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ANALYTICAL METHOD FOR THE ESTIMATION OF METFORMIN HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, rapid, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method for the analysis of Metformin Hydrochloride (MET) in bulk and tablet dosage form has been developed and validated. This method was performed with a Symmetry C_{18} (4.6 × 150mm, 5µm) column with 60:40 (v/v) 50mM potassium dihydrogen orthophosphate buffer : methanol as mobile phase at a flow rate of 1.0 ml/min. UV detection at 262nm; MET was eluted with retention time of 1.694 min. The method was continued and validated accordance with ICH guidelines. Validation revealed the method is rapid, specific, accurate, precise, reliable, and reproducible. Calibration curve plots were linear over the concentration ranges of MET was 100-300µg/mL. A limit of detection (LOD) was 0.15 µg/ml and limits of quantification (LOQ) was 0.5 µg/mL for MET. Statistical analysis was proves the method is suitable for the analysis of MET as a bulk and in tablet dosage form without any interference from the excipients. It was also proved study for degradation kinetics of three drugs. It may be extended for its estimation in plasma and other biological fluids.

***Keywords**: Metformin Hydrochloride (MET), RP-HPLC, Method Development and Validation.*

INTRODUCTION

Metformin Hydrochloride (MET) chemically N,N-Dimethylimidodicarbonimidic diamide Hydrochloride (**Fig. 1**). It is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function [1-3]. Its use in gestational diabetes has been limited by safety concerns although at least one study has been conducted which showed no concerns for children prenatally exposed to Metformin up to 2 years of age [4].

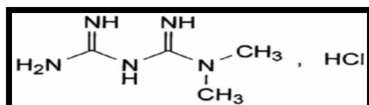

Fig. 1: Chemical structures of Metformin Hydrochloride (MET)

In the scientific literature, analysis of MET has been reported as individual ingredient [5, 6] and in combination with other compounds [7, 8].

The method described is rapid, economical, precise, and accurate and can be used for routine analysis of tablets. It was validated as per ICH guidelines [9-11].

MATERIALS AND METHODS

1. Experimental:

1.1. Materials and Methods:

Pharmaceutical grade working standards Metformin Hcl (MET) was obtained from Hetero Labs, Jedcharla, India. All chemicals and reagents were HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

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1.2. Instrumentation:

The analysis was performed using Waters-2695 (Modal Alliance) High Performance liquid chromatography, analytical balance (Mettler Toledo), PDA Detector (Standard cell) and data handling system (Empower 2), pH meter (lab India), Sonicator. The column used is Symmetry C_{18} (150×4.6mm, packed with 5µm) with the flow rate 1.0ml/min (isocratic).

1.3. Preparation of Standard Stock solution:

Accurately weighed 10 mg of MET working standard and separately transferred into a 10ml clean dry volumetric flasks, add about 7mL of Mobile Phase (60:40 v/v 50mM potassium dihydrogen orthophosphate buffer : methanol) to volumetric flask and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Calibration standards at five levels were prepared by appropriately mixed and further diluted stock standard solutions in the concentration ranges from 100-300µg/mL. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the Linearity graphs.

1.4. Preparation of Standard solution:

The above standard stock solution was containing 1000µg/mL of each MET in volumetric flask. Then transferred the 2ml of MET of prepared standard stock solution into a clean 10ml volumetric flask and made upto the mark with diluent. And finally the standard solution concentration was 200µg/mL.

1.5. Preparation of Test solution:

For the analysis of a tablet dosage form, 20 tablets were weighed individually and their average mass was determined. Then, the tablets were crushed to a fine powder. The powder equivalent to 500mg of MET was transferred to a 500mL volumetric flask and dissolved in 10mL of diluent, sonication was done for 15 min with swirling. After sonication, the solution was filtered through a membrane filter paper (#0.45µ). From the above stock solution 2mL was transferred in to 10mL volumetric flask and made volume upto the mark with diluent, the final concentration was 200µg/mL, then

injected into the chromatographic system, and analyzed quantitatively. The analysis was repeated six times and the possibility of excipient interference with the analysis was examined.

1.6. Optimization of HPLC Method:

The HPLC method was optimized and developed with a method for MET. The mixed standard solution (200mg of MET) injected in HPLC by the followed chromatographic conditions. The chromatographic separation was achieved on a Symmetry C_{18} (4.6 x 150mm, 5μm). The isocratic mobile phase consisting of 50mM potassium dihydrogen orthophosphate and Methanol in the ratio of (60:40v/v) was used throughout the analysis and the pH 2.8 adjusted with orthophosphoric acid. The flow rate of the mobile phase was 1.0ml/min. Detection was monitored at wavelength of 262nm. The column temperature was kept at ambient and injection volume was 20μl (**Table 1**).

1.7. Method validation:

The method validation was done according to the ICH guidelines. The following validation characteristic parameters are accuracy, precision, linearity, and specificity, LOD, LOQ and robustness.

1.7.1. Linearity and range: Linearity of the method was studied by the injecting the mixed standard solution with the concentration ranges of MET from 100-300μg/ml levels of target concentrations were prepared and injected six times into the HPLC system keeping the constant injection volume. The peak areas were plotted against the concentrations to obtain the linearity graphs.

1.7.2. Precision: The precision of the optimized method was evaluated by carrying out six independent assays of test sample. %RSD of six assay values was calculated. Intermediate precision was carried out the samples by using another instrument and with different analyst.

1.7.3. Limit of Detection and Quantification: The LOD and LOQ procedures were performed on samples contain very lower concentrations of analytes under the ICH guidelines. By applying the visual evaluation method, LOD was expressed by establishing the lowest concentration at which the analyte can be detected. LOQ was considered as the lowest concentration of analytes that can be detected and quantified, with acceptable accuracy and precision.

1.7.4. Robustness: Robustness was studied by evaluating the effect of small variations in the chromatographic conditions. The conditions studied were flow rate altered by ±0.1ml/min, mobile phase composition with methanol ±5ml.

1.7.5. System suitability: The system suitability parameters with respect of tailing factor, theoretical plates and repeatability of MET peak was defined.

1.7.6. Specificity: The specificity of the analytical method is the ability of the method to estimate the analyte response in the presence of additional components such as impurities, degradation products and matrix [12]. The peak purity of MET was assessed by comparing the Retention time of standard MET good correlation was obtained between the Retention time of standard and sample of MET.

The specificity method was also evaluated to ensure that there were no interference products resulting from forced degradation studies.

1.7.6.1. Forced degradation study: Forced degradation or Stress testing of a drug substance will help to identify the degradation products, which can help to establish the intrinsic stability of the molecule.

All stress decomposition studies were performed at an initial drug concentration 200μg/mL.

The Stability indicating study of MET was undergoes acid, alkali and oxidation degradation, photolysis and heat condition.

Placebo Interference: The placebo (in the present of excipients in tablet) sample were prepared as per the test method and analyzed in the HPLC. It expressed there is no additional peaks at the retention time of MET in the chromatograph it indicates that there is no placebo interference.

Acid Degradation: Sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume upto the mark with biorelevant media and analyzed using HPLC.

Alkali Degradation: Sample was treated with 3ml of 1N sodium hydroxide and kept for 10hr. After 10hr the solution was neutralized with 3ml of 1N hydrochloric acid, made the volume upto the mark with biorelevant media and analyzed using HPLC.

Oxidative Degradation: MET solution of 200 was mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume upto the mark with biorelevant media and analyzed using HPLC.

Photolytic Degradation: The **s**amples were kept under UV light for different time intervals (15mins – 7days) and made the volume upto the mark with diluent and analyzed using HPLC.

Thermal Degradation: Samples were heated at 80^0 C for 15mins - 60mins and 220^0 C for 2-5mins and analyzed.

1.7.7. Accuracy: Accuracy was carried out by applying the method to drug sample to which known amounts of MET standard powder corresponding to 50, 100 and 150% of label claim was added, mixed and the powder was extracted and determined by the system in optimized mobile phase. The experiment was performed in triplicate and percentage recovery, % RSD was calculated.

1.7.8. Analysis of marketed formulation: The marketed formulation was assayed by above description. The peak areas were monitored at 262nm, and determination of sample concentrations were using by multilevel calibration developed on the same HPLC system under the same conditions using linear regression analyzed for MET in the same way as described above.

RESULTS AND DISCUSSION

The method estimation of MET was done by RP-HPLC and in the optimized method the mobile phase consists of buffer (600 volumes of phosphate buffer and 400 volumes of Methanol and the pH was adjusted to be 2.8. Then finally filtered using 0.45μ membrane filter paper and degassed in sonicator for 15 minutes. The detection is carried out using PDA detector at 262nm. The solutions are following at the constant flow rate of 1.0 ml/min.

The retention time of MET was 1.694 minutes respectively. Linearity ranges of MET was 100-300μg/mL and the results was found for in the acceptable as (R^2) = 0.9995 for MET. LOD was 0.15 μg/ml and LOQ was 0.5 μg/mL. The all parameters value of RSD is less than 2.0% indicating the accuracy and precision of the method. The percentage recoveries were found 100.15-100.46%.

1. Method Development and Optimization:

The HPLC procedure was optimized with a view to develop a suitable LC method for the analysis of MET in fixed dose for bulk and combined dosage form. It was found that 60:40 v/v (50mM) potassium dihydrogen orthophosphate buffer: methanol gave acceptable retention time (1.694 min), plates, and good resolution of MET at the flow rate of 1.0ml/min (**Table. 1**; **Fig. 2 & 3**).

Table No. 1: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (column)	Symmetry C18 (4.6 x 150mm, 5µm)
Mobile Phase	60:40v/v, (50mM Phosphate Buffer : Methanol)
pH	2.8 ± 0.02
Flow rate (ml/min)	1.0
Run time (minutes)	8.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	262
Drugs RT (min)	1.694 & 3.344

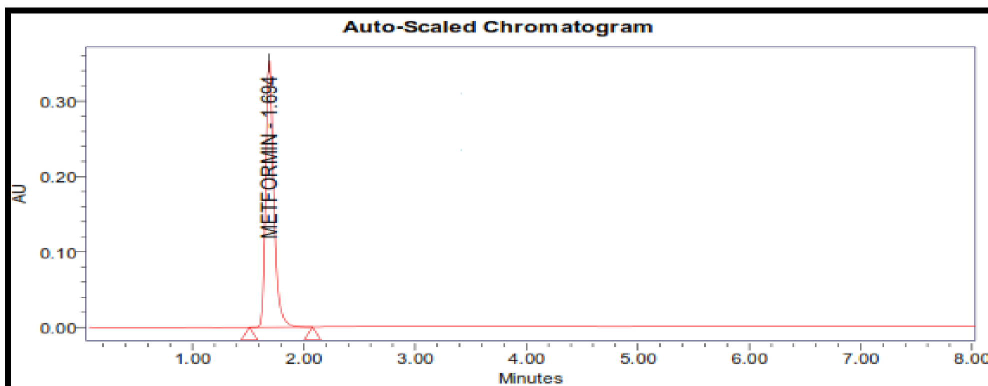

Fig. 2: Chromatogram of MET at 262nm from bulk drug

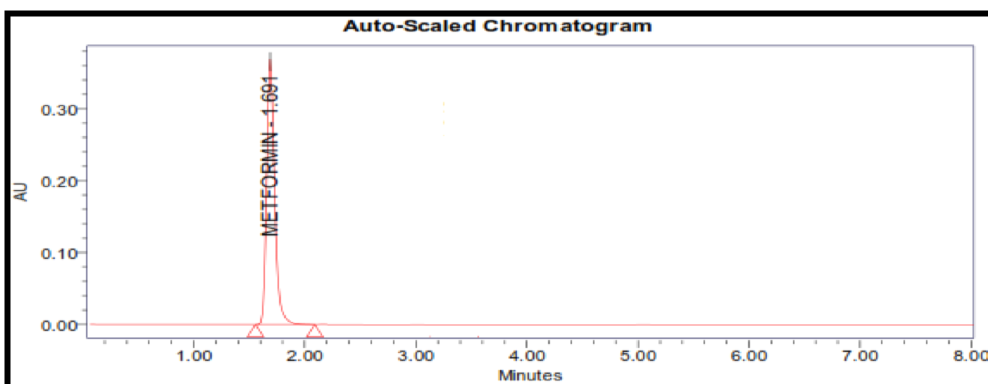

Fig. 3: Chromatogram of MET at 262nm from pharmaceutical formulation

2. Validation of Developed method:

2.1. Linearity:

Linearity was evaluated by analysis of working standard solutions of MET of five different concentrations. The range of linearity range for MET from 100-300µg/ml (**Table. 2**). The result of correlation coefficients of MET = 0.9995 (**Fig. 4 & 5**).

Table 2: Data for linearity

Analyte	Concentration range (µg/mL)	Correlation Coefficient (R^2)	Slope	Intercept
MET	100-300	0.9995	4845.x	81465

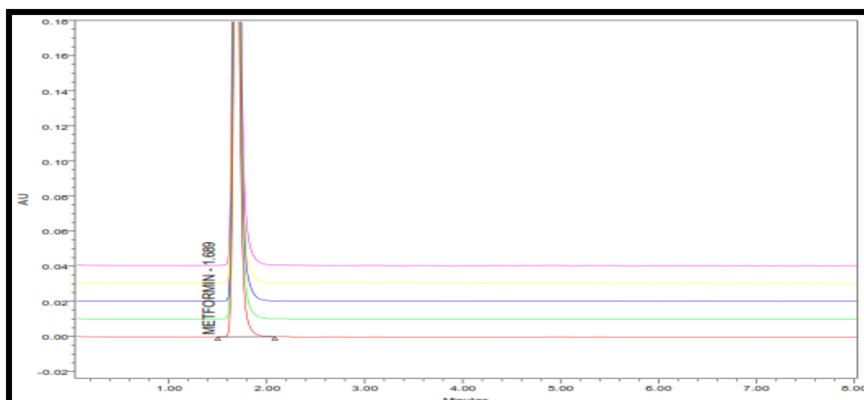

Fig. 4: Overlay linearity Chromatogram for MET

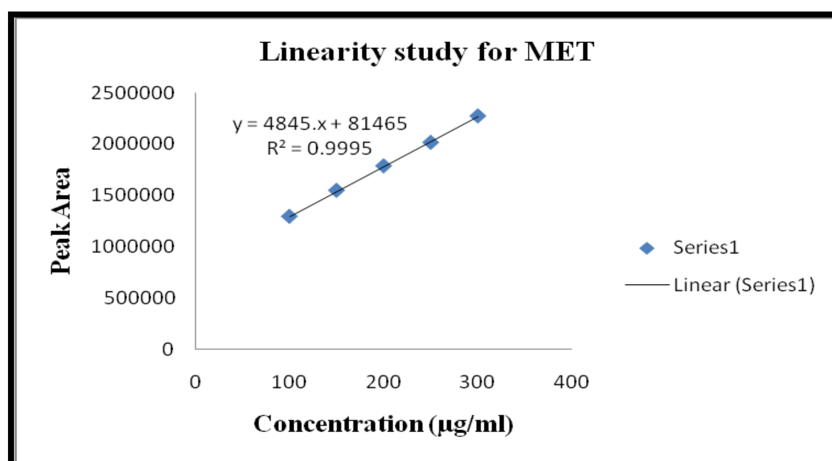

Fig. 5: Linearity Curve of Standard Metformin Hydrochloride (MET)

2.2. Precision:

The results of precision method were evaluated by carrying out six independent test samples of MET. The percentage of RSD of six sample peak area values was calculated. Different analyst from the same laboratory conditions analyzed the intermediate precision for the optimized method. The RSD values of intra-day and inter-day studies for MET confirming good precision of the optimized method (**Table. 3**).

Table No. 3: Intra-day and inter-day Precision results of MET from tablets

No. of Preparation	MET	
	Intra-day precision	Inter-day precision
Pre-1	1819456	1814518
Pre-2	1822446	1818639
Pre-3	1824679	1820266
Pre-4	1825211	1821936
Pre-5	1826102	1817838
Mean	1823578.8	1818639
St. dev.	2670.28888	2790.232
% RSD	0.146	0.153

2.3. LOD and LOQ Peaks are not correct:

The LOD and LOQ values were found to be 0.15 and 0.5µg/mL for MET (**Table. 5**).

2.4. Specificity:

Injected the extracted solutions commonly used excipients were performed to demonstrate for the absence of interaction with the drug. These results are expressed that there was no interference from the other excipients in the tablet formulation; therefore, confirm the method was specific.

2.5. System suitability:

System suitability parameters such as the theoretical plates count, resolution, % RSD and peak tailing factors are determined (**Table. 5**).

Table No. 5: System suitability parameters for MET

System suitability parameters	MET
Retention time (min)	1.694
Repeatability of retention time; %R.S.D (n=5)	0.391
Repeatability of peak area; %R.S.D= (S.D./Mean)×100	0.146
Resolution (Rs)	-
Tailing factor (asymmetric factor)	1.29
USP plate count	4761
LOD (µg/mL)	0.15
LOQ (µg/mL)	0.5

2.6. Robustness:

To ensure the insensitivity of the optimized RP-HPLC method to small alteration in the experimental conditions. The conditions studied were flow rate altered by ±0.1ml/min, mobile phase composition with methanol ±5ml (**Table. 6**).

Table No. 6: Robustness study for analytical method validation of MET tablets

	Parameters	Adjusted to	Mean Area [a]	Mean RT	SD	% RSD
MET	**Flow Rate As per method 1.0ml/min**	**0.9 ml/min**	2002121	1.86	489.69	0.23
		1.1ml/min	1627162	1.54	1748.28	0.57
	Mobile Phase (60:40) (Buffer:Methanol)	**65:35**	1797951	1.73	824.87	0.22
		55:45	1829674	1.67	1657.58	0.46

[a] = 5 *Replicates*

2.7. Solution stability studies:

Three different concentrations of MET (200µg/mL) was prepared from the sample solution and stored at room temperature for 24 hrs. Then injected into the HPLC system and the additional peaks were not found in the chromatograms so, it was indicating the stability of MET tablet in the solution (**Table. 7**).

2.8. Recovery studies:

Good recovery of the MET was obtained at different added concentrations for the tablets (**Table. 8**).

Table No. 7: Solution stability study for analytical method validation of MET tablets

Name	*Replicate (n = 5)*	*Initial*	*After 3 hrs*	*After 6 hrs*	*After 12 hrs*	*After 24 hrs*
MET	**Mean**	1823579	1820097	1817286	1813233	1803911
	SD	2670.289	2245.163	3143.457	3287.498	3788.219
	% RSD	0.146	0.123	0.172	0.181	0.21

Tablet No. 8: Accuracy Results of MET tablets

Analyte	Recovery levels	Actual Conc. (µg/mL)	Added Conc. (µg/mL)	Theoretical Conc. (µg/mL)	Found Conc. (µg/mL)	% Recovery	% RSD	% Error [a]
MET	50 %	100	50	150	150.12	100.08	0.272	0.08
	100 %	100	100	200	200.07	100.03	0.147	0.035
	150 %	100	150	250	249.92	99.96	0.714	-0.032

[a][found conc. – theoretical conc./theoretical conc.] x 100.

2.9. Analysis of a commercial formulation:

Experimentally the results for the amount of MET in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interaction from the excipients which are commonly present in formulation of tablets.

2.10. Degradation study:

Acid degradation study: In acidic degradation study, sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs at 60ºC. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume upto the mark with diluent and analyzed using HPLC. The drug content was found to be degrading up to 5.824% in acidic condition (**Fig. 6 & 7, Table. 9 & 10**).

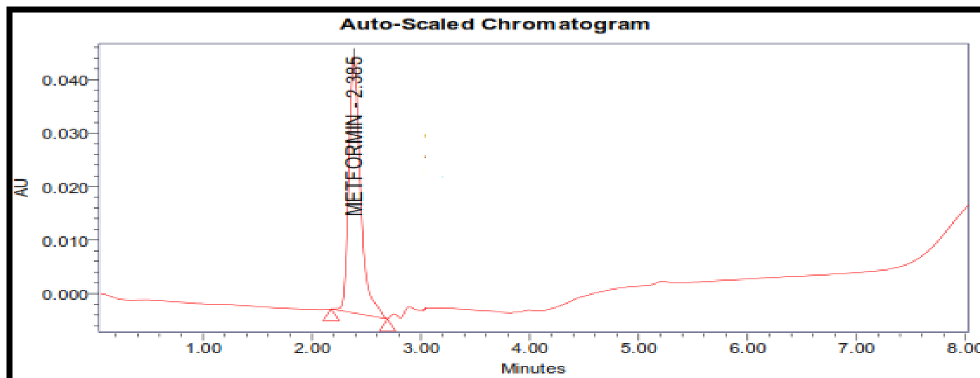

Fig. 6: Chromatogram of acidic forced degradation of MET

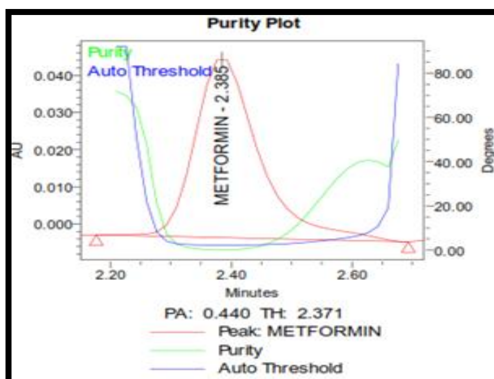

Fig. 7: Purity Plot of MET in acidic forced degradation

Alkaline degradation study: Alkaline degradation study was performed by the sample was treated with 3ml of 1N sodium hydroxide and kept for 10hr. After 10hr the solution was neutralized with 3ml of 1N hydrochloric acid, made the volume upto the mark with diluent and analyzed using HPLC. In alkali degradation, it was found that around 34.241% of the drug degraded (**Fig. 8 & 9, Table. 9 & 10**).

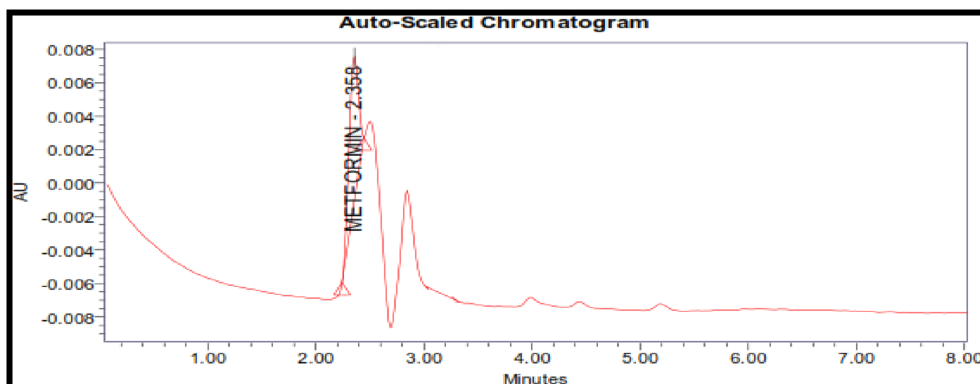

Fig. 8: Chromatogram of alkali forced degradation of MET

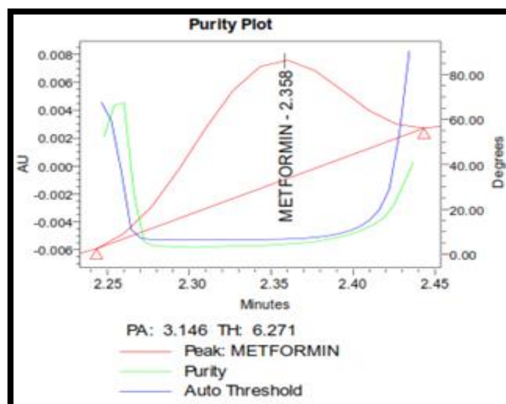

Fig. 9: Purity Plot of MET in alkali forced degradation

Oxidative degradation study: Oxidation degradation study was performed by the sample solutions were mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume upto the mark with diluent and analyzed using HPLC. In oxidative degradation, it was found that around 4.765% of the drug degraded (**Fig. 10 & 11, Table. 9 & 10**).

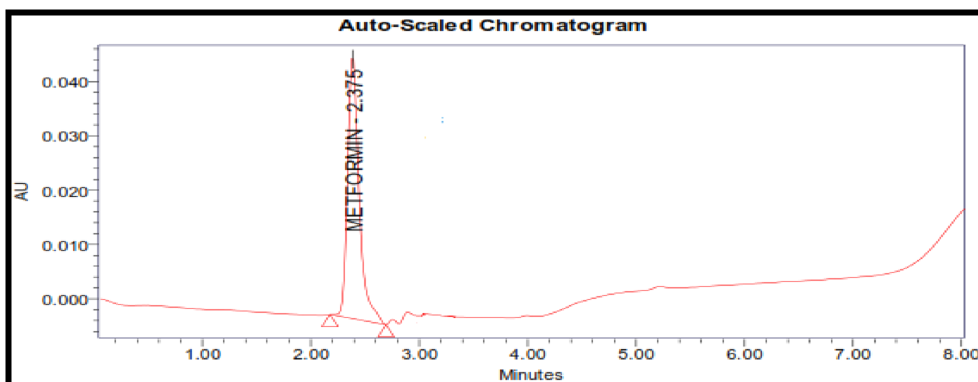

Fig. 10: Chromatogram of oxidative forced degradation of MET

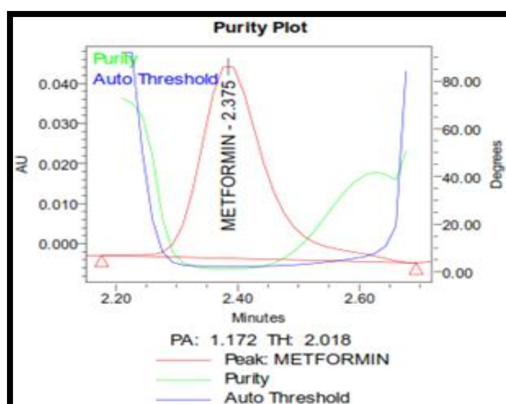

Fig. 11: Purity Plot of MET in oxidative forced degradation

Photolytic degradation study: Photolytic degradation study was performed by exposing the drug content in UV light for 15mins to 7days. There is 2.679% degradation observed in above specific photolytic condition (**Fig. 12 & 13, Table. 9 & 10**).

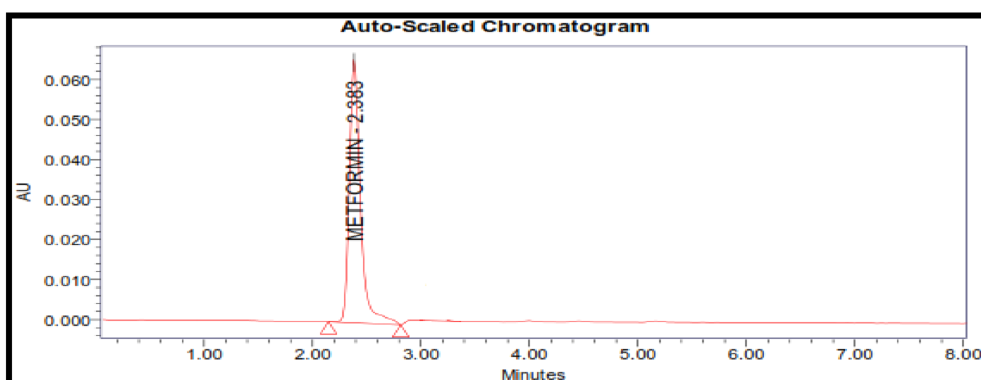

Fig. 12: Chromatogram of UV-light degradation of MET

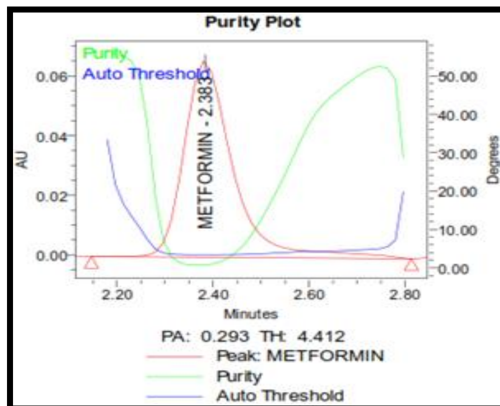

Fig. 13: Purity Plot of MET in UV-light degradation

Thermal degradation study: Thermal degradation was performed by exposing solid drug at 80°C for 15mins to 60mins and at 220°C for 2-5mins. Resultant chromatogram of thermal degradation study (**Fig. 14 & 15, Table. 9 & 10**) indicate that drug is found to be slightly stable under thermal degradation condition. Only 3.623% drug content were degraded.

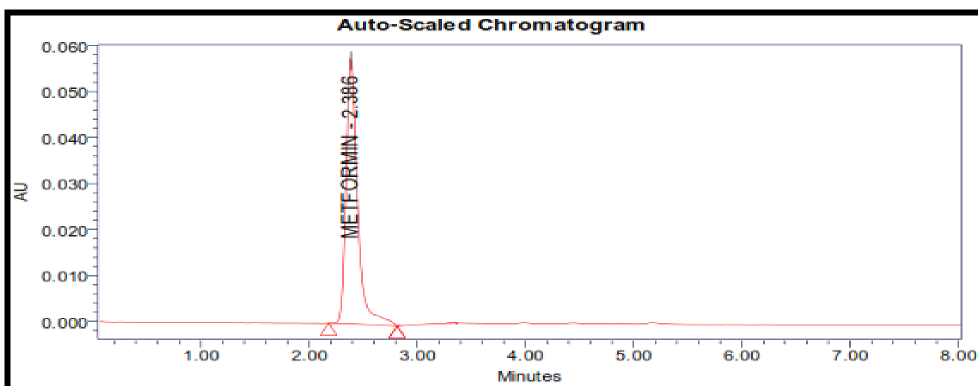

Fig. 14: Chromatogram of thermal degradation of MET

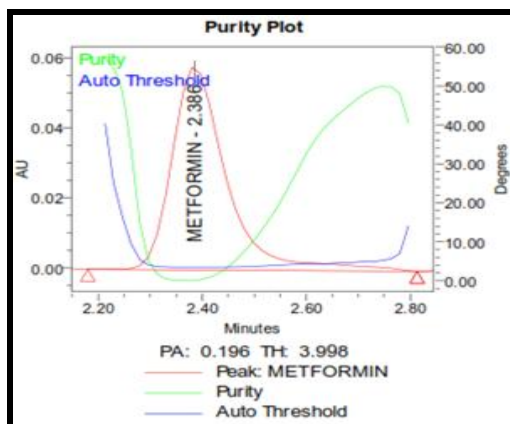

Fig. 15: Purity Plot of MET in thermal degradation

Table 9: Peak purity results of MET

Stress Condition	Purity Angle	Purity Threshold
Acid Degradation	0.440	2.371
Alkali Degradation	3.146	6.271
Oxidative Degradation	1.172	2.018
Photolytic Degradation	0.293	4.412
Thermal Degradation	0.196	3.998

Table 10: Percentage degradation of MET

Drug Name		Acid	Alkali	Oxidative	Photolytic	Thermal
MET	Std Area			1791644		
	Sample Area	1687298	1178163	1706271	1743628	1726718
	% of Degradation	5.824	34.241	4.765	2.679	3.623

CONCLUSION

A new RP-HPLC method described in this manuscript provides a simple, convenient and reproducible approach for the simultaneous estimation and quantification of Metformin Hydrochloride in routine quality control analysis.

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