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STABILITY INDICATING UV SPECTROSCOPIC METHOD FOR THE ESTIMATION OF METFORMIN HYDROCHLORIDE IN BULK AND TABLETS

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ABSTRACT: Background: Forced degradation studies are used to determine the degradation pathways and degradation products of the active pharmaceutical ingredients that could form during storage and facilitate formulation development, manufacturing and packaging. **Aim:** To develop and validate simple and precise stability indicating UV Spectrophotometric method for Metformin Hydrochloride in the bulk and tablet dosage form. **Material and Method:** Separation of the drug from its degradation products was achieved by UV Spectrophotometric method using distilled water and scanned between 200 to 400 nm. Metformin Hydrochloride was subjected to stress conditions such as hydrolysis (acid and base), oxidation, photolysis, and thermal degradation. The degraded samples were further analyzed by using this method. **Results:** The maximum absorbance was found to be at 232.2nm and found to be linear over the range 2-10 μ g/ml with good correlation coefficient (r^2) 0.998. The limits of detection and quantification were 0.5232 and 1.5856 μ g/ml, respectively. Major degradation was observed in acid hydrolysis, photolysis, and oxidative conditions. Metformin Hydrochloride was quite stable under the other stress conditions investigated. **Conclusion:** The UV Spectrophotometric method for Metformin Hydrochloride in the bulk and tablet dosage form was developed and validated. Thus, the method is proved to be stability indicating and was found to be an economical, selective, and sensitive for the desirable range.

Keywords: Metformin Hydrochloride, Forced degradation, UV Spectrophotometry

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INTRODUCTION: Metformin Hydrochloride (MET) is chemically N, N dimethylimido dicarbonimidic diamide hydrochloride **Fig. 1**. It is white crystalline powder, hygroscopic and freely soluble in water. It is official in Indian Pharmacopoeia¹, United State Pharmacopoeia² and European Pharmacopoeia³.

It is a member of the biguanide class of oral antihyperglycaemic, improves insulin sensitivity by increasing peripheral glucose uptake and utilization, decreases hepatic glucose production and intestinal absorption of glucose. MET is the first line drug for the treatment of type II diabetes, particularly in overweight or obese people and those with normal kidney function.

Evidence suggests that it may be the best choice for people with heart failure. It is also used in the treatment of polycystic ovary syndrome. Major action of MET is to increase glucose transport across the cell membrane in skeletal muscle⁴⁻⁵.

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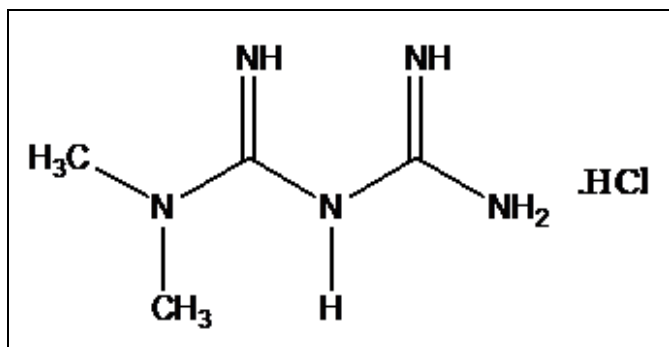


FIG. 1: STRUCTURE OF METFORMIN HYDROCHLORIDE (MET)

Literature survey reveals that methods available for estimation of Metformin hydrochloride include UV spectrophotometry⁶⁻⁷, HPLC⁸⁻⁹, HPTLC¹⁰⁻¹¹, UPLC¹², and Capillary Electrophoresis¹³. To the best of author's knowledge, there are few stability indicating RP-HPLC¹⁴, HPTLC¹⁵ and UV spectroscopy¹⁶ methods are available for MET. Forced degradation studies of Metformin by using distilled water as solvent being advantageous as plenty of availability, inexpensive, non-volatile, and relatively eco-friendly. Thus, there is a need for eco-friendly and economic stability, indicating UV spectroscopic method for the study of Metformin hydrochloride in bulk and its dosage forms.

The stability-indicating assay is a method that is employed for the analysis of stability samples in pharmaceutical industry¹⁷. Stress testing of a drug substance can help in turn establish the degradation pathways and the intrinsic stability of the molecule¹⁸⁻¹⁹. The quality of the finished product is very important from its safety, acceptability, and efficacy. Thus stability is considered as one of the most important criteria in pharmaceutical quality control as stable preparations would promise precise delivery of the drug to the patients.

The present study mainly deals with the forced degradation of Metformin hydrochloride under the stress conditions like acid and base hydrolysis, oxidation, heat and light and validation of the method for accurate quantification of Metformin in the bulk and tablet dosage form.

MATERIALS AND METHOD:

Apparatus: A Shimadzu 1800 double beam UV-VIS spectrophotometer provided with 1 cm matched quartz cell was used for absorbance measurement.

Reagents and Chemicals: Metformin HCl was obtained as a gift sample for Wockhardt Pharmaceuticals Pvt. Ltd. (Aurangabad, India). All other reagents used were of analytical grade.

Preparation of Standard solution of MET: 10 mg pure MET was transferred to a 100ml volumetric flask and diluted up to the mark with distilled water to get a concentration of 100µg/ml solution. In a 10 ml volumetric flask, pipette out 1ml from the standard stock solution and dilute it up to the mark with distilled water (10µg/ml) and scanned between 200 to 400nm and 232.20nm was found to be maximum wavelength for absorption as shown in Fig. 2.

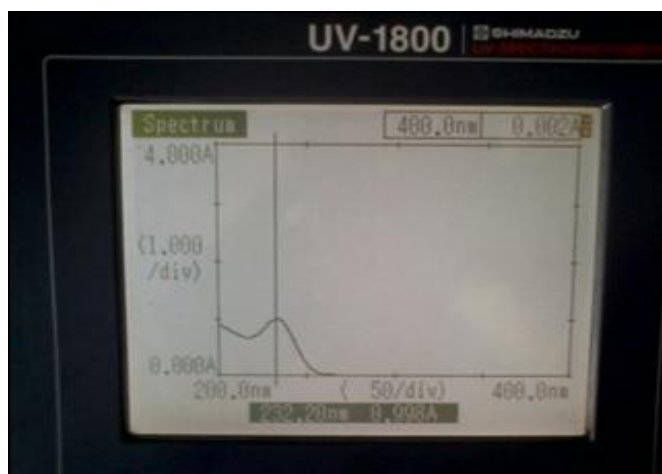


FIG. 2: STANDARD SPECTRUM OF MET AT 232.20 nm WITH 0.998 ABSORPTION

Forced Degradation Studies:

Acid and Alkaline Hydrolysis: Forced degradation in acidic media was performed by adding 10 mg of MET to 10 ml 0.01N HCl and refluxing the mixture at 80 °C for approximately 2 h. The solution was then left to reach ambient temperature, neutralized to pH 7 by addition of 0.01N NaOH. In a 10 ml volumetric flask, pipette out 1ml from above solution and dilute it up to the mark with distilled water (100µg/ml).

In a 10 ml volumetric flask pipette out 1ml from above solution and dilute it up to the mark with distilled water to get final concentration 10 µg/ml and run the spectrum as shown in Fig. 3.

Forced degradation in alkaline media was performed by adding 10 mg of MET to 10 ml 0.01 N NaOH and refluxing the mixture at 80°C for approximately 2 hours. The solution was then left

to reach ambient temperature, neutralized to pH 7 by addition of 0.01N HCl. In a 10ml volumetric flask, pipette out 1ml from above solution and dilute it up to the mark with distilled water

(100µg/ml). In a 10ml volumetric flask, pipette out 1ml from above solution and dilute it up to the mark with distilled water to get final concentration 10 µg/ml and run the spectrum as shown in Fig. 4.

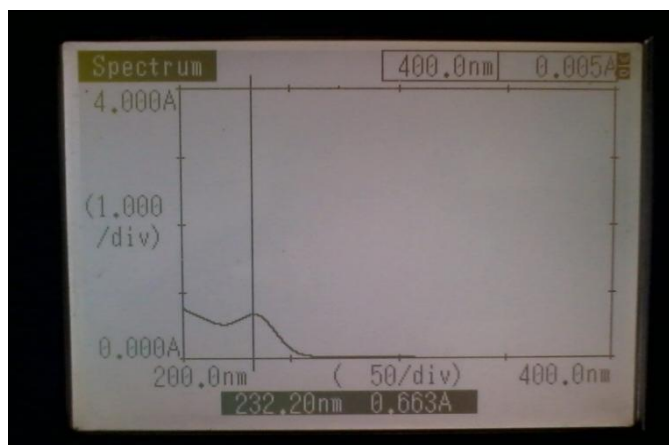


FIG. 3: ACID DEGRADED SPECTRUM OF MET AT 232.20 nm WITH 0.663 ABSORPTION

Oxidative Degradation: To study the effect of oxidizing conditions, 10 mg of MET was added to 10 ml 3% H₂O₂ solution. In a 10ml volumetric flask, pipette out 1ml from above solution and dilute it up to the mark with distilled water (100µg/ml). In a 10ml volumetric flask, pipette out 1ml from above solution and dilute it up to the mark with distilled water to get final concentration 10 µg/ml and run the spectrum as shown in Fig. 5.

Thermal Degradation: To study the effect of temperature, approximately 10 mg MET was stored

at 80°C for 2 days. Then, 10 mg taken and was dissolved in distilled water and volume was adjusted up to 10 ml with distilled water to get the concentration of 1000 µg/ml. Pipette out 1ml from above solution and dilute it up to the mark with distilled water (100 µg/ml).

In a 10 ml volumetric flask, pipette out 1ml from above solution and dilute it up to the mark with distilled water to get final concentration 10µg/ml and run the spectrum as shown in Fig. 6.

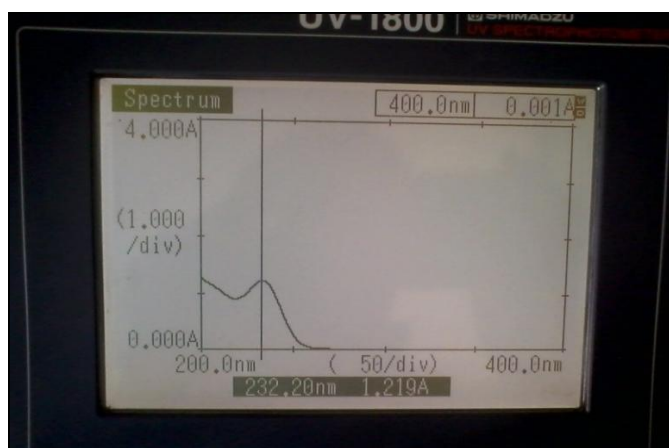


FIG. 5: OXIDATIVE DEGRADED SAMPLE OF MET AT 232.20 nm WITH 1.219 ABSORPTION

Photolysis: To study the effect of UV light, approximately 10 mg MET was exposed to short and long wavelength UV light (254 and 366nm, respectively) for 24 h, then dissolved in distilled water and made up volume 10 ml in a volumetric

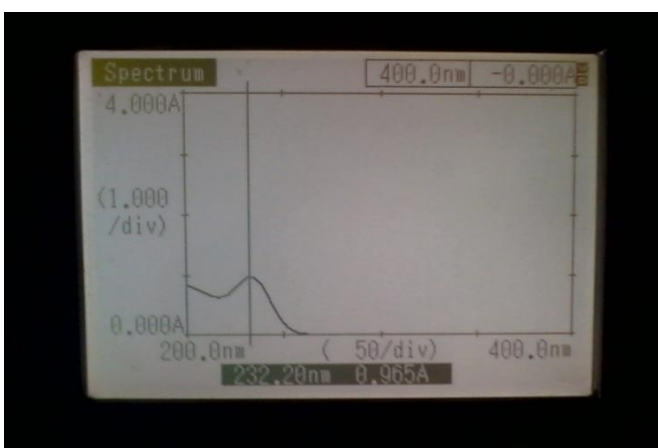


FIG. 6: THERMAL DEGRADATION OF MET AT 232.20 nm WITH 0.965 ABSORPTION

flask to get the concentration of 1000 µg/ml. Further dilution was made with distilled water to get final concentration 10µg/ml and run the spectrum, as shown in Fig. 7 and 8.

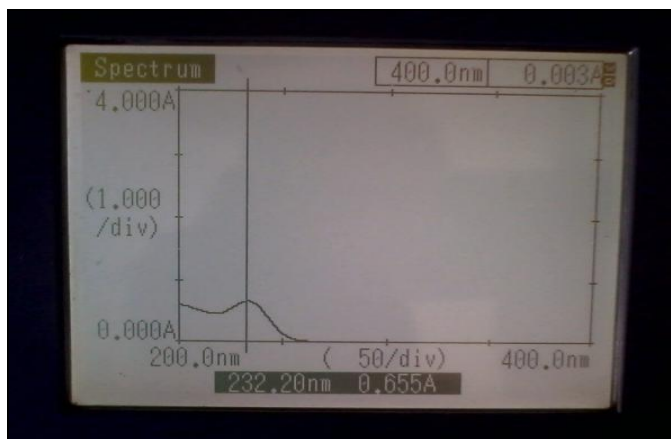


FIG. 7: UV SHORT 254nm OF MET AT 232.20nm WITH 0.665 ABSORPTION

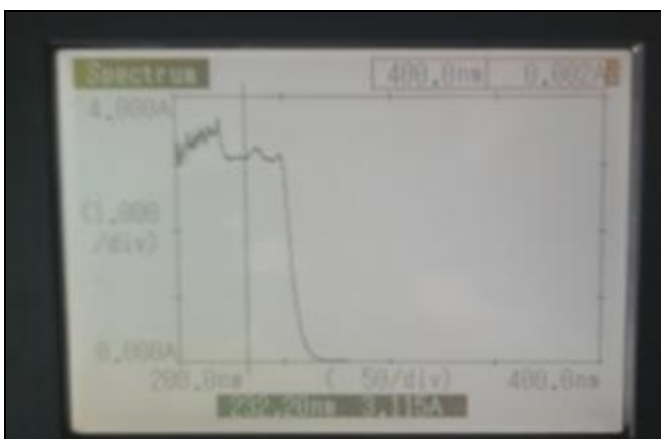


FIG. 8: UV LONG 365nm OF MET AT 232.20 nm WITH 3.115 ABSORPTION

Method Validation: The method was validated for linearity, limits of detection (LOD) and quantification (LOQ), precision, accuracy, and stability by ICH Guidelines²⁰.

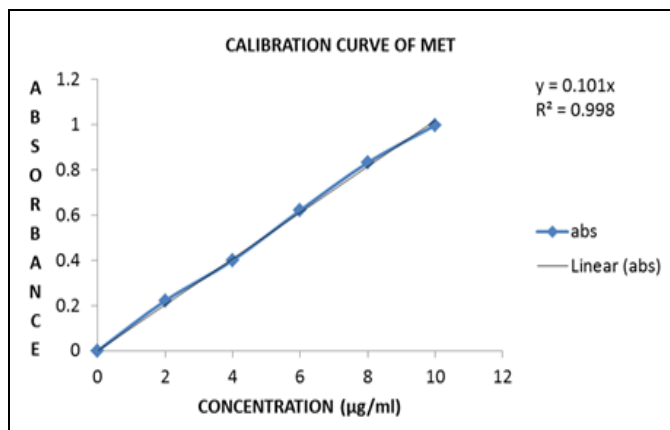


FIG. 9: CALIBRATION CURVE FOR MET AT 232.20 nm

Linearity: 10 mg pure MET was transferred to 100 ml volumetric flask and diluted up to the mark with distilled water to get a concentration of 100µg/ml solution. Aliquots of 2,4,6,8 and 10ml from a standard stock solution of MET were pipette out in 10 ml volumetric flasks, and the volume was made up to 10 ml with distilled water to get concentration 2,4,6,8 and 10µg/ml of MET respectively. The absorbance was measured in triplicate at 232.20 nm

against reagent blank. The calibration curve was constructed by plotting absorbance v/s concentration, as shown in Fig. 9.

Detection Limits: The limits of detection (LOD) and quantification (LOQ) for MET were determined, as the amounts for which signal-to-noise ratios were 3:1 and 10:1, respectively.

Precision: To determine precision, 7 days measurement (intra-days and interday) were computed with relative standard deviation (RSD %) for replicate samples (n=5) using concentration 6, 8, and 10µg/ml. Both the intra-day and inter day samples were calibrated with standard curve concurrently prepared on the same day of analysis.

Intraday Precision: Intraday precision of the test method is demonstrated by three samples of the same batch (same concentration) at initial, 24, and 48 h Table 1.

Interday Precision: Interday precision of the test method is demonstrated by three samples of the same batch (same concentration) on three successive days Table 1.

TABLE 1: EVALUATION OF INTRADAY AND INTERDAY ACCURACY AND PRECISION OF MET

MET took (µg/ml)	Intraday Accuracy and Precision			Inter-day Accuracy and Precision		
	MET found (µg/ml)	RE %	RSD %	MET found (µg/ml)	RE %	RSD %
6	6.23	0.221	0.871	6.20	0.026	1.062
8	8.32	0.040	1.420	8.24	0.054	1.634
10	10.15	0.022	0.549	10.22	0.040	0.967

Accuracy: To determine the accuracy of the proposed method, recovery study was carried out by adding the different amount (80%, 100%, and

120%) of a bulk sample of MET within the linearity range and results obtained are compiled in Table 2.

TABLE 2: RECOVERY DATA OF MET

Level	Amount of MET added (μg)	Amount of MET found (μg)	% Recovery	% RSD*
80 %	12	11.86	98.86	1.720
100 %	15	14.95	99.67	1.575
120 %	18	17.82	99.00	1.206

*An average value \pm relative standard deviation of 5 observations

RESULTS AND DISCUSSION: The proposed method for MET showed molar absorptivity of 49.301×10^3 L/mol/cm and sandell's sensitivity of $0.01002 \mu\text{g}/\text{cm}^2$ absorbance units. Linear regression of absorbance on concentration gave the equation $y=0.101x$ with a correlation coefficient (r^2) of 0.998. The relative standard deviation for intraday and interday analysis was found to be 0.9466 and 1.221, respectively.

Limit of detection and limit of quantification were found to 0.5232 and $1.5856 \mu\text{g}/\text{ml}$, respectively. The higher percentage recovery value indicates that there is no interference of the excipients present in the formulation. The optical characteristics and validation parameters of Metformin hydrochloride by UV spectroscopic method are tabulated in **Table 3**.

TABLE 3: VALIDATION PARAMETERS OF MET FOR UV-SPECTROSCOPIC METHOD

Parameter	Analytical data
Linearity Range ($\mu\text{g}/\text{ml}$)	2-10
λ max (nm)	232.2
Molar extinction coefficient, L/mol/cm	49.301×10^3
Sandell's sensitivity, $\mu\text{g}/\text{cm}^2$	0.01002
Slope	10.1×10^{-2}
Intercept	0.008
Standard deviation about regression (Sy)	± 0.0192
The standard deviation of Slope (Sb)	± 0.0023
The standard deviation of intercept (Sa)	± 0.01396
Correlation co-efficient (r)	0.998
Limit of detection (LOD, $\mu\text{g}/\text{ml}$)	0.5232
Limit of quantification (LOQ, $\mu\text{g}/\text{ml}$)	1.5856
Intraday Precision (% RSD)	0.9466
Interday Precision (% RSD)	1.221
Accuracy (% RSD)	1.5
Accuracy (% Recovery)	99.97

Degradation Behaviour: UV-Spectrophotometry studies for MET under different stress conditions

suggested the following degradation behavior **Table 4**.

TABLE 4: PERCENT DEGRADATION OF MET (10 $\mu\text{g}/\text{ml}$) AND ABSORBANCE OF DEGRADATION PRODUCT AT 232.20nm

Condition	MET	Time	Absorbance	Conc. Obtained	(%)	Degraded (%)	Observation
Acid	API	0 hrs	0.998	10.00	100	0.00	Undegraded
	API	2 hrs	0.663	6.64	66.43	33.57	Degraded
	TAB		0.840	8.41	84.16	15.84	Undegraded
Alkaline	API	2 hrs	0.809	8.10	81.06	18.94	Undegraded
	TAB		0.902	9.038	90.38	9.62	Undegraded
Thermal	API	24 hrs	0.965	9.66	96.69	3.31	Undegraded
	TAB		0.859	8.60	86.07	13.92	Undegraded
Thermal	API	48 hrs	1.029	10.31	103.10	3.10	Undegraded
	TAB		0.807	8.08	80.86	8.08	Undegraded
	API	24 hrs	0.985	9.85	98.59	1.41	Undegraded
UV ₂₅₄	TAB		0.975	9.75	97.5	2.50	Undegraded
	API	48 hrs	0.655	6.56	65.63	34.37	Degraded
	TAB		0.706	7.07	70.74	29.26	Degraded
UV ₃₆₅	API	24 hrs	1.093	10.95	109.51	9.51	Undegraded
	TAB		0.806	8.06	80.66	19.34	Undegraded
	API	48 hrs	3.115	31.81	312.12	212.12	Degraded
3% H ₂ O ₂	TAB		1.741	17.44	174.44	74.44	Degraded
	API		1.219	12.21	122.14	22.14	Degraded
3% H ₂ O ₂	TAB		1.139	11.42	114.24	14.24	Undegraded

The stability studies indicate that appreciable changes were observed by treating the drug, Metformin hydrochloride with UV light, thermal stress, oxidation, and hydrolysis along with their appreciable change in λ_{max} values. However, there was no appreciable change, thermal degradation, and alkaline. Thus, the proposed UV method proved to be stability indicating.

CONCLUSION: All these factors lead to the conclusion that the stability indicating UV spectroscopic method development is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of Metformin hydrochloride in bulk and pharmaceutical formulations without interference using distilled water.

However, there was no appreciable change with thermal and alkaline hydrolysis. The relative standard deviation (RSD) for all parameters was found to be less than two, which indicate that the validity of the method is also within the limit so the proposed method can be used for routine quantitative estimation of Metformin hydrochloride in bulk and pharmaceutical formulations.

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CONFLICT OF INTEREST: Nil

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