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Research Article

RP-HPLC force degradation profile of antidiabetic drugs

Department of Quality Assurance, Government College of Pharmacy, Kathora Naka, Amravati-444604

***Correspondence Info:**
Manoj S. Charde
Department of Quality Assurance,
Government College of Pharmacy,
Kathora Naka, Amravati-444604
E-mail: manojudps@rediffmail.com

Abstract

A force degradation profile was developed of Metformin (MET) & Pioglitazone (PIOG) in pharmaceutical combine tablet dosage form on RP-HPLCusing Comosil RP-C18 (4.6 x 150mm, 5µm) in an isocratic mode with mobile phase comprising of Acetonitrile: Pott. Dihydrogen Phosphate (pH 2.5 using OPA). The flow rate was 0.7 mL/ min and effluent was monitored at 254.0 nm. The stress conditions selected on the basis of literature review and the drug profile. For both estimations 99.81%for MET and 99.33% for PIOG mean % recovery was found with % RSD was NMT 2.0 which fully agrees by system suitability in good agreement with labeled claimed of formulation. All the parameters of system suitability were fully obeyed during force degradation profile generation.

Keywords: MET, PIOG, RP-HPLC, Stress, Force, and Degradation.

1. Introduction

Forced degradation studies are also called as stress decomposition studies, stress studies, stress testing, forced decomposition studies, etc. To determine the stability of the molecule forced degradation is carry out that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied. As indicated by ICH guideline to identify the likely degradation products that stress testing is intended which further helps in determination of the intrinsic stability of the molecule and to validate the stability indicating procedures used and establishing degradation pathways. These guidelines do not provide details about the practical approach towards stress testing because these guidelines are very general inconduct of forced degradation. Although forced degradation studies are scientific necessity during drug development and regulatory requirement, it is not considered as a formal stability program requirement. The literature review[14-20]suggested that no report of stability indicating assay for the above combination. With an objective to develop the force degradation profile for the above combination the present work was undertaken on RP-HPLC so as to support the development of stability testing process. Metformin [Fig. 1] chemically is *N, N*-dimethylimidodicarbonimidic diamide hydrochloride. White to off-white crystalline compound used as antidiabetic having solubility in methanol and freely in water, sparingly soluble in ethanol. While Pioglitazone [Fig. 2] chemically is (dl)-5-[4-2-(5-ethyl-2-pyridinyl) ethyl, phenyl -2, 4-thiazolidinedione monohydrochloride. [3, 25, 26] It is white powder and practically odorless. Used as antidiabetic having solubility in methanol and 1-propanol insoluble in water, slightly soluble in ethanol.

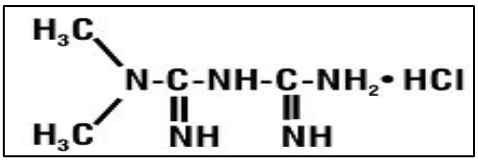

Fig. 1 Chemical Structure of Metformin

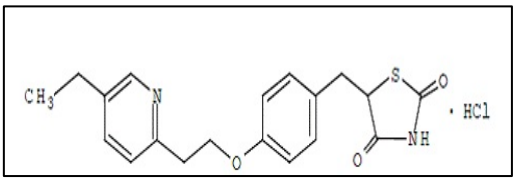

Fig. 2 Chemical Structure of Pioglitazone

2. Experimentals

Reagents & Chemicals

Standard samples of MET& PIOG were obtained as gift samples from Madras Pharmaceuticals/Maral lab (Chennai) India. The marketed formulation Pioz-

MF30 (USV LIMITED, B.S.D. Marg Govandi, Mumbai-400088) was purchased from the local market containing MET 500 mg and PIOG 30 mg and all the chemicals were used are of analytical grade.

Instruments

HPLC System of Younglin Quaternary pump with UV- VIS detector (190-990 nm)Software –Autochro. Analytical balance of citizen model CY 104 (microanalytical balance) was used for weighing purpose also the ultrasonicator servewell instruments model RC-SYSTEM MU-1700 used for sonication purpose.

Preparation of Stock solution for MET& PIOG

An accurately weighed quantity of MET working standard about 500.0 mg and PIOG working standard about 30.0 mg were transferred separately into 50.0 mL volumetric flask. About 40.0 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 10000.0 µg /mL and 600.0 µg /mL for MET and PIOG respectively.

Preparation of Working Standard Solution A

Take 1.0 mL from stock solution of PIOG and MET respectively in a 10.0 mL volumetric flask and make up the volume up to the mark with mobile phase to get 30.0 µg/mL PIOG & 500.0 µg/mL MET.

Preparation of SampleStock Solution

Take the powder weight of tablet equivalent to 500.0 mg of MET in 50.0 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 filtered it with 0.24µ to get 10000.0 µg/mL and 600.0 µg/mL of MET and PIOG respectively.

Working Sample Solution (B) Take 1.0 mL from above solution of PIOG and MET respectively in a 10.0 mL volumetric flask and make up the volume up to the mark with mobile phase to get 30.0 µg/mL PIOG & 500.0 µg/mL MET as final concentration and sonicated for 10.0 min in ultrasonicator.

Optimization of Mobile Phase and Chromatographic Conditions

Procedure The chromatographic conditions were set as per the optimized parameters. The mobile phase was allowed to equilibrate with stationary phase as was indicated by a steady baseline. Solution (A)was injected in the Rheodyne injector (20.0 µl) and the respective chromatograms were recorded.Various mobile phases were tried by permutations and combinations and also by varying column, flow rate, column temperature and type of buffers with varying pH and solvents. The various mobile phases tried are as follows.

- **Trial** -1 MEOH: KH2PO4 (30:70%, v/v), pH 2.5 with 0.05% TEA.
- **Trial** -2 ACN: KH2PO4 (50:50%, v/v), pH 2.5 with 0.05% TEA.
- **Trial** -3 ACN: KH2PO4 (50:50%, v/v), pH 2.5 with 0.05% OPA.
- **Trial** -4 ACN: KH2PO4 (50:50%, v/v), pH 2.5 with 0.05% OPA.
- **Trial** -5 ACN: KH2PO4 (20:80%, v/v), pH 2.5 with 0.05% OPA.
- **Trial** -6 ACN: KH2PO4 (40:60%, v/v), pH 2.5 with 0.05% OPA.
- **Trial** -7 ACN: KH2PO4 (30:70%, v/v), pH 2.5 with 0.05% OPA.

Above mentioned various mobile phases were tried. The mobile phase containing Acetonitrile: KH2PO4(30.0: 70.0) at pH 2.5, injection volume-20.0 µL flow rate of 0.7 mL/min was selected, due to its high resolving power, sensitivity and system suitability, for the determination of MET and PIOG. The chromatogram is shown in **Figure3**. Hence the following optimized chromatographic parameters were selected to carry out further experimentation.

- **Column** : Comosil RP-C18 (4.6 x 150mm, 5µm)
- **Flow Rate** : 0.7mL/min
- **Wavelength** : 254.0 nm
- **Injection Volume** : 20.0 µL
- **Column Temperature** : Ambient
- **Run Time** : 10.0 min
- **Mobile Phase** : Acetonitrile: Pott. Dihydrogen Phosphate (30.0:70.0 V/V)
- **pH** : 2.5 (Using OPA)

System Suitability Studies

This studies are the pharmacopoeial requirement and is used to verify, whether the reproducibility and resolution of the chromatographic system for analysis to be carried out are adequate or not. To ensure that the system is readto deliver results with acceptable accuracy and precision and operating properly this studies are performed. From five replicate injections of standard solutions the tests were performed to collecting data.

Procedure Mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline and the chromatographic conditions were set as per optimized parameters. The chromatograms were recorded for both drugs by five replicate injections of mixed working standard solution (A) were injected in to the system, and results are shown in **Table1&2.**

Analysis of Marketed Formulation

Preparation of Standard Solutions

Prepared as per the methodology adopted for laboratory mixtures

Preparation of Sample Solutions

Ten Tablets were weighed accurately and ground to fine powder. An accurately weighed quantity of Tablet powder equivalent to (500.0 mg of MET & 30.0 mg of PIOG) were transferred to 50.0 mL of volumetric flask and dissolved in sufficient amount of methanol. Then the volume was made up to the mark with methanol. The resultant solution was then filtered through whatmann filter paper (no. 41). The filtered solution was then sonicated in ultrasonicator for 10.0-15.0 min. then aliquot portions of 0.05 mL and 0.10 mL was then transferred to the three separate 10.0 mL volumetric flask and then the volume was mad up to the mark with mobile phase to get final concentration of (50.0 µg/mL, 100.0µg/mLand 3.0 µg/mL, 6.0µg/mL of MET and PIOG) respectively.

Procedure Equal volume (20.0 µL) of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The amount of drug in a Tablet was calculated using following formula

$$\text{mg/Tablet} = \frac{AT1 \times WS1 \times Ds \times P1}{AS1 \times WT \times Dt} \times \text{Avg. wt}$$

Where,

AT1 = Average area of MET/PIOG peaks in Test chromatogram

AS1 = Average area of MET/PIOG peaks in Standard chromatogram

Ds = Dilution factor for standard

Dt = Dilution factor for test

P1 = Potency of working standards of MET/PIOG peaks of % w/w basis

Avg. wt = Average weight of 10 Tablets

Further calculate the amount of MET/PIOG peaks present in % of Label claim using following formula

$$\% \text{ Label Claim} = \frac{\text{Assay (mg/Tablet)} \times 100}{\text{Label claim of MET/PIOG peaks}}$$

The results are shown in **Table3**, while chromatogram is shown in **Figure4.**

Force Degradation Studies

To determine whether the analytical method for assay was stability indicating and in order to establish the force degradation profile, the Tablet formulation of MET and PIOG were subjected to various stress conditions to perform forced degradation studies. Stress studies were carried out under the condition of acid hydrolysis, alkali hydrolysis, oxidation and neutral degradation in accordance with ICH Q1A (R2) guideline. On the basis of literature review and drug profile the selection of stress conditions was primarily depends.

The % degradation was evaluated by the following formula:

$$\% \text{ Degradation} = \frac{\text{Area of unstressed} - \text{Area of Stressed}}{\text{Area of unstressed}} \times 0$$

Approaches for Force Degradation

Acid Degradation

An accurately weighed quantity of tablet powder equivalent to (500.0 mg of MET & 30.0 mg of PIOG) was transferred to a round bottom flask to which 50.0 mL of 1.0 N HCL has been added as degradation medium. It is then subjected to reflux on water bath at 70.0^{0}C for 1.0 hr. the resultant solution was then filtered through whatmann filter paper (no. 41). Then 0.15 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (100.0 µg/mL of MET and 09.0 µg/mL of PIOG) respectively.

Alkali Degradation

An accurately weighed quantity of Tablet powder equivalent to (500.0 mg of MET & 30.0 mg of PIOG) was transferred to a round bottom flask to which 50.0 mL of 1.0N NaOH has been added as degradation medium. It is then subjected to reflux on water bath at 70.0^{0}C for 1.0 hr. the resultant solution was then filtered through whatman filter paper (no. 41). Then 0.15 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (100.0 µg/mL of MET and 9.0 µg/mL of PIOG) respectively.

Oxidative Degradation

An accurately weighed quantity of Tablet powder equivalent to (500.0 mg of MET & 30.0 mg of PIOG) was transferred to a round bottom flask to which 50.0 mL of 3.0% H_2O_2 has been added as degradation medium. It is then subjected to reflux on water bath at 70.0^{0}C for 1.0 hr. the resultant solution was then filtered through whatmann filter paper (no. 41). Then 0.15 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (100.0 µg/mL of MET and 9.0 µg/mL of PIOG) respectively.

Neutral Degradation

An accurately weighed quantity of Tablet powder equivalent to (500.0 mg of MET & 30.0 mg of PIOG) was transferred to a round bottom flask to which 50.0 mL of water has been added as degradation medium. It is then subjected into dark place at room temperature for 1.0 hr. the resultant solution was then filtered through whatmann filter paper (no. 41). Then 0.15 mL

of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (100.0 µg/mL of MET and 9.0 µg/mL of PIOG) respectively

Procedure

Equal volume (20.0 µL) of each stress sample was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area, retention time of the major peaks were measured. The respective chromatograms are shown in **Figure 5 to 8**and results were shown in **Table 4 to 8.**

3. Results and discussion

Optimization of Mobile Phase and Chromatographic Conditions

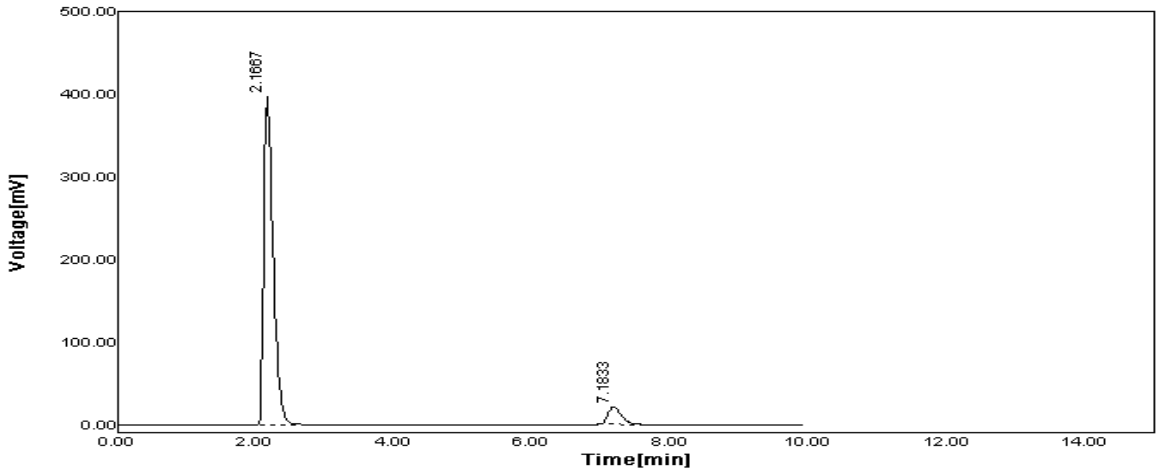

Fig. 3 Optimized Chromatogram of MET & PIOG

Observation

Good resolution with minimized tailing also proper peak shape and system suitability was observed within the limits. Hence the above chromatographic parameters are finalized.

System Suitability Studies

Table 1 Result of System Suitability Studies for (MET)

System Suitability Test (MET)					
Sr. No	**Area Reproducibility**	**Retention Time**	**Tailing Factor**	**Resolution**	**Theoretical Plates**
1	3777.33	2.1667	1.7295	0	3453
2	3777.31	2.1652	1.7289	0	3462
3	3775.24	2.1658	1.7288	0	3448
4	3776.38	2.1649	1.7291	0	3464
5	3775.36	2.1660	1.7293	0	3470
Mean	3776.324	2.16572	1.72912	0	3458.2
%RSD	0.025	0.779	1.024	0	1.723
Limit	**NMT 2%**	**NMT 1%**	**< 2**	**> 2**	**> 2000**

Observation

All the parameters of system suitability are observed within the limits for MET.

Table 2Results of System Suitability Studies for (PIOG)

System Suitability Test (PIOG)					
Sr. No	**Area Reproducibility**	**Retention Time**	**Tailing Factor**	**Resolution**	**Theoretical plates**
1	275.5991	7.1833	1.2599	19.00	4380
2	275.4384	7.1582	1.2601	19.01	4365
3	274.6804	7.1764	1.2498	19.05	4410
4	275.7506	7.1832	1.2607	18.99	4398
5	274.8702	7.1799	1.2589	19.00	4387
Mean	275.26774	7.1762	1.25788	19.01	4388.2
%RSD	0.163	0.412	1.234	0.573	0.048
Limit	**NMT 2%**	**NMT 1%**	**< 2**	**> 2**	**> 2000**

Observation

All the parameters of system suitability are observed within the limits for PIOG.

Analysis of Marketed Formulation

Table 3 Results of Marketed Formulation Analysis

Sr. No.	MET		PIOG	
	Assay (mg)	Assay (%)	Assay (mg)	Assay (%)
1	498.90	99.78	29.80	99.3
2	499.25	99.85	29.90	99.6
3	499.12	99.82	29.80	99.3
Mean	499.09	99.81666	29.83333	99.33333
SD	0.1855	0.034129	0.014275	0.512101
% RSD	0.0372	0.034188	0.514598	0.7165978

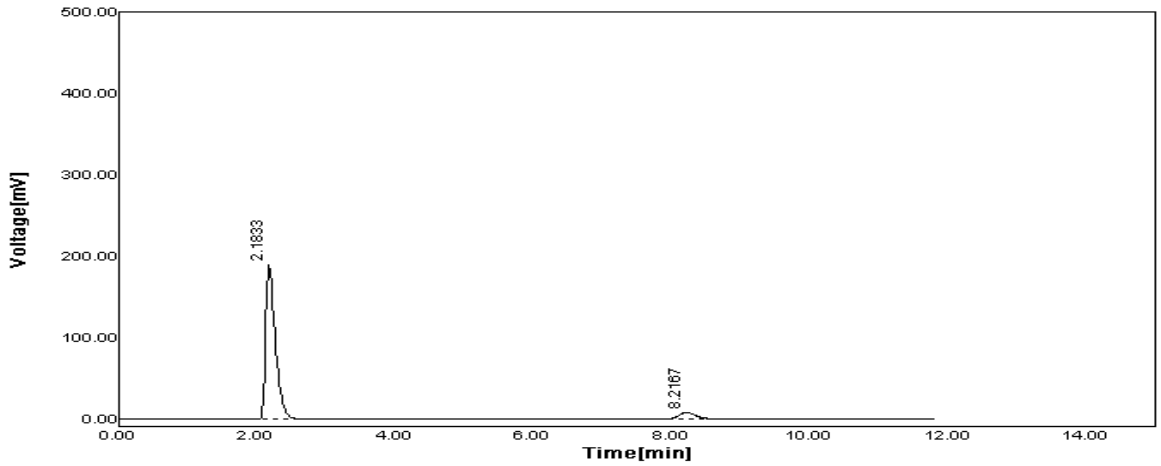

Fig. 4 Chromatogram of Marketed Formulation

The proposed method was applied to the determination of MET&PIOG in marketed formulation. The **mean % amount** found was **99.81 (MET) &99.33 (PIOG)** with **% RSD** values was **NMT 2.0%** indicates the developed method was successfully applied for analysis of marketed formulation. All the results found were in good agreement with the label content of marketed formulation.

Force Degradation Studies

Acid Degradation

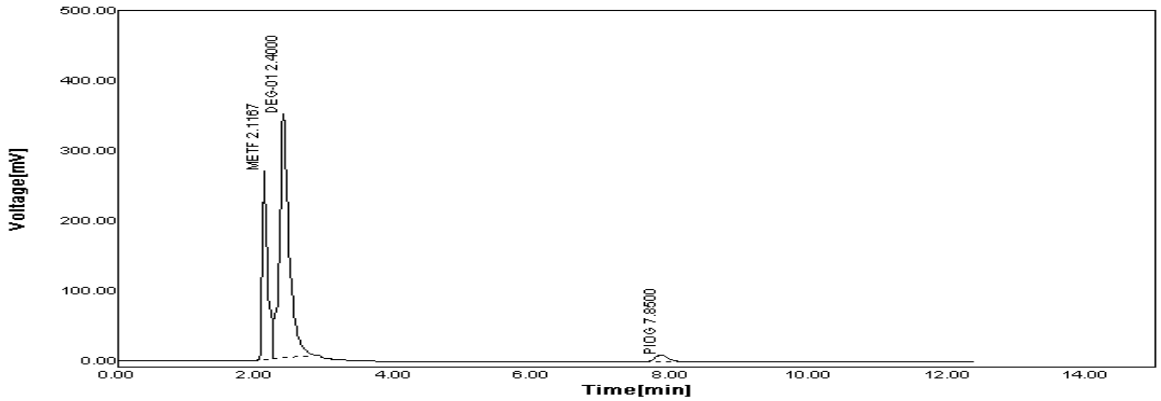

Fig. 5 Chromatogram of Acid Degradation

Acid degradation studies revels that both the drugs are capable of undergoing a strong degradation in acidic medium (1.0 N HCL) since both the drugs are basic in nature. A strong degradation was observed because of stronger ionization of both drugs under acidic medium owing to higher pKa of both the drugs.

Table 4 Overview of Acid Degradation

Name time	Retention	Area	%Area
MET	2.4000	3340.6890	68.35
Deg-1	2.1167	1416.8375	28.99
PIOG	7.8500	129.9809	2.66

Base Degradation

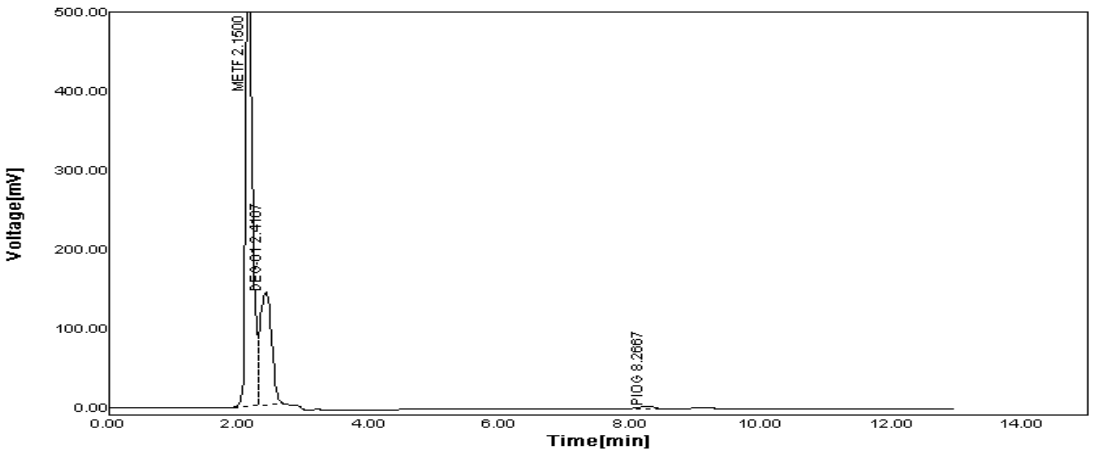

Figure 6 Chromatogram of Base Degradation

A base degradation studies revels that both the drugs are also capable of undergoing the strong degradation under basic medium (1.0 N NaOH). Since a stronger degradation peaks were reported at retention times of 2.41 min also both the drugs are strong basic in nature owing to higher pKa values.

Table 6 Overview of Base Degradation

Name time	Retention	Area	%Area
MET	2.1500	4967.2817	73.82
Deg-1	2.4167	1700.0133	25.27
PIOG	8.2667	61.1968	0.91

Oxidative Degradation

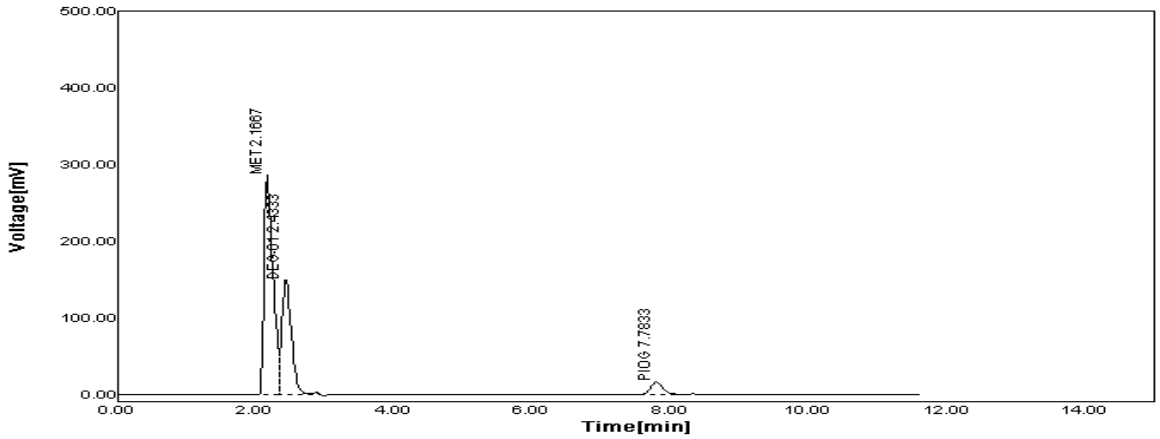

Fig. 7 Chromatogram of Oxidative Degradation

Metformin undergoes strong oxidative degradation (3.0% H_20_2) since the metformin is light sensitive in nature.

Table 8 Overview of Oxidative Degradation

Name time	Retention	Area	%Area
MET	2.1667	2584.8884	38.71
Deg-1	2.4333	1423.2639	60.90
PIOG	7.7833	0187.2104	04.64

Neutral Degradation

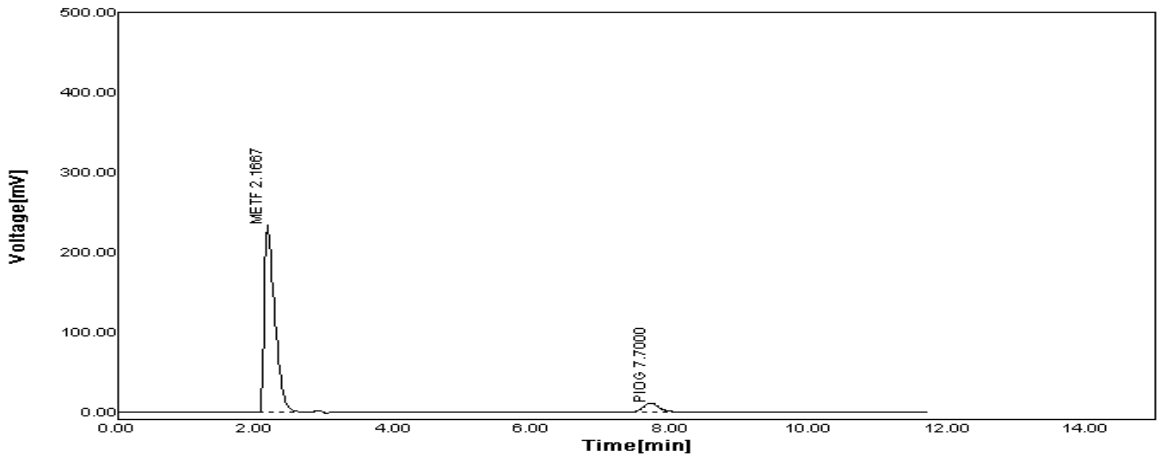

Fig. 8 Chromatogram of Neutral Degradation

A neutral degradation studies revels that the no one of both the drugs is capable of undergoing the neutral degradation (Water) owing to the water solubility of metformin.

Table 9 the summary of forced degradation study

Condition	%Assay MET	%Degradation MET	%Assay PIOG	%Degradation PIOG
Initial sample	98.85	-	98.96	-
1N HCL	95.33	3.52	97.68	1.28
1N NaOH	96.63	2.22	98.15	0.81
3% H_2O_2	71.90	26.95	96.69	2.27
Neutral	98.85	-	98.61	-

4. Conclusion

As per the ICH Q1A (R2) guideline the force degradation studies were conducted. On the primary basis of literature review and drug profile selection of stress conditions was primarily based and results of the stress studies were undergo full agreement with drug profile and review of literature. Selectivity of the developed method indicated due to the degraded products were well resolved on under optimized an chromatographic condition. Also the specificity of the developed method indicated by the results of marketed formulation analysis, hence for the stability studies on pharmaceutical preparations within pharmaceutical industry the developed method could be employed.

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