Original Article

Biopharmaceutical evaluation of formulated metformin/ rosiglitazone tablets

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ABSTRACT: The study aimed to combine two antidiabetic agents with different mechanisms of action, namely, metformin HCl and rosiglitazone maleate, in a tablet to improve glycemic control in patients with type II diabetes. The preformulation study started with development and validation of an HPLC method for the determination of both drugs in the mixture. The results of visual inspection, TLC, DSC, and FT-IR verified the absence of any physical or chemical interaction between both compounds. Four compatible excipients were selected for the formulation of the tablets by wet granulation according to a 2^2 factorial design. The prepared tablet blends were acceptable in terms of the modal size of particle distribution, bulk density, Hausner's ratio, Carr's index, and flowability. All formulations fulfilled the pharmacopoeial specifications for weight variation, content uniformity, friability, and hardness. They released 100% of the drug during the first 45 min, displaying higher dissolution efficiency than commercially available Rosiplus tablets. The tablet formulation that passed the physical and chemical stability study for 24 months at ambient conditions was tested *in vivo* on healthy volunteers in a cross-over design. Statistical analysis proved that the prepared tablets were bioequivalent to the commercial ones in terms of both the rate and the extent of absorption.

Keywords: Wet granulation, factorial design, stability study, bioavailability

1. Introduction

Non-insulin-dependent (Type 2) diabetes mellitus is a heterogeneous disorder characterized by an underlying insufficiency of insulin. This insufficiency results from defective insulin utilization and can be corrected by diet control, exercise, and administration of one or more of the currently available oral hypoglycemic agents (*1*).

Metformin (dimethylbiguanide) is an antihyperglycaemic drug used to treat non-insulin-dependent diabetes mellitus. It acts in the presence of insulin to increase glucose utilization and reduce glucose production, thereby counteracting insulin resistance. The effects of metformin include increased glucose uptake, oxidation and glycogenesis by muscle, increased glucose metabolism to lactate by the intestine, reduced hepatic gluconeogenesis, and possibly a reduced rate of intestinal glucose absorption (*2,3*). Metformin absorption is limited to the upper gastrointestinal (GI) tract, thus requiring suitable delivery systems providing complete release during stomach-to-jejunum transit (*4*).

Rosiglitazone is a thiazolidinedione. It improves insulin resistance by activating the nuclear peroxisome proliferator activated receptor-γ (PPAR-γ), resulting in increased glucose uptake in muscle and reduced endogenous glucose production. Rosiglitazone has been shown to be active as a monotherapy, in combination therapy with metformin or sulfonylureas, and even in triple therapy (*5-7*).

A review of the literature revealed a lack of data on the stability of rosiglitazone maleate in combination with metformin-HCl and the compatibility of both drugs with commonly used tablet excipients. Thus, the aim of this work was to combine those two different, yet complementary, oral antidiabetic agents in a single stable and bioavailable tablet form to improve blood sugar control in patients with type 2 diabetes and to ensure patient compliance.

2. Materials and Methods

2.1. *Materials*

Rosiglitazone maleate and metformin-HCl were obtained from Sri Venkateswara Co., Bollaram, India and Dr. Reddy Ltd., Andhra, India, respectively. PVP-K90, magnesium stearate, and talc were from Prolabo, France. Spray-dried lactose was from Meggle,

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Germany. Avicel PH 102 and starch were from FMC, PA, USA. Liquid paraffin was from El-Nasr Pharmaceutical Chemical Co., Cairo, Egypt. Methanol, butanol, and acetic acid were from E. Merck, Germany. HLPC grade solvents (methanol and acetonitrile) were from Sigma-Aldrich Chemical Co., USA. Rosiplus® tablets were obtained from Sabaa Company, Egypt, batch #10651.

2.2. *Preformulation studies*

2.2.1. *Compatibility study of rosiglitazone maleate and metformin-HCl*

Thin layer chromatography (TLC) – Five µL samples of test and standard solutions of both drugs were spotted on the loading zone of a silica gel plate at an adequate distance from each other. A solvent system consisting of a 2:4:1 mixture of distilled water:butanol:acetic acid was freshly prepared and allowed to run for 10 cm. The plate was then dried and examined under a UV lamp at a wavelength of 254 nm.

Fourier transform-infrared spectroscopy (FT-IR) – The KBr disc technique was used for sample preparation. Sample spectra were collected in the range of 4,000 and 500 cm^{-1} using a Perkin-Elmer 1600 spectrophotometer (Bruker, Coventry, UK). Collected spectra were smoothed and baseline-corrected.

Differential scanning calorimetry (DSC) – DSC thermograms of samples were recorded using a DSC-7 calorimeter (Perkin-Elmer, Norwalk, USA). Samples of 3-4 mg of the pure drugs and a 1:1 physical mixture of rosiglitazone maleate:metformin-HCl were placed in an aluminium pan and heated to a temperature of 350°C at a rate of 10°C/min, with indium in the reference pan in an atmosphere of nitrogen.

2.2.2. *HPLC determination of rosiglitazone and metformin*

In vitro determination – Rosiglitazone maleate and metformin-HCl were assayed in a mixture using a method of HPLC as previously reported (*8*). The assay was carried out using a Waters 2790 HPLC system for extraction and Shimadzu LC-10AD VP pumps for analysis. Samples were eluted on a Nova-pak C_{18} column using acetonitrile:0.01 M phosphate buffer (50:50, v/v) at a flow rate of 1 mL/min at 40°C, followed by detection at 254 nm. Calibration curves were prepared and assayed in triplicate on three different days to evaluate linearity, precision, and accuracy.

In vivo determination – The metformin content was determined using the aforementioned HPLC method used *in vitro* after generating a calibration curve using blank plasma. The plasma was spiked with different amounts of metformin-HCl in methanol. The mixture was then centrifuged at 3,000 rpm for 10 min and the supernatant (organic phase) was transferred to another clean tube and evaporated to dryness at 40°C. The residue was then reconstituted in 1 mL of methanol and the concentration of 10 µL of the final solution was determined based on the reported peak areas (*9*).

2.2.3. *Compatibility study of both drugs with different tablet excipients*

A physical mixture of rosiglitazone maleate:metformin-HCl in a 2:500 weight ratio was prepared alone and in combination with other tablet excipients in a drug-to-excipient ratio of 1:1. The tested excipients were Avicel PH 102, spray-dried lactose, PVP-K90 magnesium stearate, talc, dicalcium phosphate, and starch. The prepared mixtures were evaluated *via* visual inspection, DSC and FT-IR.

2.3. *Formulation of rosiglitazone and metformin tablets*

2.3.1. *Experimental design*

A 2^2 full factorial design was used to prepare rosiglitazone/metformin tablets using four compatible excipients. Two independent variables were used, namely, the binder type (PVP-K90, starch) and the lubricant type (Mg stearate, talc). Drug concentrations were kept constant at 2.649 mg rosiglitazone maleate (equivalent to 2 mg rosiglitazone base) and 500 mg metformin-HCl per tablet. The composition of the four prepared tablet formulations is shown in Table 1. Statistical analysis of the results was performed using statistical software (Statview Abacus Concept, version 4.57). Analysis of variance (including the sum of squares), subsequent significance tests, and the calculation of average values was done using this software.

2.3.2. *Characterization of the blends to be compressed*

The formulation blends were mixed in a mortar using the geometrical dilution technique and were characterized in terms of their particle size, %

Table 1. Composition of metformin/rosiglitazone tablets according to the experimental design used

Ingredient/tablet (mg)	Tablet formulation			
	1	a	b	ab
Metformin-HCl	500	500	500	500
Rosiglitazone maleate*	2.649	2.649	2.649	2.649
Starch	–	26	–	26
PVP-K90	26	–	26	–
Mg stearate	–	–	6	6
Talc	6	6	–	–

* 2.649 mg rosiglitazone maleate = 2 mg rosiglitazone base.

compressibility, and flowability. The particle size was determined using a laser diffraction particle size analyzer (Master sizer, Malvern, UK). The volume occupied by 5 g of each blend (bulk volume V_b) and the true volume after tapping in a graduated cylinder (tapped volume V_t) were determined and used to calculate (a) the bulk density by dividing the weight of the powder being tested by V_b, (b) Hausner's ratio given by dividing V_b by V_t, and (c) % compressibility (Carr's index) determined by $(1 - V_t/V_b) \times 100$.

The flowability of the prepared blends was calculated using the fixed height cone method. The angle of repose was calculated from the equation:

$$\tan Ø = 2h/D$$

where, D is the average diameter of the formed cone and h = 2.5 cm.

2.3.3. *Tablet compression*

Direct compression – Accurately weighed amounts of rosiglitazone and metformin were mixed using a mortar and a pestle for 5 min. The specified binder was then mixed by geometric dilution for an extra 5 min. Talc or magnesium stearate was added and the blends were compressed using a single-punch tablet machine and 20 mm oblong punches and dies (HEYNAU single station press, 5v1m, Germany). The compression force was kept constant at 10 kpsi.

Wet granulation – A mixture of the two drugs was geometrically mixed with the specified amount of the binder and kneaded using 300 mg isopropyl alcohol/ tablet. The resultant mass was passed through a 1-cm sieve to produce granules with a particle size of 780 µm and dried in a hot air oven at 60°C for 20 min. The dried mass was passed through a 1-mm sieve and finally mixed with the specified lubricant. The blend was compressed with a single punch tablet machine using 20 mm oblong punches and dies. The compression force was kept constant at 10 kpsi. A film coating was then applied to the compressed tablets using a simplified BYC-1000 coating machine, JiangSu TaiZhou Medicines Machinery Factory, in order to improve tablet aesthetics. The coat weighed 10 mg and consisted of HPMC, talc, PG, PEG, red iron oxide, and titanium dioxide after dissolution in ethanol.

2.4. *Evaluation of compressed tablets*

2.4.1. *Weight, thickness, and diameter variation*

The weight, thickness, and diameter of twenty coated tablets of each formulation were individually measured. The mean value of each measurement was calculated, with the variation serving as the relative standard deviation (% RSD).

2.4.2. *Content uniformity*

The content uniformity was determined by crushing ten tablets of each formulation. An accurately weighed amount corresponding to the weight of one tablet was dissolved in 100 mL methanol. The solution was then passed through a 0.45-µm membrane filter, properly diluted, and assayed using the previously described HPLC method.

2.4.3. *Friability*

Ten tablets of each formulation were accurately weighed and placed in the drum of a friabilator and rotated at 25 rpm for a period of 4 min. The tablets were then brushed and reweighed. The percentage loss in weight was calculated and served as a measure of friability.

2.4.4. *Hardness*

Ten tablets of each formulation were tested for hardness using a Monsanto tablet hardness tester, USA. The mean hardness in kilograms was then determined.

2.4.5. *Disintegration time*

The disintegration time for each of six tablets of each formulation was determined using a USP disintegration tester (Pharma Test, Type PTZ2, Germany) in accordance with standard testing procedures.

2.4.6. *In vitro dissolution study*

The dissolution of the prepared tablets was performed using the USP XXVIII rotating basket, at a speed of 100 rpm in 900 mL N/10 HCl (pH 1.2) and at a temperature of 37 ± 0.5°C. The study was conducted on 6 tablets for 1 h and aliquots, each of 3 mL, were withdrawn at appropriate time intervals from the dissolution medium and replaced with an equivalent amount of the freshly heated medium. The samples were analyzed for metformin and rosiglitazone content using the proposed HPLC method.

For assessment and comparison, the dissolution profiles were evaluated on the basis of the dissolution efficiency parameter at 1 h (DE_{1h}) as described by Khan *et al.* (*10*) according to the following equation:

$$DE = \int_0^t \frac{y \cdot dt}{y_{100}\, t} \times 100 \qquad \text{(Eq. 1)}$$

where the integral in equation 1 is the area under the dissolution curve up to the dissolution time *t* and *y100* is the area of the rectangle described by 100% dissolution at the same time.

Kinetic analysis of the dissolution data for all formulations was performed using the linear regression

method. The determination coefficients (r^2) according to zero-order, first-order, Higuchi (*11*), Hixson-Crowell (*12*), and Korsmeyer-Peppas (*13*) models were computed for each formulation.

2.5. *Stability study*

The four prepared tablet formulations were subjected to a long-term physical and chemical stability study (24 months at ambient temperature and humidity). Tablet samples were collected at time intervals of 2, 6, 12, and 24 months and inspected visually for any changes in colour and/or appearance. The tablets were evaluated for the percent of remaining rosiglitazone and metformin using the proposed HPLC method of analysis as well as for weight variation, thickness, diameter, hardness, and disintegration time and *in vitro* dissolution tests as previously mentioned for the freshly prepared tablets. To detect any significant variations in the dissolution profiles, the similarity factor f_2 (*14*) was calculated from the mean dissolution data according to the following equation:

$$f_2 = 50 \log \{[\, 1+\tfrac{1}{n}\textstyle\sum_{n=1}^{n} W_t\,(R_t - T_t)^2\,]^{-0.5} \times 100\} \qquad \text{(Eq. 2)}$$

where n is the number of pull points, W_t is an optional weight factor, and R_t is the reference profile that is considered similar. The value of f_2 should be between 50 and 100. An f_2 of 100 suggests that the test and reference profiles are identical. Conversely, f_2 decreases as the dissimilarity between two release profiles increases.

The formulation of choice was subjected to an accelerated stability study to predict its shelf life. Tablets were stored in ovens at temperatures of 50, 60, and 70°C. Samples were collected after 2, 4, and 6 months for each working temperature and analyzed for both active ingredients. The method of Free and Blythe was used, *i.e.*, the time required for the drug to fall to 90% of its original concentration was determined at the three temperatures by plotting the log percent of drug remaining with respect to time. The log time to $t_{90\%}$ was then plotted with respect to 1/T and the shelf life was the time at 25°C.

2.6. *In vivo study*

The performance of the selected tablet formulation in comparison to the commercial tablet formulation (Rosiplus tablets, batch No. 10651, Sabaa Pharm, Egypt) was evaluated in human volunteers. Comparison was done through the quantification of metformin in plasma. The bioequivalence between both formulations was assessed by calculating individual C_{max}, $AUC_{(0\text{-}24h)}$, $AUC_{(0\text{-}\infty)}$, and $C_{max}/AUC_{(0\text{-}24h)}$ ratios (test/reference) together with their mean and 90% confidence intervals (CIs). The inclusion of the 90% CI for the ratio in the 80 to 125% range was analyzed using ANOVA.

The study involved six healthy male volunteers with ages between 21 and 42 years (mean ± S.D., 31.3 ± 3.7 years). The height of the volunteers ranged from 145.0 to 165.0 cm (mean ± S.D., 156.7 ± 6.9 cm) and their body weight ranged from 55.1 to 87.8 kg (mean ± S.D., 71.3 ± 8.1 kg). The volunteers did not suffer from significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, or hematological diseases. The study protocol was approved by the University Committee for the Protection of Human Subjects and it complies with the Declarations of Helsinki and Tokyo for humans.

The study was conducted in an open, randomized, two-period crossover fashion with a 2-week washout period between doses. Blood samples (10 mL) from a suitable antecubital vein were collected before and 0.5, 1.5, 2, 2.5, 3, 3.5, 6, 8, 12, and 24 h after the administration of each dose. The blood samples were centrifuged at 2,500 × g for 10 min at room temperature and the plasma was decanted and stored at -20°C until assayed. All samples from a single volunteer were analyzed on the same day to avoid inter-assay variation.

3. Results and Discussion

3.1. *Preformulation studies*

3.1.1. *Compatibility study of rosiglitazone maleate and metformin-HCl*

The spots on the TLC plate of the test solutions corresponded to those of standard solutions with the same intensity and R_f, indicating the absence of interaction between the two drugs.

The DSC thermograms of rosiglitazone and metformin showed endothermic peaks at 75.96 and 241.42°C, respectively, corresponding to the melting of both drugs (Figure 1). The two peaks persisted in the DSC thermogram of the 1:1 rosiglitazone:metformin physical mixture. This indicates the absence of physical interactions between the two active ingredients.

The infrared spectra of rosiglitazone, metformin, and their 1:1 physical mixture are shown in Figure 2. The spectrum of the physical mixture exhibited characteristic bands corresponding to the functional groups of both drugs, indicating the absence of any chemical interaction between them.

3.1.2. *Determination of rosiglitazone and metformin in combination*

The retention time for rosiglitazone and metformin was approximately 2.89 and 5.21 min, respectively.

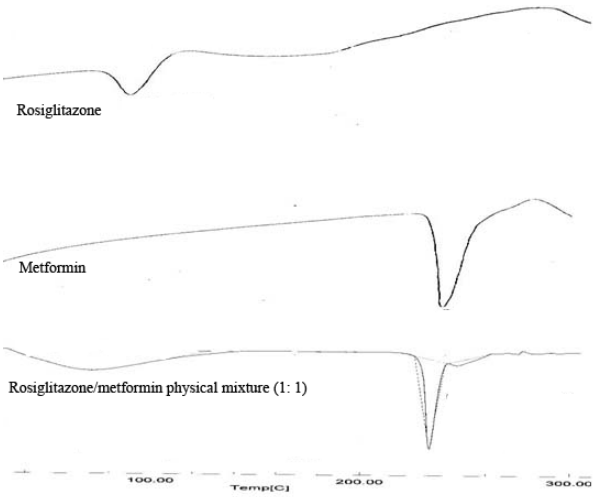

Figure 1. Compatibility study of rosiglitazone and metformin using differential scanning calorimetry.

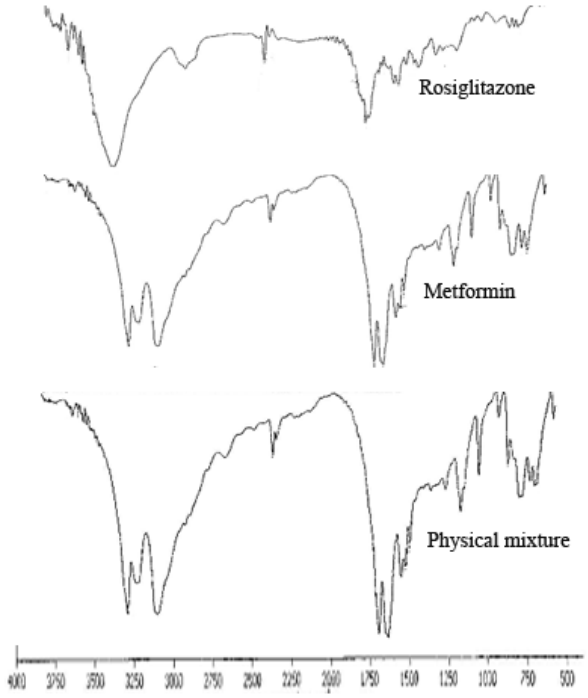

Figure 2. Compatibility study of rosiglitazone and metformin using FT-IR spectroscopy.

The proposed HPLC method was accurate, precise, and rugged, as shown in Table 2.

3.1.3. *Compatibility study of both drugs with different tablet excipients*

No changes in color or appearance (*e.g.* caking, liquefaction, and formation of clumps) were noted for any of the aforementioned pharmaceutical excipients, indicating good physical stability.

Figure 3 illustrates the DSC thermograms, where the thermal properties of the mixtures of magnesium stearate, starch, talc, and PVP-K90 with drug combination were the sum of the individual components. Few changes in the transition temperatures resulted from the mixing of the two components; the process of mixing was found to reduce the purity of each of the components and thus cause a slight lowering of the melting endotherms.

In contrast, samples of Avicel PH 102, spray-dried lactose and dicalcium phosphate either had additional peaks or the drugs' peaks were masked, so these samples were excluded from further investigations.

Similarly, the infrared spectra indicated the absence of interaction between the combined drugs with all the tested excipients except avicel, anhydrous lactose, and dicalcium phosphate (data not shown).

3.2. *Formulation of rosiglitazone and metformin tablets*

3.2.1. *Characterization of the blends to be compressed*

Analysis of random samples of the four prepared blends indicated adequate uniformity of mixing. The evaluation parameters for the four tablet blends are shown in Table 3.

The modal size of particle distribution was 77.78, 32.52, 65.43, and 59.61 μm for tablet formulations 1, a, b, and ab, respectively. PVP-K90 produced blends with markedly larger mean particle sizes than those containing starch (formulations a and ab) due to its larger particle size.

Values for Carr's index ranged from 17.89-21.69%

Table 2. Precision and accuracy of the HPLC method for the determination of rosiglitazone and metformin in a mixture

Concentration (μg/mL)	Mean	S.D.	Precision (CV)	Accuracy (%)	Mean	S.D.	Precision (CV)	Accuracy (%)
Metformin	Intraday (within batch) (n = 3)				Interday (between batches) (n = 3)			
10	9.95	0.46	4.62	99.5	9.88	0.53	5.36	98.8
20	19.71	1.664	8.44	98.55	19.04	1.72	9.03	95.2
30	31.51	1.80	5.71	105.03	30.9	1.21	3.92	103
Rosiglitazone	Intraday (within batch) (n = 3)				Interday (between batches) (n = 3)			
2	1.97	0.14	7.11	98.5	1.92	0.23	11.98	96
3	3.02	0.36	11.92	100.67	3.12	0.38	12.18	104
5	4.93	0.27	5.47	98.6	4.76	0.33	6.93	95.2

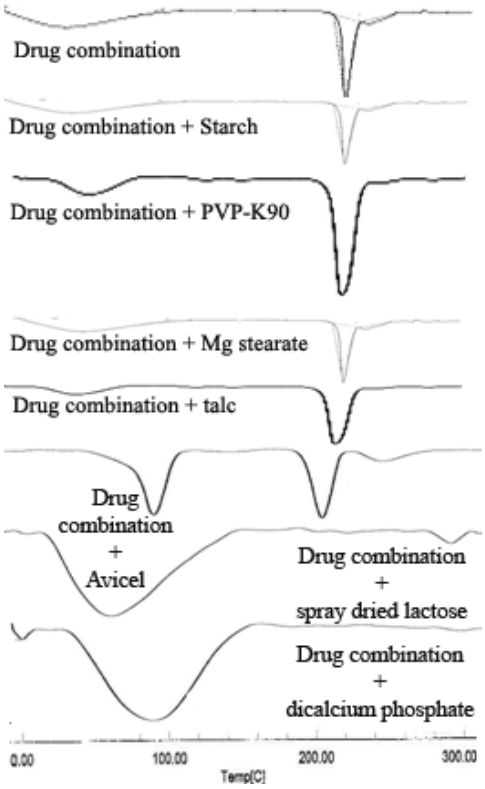

Figure 3. DSC thermograms of rosiglitazone/metformin in combination with different tablet excipients.

Table 3. Evaluation parameters for the prepared tablet blends

Formulation	Modal size of particle distribution	Bulk density (g/mL)	Hausner Ratio	Carr's index	Angle of repose (θ)
1	77.78	0.732	1.220	17.896	34.61
a	32.52	0.469	1.250	20.042	35.03
b	65.43	0.750	1.225	18.367	34.14
ab	59.61	0.461	1.277	21.687	34.45

indicating good flow characteristics. However, Carr's index is a one-point determination and does not reflect the ease or speed with which consolidation occurs. Indeed, some materials have a high index, suggesting a poor flow, but may consolidate rapidly; such a property is essential for uniform filling in a machine. Hausner's ratio relates to interparticle friction and can be used to predict powder flow properties. The recorded values ranged from 1.22 to 1.25, indicating that powders had a low interparticle friction. The tested blends had a similar angle of repose and the range of that angle indicated a reasonable flow (34.14°-35.03°).

Statistical analysis of the aforementioned data is summarized in Table 4. Results showed that the binder type, the lubricant type, and their interaction had a significant effect on both Hausner's ratio and Carr's index. Using PVP-K90 as a binder and talc as a lubricant significantly decreased Carr's index and Hausner's ratio, indicating better flowability and compressibility, respectively. Only the lubricant

Table 4. ANOVA results of factorial experiments

Source	Response (Probability > *F*)			
	Bulk density	Carr's index	Hausner's ratio	Angle of repose
Binder type	< 0.0001[a]	0.43	0.0716	< 0.0001[a]
Lubricant type	0.2005	0.374	0.834	< 0.0001[a]
Binder type * Lubricant type	0.6727[a]	0.329	0.1482	< 0.0001[a]

[a] Significant at a level of $p < 0.05$.

type had a significant effect on the bulk density. In comparison to Mg stearate, Talc significantly decreased the bulk density of the tablet blends. None of the tested variables had a significant effect on the angle of repose.

3.2.2. *Method of preparation*

Direct compression was attempted due to its advantages, like simplicity, but it produced friable tablets regardless of the excipients used and was thus excluded. Wet granulation produced tablets with good physical characteristics.

3.3. *Evaluation of the prepared tablets*

3.3.1. *Physical properties of tablets*

Relative standard deviation (% RSD) values for the mean tablet weights ranged from 0.02-0.11% for the four formulations, indicating a high level of weight uniformity. Similarly, the % RSD for the mean tablet thickness and diameter was very low (in the range of 0.04-0.08 and 0.05-0.08%, respectively).

The average drug content for all formulations complied with the pharmacopoeial limits in a range of 85% to 115% of the label claim, and the standard deviation was less than 6%. Similarly, the percent friability of all formulations was less than 0.5%, which conforms to the acceptable range for compressed tablets. All the prepared formulations had a narrow range for both the disintegration time and the hardness ranging from 7.5 to 9.1 kg.

One-way ANOVA was performed to evaluate the effect of the tested factors on the different physical properties of the prepared tablets. The binder type had a significant effect on tablet friability, hardness, and disintegration time. Starch-based tablets showed significantly greater hardness, lower friability, and slower disintegration compared to those containing PVP-K90. This could be attributed to the higher binding properties of starch. The lubricant type and its interaction with the binder type did not have a significant effect on the tested variables (data not shown).

3.3.2. *In vitro dissolution study*

The dissolution profiles of rosiglitazone and metformin from the prepared tablets and from the commercial

Rosiplus® tablets in 0.1 N HCl are illustrated in Figure 4. The dissolution efficiency (DE_{1h}) is listed in Table 5. The four tablet formulations released 100% of their loadings of both drugs in 45 min. The DE_{1h} values are in accordance with the tablets' physical properties; a higher dissolution efficiency was noted for tablets with less hardness and higher % fines (PVP-K90 based tablets).

The dissolution profile of the commercial tablets revealed a lower dissolution efficiency and the tablets did not completely disintegrate by the end of the dissolution test (1 h).

Drug dissolution data for all formulations were evaluated using various mathematical models. According to the highest determination coefficient (r^2), the dissolution of both rosiglitazone and metformin from the prepared tablets generally fits the Korsmeyer-Peppas and Higuchi diffusion models best. Results for the diffusion exponent (n) indicated that the release of both drugs was generally controlled by a non-Fickian transport mechanism that involves both diffusion and dissolution mechanisms. The exponent "n" values ranged 0.765-0.851 for rosiglitazone and 0.609-0.661 for metformin. Formulations containing starch as a binder had higher values of "n".

3.4. *Stability study*

All the stored tablets retained the same physical properties as fresh ones except for those containing talc (formulations 1 and a). Those tablets had a marked decrease in tablet hardness and an increase in their disintegration times upon storage, which could be attributed to moisture absorption. The residual drug loadings remained within the accepted official limits. All the dissolution profiles had high levels of similarity at the different time intervals, indicating good stability. The f_2 values ranged between 91.3 and 95 for rosiglitazone and 92 and 97.6 for metformin.

Based on the stability study and the *in vitro* evaluations results, formulations 1 and a were excluded due to their physical instability. Formulation b had a greater extent of dissolution in relation to formulation ab and was selected for *in vivo* evaluation.

Table 6 summarizes the accelerated stability study

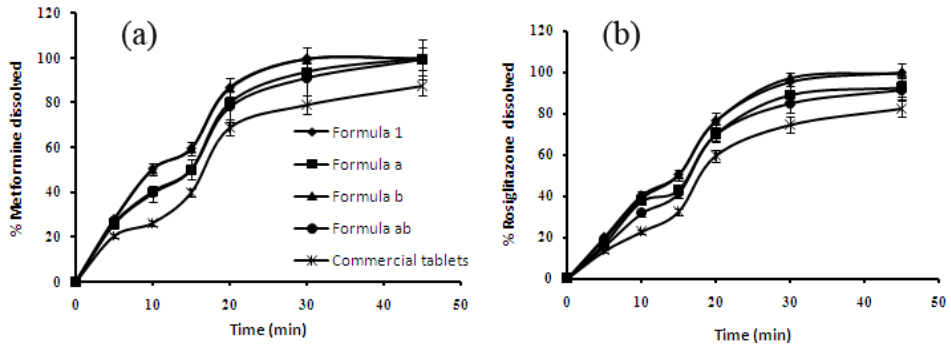

Figure 4. *In vitro* dissolution profiles of metformin (A) and rosiglitazone (B) from the prepared tablets and commercial Rosiplus tablets.

Table 5. *In vitro* evaluation parameters for the prepared rosiglitazone/metformin tablets

Formulation	Friability (% fines)*	Hardness (kg)*	Disintegration time (min)**	DE_{1h} (%)	
				rosiglitazone	metformin
1	0.161 ± 2.01	7.50 ± 0.424	8 ± 0.990	75.96	80.63
a	0.088 ± 1.11	8.95 ± 0.354	11 ± 1.414	71.13	76.69
b	0.136 ± 1.23	8.2 ± 0.707	7.5 ± 2.121	76.25	80.61
ab	0.072 ± 2.1	9.1 ± 0.707	10.1 ± 1.131	69.13	75.84

* Values are mean ± S.D., n = 10, ** n = 6.
DE_{1h} for the commercial tablets = 60.55 for rosiglitazone and 66.23 for metformin.

Table 6. Results of an accelerated stability study

Storage temperature (°C)	% drug remaining at the following time intervals			$t_{90\%}$	Shelf life (months)
	2 months	4 months	6 months		
Metformin					
50	97.2 ± 1.45	95.5 ± 2.51	93.32 ± 1.77	7.413	51.28
60	95.5 ± 2.06	89.1 ± 2.99	85.12 ± 2.43	3.98	
70	87.1 ± 2.51	79.4 ± 1.87	69.2 ± 2.55	1.86	
Rosiglitazone maleate					
50	97.7 ± 1.67	94.4 ± 1.55	91.2 ± 2.66	7.079	39.8
60	94.4 ± 2.07	88.1 ± 2.54	83.17 ± 1.67	3.65	
70	87 ± 2.66	75.8 ± 1.52	66.8 ± 2.11	1.78	

Table 7. Summary of metformin pharmacokinetic parameters and the results of statistical analysis

Parameter	Means ± S.D.		Ratio of means	
	Prepared tablets	Commercial tablets	Point estimate	90% CI
C_{max} (μg/mL)	36.535 ± 5.385	32.322 ± 4.529	113.054	111.29, 114.817
$AUC_{(0-24)}$ (μg•h/mL)	282.515 ± 4.940	261.335 ± 4.345	106.584	104.701, 108.466
$AUC_{(0-\infty)}$ (μg•h/mL)	287.870 ± 7.012	269.940 ± 6.057	–	–
t_{max} (h)	2.667 ± 0.258	3.250 ± 0.274	–	–
$t_{1/2}$ (h)	2.725 ± 0.389	2.625 ± 0.530	–	–
k (h^{-1})	0.257 ± 1.036	0.267 ± 1.058	–	–

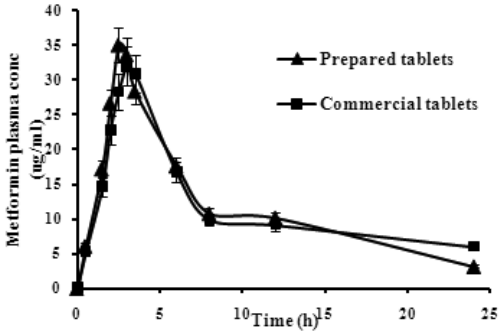

Figure 5. Mean plasma concentration-time profiles of metformin after the administration of a single dose. Error bars indicate S.D., $n = 4$.

results. The data showed that the estimated $t_{90\%}$ values at 25°C were 51.28 and 39.8 months for metformin and rosiglitazone maleate, respectively. Therefore, the shelf life for formulation b is about three years.

3.5. *In vivo study*

The calibration curve of metformin-HCl in plasma was linear in the range of (5-55 μg/mL) with $r^2 = 0.9983$. Mean values of metformin plasma concentrations are shown in Figure 5 and the pharmacokinetic parameters are listed in Table 7.

The maximum concentration reached (C_{max}) and the areas under the curve ($AUC_{(0-24h)}$) were compared. The geometric mean and 90% confidence intervals of prepared tablets/commercial tablets ratios are summarized in Table 7. Since the 90% CI for both C_{max} and $AUC_{(0-24h)}$ ratio (test/reference) was inside the 80-125% interval proposed by the US Food and Drug Administration (*15,16*), the prepared tablets were concluded to be bioequivalent to commercial tablets in terms of both the rate and the extent of absorption.

4. Conclusion

A tablet formulation containing 500 mg metformin-HCl, 2.649 mg rosiglitazone maleate, 26 mg PVP-K90, and 6 mg Mg stearate succeeded both *in vitro* and *in vivo* as a conventional stable and bioavailable tablet for the delivery of both drugs. The formulation can be easily prepared and the excipients used are cheap and available, indicating its potential for use on a larger scale.

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References

1. Thomas CR, Turner SL, Jefferson WH, Bailey CJ. Prevention of dexamethasone-induced insulin resistance by metformin. Biochem Pharmacol. 1998; 56:1145-1150.
2. Bailey CJ. Metformin--an update. Gen Pharmacol. 1993; 24:1299-1309.
3. Sirtori CR, Pasik C. Re-evaluation of a biguanide, metformin: mechanism of action and tolerability. Pharmacol Res. 1994; 30:187-228.
4. Corti G, Cirri M, Maestrelli F, Mennini N, Mura P. Sustained-release matrix tablets of metformin hydrochloride in combination with triacetyl-β-cyclodextrin. Eur J Pharm Biopharm. 2008; 68:303-309.
5. Elte JW, Blicklé JF. Thiazolidinediones for the treatment of type 2 diabetes. Eur J Intern Med. 2007; 18:18-25.
6. Ovalle F, Bell DS. Clinical evidence of thiazolidinedione-induced improvement of pancreatic β-cell function in patients with type 2 diabetes mellitus. Diabetes Obes Metab. 2002; 4:56-59.
7. Dailey GE, Noor MA, Park JS, Bruce S, Fiedorek FT. Glycemic control with Glyburide/Metformin tablets in combination with rosiglitazone in patients with type 2 diabetes: a randomized, double-blind trial. Am J Med. 2004; 116:223-229.
8. Önal A. Spectrophotometric and HPLC determinations of anti-diabetic drugs, rosiglitazone maleate and metformin hydrochloride, in pure form and in pharmaceutical preparations. Eur J Med Chem. 2009; 44:4998-5005.
9. Sambol NC, Chiang J, O'Conner M, Liu CY, Lin ET, Goodman AM, Benet LZ, Karam JH. Pharmacokinetics and pharmacodynamics of metformin in healthy subjects and patients with noninsulin-dependent diabetes mellitus. J Clin Pharmacol. 1996; 36:1012-1021.
10. Khan KA. The concept of dissolution efficiency. J Pharm Pharmacol. 1975; 27:48-49.
11. Higuchi T. Rate of release of medicaments from ointment bases containing drugs in suspensions. J Pharm Sci. 1961; 50:874-875.
12. Hixson AW, Crowell JH. Dependence of reaction velocity upon surface and agitation. Ind Engng Chem.

1931; 23:923-931.
13. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm. 1983; 15:25-35.
14. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. Guidelines for Industry: Dissolution Testing of Immediate Release Solid Dosage Forms, August 1997.
15. FDA Guidance for Industry, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products-General Considerations, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2000.
16. FDA Guidance for Industry, Statistical Approaches to Establishing Bioequivalence, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2001.

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