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STRESS STABILITY STUDY SHOWING EFFECT of ACID, BASE, H_2O_2 AND DRY HEAT ON GLIMEPIRIDE BY HPTLC METHOD

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ABSTRACT

Forced degradation studies include degradation of drug substances and drug products at conditions more severe than accelerated conditions. These studies illustrate the chemical stability of molecule which further facilitates the development of stable formulation with suitable storage conditions. Glimepiride belongs to class of second generation sulfonyl urea used to treat type –II diabetes mellitus. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors. Degradation products of Glimepiride formed under different forced conditions have been characterized through (High Performance Thin Layer Liquid Chromatography) HPTLC studies. The method was developed using TLC silica gel 60 F254 aluminum backed plate as the stationary phase and Toluene: Chloroform: Ethanol 4:4:1 v/v/v as Mobile phase taking absorbance at 234nm. The forced degradation study was carried out in accordance with the (International Conference Of Harmonization) ICH guidelines Q1A (R2) for Glimepiride in oxidative condition 6% Hydrogen peroxide for 3 hours, in acidic condition 0.1M HCl at 80˚C for 60 Minutes, in basic condition 0.1M NaOH at 80˚C for 60min and in thermal condition 60˚C for 3 hours. Degradation products were well separated by proposed method.

KEYWORDS: Glimepiride, Degradation studies, HPTLC, Accelerated conditions, ICH.

1. INTRODUCTION

Forced degradation studies provide the approach to analyze the stability of drug samples in pharmaceutical industries. ICH has revised parent drug stability testing guidelines Q1A (R2) for stress testing on the drug substances.[1-3] Test should be carried out to ascertain its inherent stability and for supporting the suitability of the proposed analytical procedures. Stability of

molecule information provides the data for selecting proper formulation, packaging, proper storage conditions and shelf life. The aim of the study was to develop inherent stability pattern of Prulifloxacin as per ICH guidelines and to develop validated stability indicating method by Normal phase HPTLC.[4,5]

Glimepiride(1-[4-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)-ethyl]phenyl]sulfonyl]-3-trans-(4-methylcyclohexyl) urea) belongs to class of second generation sulfonyl urea used to treat type –II diabetes mellitus. Molecular formula of Glimepiride is $C_{24}H_{34}N_4O_5S$ with a molecular mass of about 490.617g/mol. It belongs to class-II of Biopharmaceutical classification system. It is completely insoluble in water, acidic media and slightly soluble in various buffers and organic solvents. It is administered orally; insoluble in water, slightly soluble in methylene chloride(Dichloromethane), very slightly soluble in methanol and soluble in Dimethyl Sulfoxide (DMSO).[6-8] It acts as an insulin secretagogue. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors. Glimepiride blocks the ATP-sensitive potassium channel, Closure of those channels triggers the opening of voltage sensitive Ca2+ channels resulting in rapid influx of Ca2+ within the cytoskeleton and stimulates translocation of insulin containing granules to the plasma membrane and exocytic release of insulin.[9] Its gastrointestinal absorption is complete with no interference from meals. Significant absorption can occur within one hour and distribution throughout the body is 99.5% and bound to plasma protein. Metabolism is by oxidative biotransformation, it is hepatic and complete. The medication is metabolized to M1 metabolite by CYP2C9. M1 possesses about 1⁄3 of pharmacological activity. Excretion in the urine is about 65% and the remainder is excreted in the feces.[10-13] Glimepiride should be stored in a cool and dry place, away from moisture and should not be used after the expiry date on the package. Glimer 1 (Abbott, India), Glimulin (Glenmark) are some of the marketed preparations.

2. EXPERIMENTAL

Glimepiride is the drug used to perform stress stability studies.

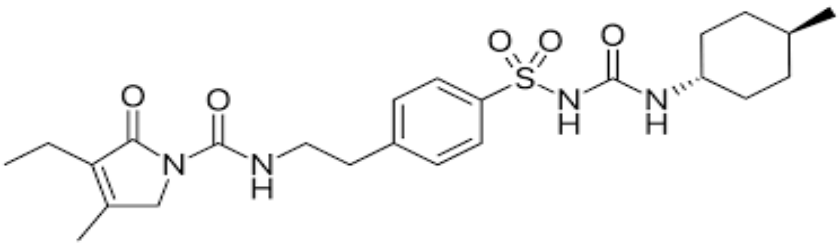

Figure 1: Glimepiride structure.

Parameters of degradation studies: The forced degradation studies for the drug substance include acid/base stress testing, H_2O_2 degradation, dry heat degradation.

Drug Authentication

1) Melting point (M.P)

Sample obtained was characterized for melting point of the substance. The melting point was determined by introducing small amount of substance in capillary and constant heat was supplied. The drug substance was tested in the temperature range of 207°C and the melting point was noted.

2) Solubility

The solubility of drug sample was tested in various solvents like acetonitrile, methanol and water. The observed results were then compared with drug profile.

3) Selection of solvent

The ideal property of a solvent should be that the drug should be completely soluble in the solvent used. The drug should be stable in the solvent used and should be economical. After suitable literature survey, practical experience and taking above factors into consideration the suitable solvents selected was Methanol.

4) Wavelength selection

Glimepiride showed maximum absorbance at 234 nm.

Development of Mobile Phase

A suitable solvent system for the composition of the mobile phase for development of chromatogram was optimized by testing different solvent mixtures of varying polarity. Various mobile phases were evaluated. Use of chloroform and ethanol as single component and a short saturation time of 15 min gave a necklace effect. So chloroform: ethanol (5:5 v/v), hexane: ethyl acetate(6:4 v/v), toluene: methanol (7:3, 8:2 v/v), toluene: chloroform: ethanol (4:4:1 v/v/v), toluene: ethyl acetate: methanol (6:5:0.5 v/v/v) were tried. The best results were obtained using toluene: chloroform: ethanol (4:4:1 v/v/v). This mobile phase showed a good resolution and a compact band of Glimepiride. Densitometric scanning of all the tracks at λmax 234 nm showed compound with Rf value 0.51 ±0.02 identified as glimepiride. The method was further used in the analysis of Glimepiride from tablet formulation without interference of the formulation excipients.

Instrumentation

Camag (Switzerland) ATS 4 applicator, a Camag Twin trough TLC Chamber. Camag TLC scanner 3, Camag Wincats Software. Hamilton (Reno, Nevada, USA) syringe (100 µL). HPTLC conditions are given in Table 1.

Table 1: HPTLC Conditions.

Stationary Phase	**TLC aluminium sheets Silica gel 60 F_{254}**
Mobile Phase	Toluene: Chloroform: Ethanol 4:4:1 v/v/v
Migration distance	70 mm
Slit Dimensions	4.00 x 0.45 mm, micro
Wavelength scanning	234 nm
R_f value of Glimepiride	0.51 ± 0.02

Chromatography

Thin layer chromatography was performed on 20×10 cm aluminum backed TLC plates coated with 250 µm layer of silica gel $60F_{254}$ (E. Merck). The plates were prewashed by methanol and activated at 105-110° for 15 min prior to use for chromatography. The samples in methanol were sprayed as 8mm wide bands at a distance of 8 mm from the bottom and 20 mm from the sides of the plate under continuous flow of air by means of ATS-4 as a sample applicator fitted with a 25µl syringe. A constant application rate of 150 nl/s was employed and track distance of 11.8 mm. The plates were then conditioned for 20 min in a pre-saturated twin-trough chamber (CAMAG) with the mobile phase toluene: chloroform: ethanol (4:4:1 v/v/v). The plate was then placed in the mobile phase and ascending development was performed upto distance of 70 mm from application position at 22°C and 40% relative humidity. After development, plates were air dried and densitometric scanning was performed at a wavelength of 234 nm.

Stress degradation Studies

In order to determine whether the analytical method was stability indicating, prulifloxacin was exposed to different stress conditions to carry out forced degradation studies, as per ICH Guidelines.[14,15]

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 F254. Toluene: Chloroform: Ethanol 4:4:1 v/v/v was used as mobile phase. Standard stock solution of Glimepiride 1mg/ml (5 µl) was applied on TLC plate. The Rf value for Glimepiride was 0.519 The typical Chromatogram of working standard solutions is

as shown in Fig.2.

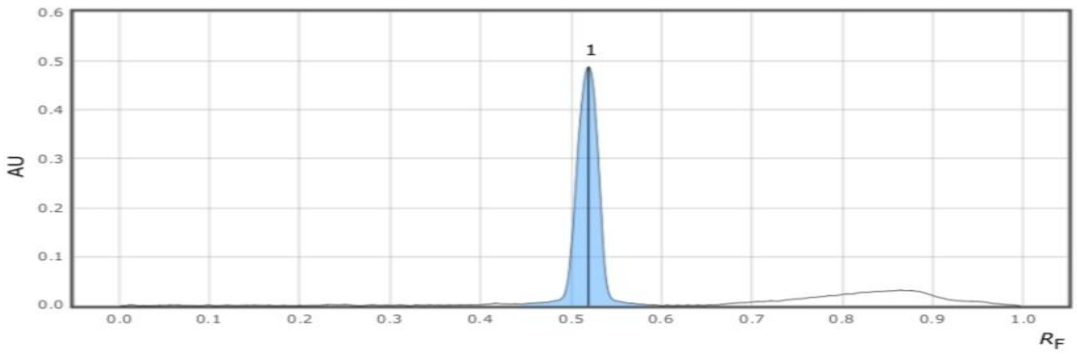

Figure 2: Chromatogram of Glimepiride.

Stress degradation in Acidic condition

A 100 mg standard Glimepiride 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, 1 ml of stock solution for Glimepiride was transferred to 10 ml volumetric flask, and volume was made up to mark with methanol. Then 5 µL of the stock solution for Glimepiride was applied to TLC plates and the chromatograms were run under the optimized chromatographic conditions. The result obtained for Glimepiride are shown in Fig. 3.

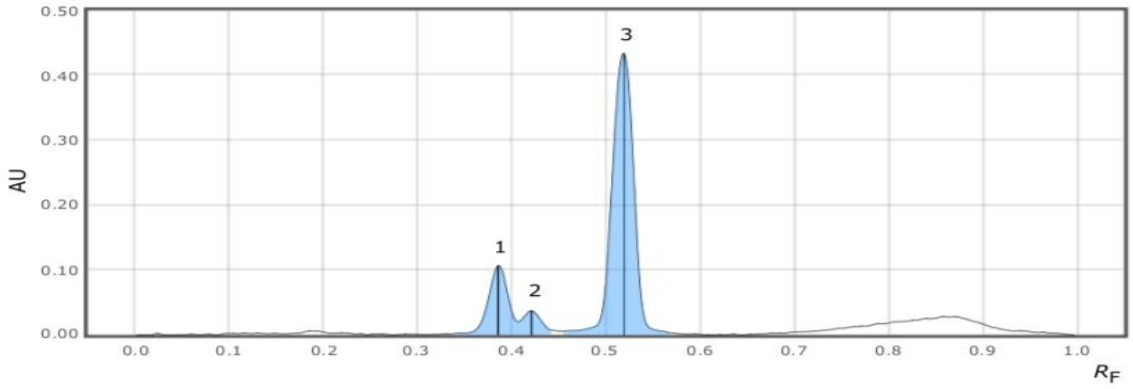

Figure 3: Chromatogram of Glimepiride and its degradation products in acid degradation study.

Stress degradation in Basic condition

A 100 mg standard Glimepiride was transferred in iodine flask and dissolved in 40 ml

methanol. 20 ml of 0.1M NaOH 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 HCl and volume was made up to the mark with Methanol. From this stock solution, 1 ml of stock solution for Glimepiride was transferred to 10 ml volumetric flask, and volume was made up to mark with methanol. Then 5 µL of the stock solution for Glimepiride was applied to TLC plates and the chromatograms were run under the optimized chromatographic conditions. The result obtained for Glimepiride are shown in Fig. 4.

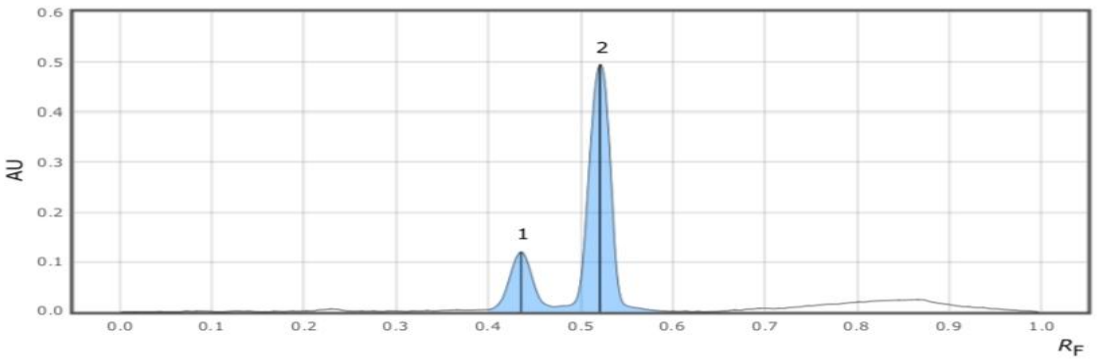

Figure 4: Chromatogram of Glimepiride and its degradation products in Base degradation study.

Stress degradation in Oxidative condition

A 100 mg standard Glimepiride was transferred to iodine flask and dissolved in 40 ml methanol. 20 ml of 6 % H2O2 was added in both the flask. The sample solution were stored at 25^{o}C (room temp.) for 3 Hrs. 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, 1 ml of stock solution for Glimepiride was transferred to 10 ml volumetric flask, and volume was made up to mark with methanol. Then 5 µL of the stock solution for Glimepiride was applied to TLC plates and the chromatograms were run under the optimized chromatographic conditions. The result obtained for Glimepiride are shown in Fig. 5.

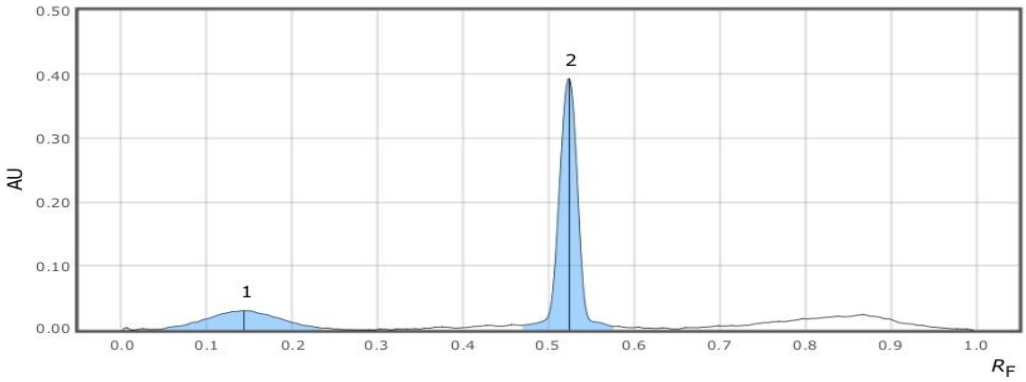

Fig. 5: Chromatogram of Glimepiride and its degradation products in Oxidative degradation study.

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 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, 1 ml of stock solution for Glimepiride was transferred to 10 ml volumetric flask, and volume was made up to mark with methanol. Then 5 µL of the stock solution for Glimepiride was applied to TLC plates and the chromatograms were run under the optimized chromatographic conditions. The result obtained for Glimepiride are shown in Fig. 6.

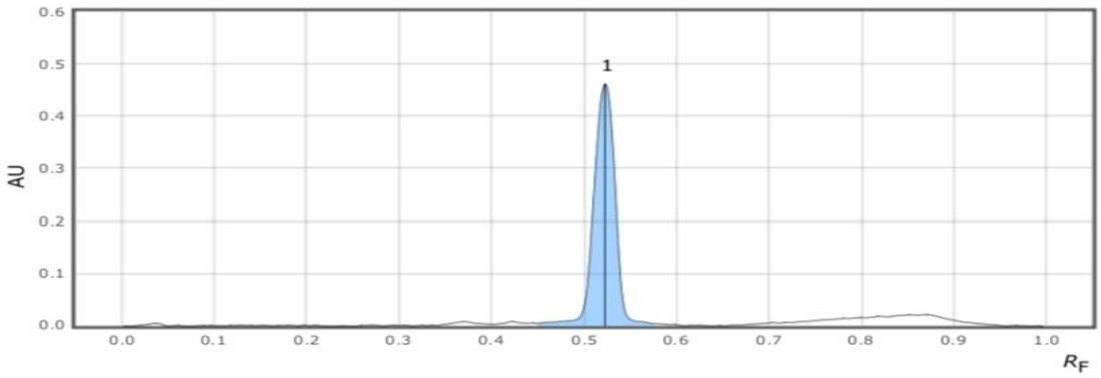

Fig. 6: Chromatogram of Glimepiride and its degradation products in Thermal degradation study.

RESULTS AND DISCUSSION

HPTLC method was validated as per ICH guidelines. The developed method was found to be linear within the range of 0.5- 3 µl with R^2 = 0.9948 for Glimepiride. The accuracy of method was determined at 80%, 100%, 120% level. The % recovery was found to be 99.6254187%.

The stress degradation studies were carried out for Glimepiride in acid, base, oxidation, thermal conditions. Summary of the results of stress degradation studies of Glimepiride are shown in Table 7.

Table 7: Summary of Stress Degradation Studies for Glimepiride.

Exposure conditions	Time (h)	Rf of degradation products	Recovery (%)
Acid, 0.1 N HCl, reflux	1	0.38, 0.42, 0.51	76.66
Base, 0.1 N NaOH, reflux	1	0.43,0.52	78.88
H_2O_2 (6 %)	3	0.14, 0.52	76.74
Dry heat (60°C)	3	-	100

CONCLUSION

The stability indicating method was developed on HPTLC for Glimepiride. Degradation product generated from forced degradation studies are potential degradation products that may or may not be the formed under relevant storage conditions but they assist in developing stability indicating method. Finally, it was concluded that the method is suitable to study stability of Glimepiride under various degradation conditions.

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