



Development and Optimization of a Green Stability Indicating HPLC Method for the Determination Metformin HCL and Glibenclamide in their Dosage Form

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Abstract

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Green analytical chemistry approach was applied in attempt to develop, optimize and validate a stability indicating RP-HPLC method for the determination of metformin HCL and Glibenclamide in their combined dosage form. The chromatographic separation was performed on C18 column (250×4.6 mm, 5µm) at a λ_{max} of 250 nm with a constant flow rate of 1.00 mL/minute. The isocratic mobile phase consisted of 70.00% organic (ethanol) to 30.00% aqueous 10.00 mM potassium phosphate buffer, pH 3.0 containing 50.00 mM octanesulphonic acid. The two drugs were stressed in acidic, basic, oxidative, and photolytic conditions. The developed method was validated according to ICH guidelines successfully. The detector response was linear in the concentration of 0.50-100.00 µg mL⁻¹ for both drugs. Metformin HCL had detection limit and quantification limit of 0.15 g mL⁻¹ and 0.50 g mL⁻¹, while glibenclamide had detection limit and quantification limit of 0.16 g mL⁻¹ and 0.49 g mL⁻¹, respectively.

Keywords: Green, Stability, HPLC, Metformin HCL and Glibenclamide

1. Introduction

Diabetes mellitus (DM), a chronic metabolic condition, is one of the most serious dangers to human health in the twenty-first century. Type 2 diabetes (T2DM) is associated with insulin resistance as a result of β-cell failure (DeFronzo et al., 1992) The number of persons with T2DM is increasing in every country, with 80 percent of people with DM living in poor and middle-income countries (Mendenhall et al., 2014). Impaired

glucose tolerance is anticipated to affect 7.5 percent (374 million) of the global population in 2019, rising to 8.0 percent (454 million) by 2030 and 8.6 percent (548 million) by 2045 (Saeedi et al., 2019) Egypt is the tenth most populous country in the world in terms of T2D patients, according to the International Diabetes Federation (IDF), diabetes is a rapidly increasing public health issue in Egypt, with considerable implications for morbidity, death, and health-care resources. In Egypt, type 2 diabetes (T2D) affects around 15.6 percent of all persons aged 20 to 79 (Hegazi et al., 2015)

Metformin alone or in combination with second-generation sulfonylureas such as gliclazide, glimiperide, glipizide, and glibenclamide are the principal oral anti-diabetic pharmacotherapies for T2DM (Haupt et al., 1991).

Metformin hydrochloride (MET) is chemically 1,1-dimethyl biguanide hydrochloride (Fig. 1) (Pharmacopoeia. 1998) and extensively used as an antihyperglycemic agent to treat T2DM (Bailey et al., 1996). It decreases the production of hepatic glucose and, by increasing peripheral glucose uptake, increases insulin sensitivity (Natali et al., 2006).

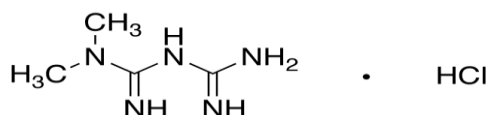


Fig.1. Chemical Structure of metformin HCl.

Glibenclamide (GLB) is a chemical sulfonylurea of the second generation, 5-chloro-N-(4-[N-(cyclohexylcarbonyl) sulfamoyl] phenethyl)-2-methoxybenzamide (Fig. 2), also known as glyburide, which is commonly used for the treatment of T2DM and gestational diabetes (Nanovskaya et al., 2006). It promotes secretion and improves the usage of insulin by sufficient tissues (Mulder et al., 1991).

A commercially available combination of (MET 500 mg & GLB 2.5 mg) and (MET 500 mg & GLB 5 mg) in Glucophage® tablets (Davidson et al., 2004)

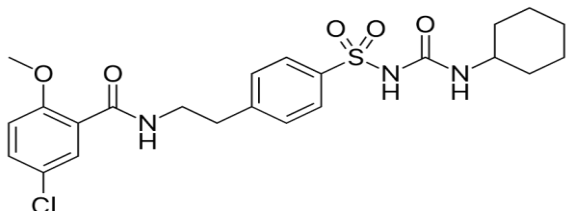


Fig.2. Chemical structure of Glibenclamide.

Environmentally, there is currently a need among analytical chemists to green RP-HPLC procedures,

particularly when toxic solvents are employed frequently. Green analytical chemistry (GAC) originated in the 2000s (Galuszka et al., 2013) with the goal of eliminating or reducing the use of hazardous compounds in analytical procedures in order to make them more environmentally friendly, cost-effective, and effective (Tobiszewski et al., 2010). NEMI labelling is a widely used four-part pictogram technique for determining an analytical method's greenness (Keith et al., 2007). In comparison to NEMI, Eco-Scale is another instrument that considers environmental effect parameters in a quantitative manner (Galuszka et al., 2012). A score is generated from 100 in this analysis, with penalty points subtracted, and a higher number (around 100) suggests a greener study. Forced degradation testing is important in the development of a stability indicating analytical tool because it helps to identify the degradation pathways and degradation products of Active Pharmaceutical Ingredients (APIs) that can form during storage and promote during formulation development, manufacturing, and packaging (Kats et al 2005; Dolan et al 2002). Degradation products or compounds are generated when the active ingredient undergoes changes due to manufacturing or storage (e.g., oxidation and hydrolysis) or when the active component reacts with an excipient or container (Aubry et al 2009; Bakshi et al 2002). To determine the effects of temperature, humidity, photostability, oxidation, and hydrolytic degradation, forced degradation studies are recommended. While there are various methods for estimating MET (Ashour et al., 2003; Tubino et al., 2010; Hegazy et al., 2009; Sultana et al., 2011; Aravne et al., 2010; El-Gindy et al., 2010; Pandit et al., 2012; Jain et al., 2008; Lakshmi et al., 2009; Yardimci et al., 2007; Ali et al., 2008; Bonfilio et al., 2013; Hamdan et al., 2010; Yardimici et al., 2005; Thomas et al., 2011; Modi et al., 2012; Abou-Dan et al 2001; Zhang et al., 2002; Zarghi et al., 2003; Koseki et al., 2005; Chen et al., 2004) and GLB (Emilsson et al., 1986; Othman et al., 1988; Havele et al., 2010; Rydberg et al., 1991; Chen et al., 2004) in the literature, only a few approaches for simultaneous estimation of MET and GLB (Salem et al., 2010; Mistri et al., 2007; Chaturvedi et al., 2008; AbuRuz et al., 2005) are accessible. HPLC stability indicating has been reported for both MET and GLB (Ashour et al., 2014). However, no stability indicating method was published for the simultaneous green analysis of two drug

combinations, whether in pure form or in pharmaceutical preparation, which became the focus of this work. The availability of a new, simple, fast approach for determining MET and GLB using environmentally friendly solvents is a significant advantage of the present method over the reported method.

2. Experimental

2.1. Chemicals and drugs

MET and GLB samples were received as gifts from Marcyrl Pharmaceutical Industries, according to company certifications, with purity claims of 99.80 and 99.75 %, respectively. Merck was the supplier of ethanol (HPLC grade). Hydrochloric (34.00 %), hydrogen peroxide (50.00 %), potassium dihydrogen phosphate, octanesulphonic acid, and sodium hydroxide were among the analytical grades purchased from (Sigma-Aldrich, St Louis, MO, USA). Deionized water was used (Milli-Q). Merck's Glucophage® film coated tablet (Batch No. 190L24) is labelled to contain 5.00 mg GLB and 500.00 mg MET.

2.2. Instrumentation

The HPLC (Hitachi LaChrom Elite, Tokyo, Japan) instrument was equipped with a model series L-2000 organizer box, a L-2300 column oven, a L-2130 pump with built-in degasser, and a Rheodyne 7725i injector with a 20 mL loop and a L-2455 diode array detector (DAD). The separation and quantitation were made on a 250× 4.6 mm (i.d.), 5 µm octadecylsilane column (Inertsil, Tokyo, Japan). UV detection was performed under scan mode (in the range of 200–350 nm with 1 nm distance) and single wavelength chromatograms at 250 nm were used for quantitative analysis.

2.3. Chromatographic condition

The isocratic mobile phase consisted of ethanol (70.00%) along 10.00 mM phosphate buffer, with pH adjusted to 3.0 and 50.00 mM octanesulphonic acid with a flow rate of 1.00 mL/min. The mobile phase was filtered through a 0.45-µm membrane and degassed prior to use. The injection volume was 20.00 µL for standards and samples. All analyses were done at ambient temperature. Single wavelength chromatograms at 250 nm were used for quantitative analysis.

2.4. Preparation of stock and standard solutions

Stock solutions of MET and GLB were prepared separately by dissolving accurately weighed 25.00 mg of each drug in 25.00 mL of ethanol to obtain a stock solution of an individual drug of 1000.00 µg mL⁻¹ for both MET and GLB, working solutions for calibration runs were prepared by diluting aliquots from each stock solution with the mobile phase to obtain concentrations (0.50-100.00 µg mL⁻¹).

2.5. Preparation of the forced degradation solutions

2.5.1. Preparation of the alkali-induced degradants

Base degradation study was carried out by taking 25.00 mg of each MET and GLB in a 50.00 mL standard volumetric flask and completed to the mark with 0.10 N NaOH and refluxed for two hours at 100.0°C then both samples were neutralized, and the final solution was injected in triplicate under optimized chromatographic conditions.

2.5.2. Preparation of the acid-induced degradants

Acid degradation study was carried out by taking 25.00 mg of each MET and GLB in a 50.00 mL standard volumetric flask and completed to the mark with 0.10N HCL and refluxed for two hours at 100.0°C then both samples were neutralized, and the final solution was injected in triplicate under optimized chromatographic conditions.

2.5.3. Preparation of oxidation induced degradants

Oxidation degradation study was carried out by taking 25.00 mg of each MET and GLB in a 50.00 mL standard volumetric flask and mixing it with 1.00 mL of 35.00% v/v hydrogen peroxide then completed to the mark with ethanol and stirred for two hours at room temperature. Under optimum chromatographic conditions, the final solution was injected in triplicate.

2.5.4. Preparation of photo induced degradants

Photolytic degradation study was carried out by taking 25.00 mg of each MET and GLB in a 50.00 mL standard volumetric flask and completed to the mark with ethanol and exposing it to direct sunlight for 24 hours. This solution was subsequently

diluted with mobile phase to achieve a concentration within the linearity range before being injected in triplicates under optimal chromatographic conditions.

2.6. Pharmaceutical dosage form preparation

Twenty tablets were weighed and finely ground in a mortar for analysis. The portions corresponding to one tablet (500.00 mg of MET and 5.00 mg of GLB) were dissolved in ethanol then transferred into 100.00 ml volumetric flask. To ensure complete extraction of the drug it was sonicated for 15 minutes, then volume was adjusted to the mark with ethanol and filtered through 0.45 μ m membrane filter. Further dilutions were performed with ethanol to reach calibration range of each compound.

2.7. Greenness of the proposed method using analytical eco-scale

Some parameters connected to the analysis

procedure are penalized on the analytical eco scale.

The penalty points are based on the amount of chemicals used and their dangers, as well as the energy efficiency of the instruments and the amount of trash created. In the previously published paper, the mechanism for calculating penalty points is detailed (Keith et al., 2007). After removing penalty points from 100, the proposed analytical method can be classified as good, acceptable, or inadequate, depending on whether the Eco Scale value is greater than 75.00, greater than 50.00, or less than 50.00.

2.8. Method validation

The method was validated according to the ICH Q2B requirements (ICH, 2005) after developing the optimal chromatographic conditions. In terms of system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, selectivity and robustness.

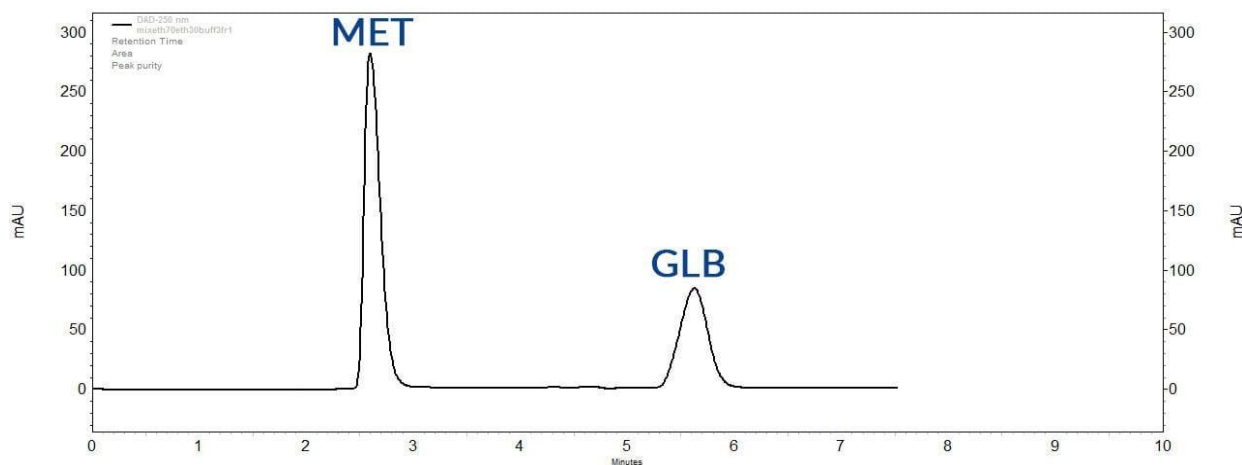


Fig.3: MET (100.00 μ g mL⁻¹) and GLB (100.00 μ g mL⁻¹) chromatogram.

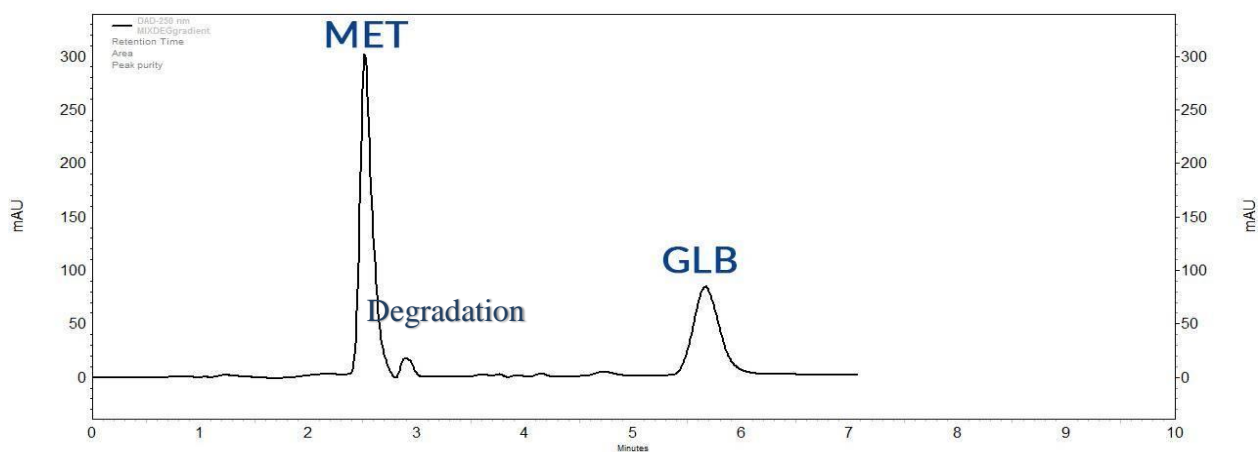


Fig.4: MET ($100.00 \mu\text{g mL}^{-1}$) and GLB ($100.00 \mu\text{g mL}^{-1}$) along with their degradation products.

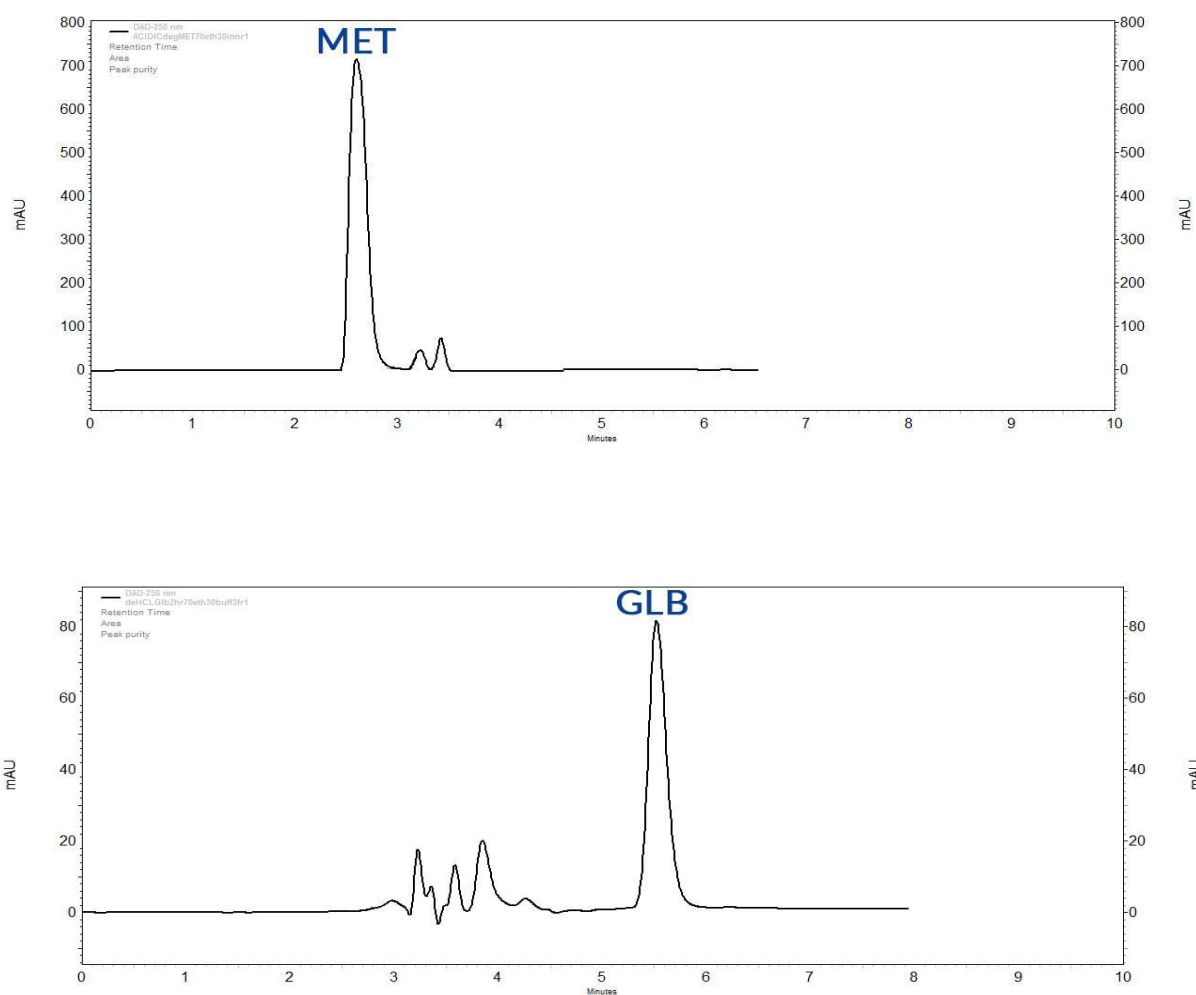


Fig.5. Acidic degradation of MET and GLB.

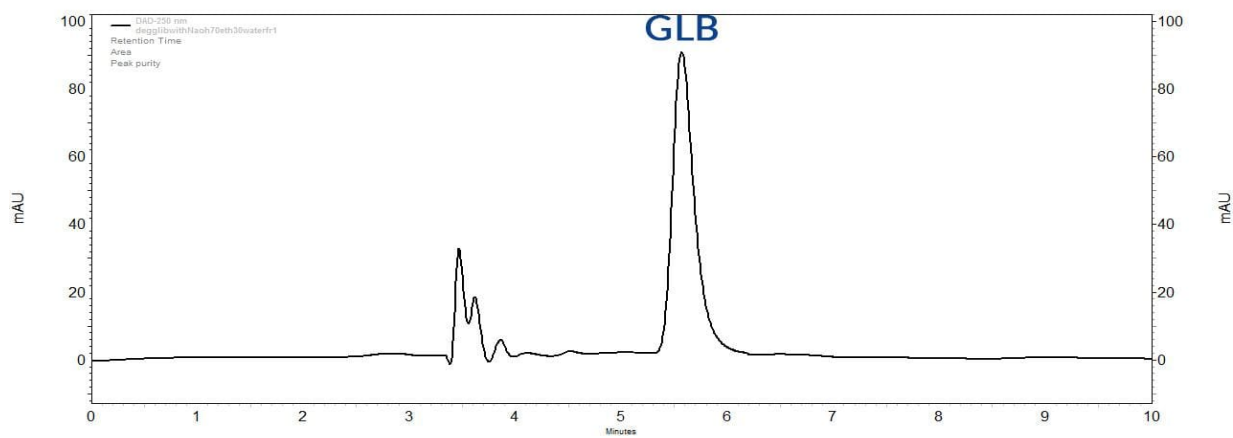
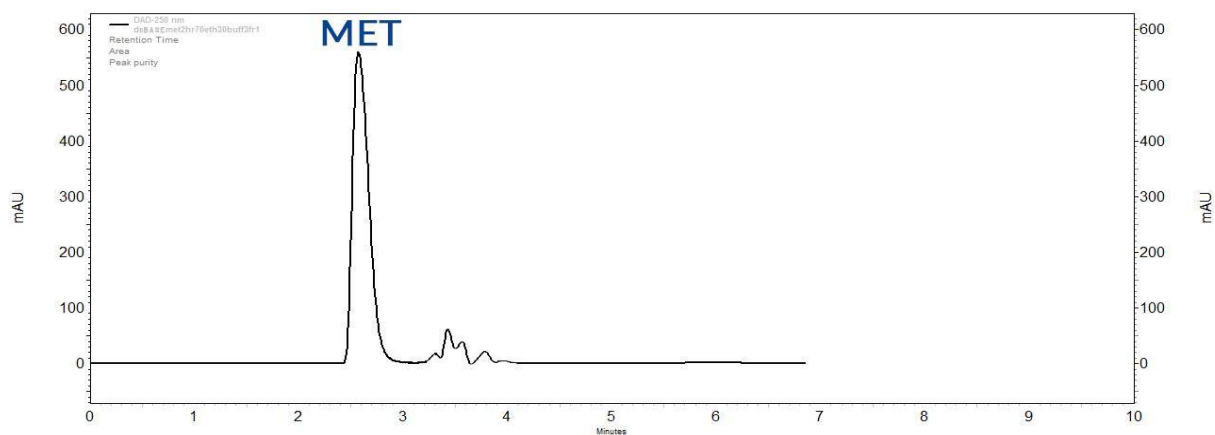


Fig.6. Basic degradation of MET and GLB.

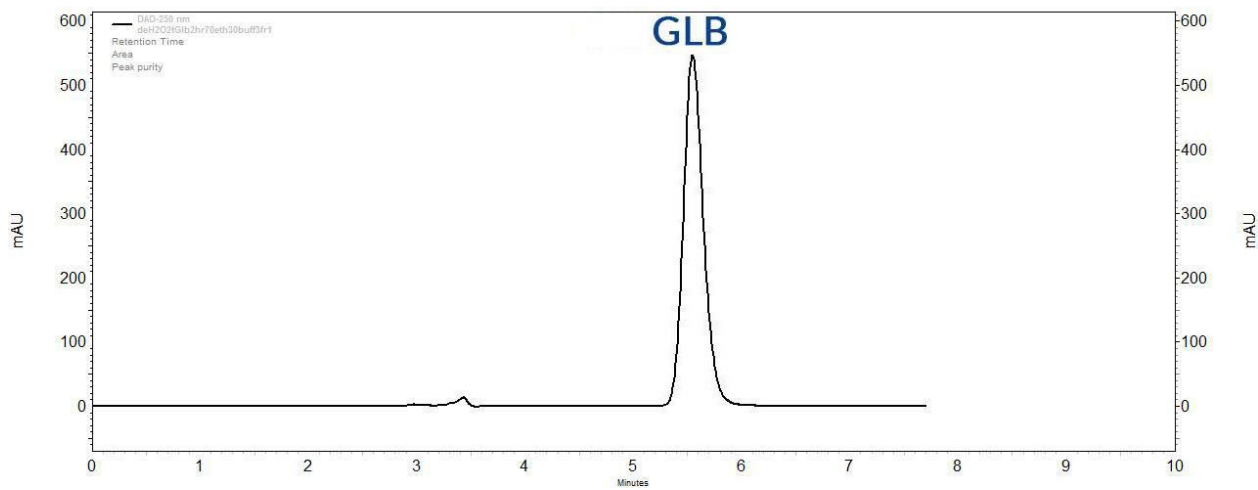
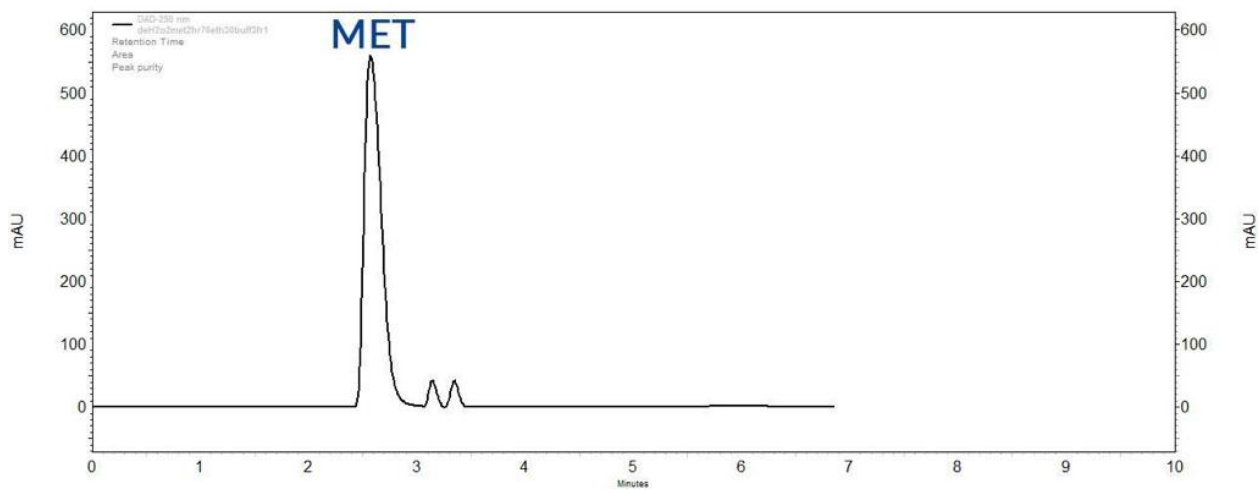


Fig.7. Oxidative degradation of MET and GLB.

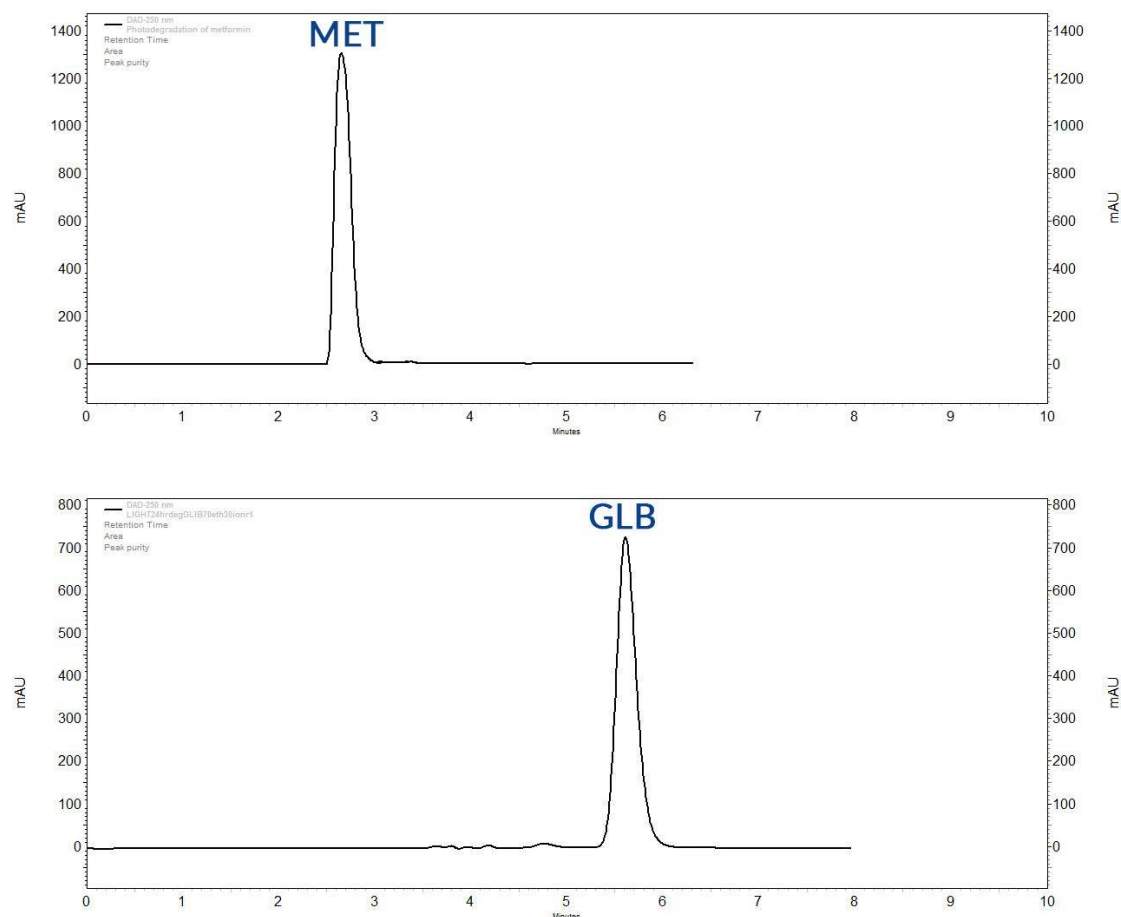


Fig.8. Photolytic degradation of MET and GLB.

Table 1: Assay parameters and regression characteristics for determination of MET and GLB by HPLC method.

Regression parameters	MET	GLB
Regression coefficient (r^2)	0.9999	0.9996
Calibration range ($\mu\text{g mL}^{-1}$)	0.50–100.00	0.50–100.00
Detection limit (LOD) ($\mu\text{g mL}^{-1}$)	0.15	0.16
Quantification limit (LOQ) ($\mu\text{g mL}^{-1}$)	0.50	0.49
Slope \pm SD	$13.45 \times 10^4 \pm 120.81$	$66.76 \times 10^3 \pm 51.59$
Confidence limit of the slope	$13.41 \times 10^4 - 13.49 \times 10^4$	$64.47 \times 10^3 - 68.49 \times 10^3$
Intercept \pm SD	$-50.00 \times 10^2 \pm 73.29 \times 10^2$	$-11.59 \times 10^2 \pm 32.95 \times 10^2$
Confidence limit of the intercept	$-28.32 \times 10^3 - 18.32 \times 10^3$	$-12.96 \times 10^4 - 97.27 \times 10^3$
number of points	5	5

Table 2: Intra-day and Inter-day precision of MET and GLB standard solutions by HPLC method.

Compound	Concentration ($\mu\text{g/ml}$)	Intra-day precision		Inter-day precision	
		Recovery% \pm S.D.	RSD%	Recovery% \pm S.D.	RSD%
MET	5.00	100.05 \pm 0.08	0.07	100.31 \pm 0.67	0.68
	50.00	101.20 \pm 0.12	0.13	101.24 \pm 0.08	0.08
	100.00	99.13 \pm 0.07	0.07	98.79 \pm 0.36	0.37
GLB	5.00	100.19 \pm 0.18	0.18	99.51 \pm 0.43	0.43
	50.00	101.06 \pm 0.76	0.77	101.03 \pm 0.70	0.71
	100.00	99.03 \pm 0.11	0.10	99.05 \pm 0.17	0.17

Table 3: Application of the standard addition technique to the analysis of MET and GLB by HPLC method.

Exp.No.	Claimed taken ($\mu\text{g mL}^{-1}$)		Amount added ($\mu\text{g mL}^{-1}$)		Recovery%	
	MET	GLB	MET	GLB	MET	GLB
1	50.00	0.50	10.00	10.00	100.19	99.84
2	50.00	0.50	20.00	20.00	99.86	99.98
3	50.00	0.50	30.00	30.00	99.75	99.99
4	50.00	0.50	40.00	40.00	98.83	100.26
5	50.00	0.50	50.00	50.00	100.38	100.03
Mean					99.80	100.02
\pmSD					0.60	0.15

Table 4: Determination of MET and GLB in laboratory prepared mixtures using the proposed HPLC method.

Exp.No.	Conc ($\mu\text{g mL}^{-1}$)		%Recovery	
	MET	GLB	MET	GLB
1	50.00	0.50	100.03	98.00
2	40.00	0.70	100.60	100.08
3	30.00	0.90	97.99	98.89
4	60.00	1.00	100.05	99.45
5	70.00	1.50	99.15	100.01
6	80.00	2.00	98.98	99.60
Mean			99.47	99.34
\pm SD			0.94	0.78

Table 5: Robustness testing of the developed HPLC method for the assay of MET and GLB.

Studied parameter	MET				GLB			
	Tested level	Retention time (min)	Tailing factor	Recovery (%)	Retention time (min)	Tailing factor	Recovery (%)	
Phosphate buffer pH	2.80	2.78	1.16	100.10	5.62	1.05	99.90	
	3.00	2.80	1.14	99.70	5.60	1.02	100.00	
	3.20	2.82	1.15	100.20	5.65	1.03	99.70	
Abs <u>EtoH</u> in the mobile phase (%)	68.00	2.66	1.17	100.03	5.63	1.06	100.60	
	70.00	2.80	1.14	100.00	5.60	1.02	99.74	
	72.00	2.79	1.32	100.20	5.71	1.04	100.14	
Flow rate (mL min^{-1})	0.90	2.91	1.11	99.60	5.64	1.04	99.66	
	1.00	2.80	1.14	99.90	5.60	1.02	100.13	
	1.10	3.72	1.13	100.10	5.58	1.05	99.80	
SD				0.21	SD		0.30	

Table 6: The system suitability test results of the developed method for determination of MET and GLB.

Comp.	Retention time (min) ^a	Capacity factor (k')	Selectivity (α)	Resolution (Rs)	Tailing factor	Plate count (N)
MET	2.80±0.13	0.73	3.51	1.02	1.14	1098
GLB	5.60±0.11	2.60			1.02	1392

^a Mean ± SD, n = 7.

Table 7: Analysis of the pharmaceutical dosage forms with the developed HPLC method.

Sample no.	Concentration ($\mu\text{g mL}^{-1}$)		%Recovery	
	MET	GLB	MET	GLB
1	50.00	0.50	99.90	99.30
2	60.00	0.60	99.60	100.20
3	70.00	0.70	99.70	99.80
4	75.00	0.75	100.30	99.10
5	80.00	0.80	99.30	100.50
6	90.00	0.90	100.10	100.30
7	100.00	1.00	100.70	100.60
Mean			99.94	99.97
±SD			0.47	0.59

Table 8: Calculations of the Analytical Eco-Scale scores for the analysis method.

Reagent	Sub-total PP	Total PP
• Ethanol (<10ml)	1 x 4	4
• Potassium dihydrogen phosphate (<1ml)	1 x 0	0
• Phosphoric acid (<1ml)	1 x 2	2
• <u>Octanesulphonic acid (<1ml)</u>	1 x 4	4
		$\Sigma = 10$
Instrument score		
• Energy		
HPLC-DAD (<0.1 KWh per sample)		2
• Transport		0
• Occupational hazard		0
• Waste (10ml)		2
• No treatment		3
		$\Sigma = 7$
Total penalty points (PP) = 17		
Analytical Eco-Scale total score = 100–17 = 83		

3. Results and Discussion

3.1. Chromatographic condition

The current work shows that reversed-phase column approach can be used to determine MET and GLB simultaneously in their combined dosage form despite large variation in the labeled claimed amount of either of them. The proposed HPLC method used conserved amounts chemicals and reagents, besides it was simple and rapid to perform. The method was able to achieve a good resolution of MET and GLB (**Fig.3**) with and no interference from degradants with total run time of less than six minutes (**Fig.4**). Changes in mobile phase composition, buffer's pH and its composition utilized in the mobile phase were initially used to tune chromatographic conditions.

The resolution between the drugs, the symmetric factor, and the number of theoretical plates were

used to optimize the mobile phase composition. Initially, ethanol and water solvents were selected regarding their environmentally benign characteristics and different ratios were tested. However, because MET had a broad peak shape, water was replaced with potassium dihydrogen phosphate buffer (10.00 mM), and several ratios of ethanol and potassium dihydrogen phosphate buffer were tried to improve the chromatographic performance. Ethanol ratio of 75.00% resulted in a broad peak of MET at around two minutes with GLB retention time of around 10 minutes. It's possible that MET's low retention is due to its strong basic character and high polarity. Different concentrations of octanesulphonic acid were added in a trial to elute GLB earlier and 50.00 mM was found optimum in terms of MET and GLB peak shapes and retention behavior. The reason GLB eluted earlier at higher octanesulphonic acid

concentrations is due to increased locking of hydrophobic tail of C18 column by the ion pairing agent that decreases the affinity of unionized GLB to stationary phase hence eluting faster.

Eventually, the mobile phase with best chromatographic performance based on peak shape and resolution was 70.00% organic (ethanol) to 30.00% aqueous 10.00 mM potassium phosphate buffer, pH 3.0 containing 50.00 mM octanesulphonic acid. After numerous preliminary investigational chromatographic runs, a flow rate of 1.00 mL/minute was chosen for further experiments. Various wavelengths from 210 nm to 300 nm were scanned for simultaneous detection of both drugs, and 250 nm was chosen as the wavelength for analysis due to better signal intensity shown by both GLB and MET

3.2. Method Validation

The developed method was validated for the parameters of system suitability, linearity, range, LOD, LOQ, precision, accuracy, selectivity, robustness, and solution stability, as detailed below.

3.2.1. Linearity

The standard solutions comprising both MET and GLB at five different concentrations were analyzed to determine linearity. For MET and GLB, the response was shown to be linear over the 0.50–100.00 $\mu\text{g mL}^{-1}$ range. The calibration equations and correlation coefficients were calculated using regression analysis of the peak area response regarding drug concentration. In all cases, a linear calibration graph was obtained with a regression equation correlation coefficient greater than 0.999 and equally distributed small residuals, and the results are given in (Table 1). The results showed that the concentration of each drug and the instrumental signal are linearly related.

3.2.2. LOQ and LOD

The detection and quantitation limits were determined using a technique based on the S.D. of the response and the slope, as recommended by the ICH [48]. The theoretical parameters were evaluated in practice and were listed in (Table 1).

3.2.3. Precision

The degree of similarity is used to define the analytical method's precision. The precision as a %RSD was used to evaluate the proposed method. (Table 2) showed the intra- and inter- day precision results. For both drugs, % RSD was less than 2 indicating that the method is precise with respect to

same day orating conditions' variations and day to day dissimilarities.

3.2.4. Accuracy

The accuracy study was carried out by addition known amounts of MET and GLB (within the linearity range concentration) with a known concentration of commercial tablets (standard addition method). The resulting combinations were tested, and the MET and GLB findings were compared to the amount added of each drug (Table 3). The high recoveries of the usual addition approach showed that the proposed method was accurate.

3.2.5. Selectivity

Selectivity was achieved by preparing different mixtures of MET and GLB within the linearity range concentration. The laboratory-prepared mixtures were analyzed according to the previous procedures using the proposed method Satisfactory results were obtained (Table 4) indicating the high selectivity of the proposed methods for determination of MET and GLB.

3.2.6. Robustness

Experimental variables (pH of the mobile phase, percentage of ethanol in the mobile phase, and flow rate) were purposefully changed to determine the robustness of the proposed approach, and the resolution between MET and GLB was evaluated. The method's robustness has been demonstrated by the degree of reproducibility of the results obtained because of tiny deliberate modifications in the procedure parameters (Table 5).

3.2.7. System suitability

The system suitability test is an important step in the development of chromatographic methods since it ensures that the system's resolution and reproducibility are sufficient for the analysis. For the combination of MET and GLB, the resolution, retention time, theoretical plate value, and symmetry were computed, and the findings were provided in (Table 6).

3.2.8. Solution stability

The stability of the method's analytical solutions was investigated by analyzing the standard and sample solutions at various time intervals. During solution stability trials, the % RSD of MET and GLB concentrations was less than 1.00%. At varied time intervals, there was no significant difference in the chromatograms of the

standard and experimental solutions. Furthermore, the lack of degradation peaks demonstrated that the material is stable in the solvent utilized in the experiment. MET and GLB solutions in ethanol showed no chromatographic analysis deviations when kept at room temperature or when stored refrigerated at 4.0 °C for 7 days.

3.3. Assay of tablet dosage form

The proposed validated method was successfully applied to determine MET and GLB in their combined tablet dosage form. Seven replicates determinations were made. Satisfactory results were obtained for each compound in a good agreement with the label claims (**Table 7**).

3.4. Greenness of the proposed method using analytical eco-scale

Calculated penalty points for different parameters of the developed HPLC-DAD method were demonstrated in (**Table 8**). The score (83 out of 100) of the developed method proves that it is an excellent method of analysis in terms of greenness.

3.5. Forced degradation results

Results of forced degradation studies indicated that MET was degraded under acidic, basic, and oxidative degradation conditions moderately. However, MET was nearly stable under sunlight photolytic conditions. GLB was degraded either slowly or moderately under all stress conditions. Both MET and GLB peaks were well resolved in the presence of all degradants. Under acid stress conditions, 96.78% and 99.39% of MET and GLB; respectively were remained intact after 2h (**Fig. 5**). Under basic stress conditions, 88.89% and 86.62% of MET and GLB; respectively were remained intact after 2h (**Fig. 6**). Under oxidative stress conditions, 86.62% and 73.33% of MET and GLB; respectively were remained intact after 2h (**Fig. 7**). Eventually, under sunlight photolytic conditions, nearly MET was found intact with no considerable degradation while 94.19% of GLB was remained intact after 24h (**Fig. 8**).

4. Conclusion

The proposed RP-HPLC method is simple, selective, rapid, accurate, precise, reproducible, robust, sensitive and stability indicating. Therefore, the method was applied to the assay of MET and GLB in tablets dosage form. The approach is also sensitive, as well as the use of an environmentally

friendly mobile phase, straightforward extraction method, a shorter retention time, and the absence of an internal standard. This approach is excellent for regular analysis of MET and GLB in commercially available formulations because of these advantages. In the field of drug/pharmaceutical analysis, the substitution of widely used hazardous solvents and chemicals with novel, harmless, and less toxic alternatives offer ecologically benign alternatives to the more hazardous substances and methods.

5. References

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