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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF GLIBENCLAMIDE AND METFORMIN IN BULK AND PHARMACEUTICAL DOSAGE USING RP-HPLC

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ABSTRACT

Objective: A New method was established for simultaneous estimation of Metformin and Glibenclamide by RP-HPLC method and Its force degradation study. **Methods**: Chromatographic separations were carried using Agilent C8, (150 X3834.6 mm, 5µm) column with a mobile phase composition of 0.1 M 0.1 M Ammonium acetate (0.1 M): Methanol (70:30) have been delivered at a flow rate of 1ml/min and the detection was carried out using waters HPLC auto sampler, separation module 2695 HPLC system with PDA detector at wavelength 254 nm. **Results**: The retention time for Metformin and Glibenclamide were 3.17 and 8.10 minute respectively. The correlation coefficient values in linearity were found to be 0.999 and concentration range 40-60 µg/ml for Metformin and 40-60 µg/ml for Glibenclamide respectively. For accuracy the total recovery was found to be 100.63 % and 101.79 % for Metformin and Glipalamide. LOD and LOQ for Metformin 2.936 and 9.78. LOD and LOQ for Glibenclamide 2.92 and 9.75. Metformin and Glibenclamide were subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. Metformin and Glibenclamide are more sensitive towards acidic and Thermal degradation. **Conclusion**: The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of of Metformin and Glibenclamide in tablet dosage form.

KEYWORDS: Metformin, Glibenclamide, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Glibenclamide is a second-generation sulfonylurea used to treat patients with diabetes mellitus type II. It is typically given to patients who cannot be managed with the standard first line therapy, metformin. Glyburide stimulates insulin secretion through the closure of ATP-sensitive potassium channels on beta cells, raising intracellular potassium and calcium ion concentrations.[1] IUPAC name is 5-chloro-N-[2-(4-{[(cyclohexylcarbamoyl)amino] sulfonyl} phenyl) ethyl]-2-methoxybenzamide. Molecular weight 494.0. Chemical formula $C_{23}H_{28}ClN_3O_5S$. Glyburide (potassium salt) is soluble in organic solvents such as DMSO and dimethyl formamide. The solubility of glyburide (potassium salt) in these solvents is approximately 10 and 2 mg/ml, respectively.

Metformin is an antihyperglycemic agent of the *biguanide* class, used for the management of type II diabetes). Currently, metformin is the first drug of choice for the management of type II diabetes and is prescribed to at least 120 million people worldwide. Metformin is considered an antihyperglycemic drug because it lowers blood glucose concentrations in type II diabetes without causing hypoglycemia.[2-4] Metformin is commonly described as an *insulin sensitizer* leading to a decrease in insulin resistance and a clinically significant reduction of plasma fasting insulin levels. Another well-known benefit of this drug is modest weight loss. Metformin is the drug of choice for obese type II diabetes patients. IUPAC name is 1-carbamimidamido-N, N-dimethylmethanimidamide. Chemical formula $C_4H_{11}N_5$. Molecular weight 129.16. Metformin hydrochloride, USP 2.0 g is soluble in 20 mL of water. The pKa of metformin is 12.4. The pH of a 1% aqueous solution of metformin hydrochloride is 6.68. It is freely soluble in water; slightly soluble in alcohol; practically insoluble in acetone and in methylene chloride.

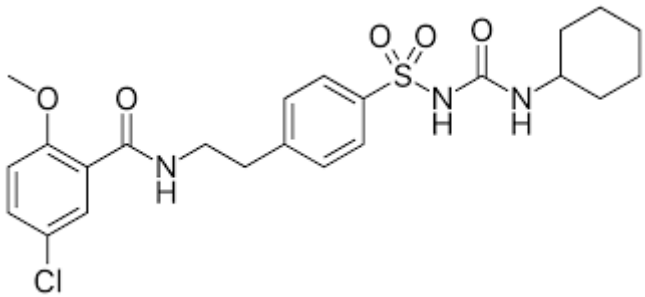


Figure 1: Structure of Glibenclamide.

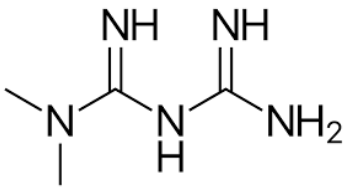


Figure 2: Structure of Metformin.

The review of literature revealed that very few ew analytical techniques were developed for the combined dosage forms of Glibenclamide and Metformin such as HPLC[5-9], spectrophotometry[10], LC /(API)MS[11] and in the present study the authors have developed two different spectrophotometric methods for the simultaneous assay of Glibenclamide and Metformin tablets and the methods were validated as per ICH guidelines.[12]

MATERIALS AND METHODS

Chemicals and Reagents: Metformin and Glibenclamide were obtained from Sun Pharma India Limited, Hyderabad. NaH_2PO_4 was analytical grade supplied by Sd Fine-chem limited, Orthophosphoric acid (Sd Fine-chem limited), and Water for HPLC (Sd Fine-chem limited), Methanol for HPLC (Sd Fine-chem limited).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 254 nm with column Agilent C8, (150 X3834.6 mm, 5µm) dimensions at 30^0C temperature. The optimized mobile phase consists of 0.1 M Ammonium acetate (0.1 M): Methanol (70:30). Flow rate was maintained at 1 ml/min and run time for 6 min.

Preparation of solutions

Preparation of buffer

Weigh and change precisely about 7.708g Ammonium acetate into a beaker containing 1000ml of water and sonicate to dissolve. Filter the solution through 0.47 µm membrane filter.

Preparation of mobile phase

The mobile stage utilized in this assessment includes a blend of Ammonium acetate and35mehonol in a ratio of 70:30. 700 ml of buffer was added and also properly combined with 300 ml of Ammonium acetate as well as an identical option is achieved. This mobile phase was fill up andsonicated for 15 minutes before utilizing in the experiment.

The diluents

The Mobile phase was used as the diluent.

Preparation of standard stock solution

Exactly determined as well as moved about 400mg of Metformin and also 10mg of Glibenclamide into a 50ml 3volumetric flask, include diluents as well as sonicate about 30ml for 30min with midway shaking (keep up the sonicator shower temperature level between20-25 ◦ C). Make up to the volume with diluent and blend. Channel a little bit of the arrangement via 0.45 film channel as well as get rid of of initial very few ml of the filtrate. Relocate 5 ml of the apart plan into a 25mlvolumetric flagon, compromise to quantity with diluent promotion mix.

Preparation of Sample stock solution

Accurately weigh 400mg of Metformin and 10mg of Glibenclamide into a 50ml volumetric jar, consist of about 30ml of diluents and sonicate for 30min with modest shaking (maintain the sonicator shower temperature between20-25 ◦ C). Make up to the quantity with diluent and also mix. Channel a part of the arrangement through 0.45 µmlayer channel and also get rid of preliminary not many ml of the filtrate. Move 5 ml of thesifted arrangement into a 25ml volumetric cup, deteriorate to volume with diluent advertisement blend.

Procedure: 10 µL of standard and sample solutions were injected into the LC-system and measure the peak areas for Metformin and Glibenclamide.

METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 6 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Agilent C8, (150 X3834.6 mm, 5µm), the mobile phase of composition 0.1 M Ammonium acetate (0.1 M): Methanol (70:30) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Metformin and Glibenclamide in their tablet dosage form. The result obtained for Metformin and Glibenclamide was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method

Linearity and Range: Solutions containing 800 µg/ml,1200 µg/ml,1600 µg/ml,2000 µg/ml,2400µg/ml, metforminconcentrations and 20 µg/ml,30 µg/ml,40 µg/ml,50 µg/ml,60µg/ml, glibencamide concentrations corresponding to 50, 75, 100, 125 and 150 percent of the concentration of the test solution, respectively, were prepared. Every solution was injected and linearity was calculated by linear-regression analysis. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Metformin and Glibenclamide and calculate the individual recovery and mean recovery values. The resulte are shown in table 4.

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1ml/min. The Organic composition in the Mobile phase was varied from 35% to 45%. The resulte are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 8.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

Forced degradation studies: Forced destruction researches were carried out on Metformin and Glibenclamide to show the stability indicating residential or commercial property of the method. The tension conditions utilized for deterioration study includes light exposure, acid hydrolysis (0.1 N HCL), base hydrolysis (0.1 N NAOH), water hydrolysis, Oxidation (3% Hydrogen peroxide), UV light exposure.

The period of time selected for destruction studies was hours. Thephotolyticdegradation was done by exposing the solid drugs to sunshine for 6 hrs. The concentration of 100 mcg/ml of each of Metformin and Glibenclamide were prepared utilizing particular solvents (NAOH, HCL, water and also Hydrogen peroxide) separately. The final concentration of 2.5 mcg/ml Metformin as well as Glibenclamide were prepared from the above discussed stock solutions after making up with the mobile stage. The final focus of these medications are analyzed in the HPLC. The % destruction of both the 3drugs was found to be more in the alkali hydrolysis. Force degradation results are shown in table 9.

RESULTS AND DISCUSSION

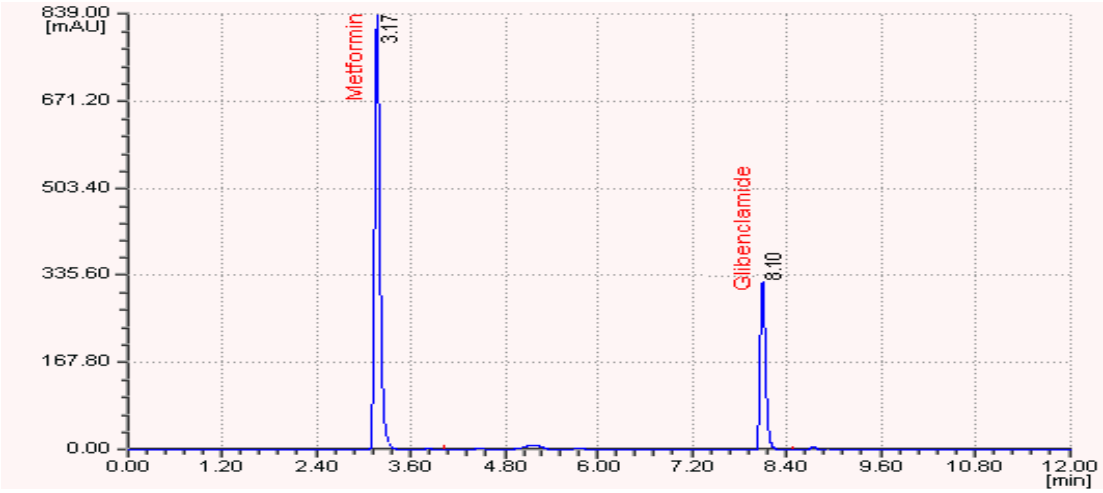


Figure 3: Standard chromatogram.

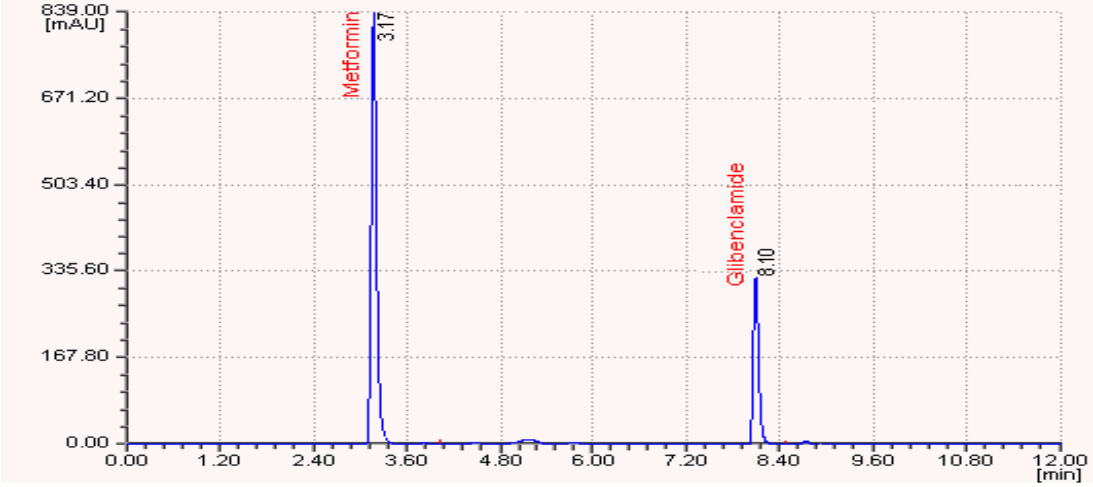
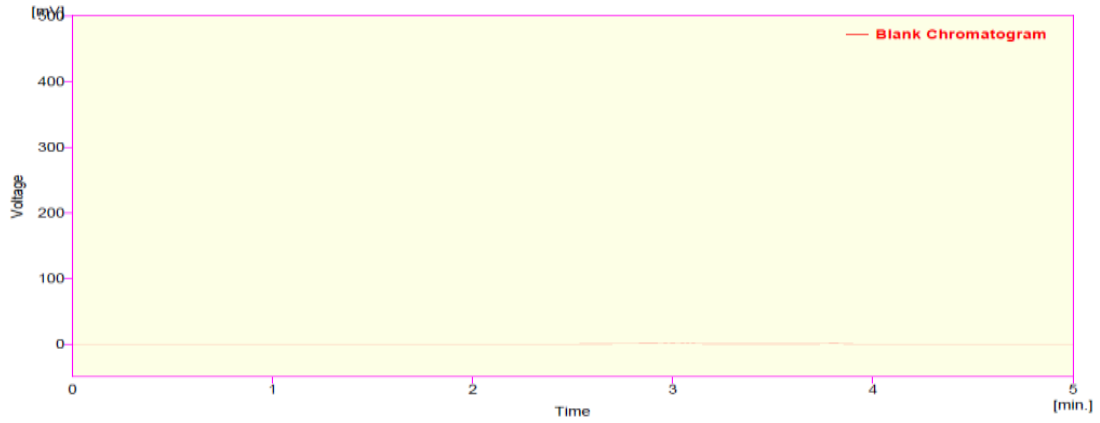


Figure 4: Sample chromatogram.



Result Table (Uncal - Blank Chromatogram)

Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
No peak to report			

Figure 5: Blank chromatogram.

Table 1: System suitability parameters.

Parameters	Metformin	Glibenclamide
Retention time	2.936	9.786
USP Plate count	5986	7522
USP Tailing	1.39	1.30

Table 2: Assay results for Metformin and Glibenclamide.

	Label Claim (mg)	% Assay
Metformin	150	101.03
Glibenclamide	300	99.95

Table 3: Linearity results for Metformin and Glibenclamide.

S.No:	Concentration(µg/ml)	Peak Area
1	20ppm	463725
2	30ppm	666868
3	40ppm	899612
4	50ppm	1138221
5	60ppm	1375426
6	70ppm	1593767
Mean		1134843
Coefficient of Corelation		0.998

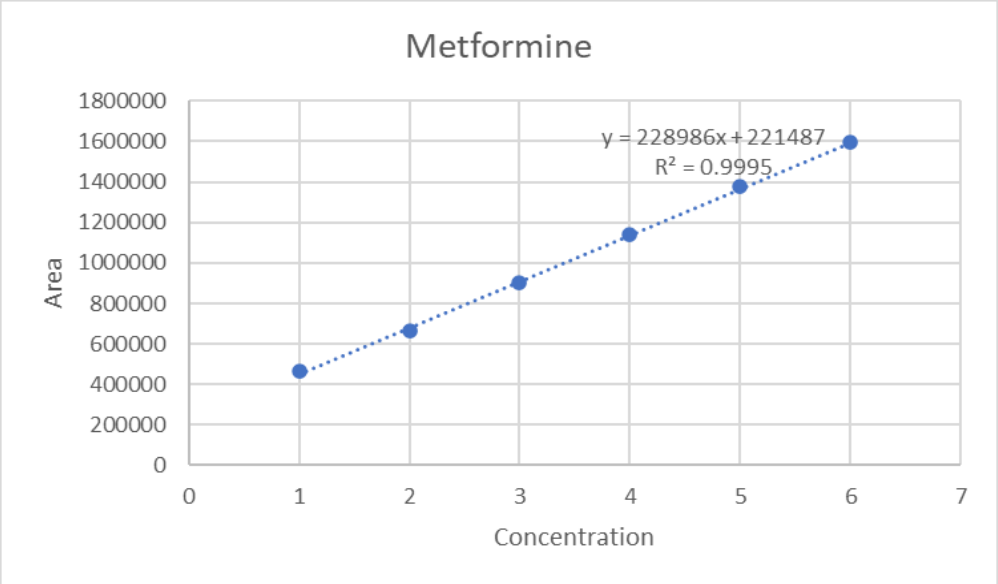


Figure 4: Linearity graph for Metformin.

S.No:	Concentration(µg/ml)	Peak Area
1	20ppm	467525
2	30ppm	668668
3	40ppm	899412
4	50ppm	1128421
5	60ppm	1365426
6	70ppm	1594287
Mean		1131243
Co-relation Coefficient		0.999

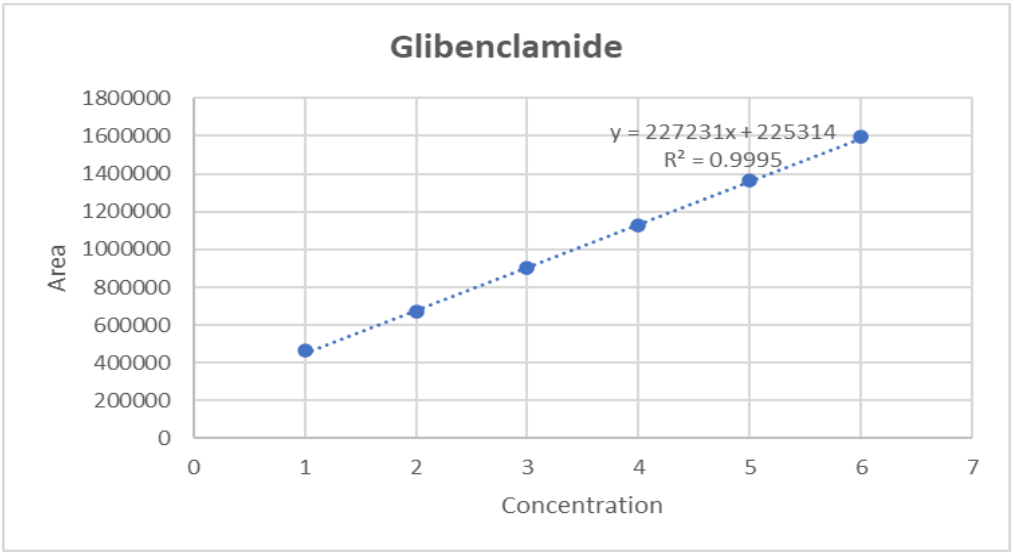


Figure 5: Linearity graph for Glibenclamide.

Table 4: Showing accuracy results for Metformin and Glibenclamide.

Sample ID	Concentration (µg/ml)	Amount Found	Rt	Peak Area	USP Plate Count	USP Tailing	Pure drug	% Recovery
S1 : 50 %	40	40.71	3.459	935684	3451	1	101.775	Mean= 101.440%
S2 : 50 %	40	40.272	3.46	925689	3465	1.1	100.68	
S3 : 50 %	40	40.747	3.461	936523	3448	1	101.867	% R.S.D.= 0.65098%
S4 : 100 %	50	50.778	3.462	1165243	3964	1.4	101.556	Mean= 101.556%
S5 : 100 %	50	50.784	3.461	1165382	3985	1.3	101.568	
S6 : 100 %	50	50.772	3.459	1165121	3958	1.4	101.544	%R.S.D.= 0.01181%
S7 : 150 %	60	59.559	3.464	1365482	3797	1.6	99.265	Mean= 100.243%
S8 : 150 %	60	60.436	3.463	1385462	3746	1.6	100.726	
S9 : 150 %	60	60.443	3.464	1385643	3789	1.6	100.738	%R.S.D. = 0.84494%

Table 5: Precision results for Metformin and Glibenclamide.

HPLC Injection	Rt	Peak Area	USP Plate Count	USP Tailing
Replicates of glibencamide and metformin				
Replicate – 1	3.461	1065243	3986	1.5
Replicate – 2	3.46	1056842	3956	1.4
Replicate – 3	3.459	1065341	3987	1.5
Replicate – 4	3.461	1064512	3926	1.4
Replicate – 5	3.46	1056864	3963	1.5
Replicate – 6	3.459	1056845	3951	1.4
Mean	3.46	1060941	3961.5	1.45
Standard Deviation	0.000894	4490.439		
% RSD	0.02585	0.42325		

Table 6: Intermediate precision resultes for Metformin and Glibenclamide.

S.No	Sample Weight	GPT	NTL	GPT	NTL
		sample area	Sample area	% Assay	% Assay
1	765.55	3364530	4601901	99.01	99.68
2	765.55	3364541	4606209	99.01	99.77
3	765.55	3365736	4603520	99.04	99.71
4	765.55	3371195	4614415	99.2	99.95
5	765.55	3371160	4609760	99.2	99.85
6	765.55	3370202	4614992	99.17	99.96
Avg Assay:				99.11	99.82
STD				0.1	0.12
% RSD				0.1	0.12

Table 7: Robustness results for Metformin and Glibenclamide.

S.No	Parameter changing	Area	USP Tailing	USP Plate count	metformine	glibencamide
1	Temp1	4686059	1.36	8709	3.59	---
2	Temp2	4691248	1.36	8734	3.59	---
3	Flow1	6073408	1.37	9828	4.48	---
4	Flow2	3883341	1.33	8081	2.99	---
5	Temp1	3236283	1.51	8648	---	2.67
6	Temp2	3278704	1.49	8690	---	2.67
7	Flow1	4162866	1.55	9585	---	3.35
8	Flow2	2683053	1.48	8363	---	2.22

Table 8: LOD, LOQ of Metformin and Glibenclamide.

Drug	LOD	LOQ
Metformin	2.936	9.786
Glibenclamide	2.927	9.756

Table 9: Degradation results for Metformin and Glibenclamide.

Stress Condition	Time	Assay of Active Substance	Assay of Degraded Products	Mass Balance (%)
Standard drug	-----	100	-----	100
Acid Hydrolysis (0.1N HCl)	24Hrs.	99.06	0.94	100
Basic Hydrolysis (0.IN NaOH)	24Hrs.	90.54	9.46	100
Thermal Degradation	24Hrs.	99.13	0.87	100
UV (254nm)	24Hrs.	89.5	10.5	100
3 % Hydrogen peroxide	24Hrs.	94.51	5.49	100

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Metformin and Glibenclamide in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Metformin and Glibenclamide in bulk and pharmaceutical dosage forms.

REFERENCES

1. Gribble FM, Reimann F: Sulphonylurea action revisited: the post-cloning era. Diabetologia, 2003 Jul; 46(7): 875-91. doi: 10.1007/s00125-003-1143-3. Epub 2003 Jun 18.
2. Witters LA: The blooming of the French lilac. J Clin Invest, 2001 Oct; 108(8): 1105-7.
3. UNGAR G, FREEDMAN L, SHAPIRO SL: Pharmacological studies of a new oral hypoglycemic drug. Proc Soc Exp Biol Med., 1957 May; 95(1): 190-2.
4. Lord JM, Flight IH, Norman RJ: Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ. 2003 Oct 25; 327(7421): 951-3.
5. Subhashini Edla and B. Syama Sundhar. New analytical method development and validation for the simultaneous estimation of Metformin and Glibenclamide in bulk and tablet dosage form using RP-HPLC. Rasayan Journal of Chemistry, 2014; 7(1): 55-63.
6. Asit Kumar De, Ayan Kumar Dey and Angshuman Biswas. Simultaneous estimation of Metformin hydrochloride and Glibenclamide by RPHPLC method from combined tablet dosage form. International Journal of Science Inventions Today, 2012; 1(2): 98-105.
7. Bhavika RC, Patil PB, Bavaskar SR, Barhate SD. Development and validation of analytical method for simultaneous estimation of Glibenclamide and Metformin HCl in bulk and tablets using UV – Visible Spectroscopy. World Journal of Pharmaceutical Research, 2015; 4(11): 1257-1266.
8. Waje MK, Barge VU and Talole B. Development and validation of stability indicating assay method for simultaneous estimation of Glibenclamide and Metformin. International Journal of Pharmaceutical Quality Assurance, 2017; 8(3): DOI: 10.25258/ijpqa.v8i03.957
9. B.Venkata Siva Rao and K. Anil Kumar. Analytical method development and validation for the assay of Metformin and Glibenclamide in bulk form by using RP-HPLC method. International Journal of Trends in Pharmacy and Life Sciences, 2015; 1(5): 676-682.
10. Nabil. A. F. Alhemiarya. Derivative spectrophotometric and HPLC validated methods for simultaneous determination of Metformin and Glibenclamide in combined dosage form. Oriental Journal of Chemistry, 2014; 30(4): 1507-1516.
11. Georgita C, Albu F, David V, Medvedovici A. Simultaneous assay of Metformin and Glibenclamide in human plasma based on extraction-less sample preparation procedure and LC/(APCI)MS. J Chromatogr B Analyt Technol Biomed Life Sci., 2007; 854(1-2): 211-218.
12. ICH Validation of analytical procedures: Text and methodology, Q2 (R1), International Conference on Harmonizati.