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A VALIDATED STABILITY INDICATING RP-HPLC METHOD OF ESTIMATION OF ROSIGLITAZONE IN DOSAGE FORM

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ABSTRACT

A simple, specific, precise, and accurate RP-HPLC method has been developed and validated for the estimation of Rosiglitazone maleate in bulk and tablet dosage form. Chromatographic separation was achieved on Hypersil BDS, C_8 250 x 4.6 mm, 5 column using 0.01M potassium phosphate buffer and acetonitrile PH(4.0) (30:70v/v) as mobile phase, methanol as diluent in isocratic mode. Flow rate of 1.0ml/min was optimized with detection wavelength at 230 nm. The retention time (Rt) was around 4.72±0.2 min. The method was validated with respect to specificity, selectivity, linearity, accuracy, precision, and robustness as per ICH guidelines. The assay method was observed linear in the concentration range of 0.079-0.318 mg/ml with a Correlation coefficient (r^2) 0.9999. The percentage recovery of active pharmaceutical ingredient from tablet dosage form ranged from 99.40-100.40%. Stress conditions of degradation in acidic, alkaline, peroxide, thermal and UV radiation were studied.

KEYWORDS: Rosiglitazone, HPLC, validation, Forced Degradation studies.

INTRODUCTION

Rosiglitazone Maleate is an antihyperglycemic agent that, in the presence of insulin resistance, increases hepatic and peripheral insulin sensitivity, thereby inhibiting hepatic gluconeogenesis and increasing peripheral and splanchnic glucose uptake. It is a potent and highly selective agonist for the nuclear receptor, peroxisome proliferator-activated receptor gamma (PPAR- γ). PPARs are found in tissues like adipose tissues, skeletal muscle and liver, which are critical to insulin action. Activation of (PPAR- γ) modulates the transcription of a number of insulin-responsive genes involved in the control of glucose and lipid metabolism.[1,2] It is administered orally; insoluble in water and ether; slightly soluble in acetone, acetonitrile and alcohol; and soluble in dimethylformamide and dimethyl sulfoxide. It electively stimulates the nuclear receptor peroxisome proliferator activated receptor-gamma (PPAR-γ) and to a lesser extent PPAR-α. It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the muscle, adipose tissue and the liver. As a result, Rosiglitazone maleate reduces insulin resistance in the liver and peripheral tissues, increases the expense of insulin dependent glucose, decreases withdrawal of glucose from the liver, and reduces quantity of glucose, insulin, and glycosylated hemoglobin in the bloodstream It is not chemically or functionally related to the alpha-glucosidase inhibitors, the biguanides, or the sulfonylureas. It addresses main pathophysiological defect i.e., insulin resistance, so it is used alone or in combination with insulin, metformin, or a sulfonylureas (glimepride and glibenclamide) as an agent to treat diabetes. It reduces peripheral and hepatic resistance to insulin, resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Rosiglitazone maleate is generally well tolerated, weight gain and oedema are the most common emergent adverse events, and there are no known drug interactions between Rosiglitazone maleate and other drugs**.** Rosiglitazone maleate was also effective in reducing some measures of cardiovascular risk and arteriosclerosis.

Rosiglitazone maleate thus offers an effective treatment option for the management of patients with type 2 diabetes. It is chemically (±) 5-[[4-[2-(5-Ethyl-2-pyridinyl)ethoxy] phenyl] methyl]-2,4] thiazolidinedione monohydrochloride. Its molecular weight is $C_{19}H_{20}N_2O_3S$•HCl The molecular weight is 392.90 Da.[3] Influence of Rosiglitazone maleate on DNA oxidative damage and metabolism of SOD (super oxide dismutase) was evaluated.[4] Effect of drug on lipid metabolism and glucose metabolism in type 2 diabetic patient showed significant decrease in the plasma glucose level and improved tissue being sensitive to insulin.[5] The solid Rosiglitazone maleate dispersion study was developed which proposed to improve the rate of dissolution and to develop tablets of Rosiglitazone maleate with effective and fast dissolution characters.[6] Rosiglitazone maleate is an oral anti-hyperglycemic agent. It is used for the

treatment of diabetes mellitus type 2. It selectively stimulates nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-gamma). It was the tenth-best-selling drug in the U.S. in 2008.[7] Attempts were made to study whether the usage of Rosiglitazone maleate as an antidiabetic increases the risk of cancer. This study revealed that the use of the drug did not show any significant risk in causing bladder cancer but is associated with the increase in the risk of prostate cancer and pancreatic cancer.[8] FDC (fixed-dose combination) of 15mg of the drug Rosiglitazone maleate and 850mg of metformin was used for 24weeks in the type 2 diabetic patients to study the anti-diabetic property and patients were not prescribed with or medicated with any diabetic drug.[9] Simultaneous estimation of drugs glimeperidine, Rosiglitazone and metformin Hcl was done by using derivative spectrophotometry method.[10] Chromatographic separation was achieved on a C_8 column. The mobile phase was methanol–water 45:55 % (*v*/*v*) containing 0.2 % (*w*/*v*) *n*-heptane sulfonic acid and 0.2 % (*v*/*v*) triethylamine; the pH was adjusted to 3.0 with orthophosphoric acid. The flow rate was 1 mL min^{-1} and the photodiode-array detection wavelength was 267 nm.[11] Rosiglitazone maleate in the tablet form was analysed and validated by HPLC with C18 column, wave length 245nm, mobile phase 50% of 10mM phosphate buffer and 50% of acetonitrile. Recovery was more than 90% with LOD and LOQ value being 10ng/ml and 2.5ng/ml respectively. The linear regression coefficient being 0.9921.[12]

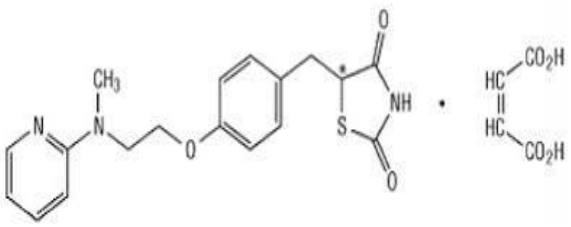


Figure.1: Structure of Rosiglitazone.

The present study was aimed for establishing a simple, accurate, and rapid RP-HPLC method for determination of Rosiglitazone in presence of its degradation products or other pharmaceutical excipients. The method was validated following analytical performance parameters suggested by ICH guidelines[13].

MATERIAL AND METHODS

Chemicals and reagents

The working standardof Rosiglitazone was procured from PADM124 and the sample of Rosiglitazone PIO/27030004.HPLC grade acetonitrile, and methanol were purchased from Rankem. Milli Q water from Merck India Pvt Ltd. Potassium dihydrogen phosphate obtained from S.D. Fine Chemicals Ltd**.**

Preparation of Solutions

Standard solution: Weigh accurately 100 mg of Rosiglitazone standard in a 50 ml volumetric flask. Add about 30 ml of Methanol and sonicate for 3 minutes. Dilute to volume with Methanol and mix. Dilute 5.0 ml of this solution to 50 ml Methanol.

Sample solution: Weigh accurately 100 mg of Rosiglitazone sample in a 50 ml volumetric flask. Add about 30 ml of Methanol and sonicate for 3 minutes. Dilute to volume with Methanol and mix. Dilute 5.0 ml of this solution to 50 ml Methanol.

Procedure

Separately inject 10 µL of standard and sample solution into the chromatograph, record the chromatograms and measure the area for the major peaks.

Apparatus and chromatographic conditions

The analytical technique was developed using Waters HPLC equipment, Model- HPLC-269, fitted with a Hypersil BDS C_8 (250 x 4.6 mm, 5 µ). The mobile phase consisted of a mixture of 0.01M potassium dihydrogen phosphate buffer and acetonitrile in the ratio 40:60. The mobile phase was filtered through a 0.22-mm nylon filter and degassed using ultrasonic bath sonicator for 30 min before running the experiment. All experiments conducted on the HPLC were carried out in isocratic mode. Injection volume was 10µL with a flow rate of 1.0 mL/min. The column temperature was maintained at 30^{o}C and elution was monitored at 225 nm using a UV detector. All chromatographic data were acquired and processed with the Empower 3 software.

Validation of the analytical method

The developed method was validated as per the ICH guidelines for linearity, accuracy and precision and specificity. Limit of detection (LOD) and limit of quantification (LOQ) were determined using the serial dilution method.

Linearity: Different aliquots of standard solution of Rosiglitazone was transferred into set of 50 ml volumetric flasks and diluted up to the mark with diluent such that the final concentrations of Rosiglitazone maleate were in the linearity range of 0.08-40.32 mg/ml. Evaluation of the drug was performed with PDA detector at 225 nm and peak area was recorded. The response for the drug was linear and the regression equation was found to be y=22750541, 96495x+39279.17 and correlation coefficient value of Rosiglitazone was found to be 0.9999. The results showed that an excellent correlation exists between peak area and concentration of drug within the specified range.

Accuracy: Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Rosiglitazone in the drug product. The study was carried out in triplicate at 50, 100 and 150 %. The percentage recovery in each case was calculated. The percentage recovery ranges from 99.40-100.40% and the mean recovery of Rosiglitazone maleate was 99.73% that shows there is no interference

from excipients and the lower values of %RSD of assay indicates the method is more accurate.

Precision: The precision was determined for Rosiglitazone in terms of system precision, method precision and intermediate precision. For system precision evaluation, a standard solution of fixed concentration was injected six times at different time intervals. Method precision was studied on six test solutions of single batch were analysed, the inter-day precision was studied by injecting the same concentration of standard solution on consecutive days and the %RSD for Rosiglitazone maleate was 0.0%, 0.3 and 1.1% (limit %RSD < 2.0%).

System suitability
System suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

Robustness
Robustness of the method was determined by making slight changes in the chromatographic conditions like flow rate, detection wavelength and organic phase in the mobile phase composition. It was observed that there were no marked changes in the chromatograms, which demonstrated that the developed HPLC method is more robust.

Solution stability: The stability of solution under study was established by keeping the solution at room temperature for 24 hrs. The result showed no significant change in concentration and thus confirms the stability of the drug in the solvent used for the analysis.

Forced degradation studies
Forced degradation study was carried out on Rosiglitazone. Conditions employed, and the results obtained from forced degradation studies are summarized below.

Acid Degradation: Sample was separately treated with 10mL of 4 N Hydrochloric acid at room temperature for 30 minutes. Cooled and neutralised with 10mL of 4 N sodium hydroxide solution. Further analysed by the proposed method.

Base Degradation: Sample was separately treated with 10mL of 4 N sodium hydroxide solution at room temperature for 30 minutes. Cooled and neutralised with 10mL of 4 N Hydrochloric acid. Further analysed by the proposed method.

Peroxide Degradation: Sample was separately treated with 5mL of 30 % v/v H_2O_2 at Room temperature for 30 minutes. Further analysed by the proposed method.

Thermal Degradation: Thermal degradation study was carried out by exposing the sample was subjected to thermal degradation by keeping at 105°C for 48 hours followed by analysis by the proposed method.

Humidity Degradation: Humidity degradation study was carried out by exposing the sample at 25°C/90% RH for 7 days.

Photolytic Degradation: Photolytic degradation study was carried out by exposing the sampleto light providing an overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours/square meter.

The results obtained are given in table 3. The chromatograms obtained are presented in list of Annexures (D to I).

RESULTS AND DISCUSSIONS

In the present work a simple reverse phase high performance liquid chromatographic method has been developed, optimized and validated for the estimation of Rosiglitazone in pharmaceutical formulations. Chromatographic separation was achieved on Hypersil BDS, C_8 250 x 4.6 mm, 5 column using 0.01M Potassium dihydrogen phosphate buffer and acetonitrile (40:60) as mobile phase, methanol as diluent in isocratic mode. Flow rate of 1.0ml/min was optimized with detection wavelength at 225 nm. The retention time (Rt) was around 4.72±0.2 min. The method was validated with respect to specificity, selectivity, linearity, accuracy, precision, and robustness as per ICH guidelines. The assay method was observed linear in the concentration range of 0.079-0.318 mg/ml with a Correlation coefficient (r^2) 0.9999. The linearity and Chromatogram of Standard Chromatogram were shown in Figure 2 and 3. The percentage recovery of active pharmaceutical ingredient from tablet dosage form ranged from 99.40-100.40%. The results were shown in Table 1 and 2.The %RSD for method precision and inter-day precision for Rosiglitazone maleate were found to be NMT 2 which indicate the method is precise. The results of precision studies were shown in Table 3 and 4. A system suitability test was performed to evaluate the chromatographic parameters. The typical chromatograms of degradation behaviour of Rosiglitazone in different stress conditions were shown from Figure 4 to 8. During the acidic and alkaline degradation, 24.5% and 18.1% was decomposed respectively. Rosiglitazone maleate has undergone oxidative 2%, thermal 1.9% and photo stability was 4.5%. The results of degradation studies were shown in Table 5. The summary of Validation parameters were shown in Table 6.

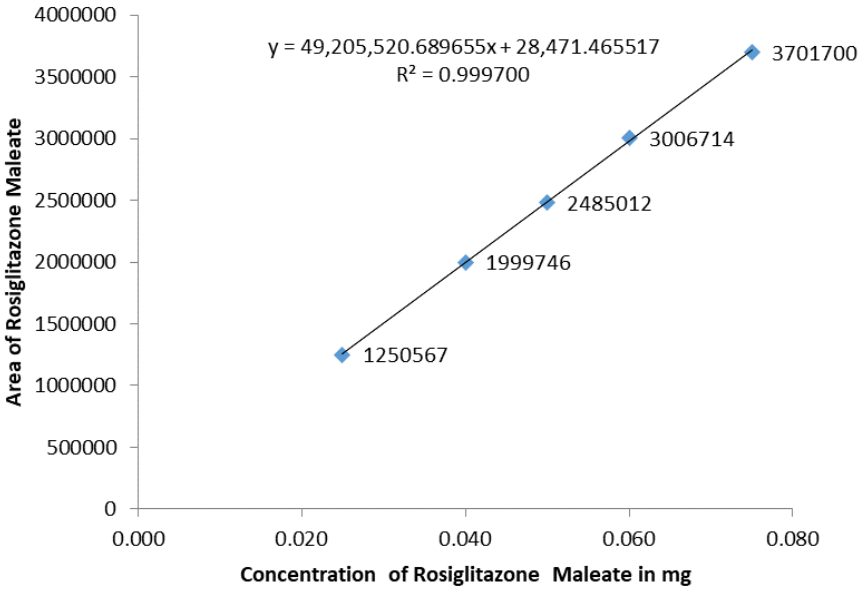


Figure 1: Linearity plot of Rosiglitazone Maleate.

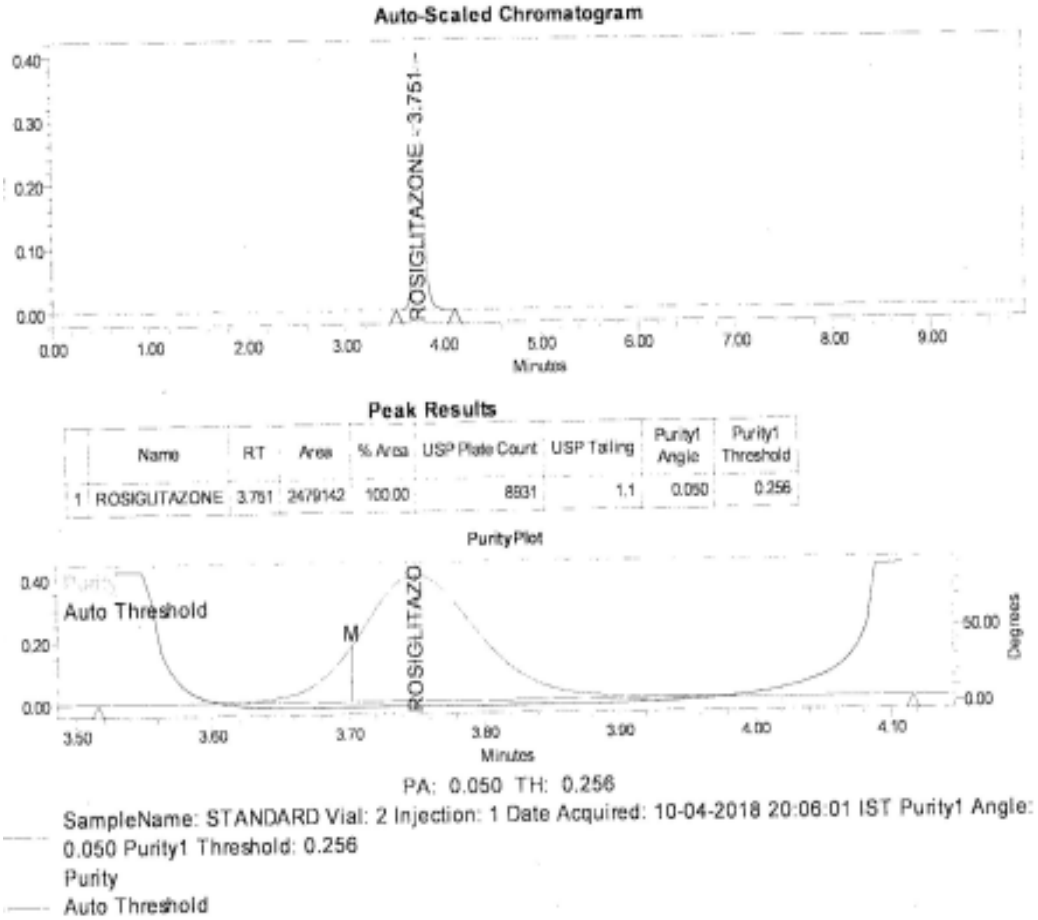
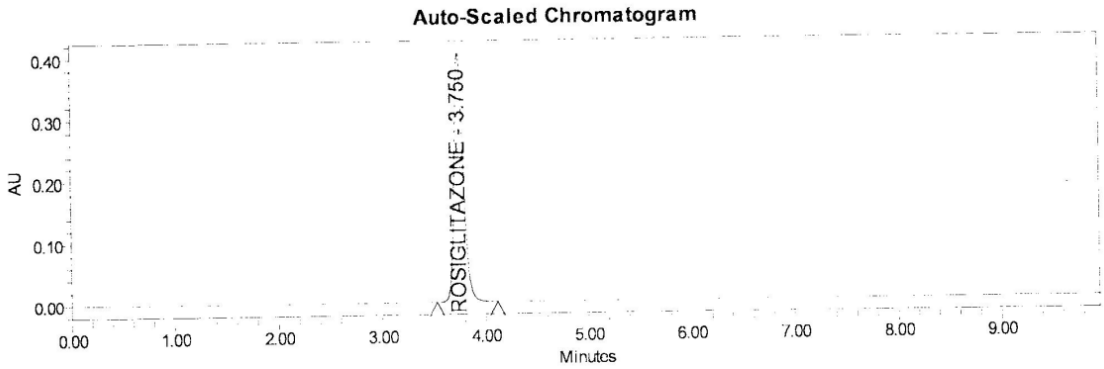
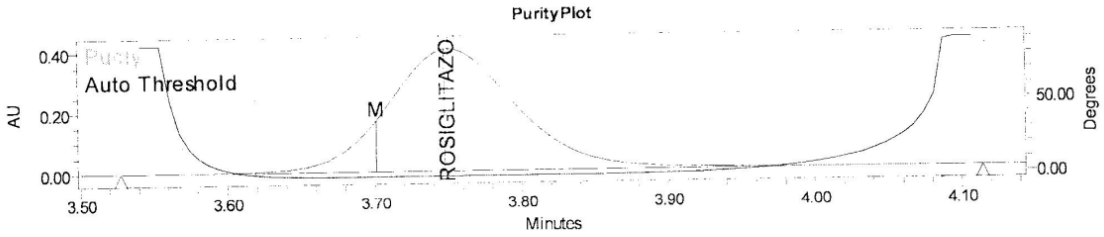


Figure 2 Linearity graph of Rosiglitazone.



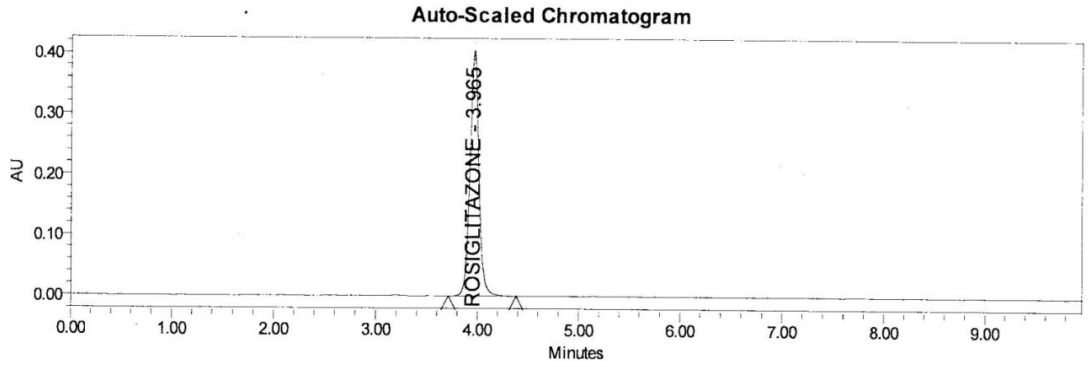
Peak Results

	Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	ROSIGLITAZONE	3.750	2485376	100.00	8952	1.1	0.048	0.256



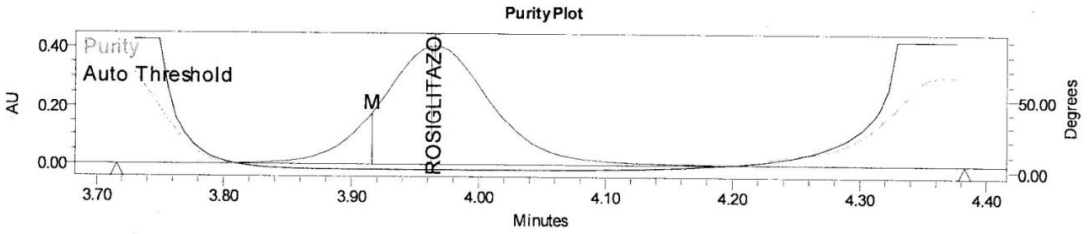
PA: 0.048 TH: 0.256

SampleName: SAMPLE Vial: 3 Injection: 1 Date Acquired: 10-04-2018 21:14:06 IST Purity1 Angle: 0.048 Purity1 Threshold: 0.256
Purity
Auto Threshold



Peak Results

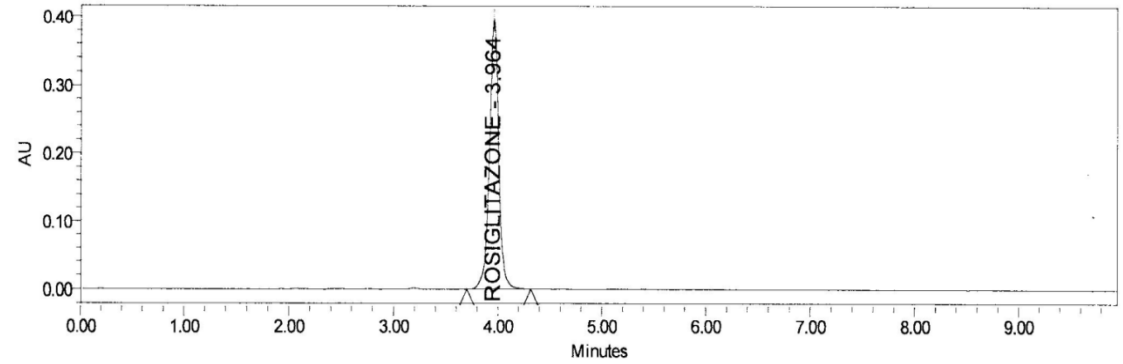
	Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	ROSIGLITAZONE	3.965	2486723	100.00	10333	1.1	0.059	0.265



PA: 0.059 TH: 0.265

SampleName: ACID STRESS_SAMPLE Vial: 5 Injection: 1 Date Acquired: 19-09-2018 17:32:05 IST Purity1 Angle: 0.059 Purity1 Threshold: 0.265
Purity
Auto Threshold

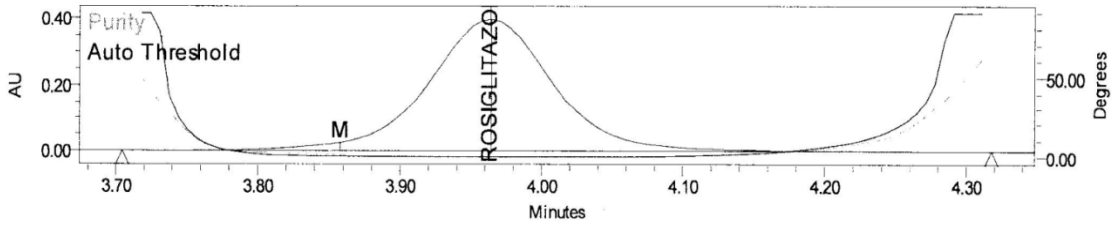
Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	ROSIGLITAZONE	3.964	2451214	100.00	10352	1.0	0.096	0.261

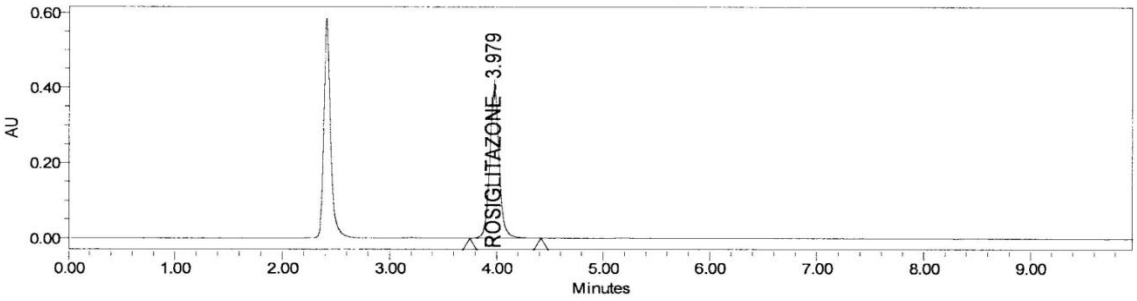
Purity Plot



PA: 0.096 TH: 0.261

SampleName: BASE STRESS_SAMPLE Vial: 6 Injection: 1 Date Acquired: 19-09-2018 17:55:16 IST
Purity1 Angle: 0.096 Purity1 Threshold: 0.261
Purity
Auto Threshold

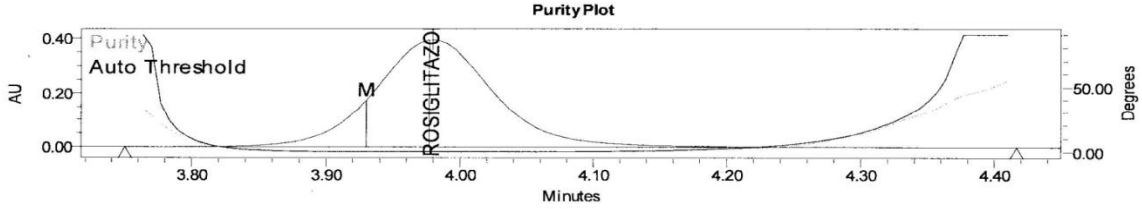
Auto-Scaled Chromatogram



Peak Results

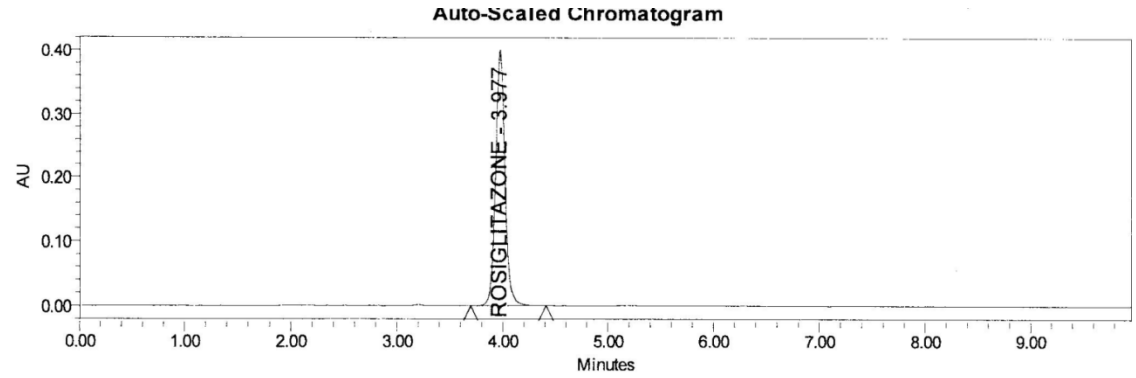
	Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	ROSIGLITAZONE	3.979	2452627	100.00	10213	1.1	0.057	0.264

Purity Plot



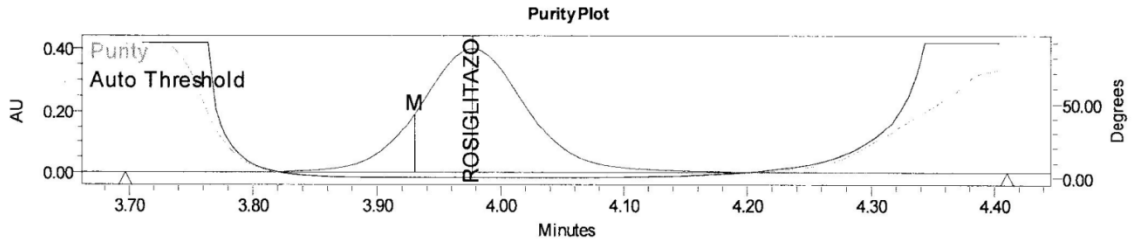
PA: 0.057 TH: 0.264

SampleName: OXIDATION STRESS_SAMPLE Vial: 11 Injection: 1 Date Acquired: 19-09-2018 19:28:48 IST Purity1 Angle: 0.057 Purity1 Threshold: 0.264
Purity
Auto Threshold



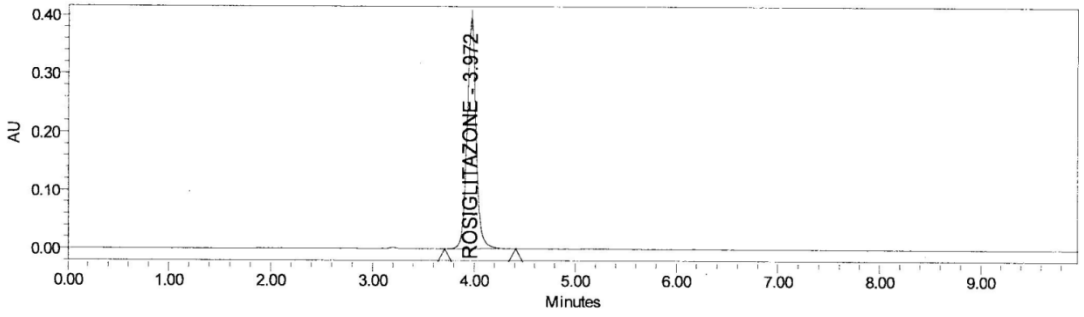
Peak Results

	Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	ROSIGLITAZONE	3.977	2450635	100.00	10368	1.1	0.062	0.271



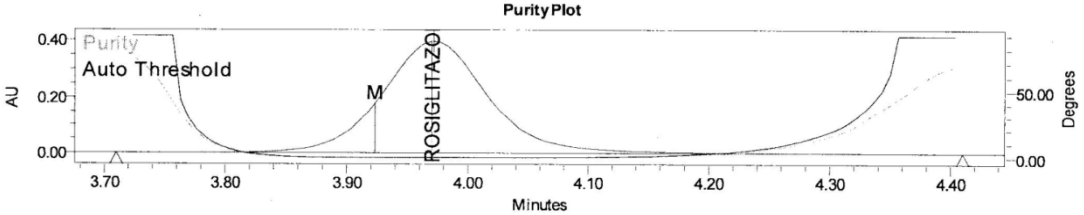
PA: 0.062 TH: 0.271

SampleName: HUMIDITY STRESS_SAMPLE Vial: 9 Injection: 1 Date Acquired: 19-09-2018 19:05:20 IST Purity1 Angle: 0.062 Purity1 Threshold: 0.271
Purity
Auto Threshold



Peak Results

	Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	ROSIGLITAZONE	3.972	2444294	100.00	10280	1.1	0.060	0.268



PA: 0.060 TH: 0.268

SampleName: PHOTOLYTIC STRESS_SAMPLE Vial: 8 Injection: 1 Date Acquired: 19-09-2018 18:41:55 IST Purity1 Angle: 0.060 Purity1 Threshold: 0.268
Purity
Auto Threshold

Table.1. Linearity of standard Rosiglitazone.

S. No.	Level	Rosiglitazone maleate Concentration(in mg)	Area	Mean Area
1.	40%	0.079	1832362	1833156
			1833949	
2.	80%	0.159	3639929	3637948
			3635967	
3.	100%	0.198	4572379	4577566
			4582753	
4.	120%	0.238	5452922	5450697
			5448472	
5.	160%	0.318	7267528	7265567
			7263606	
Correlation Coefficient (r)			**0.999951977**	
Slope			**22750541.96495**	
Intercept			**39279.17415**	
% deviation of y-intercept			**0.7**	

Table 2: Results obtained from Accuracy for Rosiglitazone (% Recovery).

Accuracy Level	Amount added (mg)	Amount found (mg)	% Recovery		%RSD
			Individual	Mean	
Accuracy solution (50%)-1	49.940	50.085	100.3	**100.5**	**0.2**
Accuracy solution (50%)-2	49.969	50.254	100.6		
Accuracy solution (50%)-3	50.049	50.288	100.5		
Accuracy solution (100%)-1	99.779	99.831	100.1	**100.2**	**0.2**
Accuracy solution (100%)-2	99.859	100.033	100.2		
Accuracy solution (100%)-3	99.690	100.114	100.4		
Accuracy solution (150%)-1	149.689	150.071	100.3	**100.1**	**0.2**
Accuracy solution (150%)-2	149.570	149.370	99.9		
Accuracy solution (150%)-3	149.659	149.959	100.2		

Table 3: Results of System Precision.

Injection	Area of Rosiglitazone Maleate
1	2478986
2	2478659
3	2478961
4	2481028
5	2478089
6	2478769
Mean	**2479082**
SD	**1007.345**
% RSD	**0.04**

Table 4: Results obtained from six sample preparations from Method Precision.

Sample Number	% Assay (On anhydrous basis)
1	100.8
2	100.9
3	101.0
4	100.9
5	101.1
6	100.8
Mean	**100.9**
SD	**0.117**
%RSD	**0.1**

Table.5. Results obtained from forced degradation study.

Mode of degradation	Condition	Purity Angle	Purity Threshold	Rosiglitazone Maleate	
				% Assay	% Degradation
Control	NA	0.149	0.275	98.1	NA
Acid stress	10 mL, 4 N HCl for 30 min. at RT	0.392	1.00	74.1	24.5
Humidity stress	25°C/90% RH for NLT 7 days	0.165	0.281	97.6	0.5
Photolytic stress	1.2 million lux hrs / 200-watt hrs / square meter	0.137	0.273	93.7	4.5
Base stress test	10 mL, 4N NaOH for 30 mins. at RT	0.335	1.005	80.3	18.1
Oxidation stress	5mL, 30% v/v H_2O_2 for 30 min at RT	0.155	0.278	96.1	2.0
Thermal stress	105°C for 48 hours	0.151	0.276	96.2	1.9

Table No.6: Summary of Validation Parameters.

S. No.	Parameter	Experiment	Acceptance criteria	Results			
1	Specificity & Forced degradation	Blank, standard solution and test solution	There should not be any interfering peaks due to diluent at the retention time of Rosiglitazone maleate peak.	There is no interference due to diluent at the retention time of **Rosiglitazone Maleate** peak in standard and Sample Solution.			
				Name	**Retention time (min)**		
					Rosiglitazone Maleate		
				Standard solution			
				Control sample			
			Peak purity angle should be less than Peak purity threshold of Rosiglitazone maleate peak in standard, and test solution. There should be no tick mark in the purity flag column.	**Name**	**Rosiglitazone Maleate**		
				Sample	**Purity Angle**	**Purity Threshold**	**Purity flag**
				Standard solution	0.05	0.256	No
				Test solution	0.048	0.256	No
		Blank, standard solution, test solution and Stressed solutions	There should not be any interfering peaks due to diluent at the retention time of Rosiglitazone Maleate peak. Peak purity angle should be less than Peak purity threshold for Rosiglitazone Maleate peak in standard, test solution and stressed solution. There should be no tick mark in the purity flag column.	There is no interference due to diluent at the retention time of Rosiglitazone maleate peak in standard and stressed solution.			
				Name	**Rosiglitazone**		
				Sample	**Purity Angle**	**Purity Threshold**	**Purity Flag**
				Standard	0.065	0.273	No
				Control Sample	0.059	0.266	No
				Acid Stress	0.059	0.256	No
				Base Stress	0.096	0.261	No
				Oxidation stress	0.057	0.264	No
				Thermal stress	0.060	0.267	No
				Photolytic stress	0.060	0.268	No
				Humidity stress	0.062	0.271	No

CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guidelines. The method was found to meet all the predetermined acceptance criteria. The validated HPLC method for Rosiglitazone is specific, stable, linear, accurate, precise, rugged and robust. Based on the validation study results, it has been concluded that the HPLC method for Rosiglitazone is suitable for intended purpose. At the same time the chromatographic elution step is undertaken in a short time (< 5 min). There is no interference from any components of pharmaceutical dosage form. The chromatograms of diluent (blank) indicate that there is no interference at the retention time Rosiglitazone peak. The peak purity angle was less than the peak purity threshold and there was no tick mark in the purity flag column for Rosiglitazone Maleate peak in all final stressed test solutions. Peak purity plot of stressed sample solutions indicates that Rosiglitazone Maleate peaks is homogeneous and has no co-eluting peaks ensuring specificity of the method. Hence it can be successfully applied to perform the routine analysis of the drug in pharmaceutical formulations.

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