

Development of stability indicating assay method for the simultaneous estimation of metformin hydrochloride and glipizide by RP-HPLC method

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Abstract

A simple, sensitive and isocratic RP-HPLC method for the estimation of Metformin Hydrochloride (MET) and Glipizide (GPZ) in combined dosage form using Inertsil C8 column (250×4.6 mm, 5 μ) in an isocratic mode with mobile phase comprising Acetonitrile: Water (70:30) & one drop of Triethylamine. The flow rate was 1.0 ml/min and effluent was monitored at 222 nm. The retention times were found to be 3.40 min for MET and 4.44 min for GPZ. The assay exhibited a linear dynamic range of 100-500 μg/ml of MET and 1-5 μg/mL for GPZ. The calibration curves were linear ($r = 0.9985$ for MET and $r = 0.9955$ for GPZ) over the entire linear range. Recovery was found to be $99.94\% \pm 0.17$ for MET and $99.61\% \pm 0.89$ for GPZ. % RSD of system precision were observed 0.0429 for MET & 0.7212 for GPZ.

Keywords: Metformin Hydrochloride, Glipizide, High Pressure Liquid Chromatography, Validation

1. Introduction

Metformin Hydrochloride (MET) is the first-line drug of choice for the treatment of type 2 diabetes, particularly in overweight and obese people and those with normal kidney function. Chemically it is *N,N*-dimethylimidodicarbonimidic diamide. Metformin hydrochloride (Fig.1) is a white crystalline powder that is odourless and has a slightly bitter taste and hygroscopic, freely soluble in water, slightly soluble in alcohol and practically insoluble in acetone, ether and chloroform. Metformin improves hyperglycemia primarily through its suppression of hepatic glucose production (hepatic gluconeogenesis). The "average" person with type 2 diabetes has three times the normal rate of gluconeogenesis; metformin treatment reduces this by over one third. Metformin 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's inhibitory effect on the production of glucose by liver cells. Research published in 2008 further elucidated metformin's mechanism of action, showing that activation of AMPK is required for an increase in the expression of SHP, which in turn inhibits the expression of the hepatic gluconeogenic genes PEPCK and Glc-6-Pase. Glipizide (GPZ) is type 2 diabetes mellitus drug. Chemically it is *N*-(4-[*N* (cyclohexylcarbonyl) sulfamoyl] phenethyl)-5-methylpyrazine-2-carboxamide. It is whitish, odorless powder, which is water solubility-37.mg/ml. insoluble in alcohol, soluble in 0.1N NaOH, freely soluble in dimethylformamide. Glipizide (Fig.2) is an oral medium-to-long acting anti-diabetic drug from the sulfonylurea class. It is classified as a second generation sulfonylurea, which means that it undergoes enterohepatic circulation. The structure on the R2 group is a much larger cyclo or aromatic group compared to the 1st generation sulfonylureas. This

leads to a once a day dosing that is much less than the first generation, about 100 fold. Mechanism of action is produced by blocking potassium channels in the beta cells of the islets of Langerhans. By partially blocking the potassium channels, it will increase the time the cell spends in the calcium release stage of cell signaling leading to an increase in calcium. The increase in calcium will initiate more insulin release from each beta cell.

The analytical chemistry hence has challenge in developing the methods for their analysis with the help of number of analytical techniques, which are available for the estimation of the drugs and their combination. literature survey^{2-6,13} was carried out that there are few methods reported for estimation of selected drugs singly, however, no stability indicating assay method has been reported for estimation of MET and GPZ drugs in combined dosage form. Market survey revealed that the selected drug MET & GPZ is available commercially as combination in tablet dosage form and It is used in diabetes. The present work is undertaken with an objective to develop economical, simple, precise, accurate and reproducible stability indicating method for estimation of these antidiabetic drugs in their combined dosage form.

Fig 1: Structure of Metformin Hydrochloride

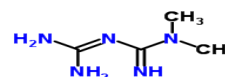
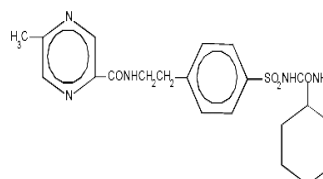


Fig 2: Structure of Glipizide



2. Methods and Materials^{1,7,11}

2.1 Chemicals and Reagents

All the solvents and chemicals used were of HPLC and analytical grade. Milli Q water and 0.45 μm Teflon filter was used throughout the experimental work. The gift drug samples of MET and GPZ were provided by Cipla Pharma Ltd. (Mumbai, India). Chemicals and Reagents Used are Hydrogen Peroxide 30%, Ortho-Phosphoric acid, Concentrated Hydrochloric Acid, Potassium Dihydrogen Orthophosphate, Sodium Hydroxide Pellets, Acetonitrile, Water, Methanol.

2.2 Instrument

The chromatographic separation performed using Jasco HPLC System with UV detector, model 2080.31. Software used to monitor was Borwin and Quaternary pump is applied. Analytical balance is used, Make Sartorius (Model AB - 20.04). pH meter was also used, Labindia Make, Model pH System 362.

2.3 Preparation of Mobile Phase

Acetonitrile (HPLC Grade) and Water (HPLC Grade) in the ratio of 60:40 was prepared. 1 drop of Triethylamine was added in that .pH was made up to the 3.0 Then resulting solution was filtered through 0.45 μm filter and degassed using sonicator. That solution was used as mobile phase.

2.4 Preparation of diluent:

Methanol of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the mixture of Acetonitrile and Water in the ratio 70:30.

2.5 Selection of Analytical Wavelength

The standard solution of MET and GPZ was scanned over the range of 200 nm to 400 nm wavelengths. The common wavelength of absorption was found to be 222 nm. So the wavelength selected for the determination of MET and GPZ was 222nm. **figure3.**

2.6 Analysis of Physical Laboratory Mixture

2.6.1 Preparation of Standard Stock Solution

An accurately weighed quantity of MET working standard about 10.0 mg and GPZ working standard about 10 mg were transferred separately into 10.0 mL volumetric flask. About 5.0 mL of methanol (HPLC Grade) was added to the volumetric flask and sonicated to dissolve the drug. The solution was cooled to the room temperature and made up to the mark with methanol (HPLC Grade) which gave the final concentrations of 1000 g/mL and 1000 g/mL for MET and GPZ respectively. Take 0.05mL of GPZ and 5.0 mL of MET from stock solution of GPZ and MET respectively in a 10 mL volumetric flask and make up the volume up to the mark with mobile phase to gate 5 $\mu\text{g/mL}$ GPZ. & 500 $\mu\text{g/mL}$ MET. **1.**

2.7 Analysis of Marketed Formulation

2.7.1 Preparation of Sample Solution

Take the powder weight of tablet equivalent to 500 mg. of MET in 100 mL of volumetric flask and add sufficient mobile phase and sonicate it for 15

min. Make up the volume up to the mark with mobile phase and filter it with 0.24 μm filter to get 5000mg/mL and 1000 $\mu\text{g/mL}$ of MET and GPZ respectively . Take 0.05mL of GPZ and 25.0mL of MET from above solution of GPZ and MET respectively in a 10.0 mL volumetric flask and make up the volume up to the mark with mobile phase to gate 5 $\mu\text{g/mL}$ GPZ. & 500 $\mu\text{g/mL}$ MET.

The results are shown in **Figure7** & discuss in the **table3.**

2.8 Optimization of Chromatographic Condition for Estimation of Drugs

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing mixture of MET & GPZ was run and different individual solvents as well as combinations of solvents have been tried to get a good separation and stable peak. Each mobile phase was filtered through 0.45 μm Teflon filter.

Finally, the optimal composition of the mobile phase, Acetonitrile:Water (70:30) & one drop triethylamine was selected. It gave high resolution of MET and GPZ with minimal tailing.

2.9 Calibration Curves for MET & GPZ

Weigh accurately 10 mg of Metformin Hydrochloride in 10.0mL methanol and 10 mg of Glipizide in 10.0 ml of methanol standard in a 10.0mL volumetric flask and dissolve by sonication in sufficient mobile phase then make up the volume by mobile phase to prepare 1000 $\mu\text{g/mL}$ stock solution. The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The series of concentrations from 1-5 $\mu\text{g/mL}$ of Glipizide, 100-500 $\mu\text{g/ml}$ of Metformin Hydrochloride std. solutions were injected and peak area was recorded.

2.10 System Suitability Test

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions. An accurately weighed quantity of MET working standard about 10.0 mg and GPZ working standard about 10.0 mg were transferred separately into 10.0 mL volumetric flask. About 5.0 mL of Methanol (HPLC Grade) was added to the volumetric flask and sonicated to dissolve the drug. The solution was cooled to the room temperature and made up to the mark with Methanol (HPLC Grade) which gave the final concentrations of 1000 g/ml and 1000 g/ml for MET and GPZ respectively.

2.11 Validation Parameters⁸⁻⁹

2.11.1 Accuracy:

To study the accuracy, 10 tablets were weighed and powdered. Analysis of the same was carried out. Recovery studies were carried out by standard addition method by adding the known amount of MET and GPZ separately to the reanalyzed sample at three different concentration levels i.e.

50%, 100%, and 150% of assay concentration and percent recoveries were calculated. 1 ml of the sample solution was pipetted out and transferred to three 10 ml volumetric flask separately along with this 0.5, 1, 1.5 ml of aliquots from the stock solution of MET and GPZ. All the solutions were filtered through 0.45 µm Nylon-66 filter and injected into HPLC system. Peak areas were recorded for all the peaks.

2.11.2 Precision :

Demonstrate the method precision by preparing 6 samples (sample preparation) as per the test method representing a single batch were applied in triplicate and injected this sample preparation, but before diluent, placebo, and standard solution in six replicates injected in HPLC system. Determine the assay of these samples and evaluate the precision of the method by computing the % RSD of the assay results.

2.11.3 Linearity and Range

The concentration ranges; 1, 2, 3, 4, 5 µg/mL for GPZ and 100,200,300,400,500 µg/mL for MET were selected as linearity range.

2.11.4 Ruggedness :

The studies were carried out for two different parameters.

a) Intra-day and Inter-day precision: The samples were analyzed at different times on same day and on different days. The percent of labeled claim was calculated and the results are shown in table 4.5

b) Different Analyst: The samples were analyzed by three different analysts by the proposed method. The results are shown in table 4.6

2.11.5 Forced degradation study:

For deciding that whether the analytical method for the assay was stability indicating, tablets of MET and GPZ were subjected to various stress conditions to conduct forced degradation studies. Stress studies were carried out under the conditions of acid/base hydrolysis, oxidation, photolytic and thermal degradation in accordance with ICH Q1A (R2). Several trials with different severity of each stressed condition were conducted, so that upto 10-30% degradation was achieved.

$$\% \text{ Degradation} = \frac{\text{Area of unstressed} - \text{Area of stressed}}{\text{Area of unstressed}} \times 100$$

Acid degradation: Tablets were crushed to fine powder and powder equivalent to 500 mg of MET and 5.0 mg of GPZ was taken into 250 mL round bottom flask. 20 mL of 0.1 N HCL was added to the flask and refluxed on heating mantle for 45min at 100 °C.

Basic degradation: Tablets were crushed and powder equivalent to 500 mg of MET and 5mg of GPZ was taken into 250 ml round bottom flask. 20 mL of

0.01N NaOH was added to the flask and refluxed on heating mantle for 2 hr at 100 °C.

Oxidative/ peroxide degradation: Tablets were crushed and powder equivalent to 500 mg of MET and 5mg of GPZ was taken into 250 mL round bottom flask. 20 mL of 3% H₂O₂ was added flask and refluxed on heating mantle for 30 min at 100°C.

2.11.2 Thermal degradation: Tablets were crushed and powder equivalent to 500 mg of MET and 5mg of GPZ was taken into 250 mL round bottom flask. Degradation was achieved by heating sample at 105°C.

2.11.3 Photo degradation: studies were carried out on fine Tablet powder. In a petri plate 1mm layer was applied and exposed to direct UV radiation for 24 hours in U.V chamber. The withdrawn samples were dissolved and then diluted with mobile phase as per sample preparation.

2.11.4 Robustness: The Robustness of the method was evaluated by changing the flow rate by ± 10%, by changing the column oven temperature by ± 5°C, by changing the wavelength by ± 2nm, by changing the organic content of mobile phase by 2% absolute, by changing the pH by ± 0.1, system suitability was done for each condition.

3 Results and Discussion

3.1 Optimization of Chromatographic Condition for Estimation of Drug

Column: Inertsil C8, 4.6 × 250mm, 5µm

Flow Rate: 1.0 ml/min

Wavelength: 222 nm

Injection volume: 20µl

Column Temperature: Ambient

Run Time: 12 minutes

Mobile Phas: Acetonitrile: Water (70:30)+Teal drop

The result was shown in the Fig 4.

After establishing the chromatographic conditions, Mix standard and marketed preparation were prepared and analysed by following procedure described under experimental work. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation. % recovery of tablet formulation containing MET (500mg) and GPZ (5mg) was found to be 99.63 and 99.34 with % RSD, 0.2446 and 0.79 respectively. Linearity was shown & the R² value was found to be 0.999 for MET and 0.999 for GPZ. The result show that an excellent correlation exists between concentration and mean peak areas within the concentration range. From the studies carried out and result obtained the proposed methods are compared in terms of statistical data, ease of application and reliability. Thus the method developed is accurate, precise, specific, & linear hence it can be said that, RP-HPLC is the most sensitive, accurate, precise and reproducible among all methods.

Fig 3: Overlain Spectra of MET & GPZ

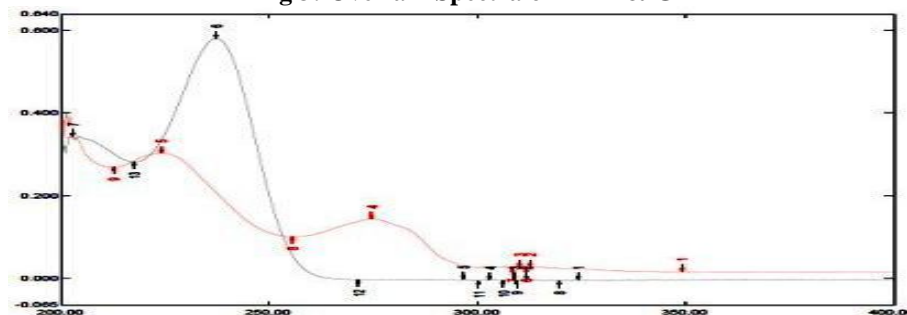


Fig 4: Chromatogram of MET and GPZ

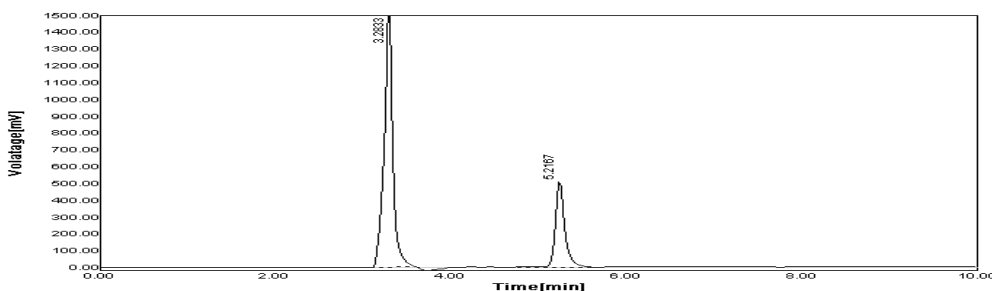


Fig 5: Chromatogram for Laboratory mixture of MET and GPZ showing retention time for MET 3.3333 min, GPZ 4.4167 min

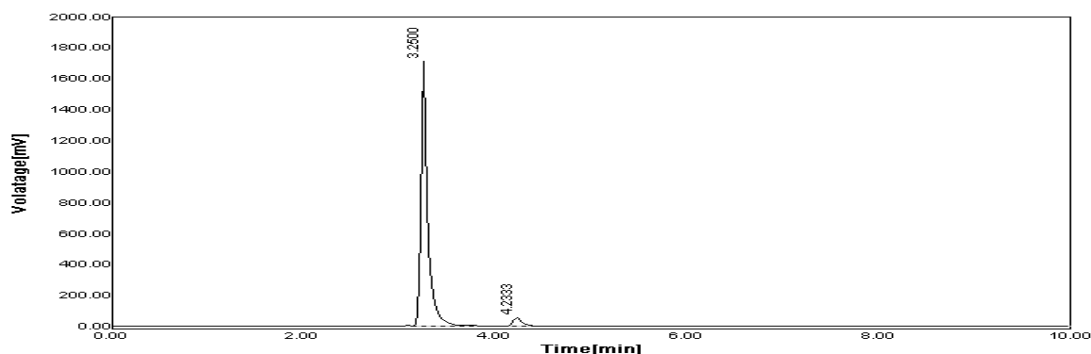


Fig 6: Chromatogram for Marketed Formulation of MET and GPZ showing retention time for MET 3.3231 min, GPZ 4.4231 min.

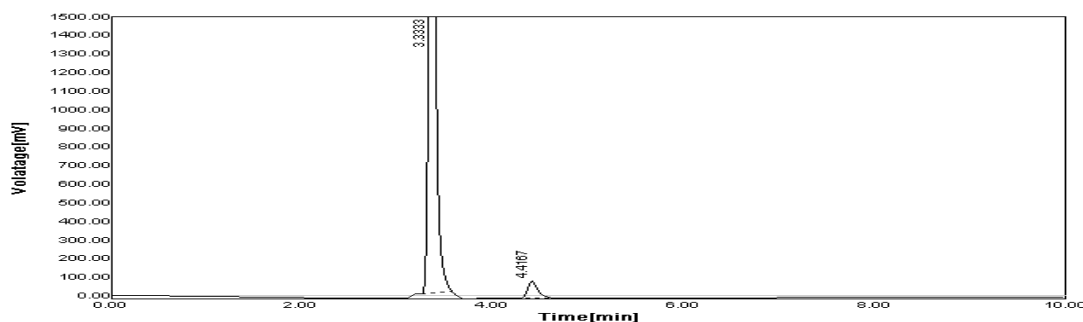


Fig 7: Calibration curve of MET

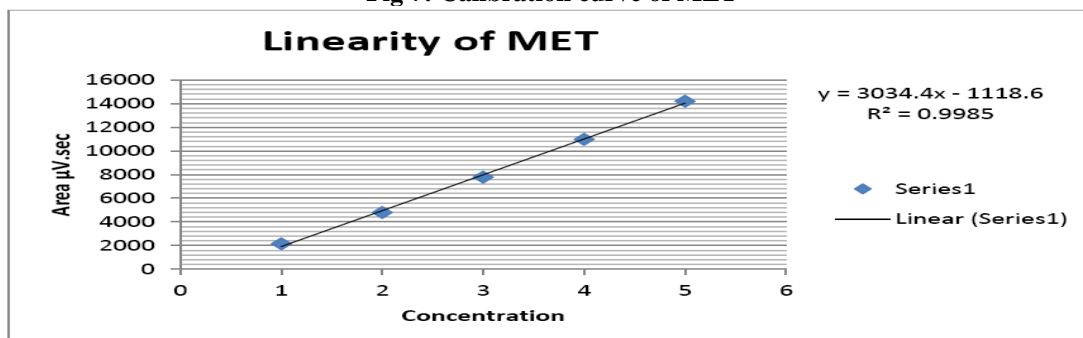


Fig 8: Calibration curve of GPZ

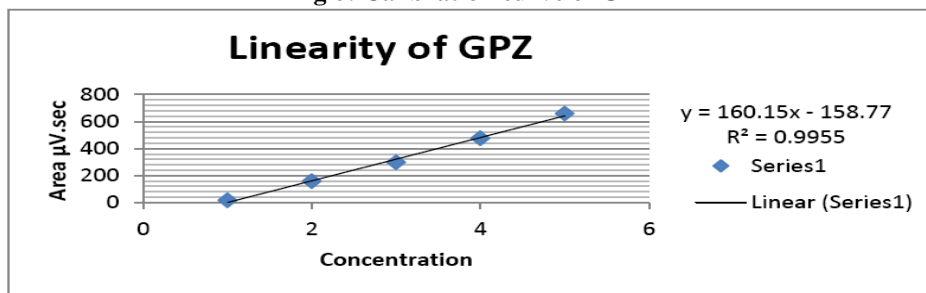


Table 1: System Suitability For MET

Sr. No.	Area Reproducibility	Retention Time	Tailing Factor (Asymmetry)	Theoretical Plates	Resolution
1	17594.1281	3.3923	1.813	8872.4	0.0000
2	17515.1131	3.4274	1.790	8912.3	-
3	17711.1391	3.3892	1.821	8862.34	-
4	17425.1591	3.4113	1.822	8772.6	-
5	17615.1191	3.4133	1.783	8872.3	-
Mean	17572.13	3.4067	1.8058	8858.388	-
% RSD	0.6	0.46	1.002	0.58	-

Table 2: System Suitability for GPZ

Sr. No.	Area Reproducibility	Retention Time	Tailing Factor (Asymmetry)	Theoretical Plates	Resolution
1	659.1344	4.5127	1.4167	6084.6	5.1002
2	658.3263	4.4477	1.5112	6184.6	5.0004
3	656.3421	4.4267	1.3911	6084.7	4.9001
4	657.2233	4.3967	1.4147	6074.4	5.0122
5	649.2312	4.4167	1.4127	6084.6	5.0120
Mean	656.0515	4.4401	1.4162	6102.58	5.0049
% RSD	0.6	1.00	0.73	0.08	1.5

Force Degradation Studies:

Fig 9: Chromatogram of Acid degradation

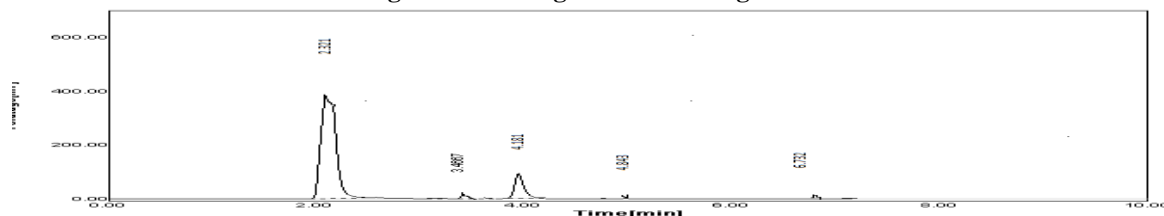


Figure 10: Chromatogram of base degradation

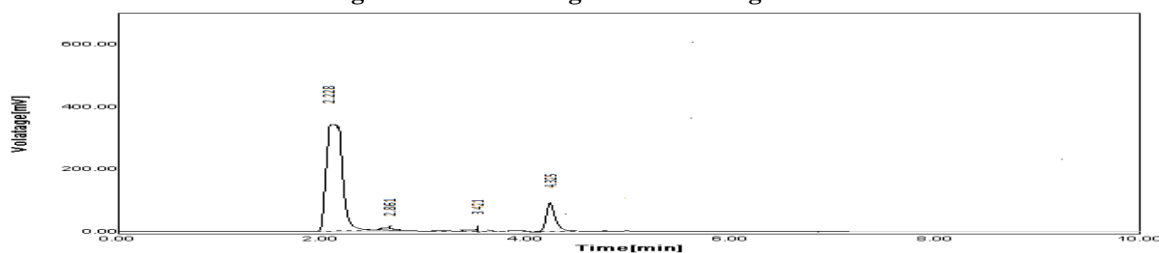


Figure 11: Chromatogram of oxidative degradation

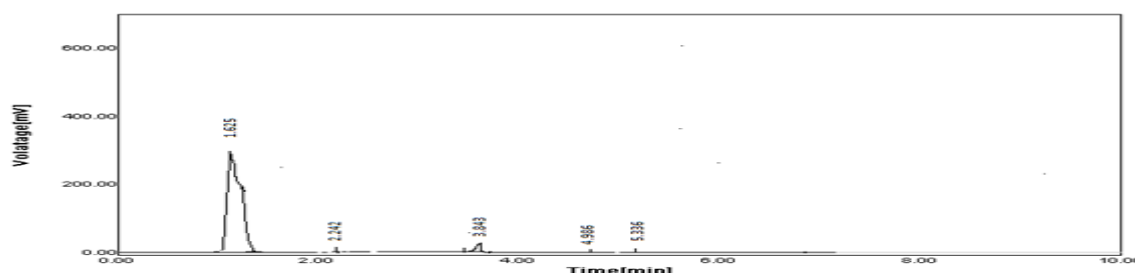


Figure 12: Chromatogram of heat degradation

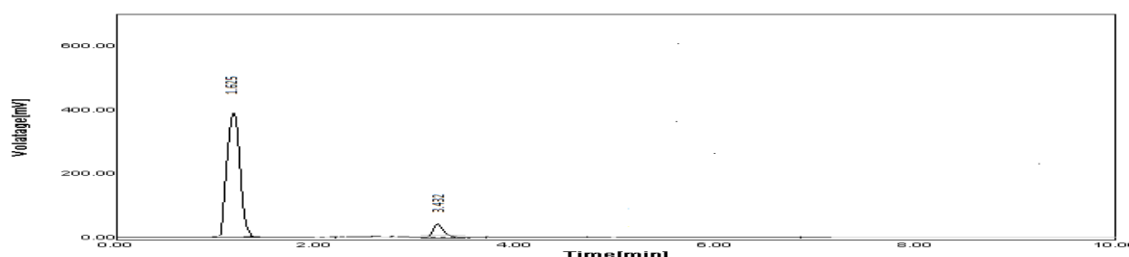


Figure 13: Chromatogram of Photodegradation

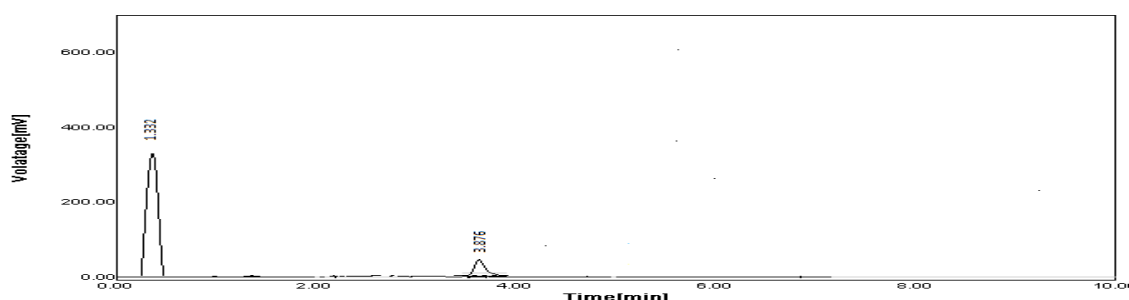


Table 3: Summary of force degradation of MET

Condition	% Assay of MET	% Degradation
Control sample	98.44	-
Acid degradation	95.53	2.91
Base degradation	87.67	10.77
Oxidative degradation	91.2	7.24
Thermal Degradation	91.85	6.59
Photolytic Degradation	97.44	1.00

Table 4: Summary of force degradation of GPZ

Condition	% Assay of GPZ	% Degradation
Control sample	99.28	-
Acid degradation	98.58	0.7
Base degradation	98.78	0.5
Oxidative degradation	73.28	26
Thermal Degradation	99.26	-nill-
Photolytic Degradation	99.24	-nill-

4. Conclusion

The developed stability indicating assay method was found to be simple, accurate, sensitive, precise, specific and rapid. Results obtained in validation exercise were fulfilling the acceptance criteria. This method can be applied for routine quantitative and qualitative analysis of MET and GPZ in bulk and pharmaceutical formulations like tablets. This method was also used to check quality of product after different storage condition and when stress degradation is carried out.

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