development of USP Apparatus 3 Dissolution Method with IVIVC for Extended Release Tablets of Metformin Hydrochloride and Development of a Generic Formulation

Thamara de Carvalho Mendes, Alice Simon, Jaqueline Correia Villeça Menezes, Eduardo Costa Pinto, Lucio Mendes Cabral, and Valeria Pereira de Sousa*

Department of Drugs and Pharmaceutics, Faculty of Pharmacy, Federal University of Rio de Janeiro; Rio de Janeiro, RJ 21941–902, Brazil.

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Metformin is a euglycemic drug for the treatment of type 2 diabetes mellitus. To date, there are 13 dissolution methodologies described in the U.S. Pharmacopoeia (USP) to evaluate the release profile of metformin from extended-release tablets utilizing either a USP apparatus 1 (basket) or 2 (paddle). In the absence of a protocol for a USP apparatus 3 (reciprocating cylinder), the goal of this work was to develop an in vitro dissolution method for metformin extended-release tablets based on an in vivo–in vitro correlation (IVIVC). Following a systematic evaluation, a final dissolution method, M4, was defined. It applied 30 dips per minute (dpm) over a total period of 10h into a series of solutions that included 2h in HCl media (pH 1.2), 1h in an acetate buffer solution (pH 4.5), 1h in phosphate buffer solution (PBS) (pH 5.8) and 6h in PBS (pH 6.8). This method showed a significant IVIVC with a calculated $R^2 > 0.98$ (point-to-point correlation, Level A) and it was successfully used as a tool to assist in the development of generic extended release formulations for metformin consisting of a lipophilic matrix system.

Key words metformin hydrochloride; United States Pharmacopoeia apparatus 3; dissolution; oral extended-release formulation; in vivo–in vitro correlation

Introduction

Metformin (MTF) is a first-line euglycemic drug for the treatment of type 2 diabetes mellitus. Its main therapeutic action is to improve the uptake of peripheral glucose that reduces plasma glucose levels and increases insulin sensitivity without causing hypoglycemia. The drug is highly hydrophilic and is classified as class III according to the Biopharmaceutical Classification System (BCS) with high solubility and low permeability. Its $pK_a$ value is 11.5, the log$P$ is $-1.43$ and the drug is ionized at physiological pH. An extended-release formulation (ER) of MTF was developed in tablet form to decrease its observed gastrointestinal side effects. The ER tablet has a hydrophilic matrix system primarily composed of hydroxy propyl methylcellulose (HPMC) polymer, which controls the dissolution rate from the matrix. In vitro dissolution tests are essential tools for assessing the quality performance of a finished product. Through suitable mathematical models, in vitro dissolution data can be correlated with in vivo pharmacokinetic data to establish an in vivo–in vitro correlation (IVIVC). A high level of correlation is achieved when there is a point-to-point relationship between the in vitro dissolution data and in vivo absorption data, which is defined as a level A correlation. The development of discriminative dissolution tests requires the selection of suitable hydrodynamic conditions for each type of formulation. Among the dissolution apparatuses described in USP, USP 1 and USP 2 have been widely used to evaluate the dissolution of solid oral dosage forms. However, for ER formulations, the USP 3 apparatus (USP 3; reciprocating cylinder) is more suitable because of its ability to expose the product to a series of solutions with different pHs and immersion rates. This apparatus provides more adequate hydrodynamic conditions compared to USP 1 and 2, and can better mimic the various conditions of the GIT. Therefore, the goal of this work was to develop an in vitro dissolution methodology for MTF ER tablets using USP 3 based on IVIVC. In addition, the method that showed the highest level of IVIVC was used as a tool to assist in the development of generic ER formulations for MTF using a lipophilic matrix system.

Experimental

Chemical and Reagents A reference standard of metformin hydrochloride, with 99.7% purity, was purchased from...
Drug Solubility  The solubility of MTF was determined in hydrochloric acid (HCl) pH 1.2, sodium acetate buffer solution (ABS) pH 4.5 and potassium PBS pH 5.8, 6.8 and 7.2. Saturated solutions were obtained by adding a known excess of MTF to beakers containing 5 mL of each solution that had been preheated to 37°C and maintaining a constant stirring for 24 h. Next, samples were filtered through a 0.45 µm membrane, volumetrically diluted and their absorption measured at 232 nm in a UV-Vis absorption spectrometer (Vankel, 50, Varian Inc., Palo Alto, CA, U.S.A.). Concentrations were calculated from a standard curve prepared for each condition. The solubility assays were performed in triplicate.

Evaluation of Dissolution Profile with USP 1 and 2  An in vitro dissolution study of the reference product Glifage® XR 750 mg was performed in sextuplicate using USP 1 (basket apparatus) and USP 2 (paddle apparatus) in a Hanson Research SR6 dissolver (Hanson Research Corp.). The dissolution media consisted of 1000 mL of 0.1 M PBS (pH 6.8) at 37 ± 0.5°C and a rotational speed of 100 rpm. Aliquots of 10 mL were collected hourly without media replacement for a total period of 10 h through an automatic manifold system (AutoPlus MultiFill Hanson) using a 10 µm cannula filter. Samples were filtered through a 0.45 µm membrane, diluted and quantified by UV as described above to determine the percentage of MTF released.

Development of a Dissolution Method with USP 3  A dissolution method was developed with reciprocating cylinders (USP 3; BIODIS Varian, Varian Inc.) using Glifage® XR 750 mg tablets. The dissolution conditions consisted of 250 mL of media maintained at 37 ± 0.5°C. Media included buffered solutions of HCl (pH 1.2), ABS (pH 4.5) and PBS (pH 5.8, 6.8 or 7.2) to create a pH gradient that simulated the absorption of an ER tablet through the GIT. The top and bottom of the inner tubes had a #20 mesh (840 µm) of polypropylene. A programmed drain time of 10 s was set for the process of transferring to the next row of vessels containing new media. Aliquots of 5 mL were manually collected hourly without media replacement for 10 h using a 10 µm cannula filter. For sampling, the cylinders were held above the dissolution medium for 30 s. Samples were filtered through a 0.45 µm membrane, diluted and quantified by UV as described above. The cumulative percentage of MTF was calculated from a linear standard curve. All experiments were performed in sextuplicate. The best dissolution method was applied for the analysis of commercially available tablets in dosages ranging from 500mg to 1 g and novel formulations produced in this study. The commercial formulations consisted of four different generic formulations and one similar formulation. In Brazil, similar formulations are medicines that are considered as brand-name products composed of the same active pharmaceutical ingredient as the generic and reference formulations. The Brazilian Health Surveillance Agency (ANVISA) accepts registrations of pharmaceutical products in one of the three types, either reference, generic or similar.

In Vitro–in Vivo Correlation  The IVIVC study was performed with data from the in vitro dissolution profiles of Glifage® XR tablets and the in vivo plasma concentration data of the drug after oral administration in fasting individuals obtained from previously reported pharmacokinetic studies. The absorbed fraction (% Fa) of MTF was calculated by mathematical deconvolution of the in vivo absorption data using the mathematical equation of Loo-Riegelman (Eq. 1).

$$f = \frac{C_p + K_d \int_0^\infty C_{dr} \, dt}{1 + K_d \int_0^\infty C_{dr} \, dt} \left( \frac{X_p}{V_s} \right)$$

Where, $C_p$ is the plasma concentration of MTF in time (t), $K_d$ is the elimination constant, $J_0$ is the area under the curve at time zero, $f_p$ is the area under the curve from time zero to infinity, $X_p$ is the amount of drug in the peripheral compartment after oral administration and $V_s$ is the apparent volume of distribution in the central compartment. The in vitro and in vivo fractions were plotted as independent ($x$) and dependent ($y$) variables, respectively. Linear regressions were calculated using Microsoft Office Excel 2010.

Formulation of Generic Tablets  A wet granulation method was used to formulate various tablets wherein the granules were produced using different matrices to obtain ER systems. The materials used included the clays Viscogel S4, S7 and B8, lipid matrices containing glyceryl monostearate, stearic acid and cetostearyl alcohol and lipid matrices associated to HPMC. Water or ethanol (96%) were used as the binder solution. Glifage® XR 750 mg was the reference for the development of the formulations, so that the amount of MTF per tablet and the average weight were equivalent. Initially, MTF and all excipients were separately passed through a 250 µm size sieve. Next, the active pharmaceutical ingredient was mixed with the excipients comprising each of the formulations for 10 min prior to the addition of binder solution. This wet blended mixture was sieved (420 µm) to form granules that were subsequently oven dried at 65°C (Quimis Q-317B) followed by a second pass through a 420 µm sieve. Magnesium stearate was added as a lubricating agent and mixed with the dried granules for 10 min. Tablets were made with a single compressor (Erweka, Heusenstamm, Germany) using 16 mm punches at the top and bottom with a compression force of 3000 kgf to produce biconvex tablets weighing approximately 1.080 g each.

Quality Control of the Developed Formulations  An analysis of the average weight, hardness and friability was performed on 10 tablets as recommended by the Brazilian Pharmacopoeia. A disintegration test (Erweka, ZT 53) was performed with data from the in vitro dissolution profiles of Glifage® XR tablets and the in vivo plasma concentration data of the drug after oral administration in fasting individuals obtained from previously reported pharmacokinetic studies. The absorbed fraction (% Fa) of MTF was calculated by mathematical deconvolution of the in vivo absorption data using the mathematical equation of Loo-Riegelman (Eq. 1).
performed with 700 mL of media at 37°C. Media included water and 0.1 M HCl solution using 6 tablets in each. \textsuperscript{17} Tablet content was evaluated for the extraction of MTF from a homogeneous mixture of pre-weighed and crushed tablets. Different samples were diluted, filtered through a 0.45 µm membrane and quantified according to the methodology described in USP 40 \textsuperscript{18} on a HPLC system Elite LaChrom HPLC system, Merck-Hitachi (Darmstadt, Germany) coupled to a photodiode detector (DAD L-2130), quaternary pump (L-2455), column oven (L-2350), automatic sampler (L-2200) and Eze-Chrom software. The MTF analysis was isocratically achieved at 30°C with a Kromasil 100-5 C18 column (4.6 × 150 mm, 5 µm). The mobile phase consisted of a mixture of PBS (pH 3.85) and acetonitrile at a ratio of 90:10 (v/v) and a flow rate of 1.2 mL/min with an UV detection at 218 nm. The total run time was 6 min and the retention time of MTF was approximately 4.5 min. The dissolution test was performed as previously reported (Development of a Dissolution Method with USP 3) using the USP 3 method that presented the highest IVIVC.

**Statistical Analysis** The dissolution profiles of MTF tablets were evaluated by statistical analysis using independent mathematical models: difference factor ($f_1$), similarity factor ($f_2$) and ANOVA (multiple variance analysis of Tukey). Statistical analyzes of variance were performed with GraphPad Prism (San Diego, CA, U.S.A.) version 5.0 for Windows.

### Results and Discussion

**Solubility** Different dissolution media compositions over a physiological pH range from 1.2 to 7.2 were evaluated during the course of development of a dissolution method to obtain a discriminative dissolution media. \textsuperscript{19} The results are summarized in Table 1 and indicate a high solubility of MTF in all media. As a general observation, the solubility decreased as the pH increased, which is in agreement with previous reports. \textsuperscript{20,21} It was also possible to verify that MTF was stable in all media for the duration of the 24 h solubility test. Sink conditions were maintained in all media.

**Evaluation of Dissolution Profile with USP 1 and 2** USP 40 describes 13 official dissolution methods for MTF ER tablets using USP 1 or USP 2. \textsuperscript{22} The main difference between the methods is related to the sampling times and total analysis time. Here, two tests were proposed that aimed to apply the methods is related to the sampling times and total analysis time. Figure 1 shows that a dissolution percentage greater than 85% was reached in 9 h using USP 1, whereas USP 2 achieved a dissolution percentage greater than 85% in 8 h. Statistical analyses did not show any significant difference between the dissolution profiles ($f_1 = 3.26$ and $f_2 = 81.32$; $p > 0.05$).

Intercurrences occurred during the analysis in both apparatuses that did not affect data uniformity (relative standard deviation (RSD) <10%). In the USP 1, matrix swelling and the formation of a gel layer was observed, which could limit drug delivery to the sampled volume by impeding passage through the basket and/or interrupt the hydrodynamics of the dissolution. In the USP 2, the tablet was seen to float in the dissolution vessel during the analysis, which was probably due to hydration of the non-disintegrating polymer matrix and a swelling of the system. The in vitro dissolution data from USP 1 and 2 and the in vivo data from Glifage® XR were used to generate an IVIVC. For the USP 1, the slope value was 1.755 and $R^2$ was 0.9795, while for the USP 2, the slope was 1.839 and $R^2$ was 0.9734. These values are consistent with U. S. Food and Drug Administration (FDA) recommendations ($R^2 >0.95$) and indicate a suitable IVIVC. \textsuperscript{23} However, to achieve more biorelevant conditions, a dissolution method was pursued utilizing the USP 3 for this ER formulation, since USP 3 can create hydrodynamic conditions which, together with a series of several dissolution media, can simulate the environments experienced during passage of the dosage form through the GIT.

**Development of a Dissolution Method with USP 3** Different immersion rates and pH combinations of media were tested (Method M1; Table 2). Initially, in Method M1, the influence of the immersion rate of 5, 15, 30, 40 and 50 dpm were evaluated, as previously indicated by Borst et al. \textsuperscript{22} The comparison was conducted in a series of media at defined times; HCl (pH 1.2) - 1 h, ABS (pH 4.5) - 2 h, PBS (pH 5.8) - 1 h, PBS (pH 6.8) - 5 h, PBS (pH 7.2) - 1 h, which are based on the physiological transit time and the specific pH in each portion of the GIT. \textsuperscript{11,23}

The percentage of MTF dissolved during 10 h by Method M1 was greater than 85% for 30, 40 and 50 dpm immersion rates with the dissolved amount of drug proportional to the increase in the immersion rate, as shown in Fig. 2A. As sug-

### Table 1. MTF Solubility and Stability after 24h in Different Dissolution Media

| Dissolution media | Saturation concentration (mg/mL) ± RSD | Recovered mass (%) ± RSD |
|-------------------|----------------------------------------|--------------------------|
| Water             | 326.71 ± 0.44                          | -                        |
| HCl pH 1.2        | 429.30 ± 0.26                          | 102.61 ± 1.42            |
| ABS pH 4.5        | 318.61 ± 2.45                          | 99.88 ± 1.21             |
| PBS pH 5.8        | 304.98 ± 1.17                          | 98.32 ± 0.93             |
| PBS pH 6.8        | 317.14 ± 0.51                          | 99.86 ± 1.25             |
| PBS pH 7.2        | 253.49 ± 2.62                          | 98.85 ± 1.18             |

ABS, acetate buffer solution; PBS, phosphate buffer solution; $n = 3$.}

![Fig. 1. Dissolution Profiles of Metformin HCl Obtained from ER Reference Tablets at Doses of 750 mg](image)
ggested by Khamanga and Walker, higher agitation rates can destruct the polymer network and consequently the drug can be released more quickly from the matrix.24 Adhesion of the tablet to the cylinder was observed at rates of 5 and 15 dpm due to the lower immersion rate. This fact may have contributed to the lower dissolved amount of MTF observed at both speeds (<85%). A gel layer was seen to form around the tablets, which generated a high RSD in the dissolution profile at 5 dpm. These velocities were considered inadequate to evaluate the dissolution of the MTF through the USP 3. In contrast, the immersion rate of 50 dpm was considered to be overly abrupt and could decrease the discriminating power of a method based on this rate. At 30 and 40 dpm, a free movement of the tablet in the cylinder was observed without the formation of a gel layer around its outside. While the statistical analysis showed similarities (p > 0.05) between the different immersion rates, 30 and 40 dpm were chosen for further evaluations.

Next, the influence of pH on dissolution was evaluated at 30 and 40 dpm, varying the resident time of the tablet in each media (Methods M2–5; Table 2). With an immersion rate of 30 dpm, the M4 method showed similarity to the M2 and M5 methods (p > 0.05), while M3 method was statistically different from the others (p < 0.0001). At the higher rate of 40 dpm, methods M2 and M4 were similar; M3 and M5 also did not show a statistical difference (p > 0.05). These data suggest that a velocity of 30 dpm has a greater discriminate power for an evaluation of the dissolution of MTF from formulations. The increased agitation at 40 dpm most likely attributed to a more rapid release of drug that has been observed by others, contributing to the lower dissolved amount of MTF observed 15 dpm due to the lower immersion rate. This fact may have an influence on the overall dissolution efficiency.26,27

**In Vitro–in Vivo Correlation** The data obtained from different dissolution conditions were evaluated to establish an IVIVC for Glifage® XR tablets.14–16 The analysis of the drug dissolved in different dissolution media was important to evaluate the potential amount of MTF present in each portion of the GIT. The rate and site of drug release can influence the magnitude and duration of the pharmacological response and directly affect the efficacy of a controlled release formulation.28,29 In accordance with its multi-compartmental distribution model,27 the absorption of MTF by different compartments of the GIT, as well as the liver, is of great importance for the desired hypoglycemic action and influences its overall systemic effect. In addition, pharmacokinetic and pharmacodynamic data suggest that an appropriate oral formulation of MTF should initiate drug release in the upper parts of the GIT.27

The dissolved percentage of MTF in different media utilizing USP 3 were plotted against the absorbed fraction according to Loo-Rielgeman to account for the multicompartmental distribution of metformin.37 Since the absorption of MTF in the GIT decreases as the dose increases, it suggests an absorption mechanism that can be saturated or is limited by the transit time through the GIT.28 After absorption, MTF is rapidly distributed and does not bind to plasma proteins.2,28 The mean elimination half-life in vivo is between 3.5 and 5 h, reaching a maximum plasma concentration at 4 to 7 h after administration of the ER formulation.14,15 Our results showed a satisfactory release of MTF from the ER tablet in vitro that ranged from 85–100% of dissolution for 30, 40 and 50 dpm of immersion rate with USP apparatus 3.

All dissolution methods tested in this work were evaluated for their IVIVC. The values for slope, intercept and R² are presented in Table 3. The data show a negative intercept with values between −36.125 and −22.072, which can be attributed to a lag time in drug absorption that can be related to the absorption-limited rate of MTF. In the BCS, MTF is a class III drug in and presents low permeability.9,29,30 The slope values are between 1.409 and 2.155, which agree with the intercept values. The high values of R² indicate a linear correlation with in vivo data.

The method M4 (USP 3, 30 dpm) showed a significant linear correlation with the in vivo absorption data for the 750 mg tablets with an R² = 0.9860 (point-to-point correlation). As a level A correlation, this condition was used to evaluate the dissolution profile of tablets containing 500 mg and 1 g of MTF. Both doses also presented with an R² > 0.98 (Table 3), demonstrating that the method was discriminative and able to simulate the passage of the tablet by GIT. In addition, the high correlation for all drug doses makes the dissolution method more accurate for use in quality control and bioequivalence studies.31

| Method | DPM | Dissolution media/time (h) |
|--------|-----|---------------------------|
| M₁     | 5, 15, 30, 40, 50 | HCl pH 1.2 | ACB pH 4.5 | PBS pH 5.8 | PBS pH 6.8 | PBS pH 7.2 |
| M₂     | 30/40 | HCl pH 1.2 | PBS pH 6.8 |
| M₃     | 30/40 | HCl pH 1.2 | ACB pH 4.5 | PBS pH 6.8 |
| M₄     | 30/40 | HCl pH 1.2 | ACB pH 4.5 | PBS pH 5.8 | PBS pH 6.8 | PBS pH 7.2 |

ABS, acetate buffer solution; PBS, phosphate buffer solution.
indicates that the generic product B was not a true equivalent. The other formulations were similar to the reference tablet ($f_2<15$ and $f_2>50$). Generic product A containing either 500 or 750 mg (Figs. 3A and 3B, respectively) displayed approximately 10% greater level of dissolved drug percentage than Glifage® XR after 8 h of digestion (Fig. 3B). Despite differences in the percent dissolved, the generic products A of 500 and 750 mg, generic product B of 750 mg along with the similar tablet of 500 mg obtained dissolution profiles equivalent to Glifage® XR, demonstrating the applicability of the method in the dissolution profile analysis of MTF ER tablets.

**Formulation of Generic Tablets** The dissolution method with a higher IVIVC (M4, USP apparatus 3, 30 dpm) was applied to develop a generic ER formulation for MTF. The formulations were based on hydrophobic material, which in general differ from the hydrophilic polymers in relation to the mechanism and the release kinetics. The choice of hydrophobic excipients sought to guarantee the extended release of MTF. Clays (Viscogel S4, S7 and B8) and lipid material (glyceryl monostearate, stearic acid and cetostearyl alcohol) were used in the development of the formulation. The clays show efficient release control due to their surface area and the cation exchange capacity, thus retaining large amounts of drugs, especially basic drugs such as MTF. The choice of lipid materials provide advantages for highly water soluble drugs from their chemical inertia compared to other materials, good stability at different pHs and their resistance to alteration by foods present in the GIT.

Studies have reported that a more efficient release may occur with the association of hydrophobic materials with hydrophilic polymers. In general, the various lipid materials differ in relation to the degree of lipophilicity and can generate different release profiles depending on the proportions used. Some studies report the use of lipidic materials to promote the prolonged release of MTF, using techniques such as wet granulation, direct compression and hot melt granulation.

In this work, the properties of formulations containing clays, formulations containing only lipid materials and formulations containing lipid materials associated with hydrophilic polymers were evaluated. The goal was to promote a prolonged release of MTF equivalent to Glifage® XR. Wet granulation was used for the preparation of the tablets because of the advantages of improving the flowability of powder mixtures, reducing the cohesion of the particles and increasing the fluidity of the mixture. This is particularly important for high-weight formulations such as MTF. The formulations were divided into three groups according to the matrix components. Group A consists of clays, Groups B is composed of mixed matrix (lipid material and polymer) and group C is formed only by lipid matrix. In addition, HPMC and shellac were evaluated as components of the binder solution of the Group B formulations as described in Table 4.

**Quality Control of the Developed Formulations** The characteristics of Glifage® XR 750 mg, the reference medicine, were used as the basis for the quality control of produced formulations. As Glifage® XR has a non-disintegrating matrix system (average weight of ca. 1.080 g), non-disintegrating tablets were selected as the initial criterion. All tablets from Group A and the formulation F7 from Group B totally disintegrated in less than 30 min, which eliminated them from further consideration. Based on weight, all formulations were similar to the reference drug with only the formulation F5 display-
ing a lower mean weight (Table 5). Yet, none of the tablets exceeded the variation limit of ±5.0%, as described by the Brazilian Pharmacopoeia for uncoated tablets with an average weight above 250 mg.

The hardness variation between the tablets of each group was low. The formulation F5 from group B showed the lowest hardness, which was proportional to its lower average weight value. All formulations had hardness lower than Glifage® XR.

The hardness and friability of the tablet are related to the particle size and properties of the compression process, such as compression force, technique used and tablet shape. Glifage® XR is a large oblong/cylindrical tablet, different from the wet granulation formulations of this study that have a smaller diameter and oblong/rounded shape. All the tablets had a mass loss of less than 1.5% in the friability test, within the limits specified by the Brazilian Pharmacopoeia. In the determination of the content, only the formulation F5 (Group B) presented inadequate MTF content (90–110%), with 85.3 ± 1.25%.

The dissolution profiles of tablets from Groups B and C are shown in Fig. 4. The percentage of MTF released during the 10h test was greater than 85% in all formulations. Formulations F4, F5 and F6 from Group B showed rapid release at pH 1.2 with around 60–70% of the drug detectable within 2h. This result indicates that glyceryl monostearate or its association with HPMC were not sufficient to retain the drug within the matrix for a prolonged time course. The formulations in which HPMC was replaced by shellac in the binder solution also displayed a rapid drug release along with a total disintegration as observed in formulations F6 and F7. This was most probably due to the instability of shellac in alcohol containing solutions, which could have led to a poor cohesion of the granules.

All formulations from Group C presented a dissolution percentage of MTF less than 35% during the first hour at pH 1.2, as presented in Fig. 4B. It appeared that the association of lipid materials proved to be effective in controlling the release of the drug in a constant and prolonged manner at acidic pH. It was observed that the ethanolic component content in the

Table 3. Correlation Coefficients (R²), Slopes and Intercept Values from in Vivo–in Vitro Correlation Established for the Dissolution Profile Reference Tablets with the Application of the USP Apparatus 1, 2 and 3 and the in Vivo Data of Fa% Obtained after Mathematical Deconvolution

| Formulation Method | Agitation rate | IVIVC | Slope | Intercept | R² |
|--------------------|----------------|-------|-------|-----------|-----|
| USP 1 750 mg XR    | 100 rpm        |       | 1.755 | −27.922   | 0.9795 |
| USP 1 M1           | 5 dpm          |       | 1.839 | −36.125   | 0.9734 |
| USP 1 M2           | 15 dpm         |       | 2.155 | −36.267   | 0.9717 |
| USP 1 M3           | 30 dpm         |       | 1.853 | −29.081   | 0.9769 |
| USP 1 M4           | 40 dpm         |       | 1.609 | −29.626   | 0.9768 |
| USP 1 M5           | 50 dpm         |       | 1.434 | −25.429   | 0.9838 |
| USP 2 750 mg XR    | 30 dpm         |       | 1.698 | −30.207   | 0.9742 |
| USP 3 750 mg XR    | 30 dpm         |       | 1.787 | −31.460   | 0.9836 |
| USP 3 M1           | 30 dpm         |       | 1.640 | −28.815   | 0.9860 |
| USP 3 M2           | 30 dpm         |       | 1.642 | −29.793   | 0.9677 |
| USP 3 M3           | 30 dpm         |       | 1.620 | −33.594   | 0.9821 |
| USP 3 M4           | 30 dpm         |       | 1.676 | −34.436   | 0.9771 |
| USP 3 M5           | 30 dpm         |       | 1.409 | −22.072   | 0.9717 |
| USP 3 M6           | 30 dpm         |       | 1.729 | −37.437   | 0.9785 |
| 500 mg XR M1       | 30 dpm         |       | 1.722 | −26.834   | 0.9891 |
| 500 mg XR M2       | 30 dpm         |       | 1.849 | −24.744   | 0.9943 |

Fig. 3. Dissolution Profile of ER Reference, Generic and Similar Drugs, through M4 Method under Agitation Rate of 30dpm Using Bio-Dis
(A) Dissolution profile of ER reference, generic and similar drugs at 500 mg; (B) Dissolution profile of ER reference and generic drugs at 750 mg. Each point represents the mean result (n = 6) and DPR of metformin HCl dissolved percentage at each time point.
matrix influenced the release of the drug, since a decrease in cetostearyl alcohol of the formulation F9 led to more than 85% drug release within 5 h. In addition, the substitution of water by ethanol in the binder solution of formulation F10 provided an increase in the lipophilicity of the system and therefore a lower release of the drug from the matrix.40)

The mechanism of drug release from Glifage® XR is by diffusion.3) Tablets from Groups B and C also showed drug release by diffusion; however, they suffered surface erosion at the final dissolution times (8–10 h). In this study, the kinetics

Table 4. Composition of the Formulations (%)

| Formulation | Raw material (% per tablet) | Internal phase | External phase | Binder solution |
|-------------|-----------------------------|----------------|---------------|----------------|
|             | MET | GMS | HPMC 50cPs | VS4 | VS7 | VB8 | Stearic acid | Cetostearyl alcohol | HPMC 100,000 cPs | Shellac | Magnesium Stearate | Distilled water | Ethanol 96% |
| Group A     |     |     |         |     |     |     |             |             |                   |             |               |                |             |
| F1          | 75  | —   | —       | 30  | —   | —   | 0.1        | —             | 1.5              | x             | —               |                |             |
| F2          | 75  | —   | —       | —   | 30  | —   | 0.1        | —             | 1.5              | x             | —               |                |             |
| F3          | 75  | —   | —       | 30  | —   | —   | 0.1        | —             | 1.5              | x             | —               |                |             |
| Group B     |     |     |         |     |     |     |             |             |                   |             |               |                |             |
| F4          | 75  | 30  | —       | —   | —   | —   | 0.1        | —             | 1.5              | x             | —               |                |             |
| F5          | 75  | 20  | 10      | —   | —   | —   | 0.1        | —             | 1.5              | x             | —               |                |             |
| F6          | 75  | 20  | 10      | —   | —   | —   | 0.1        | 0.2           | 1.5              | —             | x               |                |             |
| F7          | 75  | 20  | 10      | —   | —   | —   | 0.1        | 0.4           | 1.5              | x             | —               |                |             |
| Group C     |     |     |         |     |     |     |             |             |                   |             |               |                |             |
| F8          | 75  | 20  | —       | —   | —   | —   | 20         | 0.1           | 1.5              | x             | —               |                |             |
| F9          | 75  | 27  | —       | —   | —   | —   | 13         | 0.1           | 1.5              | x             | —               |                |             |
| F10         | 75  | 27  | —       | —   | —   | —   | 13         | 0.1           | 1.5              | —             | x               |                |             |
| F11         | 75  | 20  | —       | —   | —   | —   | 20         | 0.1           | 1.5              | x             | —               |                |             |

Values expressed as $n = 6 \pm S.D.$ Statistical analysis by independent model methods, difference ($f_1$) and similarity factors ($f_2$) and ANOVA.

Table 5. Quality Control and Statistical Analysis of the Dissolution Profiles Obtained by M4 Method under Agitation Rate of 30 dpm Using USP Apparatus 3 between the XR Reference Tablets at the 750 mg Dose and the Formulations of Groups B and C

| Formulation | Average weight | Hardness | Friability | Drug content | Statistical analysis |
|-------------|----------------|----------|------------|--------------|----------------------|
| Reference   | 1.0899 ± 0.01  | 209.2 ± 4.52 | 0.009      | 100.46 ± 3.08 | $f_1$ | $f_2$ | ANOVA |
| Group B     |                |           |            |              | $f_1$ | $f_2$ |          |
| F4          | 1.0810 ± 0.0056 | 82.8 ± 3.68 | 0.002      | 93.79 ± 1.37 | 23.46 | 36.38 | $p < 0.0001$ |
| F5          | 1.0064 ± 0.0025 | 72.0 ± 4.57 | 0.003      | 85.27 ± 1.07 | 14.93 | 45.80 | $p > 0.05$ |
| F6          | 1.0665 ± 0.0035 | 91.1 ± 8.86 | 0.009      | 94.92 ± 2.35 | 20.45 | 38.34 | $p < 0.001$ |
| Group C     |                |           |            |              | $f_1$ | $f_2$ |          |
| F8          | 1.0799 ± 0.03  | 97.5 ± 5.89 | 0.001      | 108.68 ± 7.08 | 17.41 | 51.07 | $p > 0.05$ |
| F9          | 1.0662 ± 0.02  | 91.4 ± 9.36 | 0.002      | 94.88 ± 0.80 | 12.57 | 52.78 | $p < 0.05$ |
| F10         | 1.0728 ± 0.01  | 93.2 ± 2.20 | 0.001      | 105.98 ± 1.53 | 19.60 | 47.82 | $p < 0.001$ |
| F11         | 1.0714 ± 0.01  | 132.8 ± 8.83 | 0.017     | 100.34 ± 4.78 | 10.14 | 60.61 | $p > 0.05$ |

Fig. 4. Dissolution Profile Obtained from ER Formulations Containing 750 mg of Metformin HCl, Based on Mixed and Lipid Matrices, through M4 Method under Agitation Rate of 30 dpm Using Bio-Dis

(A) Formulations F4, F5 and F6 (Group B), (B) Formulations F8, F9, F10 and F11 (Group C). Each point represents the mean result ($n = 6$) and DPR of metformin HCl dissolved percentage at each time point.
of MTF release from Glifage® XR and the developed tablets was best expressed by the Korsmeyer–Peppas kinetic model, with an $R^2 > 0.98$. The mechanism of drug release appears to be by non-Fickian diffusion (anomalous transport) and controlled by the relaxation of the polymer chains, since the exponent $n$ expressed by the equation of Korsmeyer–Peppas presented a value between 0.5 and 1.0. This indicated that drug release depended on the mechanisms of swelling and diffusion of the dosage form.\(^{32}\) In fact, this kinetic model is reported as the most adequate to evaluate the release of drugs from matrix systems since it accounts for the geometry and the characteristics of the polymers.\(^{32}\)

A statistical analysis showed that formulation F11 generated a dissolution profile most equivalent to the reference tablet ($f_1 = 10.14$; $f_2 = 60.61$, $p > 0.05$), as presented in Table 5. In addition, this formulation presented similar results to the reference drug in the quality control analyses and lower RSD values in the dissolution studies. Therefore, F11 can be considered as a possible generic ER formulation for MTF. It should be emphasized that the use of the USP apparatus 3 was essential to develop a method able to predict the behavior of the MTF release in vivo. The dissolution method was efficient in assisting the development of ER tablets by guiding the choice of excipients and their proportions suitable for an efficient drug delivery.

**Conclusion**

A suitable release profile of MTF from ER tablets was achieved with a method developed utilizing a USP apparatus 3 that resulted in the release of more than 85% of the drug within 10h. The final method, M4, included buffers with four different pHs to simulate passage through the GIT. Applying a rate of 30dpm to the reciprocating basket of the USP 3 displayed a high correlation coefficient ($R^2 > 0.98$) for all doses of the drug and was highly discriminative. We suggest that this method can be used for MTF quality control, testing post-approval changes and equivalence studies. In addition, the IVIVC of the method suggests it could guide the development of generic formulations containing MTF and for the choice of suitable excipients.

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**Conflict of Interest** The authors declare no conflict of interest.

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