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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR ESTIMATION OF GLIMEPIRIDE AND METFORMIN HYDROCHLORIDE

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Keywords:

Glimepiride, Metformin hydrochloride, HPTLC, Validation, Stability-indicating, Degradation

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ABSTRACT: An Accurate, sensitive, precise and stability indicating high performance thin layer chromatographic method has been developed and validated for the estimation of Glimepiride and Metformin hydrochloride. The method was developed using TLC aluminium plates precoated with silica gel $60F_{254}$ as the stationary phase and 0.5% Ammonium Sulfate: Methanol (7.5:2.5 v/v) as mobile phase. Densitometric analysis of Glimepiride and Metformin hydrochloride was carried out in the absorbance mode at 228 nm. The two drugs were satisfactorily resolved with R_F values 0.73 and 0.45 for Glimepiride and Metformin hydrochloride respectively. Linearity was found to be 600-2100 ng/band for Glimepiride and 200-700 ng/band for Metformin hydrochloride. The method was found to be accurate, precise, and robust according to acceptance criteria. The limit of detection (LOD) was found to be 0.05 ng/band and 0.32 ng/band for Glimepiride and Metformin hydrochloride respectively. The limit of quantification (LOQ) was found to be 0.16 ng/band and 0.96 ng/band for Glimepiride and Metformin hydrochloride respectively. The proposed HPTLC method was successfully applied for the forced degradation study of Glimepiride and Metformin hydrochloride. Forced degradation study was carried out for both the drugs in oxidative condition 0.3% H2O2 for 30mins, in acidic condition 0.1 M HCl at 80˚C for 60min, in basic condition 0.01 M NaOH at 80˚C for 30 min, in thermal condition 60˚C for 24 hours and in photolytic condition for 24 hours. Degradation products were well separated by proposed method.

INTRODUCTION Glimepiride is widely used in the treatment of non-insulin dependent Type II diabetes mellitus 1. It acts by stimulating insulin secretions from the beta cells of pancreas and is also known to increase peripheral insulin sensitivity thereby decreasing insulin resistance. It can be used in combination with metformin, thiazolidinediones, alpha-glucosidase inhibitors and insulin 2. After oral administration, it is completely absorbed from the gastrointestinal tract. Peak plasma concentration is reached 2 - 3 hrs after dosing. Its bioavailability changes a little with food and glimepiride (99.5%) are bound to proteins. Glimepiride is completely metabolised in liver.[1-2] The structure of glimepiride is shown in **Fig.1.**

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Metformin is a Biguanides derivative producing an anti hyperglycemic effect which can only be observed in man or in the diabetic animal and only when there is insulin secretion. Metformin, at therapeutic doses, does not cause hypoglycemia when used alone in man or in the non-diabetic animal, except when using a near lethal dose. Metformin has no effects on the pancreatic beta cells.

The main mechanism of action of metformin is reduction of hepatic glucose output, largely by inhibiting hepatic gluconeogenesis. Metformin also slows intestinal absorption of sugars and improves peripheral glucose uptake and utilization. A very important property of this drug is its ability to modestly reduce hyperlipidemia (low-density lipoprotein [LDL] and very-low-density lipoprotein [VLDL] cholesterol concentrations fall, and high-density lipoprotein [HDL] cholesterol rises). These effects may not be apparent until 4 to 6 weeks of use. The patient often loses weight because of loss of appetite. The ADA treatment algorithm

recommends metformin as the drug of choice for newly diagnosed Type 2 diabetics. Metformin may be used alone or in combination with one of the other agents, as well as with insulin. Hypoglycemia has occurred when metformin was taken in combination. [3 - 4] The structure of Metformin hydrochloride is shown in **Fig. 2.**

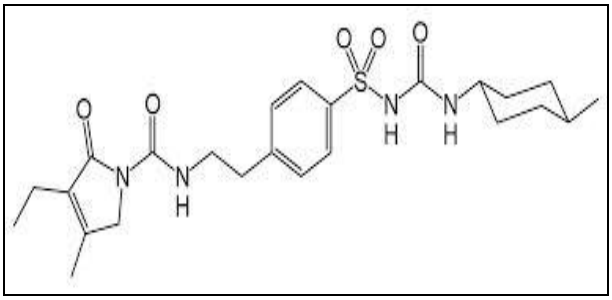

FIG.1: GLIMEPIRIDE

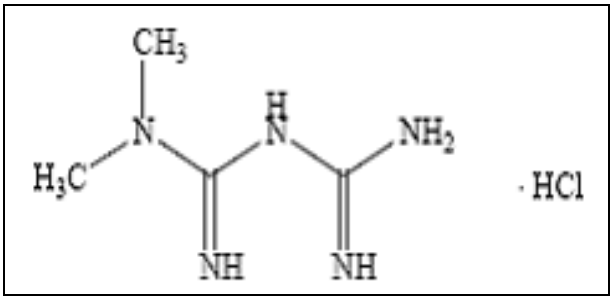

FIG. 2: METFORMIN HYDROCHLORIDE

Glimepiride and Metformin hydrochloride are used in the treatment of type 2 Diabetes mellitus. Combination of these two drugs can lower the blood glucose level in type 2 diabetes mellitus. Literature survey reveals that HPLC [5-10] methods, UV Visible Spectrophotometric [7, 10] method and HPTLC [11-12] method have been reported for the combined dosage form.

MATERIALS AND METHODS:

Material: Pure Glimepiride and Metformin hydrochloride sample were procured as gift sample from Triveni Chemicals,Vapi and Abhilasa pharma pvt Ltd., Ankleshwar respectively. The solvent used methanol (AR grade), NaOH (AR grade), HCl (AR grade) and H2O2 (AR grade). These chemicals were purchased from Merck Chemicals (Mumbai, India). Ammonium sulfate (AR grade) was purchased from Allied chemical Corporation, Vadodara, Gujarat, India. Tablets were procured from local market.

Equipment: Camag HPTLC system consisting Linomat 5 applicator, camag TLC scanner 3 and Win CATS software V-1.3.4 was used for chromatographic separation. Spotting of samples was done by using Hamilton microliter syringe.

Methods:

Preparation of standard stock solution: Mixed standard stock solution of 500 µg/ml of each GLM and MET were prepared. From this solution 1 – 6 µl solutions were applied on the TLC plate. After chromatographic development, bands were scanned over the range 200-400 nm spectrum at scan speed 100 nm/s and the spectra were recorded.

Validation of Analytical Method:

Linearity: Mixed standard stock solution having concentration 150µg/ml GLM and 50µg/ml MET were prepared. From this stock solution 4, 6, 8, 10, 12, 14µl was applied in form of band of desired concentration range 600 to 2100 ng/band for GLM and 200-700 ng/band for MET. Each concentration was applied six times to the TLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves. The calibration curve for Glimepiride and Metformin hydrochloride are shown in **Fig. 3** and **Fig. 4.**

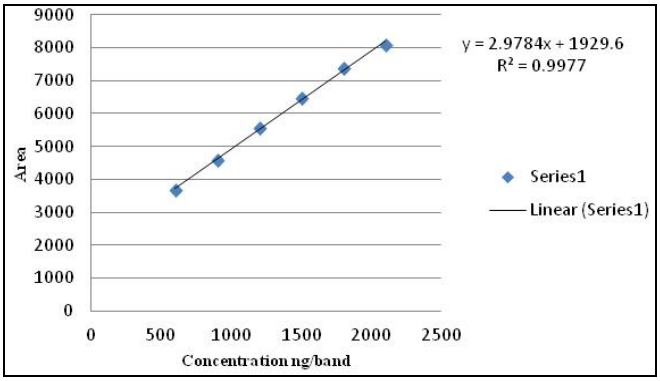

FIG 3: CALIBRATION CURVE FOR GLIMEPIRIDE

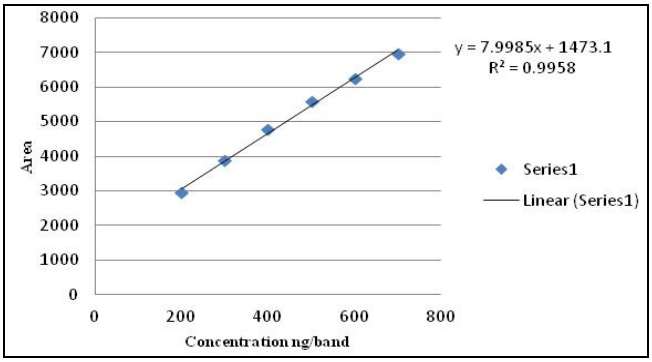

FIG 4: CALIBRATION CURVE FOR METFORMIN HYDROCHLORIDE

Precision: The precision of the method was demonstrated by intra-day and inter-day variation studies. Intra-day precision was determined by analyzing the combined standard solutions of GLM (600, 900, 1200 ng/band) and MET (200, 300, 400 ng/band) in linearity range at three different time intervals on same day. Inter-day precision was determined by analyzing the combined standard solutions of GLM (600, 900, 1200 ng/band) and MET (200, 300, 400 ng/band) in linearity range at three consecutive days. The percentage RSD was calculated. The result obtained for Glimepiride and Metformin hydrochloride are shown in **Table 1.**

Accuracy: Accuracy was determined at three different level 80%, 100% and 120% of the target concentration 900 ng/band of GLM and 300 ng/band of MET in triplicate. The result obtained for Glimepiride and Metformin hydrochloride are shown in **Table 2.**

TABLE 1: PRECISION STUDIES FOR GLIMEPIRIDE AND METFORMIN HYDROCHLORIDE

Conc.[ng/band] GLM	GLM			Conc.[ng/band] MET	MET		
	Area Mean (n=3)	SD	%RSD		Area Mean (n=3)	SD	%RSD
			Intra – day Precision				
600	3673.3	9.53	0.25	200	2952.6	7.5	0.25
900	4579.7	10	0.21	300	3896.5	7.97	0.2
1200	5530.2	11.35	0.2	400	4781.4	9.01	0.18
			Inter – day Precision				
600	3668.6	10.06	0.27	200	2952.3	9.71	0.32
900	4586	11.93	0.26	300	3897.5	11.26	0.28
1200	5542.2	12.52	0.22	400	4770.4	10.69	0.22

TABLE 2: RECOVERY STUDIES FOR GLIMEPIRIDE AND METFORMIN HYDROCHLORIDE

Drugs	Level	Conc [ng/band]	Total conc [ng/band]	Mean total conc found (n=3)[ng/band]	% Recovery mean (n=3)
GLM	80%	900+720	1620	1412.18	100.2
	100%	900+900	1800	1592.37	100.26
	120%	900+1080	1980	1774.76	100.4
MET	80%	300+240	540	541.49	100.1
	100%	300+300	600	599.07	99.32
	120%	300+360	660	660.01	99.69

Limit of Detection & Limit of Quantification: LOD and LOQ of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations as per ICH guideline.

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response.

S = slope of the calibration curve.

Specificity: Specificity of the method was ascertained by analyzing standard drug and sample. The spot for GLM and MET was confirmed by comparing the R_f and spectra of the spot with that of standard, indicating absence of other interference at that R_f.

Robustness: The effect of small, deliberate variation of the analytical conditions on the peak areas of the drugs was examined. Change in chamber saturation time and change in volume of mobile phase were investigated and %RSD was calculated.

Analysis of marketed formulation:

Twenty tablets were weighed, their mean weight determined and finally powdered. An accurately weighed tablet powder equivalent to 500 mg of MET and 2 mg GLM was transfer into 100 ml volumetric flask containing 50 ml of Methanol, sonicated for 10 minute and volume was made up to the mark with Methanol, the resulting solution was filtered using 0.45 µm filter. Then 0.6 ml of the above filtered solution was diluted to produce a concentration 300µg/ml for MET from which 1µl

of the spot was applied which gave final concentration of 300ng/spot.

Then preparation of sample solution of GLM, an accurately weighed tablet powder equivalent to 500 mg of MET and 2 mg of GLM was transfer in to 100ml volumetric flask containing 50 ml of methanol, in this solution 100mg of pure GLM powder was spiked, sonicated for 10 minute and volume was made up to the mark with Methanol, the resulting solution was filtered using 0.45 µm filter. Then 8.8 ml of the above filtered solution was diluted to produce a concentration 900µg/ml for GLM from which 1µl of the spot was applied which gave final concentration of 900ng/spot. The amount of MET and GLM was determined. The result obtained for Glimepiride and Metformin hydrochloride are shown in **Table 3.**

TABLE 3: ASSAY RESULTS FOR GLIMEPIRIDE AND METFORMIN HYDROCHLORIDE

Formulation	Tablet formulation	
	GLM	MET
Concentration [ng/band]	900	300
Concentration found [ng/band] (n=3)	897.96	298.73
%Purity	99.77	99.57

Stress degradation Studies:

Densitogram of Glimepiride and Metformin hydrochloride:

Methanol was used as a solvent for solution preparation. Stationary phase was aluminium TLC plate (10×10cm) precoated with silica gel F_{254}. 0.5% Ammonium sulfate: Methanol (7.5:2.5 v/v) was used as mobile phase. Mixed standard stock solution of Glimepiride and Metformin hydrochloride 10µl (1500ng/band and 500ng/band) was applied on TLC plate. The Rf value for Glimepiride and Metformin hydrochloride was 0.67 and 0.43. The typical densitogram of working standard solutions is as shown in **Fig.5.**

Stress degradation in acidic condition: A 100 mg standard Glimepiride and 100 mg Metformin hydrochloride was transferred to two separate iodine flask and dissolved in 40 ml methanol. 20 ml of 0.1M HCl was added in both the flask. Refluxed it at 80˚C for 60 min in water bath. After exposure to degradation condition, they were transferred to 100 ml volumetric flask, neutralised it with 0.1M NaOH and volume was made up to the mark with Methanol.

From this stock solution, 5 ml of stock solution for Metformin hydrochloride (500 ng/band for MET) was transferred to 10 ml volumetric flask, and volume was made up to mark with methanol. Then 1.5 µL of the stock solution for Glimepiride (1500 ng/band for GLM) and 1 µL of stock solution of Metformin hydrochloride were applied to TLC plates and the chromatograms were run under the optimized chromatographic conditions. The result obtained for Glimepiride and Metformin hydrochloride are shown in **Fig. 6** and **Fig. 7.**

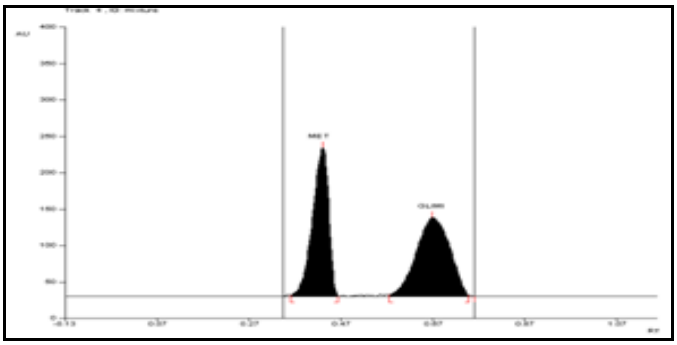

FIG. 5: DENSITOGRAM OF WORKING STANDARD SOLUTIONS OF GLIMEPIRIDE (1500 ng/band) AND METFORMIN HYDROCHLORIDE (500 ng/band)

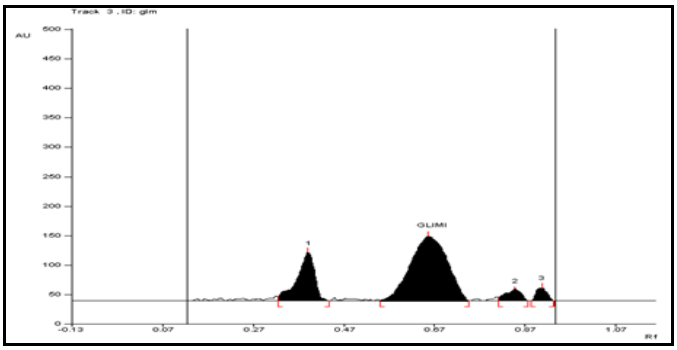

FIG. 6: DENSITOGRAM OF GLIMEPIRIDE AND ITS DEGRADATION PRODUCTS IN ACID DEGRADATION STUDY

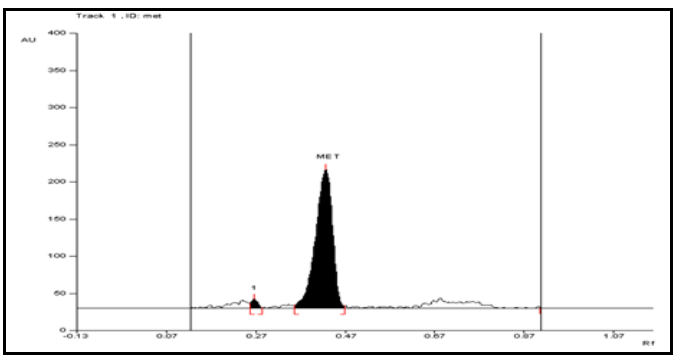

FIG. 7: DENSITOGRAM OF METFORMIN HYDROCHLORIDE AND ITS DEGRADATION PRODUCTS IN ACID DEGRADATION STUDY

Stress degradation in basic condition: A 100 mg standard Glimepiride and 100 mg Metformin hydrochloride was transferred to two separate iodine flask and dissolved in 40 ml methanol. 20 ml of 0.01M NaOH was added in both the flask. Refluxed it at 80˚C for 30 min in water bath. After exposure to degradation condition, they were transferred to 100 ml volumetric flask, neutralised it with 0.01M HCl and volume was made up to the mark with Methanol.

From this stock solution, 5 ml of stock solution for Metformin hydrochloride (500 ng/band for MET) was transferred to 10 ml volumetric flask, and volume was made up to mark with methanol. Then 1.5 µL of the stock solution for Glimepiride (1500 ng/band for GLM) and 1 µL of stock solution of Metformin hydrochloride were applied to TLC plates and the chromatograms were run under the optimized chromatographic conditions. The result obtained for Glimepiride and Metformin hydrochloride are shown in **Fig. 8** and **Fig. 9.**

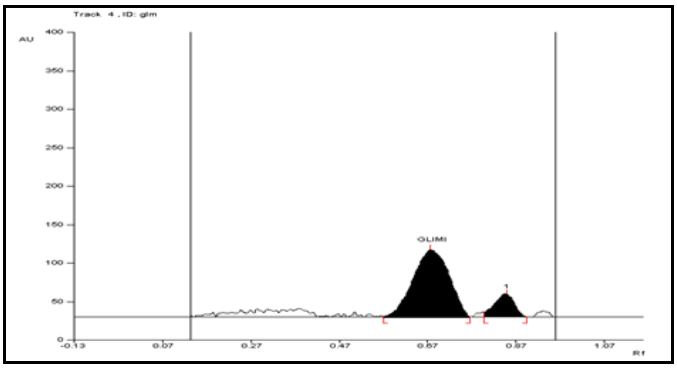

FIG. 8: DENSITOGRAM OF GLIMEPIRIDE AND ITS DEGRADATION PRODUCTS IN BASE DEGRADATION STUDY

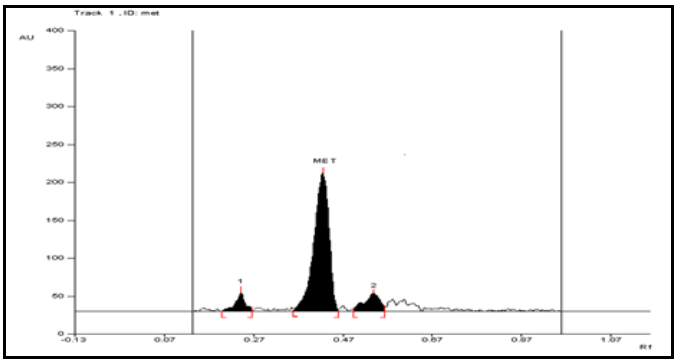

FIG. 9: DENSITOGRAM OF METFORMIN HYDROCHLORIDE AND ITS DEGRADATION PRODUCTS IN BASE DEGRADATION STUDY

Stress degradation in photolytic condition: Standard drugs were taken in petri dish and exposed in UV chamber for 24 hrs. A 100 mg standard Glimepiride and 100 mg Metformin hydrochloride was transferred to two separate 100 ml volumetric flask and dissolved in methanol and volume was made up to the mark with Methanol.

From this stock solution, 5 ml of stock solution for Metformin hydrochloride (500 ng/band for MET) was transferred to 10 ml volumetric flask, and volume was made up to mark with methanol. Then 1.5 µL of the stock solution for Glimepiride (1500 ng/band for GLM) and 1 µL of stock solution of Metformin hydrochloride were applied to TLC plates and the chromatograms were run under the optimized chromatographic conditions. The result obtained for Glimepiride and Metformin hydrochloride are shown in **Fig. 10 and Fig. 11.**

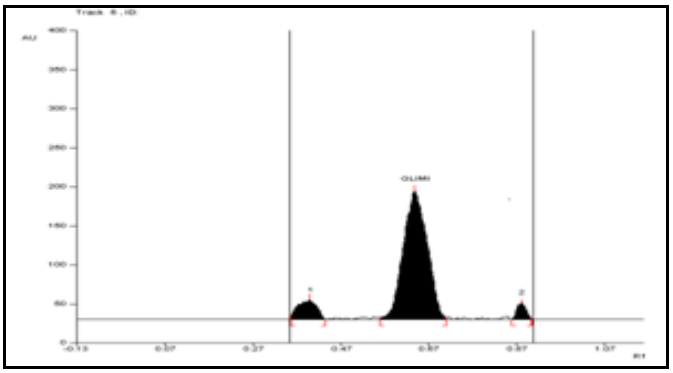

FIG. 10: DENSITOGRAM OF GLIMEPIRIDE AND ITS DEGRADATION PRODUCTS IN PHOTOLYTIC DEGRADATION STUDY

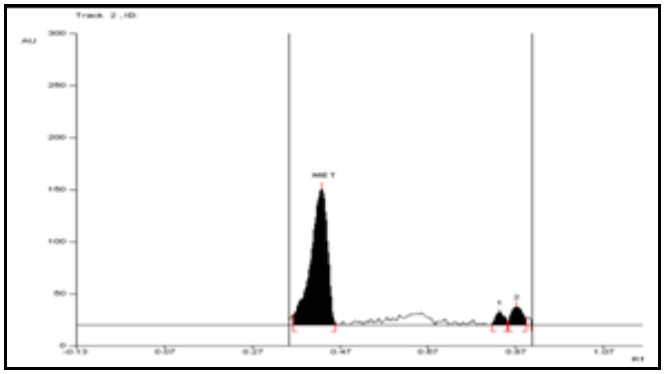

FIG. 11: DENSITOGRAM OF METFORMIN HYDROCHLORIDE AND ITS DEGRADATION PRODUCTS IN PHOTOLYTIC DEGRADATION STUDY

Stress degradation in oxidative condition: A 100 mg standard Glimepiride and 100 mg Metformin hydrochloride was transferred to two separate iodine flask and dissolved in 40 ml methanol. 20 ml of 0.3% H_2O_2 was added in both the flask. The

sample solution were stored at 25°C (room temp.) for 30 min. After exposure to degradation condition, it was transferred to 100 ml volumetric flask and volume was made up to the mark with Methanol.

From this stock solution, 5 ml of stock solution for Metformin hydrochloride (500 ng/band for MET) was transferred to 10 ml volumetric flask, and volume was made up to mark with methanol. Then 1.5 µL of the stock solution for Glimepiride (1500 ng/band for GLM) and 1 µL of stock solution of Metformin hydrochloride were applied to TLC plates and the chromatograms were run under the optimized chromatographic conditions. The result obtained for Glimepiride and Metformin hydrochloride are shown in **Fig. 12** and **Fig. 13.**

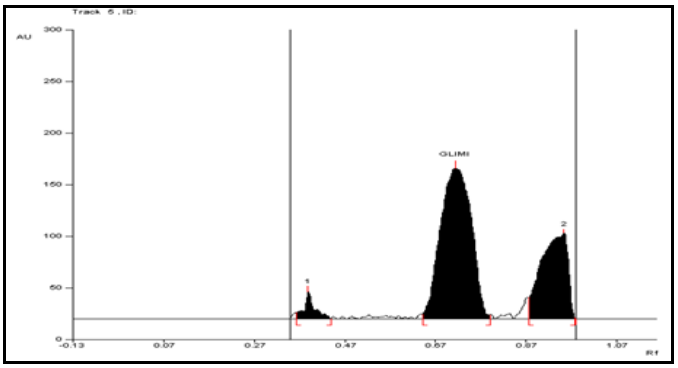

FIG. 12: DENSITOGRAM OF GLIMEPIRIDE AND ITS DEGRADATION PRODUCTS IN OXIDATIVE DEGRADATION STUDY

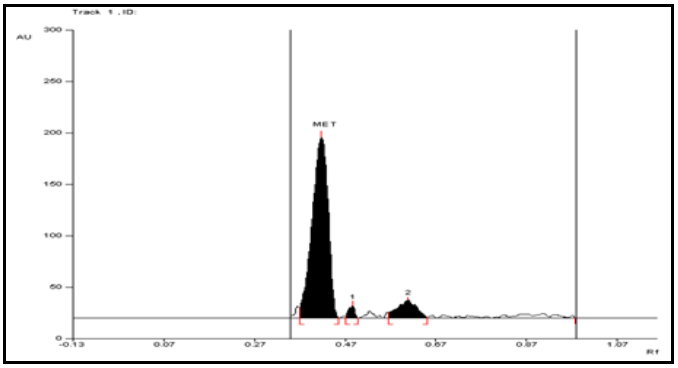

FIG. 13: DENSITOGRAM OF METFORMIN HYDROCHLORIDE AND ITS DEGRADATION PRODUCTS IN OXIDATIVE DEGRADATION STUDY

Stress degradation in thermal condition: Standard drugs were taken in porcelain dish and exposed to a temperature of 60ºC for 24 hour in hot air oven. A 100 mg standard Glimepiride and 100 mg Metformin hydrochloride was transferred to two separate 100 ml volumetric flask and dissolved in methanol and volume was made up to the mark with Methanol.

From this stock solution, 5 ml of stock solution for Metformin hydrochloride (500 ng/band for MET) was transferred to 10 ml volumetric flask, and volume was made up to mark with methanol. Then 1.5 µL of the stock solution for Glimepiride (1500 ng/band for GLM) and 1 µL of stock solution of Metformin hydrochloride were applied to TLC plates and the chromatograms were run under the optimized chromatographic conditions. The result obtained for Glimepiride and Metformin hydrochloride are shown in **Fig. 14 and Fig. 15.**

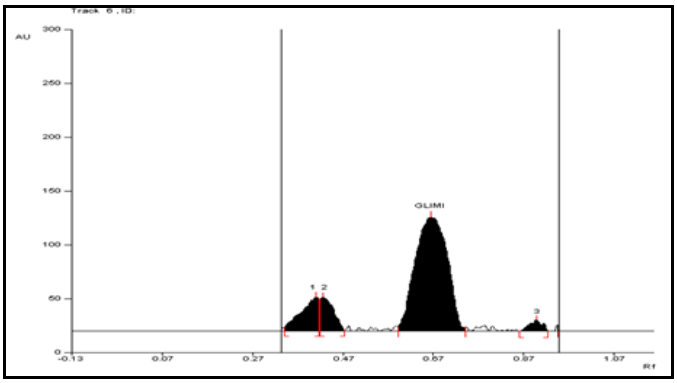

FIG. 14: DENSITOGRAM OF GLIMEPIRIDE AND ITS DEGRADATION PRODUCTS IN THERMAL DEGRADATION STUDY

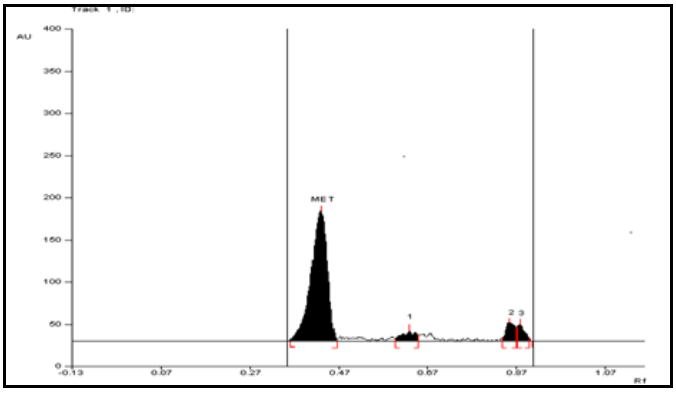

FIG. 15: DENSITOGRAM OF METFORMIN HYDROCHLORIDE AND ITS DEGRADATION PRODUCTS IN THERMAL DEGRADATION STUDY

RESULTS AND DISCUSSION:

HPTLC method was validated as per ICH guidelines. The developed method was found to be linear within the range of 600 – 2100ng/band with R^2=0.9977 for Glimepiride and 200-600 ng/band with R^2 = 0.9958 for Metformin hydrochloride. The accuracy of method was determined at 80%, 100%, 120% level. The % recoveries were found to be

100.2%-100.4% for Glimepiride and 99.32%-100.1% for Metformin hydrochloride.

The LOD for Glimepiride was found to be 0.05ng/band and for Metformin hydrochloride 0.32 ng/band and the LOQ Glimepiride was found to be 0.16ng/band and for Metformin hydrochloride 0.96 ng/band indicating the sensitivity of the method. The developed method was found to be precise as the % RSD values for intra-day and inter-day were found to be less than 2%. The method was also found to be robustness indicated by the % RSD values which are less than 2 %. The summary of validation parameters of proposed HPTLC method is shown in **Table 4.** The stress degradation studies was carried out for both the drugs in acid, base, photolytic, oxidation, thermal conditions. Summary of the results of stress degradation studies of Glimepiride and Metformin hydrochloride are shown in **Table 5.**

TABLE 4: SUMMARY OF VALIDATION PARAMETERS

Parameters	GLM	MET
Linearity [ng/band]	600-2100	200-700
%Recovery	100.2 - 100.4	99.32 – 100.1
Precision (%RSD)		
Intra-day (n=3)	0.2-0.25	0.18-0.25
Inter-day (n=3)	0.22-0.27	0.22-0.32
LOD [ng/band]	0.05	0.32
LOQ [ng/band]	0.16	0.96
Specificity	Specific	Specific
Robustness (%RSD)		
Mobile phase composition (±0.01mL)	0.5	0.8
Chamber saturation time (±0.01mL)	0.3	0.9

TABLE 5: SUMMARY OF STRESS DEGRADATION STUDIES FOR GLIMEPIRIDE AND METFORMIN HYDROCHLORIDE

Sr. No.	Stress degradation parameter	R_f of degradation product		% of drug degraded (based on area)	
		GLM	MET	GLM	MET
1	ACID	0.66	0.43	15.08%	11.71%
2	BASE	0.64	0.44	16.60%	17.18%
3	PHOTOLYTIC	0.67	0.45	19.12%	14.40%
4	OXIDATION	0.73	0.42	13.32%	19.25%
5	THERMAL	0.64	0.43	18.52%	12.35%

CONCLUSION: The proposed methods are precise, specific, accurate, robust and stability-indicating ones. Glimepiride and Metformin hydrochloride can be determined in bulk and pharmaceutical formulation and percentage degradation. ICH guidelines were fallowed throughout the study for method validation and stress testing.

ACKNOWLEDGEMENTS: The authors are thankful to Triveni Chemicals, Vapi and Abhilasa pharma pvt Ltd., Ankleshwar for providing gift sample of standard Glimepiride and Metformin hydrochloride . We are also thankful to the Principal, A.R. College of Pharmacy and G.H. Patel Institute of Pharmacy, who had helped us throughout the entire work.

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