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Original Article

A NEW STABILITY INDICATING UPLC METHOD FOR THE DETERMINATION OF TWO ANTIDIABETIC DRUGS IN COMBINATION: APPLICATIONS TO BULK AND TABLET FORMULATION

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ABSTRACT

Objective: This work was intended to develop a rapid and sensitive stability-indicating ultra-performance liquid chromatographic (UPLC) method for the determination of Metformin and Gliclazide simultaneously in their pharmaceutical bulk and tablet formulation.

Methods: Separation was performed on Lunna C18 (100 mm x 2.6 mm, 1.6µ) column by using trifluoroacetic acid buffer: acetonitrile (70: 30, v/v) as a mobile phase at a flow rate of 1 ml/min and a wavelength of detection of 227 nm. Method validation and forced degradation studies were conducted per the respective guidelines of the International Conference on Harmonization.

Results: Retention times under the optimized condition were 1.719 min and 2.845 min for Metformin and Gliclazide, respectively. Linearity ranged between 25.0-375.0 µg/ml for Metformin and 4.0-60.0 µg/ml for Gliclazide with a coefficient of determinations (r^2) of greater than 0.99. The limit of detection values was 0.25 µg/ml for Metformin and 0.04 µg/ml for Gliclazide. Recovery results ranged from 99.63-101.23 %, and the % RSDs for the precision studies were less than 1.11% for both drugs. The % degradations at various stress conditions ranged from 14.0-5.0% for Metformin and 13.3-2.4% for Gliclazide. The analyte peaks were clearly resolved from the degradant peaks in forced degradation studies.

Conclusion: A fast, sensitive and efficient ultra-performance liquid chromatographic method was successfully developed and validated for the concurrent estimation of Metformin and Gliclazide in their combination, and thus the proposed method can be effectively applied for routine quality control works.

Keywords: Gliclazide, Metformin, Stability-indicating, UPLC

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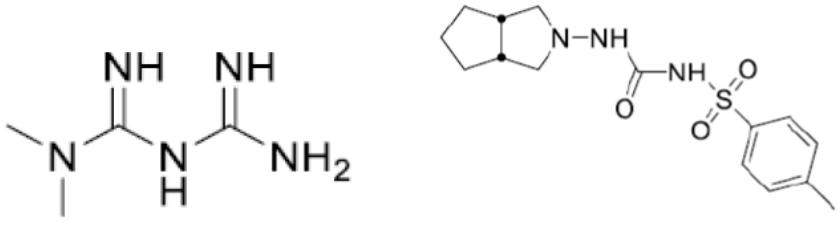
INTRODUCTION

Type 2 diabetes mellitus (T2DM) is among the main public health concerns globally and it accounts for approximately 90% of the total cases of diabetes [1]. Oral hypoglycemics are the mainstream therapeutic requirements for T2DM and currently, several individual agents are marketed from seven major classes, including sulfonylureas, biguanides, meglitinides, thiazolidinediones, *dipeptidyl peptidase-4* inhibitors (DPP-4I), sodium-glucose cotransporter-2 inhibitors (SGLT), and *alpha-glucosidase* inhibitors [2, 3]. In many cases, therapy with a single glucose-lowering agent does not provide adequate glycemic control and consequently, most patients with T2DM require therapy with multiple oral antidiabetics. For patients requiring multiple oral hypoglycemics, the drugs may be given as multiple pills, or as single-pill fixed-dose combinations (FDCs). FDCs have certain advantageous over multiple pills, including convenience, ease of administration, and a reduction in the pill burden. Thus, FDCs potentially improve patients' treatment adherence and optimize the achievement and maintenance of glycemic targets [4, 5]. Among the recently marketed combined oral hypoglycemics, the FDC of Metformin and Gliclazide is most commonly utilized in therapeutic settings [6-8].

Metformin is among the biguanides and is chemically, 3-(diaminomethylidene)-1,1-dimethylguanidine; hydrochloride (fig. 1a). Its hypoglycemic effect is mainly due to the reduction of gluconeogenesis in the liver and enhancement of glucose uptake into the peripheral tissues. Its therapeutic indication is for type II diabetic cases, especially for those obese and individuals with normal kidney function. Gastrointestinal (GI) upset, lactic acidosis, weakness, and muscle pain are among the frequently reported side effects of Metformin [9, 10]. Gliclazide is among the sulfonylureas and is chemically 1-(3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrol-2-yl)-3-(4-methylphenyl)sulfonylurea (fig. 1b). Gliclazide acts mainly on the pancreatic beta cells and thereby increases insulin secretion, which consequently results in reducing blood glucose levels and is mainly indicated for T2DM. The frequently notable adverse effects of Gliclazide are hypoglycemia, gastrointestinal disturbances, rash, and elevation of serum creatinine [11-13].



a b

Fig. 1: Chemical structure of (a) Metformin, (b) Gliclazide

A thorough literature survey disclosed that several analytical and bioanalytical method development and validation reports were available for the assay of Metformin and Gliclazide simultaneously using ultraviolet (UV) spectrophotometry in bulk and formulations [14, 15], High-Performance Liquid Chromatography (HPLC) in bulk and formulations [16-21], High-performance Thin-layer chromatography (HPTLC) in bulk and formulation [22], and HPLC in biological fluids [23]. However, there was no sufficient information reported elsewhere in the literature for the simultaneous determination of Metformin and Gliclazide in bulk and their fixed-dose formulations using UPLC. Hence, we attempted to develop a validated stability-indicating UPLC method for the estimation of Metformin and Gliclazide simultaneously in bulk and dosage formulation because UPLC has better separation efficiency and sensitivity, and it allows faster analysis than the HPLC method [24, 25].

MATERIALS AND METHODS

Chemicals and reagents

The working standards of Gliclazide and Metformin (99.3±0.2 %purity, against pharmacopeial reference standard) were procured from Biocon, Bangalore. Anclazide-M® tablets, labeled to contain Gliclazide (80 mg) and Metformin (500 mg), manufactured by AN pharmaceuticals Pvt Ltd. were purchased from local pharmacy stores. HPLC grade acetonitrile and analytical grade chemicals such as trifluoroacetic acid (TFA), hydrochloric acid, hydrogen peroxide, and sodium hydroxide were procured from E. Merck Limited, Mumbai. Purified water was prepared by using the Borosil double distillation apparatus.

Apparatus and instrumentation

A Waters UPLC system equipped with a photodiode array (PDA) detector and auto sample injector was employed in the study. Waters Empower 2 software was employed to monitor and integrate the output signals. Other instruments and apparatus such as Metler Toledo ME204 analytical balance, Hover Labs LMPH-9 pH meter, Remi ultrasonicator, Millipore vacuum filtration unit, Borosil double distillation apparatus, and Kemi hot air oven were used.

Chromatographic conditions

Analysis was performed on Lunna C18 (100 mm x 2.6 mm, 1.6µ) column with 0.1%TFA buffer: acetonitrile (70: 30, v/v) as a mobile phase. The determinations were carried out using 10 µl injection volume, and the flow was rated at 1.0 ml/min isocratically at 25 ℃, with a total run time of 3.5 min. Analytical outputs were monitored at 227 nm.

Preparation of buffer, mobile phase, and diluent

A 1 ml TFA was added to 1 L purified water, filtered, and sonicated to produce a 0.1% buffer solution of trifluoroacetic acid. Mixing the buffer (0.1% TFA) and acetonitrile in a 70: 30 (v/v) ratio constituted the mobile phase. The same solvent mixture as above was used as the diluent.

Preparation of laboratory prepared mixture

Accurately weighed and transferred 250 mg of Metformin, and 40 mg of Gliclazide pure powders to a 100 ml calibrated volumetric flask. Added approximately 70 ml of diluent, sonicated for 15 min, and then diluted to the volume with the diluent to give a mixture of stock solution containing 0.4 mg/ml, and 2.50 mg/ml of Gliclazide and Metformin, respectively. Transferred 5 ml from the above stock solution into a 50 ml flask, made up to the volume with diluent, and filtered to give a mixed working standard solution containing 40 µg/ml and 250 µg/ml of Gliclazide and Metformin respectively.

Preparation of sample solution

Accurately weighed and transferred 10 tablets labeled to contain 500 mg of Metformin and 80 mg of Gliclazide per tablet to a mortar and finely powdered with a pestle. Transferred a powder equivalent to 250 mg of Metformin and 40 mg of Gliclazide to a 100 ml calibrated volumetric flask, added approximately 70 ml of diluent, sonicated for 15 min, and then diluted to the volume with diluent to give sample stock solution containing a mixture of 0.4 mg/ml, and 2.50 mg/ml of Gliclazide and Metformin respectively. Transferred 5 ml of the resultant solution to a 50 ml volumetric flask, diluted, and filtered to give a working sample solution containing a mixture of 250 µg/ml and 40 µg/ml of Metformin and Gliclazide respectively.

Method validation

The optimized method was validated for linearity, accuracy, precision, robustness, detection limit, quantitation limit, and system suitability per ICH Q2 (R1) guideline [26].

System suitability test

The system suitability test was carried out by performing six replicate injections of a working standard solution containing a mixture of 250 µg/ml of Metformin, and 40 µg/ml of Gliclazide. System suitability parameters were computed to check the resolution and reproducibility of the method.

Specificity

The specificity was checked by performing the analysis of the drugs in laboratory prepared mixtures and sample solutions to examine interfering peaks (if any) at the retention times of the analyte peaks in the chromatograms of blank and placebo.

Linearity

An appropriate volume of aliquots from a mixture of Metformin and Gliclazide standard stock solutions were transferred to seven different volumetric flasks to prepare the calibration solutions. The volumes were adjusted to the point with diluents to give Metformin calibration solutions ranging from 25.0-375.0 µg/ml, and Gliclazide calibration solutions ranging from 4.0-60.0 µg/ml.

Accuracy

Concentrations of both drugs at levels of 50 %, 100%, and 150 % of working standard solutions were prepared and spiked to pre-analyzed sample solutions and performed UPLC analysis each in triplicate. The % mean recovery at each of the concentration levels was computed to determine the accuracy.

Precision

Precision was assessed in terms of repeatability and intermediate precision. For repeatability, six independent determinations of the same homogeneous test sample solution containing a mixture of 250 µg/ml of Metformin, and 40 µg/ml of Gliclazide were conducted against the standard solution. For evaluating the intermediate precision, six independent determinations of test sample solutions containing a mixture of 250 µg/ml of Metformin, and 40 µg/ml of Gliclazide were performed against the reference standard on two consecutive days. The %RSDs were computed to evaluate precision.

Robustness

The robustness was assessed by analyzing working standard solutions of the drugs at variable chromatographic conditions. Solutions were analyzed by the UPLC system at variable flow rate conditions of±0.1 ml/min and organic mobile phase composition of±5%.

Solution stability

To reveal solutions stability during analysis, sample and stock standard solutions were kept in tightly capped volumetric flasks on the laboratory benchtop for 24 h. The same solutions were analyzed at the initial (0 hr) and immediately after 24 h.

Forced degradation study

Forced degradation studies were conducted according to ICH guidelines Q1A (R2) [27]. Acid, alkali, thermal, peroxide, and photo stress conditions were employed in the samples containing a mixture of Metformin and Gliclazide in different ratios. For the acid degradation study, 1 ml of 1N HCl was added to 1 ml sample stock solution; the solution was refluxed at 60 ℃ for 30 min, then cooled to room temperature and neutralized with 1 ml of 1N NaOH. The resultant solution was transferred to a 10 ml volumetric flask, diluted to the volume with diluent, and filtered to yield a working concentration of sample solution containing a mixture of 40 µg/ml,

and 250 µg/ml of Gliclazide, and Metformin, respectively. In the case of alkali stress study, 1 ml of 1N NaOH was added to 1 ml sample stock solution; the solution was refluxed at 60 ℃ for 30 min, then cooled to room temperature and neutralized with 1 ml of 1N HCl. The resultant solution was transferred to a 10 ml volumetric flask, diluted to the volume with diluent, and filtered to yield a working concentration of sample solution containing a mixture of 40 µg/ml, and 250 µg/ml of Gliclazide, and Metformin, respectively. For the oxidative stress study, 1 ml of 30 % H_2O_2 was added to 1 ml sample stock solution; the solution was refluxed at 60℃ for 30 min, and then cooled to room temperature. The resultant solution was diluted in the same way as above in the case of acid and alkali-induced studies. To carry out the thermal stress studies, a sample powder equivalent to 40 mg of Gliclazide and 250 mg of Metformin was placed in an oven at 60 °C for 2 h. Then the powder was transferred to a 100 ml volumetric flask containing 75 ml of diluent, sonicated for 15 min, cooled to room temperature, and made up to the volume with a further quantity of diluent. Transferred 5 ml from the above stock solution into a 50 ml flask, made up to the volume with diluent, and filtered to give a working sample solution containing a mixture of 40 µg/ml, and 250 µg/ml of Gliclazide, and Metformin, respectively. For the photostability study, a sample powder equivalent to 40 mg of Gliclazide, and 250 mg of Metformin was kept in a UV chamber for 24 h. Then the powder was transferred to a 100 ml volumetric flask containing 75 ml of diluent, sonicated for 15 min, and made up to the volume with a further quantity of diluent. Transferred 5 ml from the above solution into a 50 ml flask, made up to the volume with diluent, and filtered to give a working sample solution containing a mixture of 40 µg/ml, and 250 µg/ml of Gliclazide, and Metformin, respectively. Following stress exposure, aliquots of all stressed sample solutions were injected into the UPLC system to check the degradation behavior of the drugs and the stability-indicating capability of the method. Peak purity was determined by computing the purity angle and the threshold angle by employing Empower 2 software. The average value of the angle between each spectrum of the peak and the spectrum at the top of the peak was taken as the purity angle and the sum of the solvent angle and the noise angle was taken as the purity threshold for each analysis.

RESULTS

Method development and optimization

The prime point of focus in the development of this new method was obtaining an optimized chromatographic condition that results in acceptable retentions and better chromatographic peaks in terms of sharpness, symmetry, and resolution within a short run time. After several systematic trials the optimized condition was obtained on Lunna C18 (100 mm x 2.6 mm, 1.6 µ) column, with a mobile phase composed of a mixture of 0.1%TFA and acetonitrile (70:30, v/v) in an isocratic flow at a rate of 1.0 ml/min. The volume of auto sampler injection was adjusted to 10 µl, column temperature was set at 25 ℃, and detection wavelength was chosen at 227 nm. Metformin and Gliclazide were successively eluted at the retention time (RT) of 1.719 min and 2.845 min, respectively, using the optimized condition. The optimized chromatogram is presented in fig. 2.

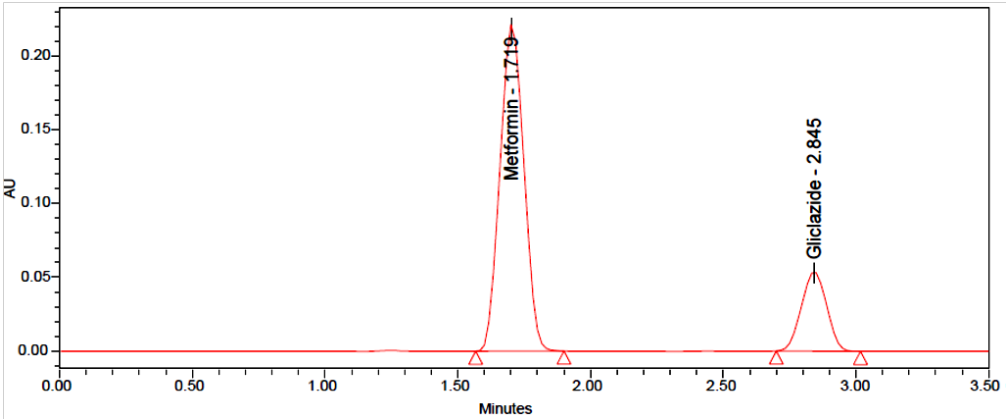


Fig. 2: Chromatogram of the optimized method: Metformin (250 µg/ml) and Gliclazide (40 µg/ml)

Method validation

System suitability test

From the system suitability test, parameters like retention time (RT), capacity factor (k'), selectivity (α), resolutions(R), tailing factor (T), the number of theoretical plates (N), and % RSD were computed and the findings were within the acceptable limit (table 1). The % RSD values for retention times never exceeded 0.52% for both drugs.

The tailing factors were less than 1.2, the number of theoretical plates was greater than 3000, and the resolution was more than 6. The result of the system suitability test is presented in table 1.

Specificity

The specificity chromatograms of the blank and placebo depicted in fig. 3 and 4, respectively indicate that no interfering peaks were observed at the retention times of Metformin and Gliclazide.

Table 1: System suitability data of the proposed method

Parameter	Metformin	Gliclazide	Acceptance criteria [26,28]
Retention time	1.716±0.003	2.845±0.003	
Capacity factor (k')	2.26±0.002	4.41±0.01	*NLT 1
Selectivity (α)	-	1.95±0.002	*NLT 1
Theoretical plate (N)	8463±9.9	3886±11.9	*NLT 2,000
Tailing factor (T)	1.03±0.02	1.15±0.04	**NMT 2
Resolution	-	6.49±0.07	*NLT 2
% RSD	0.52	0.36	**NMT 2

mean±Standard Deviation (SD), n= 6. * Not Less Than, ** Not More Than

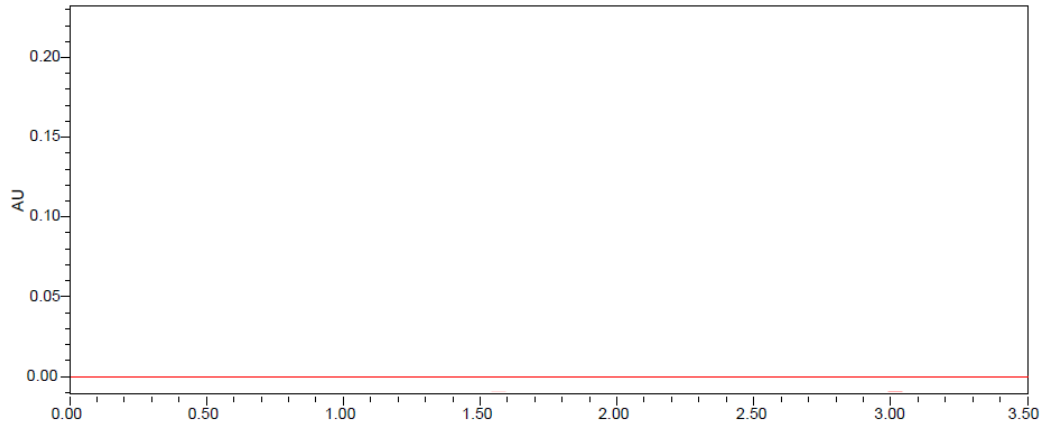


Fig. 3: Specificity chromatogram of blank

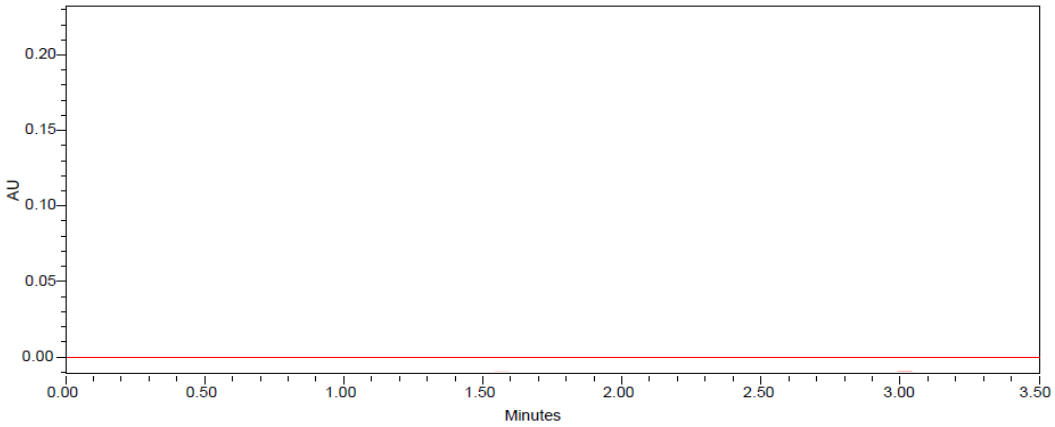


Fig. 4: Specificity chromatogram of placebo

Linearity

The proposed method was linear for both drugs in the investigated concentration ranges of 25.0-375.0 µg/ml for Metformin, and 4.0-60.0 µg/ml for Gliclazide. The linearity equation for Metformin was, y = 94071x+52581 (coefficient of determination, r^2 =0.9993) and the linearity equation for Gliclazide was, y= 12592x+3384 (r^2 =0.9998), where y denotes peak area and x represents the corresponding concentration. The linearity plots for Metformin and Gliclazide are presented in fig. 5 and 6, respectively.

Accuracy

The accuracy of the proposed method was evaluated by performing recovery studies using the standard addition method by spiking the known quantities of working standards at 50, 100, and 150 % each in triplicate to the pre-analyzed samples of Metformin and Gliclazide. The recoveries were found to be 99.63-101.23% for Metformin, and 99.67-100.473% for Gliclazide with % RSD values of less than 1.48. The results demonstrated that the proposed method was accurate. The accuracy result is presented in table 2.

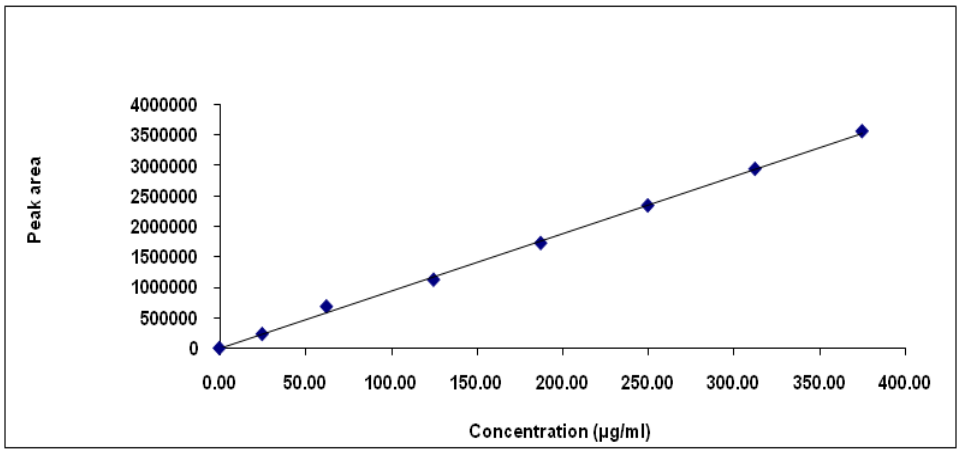


Fig. 5: Standard calibration graph of metformin

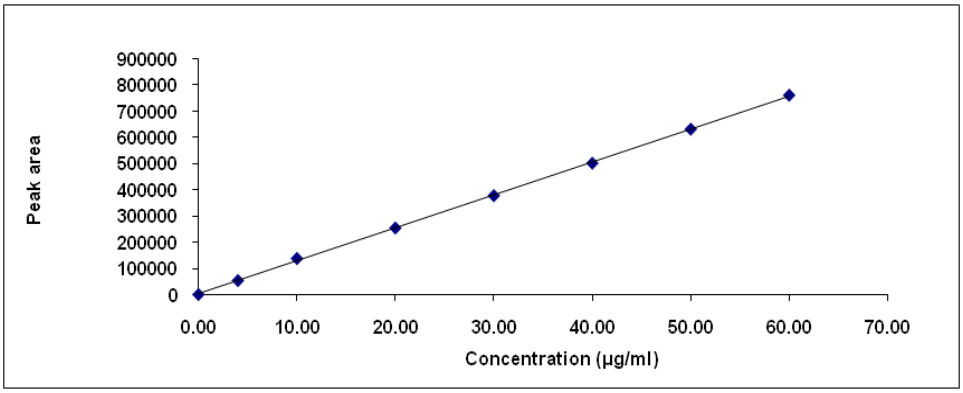


Fig. 6: Standard calibration graph of gliclazide

Table 2: Accuracy data of the proposed method

Recovery level	Amount added (µg/ml)	Peak area	Amount recovered (µg/ml)	% Mean recovery±SD	% RSD
Metformin					
50%	125.0	1159277	124.6	99.63±1.46	1.47
100%	250.0	2336841	251.13	100.43±1.00	0.97
150%	375.0	3531960	379.59	101.23±0.55	0.54
Gliclazide					
50%	20.0	255687	20.14	100.73±0.67	0.66
100%	40.0	503996	39.70	99.73±0.24	0.61
150%	60.0	758785	59.78	99.67±0.71	0.73

mean±SD (n= 3)

Precision

The chromatograms of six injections for method precision studies and six injections for intermediate precision studies were recorded and % RSD values were calculated (table 3). The % RSD of assay values for method precision were 0.91% for Metformin, and 1.11% for Gliclazide, while the % RSD of intermediate precision for the two consecutive days of both drugs was less than 1.1%. The results demonstrated the appropriate precision of the developed method.

Limit of detection (LOD) and limit of quantification (LOQ)

The sensitivity of the proposed method was demonstrated in terms of LOD and LOQ based on the signal-to-noise(S/N) ratio method. analyte solutions at lower concentrations were prepared and analyzed in triplicates. LOD was established by identifying the concentration, which gave a signal-to-noise (S/N) ratio of 3, whereas LOQ was established by identifying the concentration, which gave an S/N ratio of 10. LOD and LOQ result is presented in table 4.

Table 3: Precision data of the proposed method

Injection	Method precision (% Assay)		Intermediate precision (% Assay)			
	Metformin	Gliclazide	Metformin		Gliclazide	
			Day 1	Day 2	Day 1	Day 2
1	101	98.8	100.8	100.5	99.7	99.5
2	99.6	99.4	99.4	99.4	100.7	100.6
3	100.1	101	99.0	98.9	99.5	99.8
4	100.8	99.2	101.6	101.7	101.4	101.5
5	101.7	100.4	100.1	100.2	98.9	99.2
6	99.3	101.6	100.2	99.6	100.3	99.6
Mean	100.4±0.9	100.1±1.1	100.4±1.0	100.3±0.8	100.1±0.9	100.0±0.9
% RSD	0.91	1.11	1.02	0.82	0.9	0.86

mean±SD (n= 6)

Table 4: LOD and LOQ data of proposed method

Parameter	Measured values (µg/ml)	
	Metformin	Gliclazide
LOD	0.25±0.001	0.04±0.001
LOQ	0.825±0.003	0.132±0.0

mean±SD (n= 3)

Robustness

At the studied robust conditions, such as flow rate conditions of±0.1 ml/min and organic mobile phase composition of ±5% no significant change was observed in the analytical output of the method. The system suitability parameters in all the robust studied conditions were within the acceptable limit [26, 28]. Also, % the RSDs of retention times ranged between 0.45%-1.09%,

demonstrating the proposed method was robust. Robustness data is presented in table 5.

Table 5: Robustness data of the proposed method

Parameter	Optimized condition	Robust condition	RT	N	T	R	% RSD
Metformin							
Flow rate	1 ml/min	Less flow (0.9 ml/min)	2.235	2542	1.09	-	0.62
		More flow (1.1 ml/min)	1.394	2249	1.09	-	1.09
Mobile phase composition	Acetonitrile: buffer (30:70, v/v)	Less organic (25: 75%)	2.263	2712	1.11	-	0.70
		More organic (35, 65)	1.404	2617	1.07	-	0.45
Gliclazide							
Flow rate	1 ml/min	Less flow (0.9 ml/min)	3.770	6708	1.02	8.51	0.81
		More flow (1.1 ml/min)	2.732	7561	1.03	7.63	0.75
Organic phase composition	Acetonitrile: buffer (30:70, v/v)	Less organic (25: 75%)	3.785	6376	1.08	8.58	0.50
		More organic (35, 65%)	2.716	7648	1.01	7.48	0.45

Solution stability

The results of stability of solutions confirmed that no significant degradation within the indicated test period (24 h) was observed (<2%). No significant difference was also observed in chromatographic responses, such as peak shape and retention time. Thus, the stock solution can be regarded as stable for at least 24 h.

Forced degradation study

The degradation study results showed that degradant peaks were observed when the drug samples were stressed with alkali, and peroxide, while no apparent degradant peaks were seen in photo and acid degradation. The % degradation for Metformin ranged from 14.0-5.0%, the maximum and minimum degradation was recorded in alkali and photo stress conditions, respectively. For Gliclazide the % degradation ranged from 13.3-2.4%, the maximum, and minimum were observed in acid and photo stress conditions, respectively. All peaks in the degradation studies were clearly resolved, and no co-eluted peaks were observed. The system suitability parameters of the parent drug peaks in all stressed conditions were within the acceptable limit. Results of forced degradation data are presented in table 6 and chromatograms for acid, alkali, and photodegradation studies are presented in fig. 7-9.

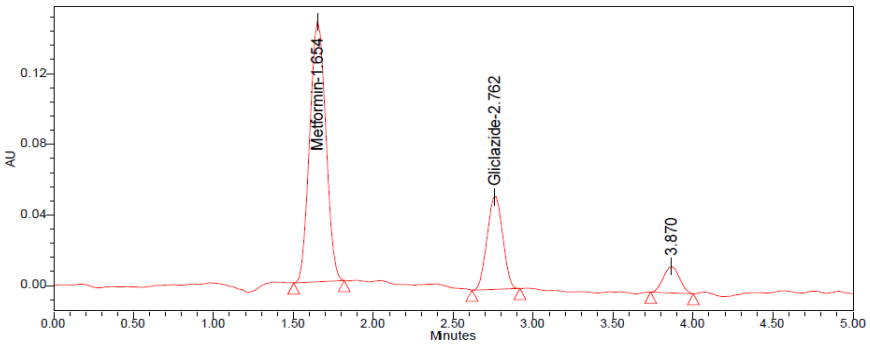


Fig. 7: Chromatogram of acid degradation study

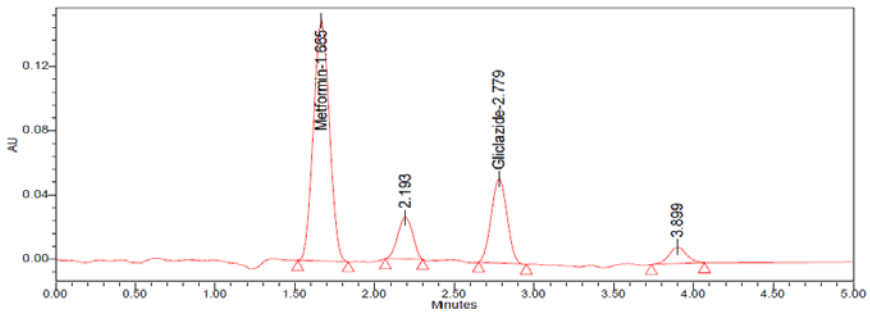


Fig. 8: Chromatogram of alkali degradation study

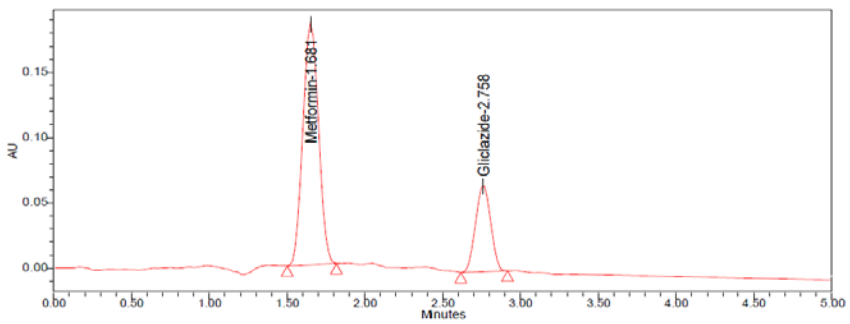


Fig. 9: Chromatogram of photodegradation study

Table 6: Forced degradation study data of the proposed method

	Stress condition					
	Control	**Acid**	**Alkali**	**Photo**	**Peroxide**	**Thermal**
Metformin						
% degradation	-	11.9	14.0	5.0	12.1	13.1
Purity angle	0.138	0.303	0.346	0.352	1.184	0.353
Purity threshold	10.826	10.358	10.346	10.868	10.321	10.863
Plate count	3456	3194	3570	3127	3434	3656
Tailing	1.18	1.08	1.19	1.20	1.04	1.15
Gliclazide						
% degradation	-	13.3	12.6	2.3	11.1	8.8
Purity angle	4.185	2.965	2.929	4.166	2.537	4.155
Purity threshold	10.719	10.576	10.548	10.741	10.616	10.752
Plate count	8154	8596	8637	8508	8028	8175
Tailing	1.06	1.01	1.11	1.13	1.06	1.09
Resolution	5.87	5.89	3.25	6.15	3.03	6.02

DISCUSSION

In comparison to previously reported HPLC methods, the proposed UPLC method is fast and requires less solvent consumption as the total run time was 3.5 min, whereas, in previously reported HPLC methods the total run times were approximately ranged from 8-20 min [16, 18, 20]. The method development result from this study thus supports the theoretical principles and practical observations that describe the advantages of UPLC over HPLC in terms of faster analysis time and cost-effectiveness due to less solvent consumption [24, 25]. More acceptable retention and well separation of the two peaks within a short run time expresses the better separation efficiency and resolution of the UPLC method. Moreover, the proposed UPLC method has a relatively lower limit of detection which suggests its better sensitivity as compared to HPLC methods reported earlier [15-18].

To optimize the proposed method several trials were conducted systematically on chromatographic conditions like column type, PDA wavelength, and mobile phase conditions. PDA detection wavelength screening tests demonstrated that a good peak response of both drugs was recorded at 227 nm, and thus this wavelength was chosen to monitor the output signals. Reverse phase solvents like mixtures of water: organic solvent and buffer: organic solvent were studied at different ratios on UPLC columns to optimize the proposed method. Co-elution of analytes and poor resolution were the majorly encountered problems with trails which included pure water as a mobile phase composition. The incorporation of phosphate and formic acid buffer as an aqueous phase composition of the mobile phase improved the resolution but peak tailings were beyond the acceptable limit. Consequently, 0.1%TFA buffer and acetonitrile were tried in isocratic mode (1 ml/min) at various compositions, and the desired peaks fulfilling all the parameters including sharp, symmetric, and well-resolved peaks were obtained with a 70:30, v/v ratio of the buffer and organic solvent (acetonitrile). Eventually, the use of Lunna C18 (100 mm x 2.6 mm, 1.6 µ) column with the optimized mobile phase composition at ambient column temperature conditions gave good chromatographic results for Metformin and Gliclazide.

As per the validation results, all the parameters were within the acceptable limits of ICH guidelines [26, 28]. The system suitability test results evidenced that more efficient separation (N>2000), with well-resolved (R>2) and symmetric peaks (T<2) were obtained consistently. Moreover, the analytical output was reproducible since the %RSD for the retention time of six system suitability determinations was less than 2%. The method was found to be selective because formulation excipients didn't interfere with blank and placebo determinations. The r^2 obtained from the least square regression analysis for both drugs was closer to 1 which indicates the better linearity of the developed method. Falling of the recovery results within 98%-102% reveals the accuracy of the method and, the %RSD values for repeatability and intermediate precision determinations never exceeded 1.11% showing the better precision of the proposed UPLC method. Analytical results from a deliberate slight variation on the optimized method condition indicated that variations have no significant influence on the analytical output and thus the method was robust.

No degradant peak was co-eluted with the analyte peaks in the chromatograms of stressed studies (fig. 7-9). The drug peaks were pure since purity angle values were less than purity threshold values in all the stressed conditions. From the results of forced degradation studies, it can be recommended that the method is capable to separate and quantify the analytes in the presence of their degradation products [27]. Thus, the validated UPLC method was stability-indicating.

CONCLUSION

A new ultra-performance liquid chromatographic method was successfully developed and validated for the quantification of Metformin and Gliclazide simultaneously in bulk and formulation. The method was demonstrated to be stability-indicating, fast, sensitive, accurate, precise, and robust. Thus, the proposed method can be effectively applied for routine quality control works.

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AUTHORS CONTRIBUTIONS

Both the authors have contributed equally.

CONFLICT OF INTERESTS

The authors have declared that no conflict of interest exists.

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