

Dissolution Test of Patent and Generic Drugs of Metformin Hydrochloride

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ABSTRACT

Type 2 diabetes is a chronic, multifactorial disease with a genetic predisposition caused by a deficit in the action or secretion of insulin, resulting in hyperglycemia, which is characterized by increased blood glucose levels. Metformin hydrochloride is the most frequently used medication. There is a wide variety of generic metformin products in the Mexican market, and these products can vary in quality depending on the manufacturing process and formulation. The purpose of this study was to assess the dissolution properties of 850-mg immediate-release metformin hydrochloride tablets from two batches of three different brands available in the Mexican market (in Mexico City): patent Dabex, interchangeable/generic Dinorax, and similar Setebaid. In vitro dissolution studies were performed in accordance with the *Pharmacopoeia of the United Mexican States* (FEUM). For the titration test, Dinorax exceeded the specifications (95–105%) as well as batch 2 of the Setebaid brand. For the dissolution tests, Dinorax and Setebaid passed but not Dabex. For weight variation, all brands were within the limits of acceptance criteria. The Dabex brand, which is considered a reference by FEUM, did not pass the dissolution test.

Keywords: Dissolution profiles, metformin hydrochloride, diabetes mellitus type 2, generic drugs

INTRODUCTION

In Mexico, the prevalence of diabetes is 13.7%, of which 68.2% of those diagnosed have glycemic uncontrol (1). The number of deaths from diabetes mellitus is higher in the population aged 60 and over (2).

Type 2 diabetes is a chronic, multifactorial disease with a genetic predisposition caused by a deficit in the action or secretion of insulin, resulting in hyperglycemia. A widely used drug in the management of type 2 diabetes is metformin, which is a biguanide (dimethylbiguanide). This oral antidiabetic medication is most frequently used for patients with diabetes; around 150 million patients per year worldwide have used this drug over the last 60 years (3, 4). The drug comes from guanidine, which is derived from the *Galega Officinalis* plant that has been used since medieval times (5, 6). This drug acts as an antihyperglycemic agent (but does not predispose to hypoglycemia), which decreases hepatic glucose production by inhibiting gluconeogenesis and glycogenolysis, resulting in improved insulin action in the liver, increased uptake of glucose by the muscle, decreased absorption of glucose in the gastrointestinal tract, restored insulin resistance, and improved dyslipidemia profiles (5–8). According to the Biopharmaceutical Classification System (BCS), metformin is a highly hydrophilic drug and is classified as class 3, with high solubility and low permeability. It has a pKa value of 11.5, log P is -1.43, and it ionizes at physiological pH (9).

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Dissolution, also called mass transfer, is the process of a solid dissolving in a solvent or medium. Dissolution is a kinetic process, and the rate of dissolution indicates its performance (10). Dissolution tests can help guide the development of new formulations and proper selection of excipients, evaluate characteristics of active substances, and compare the quality and stability of different batches. Dissolution testing also helps to predict the in vivo performance of a drug and minimize the need to perform bioequivalence (BE) studies (11).

The gastrointestinal mucosa absorbs metformin as soon as it is administered. The small intestine is the main site of absorption of immediate-release metformin, and little absorption occurs in the stomach and colon (12). Therefore, metformin must have a rapid dissolution rate to increase the contact time of the dissolved drug with the absorption mucosa (13, 14). The dissolution test is a physical test that measures the ability of both the pure drug (intrinsic dissolution) and the one contained in a solid pharmaceutical form to dissolve in a given medium under controlled experimental conditions. The titration percentage of the test drug must be within the pharmacopoeial limits and should not differ by more than 5% from the reference drug, according to the metformin tablets monograph of the current FEUM (15).

The objective of this study is to compare the results of dissolution profiles and titration tests carried out with two batches (and lots) of three different brands of immediate-release metformin tablets (850 mg) available in the Mexican market (Mexico City): Dabex (reference), Dinorax, and Setebaid.

METHODS

The metformin hydrochloride standard was obtained from the FEUM (lot 100249). Three commercial brands of 850-mg metformin hydrochloride immediate-release tablets were obtained from a commercial pharmacy chain in Mexico City. For each brand, two batches were analyzed (Table 1). All reagents used were analytical grade.

Weight Uniformity

Twenty tablets were randomly selected and individually weighed on an analytical balance. The individual weights were compared with the average weight of each brand to determine the weight variation and compare it with the permissible limits (16).

Quantitative Assay

The assay for the quantification of metformin hydrochloride in the tablets was carried out according to the *FEUM* and *USP-NF* (15, 17). A standard solution of metformin hydrochloride in distilled water was prepared at a concentration of 0.01 mg/mL. For preparation of the sample, 20 tablets of each brand and batch were separated, finely crushed, and mixed. A sample of accurately weighed powder, equivalent to approximately 100 mg metformin hydrochloride, was transferred to a 100-mL volumetric flask. A 70-mL volume of water was added and stirred for 15 min, made up to 100 mL with water, then filtered, discarding the first 20 mL of the filtrate. From the filtered solution, an aliquot of 10 mL was taken and adjusted to a volume of 100 mL with water; from this solution a second aliquot of 10 mL was taken and adjusted to 100 mL with water to obtain a solution of the sample at 0.01 mg/mL. The sample was analyzed by UV/Vis spectrophotometry (DU7500i, Beckman) at 232 nm.

Table 1. Metformin Hydrochloride Drug Information

| Commercial brand | Manufacturer | Batch ID | Lot number | Expiration date |
|-----------------------------|------------------------|----------|------------|-----------------|
| Dabex (Patent/Reference) | MERCK, S.A. DE C.V. | 1 | M24861-1 | 09/2016 |
| | | 2 | M28861-2 | 09/2016 |
| Dinorax (Generic) | BRULUART, S.A. DE C.V. | 1 | 211243 | 12/2014 |
| | | 2 | 301159 | 01/2015 |
| Setebaid (Generic) | BEST, S.A. DE C.V. | 1 | 1211261 | 12/2014 |
| | | 2 | 1304202 | 04/2015 |

Dissolution Tests

The FEUM method for performing dissolution tests with immediate-release metformin tablets was followed (15). A USP type I apparatus (RC-8DS, Biobase) with a speed of 100 rpm at 37 ± 0.5 °C was used to test six tablets for each brand and lot. The dissolution medium was 900 mL of phosphate buffer at pH 6.8, and 10 mL of sample solution was extracted from each container. The collected samples were filtered through Whatman #41 filter paper. Filtered samples were appropriately diluted (100-fold dilution), and absorbance readings were taken with a UV/visible spectrophotometer at 232 nm. A standard solution with a concentration of 10 µg/mL was also prepared, and the absorbance was measured. The sample concentration was calculated by comparing absorbance of the sample solution with the standard solution. Finally, the data were expressed as the percentage of drug dissolved at 45 min. For immediate-release tablets, tolerance should be greater than 70% of the declared amount of metformin within 45 min of dissolution (15).

Dissolution Rate Determination

To determine the dissolution profile, 12 tablets of each brand and lot were subjected to dissolution testing as described above. Sample aliquots (3 mL) of the medium were taken at 10, 20, 30, 45, and 60 min, without replacing the dissolution medium. Each sample was filtered and diluted, then absorbance was measured at 232 nm using a UV-visible spectrophotometer. Metformin concentrations were determined by interpolating the absorbance on a calibration curve with a range of 2–16 µg/mL, and the cumulative percentage dissolved at each timepoint was calculated.

The analytical method and dissolution method were validated according to NOM-177-SSA1-2013 and the U. S. Food and Drug Administration guidance (18, 19) (supplemental data).

Spectroscopy Raman

The active pharmaceutical ingredient (API) was placed in the sample crucible, directly from its container. For each brand and batch of metformin tablets, one tablet was placed in an agate mortar and ground for 5 minutes, then placed in the sample crucible to perform Raman Spectroscopy under the following conditions: Raman laser frequency: 18792.7734; data spacing: 0.964233DATA; exposure time: 5.00 sec, number of exposures: 3, number of background exposures: 3; wavelength: 532 nm, laser polarization: parallel, grating: 900 lines/mm, spectrograph aperture: 50 µm slit; sample position: microscope; camera temperature: -20 C; CCD rows binned: 1-1; CCD binning: on chip; RIM position: mirror; polarization analyzer: Out; illuminators: on.

Statistical Analyses

Data were presented as mean \pm standard deviation. The DDSolver Excel plugin was used to calculate the similarity factor (f_2), dissolution efficiency (DE), mean dissolution time (MDT), and area under the release curve (AUC). The f_2 value was calculated only if the reference or test product released less than 85% of the API in 15 min with $n = 12$; all other dissolution tests were $n = 6$.

RESULTS AND DISCUSSION

Weight variation was within the limits of the acceptance criteria according to the Mexican Pharmacopoeia for all three brands of metformin tablets. Both batches of Dinorax and batch 2 of Setebaid exceeded the specifications the assay (95–105% of label claim). Dinorax and Setebaid passed the dissolution test, because more than 70% of the metformin hydrochloride dissolved after 45 min, whereas Dabex did not achieve this limit. These results are summarized in Table 2.

Figure 1 shows the comparative dissolution profiles of the drugs under study. Although Dabex is considered the reference drug, the dissolved percentage was lower than the generic drugs at 45 mins, and Dabex did not release 70% of the API during the test. Batch 1 of Dabex had dissolved around 40% of drug after 45 min, whereas batch 2 dissolved 68–80% at 45–60 min. Batch 1 of Dinorax had the highest drug release from the first sampling time,

followed by batch 2 of Setebaid. The two batches of Dinorax had a difference of around 20% dissolution after 20 mins. With Setebaid, the difference between batches was less. Umeta et al. reported that the necessary amount of drug substance released after 30 minutes as indicated in the *USP* (> 80% cumulative dissolution) is one of the most important quality control tests that helps to guarantee uniformity and equivalence between different batches (13, 17). Only two batches (Dinorax batch 1 and Setebaid batch 2) satisfied the *USP* criteria for an immediate-release dosage form (Fig. 1).

Table 2. Results of Pharmacopeial Tests of Metformin Hydrochloride Tablets

| Parameters | Dabex (Reference) | | Dinorax (Generic) | | Setebaid (Generic) | | Acceptance criteria (15, 16) |
|------------------------------|-------------------|--------------|-------------------|--------------|--------------------|---------------|------------------------------|
| | Batch 1 | Batch 2 | Batch 1 | Batch 2 | Batch 1 | Batch 2 | |
| Tablet weight | 899.4 ± 5.3 | 889.0 ± 8.0 | 972.4 ± 2.0 | 972.9 ± 7.2 | 991.4 ± 9.6 | 1012.8 ± 19.2 | Percentage deviation < 5% |
| Weight variation (% range) | -1.3 to 1.0 | -1.0 to 1.8 | -0.3 to 0.4 | -0.9 to 1.8 | -1.9 to 1.7 | -3.7 to +4.1 | |
| Assay (% per tablet) | 104.7 ± 14.0 | 100.5 ± 17.8 | 108.8 ± 10.2 | 107.1 ± 12.8 | 101.6 ± 17.3 | 115.7 ± 12.6 | 95–105% of label claim |
| Dissolution (% drug release) | 40.3 ± 2.6 | 60.9 ± 6.4 | 93.3 ± 5.4 | 73.7 ± 5.3 | 74.7 ± 9.2 | 86.2 ± 6.9 | ≥ 70% in 45 minutes |

Data are mean ± SD.

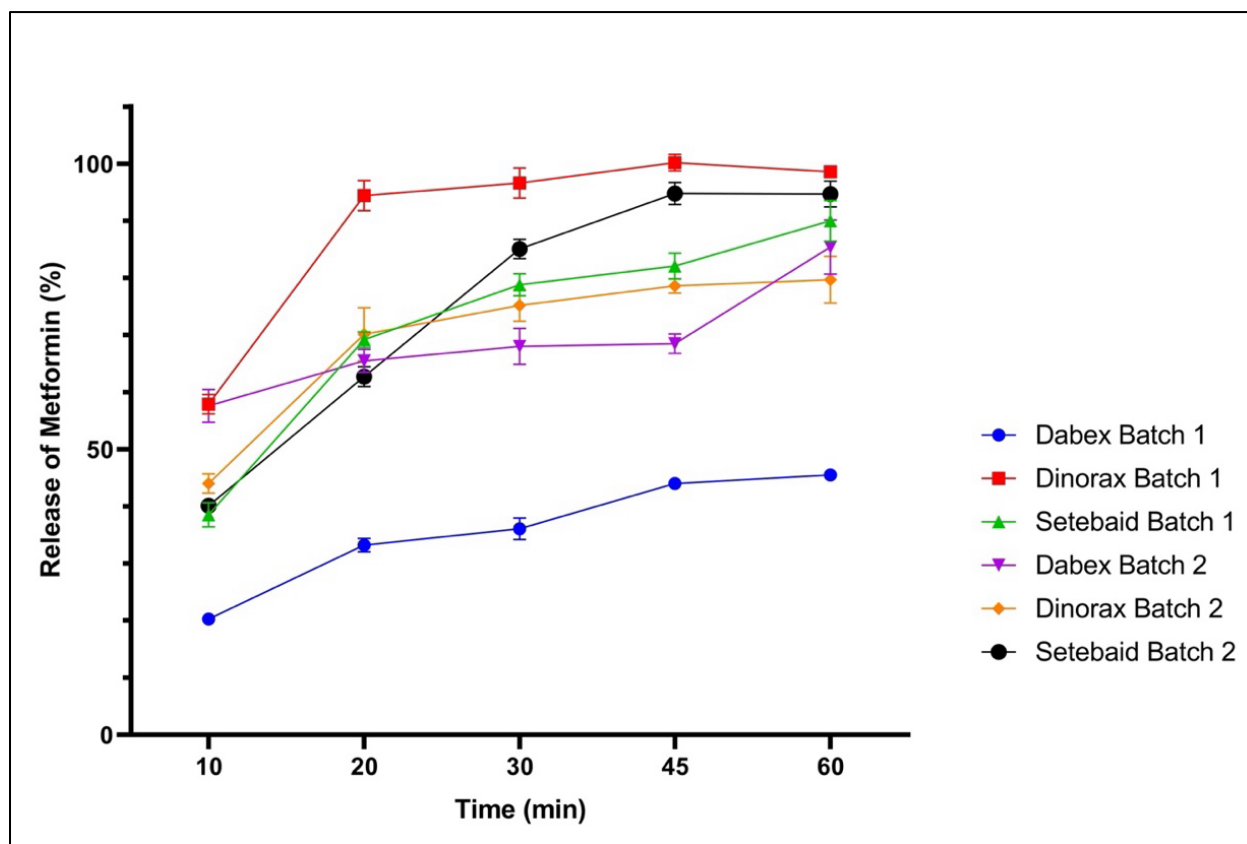


Figure 1. Comparative dissolution profiles of metformin 850-mg tablets between reference (Dabex) and generic products (Dinorax and Setebaid). Data are presented as mean ± standard error (n = 12).

As shown in Table 3, most of the products studied contained more than 100% of the API; however, the dissolution behavior showed that the reference product (Dabex) had a lower AUC compared to the generic products (Dinorax and Setebaid). Even between the batches of Dabex, a difference was observed (smaller difference in lot 1 compared to lot 2). These results agree with the MDT, where the values were higher for the reference compared to the generic products; a higher MDT indicates a slower rate of drug release from the dosage form (13). Thus, Dabex presented a slower dissolution rate than the other products. For two drugs to be used interchangeably, according to Anderson et al., the DE of two products must be $\pm 10\%$ (20). In this study, DE for Dabex was much lower in batch 1 and much higher in batch 2 (33% vs 83%) (Table 3). These findings indicate that the two batches of Dabex cannot be used interchangeably with each other or with the other brands, despite Dabex being the reference drug. Furthermore, all f_2 values were less than 50 (Table 4), indicating no similarity between the products, except for batch 2 of Dinorax.

Figure 2 shows the Raman spectrum for the API and the three brands of metformin hydrochloride tablets. In the spectra, the bonds that belong to metformin hydrochloride – methyl bonds (CH₃), nitrogen hydrogen bonds (N-H), carbon nitrogen bonds (C-N), and carbon nitrogen bonds (C-N) – with out-of-plane bending are identified. Coinciding with Mamisashvili, all metformin tablets showed the same peaks in the region of 400–3300 cm^{-1} ; the highest signals correspond to pure metformin (21). The excipients present a signal with lower intensity peaks.

Table 3. Parameters Characterizing the Drug Release Curve for 850-mg Metformin Hydrochloride Tablets in a pH 6.8 Phosphate Buffer

| Product | AUC (min%) | MDT (min) | DE (%) |
|--------------------|------------|-----------|--------|
| Dabex Bach 1 (Ref) | 1987.0 | 16.51 | 33.12 |
| Dabex Bach 2 (Ref) | 3750.4 | 16.09 | 82.89 |
| Dinorax Bach 1 | 4973.5 | 9.54 | 66.13 |
| Dinorax Bach 2 | 3857.2 | 11.59 | 62.51 |
| Setebaid Bach 1 | 3967.6 | 15.91 | 64.29 |
| Setebaid Bach 2 | 4223.8 | 15.39 | 70.40 |

AUC, area under the release curve; MDT, mean dissolution time; DE, dissolution efficiency.

Table 4. Summary of Similarity Factor, f_2 for Generic Brands Compared to the Reference (Dabex) in pH 6.8 Phosphate Buffer

| Reference | Dinorax Bach 1 | Dinorax Bach 2 | Setebaid Bach 1 | Setebaid Bach 2 |
|--------------|----------------|----------------|-----------------|-----------------|
| Dabex Bach 1 | 13.21 | 23.34 | 21.53 | 19.02 |
| Dabex Bach 2 | 31.17 | 52.51 | 46.31 | 38.94 |

$f_2 \geq 50$ represents similarity.

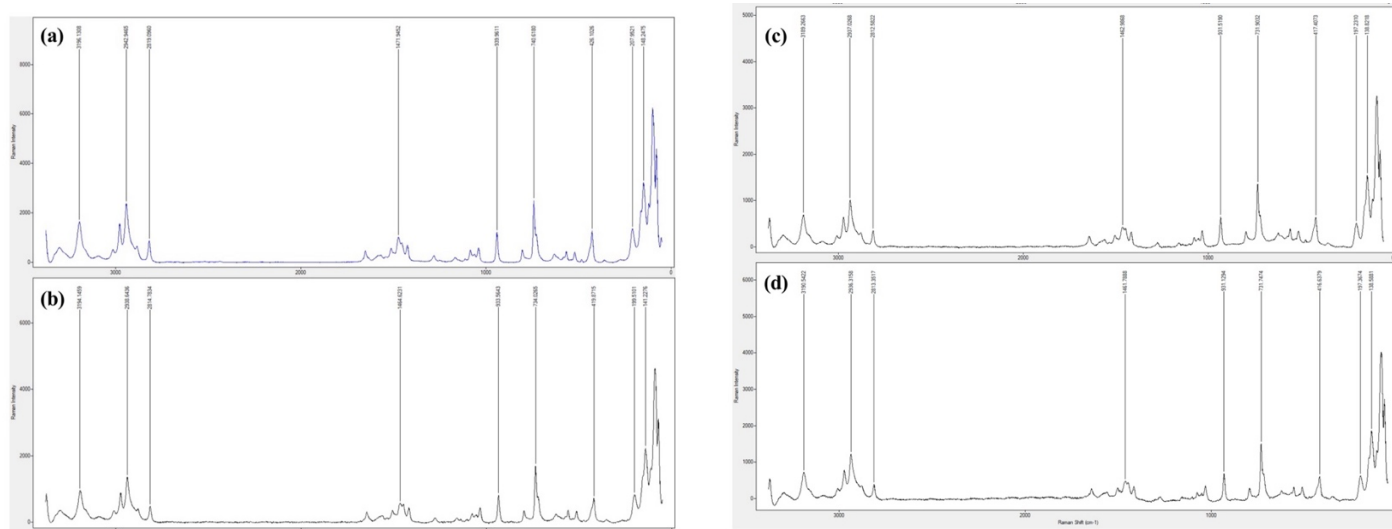


Figure 2. Raman spectrum of the pure active ingredient (a), Dabex (b), Dinorax (c), and Setebaid (d).

CONCLUSION

In the present study, the quality parameters of three Mexican brands of 850-mg immediate-release metformin hydrochloride were examined, including dissolution, weight variation, assay, and Raman spectroscopy. The weight variation was within the limits of the acceptance criteria. Dinorax (both batches) and Setebaid (batch 2) exceeded the assay specifications of 95–105%. Dissolution testing revealed that only the generic products released $\geq 70\%$ of the API within 45 mins, whereas Dabex (reference) did not pass. There was no similarity between the dissolution profiles, based on f_2 values. In the Raman spectrum, the main bands for metformin hydrochloride were detected in all cases. Differences in dissolution behavior can impact therapeutic performance and significantly affecting the consumer.

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article and may be requested by contacting the corresponding author.

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DISCLOSURES

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