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Development And Validation of Rp-Hplc Method for The Estimation of Metformin Hydrochloride and Rosiglitazone in Pharmaceutical Dosage Forms.

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ABSTRACT

The working condition for the RP-HPLC method was established for Metformin HCL and Rosiglitazone then was applied on pharmaceutical dosage forms. A simple reverse phase liquid chromatographic method has been developed and subsequently validated.The separation method was carried out by using a mobile phase consisting of 0.02M dipotassium hydrogen phosphate and acetonitrile in the ratio 70:30%v/v. The detection was carried out by using UV – Visible SPD 20 A at 229 nm. The column was phenominex Gemini C18 (250×4.6mm×5µ).The flow rate was selected as 1ml/min.The retention time of Metformin HCL and Rosiglitazone was found to be 3.614 and 6.390 respectively. The asymmetry factor or tailing 1.49 and 1.68 respectively, which indicates symmetrical nature of the peak. The number of theoretical plates of Metformin HCL and Rosiglitazone was found to be 7488 and 6583 respectively, which indicates the efficiency performance of the column.

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use under acidic, alkaline, oxidative, thermal, photostability, and neutral conditions. This method was successfully validated for all the parameters and could detect the correct amounts of active drug substance in formulations that are available in the market. This developed method in the present study could be successfully employed for the simultaneous estimation of Metformin hydrochloride and Rosiglitazone in pharmaceutical dosage form.

Key words: Metformin, Rosiglitazone, RP-HPLC, validation, force degradation, stability

INTRODUCTION

Metformin (MET) is chemically 1-carbamimidamidoN,N-dimethylmethanimidamide. It belongs to the biguanide class of antidiabetic drugs. It is the first line drug of choice for the treatment of type-2 diabetes. It activates adenosine monophosphate activated protein kinase, a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and metabolism of glucose and fats.[3-5] Rosiglitazone, 3 5-[[4-[2-[methyl(pyridin-2-yl)amino]ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione are used in the treatment of type 2 diabetes. Rosiglitazone hydrochloride has been shown to affect abnormal glucose and lipid metabolism associated with insulin resistance by enhancing insulin action on peripheral tissues. Many patients suffering from type 2 diabetes require treatment with more than one antihyperglycemic drug to achieve optimal glycemic control..

A literature survey reveals a good number of analytical methods for the estimation of metformin hydrochloride and Rosiglitazone individually or in combination with other drugs using ultraviolet (UV) spectrophotometry,[6] high performance liquid chromatography (HPLC),[7] HPTLC,[8] and LC-MS/MS[9] Hence, we tried to develop a simple stability indicating HPLC method for the estimation of the selected drugs. The developed method has been validated as per the guidelines of the ICH.[10] To establish the stability indicating nature of the method forced degradation studies were planned for the proposed method under acidic, alkaline, oxidative, thermal, photostability, and neutral conditions.

The combination of Metformin HCL and Rosiglitazone was selected for the present study. According to the literature survey conducted, it was observed that no method was reported in RP-HPLC for the estimation of individual drug carried out. Hence present study aims to develop an accurate, precise, specific, linear, simple, rapid, validated and cost effective analytical method for Metformin HCL and Rosiglitazone in tablet dosage form by RP-HPLC method.

METHODS AND MATERIAL

Chemicals and Reagents: Pure Metformin and Rosiglitazone were obtained as gift samples

from Glenmark Pharmaceutical Ltd. Aurangabad. HPLC grade Acetonitrile and Methanol from Merck, AR grade ortho phosphoric acid from Qualigens, and AR grade Potassium Dihydrogen Orthophosphate from Emplura, UV-1100 Shimadzu, HPLC, Sonicator from PCI.

Instrumentation

Separation was performed with Shimadzu HPLC equipped with a pump 2695, auto sampler and UV detector. HPLC workstation software was applied for data collecting and processing. UV – Visible SPD 20 A at 240nm.The column was phenominex Gemini C18 (250×4.6mm×5µ).The flow rate was selected as 1ml/min. The injection volume was 20 µL and all the experiments were performed at temperature 300C. The run time was set at 10.20 min. Mixture of Methanol and Water in the ratio of 10:30% v/v is used as a solvent which is sonicated to degas. HPLC grade water was obtained from a Milli – Q water purification system.

Methodology

Selection of wavelength for detection of components

Solution of Metformin HCL and Rosiglitazone were scanned in the UV region and spectrum was recorded .The solvent used was 0.02M dipotassium hydrogen phosphate,and acetonitrile in the ratio 55:45. It was seen that at 260nm all compounds have good absorbance, which can be used for the estimation of compounds by HPLC.

Selection of chromatographic method

Proper selection of the method depends on the nature of the sample (ionic or ionisable or neutral molecules), its molecular weight, pka value and stability .The drugs selected in the present study are polar and so reversed phase or ion exchange chromatography can be used. The reverse phase HPLC was selected for the initial separation because of its simplicity and suitability.

For the literature survey and with knowledge of properties of the selected drugs, Phenominex Gemini C18 (250 × 4.6mm) 5µ column was chosen as stationary phase and mobile phase with different compositions such as Acetonitrile was used. The separations were not observed so use of buffer was finalized.

For all the data observed, obtained and available the initial separation condition were set to work around.

Chromatographic condition Use suitable High Performance Liquid Chromatography equipped

Instrument	**Shimadzu prominence**
Column	Phenomenex Gemini C18 (250 × 4.6mm), 5µ.
Column oven temperature	Ambient
Wavelength	229 nm
Flow rate	1.0 ml/min
Injection volume	20µl
Run time	10 min
Mobile phase	Solvent A – Buffer Solvent B –Acetonitrile
Solvent Ratio	50:50% $^{V}/_{V}$ of A: B

Preparation of Standard Stock Solution:

Standard stock solution of Metformin and Rosiglitazone was prepared by dissolving 100 mg of Metformin and 10 mg of Rosiglitazone respectively in 100 mL mobile phase. The solutions are sonicated to dissolve the drugs. Further these solutions were diluted to prepare concentrations of 500 µg/ml and 2 µg/ml of Metformin and Rosiglitazone respectively.

Preparation of Test Solution: Test stock solution of Metformin and Rosiglitazone was prepared by dissolving about 2.0 g of test sample (Brand Name: Glimestar M) (which is equivalent to 100 mg Metformin and 10 mg of Rosiglitazone) into 100 ml volumetric flask. The solutions are sonicated to dissolve the drugs. Further these solutions were diluted to

prepare concentrations of 500 µg/ml and 2 µg/ml of Metformin and Rosiglitazone respectively. Preparation of Potassium Dihydrogen Phosphate Buffer: Weighed about 272.1 mg of Potassium Dihydrogen phosphate and dissolved in 100 ml of water and pH was adjusted to 3.0 with Phosphoric acid and then filtered through 0.45 µ nylon membrane filter.

Method Validation

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. The other component may include excipients, impurities, degradation product etc. Peak purity test may be useful to show that the analyte chromatographic peak is not contributed by more than one component (e.g. .diode array, mass, spectroscopy).

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line. Linearity should be evaluated by visual inspection of a plot of signal as a function of analyte concentration. The correlation coefficient, y- intercept, slope of the regression line and the residual sum of squares should be calculated.

System precision

The system precision was evaluated by measuring the peak response of Metformin HCL and Rosiglitazone, WS solution prepared as per the proposed method and chromatograms were recorded.

Accuracy

To document accuracy, the ICH guideline on methodology recommends collecting data from a minimum of nine determinations over a minimum of three concentration levels covering the specified range.

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters.

The robustness of a method is evaluated by varying method parameters such as percent organic solvent, pH, ionic strength or temperature and determining the effect on the results of the method. Robustness tests were generally introduced to avoid problems in linear laboratory studies and toidentify the potentially responsible factors.

Ruggedness

To determine the degree of reproducibility of the results by this method involved the studies of the analyst to analyst and day to day; that is to carry out precision study in six replicate of an assay of a single batch sample bytwo different analysts on two different days.

LOD and LOQ

ICH has recommended some method for determining the limit of detection. The method may be either instrumental or non-instrumental. . It is calculated using formula

LOD = 3.3 σ/S; where, σ = S.D; S =Slope

Limit of Quantitation (LOQ) is also based on standard deviation of the response and the slope of calibration curve.

LOD = 3.3s / S

Where, s = Standard deviation of the response S = Slope of calibration curve

Force Degradation Studies

Performed the forced degradation of test method to demonstrate the noninterference of impurities, degradation products in quantification of analyte by various stress conditions like acid, base peroxide and thermal.

RESULT AND DISCUSSION

The working condition for the RP-HPLC method was established for Metformin HCL and Rosiglitazone and was applied on pharmaceutical dosage forms. A simple reverse phase liquid chromatographic method has been developed and subsequently validated.

The separation method was carried out by using a mobile phase consisting of 0.02M dipotassium hydrogen phosphate and acetonitrile in the ratio 70:30%v/v.the detection was

carried out by using UV – Visible SPD 20 A at 229 nm. The column was phenominex Gemini C18 (250×4.6mm×5µ).The flow rate was selected as 1ml/min.

The retention time of Metformin HCL and Rosiglitazone was found to be 3.614 and 6.390 respectively. The asymmetry factor or tailing 2.099 and 3.285 respectively, which indicates symmetrical nature of the peak. The number of theoretical plates of Metformin HCL and Rosiglitazone was found to be 7488 and 6583 respectively, which indicates the efficiency performance of the column.

Linearity: The linearity responses in the concentration range of 80-730 µg/mL for Metformin hydrochloride and 8-70 µg/mL for Rosiglitazone was determined were found to obey linearity with a correlation coefficient of 0.9996 and 0.9987 respectively. The linearity range of metformin HCL and Rosiglitazone were shown in table. The calibration curves were plotted as peak area vs concentration of the standard solution. The calibration graph show linear response over the range 80 to 120 ug/ml.

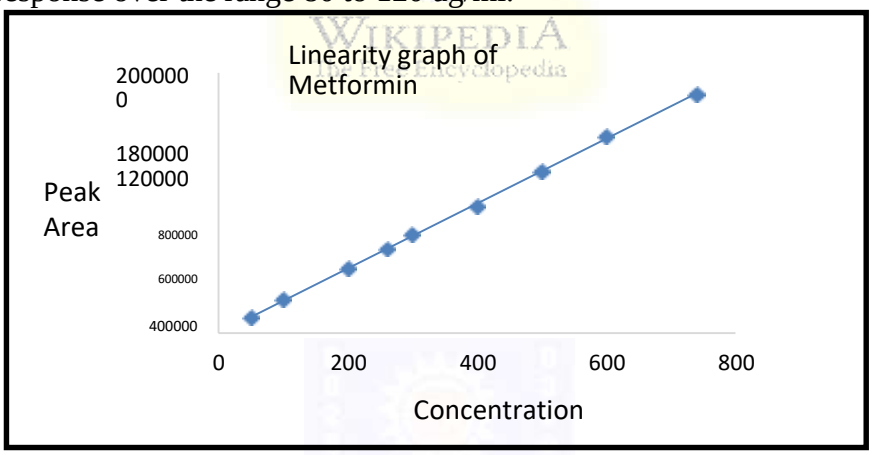


Figure No. 1: Linearity graph of Metformin HCl.

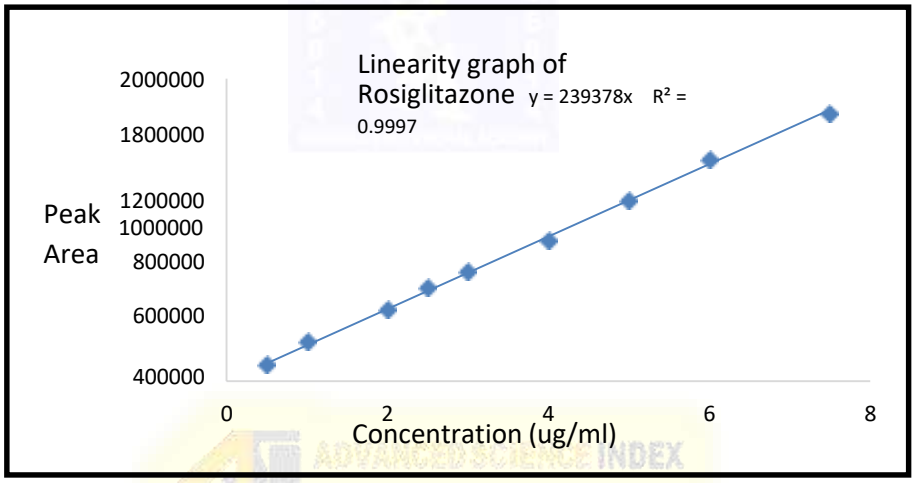


Figure No. 2: Linearity graph of Rosiglitazone .

From the linearity studies, specified concentration levels were determined .It was observed that Metformin HCL and Rosiglitazone was linear in the range of 80% to 120% for the target concentration .The linearity range of 10-50mg/ml for Metformin HCL and Rosiglitazone were found to obey linearity with a correlation coefficient of 0.999 and 0.999 respectively.

The validation of proposed method was verified by recovery studies. The percentage recovery range was found to be satisfied which represent in results. The robustness studies were performed by changing the pH and wavelength. The ruggedness study was also performed.

The analytical method validation was carried as per ICH guidelines and given below are the tables are the summary of the result.

ACCURACY: The accuracy of the method was determined by recovery experiment; a

known quantity of the pure drug was added to the pre-analyzed sample formulation at 80%, 100% and 120%. The recovery studies were carried out three times of each level and the percentage recovery and percentage relative standard deviation were calculated and given in table 8 and 9. The percentage recovery of metformin HCL and Rosiglitazone were found to be in the range 99.78, 99.24, 101.18 and 99.92, 99.05, 100.03 respectively.

Table No.1 : Percent Recovery of Metformin and Rosiglitazone

S.NO	Level	% Recovery for Metformin hydrochloride	%Recovery for Rosiglitazone
1	50%	99.6	99.6
2	100%	100.10	98.8
3	150%	99.7	99.8

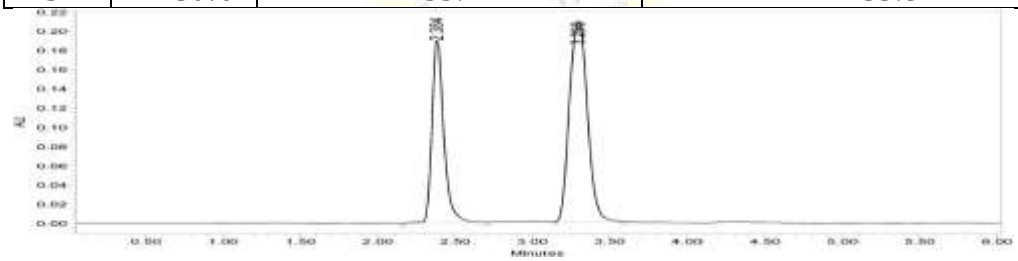


Figure 3: Accuracy graph (50 %) of MET and RZG

PRECISION: Precision is the measure of the degree of repeatability of an analyte method under normal operation and is normally expressed as percent relative standard deviation for a significant number of the samples. According to the ICH precision should be performed at three different levels: Repeatability, Intermediate precision, Reproductibility.

Table No. 2 : Precision of Metformin and Rosiglitazone

Drug	Concentration added, µg mL	Intra-day precision		Inter-day precision	
		Mean amount found, µg/mL	% RSD (n = 6)	Mean amount found, µg/mL	% RSD (n = 6)
Metformin hydrochloride	500	499.33±0.61	0.41	499.9±0.31	0.50
Rosiglitazone	5	4.95.8 ±0.52	0.35	4.72±0.54	0.39

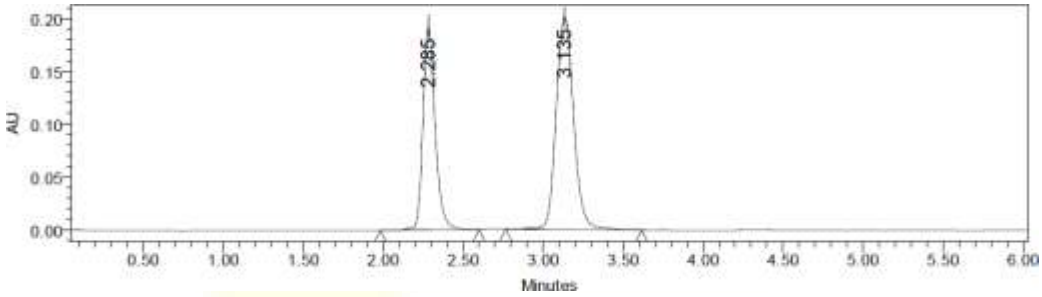


Figure 4: Precision graph of MET and RZG

LIMIT OF DETECTION (LOD): The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, though not necessarily quantitated. It is a limit test that specifies whether or not an analyte is above or below a certain value. ICH has recommended some methods for determining the limit of detection. The method may be either instrumental or non-instrumental.

Table No. 3: LoD of Metformin and Rosiglitazone

LOD	Metformin HCL:(ug)	Rosiglitazone (ug)
1.	1.05	1.12

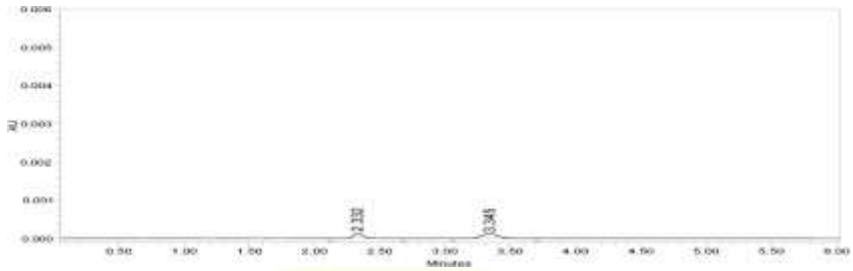


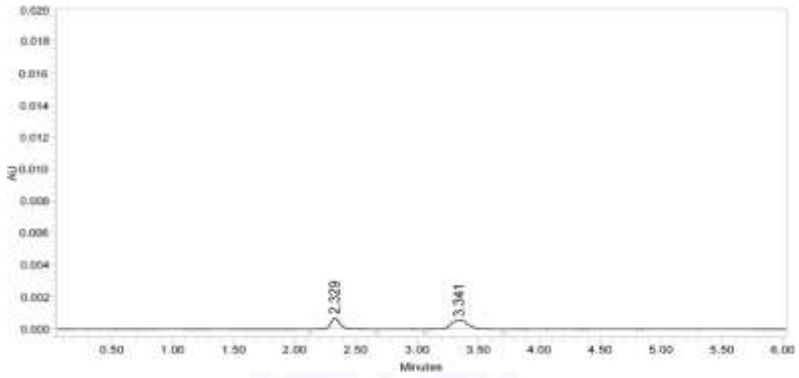
Figure 5: LOD graph of MET and RZG

LIMIT OF QUANTITATION (LOQ): The limit of Quantitation (LOQ) is defined as the lowest concentration of the analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.Limit of Quantitation (LOQ) is also based on standard deviation of the response and the slope of the calibration curve.

Table No. 4: LOQ of Metformin and Rosiglitazone

LOQ	Metformin HCL (ug)	Rosiglitazone (ug)
1.	5.6	3.5

Figure 6: LOQ graph of MET and RZG

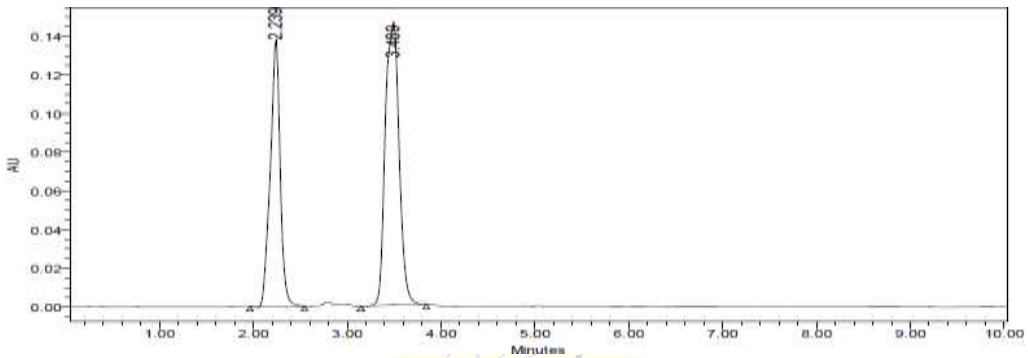


ROBUSTNESS: Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. The robustness of a method is evaluated by varying method parameters such as percent organic solvent, pH, ionic strength or temperature and determining the effect on the results of the method. Robustness tests were generally introduced to avoid problems in linear laboratory studies and to identify the potentially responsible factors.

Table No. 5: Robustness Study for Metformin HCL and Rosiglitazone

Robustness Criteria	RT of Metformin	RT of Rosiglitazone
Change in flow +0.2	3.305	6.075
Change in flow -0.2	3.890	6.860
Change in Wavelength by -PH	3.327	6.644
Change in Wavelength by +PH	3.435	6.715

Figure 7: Robustness graph of MET and RZG



6.2.6 RUGGEDNESS: Ruggedness of analytical method is the degree of reproducibility of the results obtained by the analysis of the same samples under a variety of test conditions such as different laboratories, analysts, instruments, temperature, different days etc.

Table No. 6: Ruggedness Interday Analysis study

Sample No.	% Assay of Metformin HCL	% Assay of Rosiglitazone
Analyst- 1	98.2	101.0
Analyst- 2	99.3	101.2
Analyst- 3	99.2	101.4
Analyst- 4	99.4	101.2

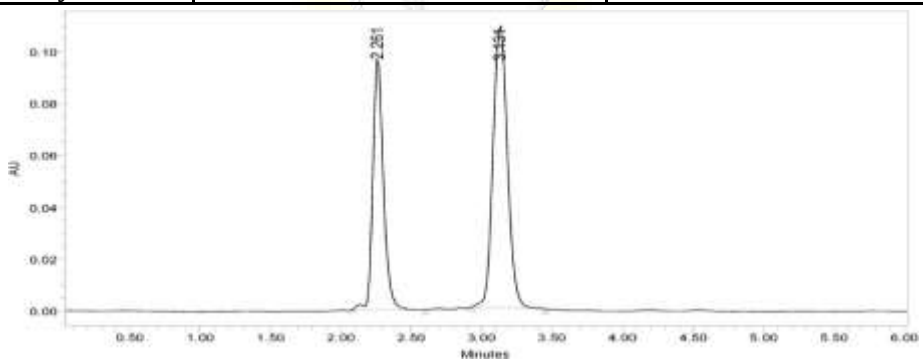


Figure 8: Ruggedness graph of MET and RZG

SYSTEM SUITABILITY: To verify whether the analytical system is working properly or it can give accurate and precise results, the system suitability parameters are to be set. Inject separately 20 µ L each of the following solutions into the HPLC.

Table No. 7: System suitability study

System Suitability Parameter	Metformin HCL	Rosiglitazone
Tailing factor	1.29	1.08
No. of Theoretical Plates	6583	7488
Resolution	3.551	

DEGRADATION STUDY:

Forced degradation studies of both drugs were carried out under various stress conditions as follows:

Effect of Acid, Alkaline and Neutral Hydrolysis: Metformin and Rosiglitazone were found to undergo 8.35% & 10.36% decomposition under acidic stress condition with a degradation product at retention time of about 2.88 min and 8.30 min respectively and minute decomposition about 7.32 % & 8.72% under basic stress condition with a degradation product at retention time of about 2.55 min. and 8.80 min respectively. Under neutral degradation condition, no degradation was observed.

Effect of Oxidation: In oxidation stress condition, almost 12.49% & 9.65% of Metformin and Rosiglitazone were degraded and degradation peak appeared in chromatogram.

Effect of Heat: Under dry thermal stress condition, Metformin and Rosiglitazone were degraded about 1.06% & 1.35% with degradation product.

Effect of light: When Metformin and Rosiglitazone in solution state were exposed to sun light; and Metformin and Rosiglitazone in powder state were exposed to UV light, no degradation was observed, respectively.

The samples exposed to acidic, alkaline, neutral, oxidative, thermal and photolytic conditions were colorless. In Photolytic stability, Metformin and Rosiglitazone were found to be stable showing no degradation. All degradate were resolved from Metformin and Rosiglitazone peaks and the percentage degradation for each condition indicated

that there were no interference from degradate in determination of the Metformin and Rosiglitazone in tablet dosage form. Thus, the proposed, method was found to be "Stability Indicating"

Table No. 8: Forced degradation study

Stress conditions/duration	% Degradation d Metformin	% Degradation of Rosiglitazone
Acidic/0.1N HCl	8.35	10.36
Alkaline/ 0.1N NaOH	7.32	8.72
Oxidative/ 3% H202	12.49	9.65
Thermal 60°C	1.06	1.35

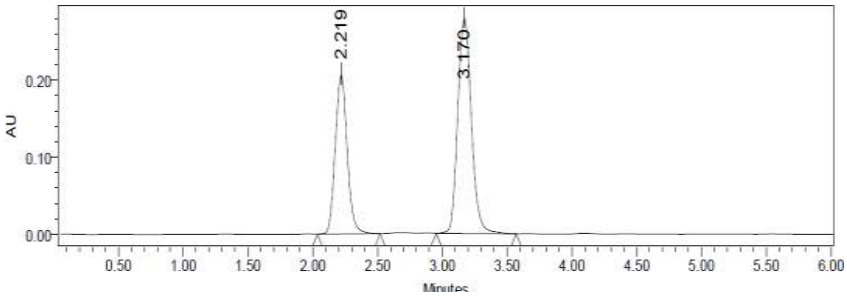


Figure 9: Acid Degradation Chromatogram for Metformin hydrochloride and Rosiglitazone

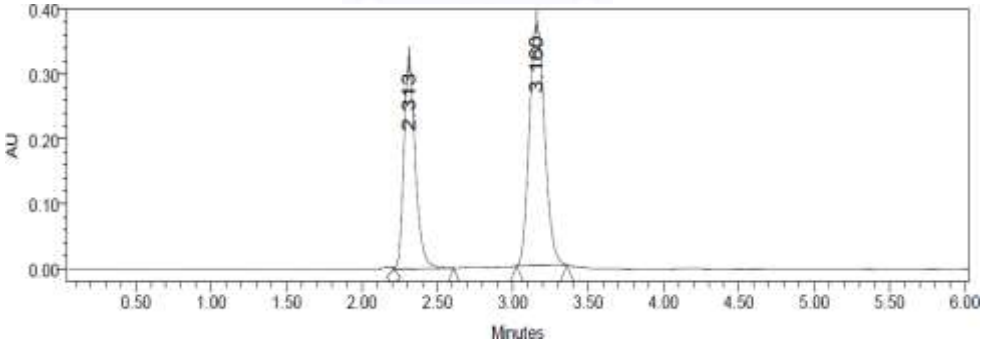


Figure 10: Alkali Degradation Chromatogram for Metformin hydrochloride and Rosiglitazone

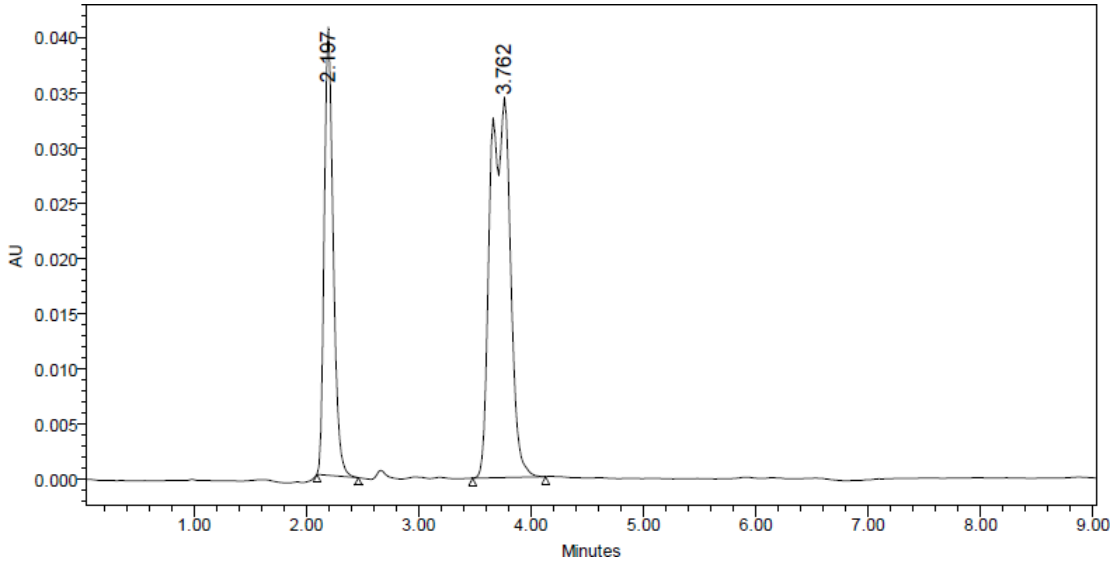


Figure 11: Peroxide Degradation Chromatogram for Metformin hydrochlorideand Rosiglitazone

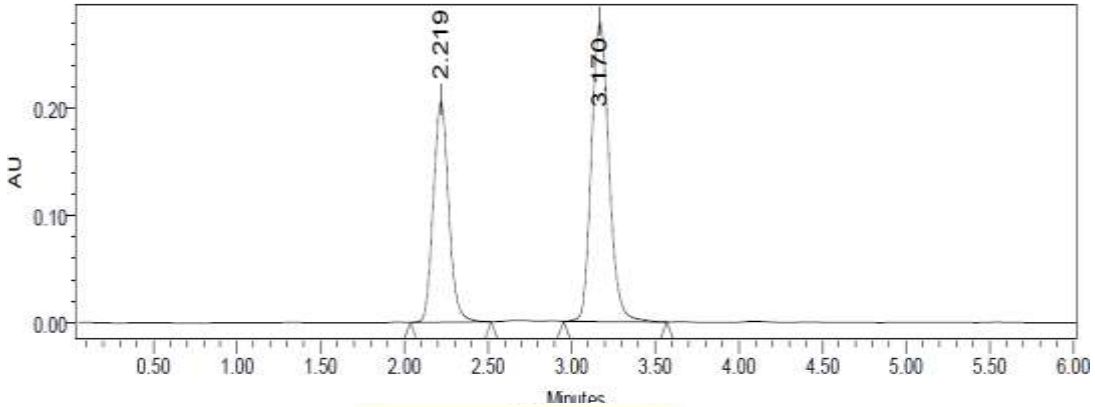


Figure 12: Reduction Degradation Chromatogram for Metformin hydrochlorideand Rosiglitazone

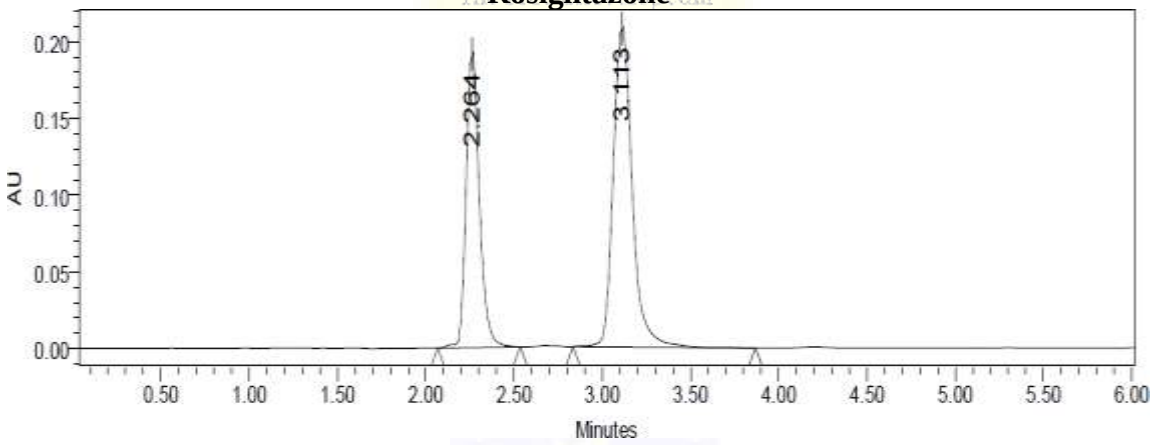


Figure 13: Thermal Degradation Chromatogram for Metformin hydrochlorideand Rosiglitazone

CONCLUSION

RP-HPLC method was developed. It was validated for the estimation of Metformin HCL and Rosiglitazone in tablet dosage form using HPLC Shimadzu Prominence with UV-Visible SPD 20A Detector and Phenominex C18 (250x4.6mm, 5µ) column, injection of 20 µl is injected and eluted with the mobile phase of dipotassium hydrogen phosphate buffer, and acetonitrile in the ratio 70:30% v/v, which was pumped at a flow rate of 1.0 ml/min at 229 nm. The peak of Metformin HCL and Rosiglitazone are found well separated at 3.614 and 6.340 respectively. The developed method was validated for various parameters as per ICH guidelines like Accuracy, Precision, Linearity, Specificity, Ruggedness, Robustness, LOQ and LOD

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