



Physicochemical stability of three generic brands of metformin in solution

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ABSTRACT

Pharmacological management of insulin resistance and dyslipidemias in children and adolescents is mandatory to prevent metabolic syndrome (MS) and type 2 diabetes mellitus (T2DM).

Material and method: extemporaneous formulations were developed from 500 mg tablets of three brands of generic metformin: Medimart®, Farmacias del Ahorro®, and Primer Nivel®, with agreeable flavor, color, and consistency and dose adjusted for ease of administration. Their physicochemical integrity was determined under different storage conditions: 25°C with light, 25°C in darkness, at 4°C and 40°C. The stability of the drug was determined by ultra performance liquid chromatography with ultraviolet detection (UPLC-UV) and by measuring the pH of the stored solution; the mobile phase was phosphate buffer (KH₂PO₄) 0.1 M, pH = 6.5, sodium dodecyl sulfate (SDS) 4.6 mM and acetonitrile (63:7:30) at 0.8 mL/min, VARIAN Pursuit C8 150 × 3.9 mm column tempered at 40°C, with detection at 236 nm.

Results: All the commercial brands of metformin were stable under all storage conditions for up to 30 days. They retained more than 90% of the initial quantity of the active drug, with pH of 7.4 ± 0.3.

Conclusion: extemporaneous formulations of metformin can be made with both the innovative drug and with generics; money can be saved, with the certainty that they will retain their physicochemical properties.

Key words: generic metformin, innovative metformin, extemporaneous formulation, physicochemical stability, UPLC-UV.

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Due to the increase in child obesity, insulin resistance, and dyslipidemias,¹ it has been suggested that obese children and adolescents are at risk of developing type 2 diabetes mellitus and metabolic syndrome (MS),^{2,3} and in response drug treatment with metformin has increased recently in pediatric endocrinology services, to ensure the greatest possible success of exercise and diet based therapy. Metformin is a antihyperglycemic drug which lowers blood sugar and sensitizes peripheral tissues to the action of insulin without causing hypoglycemia.^{4,5} Also, it offers mechanisms complementary to sulfonylureas in control of T2DM and may be useful in management of comorbidities associated with overweight and obesity,⁶ because it also interferes with oxidation of free fatty acids.⁷ Metformin is the drug of choice for treating T2DM and its comorbidities; however, aside from the United States and Canada (where Riomet™ is used), there is no commercial presentation with adjusted dose and easy administration for children. The aim of this study was to make extemporaneous formulas of metformin from tablets of three generic brands, and evaluate their physicochemical stability to determine the best conditions in which it can be prepared, transported, and stored for efficient pediatric use, and maintain its properties of agreeable flavor and no calories.

MATERIALS AND METHODS

Reagents

The acetonitrile (Caledon™, Canada) was HPLC grade; the other reagents used in chromatography, such as sodium dodecyl sulfate (SDS), sodium hydroxide (NaOH), and potassium phosphate monobasic (KH₂PO₄) were reagent grade. The primary standards of metformin and ranitidine were obtained from MP Biomedicals. The innovative medicine, Roche Glucophage®, as well as the generics, Medimart®, Farmacias

del Ahorro®, and Primer Nivel® (metformin hydrochloride, 500 mg tablets) were obtained from a local pharmacy. The extemporaneous formulation of metformin was made with brand label bottled drinking water (Danone® Bonafont®).

Preparation of sweetened solution

For each brand of generic metformin the individual weight of 20 tablets was recorded, using a previously reported method.⁸ The tablets were crushed with a porcelain mortar and pestle, and by the quantity of powder containing 500 mg of active compound was calculated by linear correction, which was around 94.4% (average weight 0.53 ± 0.015 g); this indicates a minimal quantity of pharmaceutical excipients. The powder was placed in a sterile, 250 mL Erlenmeyer flask. Separately, the vehicle for the formula was prepared with bottled drinking water.

The bitter flavor of the solution was masked by adding Splenda® (sucralose, Johnson & Johnson), a calorie-free commercial sweetener, in a proportion of 10 g of sweetener per 100 mL of water (10%).

To 25 mL of water sweetened with Splenda® was added the powder from tablets of each brand (equivalent to 500 mg of metformin) so that every 5 mL of the resulting solution contained 100 mg of the active substance. This corresponds to one fifth of the recommended initial dose for adults, which could be used to initiate treatment in children and adolescents.

Pattern curve and control points in metformin standard solution

A mother solution was made with the metformin standard at 1 mg/mL and from that subsequent dilutions were made in deionized water to obtain the following calibrators: 20, 40, 60, 80, 100, and 200 mg/mL, as well as solutions of intermedi-

ate concentrations on the curve, which were the quality control points: 30, 70, and 150 mg/mL. Of each calibrator or control solution 190 mL was taken and 10 mL of the external standard (ranitidine, 1 mg/mL) was added; of this mixture 10 mL was injected in the chromatography system.

Chromatographic conditions

The mobile phase consisted of potassium phosphate monobasic (KH_2PO_4) buffer 0.1 M, adjusted with NaOH 5 M to pH = 6.5 (252 mL), to which SDS 4.6 mM (28 mL) was added, then mixing with ACN (120 mL) for a final volume of 400 mL (KH_2PO_4 :SDS:ACN ratio equal to 63:7:30); this was repeated daily for each run. All the samples were injected in the UPLC-UV machine (Acquity™, Waters) in a volume of 10 mL, eluted at a rate of flow of 0.8 mL/min in a VARIAN Pursuit C8 150 × 3.9 mm column, conditioned at 40°C, and detection was at 236 nm. The data was analyzed using Empower™ version 2.0 software.

Preparation of the sweetened solution

Metformin solutions were prepared with tablets of each of the brands. Three working solutions were prepared at concentrations usable in clinical practice (75-375 mg/5 mL): 1.5, 3.5, and 7.5 g/100 mL. Each solution was prepared by placing: 6 tablets (3,000 mg of active substance) in a flask, 14 tablets (7,000 mg) in another flask, and in a third flask 30 tablets (15,000 mg). To each flask was added 200 mL of calorie-free sweetened water (sucralose 10%) for solutions with 15, 35, and 75 mg/mL. They were agitated manually at room temperature until dissolution. Because the quantity of excipients is very small and metformin is completely soluble in water it was not necessary to crush the tablets previously.

Evaluation of physicochemical stability

Of each of the three working solutions 2 mL samples were taken and placed in 15 mL conical tubes (Falcon®) and distributed at four storage sites, to wit: 25 ± 2°C exposed to light and in darkness, 4°C in darkness, and 40°C in darkness. The solutions were stored under these conditions for 30 days after they were made.

Physicochemical stability was evaluated by determining the drug concentration by UPLC-UV at 0, 3, 7, 15, and 30 days of storage. For quantification 10 µL of each solution was taken and diluted in a final volume of 5 mL of deionized water (1:500) to adjust the concentration to allow quantification with the pattern curve. Of each dilution 190 mL was taken and mixed with 10 µL of the external standard (ranitidine, 1 mg/mL). Of the mixture, 10 µL was injected in the chromatography system. The pH of the solutions was determined with a UB-10 potentiometer (Denver Instruments, USA). Change in color, possible foreign particles, and turbidity were determined visually.

RESULTS

Validation of analytical method to measure quantity of metformin

The ultra performance liquid chromatography with ultraviolet detection (UPLC-UV) method used in this study was validated in accordance with the domestic guidelines described in Official Mexican Standard NOM-177-SSA1-2013 and following good laboratory practices. The range was linear in an interval of 20 to 200 µg/mL with a coefficient of correlation $r = 0.9997$. The method was repeatable and reproducible because the control or nominal concentrations (PC-1, PC-2, and PC-3) had a coefficient of variation less than or equal to 15% ($\text{CV} \pm 15\%$) when

calculated based on the pattern curve; such values are acceptable in accordance with Official Mexican Standard NOM-177-SSA1-2013⁹ to conclude that the solutions are physicochemically stable. The results can be seen in Table 1.

Table 1. Validation parameters of ultra performance liquid chromatography with ultraviolet detection (UPLC-UV) for determining the quantity of metformin in a solution

Linearity	Interval of concentrations	Coefficient
	20–200 µg/mL	r = 0.9997 r ² = 0.9996
Precision and accuracy	Mean ± SD (ug/mL)	C.V. (%) [†]
a) Repeatability (n = 5 series)	30 30.81 ± 1.78	5.8
	70 70.15 ± 2.10	2.9
	150 153.72 ± 1.52	0.9
b) Reproducibility (n = 3 series)	30 29.11 ± 0.34	1.2
	70 71.29 ± 0.37	0.5
	150 151.61 ± 2.51	1.7
Limit of quantification (n = 5)	Concentration	
	20 µ/mL	
Limit of detection (n = 3)	1 µ/mL	
Absolute recovery (n = 3 series)	Mean ± SD (µ/mL)	Recovered (%) [‡]
	30 29.96 ± 0.94	99.8
	70 70.65 ± 0.42	99.8
	150 150.28 ± 5.54	100.9
		100.2

Physicochemical stability of the sweetened solution

Figure 1 shows the three concentrations assayed: PC-1, PC-2, and PC-3 (quality control points) of metformin in solution of the three generic brands compared with the innovative brand reported previously. The three generic brands of metformin were stable for 30 days under the four storage conditions tested in this study (Table 2). The acceptance criterion to determine physicochemical stability was that the solutions at the quality control points have metformin concentrations in the following ranges of concentration: for PC-1, 30 ng/mL ± 15, or 25.5 to 34.5; for PC-2, 70 ng/mL ± 15%, or 59.5 to 80.5; and for PC-3, 150 ng/mL ± 15, or 127.5 to 172.5.

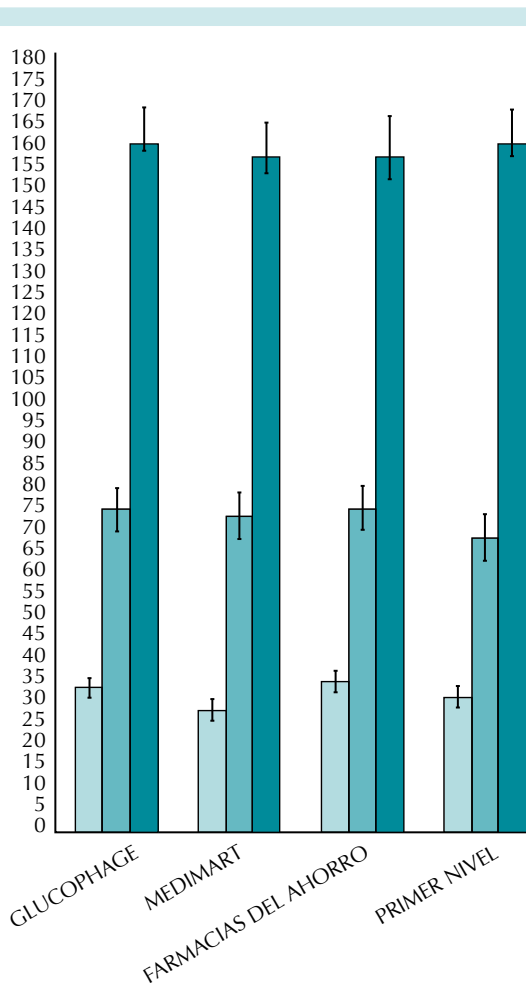


Figure 1. Physicochemical stability of three generic brands of metformin in calorie-free sweetened solution, stored for 30 days at 25 ± 2°C and exposed to light. Light bars: PC-1 (30 ng/mL ± 15%); medium bars: PC-2 (70 ng/mL ± 15%); dark bars: PC-3 (150 ng/mL ± 15%). The similarity in values for the problem concentrations among all the brands indicates that they are all stable under these storage conditions.

DISCUSSION

The costs of treating T2DM in Mexico and Latin America represent approximately 14% of the annual salary most workers earn.

Table 2. Physicochemical stability of three generic brands of metformin in solution stored for 30 days under different environmental conditions

Storage	MEDIMART			FARMACIAS DEL AHORRO			PRIMER NIVEL		
	PC-1	PC-2	PC-3	PC-1	PC-2	PC-3	PC-1	PC-2	PC-3
RT LIGHT	28.3 ± 1.2	73.2 ± 2	155.2 ± 5.9	34.1 ± 2.4	74.9 ± 4.9	156.4 ± 8.4	31.6 ± 1.7	67.9 ± 2.6	157.9 ± 4.2
RT DARK	33.4 ± 2.6	79.4 ± 4	156.6 ± 8.2	33.7 ± 0.5	72.3 ± 3.3	157.2 ± 0.6	29.8 ± 0.8	70 ± 1.4	154 ± 6.3
4°C	32.7 ± 0.2	74 ± 1.6	157.9 ± 2.2	32.6 ± 3.4	73.7 ± 4.3	153.5 ± 2.6	32.6 ± 2.8	71.1 ± 1.6	158.2 ± 4.8
40°C	33.5 ± 3.7	75 ± 1.7	161.1 ± 3.8	30.6 ± 1.3	73.6 ± 3	151.9 ± 2.6	33.3 ± 1.5	69.6 ± 0.6	156.6 ± 3.4

RT LIGHT: room temperature exposed to light; RT DARK: room temperature in darkness; 4°C in refrigeration; 40°C in incubator.

Further exacerbating this problem, T2DM is appearing at increasingly early ages,¹⁰ in adolescence and even in childhood. This will increase the costs of treatment and reduce years of healthy life and working capacity in the population.¹¹ These issues underscore the need to implement appropriate pharmacological treatment when the first warning signs appear. The use of metformin in the pediatric population, slender or obese with insulin resistance, promises to be part of a successful preventive therapeutic plan because its mechanism of sensitizing tissues to the action of insulin¹² helps the body respond favorably to hyperglycemia before the onset of diabetes mellitus, in contrast with other drugs such as glibenclamide which, due to its active mechanism, is preferred for management of T2DM in the adult population rather than for prevention. In fact, in Mexican adults with recent diagnosis of T2DM glibenclamide is more successful and less costly than metformin,¹³ which further underscores metformin's potential for prevention of T2DM.

Because extemporaneous preparations represent a therapeutic alternative in pediatrics,¹⁴ it is important to show that they are of good quality, stable, bioavailable, effective, and safe.¹⁵

An oral preparation preferably should be well tolerated, effective, and stable and have an acceptable flavor.¹⁶ All these characteristics, taken together, contribute to the success of a treatment because administration of a formulation with

an unpleasant flavor can cause abandonment.¹⁷

Because children have a known preference for sweet foods and beverages, sweeteners like saccharine, aspartame, or sucralose are added in pediatric formulations. Although the most widely used sweetening compound is sucrose, it is recommended to avoid it in medicines designed for long-term therapies because it is associated with dental problems,¹⁸ which is why sucralose is preferred in formulating pediatric preparations of medicines.^{18,19}

In prior reports, a calorie-free sweetened metformin solution made from generic brand tablets (Glucophage®) was physicochemically stable for up to 30 days.⁸ In this study we show that extemporaneous solutions made with the same medicine, but of three different generic brands, are equally stable under the same storage conditions and for the same time as the innovative product. This offers advantages for treating pediatric patients who need metformin dose adjustment, because solutions of the drug can be made from less costly brands. This represents a savings for the parents of family members responsible for children in treatment, who also have more options to make a solution without depending on using the innovative drug, which is not available at some local pharmacies.

The extemporaneous liquid solutions of metformin made in our laboratory are currently under

evaluation, conducting comparative bioavailability and safety tests in healthy volunteers to corroborate that plasmatic concentrations similar to those obtained with fractioned tablets can be reached. We seek to prove that they are safe as a means of adjusting dosage in the pediatric population in the short term.

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