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STABILITY INDICATING RP – HPLC METHOD FOR DETERMINATION OF GLIBENCLAMIDE & METFORMIN HCL IN PURE AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the determination of Glibenclamide & Metformin HCL in pure and tablet forms. The method showed a linear response for concentrations in the range of 10-50µg/ml & 500-2500µg/ml using Methanol: Water solution in the ratio (70:30) as the mobile phase with detection at 226 nm and a flow rate of 1 ml/min and the retention time for Glibenclamide and Metformin was found to be 9.2 and 3.8 min respectively.. The value of correlation coefficient were 0.999, &0.998. The method was validated for precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, peroxide and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability indicating one.

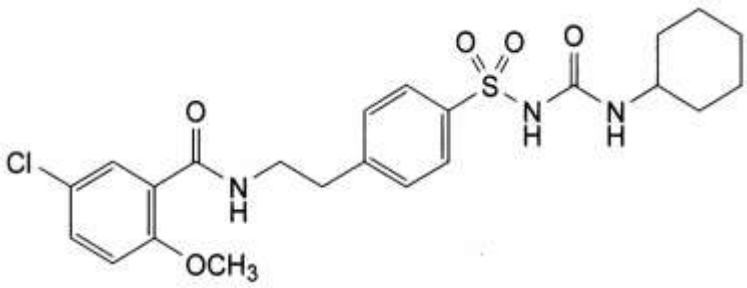
KEYWORDS: Glibenclamide, Metformin HCL, RP-HPLC, Degradation studies.

INTRODUCTION

Glibenclamide

Glibenclamide acts as anti-diabetic drug belongs to the class of sulfonylureas commonly known as sulfa drugs. The drug is effectively used in the treatment of type 2 diabetes. It is also helpful in improving the outcoming results on animal stroke models by preventing brain swelling and enhancing neuroprotection. The mechanism of action of drug is illustrated by

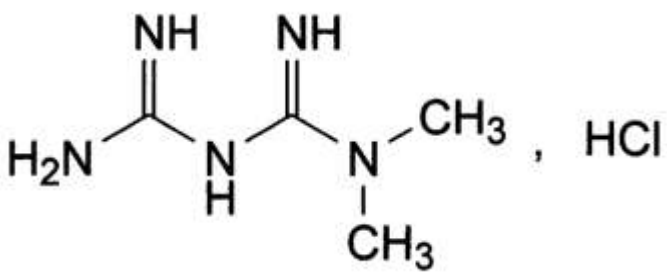
binding and activation of the sulfonylurea receptor 1 (SURI 1), which is considered as regulatory subunit of the ATP-sensitive potassium channels, in pancreatic beta cells (KATP). This leads to cell membrane depolarization opening voltage-dependent calcium channel due to inhibition. The increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release is resulted by the membrane depolarization of the cell membrane of pancreas. It restricted to patients suffering with G6PD deficiency as it may cause acute haemolysis. Recent studies reveled that Glibenclamide is associated with significantly higher annual mortality when combined with metformin than other insulinsecreting medications, and has potential to lower some of side effects. The common side effects of the intaking drug are hypoglycemia and Cholestatic jaundice. of glucose.



Chemical Structure of Glibenclamide

Metformin Hydrochloride

Metformin Hydrochloride is 1,1-Dimethylbiguanide hydrochloride, and is used in the treatment of diabetes mellitus. It is completely different from the hypoglycemic sulfonamides both in its structure and its mode of action. It possibly interferes with mitochondrial respiratory chains and promotes peripheral glucose utilization by enhancing anaerobic glycolysis or it enhances binding of insulin to its receptors and potentiates its action. Other explanation is that it suppresses hepatic gluconeogenesis and inhibits intestinal absorption of glucose. It causes little or no hypoglycemia in non diabetic patients.



Chemical Structure of Metformin HCL

EXPERIMENTAL

MATERIALS AND METHODS

Gift sample of Glibenclamide &Metformin HCL ware received from Emcure Pharmaceutical PVT.LTD. Commercial formulations Daonil-M containing 5mg of Glibenclamide & 500mg of Metformin HCLwere purchased from the local market.

INSRUMENTATION

The analysis of the drug was carried out on a Younglin (S.K) Gradient System UV Detector. Equipped with a Reverse phase (Thermo) C18 Column (4.6mm x 250 mm; 5µm), a SP930D pump, a 20µl injection loop and a UV730D Absorbance detector and running on Autochro-3000 software.

Chromatographic conditions

Mobile phase consist mixture of methanol-water in the ratio of 70:30%v/v (PH was adjusted with 0.1% Triethanolamine). The mobile phase was pumped from the solvent reservoir to the column at flow rate 1ml/min. column temperature was maintained at Ambient. UV Detection performed at 226nm.the mobile phase was degassed by an ultrasonic water bath for 5min.filter through 0.45µ filter under vacuum filtration. The column was equilibrated for at least 30min with mobile phase flowing through the system. Mobile phase used as diluents during the standard and test sample preparation.

Selection of detection wavelength

UV detector was selected, as it is reliable and easy to set at constant wavelength. A fix concentration of analyte were analysed at different wavelengths. As per the response of analyte, 226 nm Wavelength was selected.

Preparation of Standard Solutions

Accurately weigh and transfer 10mg of Glibenclamide & Metformin HCL working standard into two seperate 10 ml volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent To get 1000µg/ml standard (Stock solution) Further An aliquots portion of Glibenclamide stock solution &Metformin stock solution in the ratio of 1:100 were mixed in volumetric flask (10.0 ml) and volume was adjusted up to mark with methanol as mobile phase Mix well and filter through 0.45µm filter**.** The simple chromatogram are shown in fig no. 1.

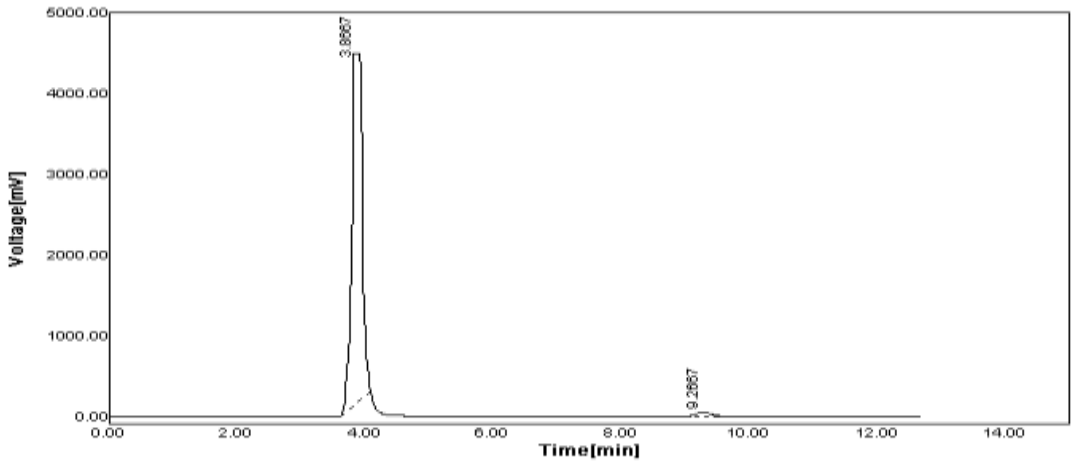


Fig No.1 The Simple Chromatogram of Standard Glibenclamide & Metformin HCL

Preparation of calibration graph

The linearity of response for Glibenclamide & Metformin assay method were determined by preparing and injecting solutions with concentrations of about 10,20,30,40,50µg/ml of Glibenclamide &500,1000,1500,2000,2500µg/ml . Linearity curves of Glibenclamide & Metformin HCL shown in fig. no.2 & 3.

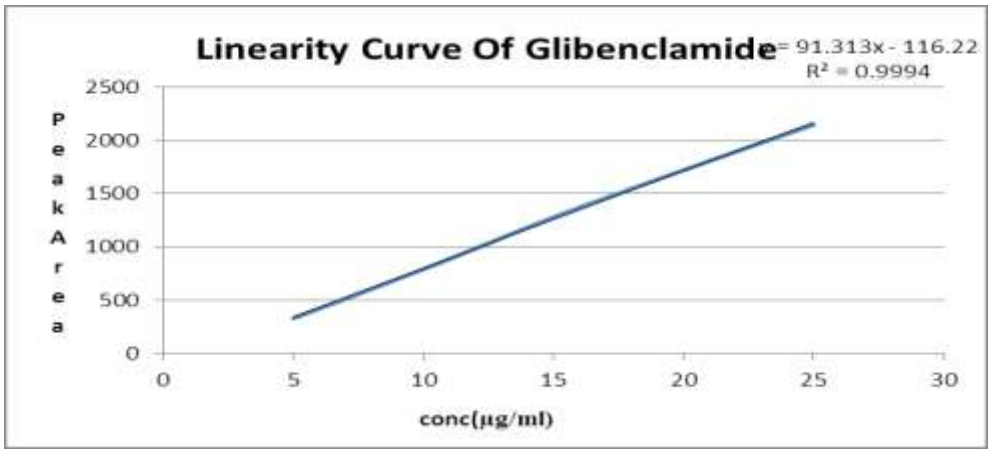


Fig.No.2 Linearity curve of Glibenclamide

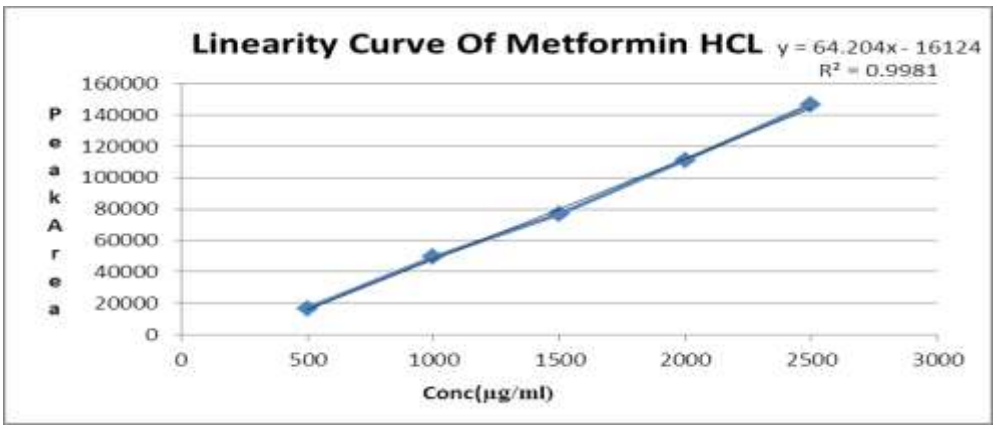


Fig.No.3 Linearity curve of Metformin HCL

Sample Solution Preparation

Twenty tablets each containing 5 mg of Glibenclamide and 500 mg of Metformin Hydrochloride were weighed and finely powdered, a quantity of 10 mg of Glibenclamide and 10 mg Metformin Hydrochloride was weighed and transferred to 10 ml volumetric flask and 7 ml diluent was added to it. The solution was then sonicated for 10 minutes and finally volume was made upto the 10 ml mark with diluent. Solution was filtered through 0.45µm filter. The final concentration of Glibenclamide 1mcg/ml and Metformin HCl 100mcg/ml and this solution was used for the estimation.

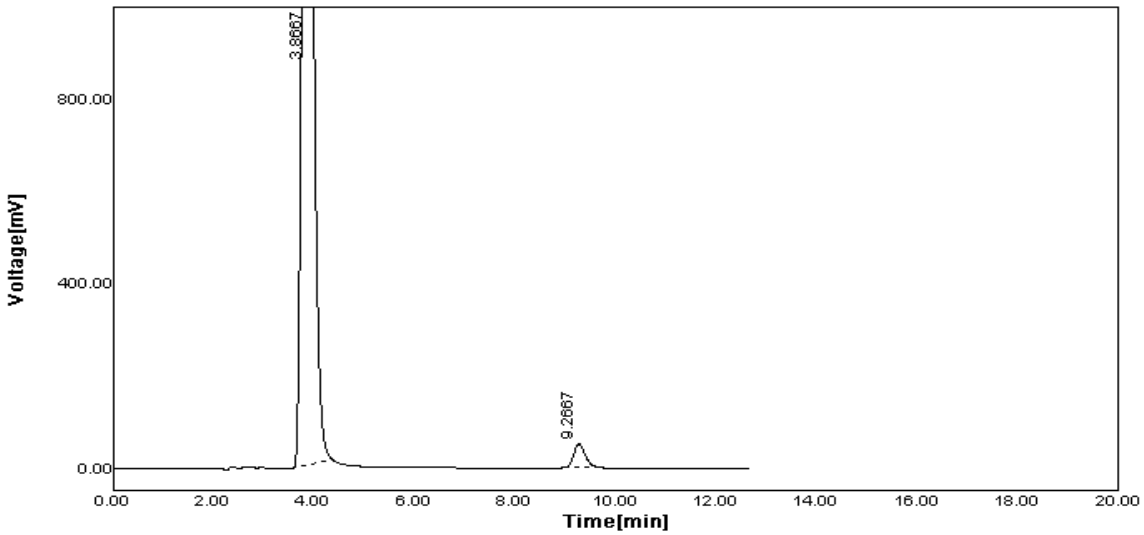


Fig. no.4 Simple Chromatogram of test Glibenclamide& Metformin HCL

FORCED DEGRADATION STUDIES

To evaluate the stability indicating properties of the developed HPLC method, forced degradation studies were carried out in accordance to the ICH guidelines , to produce the possible relevant degradants and test its chromatographic behavior. Intentional degradation was attempted to stress conditions of photolytic degradation, acid hydrolysis (using 0.1N HCl), base hydrolysis (using 0.1N NAOH), oxidative degradation (using 3.0% H2O2) and thermal treatment to evaluate the ability of the proposed method to separate Glibenclamide & Metformin HCL from its degradation products. Glibenclamide & Metformin at a concentration of 1:100 µg/ml was used in all the degradation studies. After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase.

Acid Degradation

From the prepared Stock Solution pipette out 0.01 ml of Glibenclamide&1 ml of Metformin into a 10ml clean and dry volumetric flask, then 3 ml of 0.1N HCl was added. The volumetric flask was kept at normal condition for 60 minutes and further it was neutralized. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain a final concentration of 1:100 µg/ml. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials. chromatograms for acid degradation studies were shown in fig 5.

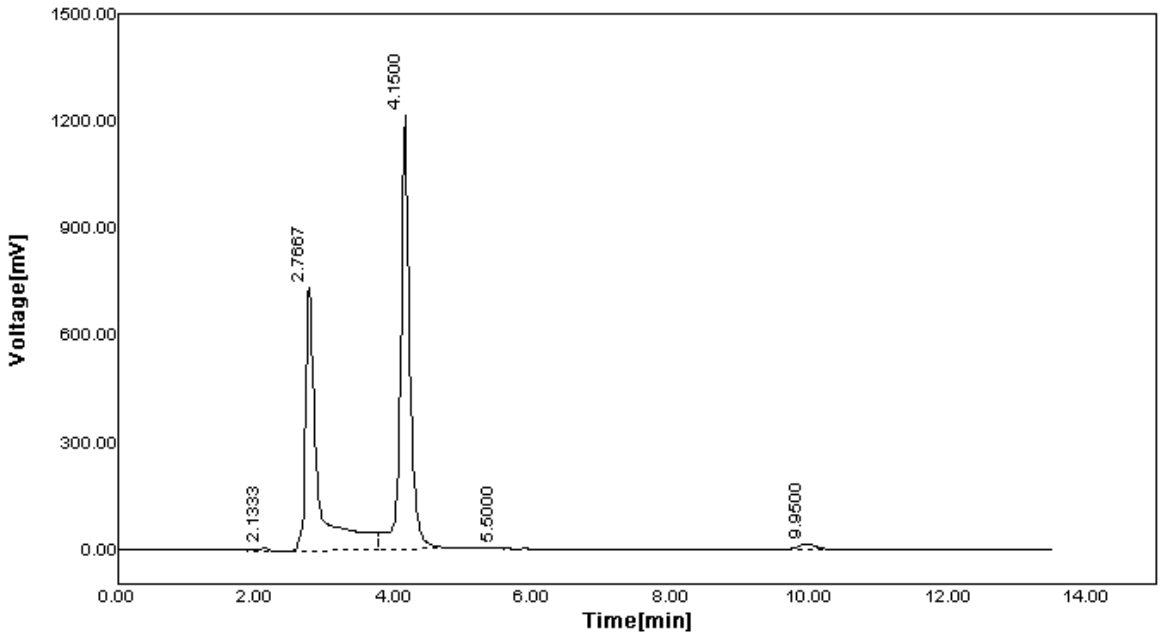


Fig no.5 Chromatogram of Acid Degraded Sample

Base Degradation

From the prepared Stock Solution pipette out 0.01 ml of Glibenclamide&1 ml of Metformin into a 10ml clean and dry volumetric flask, then 3 ml of 0.1N NaOH was added. The volumetric flask was kept at normal condition for 60 minutes and further it was neutralized. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain a final concentration of 1:100 µg/ml. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials. chromatograms for acid degradation studies were shown in fig 5. Forced degradation in alkaline media was performed using 0.1N NaOH. The representative Chromatograms for alkaline degradation studies were shown in fig-6 respectively.

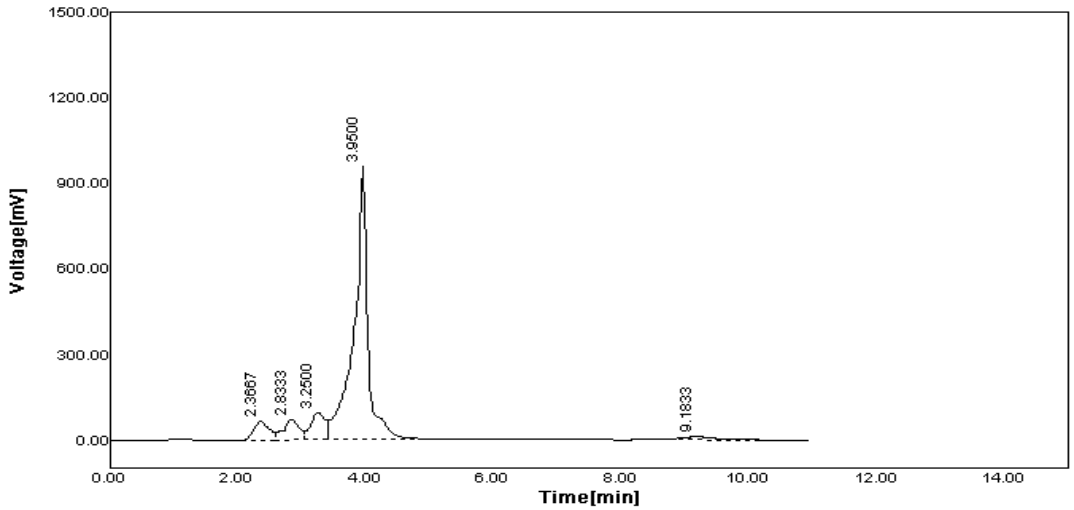


Fig no.6 Chromatogram of Base Degraded sample

Neutral Degradation

Neutral degradation in alkaline media was performed using water. The representative chromatograms for Neutral degradation studies were shown in fig-7 respectively.

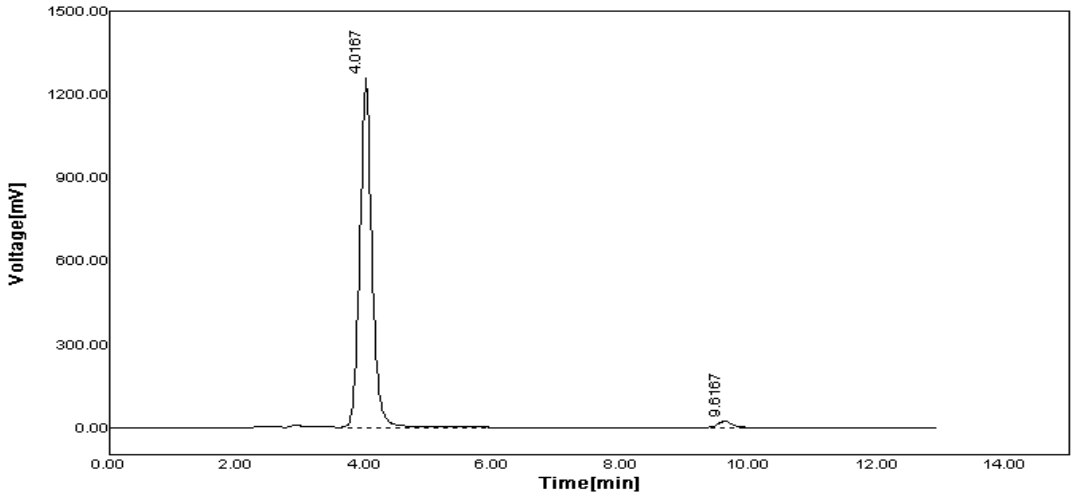


Fig no. 7 Chromatogram of Neutral Degraded sample

Oxidative degradation

From the prepared Stock Solution pipette out 0.01 ml of Glibenclamide&1 ml of Metformin into a 10ml clean and dry volumetric flask, then 1 ml of 3 % w/v of hydrogen peroxide was added. The volumetric flask was kept at normal condition for 60 minutes and further it was neutralized. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain a final concentration of 1:100 µg/ml. The resultant solution was filtered with

0.45 microns syringe filters and placed in the vials. Oxidative degradation was performed using 3 % w/v of hydrogen peroxide. The representative Chromatograms for alkaline degradation studies were shown in fig-8 respectively.

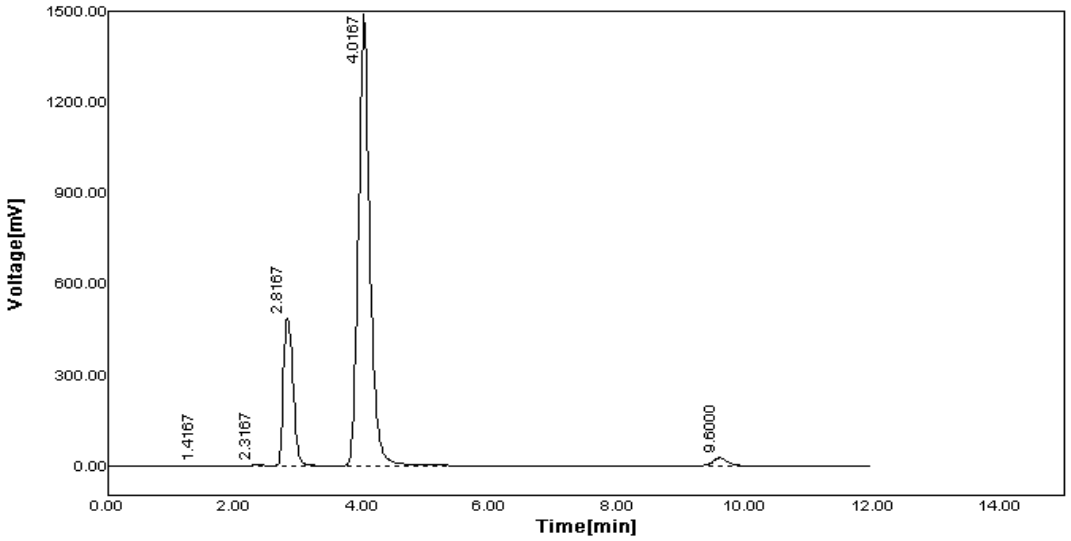


Fig. no. 8 Chromatogram of Oxidative degraded sample

Table No.1 Stress Study of Glibenclamide & Metformin HCL

Stress condition	Retension Time (min)		% Assay	
	GLB	MET	GLB	MET
Acid hydrolysis (0.1N HCL)	4.150	9.950	85.75	80.74
Base hydrolysis (0.1N NAOH)	3.950	9.183	94.95	100.65
Oxidation (3%H2o2)	4.016	9.600	109.83	109.85
Neutral	4.016	9.616	102.18	113.50

Validation of the Method

The analytical method was validated with respect to parameters such as linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity, recovery and robustness/ruggedness.

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve Shown in fig no.2 & 3.The constructed calibration curves were linear over the concentration range of 10–50µg/ml for Glibenclamide & 500-2500µg/ml for Metformin HCL. Peak areas of Glibenclamide & Metformin HCL was plotted against their respective concentrations and linear regression analysis was performed on the resultant curve Correlation coefficient (n=3) was found to be more than 0.999 & 0.998 with %RSD

values were less than 2% across the concentration ranges studied.Typically, the regression equation were: y = 91.31x-116.2 & y = 64.20x-16124

Repeatability

Repeatability was ascertained by getting the sample analyzed by different analyst and carrying out analysis for no. of times. The results are shown in table no.2.

Table No.2 Repeatability

Sr.No.	Conc		Peak Area		Amt Found		% Amt Found	
	GLB	MET	GLB	MET	GLB	MET	GLB	MET
1	20	2000	1728.151	111141.6	20.17	1983.82	100.86	99.19
2	20	2000	1727.149	111146.9	20.16	1983.91	100.80	99.20
3	20	2000	1726.154	111144.8	20.15	1983.88	100.75	99.19
4	20	2000	1728.134	111147.5	20.17	1983.92	100.85	99.20
5	20	2000	1727.165	111143.5	20.16	1983.85	100.80	99.19
6	20	2000	1728.165	111142.3	20.17	1983.84	100.86	99.19
7	20	2000	1726.154	111143.2	20.15	1983.85	100.75	99.19
8	20	2000	1728.165	111145.3	20.17	1983.88	100.86	99.19
9	20	2000	1727.165	111146.2	20.16	1983.90	100.80	99.19
10	20	2000	1727.146	111145.3	20.16	1983.88	100.80	99.19
	MEAN		1727.355	111144.7	20.16	1983.87	100.81	99.19
	SD		0.74	1.87	0.01	0.03	0.04	0.00
	% RSD		0.39	0.98	0.04	0.01	0.02	0.001

Limit of Detection and Limit of Quantitation

The limits of detection and quantification were calculated by the method based on standard deviation (σ) and slope (S) of the calibration plot using the formula LOD = 3.3 σ/S and LOQ =10 σ/S. The limit of detection (LOD) of the Glibenclamide was found to be 0.012 µg/ml). The limit of quantitation (LOQ) of was found to be 0.032µg/ml. The limit of detection (LOD) of the metformin was found to be 0.24 µg/ml & The limit of quantitation (LOQ) was found to be 0.49µg/ml.

Precision

Precision was evaluated in terms of Intraday and Interday precisions. Intraday precision was determined by analyzing sample solutions of from Daonil m formulations at three levels covering low, medium, and higher concentrations of calibration curve for five times on the same day. Inter day precision was determined by analyzing sample solutions of GLB & MET at three levels covering low, medium, and higher concentrations over a period of seven days (n=5). The peak areas obtained were used to calculate mean and %RSD (relative SD) values.

Table No.3 Precision

Compound	Conc(µg/ml)	Intraday		Interday	
		Mean±SD	%Amt Found	Mean±SD	%Amt Found
Glibenclamide	10	800.95±0.70	100.37	790.67±0.02	99.24
	15	1285.36±0.71	100.21	1279.26±0.70	101.76
	20	1764.72±0.71	102.85	1744.75±0.70	101.76
Metformin	1000	46553.32±1.41	97.78	49655.85±0.72	102.61
	1500	77372.14±0.70	97.19	76371.14±0.70	96.15
	2000	111168.0±0.64	99.21	111156.90±0.71	99.20

*mean of each 3 reading

Accuracy

Accuracy data for the assay following the determination of the compound of interest is summarized in Table-4.Accuracy was determined by interpolation of replicate (n=5) peak areas of three accuracy standards of different concentration, from a calibration curve that had been prepared as previously described. In each case, the percent relevant error and accuracy was calculated in Table no.4. Accuracy data (n=5)

Table No.4 Accuracy

Amount of Sample		Amount of Drug added		Amount Recovered		% Recovery	
GLB(mg/ml)	MET(mg/ml)	GLB(mg/ml)	MET(mg/ml)	GLB(mg/ml)	MET(mg/ml)	GLB(%)	MET(%)
5	500	4	400	3.71	399.58	92.66	99.90
5	500	5	500	4.76	508.42	95.18	101.68
5	500	6	600	6.17	606.73	102.77	101.12

Specificity

The results of stress testing studies in addition to that of monitoring standard solutions of the drug in the presence of their impurities indicated a high degree of specificity of this method. The degradation product(s) of the parent compound was found to be similar for both the tablets and API powder. All the degradation products formed during forced decomposition studies were well separated from the analyte peak demonstrates that the developed method was specific and stability-indicating.

Ruggedness/Robustness

As recommended in the ICH Guidelines, a robustness assessment was performed during the development of the analytical procedure. The ruggedness of the method is assessed by comparison of the intra- and inter-day assay results that has been performed by two analysts. The %RSD values for intra- and inter-day assays of Daonilm tablets performed in the same laboratory by two analysts did not exceed 1.8%, indicating the

ruggedness of the method. In addition, the robustness of the method was investigated under a variety of conditions including changes of flow rate, wavelength, mobile phase composition and column temperature,. The degree of reproducibility of the results obtained as a result of small deliberate a variations in the method parameters has proven that the method is robust and the data was summarized in Table no. 5.

Table No. 5 Ruggedness/Robustness

sample	Parameters	Conc.	Amount of drug detected (mean ±SD)	%RSD
Glibenclamide	Mobile phase composition-(69:31)	5	401.06 ± 0.81	0.204
	Mobile phase composition-(71:29)	5	390.866 ± 1.22	0.01
	Wavelength change225nm	5	374.24± 0.768	0.01
	Wavelength change227nm	5	411.24±0.69	0.16
	Flow rate change(1.1ml)	5	371.80 ± 0.89	0.21
	Flow rate change(0.9ml)	5	471.01 ± 0.81	0.01
Metformin HCL	Mobile phase composition-(69:31)	500	17261.22 ± 0.89	0.21
	Mobile phase composition-(71:29)	500	16898.67 ± 1.16	0.01
	Wavelength change225nm	500	17108.39 ± 1.49	0.01
	Wavelength Change 227nm	500	19256.9 ± 0.81	0.00
	Flow rate change(1.1ml)	500	16382.68 ± 0.76	0.00
	Flow rate change(0.9ml)	500	19667.9 ± 0.31	0.00

Table no.6 System suitability parameter

SR.NO.	Parameters	Result	
		GLB	MET
1	Range(µg/ml)	10-50	500.2500
2	Detection wavelength	226	233
3	Mean R^2 value	0.999	0.998
4	Slop(m)	91.31	64.20
5	Intercepts(c)	116.2	16124
6	Retention Time (min.)	9.2	3.8
7	Theoretical plate (N)	7456.3	8963.3
8	Tailing Factor	1.115	1.105
9	LOD	0.012	0.24
10	LOQ	0.032	0.49

Assay

The validated method was applied to the determination of Glibenclamide & Metformin HCL in commercially available Daonil m tablets. Figure 1 and Figure 4 illustrates two typical HPLC chromatograms obtained from Glibenclamide & Metformin HCL standard

solution and from the assay of Daonil m tablets respectively. The results of the assay (n = 9) undertaken yielded 101.17 & 99.96% (%RSD = 0.72 & 0.67%) of label claim for Glibenclamide & Metformin HCL. The mean retention time of Glibenclamide & Metformin HCL was 9.2 & 3.8 min. The results of the assay indicate that the method is selective for the analysis of Glibenclamide & Metformin HCL without interference from the excipients used to formulate and produce these tablets.

Table no.7 Label Claim

Sample	Label Claimed	% Label Claim* ± SD	%RSD
DAONIL-M	Glibenclamide 5mg	101.17± 0.73	0.72
	Metformin HCL500mg	99.96± 0.65	0.67

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug GLB & MET HCL preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the Methanol and water were optimized to give symmetric peaks with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol : Water 70:30(v/v). The retention time of GLB & MET HCL was found to be 9.2 &3.8 min, respectively. The RSD values for accuracy and precision studies obtained were less than 2%which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 6. The LOD and LOQ were found to be µg/ml and µg/ml respectively. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust. GLB & MET HCL were found to be relatively stable following photolysis and Thermal degradation. Considerable degradation was observed for both in oxidation, acid and base hydrolysis. The validated method was applied to the determination of GLB &MET HCL in commercially available Daonil m tablets. The results of the assay indicate that the method is selective for the analysis of Glibenclamide & Metformin HCL without interference from the excipients used to formulate and produce these tablets.

CONCLUSIONS

A simple, rapid, accurate and precise stability indicating HPLC analytical method has been developed and validated for the routine analysis of Glibenclamide & Metformin HCLin API and tablet dosage forms. The results of stress testing reveal that the method is selective and stability indicating. The proposed method has the ability to separate the analyte from their degradation products, related substances; excipients found in tablet dosage forms and can be applied to the analysis of samples obtained during accelerated stability experiments.

REFERENCES

1. Martindale: The Complete Drug Reference,Pharmaceutical Press, 2007; 411.
2. Seema M. Dhole, Pramod B. Khedekar, Nikhil D. Amnerkar, UV Spectrophotometric Absorption Correction Method for the Simultaneous Estimation of Pioglitazone HCl, Metformin HCl and Glibenclamide in Multicomponent Formulation, Int.J.Analytical&Bioanalytical Chem, 2013; 3(1): 18-22.
3. 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, RJC, 2014; 7(1): 55-63.
4. Narendra Nyola, and Govinda Samy Jeyabalan, Method Development Of Simultaneous Estimation Of Sitagliptin And Metformin Hydrochloride In Pure And Tablet Dosage Form By Uv-Vis Spectroscopy, WJPPS, 2012; 1(4): 1392-1401.
5. Patil Sudarshan S., Bonde C. G., Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and MetforminHCl in Bulk and Tablets using UV – visible spectroscopy, Int.J. ChemTech Res, 2009; 1(4):905-909.
5. Narsimha rao. Doredla ,Shanmugasundaram. P, Hemant vaishnav, Method Development and Validated of Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide in Pure and Tablet dosage form by Spectrophotometric Multi component Method, Int.J. ChemTech Res, 2011; 3(4): 2011-2017.
6. Abida Bilal, Kanwal Rehman, Muhammad Sajid Hamid Akash, Khalid Hussain, Muhammad Ibrahim and Syed Saeedul Hussan, Development and Validation of Analytical Method for Qualitative and Quantitative Determination of Glibenclamide in Different Brands of TabletDosage form Using UV-Visible Spectroscopy, J Mol Genet Med, 2013; 7(3): 1-6.

7. Shweta s. Havele,sunil r. Dhaneshwar, Determination Of Glibenclamide,Metformin Hydrochloride And Rosiglitazone Maleate By Reversed Phase Liquid Chromatographic Technique In Tablet Dosage Form, Chem. Ind. Chem. Eng. Q., 2014; 20(1): 39−47.
8. Amruta B. Loni, Minal R. Ghante, S. D. Sawant, Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin phosphate and Metformin hydrochloride in Bulk and Tablet Dosage Form, Der Pharma Chemica, 2012; 4(3): 854-859.
9. Asit Kumar De, Ayan Kumar Dey, Angshuman Biswas,Simultaneous Estimation Of Metformin Hydrochloride And Glibenclamide By Rphplc Method From Combined Tablet Dosage Form, IJSIT, 2012; 1(2): 98-105.
10. Safwan Ashour*, Amir Alhaj Sakur , Manar Kudemati, Development and Validation of Stability Indicating HPLC Method for Simultaneous Determination of Antidiabetic Drugs Metformin Hydrochloride and Glyburide in Tablets, International Research Journal of Pure & Applied Chemistry, 2014; 4(6): 605-620.