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Comparison of Generic Furosemide Products by In Vitro Release Studies using USP Apparatus 2 and 4

José Raúl Medina-López[1*], Alexander Domínguez-Reyes[1], and Marcela Hurtado[1]
[1]*Departamento Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, Mexico City, Mexico.*

e-mail: rmlopez@correo.xoc.uam.mx

ABSTRACT

Dissolution studies are essential for comparing the quality of generic drugs to their reference products. The objective of this work was to evaluate the in vitro release of furosemide in tablets under official dissolution conditions and using the flow-through cell method. To this end, two USP apparatus were used: apparatus 2 (paddle at 50 rpm) containing 900 mL of phosphate buffer solution pH 5.8, and apparatus 4 (flow-through cell at 16 mL/min) with the same medium. The dissolved drug was quantified by UV spectrophotometry at 274 nm for 60 min. Although the results at a single time point met the acceptance criterion of Q = 80% at 60 min, the dissolution profiles of generic drugs were not similar to the reference product. The similarity factor ($f_2 < 50$), mean dissolution time, dissolution efficiency, $t_{50\%}$, $t_{80\%}$, and *Td* values corroborate the difference between the profiles ($p < 0.05$). In vitro release testing demonstrates that, for furosemide tablets available in Mexico, the generic formulations perform differently from the reference products. These differences could affect in vivo absorption, which could yield different therapeutic effects. More evaluation of generic furosemide tablets manufactured in Mexico is needed.

KEYWORDS: Furosemide, generic drug products, flow-through cell method

INTRODUCTION

Generic drugs represent inexpensive options for patients and health systems, and they must exhibit the same quality, safety, and efficacy as reference drugs. Most generic formulations properly fulfill the function they were prepared for; however, sometimes, the formulation's in vivo performance can result in a lack of therapeutic effect (*1*). The absorption of poorly water-soluble drugs can be limited by the rate of dissolution, so both the formulation and manufacturing process play an important role in the full and timely release of the active pharmaceutical ingredient from the dosage form.

Pharmacopeial dissolution testing for solid and some semi-solid dosage forms is mostly performed using USP apparatus 1 and 2 (basket and paddle, respectively). The procedures employed in these units are widely known, and the hydrodynamic environment created in the in vitro test continues to be a subject matter of research for several authors (*2*). Despite their extensive use, neither apparatus completely reproduces the process of dissolution taking place in vivo due to the complex nature of the gastrointestinal tract.

The flow-through cell (USP apparatus 4) was introduced as an alternative to the basket and paddle apparatus. The following advantages have been recognized (*3*):

1. There are very few apparatus parameters that affect the test and have to be standardized.
2. Ideal hydrodynamics conditions for turbulent and laminar solvent flow conditions exist.
3. Working with an unlimited amount of solvent is possible, thus overcoming problems due to non-sink conditions.
4. pH changes may be easily performed stepwise since the medium is exchanged very rapidly in the low volume cells (this allows adaptation of test parameters to physiological conditions).
5. Apparatus 4 allows for easy positioning and consistent testing of a wide variety of sample types including powders, granules, implants/microcapsules, suppositories, granules, and soft gelatin capsules as well as conventional tablets and coated tablets.

* *Corresponding author.*

6. Tests can be run either in an open or closed system (e.g., fixed or unlimited solvent).

USP Apparatus 4 has been useful in developing more discriminative dissolution methods than those that employ apparatus 1 or 2, as was demonstrated with commercial tablets of albendazole and carbamazepine, both having low solubility (*4, 5*). Despite the advantages of apparatus 4 over the basket and paddle equipment, little information is available on its application in the evaluation of generic drug products with solubility problems.

Furosemide has low solubility and low permeability and is classified in the Biopharmaceutics Classification System as a class IV drug. Furosemide tablets are used to treat hypertension and edematous states associated with renal or liver failure (*6*). Furosemide is mainly absorbed in the stomach and small intestine, which means it has a narrow absorption window. For this reason and its physicochemical characteristics, absorption of furosemide is variable and erratic (20–60%) (*7*). To evaluate the quality of commercial furosemide tablets, pharmacopeial dissolution testing indicates the use of USP apparatus 2 at 50 rpm with 900 mL of phosphate buffer solution pH 5.8 at 37 ± 0.5 °C as a dissolution medium. Under such conditions, not less than 80% of the drug should be dissolved in 60 min (Q = 80%) (8). However, to date, there is no published scientific information that reports an in vitro/in vivo correlation (IVIVC) for furosemide tablets using official conditions.

The present study's objective was to compare the furosemide dissolution profile of generic tablets with the reference product using the official pharmacopeial method (apparatus 2) and an alternative, flow-through cell method (apparatus 4). The study also aimed to validate the applicability of both apparatus in the evaluation of biopharmaceutical quality of generic furosemide tablets and potential for bioavailability problems.

MATERIAL AND METHODS

Three generic drug products (randomly encoded as A, B, and C) and the reference drug (R), Lasix (Sanofi-Aventis de Mexico SA de CV, Ocoyoacac, Mexico), were used in the same doses of 40 mg. The generic drug manufacturers in alphabetical order were Biomep SA de CV (Naucalpan de Juarez, Mexico); Bioresearch de Mexico, SA de CV (Naucalpan de Juarez, Mexico); and Buffington's de Mexico SA de CV (Mexico City, Mexico). Mexican health authorities have established Lasix as a reference drug in dissolution and bioequivalence studies (*9*). The monobasic and dibasic phosphates were purchased from J. T. Baker-Mexico (Xalostoc, Mexico). The furosemide standard was purchased from Sigma-Aldrich Co. (St. Louis MO, USA).

Dissolution tests were performed using an automatic USP apparatus 2 (Sotax AT7-Smart, Switzerland) at 50 rpm with 900 mL of dissolution medium and apparatus 4 (Sotax CE6, Switzerland) with 16 mL/min laminar flow. In both apparatus, phosphate buffer solution pH 5.8 at 37.0 ± 0.5 °C was employed as a dissolution medium. The dissolved furosemide was determined at 274 nm in relation to a calibration curve prepared on the day of analysis.

Content Uniformity and Assay

The content uniformity and assay tests were carried out on all drug products under study in accordance with the procedures described in the USP (*8*).

Analytical Method Validation

The analytical method was validated following the ICH guidelines (*10*).

Linearity

To demonstrate linearity of the system, two calibration curves were prepared with five solutions of furosemide (1.25–20 µg/mL) in phosphate buffer solution pH 5.8. The absorbance was determined at 274 nm with 1-cm quartz cells. The obtained data were fitted to the equation of a straight-line (y = bx + a), and the regression coefficients, regression analysis of variance, and 95% confidence interval ($CI_{95\%}$) were calculated for the intercept.

Accuracy and Precision

To discard matrix effects, accuracy and precision were determined by the standard addition method. Twenty (*20*) tablets were accurately weighed and ground in a mortar. The powder (including 10 mg of standard with an equivalent to 80, 100, and 120% of drug) was dissolved in 900 mL of dissolution medium. This medium was previously vacuum degassed. Apparatus 2 was used at 50 rpm for 60 min, then a sample was taken, and the amount of dissolved furosemide was calculated. Each sample was run in triplicate. The percentage of relative error (RE, calculated with the following equation: [(amount found – amount added) / amount added] ×100) was considered as a measure of method's accuracy, and the coefficient of variation (CV, calculated as standard deviation divided by mean) as a measure of its precision. The experiments were performed on 3 consecutive days.

Solution Stability

The stability of furosemide standard solution was evaluated by analyzing two solutions of known drug

concentration in phosphate buffer solution pH 5.8 (3 and 15 µg/mL). The absorbance of all solutions was determined before and after storage for 24 and 48 h at 4 °C and 25 °C. The percentage of absolute difference (AD) was calculated as follows: AD = [(initial response – final response) / initial response] × 100).

Dissolution Studies

Dissolution profiles of furosemide drugs under study were determined under pharmacopeial conditions using apparatus 2 at 50 rpm with 900 mL of phosphate buffer solution pH 5.8 at 37.0 ± 0.5 °C (n = 12) and with apparatus 4 with the laminar flow at 16 mL/min and 22.6-mm cells (*8*). Both units of equipment were programmed to take samples automatically every 5 min for 60 min.

Data Analysis

Dissolution profiles of generic drug products were compared with the reference drug by calculating the similarity factor, f_2. The profiles were considered similar if the value of f_2 was between 50 and 100 (*11*). The dissolution data were used to calculate the model-independent parameters, mean dissolution time (MDT) and dissolution efficiency (DE). MDT coincides with *Td* value (this is the last value calculated with α and β parameters of the Weibull function), which is the time interval necessary to dissolve 63.2% of the drug (*12*). MDT can be calculated by Equation 1 (*13*):

$$\mathrm{MDT} = \frac{\sum_{j=1}^{n} \hat{t}_j \Delta M_j}{\sum_{j=1}^{n} \Delta M_j}, \qquad \text{Eq. (1)}$$

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

DE is defined as the area under the dissolution curve up to a certain time, t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. It can be calculated by Equation 2 (13):

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

where y is the drug percent dissolved at time t, and dt is the time differential.

The model-dependent parameters, $t_{50\%}$ and $t_{80\%}$, which represent the time at which 50% and 80% of the dose is dissolved, respectively, were also calculated. The values of MDT and DE were calculated using DDSolver (version 1.0), a menu-driven add-in program for Microsoft Excel (Mountain View, CA, USA) (*14*). The $t_{50\%}$ and $t_{80\%}$ parameters were obtained with data fitting to the hyperbole equation (y = ax/b + x) using the Sigmaplot software (version 11.0, Systat Software Inc, San Jose, CA, USA). To compare data for the generic and reference drugs, a one-way ANOVA was performed, followed by a Dunnett's multiple comparison test. Values with $p < 0.05$ were considered statistically significant differences.

Moreover, to describe the drug release process within the formulation in the best possible way, dissolution data were fitted to different mathematical models, frequently used in in vitro studies. These models included Makoid-Banakar, Peppas-Sahlin, Weibull, Logistic, Gompertz, and Probit models. The best fit model was the one exhibiting a higher value of adjusted correlation coefficient ($R^2_{adjusted}$) and a lower value of the Akaike Information Criterion (AIC) (*13*). For this analysis, which was considered to be model dependent, DDSolver was used.

RESULTS

Content Uniformity and Assay

The content uniformity and assay tests were performed, and all drugs met the established pharmacopeial criteria. The results are shown in Table 1.

Table 1. Results of Content Uniformity and Assay Tests for Reference (Ref) and Generic (A–C) Furosemide Tablets

Product	Content Uniformity (%), Range	Assay (%)
Ref	97.4–100.31	100.79
A	96.71–99.83	100.42
B	98.49–101.17	99.96

Data are mean values (n = 10).

Dissolution Method Validation

The linearity of the furosemide in phosphate buffer pH 5.8 solutions at 274 nm is shown in Figure 1. The 95% confidence interval for the intercept was -0.0027 to 0.0012.

Accuracy and precision of the method were evaluated by analyzing the three samples of ground tablets, with different drug percentages, for 3 days. The results of these tests are given in Table 2. The CV ranged from 1.65 to 2.29%, and RE values did not exceed 2.20%, indicating that the dissolution method employed has good accuracy and precision. Solution stability was evaluated with the analysis of two solutions at different times and temperatures. The AD values ranged from -2.47 to 1.81%, indicating that the furosemide solutions remained stable under all conditions used.

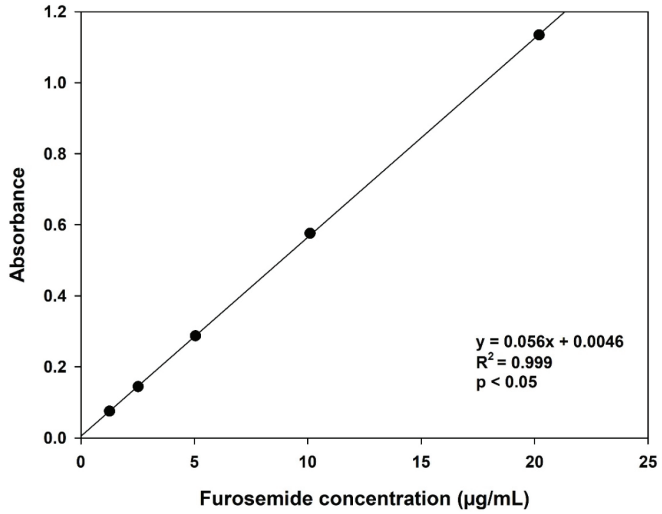

Figure 1. Linearity of furosemide standard solutions at 274 nm.

Dissolution Studies

Dissolution profiles of furosemide obtained under pharmacopeial conditions (USP Apparatus 2) and using the flow-through cell (apparatus 4) are shown in Figure 2.

In apparatus 2, all drugs met the pharmacopeial specification (Q = 80% at 60 min). In apparatus 4, all generic products met the specifications but the reference product did not surpass 40% of the dissolved drug.

The percentages of furosemide dissolved at 60 min of all study drugs are given in Table 3. Differences in drug release compared the reference were statistically significant for one generic product (C) in apparatus 2 and for all three generic formulations in apparatus 4 ($p < 0.05$). When the similarity factor, f_2, was used as a comparative measure, the dissolution profiles of the generic drugs were not similar to the reference profile in either apparatus ($f_2 <$ 50). Moreover, due to the limited rate and degree of drug dissolution from the reference, the lowest values of f_2 (6.91–12.26) were obtained when apparatus 4 was used.

Model-Independent and Model-Dependent Comparisons

Data on dissolved furosemide as a function of time were used to calculate the values of model-independent parameters, MDT and DE, and model-dependent parameters, $t_{50\%}$ and $t_{80\%}$. The results are reported in Table 3.

Significant differences were found for all MDT values with both apparatus and for DE values in apparatus 4. Significant differences were also found for most model-dependent parameters in apparatus 2 ($p < 0.05$). The exception was the $t_{80\%}$ value of generic product C. Due to the limited dissolution of drug contained in the reference product (< 40% at 60 min) with the use of apparatus 4, it was not possible to calculate $t_{50\%}$ and $t_{80\%}$ values or compare the dissolution profiles of generic drugs with the reference.

To compare the dissolution profiles using apparatus 4 and model-dependent parameters, data were fitted to different models that allow a mathematical explanation of the dissolution process. The selected models represent some of the most commonly used equations for this kind of study. The fit results are shown in Table 4.

In accordance with the established criteria (highest R_2 adjusted value and lowest AIC value), the best fit model for all dissolution data of furosemide was the Weibull model. The expression of this function is given in Equation 3 (14):

$$F = F_{\max}\left[e^{-\frac{(t-Ti)^{\beta}}{\alpha}}\right], \qquad \text{Eq. (3)}$$

where F is the percent of drug dissolved vs. t time, $F_{\max}$ is the maximum percent of drug dissolved at infinite time, α is the scale factor of the process, β is the shape factor, and Ti is a location parameter time in which the drug begins to dissolve.

The furosemide dissolution data for all formulations obtained with apparatus 2 and 4 were adjusted to fit the Weibull model, and the resulting dissolution profiles were statistically compared using *Td* values derived from the equation. The *Td* value is a model-dependent parameter frequently used to compare dissolution profiles (*15*). The mean values of α, β, Ti, $F_{\max}$, and *Td* are shown in Table 5.

Comparison of *Td* values for all generic drugs with the reference, significant differences were found with both apparatus ($p < 0.05$). The shape factor of the Weibull function, β, characterizes the dissolution profile as exponential (β = 1) (case 1), sigmoid S shape with upward curvature followed by a turning point (β > 1) (case 2), or as parabolic with a steeper initial slope that is consistent with the exponential (β < 1) (case 3) (*13*). In this work, generic formulations A and B had β < 1 in both apparatus, but the β value for formulation C and the reference varied depending on the hydrodynamics of apparatus 2 and 4.

DISCUSSION

In this in vitro study, all furosemide products met the Q criterion stipulated in the USP. The official dissolution test evaluates the degree of drug dissolved at a single time point. For highly soluble drugs, this test may be

Table 2. Accuracy and Precision of Dissolution Method Used to Determine Drug Content in Tablets Containing Furosemide

	Inter-day (n = 3)			Intra-day (n = 9)		
Added (mg)	Found (mg)	CV (%)	RE (%)	Found (mg)	CV (%)	RE (%)
32.0	32.11 ± 0.32	1.71	0.35	32.46 ± 0.18	1.65	1.43
40.0	39.75 ± 0.44	1.91	-0.63	40.15 ± 0.29	2.19	0.38
48.0	49.06 ± 0.58	2.06	2.20	48.27 ± 0.37	2.29	0.57

Data are mean values ± standard deviation. CV - coefficient of variation; RE - relative error.

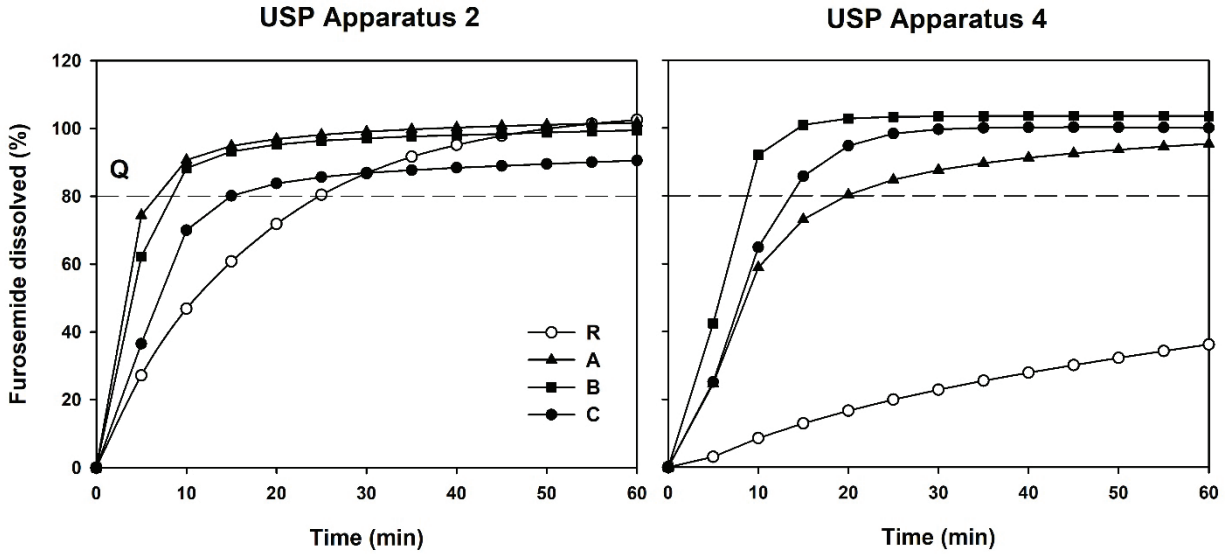

Figure 2. Dissolution profiles of furosemide from generic (A-C) and reference (R) products obtained using USP apparatus 2 and 4. For clarity, error bars have been omitted. Data are mean values (n = 12).

Table 3. Model-Independent and Model-Dependent Parameters Calculated to Compare Dissolution Profiles of Furosemide in Generic (A–C) and Reference (Ref) Products

Product	Diss. at 60 min (%)	MDT (min)	DE (%)	$t_{50\%}$ (min)	$t_{80\%}$ (min)
USP Apparatus 2					
Ref	102.55 ± 0.61	15.61 ± 0.68	75.93 ± 1.52	10.76 ± 0.80	25.74 ± 1.51
A	101.69 ± 0.52	5.51 ± 0.10*	92.35 ± 0.59*	1.76 ± 0.12*	6.15 ± 0.46*
B	99.45 ± 0.88	6.01 ± 0.33*	89.53 ± 1.21*	2.55 ± 0.34*	8.80 ± 1.17*
C	90.57 ± 1.02*	8.49 ± 0.42*	77.76 ± 1.12	5.58 ± 0.45*	21.15 ± 1.75
USP Apparatus 4					
Ref	36.15 ± 1.04	25.14 ± 0.39	20.98 ± 0.57	-	-
A	95.33 ± 1.56*	11.84 ± 0.18*	76.53 ± 1.35*	8.07 ± 0.33	24.32 ± 1.43
B	103.53 ± 1.30*	6.19 ± 0.05*	92.86 ± 1.18*	3.38 ± 0.10	9.93 ± 0.51
C	100.05 ± 1.38*	9.05 ± 0.28*	84.93 ± 1.09*	6.13 ± 0.22	16.85 ± 0.78

Data are mean values ± standard error medium (n = 12).
**p < 0.05. Dash (-) indicates not calculated. Diss – dissolution; MDT – mean dissolution time; DE – dissolution efficiency.*

Table 4. Mathematical Models Used to Fit Dissolution Data of Generic (A-C) and Reference (Ref) Products Containing Furosemide

	Makoid-Banakar	Peppas-Sahlin	Weibull	Logistic	Gompertz	Probit
USP Apparatus 2						
$R^2_{adjusted}$						
Ref	0.9970	0.9950	0.9994	0.9527	0.9177	0.9639
A	0.9432	0.9510	0.9998	0.9417	0.9405	0.9480
B	0.8782	0.8807	0.9990	0.9742	0.9788	0.9656
C	0.8923	0.8788	0.9985	0.9202	0.9454	0.8992
AIC						
Ref	30.04	37.86	8.29	71.10	77.91	67.65
A	45.03	42.57	-27.31	39.74	39.02	39.96
B	62.16	61.76	1.72	36.38	33.16	41.92
C	70.60	72.06	13.39	65.27	59.97	68.40
USP Apparatus 4						
$R^2_{adjusted}$						
Ref	0.9975	0.9995	0.9998	0.9956	0.9979	0.9982
A	0.9464	0.9333	0.9993	0.9844	0.9920	0.9797
B	0.7908	0.7629	1.000	0.9125	0.9146	0.9101
C	0.9265	0.8936	0.9991	0.9778	0.9688	0.9799
AIC						
Ref	15.53	-6.70	-19.69	21.39	7.08	10.76
A	69.44	72.17	15.40	51.72	42.17	53.57
B	82.36	83.86	-30.74	69.83	69.49	70.05
C	75.83	80.27	-3.51	52.33	61.26	44.59

Data are mean values (n = 12). AIC – Akaike information criterion.

Table 5. Weibull Equation Parameters and Td values of Generic (A-C) and Reference (Ref) Products Containing Furosemide

	α	β	*Ti*	F_{max}	*Td* ± SEM (min)
USP Apparatus 2					
Ref	627.71	1.13	-1.98	110.39	20.89 ± 3.93
A	0.80	0.26	3.38	104.88	4.33 ± 0.18*
B	0.88	0.31	4.56	103.02	5.24 ± 0.18*
C	1.78	0.52	4.31	90.88	7.02 ± 0.39*
USP Apparatus 4					
Ref	35.18	0.82	3.12	65.97	85.89 ± 13.58
A	3.46	0.61	4.08	98.53	10.95 ± 0.24*
B	2.22	0.86	3.82	103.53	6.30 ± 0.05*
C	23.36	1.28	2.16	100.29	9.37 ± 0.22*

Data are mean values (n = 12).
**p < 0.05. Ti – location parameter which represents the lag time before the onset of the dissolution or release process and in most cases will be near zero; Td – time interval necessary to dissolve or release 63.2% of the drug present in the pharmaceutical dosage form; SEM – standard error medium.*

sufficient to estimate the formulation performance and quality; however, for poorly soluble drugs and especially those presenting bioavailability problems, the dissolution profile becomes a predictive factor for absorption and manifestation of the therapeutic effect. In this study the dissolution rate exhibited by generic furosemide products was different from that observed for the reference product.

Several authors have reported problems with dissolution of 40-mg furosemide tablets under pharmacopeial conditions. In a study evaluating 13 drugs, only four formulations were found to meet the pharmacopeial criterion of Q (*16*). In another dissolution study of nine commercial formulations, all products reached Q, but only one exhibited a similar dissolution profile as the reference (f_2 = 54.3) (*17*). All products met the pharmacopeial criterion in this work, but the dissolution profiles were not similar to the reference drug. The results are consistent with those reported by other authors who have worked with poorly soluble drugs, such as naproxen sodium and meloxicam in tablets, as well as ibuprofen suspensions (*18–20*).

Given the reported solubility of furosemide (0.27 at 0.33 mg/mL at pH 5.0) and dissolution conditions in apparatus 4 (i.e., volume and acidity of the medium), complete dissolution of the reference drug should not be a problem (*6*), which suggests a need to evaluate drugs containing furosemide as the active ingredient in three crucial aspects: quality of excipients, manufacturing process, and conditions used to determine the in vitro dissolution of the drug. Other authors have reported that the dissolution medium with pH 5.6 is the most adequate to ensure uniformity among batches and bioequivalence with drugs containing this compound (*21*).

The results indicate that not all commercially available drugs have the same drug release capacity under the same experimental conditions. It becomes critical when dealing with medications whose absorption depends on the rate of dissolution. Furosemide has been reported to have a bioavailability of 60–70% with variable and erratic absorption (*22*). Other authors report bioavailability ranging between 37% and 51%, with high inter- and intra-subject variability (*23*). Specifically, the Lasix product has been reported to have an absolute bioavailability of 56% (*24*). This value seems to be related to the low dissolution rate and degree found with the same product when using apparatus 4 in the present study (< 50%). On the other hand, 113% relative bioavailability has been reported for generic furosemide tablets (40 mg), and 129% has been reported for 500-mg tablets (the latter being compared with the Lasix product) (*16*, *25*). Data obtained using apparatus 4 are evidence of a high probability for generic formulations to exhibit supra equivalence, which would explain results obtained by other authors.

As for the dissolution rate, the time necessary to reach $t_{50\%}$ for generic drug B was 4.2 times lower than the reference drug, which is the opposite result of that reported by Stüber et al. in an in vivo study where the drug with the lowest bioavailability exhibited a $t_{50\%}$ value 4.4 times higher than the reference (*26*). In that study, the most significant in vitro differences were observed with apparatus 2 at 50 rpm and pH 5.3. Considering the MDT data of the generic formulations and apparatus 2 from our study, the difference between the time necessary to reach this value was 1.8 and 2.8 times lower than that calculated for the reference drug. Statistical comparisons of drug percentages dissolved at 60 min as well as MDT, DE, and *Td* values obtained with apparatus 4 confirm the differences observed in the dissolution profiles between both apparatus.

Parameters like MDT and DE are useful for establishing a significant IVIVC at levels B and C, respectively. At level B, MDT is associated with mean residence time or the mean time during which drug molecules remain in the body. At level C, however, a parameter that globally reflects drug dissolution is related to bioavailability parameters such as, for instance, the area under the curve of plasma profile or the C_{max} value (*27*). Some authors have found an IVIVC with furosemide drugs under four experimental conditions: (1) pH 7.8, paddle apparatus at 25 rpm; (2) pH 7.8, paddle apparatus at 50 rpm; (3) pH 5.3, paddle apparatus at 50 rpm; and (4) pH 7.8, flow-through cell (100 mL/h) (*26*).

Dissolution studies with apparatus 4 are important and necessary because it is the commercial equipment that best simulates the hydrodynamic environment of the gastrointestinal tract and allows establishing a significant IVIVC, thus facilitating the prediction of the drug's in vivo performance through in vitro data. Some authors have also reported, with this equipment, a better estimation of absorption rate with drugs containing cilostazol and diclofenac sodium, both of which have solubility problems (*28*, *29*).

Another, not less important, aspect of this paper involves considering the appropriate applicability of using a pharmaceutical product that exhibits a low rate and degree of dissolution as a reference drug. With

apparatus 4, differences in the process of dissolution studies become evident. According to international regulations, the performance of generic drugs must be compared with that of the reference formulation and may need to be reformulated if the biopharmaceutical characteristics are not consistent with the reference. However, when in vitro data for reference drugs are not favorable (appropriate dissolution rate and degree), it would be convenient to evaluate their usefulness as parameters of comparison because of the risk that generic formulations may present greater bioavailability than the reference. An incorrect design of diuretic drugs would have negative clinical consequences; for instance, cardiovascular disease represents the first cause of death (30%) in renal transplant receivers. The most common symptoms and comorbidities among patients with advanced heart failure are dyspnea, pain, depression, fatigue, and edema. Diuretics are the primary treatment for dyspnea and edemas, and furosemide is the most commonly used drug for treating these symptoms (*30*).

CONCLUSIONS

Although generic medications are available for marketing, it is important to evaluate the rate at which they release the active ingredient, especially formulations prepared with poorly soluble drugs. Generic furosemide formulations exhibited a dissolution rate and degree different from those observed with the reference drug, independently of the dissolution equipment used. These differences were more evident when using USP apparatus 4. It is possible that poor release is related to in vivo behavior; however, this should be the subject of future research. Clinical repercussions of these results must be evaluated to ensure that pharmaceutical products manufactured in Mexico containing furosemide maintain the quality, safety, and efficacy to be safely exchanged.

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CONFLICT OF INTEREST

The authors declared no conflict of interest related to this article.

REFERENCES

1. Kaló, Z.; Holtorf, A. P.; Alfonso-Cristancho, R.; Shen, J.; Ágh, T.; Inotai, A.; Brixner, D. Need for multicriteria evaluation of generic drug policies. *Value Health*. **2015**, *18*, 346–351. DOI: 10.1016/j.jval.2014.12.012.
2. Todaro, V.; Persoons, T.; Grove, G.; Healy, A. M.; D'Arcy, D. M. Characterization and simulation of hydrodynamics in the paddle, basket and flow-through dissolution testing apparatuses - A review. *Dissolut. Technol*. **2017**, *24*, 24–36. DOI: 10.14227/DT240317P24.
3. Looney, T. J. USP Apparatus 4 [flow-through method] primer. *Dissolut. Technol*. **1996**, *3*, 10–12. DOI: 10.14227/DT030496P10.
4. Hurtado y de la Peña, M.; Vargas Alvarado, Y.; Domínguez-Ramírez, A. M.; Cortés Arroyo, A. R. Comparison of dissolution profiles for albendazole tablets using USP apparatus 2 and 4. *Drug Dev. Ind. Pharm*. **2003**, *29*, 777–784. DOI: 10.1081/DDC-120021777.
5. Medina, J. R.; Salazar, D. K.; Hurtado, M.; Cortés, A. R.; Domínguez-Ramírez, A. M. 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*, 141–147. DOI: 10.1016/j.jsps.2013.02.001.
6. Granero, G. E.; Longhi, M. R.; Mora, M. J.; Junginger, H. E.; Midha, K. K.; Shah, V. P.; Stavchansky, S.; Dressman, J. B.; Barends, D. M. Biowaiver monographs for immediate release solid oral dosage forms: furosemide. *J. Pharm. Sci.* **2010**, *99*, 2544–2556. DOI: 10.1002/jps.22030.
7. Radwan, S. E.; Sokar, M. S.; Abdelmonsif, D. A.; El-Kamel, A. H. Mucopenetrating nanoparticles for enhancement of oral bioavailability of furosemide: In vitro and in vivo evaluation/sub-acute toxicity study. *Int. J. Pharm*. **2017**, *526*, 366–379. DOI: 10.1016/j.ijpharm.2017.04.072.
8. Furosemide Tablets Monograph. In *The United States Pharmacopeia USP 42–NF 37*; The United States Pharmacopeial Convention, Inc.: Rockville MD, 2019.
9. COFEPRIS Listado actualizado de medicamentos de referencia. Gobierno de México website. February 11, 2020. https://www.gob.mx/cms/uploads/attachment/file/502513/Listado_de_medicamentos_de_Referencia.pdf. (accessed January 21, 2021).
10. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. *Validation of Analytical Procedures: Text and Methodology Q2*(*R1*); ICH Harmonised Tripartite Guideline: Geneva, Switzerland, 2005.
11. Moore, J. W.; Flanner, H. H. Mathematical comparison of dissolution profiles. *Pharm. Technol*. **1996**, *20*, 64–75.

12. Tanigawara, Y.; Yamaoka, K.; Nakagawa, T.; Uno, T. New method for the evaluation of in vitro dissolution time and disintegration time. *Chem. Pharm. Bull*. (*Tokyo*). **1982**, *30*, 1088–1090. DOI: 10.1248/cpb.30.1088.
13. Costa, P.; Sousa Lobo, J. M. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci*. **2001**, *13*, 123–133. DOI: 10.1016/S0928-0987(01)00095-1.
14. Zhang, Y.; Huo, M.; Zhou, J.; Zou, A.; Li, W.; Yao, C.; Xie, S. DDSolver: an add-in program for modeling and comparison of drug dissolution profiles. *AAPS J*. **2010**, *12*, 263–271. DOI: 10.1208/s12248-010-9185-1.
15. Yuksel, N.; Kanik, A. E.; Baykara, T. Comparison of in vitro dissolution profiles by ANOVA-based, model-dependent and -independent methods. *Int. J. Pharm*. **2000**, *209*, 57–67. DOI: 10.1016/S0378-5173(00)00554-8.
16. Kaojarern, S.; Poobrasert, O.; Utiswannakul, A.; Kositchaiwat, U. Bioavailability and pharmacokinetics of furosemide marketed in Thailand. *J. Med. Assoc. Thai*. **1990**, *73*, 191–197.
17. Han, Y. K.; Simionato, L. D.; Calvo, R. G.; Mattei, M. B.; Segall, A. I. Comparison of dissolution profiles of furosemide tablets available in the Argentinian market. *J. Appl. Sol. Chem. Mod*. **2014**, *3*, 186–193. DOI: 10.6000/1929-5030.2014.03.04.1.
18. Medina, J. R.; Uribe, A.; Hurtado, M.; Domínguez-Ramírez, A. M. In vitro equivalence study of generic naproxen sodium tablets using the USP paddle apparatus and the flow-through cell method. *Int. J. Pharm. Pharm. Sci*. **2015**, *7*, 248–254.
19. Medina-López, J. R.; Orozco-Juárez, J. A.; Hurtado, M. Dissolution performance of meloxicam formulations under hydrodynamics of USP paddle apparatus and flow-through cell method. *Int. J. Appl. Pharm*. **2019**, *11*, 182–188. DOI: 10.22159/ijap.2019v11i4.33243.
20. Medina, J. R.; Cortés, M.; Romo, E. Comparison of the USP Apparatus 2 and 4 for testing the in vitro release performance of ibuprofen generic suspensions. *Int. J. Appl. Pharm*. **2017**, *9*, 90–95. DOI: 10.22159/ijap.2019v9i4.19926.
21. McNamara, P. J.; Foster, T. S.; Digenis, G. A.; Patel, R. B.; Craig, W. A.; Welling, P. G.; Rapaka, R. S.; Prasad, V. K.; Shah, V. P. Influence of tablet dissolution on furosemide bioavailability: a bioequivalence study. *Pharm. Res*. **1987**, *4*, 150–153. DOI: 10.1023/A:1016427321532.
22. Sweetman S. C. *Martindale: The complete Drug Reference*. 36th ed.; Pharmaceutical Press: London, 2009.
23. Kelly, M. R.; Cutler, R. E.; Forrey, A. W.; Kimpel, B. M. Pharmacokinetics of orally administered furosemide. *Clin. Pharmacol. Ther*. **1974**, *15*, 178–186. DOI: 10.1002/cpt1974152178.
24. Grahnén, A.; Hammarlund, M.; Lundqvist, T. Implications of intraindividual variability in bioavailability studies of furosemide. *Eur. J. Clin. Pharmacol*. **1984**, *27*, 595–602. DOI: 10.1007/BF00556898.
25. Wolf-Coporda, A.; Lovrić, Z.; Huić, M.; Francetić, I.; Vrhovac, B.; Plavsić, F.; Skreblin, M. Determination of bioequivalence of two furosemide preparations; the effect of high doses of furosemide on some pharmacokinetic parameters. *Int. J. Clin. Pharmacol. Res*. **1996**, *16*, 83–88.
26. Stüber, W.; Mutschler, E.; Steinbach, D. The pharmaceutical and biological availability of commercial preparations of furosemide. *Arzneimittelforschung*. **1982**, *32*, 693–697.
27. Demirtürk, E.; Öner, L. In vitro – in vivo correlations. FABAD *J. Pharm. Sci*. **2003**, *28*, 215–224.
28. Jinno, J.; Kamada, N.; Miyake, M.; Yamada, K., Mukai, T.; Odomi, M.; Toguchi, H.; Liversidge, G. G.; Higaki, K.; Kimura, T. In vitro-in vivo correlation for wet-milled tablet of poorly water-soluble cilostazol. *J. Control. Release* **2008**, *130*, 29–37. DOI: 10.1016/j.jconrel.2008.05.013.
29. Jantratid, E.; De Maio, V.; Ronda, E.; Mattavelli, V.; Vertzoni, M.; Dressman, J. B. 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*, 434–441. DOI: 10.1016/j.ejps.2009.03.015.
30. Sánchez, M. P.; Lorenzo, M. N. C.; Pérez, M. C.; Lorman, R. S. Palliative use of furosemide in continuous subcutaneous infusion in a renal transplant patient with heart failure. [in Spanish] *Nefrologia*. **2018**, *38*, 347–458. DOI: 10.1016/j.nefro.2017.11.020.