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DEVELOPMENT AND VALIDATIONS OF UV-VIS SPECTROSCOPY METHOD FOR THE DETERMINATIONS OF METFORMIN HYDROCHLORIDE AND ROSUVASTATINE CALCIUM IN BULK DRUG AND IN PHARMACEUTICAL SYNTHETIC MIXTURE

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ABSTRACT:

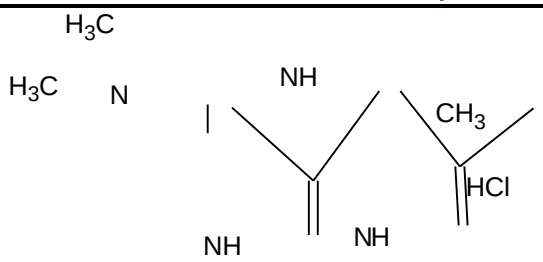
A Rapid and sensitive U.V. spectrophotometric method for the determination of Metformin Hydrochloride Hydrochloride and Rosuvastatine calcium calcium in the pharmaceutical synthetic mixture was developed. The UV spectroscopy method was performed at 232.6 nm for Metformin Hydrochloride Hydrochloride and 240.4 nm for Rosuvastatin and samples were prepared by using water as solvent. The linearity range for Metformin Hydrochloride is 5-25 µg/ml and for Rosuvastatine calcium is 0.4-2.0 µg/ml. Regression Coefficient For Metformin Hydrochloride and Rosuvastatine calcium is 0.999 and 0.998 respectively. The LOD and LOQ for Metformin Hydrochloride e was found to be 0.264 µg/ml and 0.80 µg/ml respectively. The LOD and LOQ for Rosuvastatine calcium was found to be 0.1143 µg/ml and 0.3464 µg/ml respectively. The proposed method were simple, rapid, precise, accurate and sensitive can be used for routine of the quality control in pharmaceuticals.

KEYWORDS: Metformin Hydrochloride, Rosuvastatine calcium, Q method

INTRODUCTION:

Metformin Hydrochloride e is the oral antidaibetic drug in the biguanide class. It is the first line drug for the treatment type 2 daibetes mellitus particularly in the overweight and obes people. Rosuvastatine calcium is the selective compitative inhibitors of the HMG-CoA reductase. There for the combinations of this drug are given to control the comprehensive control of Diabetes and associated Dyslipedimia.

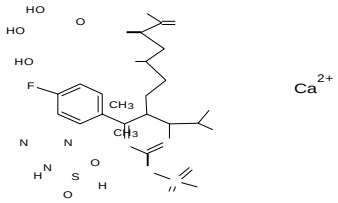
Chemically, Metformin Hydrochloride is 1, 1-dimethyl biguanide hydrochloride, Metformin Hydrochloride improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis.



Chemical structure of Metformin Hydrochloride.

Rosuvastatin calcium is calcium; (E,3R,5S)-7-[4-(4-fluorophenyl)-2 [methyl (methylsulfonyl) amino]-6-propan-2- yl pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoate. It is a Hydroxy Methyl Glutaryl-CoA-Reductase inhibitor, or statin, that reduces the plasma concentrations of LDL-cholesterol; Apolipoprotein B, and Triglycerides while increasing

HDLcholesterol levels in patients with hypercholesterolemia and those at risk for cardiovascular diseases



Chemical structure of Rosuvastatin

MATERIALS AND METHOD:

Instrumentation:

A Shimadzu model UV-1800 double beam UV-Visible spectrophotometer attached with Computer operated software UV probe 2.0 with spectral width of 2 nm, with a pair of 1 cm Matched quartz cells were used to measure absorbance of the resulting solutions. Analytical Weighing balance (AA-2200), digital and Oscar ultrasonic bath (Ultrasonic Cleaner Micro clean 103) were used during the study. HPLC grade water system (Model: Milipore-Q5)

Material:

Working standard of Metformin Hydrochloride and Rosuvastatine calcium was obtained from Wokhardt pharmaceutical Pvt. Ltd. Aurangabad and Aurbindo pharmaceutical Pvt. Ltd. Hyderabad. Other chemicals such as water, 0.1 N NaoH (AR grade), 0.1 N HCL (AR grade), H_20_2 (AR grade) , etc are provided by School Of Pharmacy, S.R.T.M.University, Nanded.

Selection of Analytical wavelength:

The working standard solution containing 10 µg/ml of MET and ROS was separately scan in the spectrum mode of 200-400 nm. Wavelengths of absorption maxima's were determined for both the drug. MET showed maximum absorbance at 232.6 nm (Fig.No.1) and ROS showed maximum absorbance at 240.4 nm (Fig.No.2). From the overlay spectra of MET and ROS two wavelength were selected that is 246.8 nm which is the isobestic wavelength at which both the drug shows absorbance and 232.6 nm absorption maxima for MET. Fig. 3 Represent the overlay spectra of both the drugs.

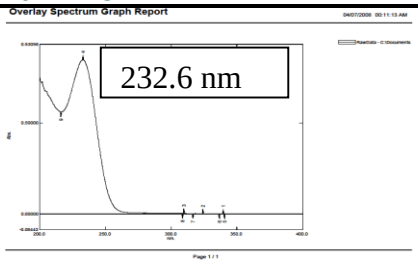


Fig 1 : Absorption spectrum of MET by Spectrophotometry.

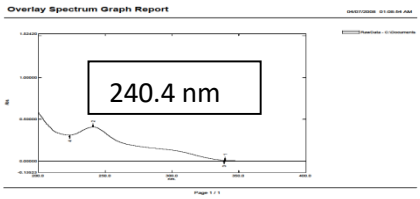


Fig 2 : Absorption spectrum of ROS by UV UV Spectrophotometry.

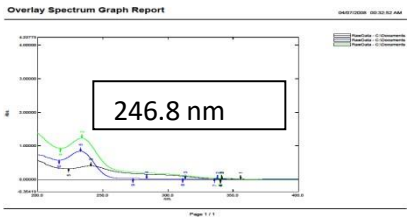


Fig 3: Isobestic point of MET and ROS.

Preparations of standard stock solutions:

Accurately 100 mg of pure MET and ROS were weighed and transferred into two separate 100 ml volumetric flasks. Sufficient water was added. Sonicate for 15 min. and volume was made up to 100ml with water. Further dilution was made up to produce 10 µg/ml & 0.8
µg/ml of stock solution for MET & ROS respectively.

Analysis of laboratory Prepared mixture by proposed method:

Accurately weighed 100 mg of MET and 100 mg of ROS were transferred into 100 ml volumetric flask and dissolved in water, then volume was made up to mark with water. Further dilution was made in 100 ml volumetric flask to get final concentration of 10 µg/ml of MET and 0.8 µg/ml of ROS .The conc of laboratory mixture carried out in such way to get the conc 10 µg/ml of MET & 0.8 µg/ml of ROS.The absorbance of resulting mix solution was measured at 232.6 nm and 246.8 nm. The concentration of both the drugs was obtained from equation 1 and 2.

$$C_x = \frac{Q_m - Q_y A}{Q_x - Q_y \quad a_1} \quad \ldots\ldots\ldots\ldots\ldots (1) \qquad C_y = \frac{Q_m - Q_x A}{Q_y - Q_x \quad a_2} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where,

C_X = concentration of MET in g/100 ml

C_Y = Concentration of ROS in g/100 ml

Q_m = Absorbance ratio of mixture at 232.6 nm and 246.8 nm Q_x = Ratio of absorbance of MET at 232.6 nm and 246.8 nm Q_y = Ratio of absorbance of ROS at 232.6 nm and 246.8 nm

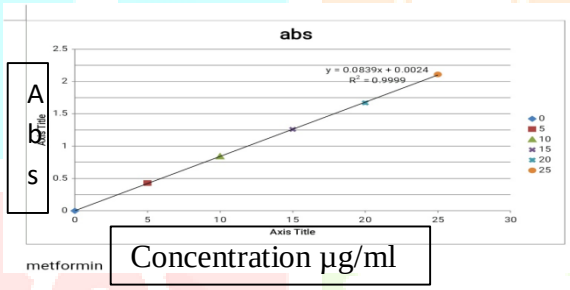
A = Absorbance of mixture at iso-absorptive wavelength i.e. 246.8 nm

a_1 & a_2 = Absorptivity of MET and ROS at iso-absorptive point i.e. 246.8 nm

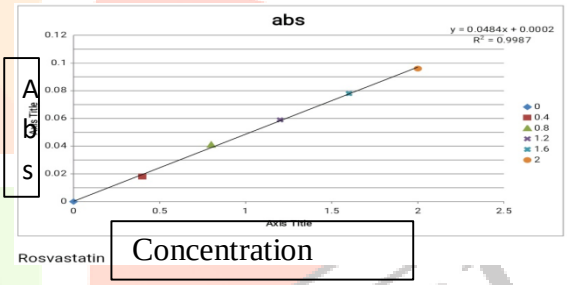
VALIDATION OF ANALYTICAL METHOD:

Linearity:

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of MET and ROS respectively. Beer-Lamberts law was obeyed in the concentration range 5-25 µg/ml and 0.4-2 µg/ml for MET and ROS respectively. The proposed methods were evaluated by its regression coefficient (r^2) value which is calculated by statistical method. The regression coefficient (r^2) was found to be 0.999 for MET and 0.998 for ROS respectively. Optical characteristics and other parameters are shown in table 1



Calibration curve of Metformin Hydrochloride



Calibration curve of Rosuvastatine calcium

Table 1: Optical characteristics' and other parameters

PARAMETERS	MET	ROS
Wavelength (nm)	232.6 nm	246.8 nm
Linearity range (µg/ml)	5 - 25 µg/ml	0.4 -2 µg/ml
Limit of detection (µg/ml)	0.264 µg/ml	0.1143 µg/ml
Limit of quantitation (µg/ml)	0.879 µg/ml	0.3806

		µg/ml
Regression equation	y = 0.083x+0.005	y= 0.048x+0.000
Slope	0.083	0.048
Intercept	0.005	0.000
Regression coefficient (r^2)	0.999	0.998

Precision:

The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation). The precision was determined at different parameter like repeatability, intermediate precision (intra-day, inter-day). Repeatability was determined by analyzing MET 10 (µg/ml) and ROS (0.8 µg/ml) for three times. The intermediate precision of the method was studied to find out intraday and interday variation in the test method of MET and ROS. Intraday precision was determined by analyzing same concentration of solution for three times within the day and interday precision was determined daily for three days. Then % RSD was calculated and it was within limit (less than 2%). Results of repeatability are shown in table 2. Results of intermediate precision study are shown in table 3.

Table 2 Results of repeatability

Sr.no.	Concentration (µg/ml)		Absorbance nm		% Amount found	
	MET	ROS	MET	ROS	MET	ROS
1	10	0.8	0.841	0.038	99.94	97.25
2	10	0.8	0.837	0.036	99.90	98.75
3	10	0.8	0.848	0.039	99.80	101.49
4	10	0.8	0.838	0.039	99.99	99.75
5	10	0.8	0.841	0.037	99.79	98.75
6	10	0.8	0.830	0.038	99.99	98.77
			% found		99.90	99.12
			S.D.		0.0893	1.407
			R.S.D.		0.0893	1.420

Table 3. Results of intermediate precision study

Sr. no	Interval of time	Concentration (µg/ml)		% Amount found	
		MET	ROS	MET	ROS
1	Intraday	10	0.8	99.94	97.25
2		10	0.8	99.99	98.75
3		10	0.8	100.37	97.05
		% found		100.01	97.68
		S.D.		0.235	0.929
		R.S.D.		0.234	0.951
Sr. no	Interval of time	Concentration (µg/ml)		% Amount found	
		MET	ROS	MET	ROS
1	Interday	10	0.8	99.94	97.25
2		10	0.8	99.99	99.75
3		10	0.8	100.18	99.41
		% found		100.01	98.47
		S.D.		0.126	1.25
		R.S.D.		0.125	1.26

Accuracy (Recovery study):

The accuracy of the proposed methods was assessed by recovery studies at three different levels (80%, 100%, and 120%). The recovery studies were carried out by adding known amount of standard drug to pre-analyzed sample. The resulting solutions were then re- analyzed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at three replicate of three concentrations levels. The results of accuracy studies are shown in table 4.

Table 4 The results of accuracy studies

Drug	Amount Present In mg	Amount Added in mg	Amount Recovered in mg	% Recovery	S.D	% R.S.D
MET	500	400	899.74	99.16	0.1184	0.1184
	500	500	1001.5	100.96	0.3667	0.3661
	500	600	1100.6	100.05	0.2426	0.2424
ROS	40	32	72.33	100.45	1.05	1.04
	40	40	81.05	101.31	0.109	0.1075
	40	48	87	98.86	0.57	0.5765

**Indicates average of 3 determinants*

Limit of detection (LOD)

LOD is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method.

The limit of detection (LOD) may be expressed as LOD =

$3.3*\sigma/ S$

Limit of quantification (LOQ)

LOQ is defined as the lowest concentration of analyte that can be determined with acceptable accuracy and precision by the analytical method.

The limit of quantitation (LOQ) may be expressed as LOQ = 10*

σ/S

Table 5: LOD and LOQ

Parameters	LOD	LOQ
MET (µg/ml)	0.264	0.1143
ROS (µg/ml)	0.879	0.3806

FORCED DEGRADATIONS STUDY

Degradation studies were carried out as per ICH guidelines. The sample solutions were subjected to acidic, basic, peroxide, water and light

1] Acid degradation.

Weight accurately 100 mg of MET and 100 mg of ROS was transferred in 100 ml of volumetric flask and dissolved in 10 ml solvent by sonication. Then make the volume up to the mark to produces conc. 1000µg/ml. Then 10 ml Stock removed and dilute up to 100 ml to get the conc. 100 µg/ml for both the drug .For MET remove 10 ml of solutions from above stock and for ROS remove 0.8 ml stock from 100 µg/ml stock solution. Add 10 ml of 0.1 N HCl for MET and Add 0.8 ml of 0.1 N HCl for ROS and reflux both the flask at 60 ^{0}C For 30 min after the reflux period stop the reactions by adding base and make the volume with water.

2] Base Degradation:

Weight accurately 100 mg of MET and 100 mg of ROS was transferred in 100 ml of volumetric flask and dissolved in 10 ml solvent by sonication. Then make the volume up to the mark to produces conc. 1000µg/ml. Then 10 ml Stock removed and dilute upto 100 ml to get the conc. 100 µg/ml for both the drug .For MET remove 10 ml of solutions from above stock and for ROS remove 0.8 ml stock from 100 µg/ml stock solution. Add 10 ml of 0.1 NaoH for MET and Add 0.8 ml of 0.1 NaoH for ROS and reflux both the flask at 60 ^{0}C For

30 min after the reflux period stop the reactions by adding acid and make the volume with water.

3] Oxidative Degradation:

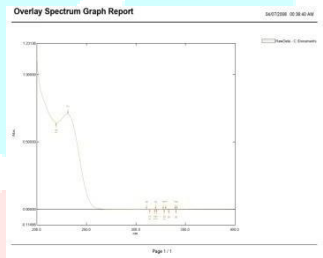
Weight accurately 100 mg of MET and 100 mg of ROS was transferred in 100 ml of volumetric flask and dissolved in 10 ml solvent by sonication. Then make the volume up to the mark to produces conc. 1000 µg/ml. Then 10 ml Stock removed and dilute up to 100 ml to get the conc. 100 µg/ml for both the drug .For MET remove 10 ml of solutions from above stock and for ROS remove 0.8 ml stock from 100 µg/ml stock solution. Add 10 ml of 3 % H2O2 for MET and Add 0.8 ml of 3 % H2O2 for ROS and reflux both the flask at For 30 min.

4] Thermal Degradation:

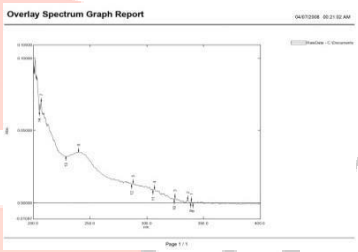
Dry heat study was performed by keeping Metformin Hydrochloride and rosuvastatin separately in oven at 60 ^{0}C for 1 hr. A sample was withdrawn at appropriate times, weighed and dissolved in water to get solution of 10 µg/ml and 0.8 µg/ml for MET and ROS respectively.

5] Photolytic degradation:

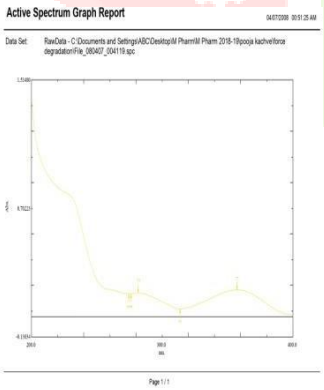
Photolysis was performed by exposing Metformin Hydrochloride and rosuvastatin separately to UV light for period of 3 hr. A sample was withdrawn, weighed and dissolved in water to get solution of 10 µg/ml and 0.8 µg/ml for MET and ROS respectively.

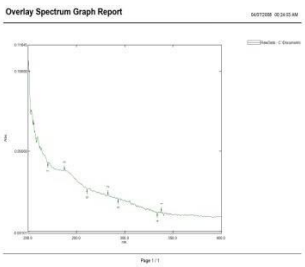


Forced degradation of MET (Acid)

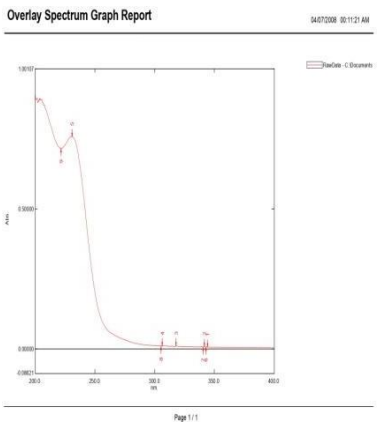


Forced degradation of ROS (Acid)

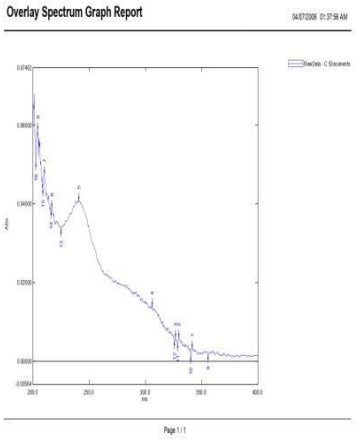




Forced degradation of MET (Base)

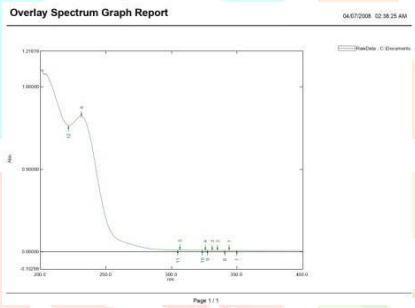


Forced degradations of ROS (Base)

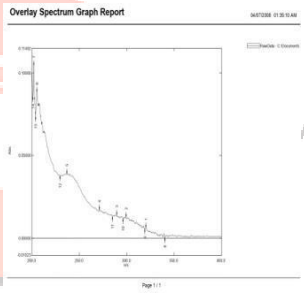


Forced degradation of MET (Thermal)

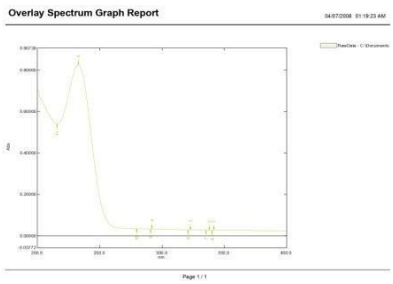
Forced degradations of ROS (Thermal)



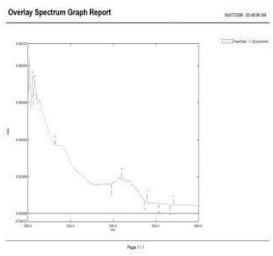
Forced degradation of Metformin Hydrochloride (H_20_2)



Forced degradations of Rosuvastatine calcium (H_20_2)



Forced degradation of MET (U.V. Light)



Forced degradations of ROS (U.V.Light)

Table no.5 Summary of Force degradation study

Stress condition/ duration	MET		ROS	
	% degradation	% recovery	% degradation	% recovery
Acid / 0.1 N HCl/ Reflux at 60ºC 30 min	17.03	82.97	15.64	85.36
Alkaline /0.1 N NaOH/ Reflux at 60ºC 30 min	10.54	89.76	12.20	87.80
Oxidative /3 % H2O2 / Reflux at 60ºC 30 min	16.08	83.92	7.32	92.68
Dry heat/ 60ºC/ 1 hr	10.72	89.28	7.32	92.68
UV light for 3h	1.43	98.57	9.19	90.81

CONCLUSION:

The developed method is simple, specific and accurate. Hence it can be used for routine quality control analysis of MET and ROS in bulk and pharmaceutical dosage forms.

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