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Stress Degradation Studies on Simultaneous Estimation of Glipizide and Metformin Hydrochloride Using Stability –Indicating Chromatographic Methods

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ABSTRACT

Two sensitive and reproducible methods are described for the quantitative determination of Glipizide and Metformin Hydrochloride in presence of its degradation products. The first method was based on high performance liquid chromatographic (LC) separation of the drug from its degradation products on the reversed phase, Cosmosil® column [C_{18} (5 µm, 4.6 x 150 mm, i.d.)] at ambient temperature using a buffer consisting of 10 mM potassium dihydrogen phosphate (pH adjusted to 2.5 with diluted o- phosphoric acid) and acetonitrile 50: 50 v/v as optimized mobile phase in a gradient program. The flow rate was 0.7 ml min^{-1} and quantitation was achieved UV determination at 225 nm based on peak area with linear calibration curves at concentration range 10-25 µg ml^{-1} and 20-50 µg ml^{-1} respectively. The second method was based on high performance thin-layer chromatographic (HPTLC) separation followed by densitometric measurement of spots at 216 nm. The separation were carried out on Merck HPTLC aluminium sheets of silica gel 60 F_{254} using Toluene: Methanol: Ethyl acetate: Formic acid in the ratio of (3:6:3:0.2, *v/v/v/v*), as mobile phase. This system was found to give compact spots for Glipizide and Metformin hydrochloride after double development (retention factor, R_F 0.08 ± 0.02 and R_F 0.74 ± 0.02 respectively). The second order polynomial regression analysis data was used forth regression line in the range of 200-1400 ng $spot^{-1}$ and 200-1400 ng $spot^{-1}$ respectively. Both the method has been successively applied to pharmaceutical formulation. No chromatographic interference from the tablet exicipients was found. Both the methods were validated in terms of precision, robustness, recovery, limits of detection and quantitation.

Keywords: Glipizide, Metformin Hydrochloride, reverse phase high performance liquid chromatography, high performance thin-layer chromatography, Quantitative Analysis, Validation, stability indicating procedure, pharmaceutical formulation.

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INTRODUCTION

Diabetes mellitus is difficult to control with a single oral hypoglycaemic agent and the rate of mono therapy failure is high. Hence, combination therapy with complementary classes of drugs that act on different aspects of glycemic control would be expected to be an effective strategy for the control of diabetes. Metformin hydrochloride (MET), 1,1-dimethylbiguanidine monohydrochloride (Figure 1); Metformin is official in IP[1] and USP,[3]. It activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats; activation of AMPK is required for Metformin hydrochloride inhibitory effect on the production of glucose by liver cells [2].

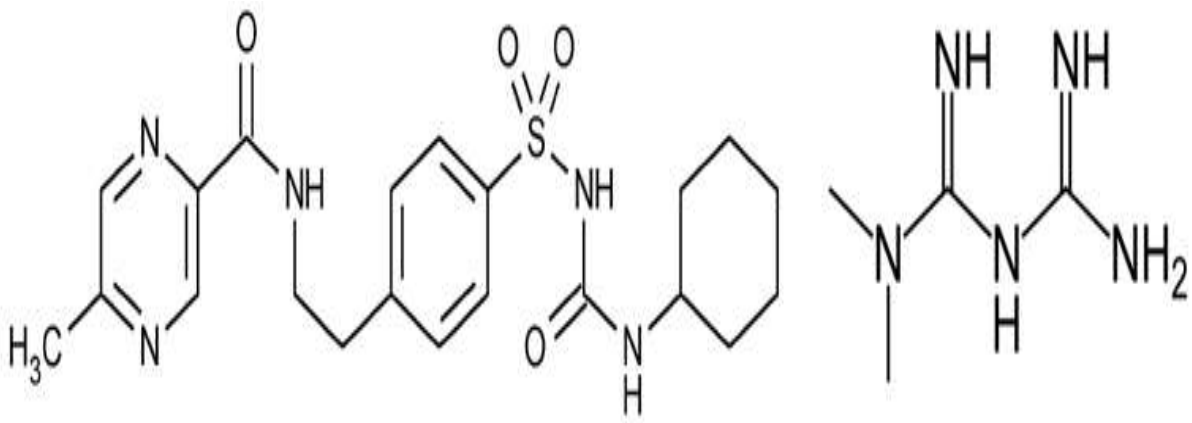


Figure 1:Structure of Glipizide and Metformin Hydrochloride

Glipizide (GPZ), N-[2-[4-[[[(Cyclohexylamino) carbonyl] amino] sulfonyl] phenyl] ethyl]-5-methylpyrazine carboxamide] (Fig 1) is an oral antihyperglycemic agent used in the treatment of noninsulin-dependent diabetes mellitus. It lowers the blood glucose level in humans by stimulating the release of insulin from the pancreas and helping the body to use insulin efficiently[5]. GPZ is included in the USP [3] and European Pharmacopoeia [4]. Among its other qualities, it has been shown to stimulate insulin action through extrapancreatic effects; to favorably influence the principal pathophysiologic abnormalities, defective secretary dynamics, and target-cell resistance to insulin observed in noninsulin-dependent diabetes; to improve control of blood glucose; and to lower the level of plasma glucose and to maintain this effect despite a short half-life [6].

Literature survey reveals; UV, HPLC [7-10] methods for analysis of Metformin as single and combined dosage forms with other drugs. Several HPLC methods are available for the determination of GPZ in plasma and dosage forms [11–19]. While, there is chromatographic method development [20-21] strategy. The reported methods either lack instability indicating study or used huge costly mass spectra for the determination. However, no article related to the stability

indicating chromatographic determination of GPZ and MET in pharmaceutical dosage form has been reported in literature. The ICH guideline entitled "stability testing of new drug substances and products" requires that stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Hydrolytic (acid, base, and neutral), oxidative, photolytic, and thermal stability are required. An ideal stability indicating method is one that quantifies the standard drug alone and also resolves its degradation products. Consequently, the implementation of an analytical methodology to determine GPZ and MET in pharmaceutical dosage form in presence of its degradation products is a pending challenge of pharmaceutical analysis. Therefore, it was thought necessary to study the stability of GPZ and MET towards hydrolytic (acid, base, and neutral), oxidative, photolytic, and thermal degradation processes. The aim of this work was to develop stability indicating chromatographic methods for determination of GPZ and MET in presence of its degradation products and related impurities for assessment of purity of bulk drug and stability of its bulk dosage forms using LC and HPTLC densitometry. The two methods are simple, accurate, specific, linear, and stability-indicating, reduces the duration of the analysis and suitable for routine determination of GPZ and MET from tablet formulation. Both the proposed methods were subjected to the International Conference on Harmonization (ICH) compliance and its updated international convention.

MATERIALS AND METHOD

Materials

Pharmaceutical grade of Glipizide and Metformin were kindly supplied as a gift sample by Glenmark pharmaceuticals Pvt ltd., Mumbai, India used without further purification and certified to contain 99.65% (w/w) and 99.03% (w/w) respectively, on dried basis. All chemicals and reagents like potassium dihydrogen phosphate buffer, acetonitrile, toluene, ethyl acetate, formic acid and methanol for chromatography were from used were of LC grade and purchased from Merck chemicals, India.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

For LC method

Chromatographic separation was achieved on a Younglin column C_{18} Cosmosil® (150 mm × 4.6 mm, 5 µm). Isocratic elution using a mobile phase consisting of potassium dihydrogen phosphate buffer (pH 3) - acetonitrile (50:50, v/v) with UV detection at 225 nm was performed. The mobile phase and buffer solution was filtered through 0.45 µm membrane filter and degassed for 30 min in an ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of

0.7 ml min^{-1}. Analyses were performed at ambient temperature and the injection volume was 20 µL.

For Planar Chromatography

It was performed on 10 × 10 cm^2 aluminum HPTLC plates precoated with 0.2 mm layers of silica gel (E.Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India). Before chromatography, the plates were prewashed with methanol and dried in an oven at 50 ^{0}C for 5 min. Samples were applied as 6-mm wide bands, under a continuous flow of nitrogen, by means of a CAMAG (Muttenz, Switzerland) Linomat V sample applicator equipped with an applicator microsyringe (Hamilton, Bonadauz, Switzerland). A constant application rate of 0.1 µL s^{-1} was used. The plates were then conditioned for 20 min in a presaturated twin-trough glass chamber (10 × 10 cm^2) with the mobile phase of toluene: methanol: ethyl acetate: Formic acid (3:6:3:0.2, *v/v/v/v*). The plates were then placed in the mobile phase and ascending development was performed to a distance of 70 mm from the point of application at ambient temperature, and the development time was 12 min. Subsequent to the development, the plates were dried in a current of air with the help of an air dryer and spots were visualized in CAMAG UV cabinet with dual wavelength UV lamp (254 and 366 nm); densitometric scanning was performed at 216 nm with CAMAG TLC scanner III operated in reflectance–absorbance mode and controlled by Wincats software (V 3.15, camag). The source of radiation utilized was deuterium lamp emitting a continuous uv spectrum between 190-400 nm. Concentrations of compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was via peak areas with second order polynomial regression. The shape of calibration curves in thin layer chromatography is generally inherently non linear due to scattering of light. Calibration curves generally comprise a pseudolinear region at low sample concentration and then departure from linearity begins at higher sample concentrations. The extent of individual ranges of the calibration curves is frequently very different for different substances. Tin some instances, the pseudo linear range may be adequate for most analytical purpose, in others no reasonable linear range exist. Scattering of light is highly dependent on the type of the TLC plate, measuring wavelength, measuring mode, molar absorptivity and concentration of the sample. The use of HPTLC plates is therefore advantageous since they are less scattering than conventional TLC plate.

STANDARD SOLUTIONS AND CALIBRATION GRAPHS

For LC method

Standard stock solutions were prepared by dissolving 100 mg of the stock solution in methanol in a 100 mL volumetric flask and then completed to volume with methanol. Injection of 20 µl by

triplicate were made six times for each concentration and chromatographed under the conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

For HPTLC densitometric method

Stock standard solution (1000 µg mL^{-1}) was prepared by dissolving 100 mg of each drug in 100 ml in methanol. For calibration, working solutions of GPZ and MET were prepared by dilution of the stock solutions and spotted on the plate in the range 200-1400 ng $spot^{-1}$ and 200-1400 ng $spot^{-1}$ respectively. Six analyses of each concentration were performed and then calibration plots (dependence of peak area on amount of each drug) were determined by linear least-squares regression. The plate was developed on previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

SAMPLE PREPARATION

To determine the content of both drugs in conventional tablet (label claim: Glynase®MF USV Pharmaceuticals (Batch No. 05001480) contains 5 mg Glipizide and 500 mg Metformin Hydrochloride per tablet), the 20 tablets were weighed their mean weight determined and they finely powdered and powder equivalent to 1000 mg was weighed. Then equivalent weight of the drug was transferred in to a 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 min and diluted to 100 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 µm filter (Millipore, Milford, MA)

For LC method

The above stock solution was further diluted to get sample solutions at three different concentrations of 10, 15, 20 µg ml^{-1} for GPZ and 15, 20, 25 µg ml^{-1} for MET respectively. A 20 µl volume of each sample solution was injected into LC, six times, under the conditions described in Section 2.2.1 the peak area of the spots were determined using multilevel calibration developed on the same LC system under the same conditions using linear regression equation.

For HPTLC densitometric method

The above stock solution was further diluted to obtain sample solutions at three different concentrations of 400, 600, 800 ng $spot^{-1}$ for GPZ and 400, 600, 800 ng $spot^{-1}$ for MET respectively. One microlitre of each sample solution was applied six times to the under the conditions described in Section 2.2.2. The peak areas of the spots were determined using multilevel calibration developed on the same plate under the same using second order polynomial regression equation.

METHOD VALIDATION

Precision

Precision of the method was determined with the product. An amount of the product powder equivalent to 100% of the label claim of GPZ and MET were accurately weighed and assayed. System repeatability was determined by six replicate applications and six times measurement of a sample solution at the analytical concentration. The repeatability of sample application and measurement of peak area of active compound were expressed in terms of % relative standard deviation (%RSD) and standard error (S.E.). Method repeatability was obtained from RSD value by repeating the assay six times in same day for intra-day precision. Intermediate precision was assayed by the assay of two, six sample sets on different days (inter-day precision). The intra-day and inter-day variation for determination of GPZ and MET was carried out at three different concentration levels for HPTLC and LC, respectively.

Robustness of the method

For LC method

To evaluate LC method robustness a few parameters were deliberately varied. The parameters included variation of flow rate, percentage of acetonitrile in mobile phase, column temperature and methanol of different lots. Robustness of the method was done at three different concentration levels 10, 15, 20 µg ml^{-1} for GPZ and 15, 20, 25 µg ml^{-1} for MET respectively.

For HPTLC-densitometric method

By introducing small changes in the mobile phase composition, the effects on the results were examined. The temperature, plate pre-treatment by washing with methanol and activated at 60 ^{0}C for 2, 5, 7 min, respectively prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 min. Robustness of the method was done at three different concentration levels 400, 600, 800 ng $spot^{-1}$ for the both drug.

Limit of detection and limit of quantitation

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and or degradation products.

For LC method

The limit of detection and limit of quantitation were separately determined at a signal to noise ratio (S/N) of 3 and 10. LOD and LOQ were experimentally verified by diluting known concentrations of GPZ and MET until the average responses were approximately 3 or 10 times the standard deviation of responses for six replicate determinations.

For HPTLC – densitometric method

The limit of detection and limit of quantitation were spotted six times following the same method as explained above. The signal to noise ratio (S/N) of 3 and 10. LOD and LOQ were experimentally verified by diluting known concentrations of GPZ and MET until the average responses were approximately 3 or 10 times the standard deviation of responses for six replicate determinations.

Specificity

For LC method

The specificity of the lc method was determined by the complete separation of GPZ and MET in presence of its degradation products along with other parameters like retention time (t_R), Capacity factor (k), tailing/asymmetrical factor (t) etc.

For HPTLC-densitometric method

According to USP 28, method, system-suitability tests are an integral part of a chromatographic analysis and should be used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis [34]. To ascertain the effectiveness of the method developed in this study, system-suitability tests were performed on freshly prepared standard stock solutions interspersed with replicate sample solutions of GPZ and MET.

The low % RSD value indicated the suitability of this method for routine analysis of GPZ and MET in pharmaceutical dosage form.

Recovery studies

For both methods recovery studies was carried out by applying the method to drug sample to which known amount of GPZ and MET corresponding to 80,100, 120% of label claim had been added (standard addition method). At each level of amount six determinations were performed and the results obtained were compared with expected results.

FORCED DEGRADATION STUDIES

A stock solution prepared as 1000 µg/mL GPZ and MET in methanol was used for forced degradation studies. This was used for forced degradation to provide an indication of the stability indicating property and specificity of proposed method.

Preparation of acid and base induced degradation product

Individually, 5 mL of standard solution was transferred to a 10 mL volumetric flask and boiled for 1 h at 60^0C after adding (a) 5 mL of 1 N HCl for acid hydrolysis (b) 5 mL of 1 N NaOH for basic hydrolysis before the analysis.

Preparation of hydrogen peroxide induced degradation product

To 5 mL of standard solution, 100 µL of 3 % H_2O_2 solution (v/v) were added and mixed. The solution was left at room temperature for 1 h in the dark.

Photochemical degradation product

The photochemical stability of GPZ and MET was studied by exposing the methanolic stock solution to direct sunlight for 8 h (from 9 AM to 5 PM at ≅ 30^0C) The chromatogram of the acid and base degraded samples for GPZ and MET showed only the spots of the pure drug.

RESULTS AND DISCUSSION

OPTIMIZATION OF PROCEDURES

Optimization of LC Method

The LC procedure was optimized with a view to develop a stability-indicating assay method. Modifications were made in above mobile phase and finally the mobile phase containing 10 mM potassium dihydrogen phosphate: acetonitrile in (50:50, *v/v*) ratio, pH 3 adjusted with o-phosphoric acid was tried. At 0.7mL/min flow rate GPZ and MET shows 6.5 and 1.9 min retention time and total time of analysis was 15 min. It was showing satisfactory results and three well-resolved peaks of GPZ and MET were recorded (Figure 9).

Optimization of HPTLC-densitometric method

Initially toluene and methanol was tried in the ratio of 5.0:5.0 *(v/v)*. The developed spots lack compactness, were discussed and R_F was considerably high. It was found that Toluene: Methanol: Ethyl acetate: Formic acid (3:6:3:0.2, *v/v/v/v*), as mobile phase gave compact spot and typical peak nature, good resolution with R_F value of 0.74 (± 0.05) for GPZ and 0.08 (± 0.05) for MET (Figure 2). Densitometric scanning was then performed with a Camag TLC scanner 3 equipped with Wincats Software Version 1.3.0 at λ_{max} = 216 nm using Deuterium light source, the slit dimensions were 6.00 X 0.45 mm. The spot appeared more compact and peak shape more symmetrical when the HPTLC plate were pretreated with methanol and activated at 100^0C for 5 min. Well defined spots of standard along with its degradation products were obtained at 30 min at room temperature. It was required to eliminate the edge effect and to avoid unequal solvent evaporation losses from the developing plate that can lead to various types of random behavior usually resulting in generally lack of reproducibility in R_F values (Table 1).

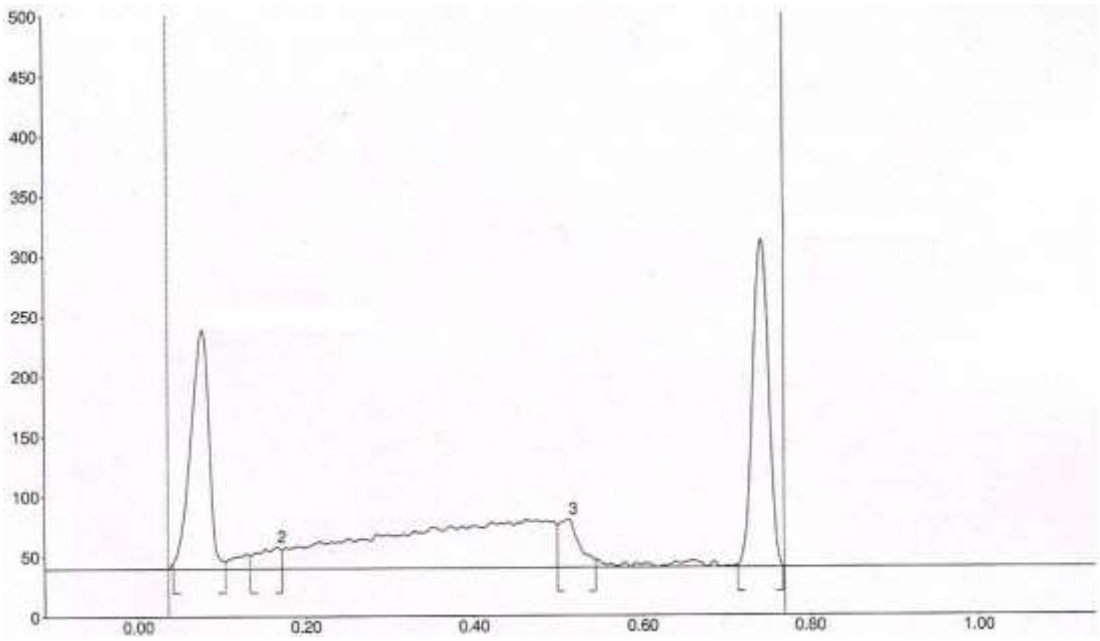


Figure. 2: Typical HPTLC Chromatogram of Standard GPZ and MET Mixture (R_f 0.74 ± 0.05 and 0.08 ± 0.05; respectively) measured at 216 nm, mobile phase: Toluene: Methanol: Ethyl acetate: Formic acid (3:6:3:0.2, *v/v/v/v*).

Linearity

Both drug showed good correlation coefficient in concentration range for as shown in (Table 1). For both proposed methods no significant difference was observed in the slopes of standard curves (ANOVA; $P< 0.05$).

Precision

For both proposed methods provides acceptable intra-day and inter day variations that were expressed in terms of % RSD values depicted in Table 1.

Table 1: Summary of validation parameters

Validation parameter	**HPLC**		**LC**	
	Metformin HCL	**Glipizide**	**Metformin HCl**	**Glipizide**
Specificity	Specific	Specific	-	-
Linear range	200-1400 ng/spot	200-1400 ng/spot	10-50 µg/ml	5-25 µg/ml
Precision (%RSD)				
Method precision (*n = 6)*	1.98	0.36	1.82	1.47
Intra-day (*n = 6*)	1.59	0.57	1.58	1.28
Inter-day (*n = 6*)	0.99	1.42	1.46	1.08
Different analyst (*n = 6*)	1.43	1.98	2.93	1.58
Limit of detection	50 ng/spot	5 ng/spot	0.10 µg/ml	0.50 µg/ml
Limit of quantification	100 ng/spot	50 ng/spot	80 µg/ml	30 µg/ml
Regression Equation	3.379 x + 1030	3.603 x + 1612	83.11x+ 19.7	100.5x +107.6
Correlation Coefficient	0.9975	0.9999	0.9991	0.9992
Resolution/Retention factor	0.08 (± 0.05)	0.74 (± 0.05)	1.91 ± 0.05	6.81 ± 0.05

Robustness of the method

For LC method

Robustness of the method was performed with change in selected parameters (factors) like flow rate, change in column temperature and change in mobile phase composition. Insignificant differences in peak areas and less variability in retention time were observed.

For HPTLC-densitometric method

Robustness of the method was performed with change in selected parameters (factors) like plate activation time, chamber saturation time, volume of mobile phase, and development distance from spot application that remained unaffected by small variations of these parameters. One factor at the time was changed to estimate the effect. The low values of %RSD as shown in (Table 2 a &b) indicated robustness of the method.

Table 2 (a): Robustness evaluation[a] of the LC method (n=6);

Chromatographic Changes				
Factor[b]	**Level**	**t^c_R**	**k[d]**	**T[e]**
A. Flow Rate				
0.5	-1	3.0	2.18	1.49
0.7	0	1.9	2.15	1.38
0.8	1	2.8	1.98	1.66
Mean ±S.D. (n=6)		2.0 ± 0.07	2.0 ± 0.06	1.35 ± 0.07
B. Percentage of Acetonitrile in the Mobile Phase (v/v)				
48	-1	2.97	2.19	1.72
50	0	2.1	2.15	1.38
52	1	2.6	2.11	1.24
Mean ±S.D. (n=6)		2.0 ± 0.04	2.15 ± 0.07	1.44 ± 0.01
C. Temperature				
24	-1	2.5	2.15	1.45
25	0	2.0	2.18	1.37
26	1	2.7	2.16	1.23
Mean ±S.D. (n=6)		2.0 ± 0.09	2.18 ± 0.02	1.35 ± 0.04

[a] Average of three concentration 10, 15, 20 µg/ml for MET only

[b] three factors were slightly changed at three levels (-1, 0, 1); each time a factor was changed from level 0 the other factors remained at level 0

[c] Retention time

[d] Capacity factor

[e] tailing factor

Table 2 (b): Robustness testing of HPTLC-densitometric method.

Method parameter/ Condition	Peak area of MET (*n=6*)%RSD		Peak area of GPZ (*n=6*) %RSD
Plate activation time[a]	20 min	1.88	1.04
	30 min	0.93	1.33
Chamber saturation time[a]	16 min	1.74	0.94
	24 min	0.85	1.54
Volume of mobile phase[b]	8.2 mL	1.56	1.62
	10.0 mL	1.84	0.28
Development distance from spot application[c]	7.2 mL	1.23	1.36
	8.8 mL	1.65	1.98

[a] ±20% change in set time

[b] ±10% change in set volume

[c] ±10% change in set distance

LOD and LOQ

For LC method

The signal-to-noise ratios 3:1 and 10:1 were considered as LOD and LOQ, respectively. The LOD and LOQ were 0.10 and 80 µg/ml for Metformin Hydrochloride and 0.50 and 30 µg/ml for glipizide.

For HPTLC –desitometric method

The LOD and LOQ were 50 and 100 ng per spot for Metformin Hydrochloride and 5 and 50 ng per spot for glipizide.

Specificity

For LC method

The peaks obtained were sharp and have clear baseline separation. All the components presents in mixture in whole wavelength range from 200 nm to 400 nm and it indicated that there is no degradation peak (hiding) under or unresolved form the analyte peak (pure drug), which also reflected the specificity of the method.

For HPTLC –desitometric method

Specificity of the method for MET and GPZ were proved from the spectral scan. The peak purity correlation (r) results for MET and GPZ in bulk and in pharmaceutical formulation indicate that there is no merging or co-elution of interfering peaks with MET and GPZ, so there is no interference from any excipients present in tablet formulations of MET and GPZ (Figure 4&5).

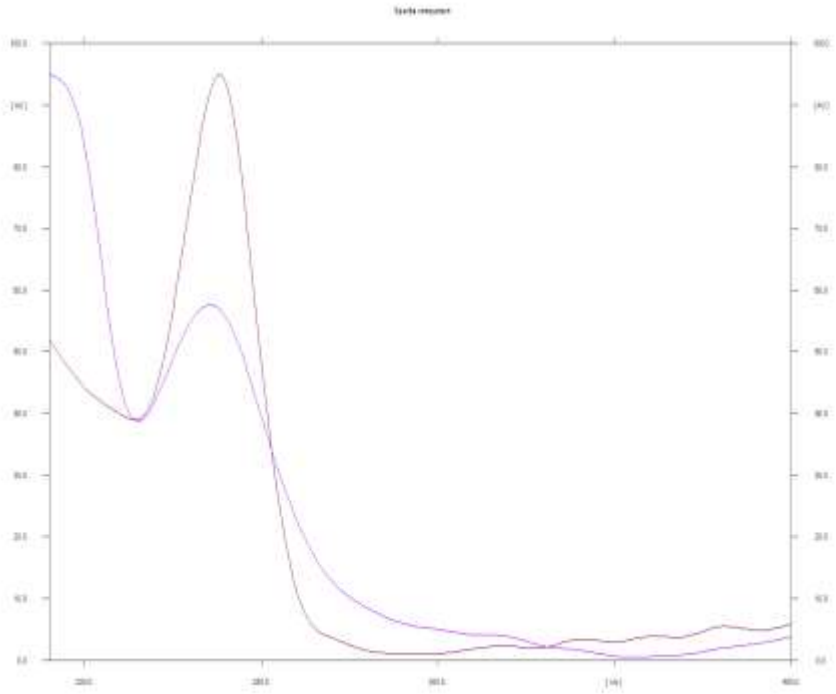


Figure. 3: Typical Overlain Spectra of GPZ and MET Standard Drug Solutions

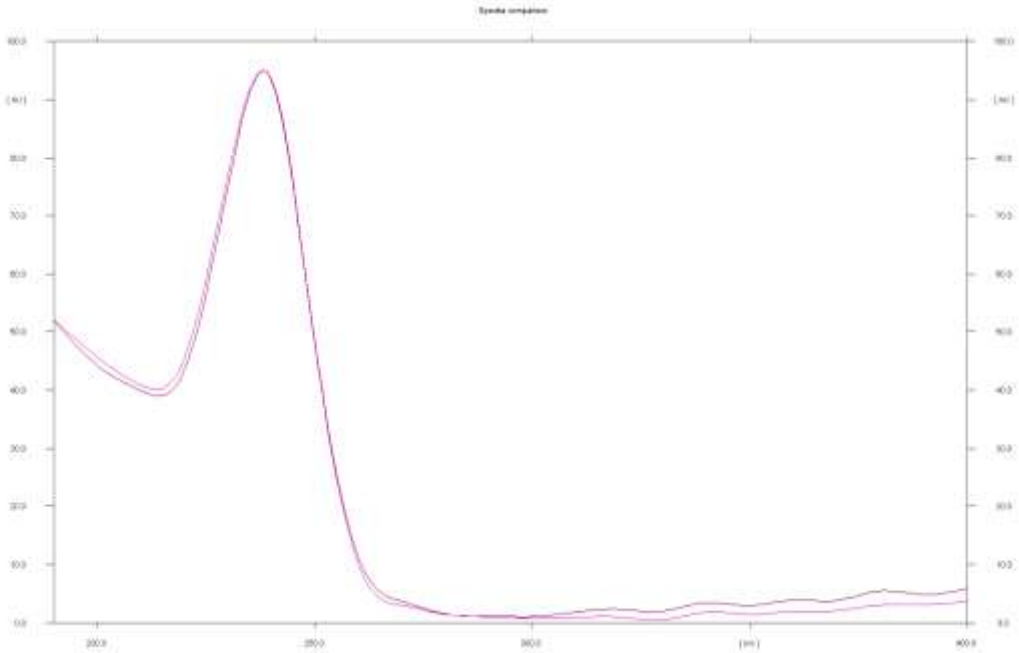


Figure. 4: Peak purity spectra of MET extracted from a GPZ -MET tablet, scanned at the peak-start, peak-apex and peak-end positions of the spot (correlation > 0.99)

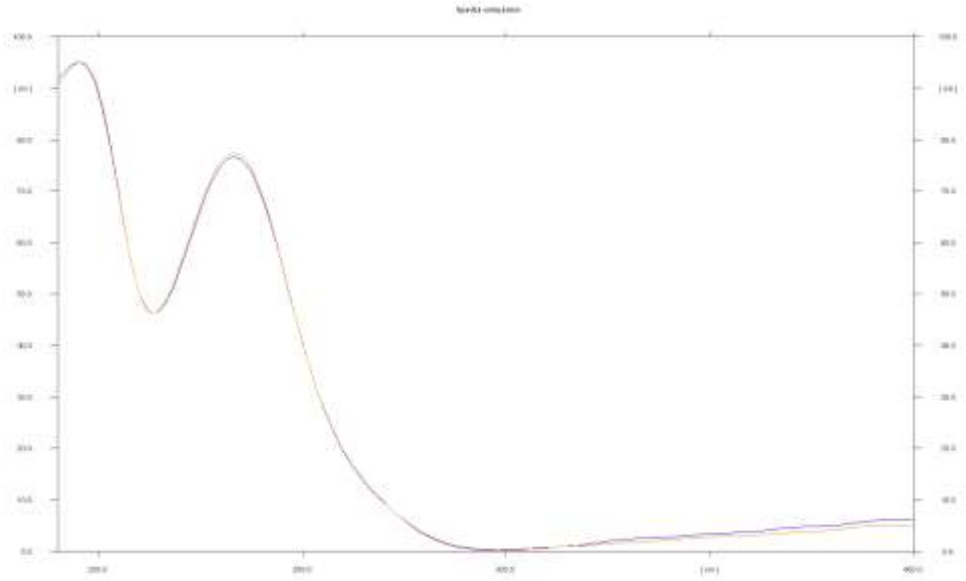


Figure. 5: Peak purity spectra of GPZ extracted from a GPZ-MET combination tablet, scanned at peak-start, peak- apex and peak-end positions of the spot (correlation > 0.99)

Recovery Studies

The both proposed methods when used for extraction and subsequent estimation of MET and GPZ from pharmaceutical dosage form after spiking with 80, 100 and 120% of additional drug afforded recovery values are given in Table 3.

Table 3. Standard addition technique for determination recovery studies by HPTLC densitometric and LC method

HPTLC densitometric						LC method					
Excess drug added to the analyte (%)	Theoretical content (ng)	Recovery (%) ± S.D.		% RSD		Excess drug added to the analyte (%)	Theoretical content (µg)	Recovery (%) ± S.D.		% RSD	
		GPZ	MET	GPZ	MET			GPZ	MET	GPZ	MET
0	800	100.70 ± 13.67	100.05 ± 22.48	0.67	1.12	0	20	99.96 ± 0.12	100.36 ± 0.15	0.61	0.51
80	1440	99.72 ± 5.65	99.83 ± 10.44	0.72	0.65	80	36	100.21 ± 0.10	101.63 ± 0.15	0.66	0.62
100	1600	99.82 ± 5.68	99.88 ± 10.66	0.28	0.53	100	40	100.40 ± 0.08	102.01 ± 0.11	0.28	0.36
120	1760	100.46 ± 10.72	100.16 ± 20.39	0.44	0.84	120	44	100.25 ± 4.06	101.34 ± 0.42	0.84	1.15

Analysis of the marketed formulation

For LC Method

Experimental results of the amount of MET and GPZ in tablets, expressed as percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any excipients, which are normally present in the tablets.

For HPTLC –densitometric method

The spot were observed in the Densitogram of the drug sample extracted from tablets. There was no interference from the excipients commonly present in the tablets. The low % R.S.D. value indicated the suitability of this method in good agreement with label claims (Table 4 a &b).

Table 4: applicability of the proposed methods for the determination MET and GPZ in Marketed Formulation

Sample	Label claim [mg/tablet]		Amount Found [%]		[%] RSD	
	MET	GPZ	MET	GPZ	MET	GPZ
T1 (by HPTLC)	500	5	497	4.98	1.52	2.07
T1 (by LC)	500	5	501	4.85	1.03	0.64

'Glynase® MF' tablets (containing 500 mg MET and 5 mg of GPZ)

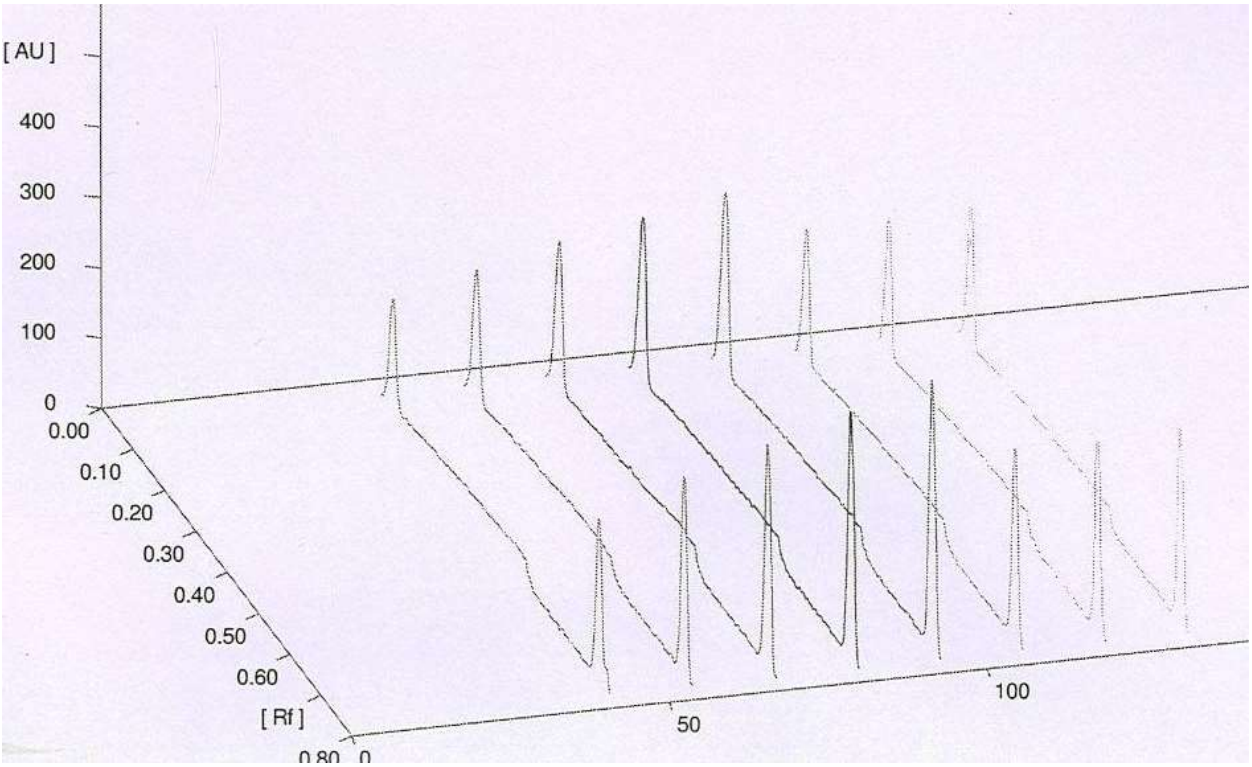
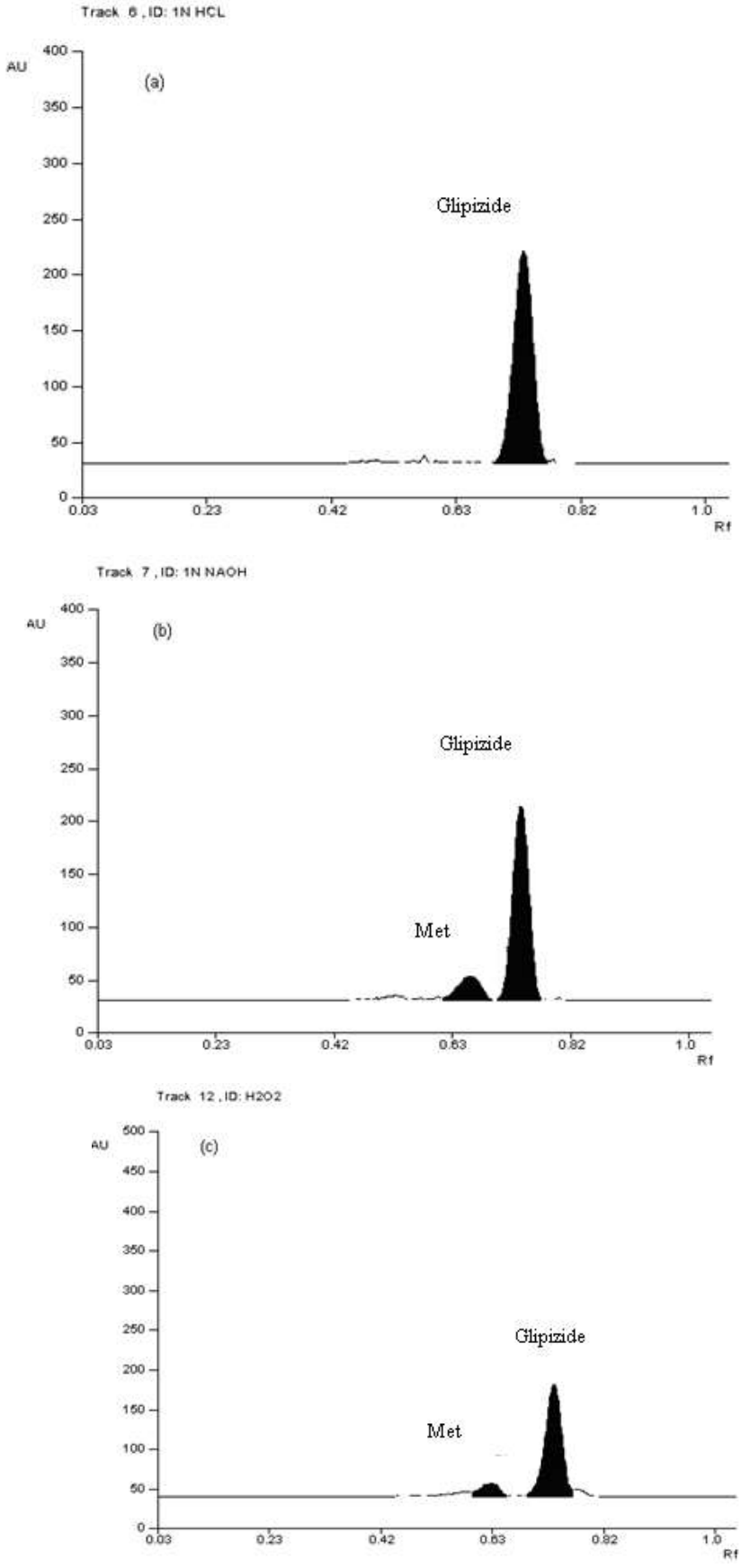


Figure. 6: 3D Linearity Spectra of GPZ and MET Standard Drug Solutions

Stability-indicating property

Acid and base induced degradation product

The concentration of the drug was found to changing from initial concentration indicating that GPZ and MET undergoes degradation under acidic and basic conditions (Figure 7 a&b; Figure 9 a, b)


Track 6 , ID: 1N HCL
AU
(a)
Glipizide
Track 7 , ID: 1N NAOH
(b)
Glipizide
Met
Track 12 , ID: H2O2
(c)
Glipizide
Met
Rf

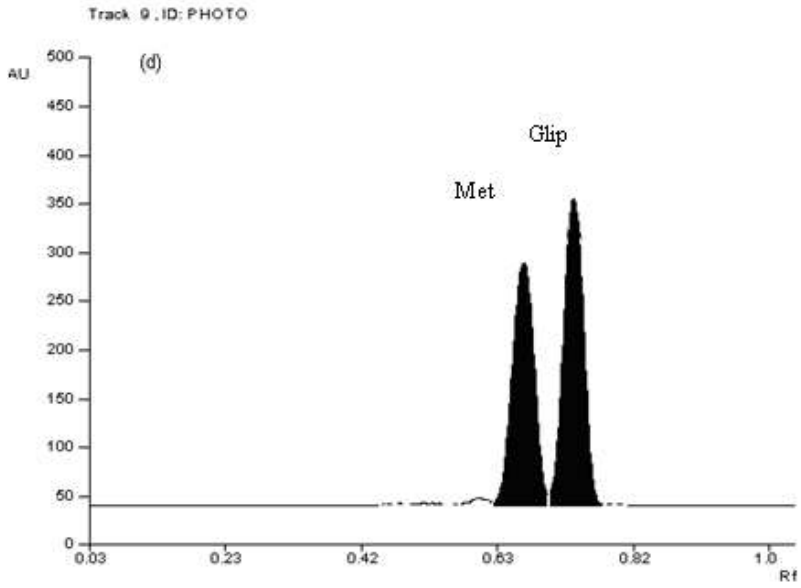


Figure. 7. Densitogram of MET and GPZ by Forced Degradation Studies

(a) acid-treated; Metformin hydrochloride (R_f : not detected), Glipizide (R_f : 0.73)

(b) base-treated; Metformin hydrochloride (R_f : 0.66), Glipizide (R_f : 0.74)

(c) Degraded with hydrogen peroxide; Metformin hydrochloride (R_f : 0.63),Glipizide (R_f : 0.73)

(d) photo-treated; Metformin hydrochloride (R_f: 0.66), Glipizide (R_f : 0.74).

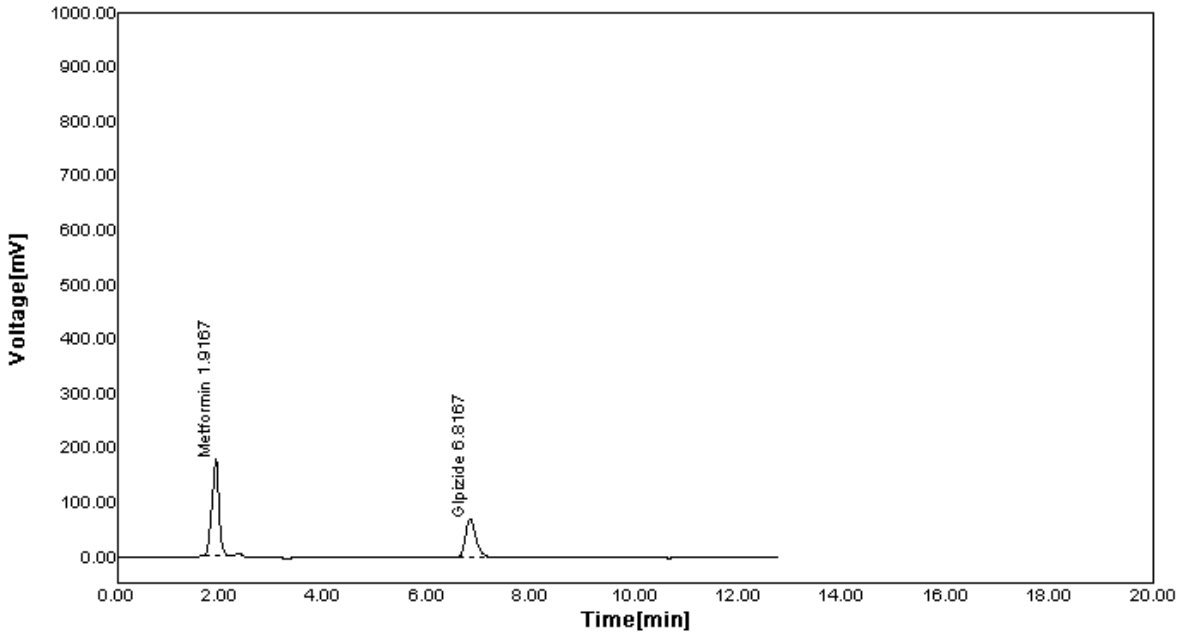


Figure 8. Chromatogram of Standard GPZ and MET Mixture (t_R 6.81 ± 0.05 and 1.91 ± 0.05; respectively) measured at 225 nm, mobile phase: 10 mM Potassium dihydrogen phosphate buffer and acetonitrile (50:50, *v/v*), pH 3 adjusted by o- phosphoric acid

Hydrogen peroxide induced degradation product

The sample degraded with 3% [w/v] hydrogen peroxide [Figure 7 c; Figure 9 c] showed one additional peak at R_f value of 0.33. The spot of degraded products were well resolved from the spot.

Photochemical and UV degradation product

In LC the photo degraded sample showed one additional peak at t_R 3.70 min when drug solution was left in day light for 44 and 7 days, respectively. The HPTLC densitogram showed one additional peak at R_f 0.53 for the photo degraded sample respectively [figure 7 d].

Neutral degradation

The LC chromatogram for neutral degradation showed decrease in peak area of standard without corresponding rise in new peak. The HPTLC densitogram for neutral hydrolysis showed one addition peak at R_f 0.09 [figure.9 d] after double development of HPTLC plates

This indicates that the drug is susceptible to acid base hydrolysis, oxidation dry and wet heat degradation and photo degradation. The results are listed in (Table 5).

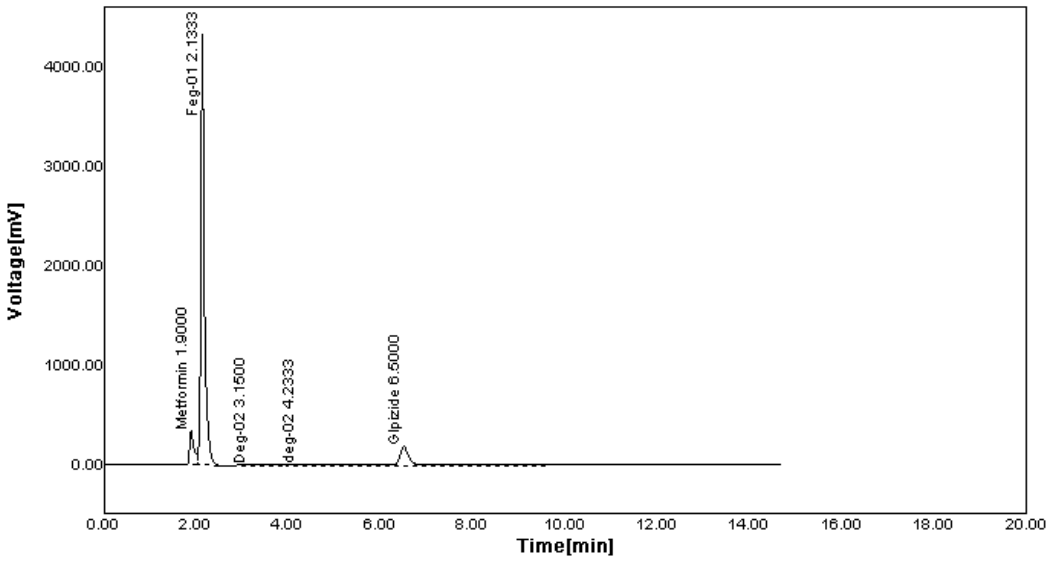
Table 5. Forced Degradation Studies using

(a) LC method

Condition	Time (hr)	Recovery (%)	
		Metformin hydrochloride	Glipizide
Acid, 0.1 N HCl 6 h (at room temperature) + at 60°C	6+1	93.14	85.80
Base, 0.1 N NaOH (at room temperature)	1	81.46	Not detected
Neutral hydrolysis (at room temperature)	2	98.60	Not detected
H_2O_2, 3% (at room temperature)	1	38.52	23.52

(b) HPTLC method

Condition	Time (hr)	Recovery (%)	
		Metformin hydrochloride	Glipizide
Acid, 1 N HCl, 60^0C	1	Not detected	72.33
Base, 1N NaOH, 60^0C	1	26.34	58.43
H_2O_2, 3 % at room temperature	1	Not detected	43.95
Daylight heat 30^0C	8	95.80	98.06


Voltage[mV]
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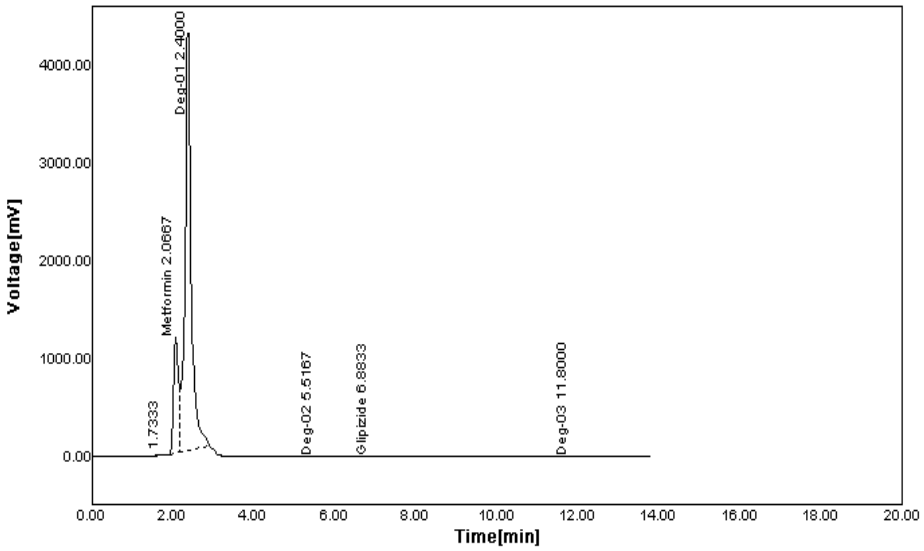
3000.00
2000.00
1000.00
0.00
Feg-01 2.1333
Metformin 1.9000
Deg-02 3.1500
deg-02 4.2333
Glipizide 6.5000
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Time[min]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Glipizide 6.8833
Deg-03 11.8000
0.00
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4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Time[min]

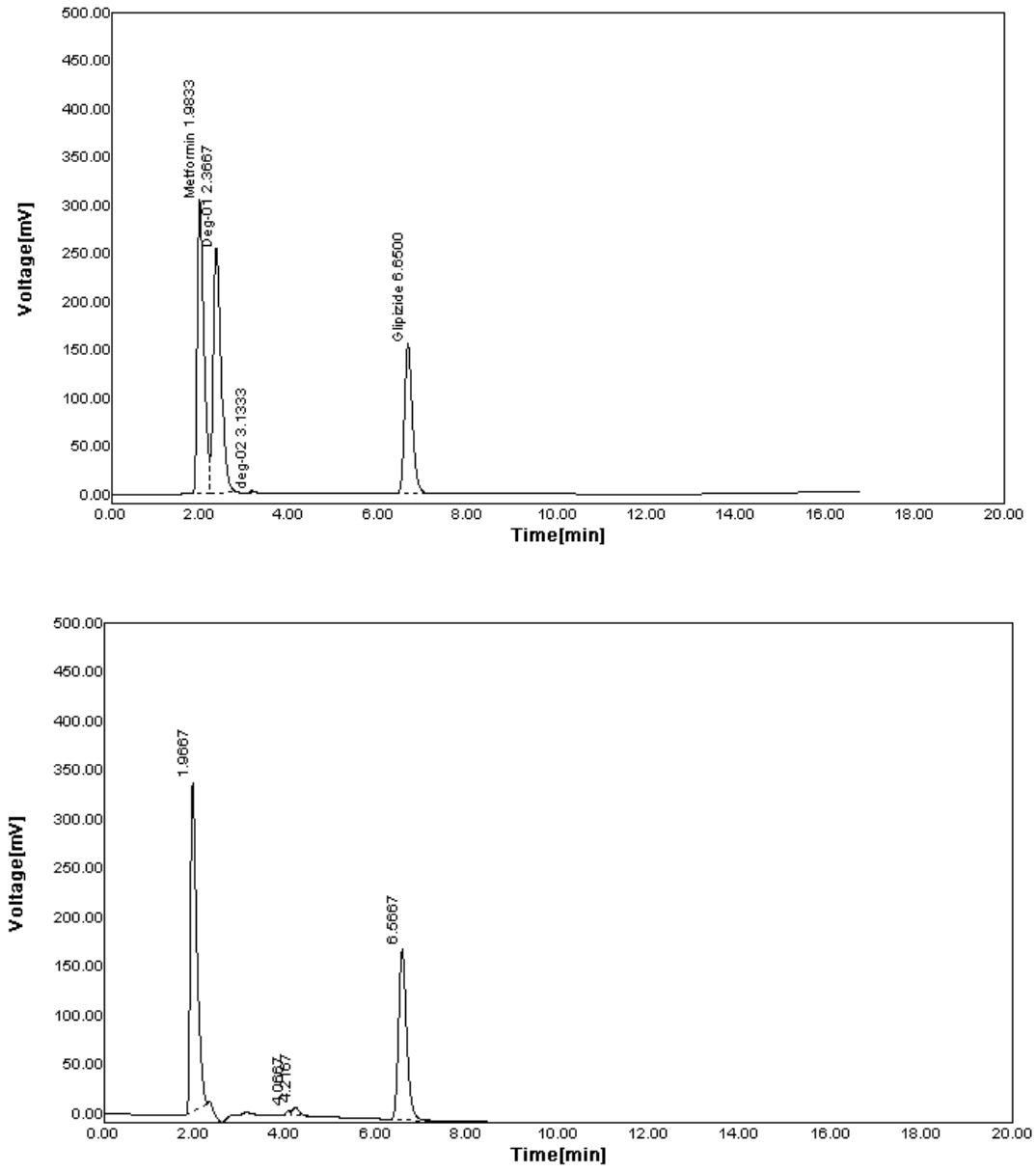

Figure. 9. Chromatogram of GPZ and MET by Forced Degradation Studies

(a) Chromatogram of acid degraded (1 N HCl, reflux for 1 hr, temp 60^0 c)

(b) Alkali hydrolysis chromatogram of GPZ and MET

(c) Oxidation chromatogram of GPZ and MET

(d) Neutral stress chromatogram of GPZ and MET

CONCLUSION

The proposed LC and HPTLC techniques are precise, specific, simple, accurate, linear, reproducible and repeatable for the estimation of MET and GPZ in pharmaceutical dosage form without any interference from the excipients and in the presence of its acidic alkaline, oxidative

and photolytic degradation products. Both the chromatographic methods were validated as per ICH guidelines. The HPTLC method offers several advantages over liquid chromatographic methods such as the possibility of simultaneous analysis of sample and standard on the same plate, short system equilibrium time, multiple/repeated scanning of chromatograms, higher mobile phase pH, large sample capacity, short run time, minimum solution consumption and no prior treatment for solvents like filtration and degassing compared to LC method. Statistical tests indicate that both the methods reduce the duration of analysis and appear to be equally suitable for routine analysis the analytes in pharmaceutical dosage form in quality control laboratories, where economy and time are essential. However, results showed the suitability of proposed method for acid, base and peroxide induced degradation kinetic studies. It was found that analyte are rapidly degraded in alkaline, oxidative conditions, while it is more stable in acidic medium. As the method separates the drug form its degradation products, it can be employed as a stability indicating one. It may be extended for quantitative estimation of said drug in plasma and other biological fluids.

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