



## Original article

# Advanced and multifaceted stability profiling of the first-line antidiabetic drugs metformin, gliclazide and glipizide under various controlled stress conditions

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## ABSTRACT

The antidiabetic drugs metformin, gliclazide and glipizide have been widely used and studied in terms of pharmacological and antidiabetic effects, and their individual stability has been studied in the literature. However, the drugs' combined stability profiling remains poorly understood, and hence the aim of this study was to investigate the collective stability profiling of different combinations at various controlled conditions. Degradation assessments were carried out on metformin, glipizide and gliclazide by applying a stability-indicating HPLC method that was developed and validated in accordance with ICH guidelines. Glipizide, gliclazide, metformin and the binary mixtures (metformin/glipizide and metformin/gliclazide) were subjected to different forced degradation conditions and were detected at 227 nm by an isocratic separation on an Alltima CN column (250 mm × 4.6 mm × 5 $\mu$ ) utilizing a mobile phase that consists of 20 mM ammonium formate buffer (pH 3.5) and acetonitrile at a ratio of (45:55, v/v). The method is linear ( $R^2 = 0.9999$ ) at the concentration range 2.5–150  $\mu$ g/ml for metformin and 1.25–150  $\mu$ g/ml for sulfonylureas respectively and offers a specific and sensitive tool for their determination in <10 min chromatographic run. All drug peaks were sharp and well separated. Stress degradation revealed that metformin has a remarkable sensitivity to alkaline stress, glipizide was more sensitive to thermal degradation while gliclazide exhibited almost full degradation in acidic, alkaline and oxidative stress conditions.

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## 1. Introduction

Glipizide and gliclazide are second-generation sulfonylureas (Tommasini,1975; Palmer and Brogden,1993). Sulfonylureas are the oldest oral therapies for type 2 diabetic patients and were mainstay treatment since 1956 (Thulé and Umpierrez,2014).

Metformin was discovered in the 1950s for treatment of diabetic patients (Bell and Hadden,1997). Metformin is the recommended first-line therapy for type 2 diabetic patients around the world, according to international guidelines (Rena et al., 2013).

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In our previous work, we have developed and validated a new HPLC method for simultaneous determination of metformin and gliclazide (Gedawy et al., 2019). In the current paper we are investigating the suitability of this new method to serve as a stability indicating tool for the analysis of gliclazide, metformin and another sulfonylurea (glipizide) when subjected to degradative stress conditions and applying the method on commercial samples of these pharmaceutical products post accelerated stability assessment. Literature survey reveals stress degradation studies that have been performed on metformin alone (Cristina Stenger et al., 2012), glipizide alone (Gupta and Bansal, 2011; Bansal et al., 2008a), gliclazide alone (Doomkaew et al., 2015; Bansal et al., 2007), gliclazide and glipizide together (Gumieniczek et al., 2014) or metformin and other agents (Sri Lakshmi et al., 2015; Vaingankar and Amin, 2016; Gite and Patravale, 2015). In one HPLC degradation study where both glipizide and metformin were studied, the authors reported glipizide degradation under various conditions while metformin did not show any degradation at the hydrolytic conditions tested (Sri Lakshmi et al., 2015), although degradation of metformin has been reported by others (Vaingankar and Amin,

2016; Ramesh and Habibuiddin, 2014). Gliclazide degradation has been reported in two papers (Gumieniczek et al., 2014; Bansal et al., 2007), though one paper stated that gliclazide could withstand alkaline degradation (Doomkaew et al., 2015). The aim of the current study was to carry out a forced degradation study to investigate the degradation behavior of the binary mixtures (metformin/gliclazide and metformin/glipizide) versus control (individual analytes) when subjected to different forms of forced degradation. This method is to our knowledge, the first stability-indicating HPLC method to compare the degradation results of metformin with two different sulfonylureas and can serve as an accurate, selective and cost-effective analytical tool for simultaneous analysis of fixed dose combinations of metformin and sulfonylureas.

## 2. Experimental

### 2.1. Materials and reagents

Metformin hydrochloride 97%, gliclazide greater than 98%, glipizide and analytical reagent grade ammonium formate were procured from Sigma-Aldrich (St. Louis, USA). Metformin<sup>®</sup> (metformin 500 mg tablets, Sandoz, Sydney, Australia), Glyade<sup>®</sup> (gliclazide 80 mg tablets, Alphapharm, Brisbane, Australia) and Melizide<sup>®</sup> (glipizide 5 mg tablets, Alphapharm, Brisbane, Australia) were obtained from a local pharmacy. Formic acid (analytical grade) was obtained from Ajax Fine Chemicals Pty Ltd (Melbourne, Australia). Acetonitrile (HPLC grade) was purchased from Thermo Fisher Scientific (Melbourne, Australia). Distilled water for buffer and sample preparations have been obtained from a Milli-Q ultra-pure water system (Millipore, Australia).

### 2.2. Instrumentation

Table 1 summarizes The HPLC system used and the chromatographic conditions of the entire study. Labsolutions version 5.82 was used for data acquisition and chromatographic integration.

### 2.3. Preparation of standard stock solutions (glipizide and metformin) for method validation

Glipizide 25 mg/100 ml (Flask A) and Metformin 25 mg/100 ml (Flask B) were prepared as described in our previous work (Gedawy et al., 2019).

### 2.4. Preparation of working solution (glipizide and metformin mixture) for method validation

Synthetic mixture of glipizide and metformin (50 µg/ml) each was prepared by transferring 2 ml from Flask A and 2 ml from Flask

B into a 10 ml flask and final volume made up with mobile phase mixture (Gedawy et al., 2019).

### 2.5. Forced degradation studies

Forced degradation studies were conducted on control samples containing glipizide 1 mg/ml (Flask C), gliclazide 1 mg/ml (Flask D), metformin 1 mg/ml (Flask E) and the combined samples of glipizide/metformin mixture 1mg/ml each (Flask F) and gliclazide/metformin mixture 1mg/ml each (Flask G). An aliquot of 10 ml from flask C, D, E, F or G were transferred into a 20 ml volumetric flask and the final volume was made up to 20 ml using 1 M HCl (in a thermostat water bath 45 °C, 24 h) for acid degradation, 1 M NaOH (in a thermostat water bath 45 °C, 24 h) for alkaline degradation, 3% H<sub>2</sub>O<sub>2</sub> (in a thermostat water bath 45 °C, 24 h) for oxidative stress and finally with the mobile phase mixture for photolytic (total of 24 h in sunlight) and thermal degradation studies (in a thermostat oven 80 °C, 24 h). These stressed samples were finally diluted to make 50 µg/ml control individual drugs or combined mixtures 50 µg/ml each. Note that oxidative and thermally stressed samples were cooled only, while acid and alkaline stressed samples were cooled and neutralized with 1 M NaOH and 1 M HCl respectively before the final dilution step.

Individually stressed glipizide, gliclazide and metformin samples (control) as well as the combined synthetic mixtures of (metformin/glipizide) and (metformin/gliclazide) were analysed in triplicates immediately after treatment and after 24 h. Note that blank acid, blank alkali and blank oxidation samples were injected to visualize and eliminate any possible unwanted new solvent peaks from the obtained results.

% Drug degradation was calculated using the formula:

#### %Drug degradation

$$= \frac{\text{Area of unstressed sample} - \text{Area of stressed sample}}{\text{Area of unstressed sample}} \times 100$$

### 2.6. Stability testing of glipizide, gliclazide and metformin tablets

Five blister strips of Melizide<sup>®</sup> (glipizide 5 mg tablets), Glyade<sup>®</sup> (gliclazide 80 mg tablets), and Metformin<sup>®</sup> (metformin 500 mg tablets) were kept at 4 °C for four weeks while five other blister strips of the same batch of each product were placed in an accelerated stability chamber at 40 °C and 75% relative humidity for the same period. After four weeks, accelerated stability samples as well as refrigerated samples were left on the bench for one hour to reach ambient temperature before they got processed for content uniformity. 20 tablets of Melizide<sup>®</sup> were weighed and crushed. 204.8 mg powder equivalent to one Melizide<sup>®</sup> tablet (5 mg glipizide) was placed in a 50 ml volumetric flask. 5mls of distilled water was then added, sonicated for 10 min and final volume was made up to the mark using the mobile phase mixture. An aliquot of 10 ml of this flask was transferred to 20 ml volumetric flask and final volume was made up to the mark using the mobile phase mixture to produce 50 µg/ml glipizide flask. 50 µg/ml metformin and 50 µg/ml gliclazide flasks from Metformin<sup>®</sup> Sandoz 500 mg tablet (average weight, 583.35 mg) and Glyade<sup>®</sup> Alphapharm 80 mg tablet (average weight, 157.3 mg) tablets were prepared as mentioned in our earlier work (Gedawy, Al-Salami and Dass, 2019). Metformin, glipizide and gliclazide content was determined as follows:

$$\% \text{Drug assay} = \frac{\text{At}}{\text{As}} \times \frac{\text{Cs}}{\text{Ct}} \times \frac{\text{Average tablet weight (mg)}}{\text{Label drug claim (mg)}} \times \frac{\text{P}}{100} \times 100$$

where, **At** is the peak area of test tablet, **As** is the peak area of reference standard.

**Table 1**  
HPLC instrumentation and the chromatographic conditions of the optimized method.

Instrument	Shimadzu HPLC, Japan equipped with a LC-20AT pump with inline degasser), (SPD-20A 5R UV detector) and (SIL-20AC HT Autosampler).
Column	Alltima CN (250 mm × 4.6 mm × 5µ), air-conditioned laboratory (25 °C)
Detector	UV
Wavelength	227 nm
Mobile phase	20 mM ammonium formate, pH 3.5 and acetonitrile, (45 :55, v/v)
Run time	10 min
Flow rate	1 ml/min
Injection volume	20 µl

**C<sub>s</sub>** is the concentration of standard, **C<sub>t</sub>** is the concentration of test tablet and **P** is the percentage purity of reference standards used.

### 2.7. Analytical method validation on metformin/glipizide mixture.

The stability indicating method for simultaneous determination of glipizide and metformin has been validated as per ICH guidelines Q2R1 (ICH, 2005) for evaluating linearity, accuracy, precision, specificity, system suitability, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

#### 2.7.1. System suitability and precision intra-day precision (repeatability) and inter-day precision (intermediate precision)

System suitability parameters with respect to number of theoretical plates, repeatability, tailing factor and resolution between glipizide and metformin peaks were assessed by injecting a blank mobile phase followed by six replicates of glipizide/metformin mixture at a concentration of 50 µg/ml each. Six independent combined samples of glipizide and metformin (50 µg/ml each) were injected on the same day under same operating conditions for system and method precision study while inter-day precision was assessed by comparing the results of 6 independent determinations on three consecutive days.

#### 2.7.2. Linearity and range

Flask A - the standard stock solution of glipizide was diluted in the concentration range (1.25–150 µg/ml). Triplicates of each concentration were analysed and plotted on a glipizide calibration curve. Metformin from (Flask B) was diluted in the concentration range of (2.5–150 µg/ml), and assayed in triplicates, then plotted on a metformin calibration curve. Intercept, slope and correlation coefficient of the calibration curves (peak area versus concentration) were determined to ensure linearity of the proposed analytical method.

#### 2.7.3. Accuracy study and recovery

Accuracy of the proposed method was confirmed by spiking the synthetic mixture of potato starch, magnesium stearate, polyvinylpyrrolidone (PVP), microcrystalline cellulose and anhydrous lactose with glipizide and metformin separately at 3 different levels 80%, 100% and 120%. Triplicate determinations of these 3 levels were recorded to obtain the mean and % RSD.

#### 2.7.4. Method sensitivity, limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ for glipizide and metformin were calculated from the linear regression equation based on the slope and standard deviation of the intercept using the formula

$$\text{LOD} = 3.3 \text{ Q/S} \quad \text{and} \quad \text{LOQ} = 10 \text{ Q/S}$$

where **Q** is the standard deviation of the intercept, and **S** is the slope of the calibration curve.

#### 2.7.5. Robustness

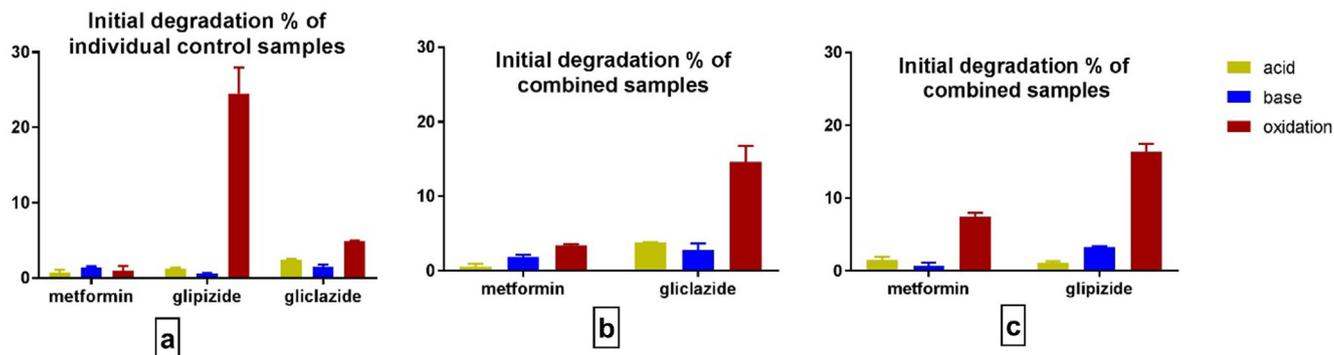
Intended small variations in the chromatographic conditions such as mobile phase composition and flow rate have been made. These variations were also evaluated for tailing factor, number of theoretical plates and resolution between glipizide and metformin peaks.

## 3. Results and discussion

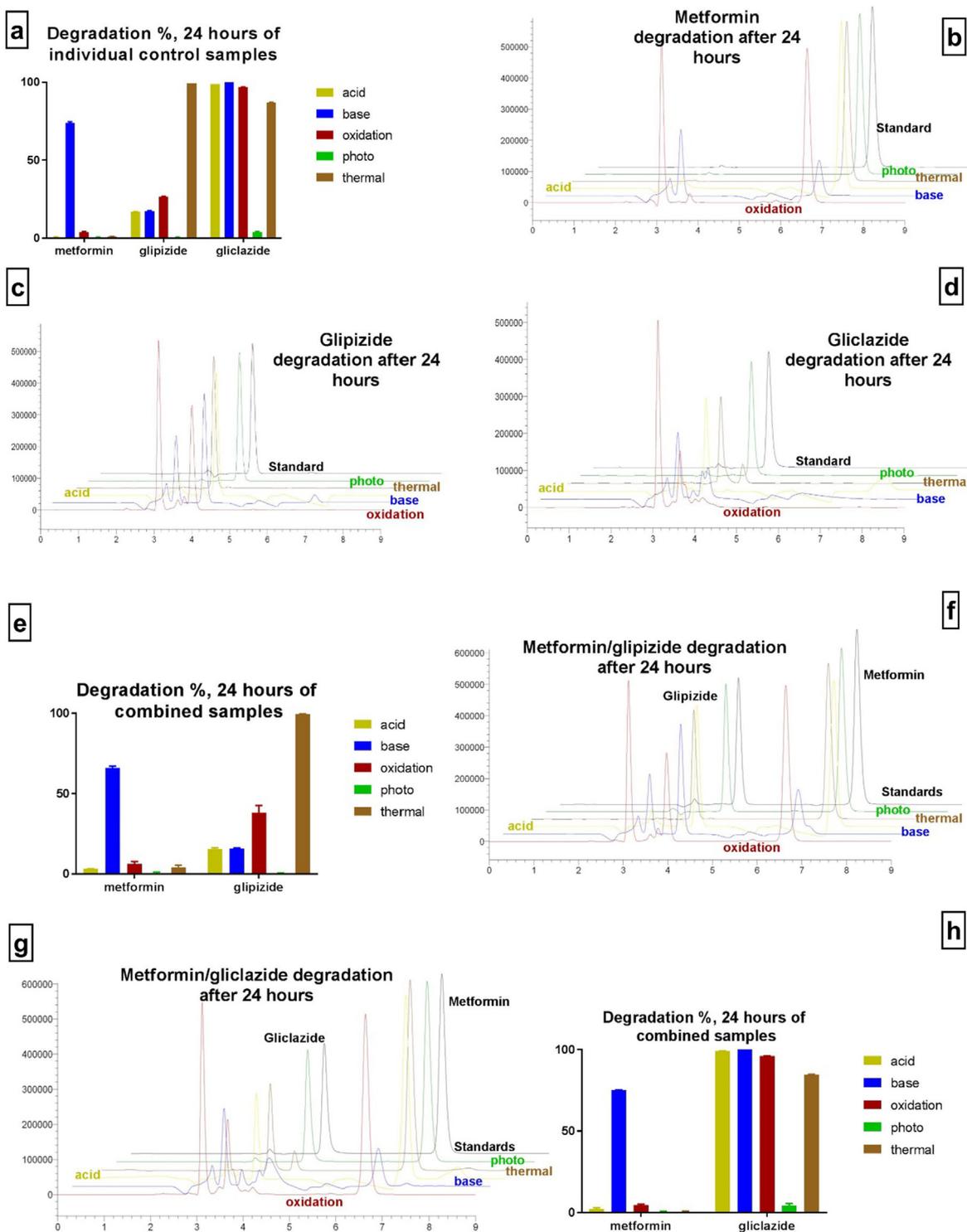
### 3.1. Forced degradation results

In the present study, it was shown that the optimized HPLC method could serve as a stability indicating procedure where it is able to detect and quantify metformin, gliclazide and glipizide in the presence of their degradation peaks (Figs. 1, 2). Immediately after acidic treatment, metformin was more stable than sulfonylureas (with <1% degradation, order of stability is metformin > glipizide > gliclazide) (Fig. 1a). On the other hand, gliclazide per se was more labile than metformin and glipizide to both acidic and basic degradation. Gliclazide also has the least stability towards acid in metformin/gliclazide sample (Fig. 1b) verifying the reported sensitivity of sulfonylureas and heterocyclic rings towards acidic and alkaline hydrolysis (Bansal et al., 2008b). Although its relative stability towards alkaline hydrolysis in control sample (Fig. 1a), glipizide has initially lower stability towards alkali when combined with metformin (Fig. 1c). Initial oxidative stress revealed that metformin is more stable than sulfonylureas in control as well as combined synthetic mixtures (Fig. 1).

Photodegradation results revealed that metformin is the most resistant to light degradation of all analytes over 24 h while gliclazide is the most sensitive to light in control samples (Fig. 2a) and in combination sample (Fig. 2h). Glipizide on the other hand, showed almost full degradation to thermal conditions after 24 h (more than 99% degradation of initial glipizide concentration) (Fig. 2a) under the specified wet thermal conditions for both control and glipizide/metformin combined samples and almost full conversion into a degradation product that appeared on the chromatogram at a retention time of 3.63 min while the original glipizide peak retained at 4.02 min (Fig. 2c, f). These results confirm the high susceptibility of glipizide to heat and humidity reported by Gupta and Bansal (Gupta and Bansal, 2011). At the end of 24 h, gliclazide exhibited more than 84% degradation under the same wet thermal conditions alone (Fig. 2a) and when combined with metformin (Fig. 2h), while metformin has negligible thermal degradation outcomes.



**Fig. 1.** Initial degradation results, immediately after treatment with acid, base and 3% H<sub>2</sub>O<sub>2</sub> of **a**, control samples of metformin (50 µg/ml)(n = 3), glipizide (50 µg/ml)(n = 3), gliclazide (50 µg/ml)(n = 3) versus **b**, combined metformin/gliclazide sample (n = 3) and **c**, combined metformin /glipizide sample (n = 3).



**Fig. 2.** Degradation results after 24 h exposure to acid, base, 3% H<sub>2</sub>O<sub>2</sub>, Sunlight and 85 °C heat of **a**, control samples of metformin (50 µg/ml)(n = 3), glipizide (50 µg/ml)(n = 3), gliclazide (50 µg/ml)(n = 3) versus **e**, combined metformin/glipizide sample (n = 3) and **h**, combined metformin /gliclazide sample (n = 3).

Metformin also was more stable than the two sulfonylureas under the tested acidic conditions alone and in combination samples while gliclazide showed more than 98.5% alone and when combined with metformin in acid (Fig. 2a, h). Glipizide exhibited close acidic degradation results (16.86%) in control sample (Fig. 2a) and (15.37%) when combined with metformin after 24 h (Fig. 2e). Despite reported gliclazide stability to alkaline hydrolysis in one paper (Doomkaew et al., 2015), full degradation of gliclazide

was noted in our experiments (Fig. 2a, h), where no gliclazide peak was detected after 24 h in either gliclazide control (Fig. 2d) or gliclazide/metformin combined sample (Fig. 2g) which confirms previous gliclazide alkaline degradation results (Gumieniczek et al., 2014; Bansal et al., 2007; Bansal et al., 2008b). Glipizide was the most resistant to basic degradative stress and recorded (17.2%) degradation in control sample (Fig. 2a) compared to (15.69%) when combined with metformin (Fig. 2e). These results

**Table 2**  
Chromatographic system suitability and precision results.

System suitability parameters (acceptance limit RSD % <2)							
	Retention time		Tailing factor		Number of theoretical plates		Resolution between both peaks
	glipizide	metformin	glipizide	metformin	glipizide	metformin	
1	4.015	6.967	1.193	1.156	6342	11,245	12.712
2	4.015	6.966	1.194	1.163	6340	11,213	12.699
3	4.012	6.965	1.194	1.162	6359	11,252	12.728
4	4.014	6.966	1.195	1.163	6358	11,244	12.721
5	4.014	6.966	1.197	1.165	6325	11,209	12.696
6	4.015	6.966	1.192	1.167	6360	11,218	12.705
Mean	4.0141	6.966	1.194	1.163	6347.33	11230.17	12.71
Standard deviation	0.0012	0.0006	0.0017	0.0037	14.08	18.86	0.0126
RSD%	0.029%	0.009%	0.144%	0.320%	0.222%	0.168%	0.099%

Precision results as peak area of different determinations on 3 different days (metformin 50 µg/ml, glipizide 50 µg/ml) (n = 6), acceptance limit RSD% <2).							
	Day 1		Day 2		Day 3		
	glipizide	metformin	glipizide	metformin	glipizide	metformin	
1	3,357,977	4,187,679	3,305,867	4,237,157	3,216,249	4,587,130	
2	3,375,004	4,216,894	3,322,221	4,238,781	3,218,643	4,569,885	
3	3,343,240	4,193,075	3,322,101	4,233,311	3,223,746	4,565,111	
4	3,345,503	4,193,694	3,300,586	4,276,047	3,229,361	4,568,475	
5	3,365,396	4,209,394	3,318,554	4,239,468	3,216,469	4,566,134	
6	3,373,302	4,223,693	3,308,166	4,285,244	3,222,432	4,577,338	
Mean	3360070.3	4204071.5	3312915.8	4,251,668	3,221,150	4572345.5	
Standard deviation	13611.05	14663.4	9241.36	22733.88	5060.04	8431.63	
RSD%	0.405%	0.349%	0.279%	0.535%	0.157%	0.184%	

confirm the stability of glipizide over gliclazide under the acidic and basic conditions reported by Gumieniczek et al (Gumieniczek et al., 2014).

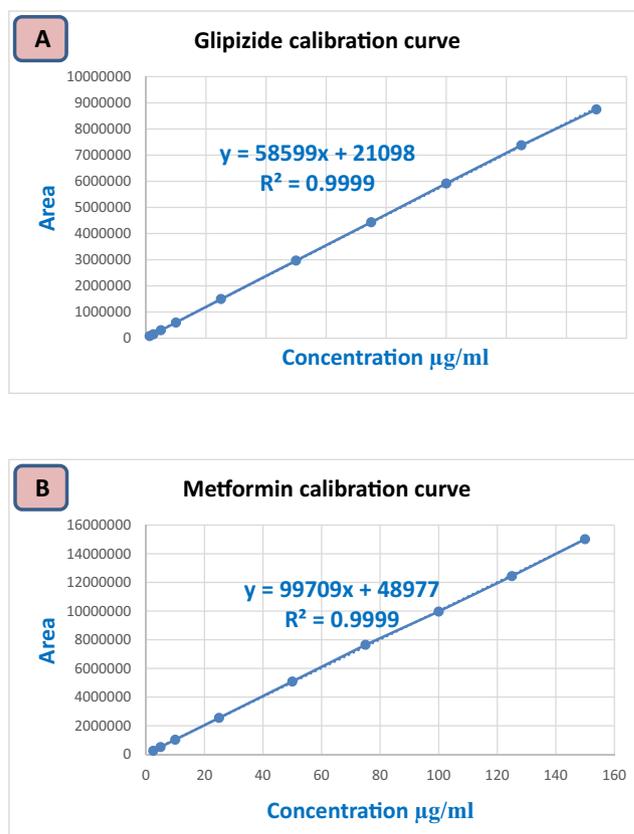
Literature survey revealed that glipizide degradation rate in acidic conditions was equivalent to that in alkaline medium (Bansal et al., 2008a; Gumieniczek et al., 2014). Our results confirm these findings, where after 24 h of stressing glipizide control sample to the specified acidic and basic conditions, almost the same degradation percentage was obtained, namely 16.86% (in acidic) and 17.2% (in alkaline) (Fig. 2a). The same pattern of glipizide degradation was noticed in the combined glipizide/metformin mixture, 15.37% (in acidic) compared to 15.69% (in alkaline) (Fig. 2e). Vulnerability of glipizide to hydrolytic degradation is attributed to the presence of amide and the sulfonylurea moiety in its chemical structure (Bansal et al., 2008a). Glipizide and gliclazide degradation has been previously reported to follow the first order kinetics (Gumieniczek et al., 2014). In our study, around 74% degradation of metformin was observed in the control sample (Fig. 2a) and more than 66% degradation in combined samples (Fig. 2e, h). The clear susceptibility of metformin towards alkaline stress than acid confirm previous results (Gite and Patravale, 2015; Vaingankar and Amin, 2016; Cristina Stenger et al., 2012; Hamdan et al., 2010) although other researchers reported its stability to all hydrolytic conditions (Sri Lakshmi et al., 2015).

Sulfonylureas showed higher sensitivity towards oxidative stress than metformin where more than 96% degradation of gliclazide was recorded in both control and gliclazide/metformin samples (Fig. 2a, h). Glipizide on the other hand, recorded 26.28% degradation in control sample (Fig. 2a), 38% degradation in glipizide/metformin sample (Fig. 2e) under same conditions, while metformin had minimal oxidative degradation.

### 3.2. Stability testing and tablet assay results

The stability indicating HPLC method was successfully used to assay three different commercial metformin, glipizide and gliclazide products in stability chamber (40 °C, 75% relative humidity) compared to control fridge samples. Metformin<sup>®</sup> (metformin

500 mg tablets), Melizide<sup>®</sup> (glipizide 5 mg tablets) and Glyade<sup>®</sup> (gliclazide 80 mg tablets) exhibited 100% content uniformity at 4 °C while stability chamber samples of Metformin<sup>®</sup>, Melizide<sup>®</sup> and Glyade<sup>®</sup> assayed 100%, 99.94% and 99.96% respectively after 4 weeks.



**Fig. 3.** Linearity curves of glipizide (A) and (B) metformin.

**Table 3**  
Recovery results for glipizide and metformin.

Glipizide recovery study (acceptance limit recovery % = 98–102%).				
Sample name	Theoretical (claimed) concentration in µg/ml	The concentration found in µg/ml	Recovery %	Statistical data
gp1 80%	41	41.59	101.44	Mean = 100.5 Standard deviation = 0.83 RSD% = 0.826%
gp2 80%		41.11	100.28	
gp3 80%		40.93	99.83	
gp1 100%	50.2	49.4	98.4	Mean = 98.8 Standard deviation = 0.38 RSD% = 0.381%
gp2 100%		49.75	99.11	
gp3 100%		49.68	98.97	
gp1 120%	59.8	59.62	99.7	Mean = 99.4 Standard deviation = 0.35 RSD% = 0.35%
gp2 120%		59.48	99.47	
gp3 120%		59.21	99.02	
Metformin recovery study (acceptance limit recovery % = 98–102%).				
mt1 80%	42	42.48	101.15	Mean = 100.46 Standard deviation = 0.597 RSD% = 0.594%
mt2 80%		42.07	100.17	
mt3 80%		42.03	100.07	
mt1 100%	51	50.89	99.79	Mean = 99.6 Standard deviation = 0.22 RSD% = 0.216%
mt2 100%		50.82	99.66	
mt3 100%		50.68	99.37	
mt1 120%	60.4	60.43	100.05	Mean = 100.03 Standard deviation = 0.28 RSD% = 0.28%
mt2 120%		60.57	100.29	
mt3 120%		60.24	99.74	

### 3.3. System suitability and precision

Results of 6 replicate injections are presented in Table 2 and the parameters tested were within the acceptable limits. Metformin and glipizide peaks were sharp (tailing factor < 1.2) and well separated (resolution greater than 2) and repeatedly retained at 6.96 min for metformin and 4.01 min for glipizide in all injections. Peak areas resulted from injecting 6 independent combined metformin and glipizide samples were precise and repeatable over 3 days, where intra and inter-day determinations verified the repeatability and precision of the proposed method. All data were compliant with ICH requirements and expressed in RSD% (acceptance limit, RSD% < 2). Results for intra and inter-day precision are given in Table 2.

### 3.4. Linearity

The analytical calibration curves constructed for both glipizide and metformin were linear in the specified ranges. The linear regression equation for glipizide was ( $y = 58599 \times + 21098$ ,  $R^2 = 0.9999$ ) Fig. 3a, and the linear regression equation for metformin was ( $y = 99709 \times + 48977$ ,  $R^2 = 0.9999$ ) Fig. 3b.

### 3.5. Recovery

98.8–100.5% glipizide recovered from the spiked excipients and 99.6–100.46% metformin recovery were recorded to prove the accuracy/trueness of the proposed analytical method.

Results for glipizide and metformin recovery are shown in Table 3.

### 3.6. Limit of detection (LOD) and limit of quantitation (LOQ)

The calculated LOD and LOQ were 0.796 µg/ml, 2.412 µg/ml for glipizide respectively and 1.069 µg/ml, 3.24 µg/ml for metformin respectively. Experimental results showed that the method was able to detect as low as 0.8 µg/ml of both glipizide and metformin and to quantify as low as 2.4 µg/ml of both too.

### 3.7. Robustness

No significant changes were noticed with small variations ensuring that the method used is robust to small intended changes

in chromatographic conditions. In all cases, metformin and glipizide peaks were symmetric (tailing factor < 2) and were well separated (resolution greater than 2) and the RSD% of metformin and glipizide retention times were < 0.2 ensuring the robustness of the proposed analytical method to small changes (data not shown).

## 4. Conclusions

The proposed stability indicating method presents a simple and rapid utility for simultaneous determination of sulfonylureas and metformin in bulk and in pharmaceutical preparations and can be used for routine quality control of single agents or metformin/sulfonylurea combination tablets. The proposed method exhibited good accuracy and robustness with a linear response behavior. Due to the mass compatibility of the mobile phase used, the method can also be optimized for LC/MS application for future work. Forced degradation results confirmed the specificity and the ability of the method to detect and quantify sulfonylureas and metformin among their degradation peaks which enables the use of this method for stability studies of glipizide, gliclazide and metformin preparations.

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