International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 12, Issue 6, 2020

Original Article

IN VITRO RELEASE PERFORMANCE OF METFORMIN HYDROCHLORIDE FORMULATIONS USING THE FLOW-THROUGH CELL METHOD

JOSE RAUL MEDINA-LÓPEZ*, FRIDA IRIANA MEDINA MORALES, RAFAEL ALONSO GALVEZ LOMELIN, JUAN CARLOS RUIZ SEGURA, MARCELA HURTADO

Departamento Sistemas Biologicos, Universidad Autónoma Metropolitana-Xochimilco, Mexico City, Mexico
Email: rmlopez@correo.xoc.uam.mx

Received: 26 Jul 2020, Revised and Accepted: 20 Aug 2020

ABSTRACT

Objective: The objective of this work was to evaluate the *in vitro* release performance of metformin hydrochloride formulations (500-mg tablets) using the hydrodynamic environment of the flow-through cell method. Results were compared with those generated by the official dissolution test (USP basket apparatus).

Methods: The reference drug product and three generic formulations were tested with phosphate buffer pH 6.8 as dissolution medium. Dissolution profiles were carried out with an automated flow-through cell apparatus using laminar flow at 16 ml/min. Drug was quantified at 233 nm during 45 min. Dissolution profiles were compared with the calculation of f_2 similarity factor, mean dissolution time, dissolution efficiency, $t_{50\%}$ and $t_{63.2\%}$. Dissolution data were adjusted to several mathematical models such as Makoid-Banakar, Peppas-Sahlin, Weibull and Logistic.

Results: With the flow-through cell method and at 45 min less than 60% of metformin hydrochloride dissolved was found, while with the USP basket apparatus, less than 75% of the drug was found. Some generic formulations showed f_2>50 with both USP apparatuses, but statistical comparisons of parameters indicated significant differences between their dissolution profiles and reference. Due to variability obtained no dissolution profiles were compared by model-dependent approach.

Conclusion: To demonstrate safe interchangeability between metformin hydrochloride generic formulations and reference bioequivalence studies should be performed. It is important post-marketing monitoring of the commercial formulations because health regulatory agencies of each country must ensure drug products with quality, safety, and efficacy at the lowest possible cost.

Keywords: Diabetes type 2, Flow-through cell method, Generic drug products, Metformin hydrochloride

DOI: http://dx.doi.org/10.22159/ijap.2020v12i6.39230. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Generics are off-patent formulations that contain the same active pharmaceutical ingredient, and the same dose, as reference drug product [1]. During the dissolution tests, many generic formulations have shown significant differences from their branded counterparts. Metformin hydrochloride is manufactured as generic drug products and by the results reported by other authors there is a need to evaluate generics even after going on sale with the aim of offering the population safe and quality medicines.

Metformin is a member of the biguanide class of drugs and is used in the treatment of type 2 diabetes mellitus [2]. This type of diabetes is characterized by abnormally high levels of glucose in the blood due to either insulin resistance of the cells or too much glucose production in the liver or a combination of both situations [3]. Metformin hydrochloride is a white to off-white crystalline compound and it is absorbed predominately from the small intestine [4]. Molecular structure of metformin hydrochloride is shown in fig. 1.

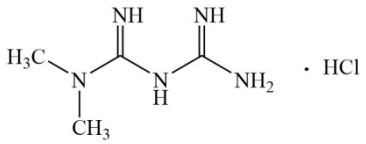

Fig. 1: Molecular structure of metformin hydrochloride

Metformin is a class III drug (high solubility/low permeability) [5] so the limiting step for its absorption is the passage through the biological membranes. For class III drugs, a biowaiver can be considered following certain criteria, one of them, more than 85% of the drug must be dissolved in at least 15 min in standard dissolution media at pH 1.2, 4.5, and 6.8 [6]. On the other hand, the USP dissolution test designed to verify the quality of metformin hydrochloride tablets point to the use of USP basket apparatus at 100 rpm with 1000 ml of phosphate buffer pH 6.8 as dissolution medium. Under these conditions, not less than 70% must be dissolved at 45 min (Q = 70%) [7].

About *in vitro* dissolution studies of metformin hydrochloride commercial formulations, several authors have reported limited works with the USP basket apparatus [8, 9]. Other manuscripts describe the use of USP paddle apparatus [10-16] and only one has reported data with the flow-through cell method [13]. Most of them agree with the use of phosphate buffer pH 6.8 as dissolution medium, while others have tested metformin hydrochloride tablets with simulated intestinal fluid pH 6.8 [10] and dissolution media within physiological pH range (pH 1.2 – 6.8) [11, 12]. These results reflect a high variability in the dissolution performance of studied formulations as well as the need to develop a common dissolution test that allows the quality of the tablets to be properly evaluated.

Several reports have found significant *in vitro*/*in vivo* correlation with data generated with the flow-through cell method [17, 18] so it is convenient to use this apparatus to evaluate the applicability of working with high solubility drugs as metformin hydrochloride tablets. The aim of this work is to document the *in vitro* release performance of metformin hydrochloride commercial formulations under the hydrodynamics of the flow-through cell method. Results could be of interest to manufacture better metformin hydrochloride formulations or to improve the *in vitro* evaluation schemes.

MATERIALS AND METHODS

Formulations and chemicals

Metformin hydrochloride tablets (500-mg) of the reference drug product Dabex® (Merck S. A. de C. V. Mexico City, Mexico) and three generic formulations (coded as A, B, and C products) were used in the study. Mexican Health authorities have established Dabex® brand as a

reference drug product for dissolution and bioequivalence studies [19]. Potassium dihydrogen phosphate and sodium hydroxide were supplied by J. T. Baker-Mexico (Xalostoc, Mexico). Metformin hydrochloride standard was purchased from Sigma-Aldrich Co. (St. Louis MO, USA).

Content uniformity and assay

Content uniformity and assay tests were performed according to the procedures described in the USP [7].

Analytical method validation

Dissolution method was validated according to ICH guidelines [20]. Method linearity, accuracy, precision, and stability were evaluated.

Linearity

Three standard calibration curves of metformin hydrochloride were prepared in the dissolution medium. Potassium dihydrogen phosphate buffer (0.68% w/v) adjusted with 1 M sodium hydroxide to pH 6.8 was used as a dissolution medium. Drug concentrations of 1.5 to 15 µg/ml were prepared. Absorbance measured at 233 nm with 1-cm quartz cells was recorded. Absorbance *vs*. drug concentration data was fitted by linear regression analysis and the coefficients of regression and regression analysis of variance (ANOVA) were calculated. Absorbance *vs.* metformin hydrochloride concentration proportionality was demonstrated by calculating the percent of relative standard deviation (RSD): [((standard deviation)/mean value) × 100] of the response factor across the calibration curve range.

Accuracy and precision

To validate these parameters, the standard addition method was used, so that matrix effects can be easily removed. Twenty tablets were accurately weighed and crushed in a mortar; then, quantities of powder of metformin hydrochloride tablets plus a quantity of metformin hydrochloride standard (10 mg) to finally give the equivalent of 80, 100, and 120% of the dose were dissolved in 1000 ml of phosphate buffer pH 6.8 at 37.0±0.5 °C. The USP basket apparatus at 100 rpm was used. At 45 min the amount of metformin hydrochloride dissolved in each vessel was calculated with reference to a standard calibration curve prepared on the day of the experiment. Each determination was performed in triplicate. The percent of relative error (RE): [((found–added)/added) × 100] was taken as a measure of the accuracy and the RSD as a measure of precision. Determinations were carried out in three consecutive days.

Stability

Drug stability was evaluated by analyzing two solutions of metformin hydrochloride prepared in phosphate buffer pH 6.8 (2 and 13 µg/ml). These solutions were analyzed at 0 h at 25 °C and at 24 and 48 h after stored at 4 and 25 °C. At 24 and 48 h (at each temperature) the percent of absolute difference (AD): [((initial–final)/initial) × 100] recovered of metformin hydrochloride was calculated.

USP basket apparatus

Dissolution profiles of metformin hydrochloride were obtained using the dissolution test described in the USP [7]. USP basket apparatus (Model AT-7 Smart, Sotax, Basel, Switzerland) at 100 rpm was used (Q = 70% at 45 min). The UV/Vis spectrophotometer (Model Lambda 35, Perkin Elmer, USA) with 1-mm flow cells was used. Equipment was controlled by specific software designed by Sotax. Metformin hydrochloride tablets were sprinkled on 1000 ml of deaerated phosphate buffer pH 6.8 at 37.0±0.5 °C. Automatic samples were taken every 5 min to 45 min (n = 12). Metformin hydrochloride dissolved was determined with a standard calibration curve.

Flow-through cell method

Dissolution profiles of metformin hydrochloride were obtained with a flow-through cell apparatus (Model CE6, Sotax AG, Basel, Switzerland) and 22.6 mm cells (i.d.). Laminar flow (originated with 6 g of glass beads) at 16 ml/min was tested. Deaerated phosphate buffer pH 6.8 at 37.0±0.5 °C was used as a dissolution medium. Automatic samples were taken every 5 min up to 45 min (n = 12). Metformin hydrochloride dissolved was determined in an UV/Vis spectrophotometer (Model Lambda 10, Perkin Elmer, USA) with 1-mm cells at 233 nm. For every trial, a standard calibration curve was prepared.

Data analysis

Dissolution profiles of reference and generic formulations were compared by model-independent and-dependent methods [21]. For model-independent comparisons, mean dissolution time (MDT) and dissolution efficiency (DE) were calculated. MDT is the time to dissolve 63.2% of drugs and it was calculated according to statistical moment's theory [22, 23]. Other authors have given the MDT a value of 62–64% [24]. DE is the area under the dissolution curve up to a certain time, t, expressed as a percent of the area of the rectangle described by 100% dissolution at the same time [25]. Both parameters were calculated with the Excel add-in DDSolver program [26]. For model-dependent comparisons, dissolution data were adjusted to the hyperbola equation and with a and b values, $t_{50\%}$, and $t_{63.2\%}$ parameters were calculated. This fit was carried out with SigmaPlot software (version 11.0). Generics *vs*. reference data comparisons were carried out with a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. Additionally, and for a complete analysis of dissolution profiles by model-dependent approach, dissolution data were fitted to Makoid-Banakar, Peppas-Sahlin, Weibull, and Logistic equation. The model with the highest adjusted determination coefficient ($R^2_{adjusted}$) 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 DD Solver program [26]. The mathematical equations of each model are given in table 1.

RESULTS AND DISCUSSION

Content uniformity and assay

Results of content uniformity and assay tests made to metformin hydrochloride formulations are shown in table 2. All commercial formulations met the content uniformity and assay standard criteria. The percentages of metformin hydrochloride content ranged from 85 to 115% and the assay test was between 90 to 110%.

Linearity

Mean regression equation from three standard calibration curves was y = 0.0836x+0.0073. linear regression was significant (R^2= 0.999, *P<0.05). The RSD value of the response factor was 2.5%.

Accuracy and precision

To evaluate the accuracy and precision of the dissolution method, analysis of several percentages of dose (80, 100, and 120%) was carried out for three different days (n = 3/d). The within-run and between-run precision and accuracy were calculated. Results are shown in table 3. RSD obtained was in the range of 0.24 – 1.45% and RE was lower than 0.72% what indicates good accuracy and precision of the dissolution method.

Table 1: Mathematical models used to fit the dissolution data

Model	Equation
Hyperbola	$y = \frac{ax}{b+x}$
Makoid-Banakar	$F = k_{MB} \cdot t^n \cdot e^{-k \cdot t}$
Peppas-Sahlin	$F = k_1 \cdot t^m + k_2 \cdot t^{2m}$
Weibull	$F = F_{max} \cdot \left[1 - e^{-\frac{t^\beta}{\alpha}}\right]$
Logistic	$F = 100 \cdot \frac{e^{\alpha+\beta \cdot \log(t)}}{1 + e^{\alpha+\beta \cdot \log(t)}}$

Table 2: Content uniformity and assay results of metformin hydrochloride formulations

Key	Content uniformity (%min-%max)[a]	Assay (%)[b]
R	95.13–106.07	99.76
A	96.09–103.99	104.85
B	100.09–115.00	98.29
C	96.16–104.53	104.03

[a]*n* = 10, [b]*n* = 3

Table 3: Accuracy and precision of the dissolution method used to determine metformin hydrochloride in commercial formulations

	Within-day[a]			Between-day[b]		
Added (mg)	Found (mg)	RSD (%)	RE (%)	Found (mg)	RSD (%)	RE (%)
400.30	397.13±0.59	0.15	-0.79	400.98±4.10	1.02	0.19
500.64	500.38±1.18	0.24	0.15	503.01±3.27	0.65	0.54
600.13	601.27±7.19	1.20	0.19	604.47±8.75	1.45	0.71

Mean value±SD, [a]*n* = 3, [b]*n* = 9. SD: Standard deviation

Stability

Stability of metformin hydrochloride in phosphate buffer pH 6.8 was evaluated, analyzing drug solutions of 2 and 13 µg/ml at different times and temperatures. Results are given in table 4. Results suggest good stability of metformin hydrochloride at both temperatures but only by 24 h. Validation of the dissolution method met the standard criteria, which generates confidence in the results obtained.

Table 4: Absolute difference values of drug solutions to test drug stability

Conc. (µg/ml)	4 °C		25 °C	
	24 h	48 h	24 h	48 h
2	-11.84	-248.85	-9.13	-256.37
13	-0.89	-46.22	-1.11	-44.25

Mean value, *n* = 8

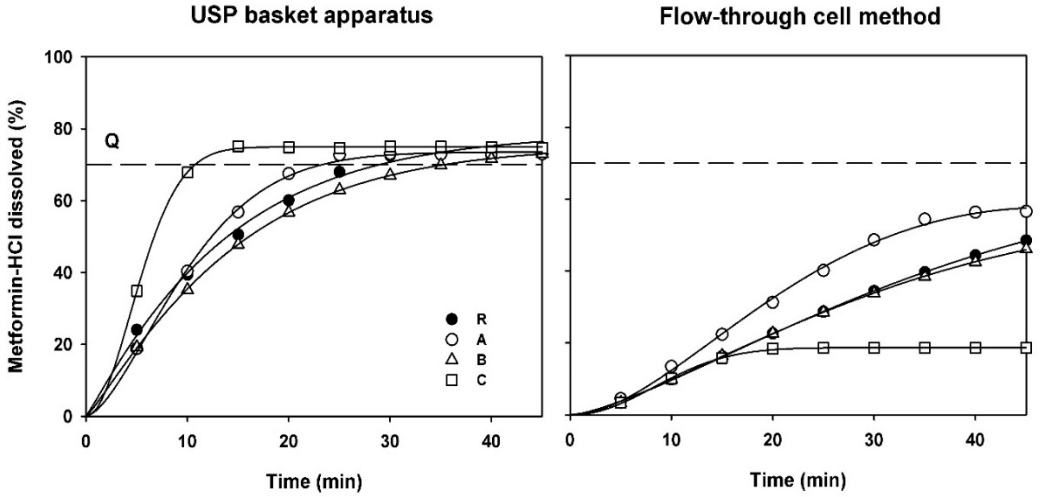

Fig. 2: Dissolution profiles of metformin hydrochloride reference (R) and generic formulations (A-C). The dashed line shows Q = 70%. For better clarity, error bars have been omitted, mean value, *n* = 12

Dissolution studies

Dissolution profiles of all metformin hydrochloride formulations, obtained with USP basket apparatus and flow-through cell method, are shown in fig. 2.

Under official conditions, all drug products met the pharmacopeial Q criteria (Q = 70% at 45 min). Metformin hydrochloride dissolved at 45 min are shown in table 5. Compliance with the above pharmacopeial tests ensures the quality of formulations. However, if the generic drug products are intended to be interchangeable with reference, none reaches>85% dissolved at 15 min so that the own biowaiver criteria for class III drugs cannot be applied. For immediate-release products manufactured with class III drugs, the assumption is that if their dissolution is very rapid under all physiological pH conditions, they can be expected to behave like oral solutions *in vivo*, since the rate-limiting step in the absorption process is permeability [11]. Data of drug dissolved at 15 min from the studied formulations are shown in table 5. Despite not having information on the dissolution performance of these drug products at pH 1.2 and 4.5 the biowaiver criteria must be met at every pH [6].

Table 5: Model-independent and-dependent parameters calculated to compare dissolution profiles of generic formulations (A-C) and reference drug product (R)

Key	Diss at 15 min (%)	Diss at 45 min (%)	MDT (min)	DE (%)	$t_{50\%}$ (min)	$t_{63.2\%}$ (min)
	USP basket apparatus					
R	50.52±0.73	74.73±0.38	11.48±0.23	55.67±0.51	14.18±0.37	23.37±0.54
A	56.81±0.86	72.96±0.45*	9.96±0.19*	56.81±0.50	13.01±0.33	21.97±0.51
B	47.81±1.13	72.97±0.54*	13.02±0.32*	51.88±0.80*	16.83±0.61*	27.52±0.95*
C	74.51±0.57	74.74±0.51	5.55±0.13*	65.49±0.53*	6.04±0.26*	11.90±0.47*
	Flow-through cell method					
R	16.27±0.10	48.51±0.36	21.98±0.05	24.81±0.17	45.57±0.33	58.07±0.49
A	22.36±0.17	56.56±1.42*	18.43±0.39*	33.26±0.40*	34.97±0.78*	48.01±1.91*
B	16.44±0.10	46.09±0.75	21.24±0.20	24.30±0.26	48.38±0.97*	63.77±1.73*
C	15.84±0.31	18.64±0.67*	9.61±0.24*	14.62±0.44*	†	†

Mean value±SEM, *n* = 12. *P<0.05, SEM: Standard error medium, †Due to limited drug dissolved no data was calculated

As can be seen in fig. 2, with the flow-through cell method, the rate and extent of metformin hydrochloride dissolved was less than the behavior obtained with the USP basket apparatus. Under the used flow-through cell conditions, no formulation showed>70% at 45 min. Usually, with the flow-through cell method, it is possible to obtain slower dissolution rates than those reported with the USP basket or paddle apparatuses [27, 28]. This performance can be explained by the hydrodynamic environment of the flow-through cell method which better reflects the natural setting of the gastrointestinal tract than other USP dissolution apparatuses [29]. Cell size, glass beads and flow rate are critical factors to form a special dissolution pattern useful to compare the *in vitro* release performance of drug products. Results agree with those reported by Hashem *et al.*, [13] where after working with four commercial products (850 and 1000-mg), phosphate buffer pH 6.8 and the USP paddle apparatus (100 rpm) and flow-through cell method (laminar flow at 8 ml/min) a slower dissolution rate was found with the flow-through cell. On the other hand, the absolute bioavailability of metformin when given orally is 50 – 60% [16]. This value can be explained, among other factors, by the low dissolution found with the flow-through cell method.

If more than 85% of the drug is dissolved within 15 min, the dissolution profiles are considered similar without further mathematical calculation [12]. As no metformin hydrochloride formulation achieved this value, dissolution profiles were compared. To compare dissolution profiles between generic formulations and reference f_1 difference and f_2 similarity factors were calculated according to equations reported by Moore and Flanner [30]. Only f_2 similarity factor is considered an official parameter to compare dissolution profiles [6]. Results are given in table 6.

Table 6: f_1 and f_2 factors calculated to compare dissolution profiles

Key	USP basket apparatus		Flow-through cell method	
	f_1	f_2	f_1	f_2
A	5.66	69.60	32.35	51.17
B	6.51	70.01	2.68	91.86
C	16.42	43.56	†	†

†Due to limited drug dissolved no data was calculated.

Considering the accepted range to similar dissolution profiles (f_1 = 0 – 15 and f_2 = 50 – 100), only generic drug products A and B showed similar dissolution profiles when the USP basket apparatus was used. Same results were obtained with the flow-through cell method but due variation out of official criteria (RSD should not be more than 20 percent at the earlier time points and should not be more than 10 percent at other time points) [6], for generic product C, no value of f_1 and f_2 were calculated. This drug product has a totally different dissolution performance in both apparatuses; apparently, its formulation (excipients and/or manufacture process) showed high sensitivity to hydrodynamic environments of the USP basket apparatus and flow-through cell method.

MDT and DE parameters as well as the percent of drug dissolved at 45 min were used to compare dissolution profiles by a model-independent approach. Results are shown in table 5. As can be seen in table 5, all generic formulations showed different dissolution profiles with the reference when USP basket apparatus was used (*P<0.05). With the flow-through cell method, only generic formulations A and C showed different dissolution profiles. Conversely, the three calculated parameters of generic product B support the result of similarity with reference. Our results agree with the results described by several authors. Villarroel Stuart *et al.*, [10] reported MDT values of 5.2 to 15.3 min. Data were obtained with the study of seven commercial formulations (500-mg) with USP paddle apparatus at 75 rpm and 900 ml of simulated intestinal fluid pH 6.8 as dissolution medium. Hashem *et al.*, [13] found DE values of 54.65 to 58.85%. They worked with two references (850-mg) and two generic products (1000-mg) using the USP paddle apparatus at 100 rpm and 900 ml of phosphate buffer pH 6.8. Additionally, the same authors reported the use of the flow-through cell method. DE values that they found were 44.0 to 57.86%. Results may differ from ours because they used 850 and 1000-mg tablets, the flow rate of 8 ml/min, and 60 min of sampling time.

Model-dependent parameters $t_{50\%}$ and $t_{63.2\%}$ were also used to compare dissolution profiles. Results are shown in table 5. With this approach, generic formulations B and C were different to reference when the USP basket apparatus was used (*P<0.05). With the flow-through cell method, no formulation was similar to reference (*P<0.05). Our results coincide with those reported by other authors. Kassahun *et al.*, [14] found $t_{50\%}$ values between 9.39 – 18.88 min. Data were obtained with the use of six commercial formulations (500-mg), USP paddle apparatus at 50 rpm and 900 ml of phosphate buffer pH 6.8. Authors claim that $t_{50\%}$ and $t_{90\%}$ are dissolution parameters that can be used to compare dissolution profiles of different brands of metformin hydrochloride tablets. In our study, it is difficult to establish similar dissolution profiles between generic formulations and reference because model-independent and model-dependent approaches, or MDT, DE, $t_{50\%}$, and $t_{63.2\%}$ comparisons, do not match.

In order to evaluate the influence of the hydrodynamic environment of the flow-through cell method and USP basket apparatus on the *in vitro* release performance of metformin hydrochloride commercial tablets, the results obtained were analyzed in two different ways. The first one with the association of $t_{63.2\%}$ values in function of MDT values and the second one with the association of $t_{50\%}$ values in

function of DE values. The first association allows visualizing the accuracy in calculating the $t_{63.2\%}$ value (time at which each formulation releases 63.2% of the drug) with its equivalent parameter MDT. The second association allows evaluating the relationship between two different approaches or comparison methods (model-dependent *vs.* model-independent). Plots for the first association are shown in fig. 3, while for the second association, only the regression equations are indicated. The regression equation for $t_{50\%}$ *vs.* DE data obtained with the USP basket apparatus was y =-0.7998x+58.473, R^2= 0.9987, with significant regression (*P<0.05). The regression equation for data obtained with the flow-through cell method was y =-1.3897x+81.13, R^2= 0.9779. For both ways of comparison, only the best results were obtained with data generated by the USP basket apparatus.

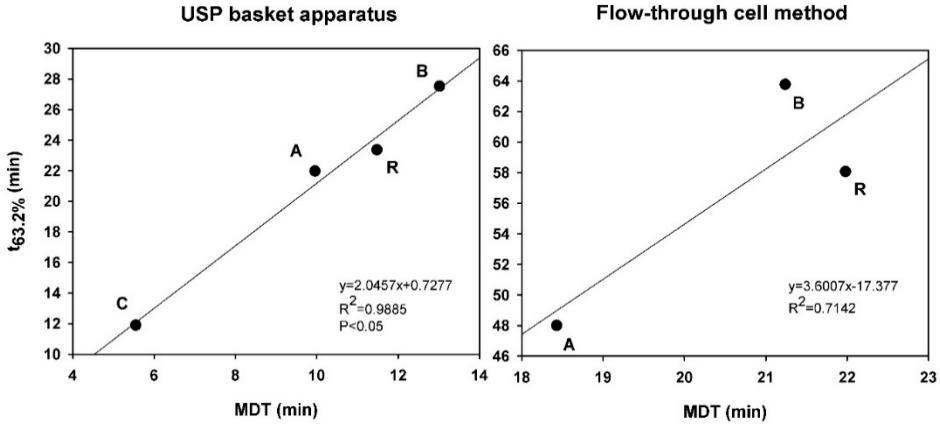

Fig. 3: Model-dependent parameter $t_{63.2\%}$ in the function of model-independent parameter MDT calculated with metformin hydrochloride formulations (R, A, B, C) obtained with both dissolution equipments. Mean value, *n* = 12

To complete comparisons by model-dependent analysis, metformin hydrochloride data were adjusted to several mathematical models commonly used to fit dissolution data. Results are shown in table 7.

Table 7: Criteria used to choose the best fit-model

Key	USP basket apparatus				Flow-through cell method			
	Makoid-Banakar $R^2_{adjusted}$	Peppas-Sahlin	Weibull	Logistic	Makoid-Banakar	Peppas-Sahlin	Weibull	Logistic
R	0.9960	0.9975	0.9912	0.9831	0.9495	0.9036	0.9997	0.9998
A	0.9885	0.9689	0.9966	0.9924	0.9958	0.9730	0.9948	0.9809
B	0.9978	0.9950	0.9986	0.9890	0.9994	0.9979	0.9994	0.9982
C	0.8294	0.7909	0.9980	0.6736	0.9495	0.9036	0.9984	0.7250
	AIC							
R	23.43	19.17	31.33	36.61	24.99	31.08	-0.78	-4.67
A	34.46	43.83	23.32	51.58	26.22	42.40	28.20	37.00
B	17.13	22.94	13.51	30.97	0.14	11.72	1.00	9.79
C	52.56	54.44	9.58	57.83	24.99	31.08	-8.96	40.03

Mean value, *n* = 12

According to established criteria to choose the best fit-model (higher value of R_2 adjusted and lower value of AIC), several mathematical models adjusted to all formulations when the flow-through cell method was used. Logistic model was the best mathematical expression to explain dissolution data of reference, Makoid-Banakar to adjust generic drug products A and B and Weibull function to explain data of generic product C. On the other hand, when the USP basket apparatus was used, Peppas-Sahlin was the best fit-model to describe dissolution data of reference drug product and Weibull function to describe data of all generic formulations. Due to variability obtained in the adjustment of metformin hydrochloride dissolution data of all formulations using both USP apparatuses, no dissolution profiles were compared by this model-dependent approach. However, it is important pointing that a great variability is observed in the adjustment of the data with the flow-through cell method as metformin hydrochloride dissolution data fitted three out of four models used.

The European Medicines Agency (EMA) and Food and Drug Administration (FDA) have pointed the criteria for the BCS-based biowaiver for class III drugs: high solubility and limited absorption for drug substance, very rapid *in vitro* dissolution rate for test and reference drug products, and no change in excipients that might affect bioavailability [6]. By its molecular characteristics, metformin is a class III drug. Membrane permeability is the rate-limiting step for its oral absorption. According to the WHO biowaiver testing procedure for class III drugs, a biowaiver can be considered if the multisource and comparator product are very rapidly dissolving (no less than 85% in 15 min at pH 1.2, 4.5, and 6.8) [31].

Different authors have provided the following information about metformin hydrochloride dissolution from commercial formulations. Akdag *et al.*, [12] worked with seven metformin hydrochloride formulations (1000-mg) using USP paddle apparatus at 50 rpm with media within the physiological pH range. Only one drug product fulfilled the criteria of BCS-based biowaiver. Oyetunde *et al.*, [11] reported a study with four formulations (500-mg) under the same conditions of Akdag *et al.*, [12]. Test and comparator samples were not very rapidly dissolving. Our *in vitro* release study covered only phosphate buffer pH 6.8 with USP basket apparatus at 100 rpm (USP conditions). Same conditions were used by Olusola *et al.*, [9] with eight formulations (500-mg) and found that three products

had>85% dissolved at 30 min and four products showed f_2>50. Criteria of rapidly dissolution has been previously established as more than 85% of drug dissolved at 30 min, respectively [6]. Meanwhile, Adegbola *et al.*, [8] studied 14 products (500-mg) and only three formulations showed>85% of drug dissolved at 15 min.

Several authors have used phosphate buffer pH 6.8 as dissolution medium to test the *in vitro* release performance of metformin hydrochloride commercial formulations with the use of USP paddle apparatus. Kassahum *et al.*, [14] used different drug products and only two of them showed>85% dissolved at 30 min. Prithi *et al.*, [15] used three formulations (500-mg) and 100 rpm as an agitation rate. All tablets comply the Q specification (>80% dissolved at 60 min). Zakeri-Milani *et al.*, [16] tested six (500-mg) and two (1000-mg) products at 100 rpm as the agitation rate. Seven formulations achieved>80% of drug dissolved at 30 min. Additionally, five formulations showed f_2>50 when were compared to the reference (500-mg) but data were calculated with six replicates of each formulation. Similar results were obtained by Hashem *et al.*, [13] that worked with four drug products and two generics achieved f_2>50 also with six replicates by drug product. One condition to calculate f_2 similarity factor is the number of dosage units, it must be calculated with an average of 12 units [6]. Villarroel Stuart *et al.*, [10] studied seven drug products and only one formulation showed<85% of drug dissolved at 15 min and the same product had f_2<50.

The use of the flow-through cell method to study metformin hydrochloride commercial formulations was only reported by Hashem *et al.*, [13]; the dissolution equipment was used with laminar flow, cells of 22.6 mm and phosphate buffer pH 6.8 as dissolution medium. Two generic formulations (1000-mg) showed f_2>50 but the reference was a drug product with a different dose (850-mg) and data were calculated with only six dosage units per drug product.

Similarity of dissolution profiles by comparison of model-dependent or model-independent parameters is not accurate since the results do not match. For this reason, it cannot be definitively concluded with this form of comparison. However, by relating parameters obtained with both approaches, better results were obtained with data generated with the USP basket apparatus than data of the flow-through cell method. In the two ways of association, significant linear regressions were obtained. Mathematical adjustment to different equations also differs and greater variability is observed with the use of the flow-through cell method.

With the brief description of all these results, a great variability is observed with commercial formulations of metformin hydrochloride around the world and the need to work in a common dissolution test to verify the quality of the drug products. Apparently, no more than 85% of drug dissolved will be achieved with a flow-through cell method so it would be necessary to test the hydrodynamics of vessels apparatuses, either basket or paddle. Our work supports better results with the USP basket apparatus instead of the flow-through cell method. It is possible that the flow-through cell method may not be a suitable option to work with metformin hydrochloride tablets. This result is opposite to that reported with class II drugs (low solubility/high permeability) where the flow-through cell method had a greater discriminatory capacity than the vessels apparatus [28, 32]. On the other hand, health regulatory agencies of each country must verify, for class III drugs, a very rapid *in vitro* dissolution rate for test and reference products as well as no change in excipients that might affect bioavailability. Block *et al.*, [33] have demonstrated that the amount and type of excipients used in metformin drug products can modify their dissolution performance.

CONCLUSION

This *in vitro* release study of metformin hydrochloride tablets had the aim of evaluating the dissolution performance of commercially available brands sold in the local market. Influence of the hydrodynamic environment of the flow-through cell method was tested on reference and generic drug products. Results were compared with data obtained using the official dissolution test and better results were found with the USP basket apparatus. No formulation evaluated achieved more than 85% of drug dissolved at 15 min so a biowaiver cannot be suggested. Considering f_2 values, two generic formulations showed similar dissolution profiles to reference but using other comparison methods all generic formulations were different. To demonstrate safe interchangeability between metformin hydrochloride generic formulations and reference bioequivalence studies should be performed. It is important post-marketing monitoring of the commercial formulations because health regulatory agencies of each country must ensure drug products with quality, safety, and efficacy at the lowest possible cost.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Ruiz ME, Gregorini A, Talevi A, Volonte MG. Dissolution studies of generic medications: new evidence of deviations from the transitive principle. Dissolut Technol 2012;19:13–24.
2. Sonal Sekhar M, Thunga G, Suhaj A. The story of metformin continues. J Pharm Pract Res 2014;44:289–90.
3. Song R. Mechanism of metformin: a tale of two sites. Diabetes Care 2016;39:187–9.
4. Hundal RS, Krssak M, Dufour S, Laurent D, Lebon V, Chandramouli V, *et al.* Mechanism by which metformin reduces glucose production in type 2 diabetes. Diabetes 2000;49:2063–9.
5. Lindenberg M, Kopp S, Dressman JB. Classification of orally administered drugs on the word health organization model list of essential medicines according to the biopharmaceutic classification system. Eur J Pharm Biopharm 2004;58:265–78.
6. Food and Drug Administration. Guidance for Industry: Waiver on *in vivo* bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system; 2017. Available from: https://www.fda.gov/media/70963/download. [Last accessed on 10 Jun 2020].
7. United States Pharmacopeia and National Formulary USP 43-NF38; The United States Pharmacopeial Convention, Inc: Rockville MD; 2020.
8. Adegbola AJ, Awobusuyi OJ, Adeagbo BA, Oladokun BS, Owolabi AR, Soyinka JO. Bioequivalence study of generic metformin hydrochloride in healthy Nigerian volunteers. J Exp Res Pharmacol 2017;2:75–81.
9. Olusola AM, Adekoya AI, Olanrewaju OJ. Comparative evaluation of physicochemical properties of some commercially available brands of metformin HCl tablets in lagos, Nigeria. J Appl Pharm Sci 2012;2:41–4.
10. Villarroel Stuart A, Clement Y, Sealy P, Lobenberg R, Montane Jaime L, Maharaj RG, Maxwell A. Comparing the dissolution profiles of seven metformin formulations in simulated intestinal fluid. Dissolut Technol 2015;22:17–21.
11. Oyetunde OO, Tayo F, Akinleye MO, Aina BA. *In vitro* equivalence studies of generic metformin hydrochloride tablets and propranolol hydrochloride tablets under biowaiver conditions in Lagos State, Nigeria. Dissolut Technol 2012;19:51–5.
12. Akdag Y, Gulsun T, Izat N, Oner L, Sahin S. Comparison of dissolution profiles and apparent permeabilities of commercially available metformin hydrochloride tablets in Turkey. Dissolut Technol 2020;27:22–9.
13. Hashem HM, Abdou AR, Mursi MN, Emara LH. Comparative *in vitro* dissolution study on metformin market products using different dissolution apparatuses. Int J Pharm Pharm Sci 2019;11:65–72.
14. Kassahun H, Asres K, Ashenef A. *In vitro* quality evaluation of metformin hydrochloride tablets marketed in Addis Ababa. Bangl J Sci Ind Res 2019;54:169–76.

15. Prithi IJ, Chowdhury SF, Chowdhury ST. Comparative *in vitro* dissolution test and other physicochemical parameters of some commercially available metformin HCl brands in Bangladesh. Pharma Innov J 2018;7:5–8.
16. Zakeri Milani P, Nayyeri Maleki P, Ghanbarzadeh S, Nemati M, Valizadeh H. *In vitro* bioequivalence study of 8 brands of metformin tablets in Iran market. J Appl Pharm Sci 2012;2:194–7.
17. Jantratid E, De Maio V, Ronda E, Mattavelli V, Vertzoni M, Dressman JB. Application of biorelevant dissolution tests to the prediction of *in vivo* performance of diclofenac sodium from an oral modified-release pellet dosage form. Eur J Pharm Sci 2009;37:434–41.
18. Jinno J, Kamada N, Miyake M, Yamada K, Mukai T, Odomi M, *et al*. *In vitro-in vivo* correlation for wet-milled tablet of poorly water-soluble cilostazol. J Controlled Release 2008;130:29–37.
19. COFEPRIS. Listado Actualizado de Medicamentos de Referencia 2020/01, Mexico. Available from: https://www.gob.mx/cms/uploads/attachment/file/534996/listado_de_medicamentos_de_Referencia_.pdf. [Last accessed on 10 Jun 2020].
20. ICH. Harmonized Tripartite Guideline. Q2B Validation of Analytical Procedures: Methodology. International Conference on Harmonization; 1996. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q2b-validation-analytical-procedures-methodology. [Last accessed on 10 Jun 2020]
21. Yuksel N, Kanik AE, Baykara T. Comparison of *in vitro* dissolution profiles by ANOVA-based, model-dependent and independent-methods. Int J Pharm 2000;209:57–67.
22. Collier PS. The interpretation of *in vivo* means dissolution time data. In: Benet L, Z, Levy G, Ferraiolo BL. eds. Pharmacokinetics. Springer-Verlang. Boston, USA; 1984.
23. Tanigawara Y, Yamaoka K, Nakagawa T, Unzo T. New method for the evaluation of *in vitro* dissolution time and disintegration time. Chem Pharm Bull 1982;30:1088–90.
24. Podczeck F. Comparison of *in vitro* dissolution profiles by calculating mean dissolution time (MDT) or mean residence time (MRT). Int J Pharm 1993;97:93–100.
25. Anderson NH, Bauer M, Bousaac N, Khan Malek R, Munden P, Sardaro M. An evaluation of fit factors and dissolution efficiency for the comparison of *in vitro* dissolution profiles. J Pharm Biomed Anal 1998;17:811–22.
26. Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, *et al*. DD Solver: an add-in program for modeling and comparison of drug dissolution profiles. AAPS J 2010;12:263–71.
27. Medina JR, Ortiz HD, Hurtado M, Dominguez Ramirez AM. Influence of dose and the USP basket and flow-through cell dissolution apparatuses in the release kinetics of metronidazole immediate-release products. Int J Res Pharm Sci 2014;5:137–46.
28. Medina JR, Salazar DK, Hurtado M, Cortes AR, Dominguez Ramirez AM. Comparative *in vitro* dissolution study of carbamazepine immediate-release products using the USP paddles method and the flow-through cell system. Saudi Pharm J 2014;22:141–7.
29. Langenbucher F, Benz D, Kurth W, Moller H, Otz M. Standardized flow-cell method as an alternative to existing pharmacopoeial dissolution testing. Pharm Ind 1989;51:1276–81.
30. Moore JW, Flanner HH. Mathematical comparison of dissolution profiles. Pharm Technol 1996;20:64–74.
31. WHO. Technical Report Series, no. 937, Annex 7. Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability; 2006. Available from: https://www.who.int/medicines/areas/quality_safety/quality_assurance/MultisourcePharmaProductsGuidelinesRegistrationRequirementsInterchangeabilityTRS937Annex7.pdf. [Last accessed on 10 Jun 2020]
32. Hurtado M, Vargas Y, Dominguez Ramirez AM, Cortes AR. Comparison of dissolution profiles for albendazole tablets using USP apparatus 2 and 4. Drug Dev Ind Pharm 2003;29:777–84.
33. Block LC, Schemling LO, Cuoto AG, Mourao SC, Bresolin TMB. Pharmaceutical equivalence of metformin tablets with various binders. Rev Cienc Farm Basica Appl 2008;29:29–35.