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LC determination of rosiglitazone in bulk and pharmaceutical formulation

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Abstract

An isocratic reversed phase liquid chromatographic (RP-LC) method has been developed and subsequently validated for the determination of rosiglitazone and its related impurities. Separation was achieved with a Symmetry C18 column and sodium phosphate buffer (pH adjusted to 6.2):acetonitrile (50:50, v/v) as eluent, at a flow rate of 1.0 ml/min. UV detection was performed at 245 nm. The method is simple, rapid, selective and stability indicating. Indole was used as internal standard for the purpose of quantification of rosiglitazone. The described method is linear over a range of 0.45–10 μg/ml for related impurities and 180–910 μg/ml for assay of rosiglitazone. The method precision for the determination of assay and related compounds was below 1.0 and 3.6% RSD, respectively. The mean recoveries of impurities were found to be in the range of 95–102%. The percentage recoveries of Active Pharmaceutical Ingredient (API) from dosage forms ranged from 99.02 to 101.30. The method is useful in the quality control of bulk manufacturing and also in pharmaceutical formulations.

Keywords: Rosiglitazone; Related impurities; Assay; Formulation; RP-LC; Validation

1. Introduction

Human diabetes is currently classified into two general categories: Type I, or insulin-dependent diabetes mellitus, and Type II, or non-insulin-dependent diabetes mellitus (NIDDM). Peripheral insulin resistance is one of the pathogenic factors that contribute to the hyperglycemic state in NIDDM [1,2].

Rosiglitazone maleate [(±)-5-[4-[2-[*N*-methyl-*N*(2-pyridyl)amino] ethoxy]benzyl]-2,4-dione thiozolidine maleate] is a potent and orally active insulin sensitizing agent that was shown to improve glycemic control in animal models of NIDDM. It was derived from a metabolite of ciglitazone and was found to be much more potent than other classes of thiozolidinediones such as pioglitazone, ciglitazone and englitazone [3].

Till now, few liquid chromatography procedures have been reported for the determination of rosiglitazone and their metabolites in biological fluids. Furthermore, Ann-Marie Muxlow reported

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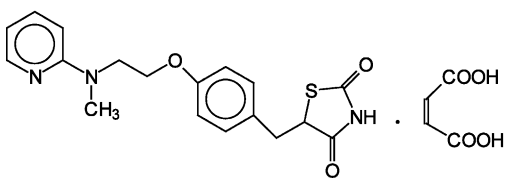
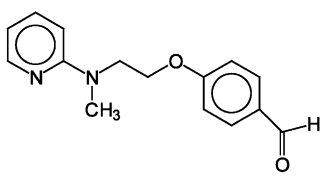
an automated HPLC method for the determination of rosiglitazone in human plasma [4]. Quantification of rosiglitazone in rat and dog plasma using fluorescence detection has been described by Gaffney et al. [5]. Abbott et al. published chiral HPLC methods for the analysis of rosiglitazone and its enantiomers in mouse, rat, dog and human plasma with fluorescence detection using α-AGP, Chiralcel OD-R and Cyclobond I SP columns [6]. However, there are no other publications concerning the analysis of rosiglitazone in bulk drug in presence of its related impurities and in pharmaceutical formulations.

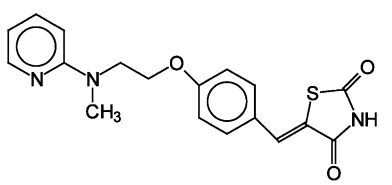
So it is felt necessary to develop a liquid chromatographic (LC) procedure which would serve as a rapid and reliable method for the determination of rosiglitazone in bulk drug with respective related impurities as well as in formulations. In the proposed method, related impurities were well separated and eluted before 15 min. Indole was used as internal standard for the purpose of quantification of rosiglitazone. Finally, the method was thoroughly validated for the assay and related compounds determination of rosiglitazone.



rosiglitazone maleate



(II)



(III)

Fig. 1. Structures of rosiglitazone maleate and its related compounds.

2. Experimental

2.1. Materials

Samples of rosiglitazone and their related substances were received from Dr. Reddy's Laboratories, Hyderabad, India. HPLC grade acetonitrile was obtained from Merck, USA. Analytical grade sodium dihydrogen phosphate, *ortho*-phosphoric acid and sodium hydroxide were purchased from Qualigens. Tablets of Avandia were purchased from the market. The average weight of Avandia tablet is about 310 mg, which contains 8 mg of rosiglitazone. High purity water was prepared by using Waters Milli-Q plus purification system.

2.2. Instrumentation

The LC system consisted of a Waters 510 pump, a Rheodyne injector equipped with a 10 μl sample loop, and a Photodiode array detector (Waters 996). The output signal was monitored and integrated using Millenium 2010 Chromatography Manager software (Waters).

2.3. Solutions

2.3.1. Mobile phase

A mixture of aqueous 0.025 M sodium di hydrogen phosphate (pH of the buffer adjusted to 6.2 with dilute NaOH) and acetonitrile in the ratio (50:50, v/v) was the mobile phase of the separation. It was filtered through a 0.45-μm nylon membrane filter prior to use.

2.3.2. Standard solutions

Solutions of rosiglitazone and its related impurities were prepared using mobile phase. The working concentration for the determination of assay and related impurities of rosiglitazone was 750 μg/ml. In each solution of rosiglitazone, the concentration of internal standard was maintained at 1.2 mg/ml.

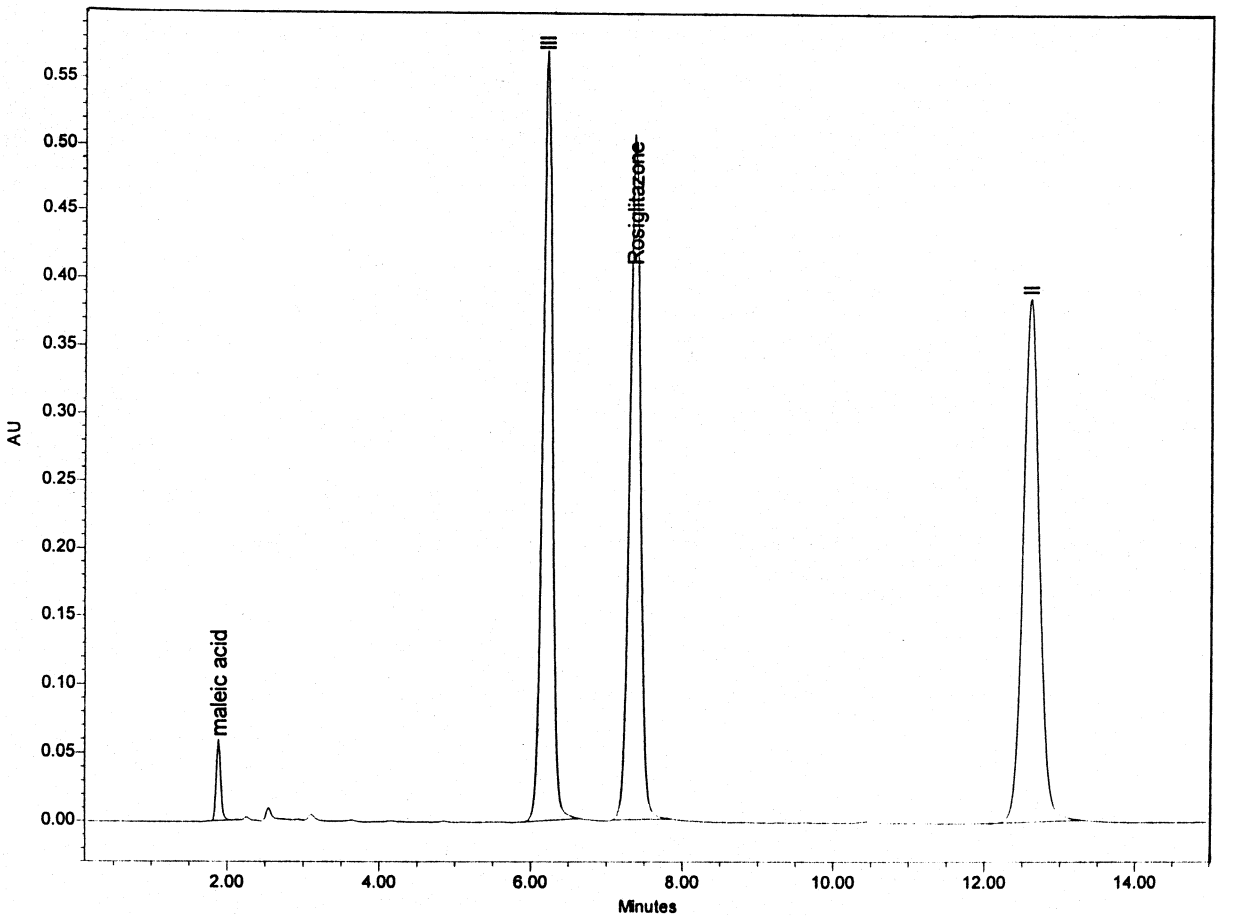


Fig. 2. HPLC chromatogram of rosiglitazone and its related compounds.

Table 1
System-suitability report

Compound	Capacity factor[a]	Selectivity[a]	Tailing factor[a]	Resolution[a]	Theoretical plates[a]
Rosiglitazone	2.9	1.26	1.004	3.1	12 951
II	5.68	1.95	1.065	15.9	16 902
III	2.29	–	1.021	24.6	11 123

[a] Number of samples analyzed are three.

2.3.3. Sample solutions

20 weighed tablets of Avandia (8 mg of rosiglitazone) were ground to a fine powder in a mortar. The amount of powder equivalent to 40 mg of the active compound was extracted with mobile phase and centrifuged. The supernatant was diluted with same solvent to required concentrations and injected into the system.

2.3.4. Degraded samples for specificity study

For acid degradation, rosiglitazone sample was refluxed with 0.1 N HCl at 60 ± 1 °C for 12 h and

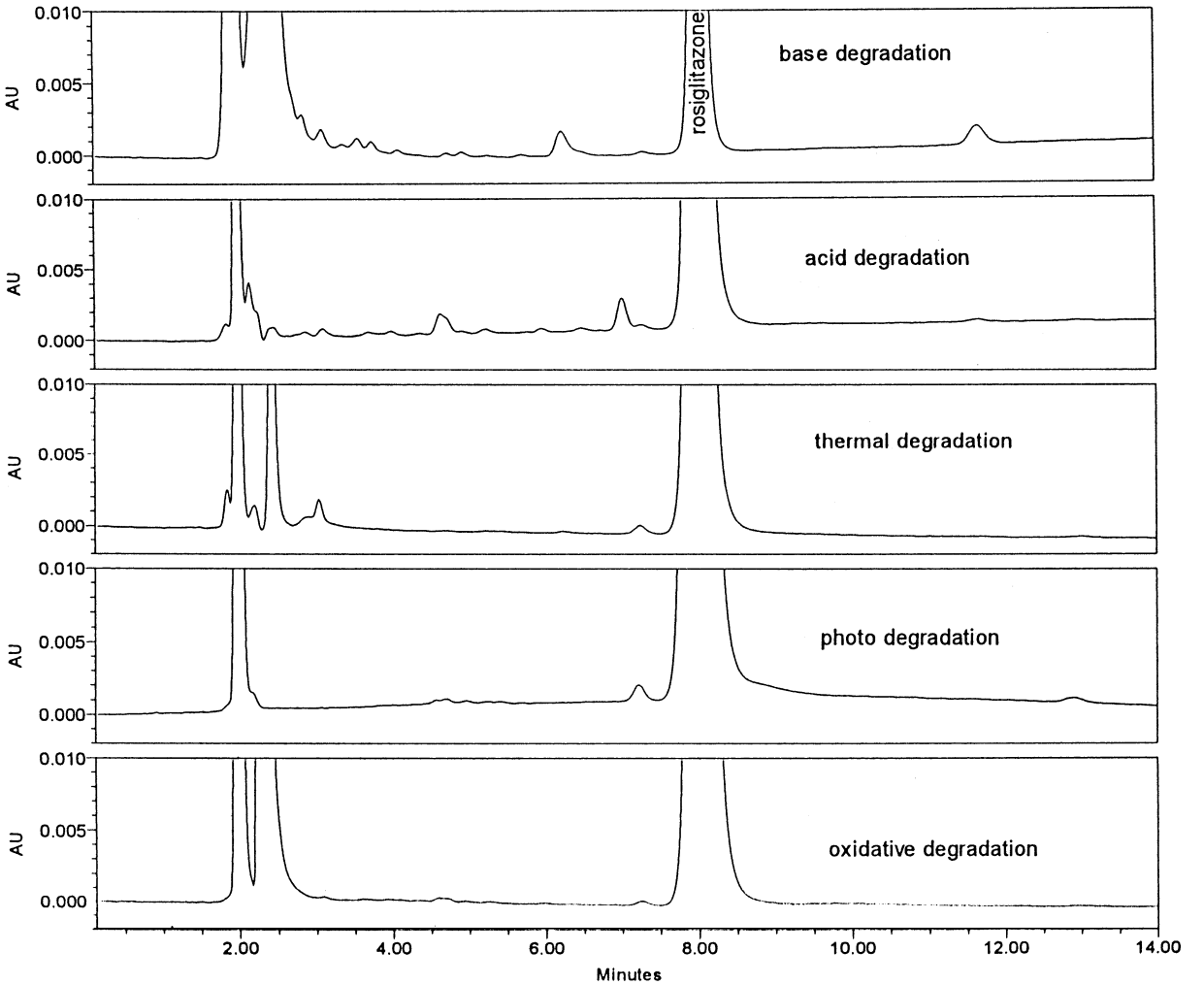


Fig. 3. Separation of the rosiglitazone from its degraded products.

Table 2
Accuracy in the assay determination of rosiglitazone

Day of analysis	Taken (mg)	Recovery (mg) ($n = 3$)	% Recovery	RME (%)	TE (%)
0 day	0.156	0.155	99.36	−0.427	−0.13
	0.282	0.282	100.12	0.118	0.538
	0.442	0.443	100.23	0.226	0.746
1 day	0.156	0.157	100.64	0.64	0.84
	0.282	0.284	100.59	0.59	0.89
	0.442	0.444	100.52	0.53	0.948
2 day	0.156	0.158	101.28	1.28	1.48
	0.282	0.285	101.06	1.06	1.26
	0.442	0.445	100.75	0.754	0.994

Table 3
Parameters of calibration plot of impurities and its recovery

Impurity	Slope	Intercept	Amount added[a] (μg)	Recovered (μg)	% Recovery
II	24 587 ± 1145	1799 ± 243	2.08	2.00	96.1
			4.16	4.03	96.8
			8.24	8.36	101.5
III	28 501 ± 1267	1856 ± 335	1.64	1.56	95.1
			3.28	3.33	101.5
			6.56	6.68	101.8

[a] $n = 3$.

then neutralized by adjusting pH to 7.0 with 0.1 N NaOH. The solution was further diluted to required concentration with the mobile phase.

For basic degradation, rosiglitazone sample was refluxed with 0.1 N NaOH at 60 ± 1 °C for 12 h and then neutralized by adjusting pH to 7.0 with 0.1 N HCl. The solution was further diluted to required concentration with mobile phase.

For oxidative degradation, rosiglitazone sample was refluxed with 3% H_2O_2 for 30 min and then diluted to required concentration with mobile phase.

For photo and thermal degradations, two separate solutions of rosiglitazone (0.5 mg/ml in diluting solution) were prepared. One solution was exposed to ultraviolet light (254 nm) and the other was kept at 70 °C temperature for 12 h.

2.4. Conditions

A Symmetry C_{18} analytical column (250 × 4.6 mm, 5 μm packing) (Waters) was used for analysis at ambient temperature. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min. The sample injection volume was 10 μl. The photodiode array detector was set to a wavelength of 245 nm for the detection.

3. Results and discussion

3.1. Method development

3.1.1. Separation of process impurities

The related compounds of rosiglitazone are shown in Fig. 1. To develop a suitable and robust LC method for the determination of rosiglitazone and its impurities, different mobile phases and columns were employed to achieve the best separa-

Table 4
Inter and intra-day assay variation of rosiglitazone

	Intra-day		
	0 day		
Mean of conc. (mg/ml) $n = 3$	0.155	0.282	0.443
SD	0.0015	0.0021	0.0026
RSD(%)	0.983	0.737	0.597
	1 day		
Mean of conc. ($n = 3$)	0.157	0.284	0.444
SD	0.001	0.0015	0.0021
RSD(%)	0.637	0.538	0.468
	2 day		
Mean of conc. ($n = 3$)	0.158	0.285	0.445
SD	0.001	0.001	0.0012
RSD(%)	0.633	0.351	0.259
	Inter-day		
Mean(of mean conc. of 3 days)	0.157	0.284	0.444
SD	0.0015	0.0015	0.001
RSD(%)	0.975	0.538	0.225

Table 5
Assay values of rosiglitazone from Avandia tablets

Amount taken (mg) ($n = 3$)	Amount recovered (mg)	% Recovery
0.154	0.156	101.29
0.231	0.234	101.30
0.308	0.305	99.02
0.385	0.388	100.78
0.462	0.465	100.65

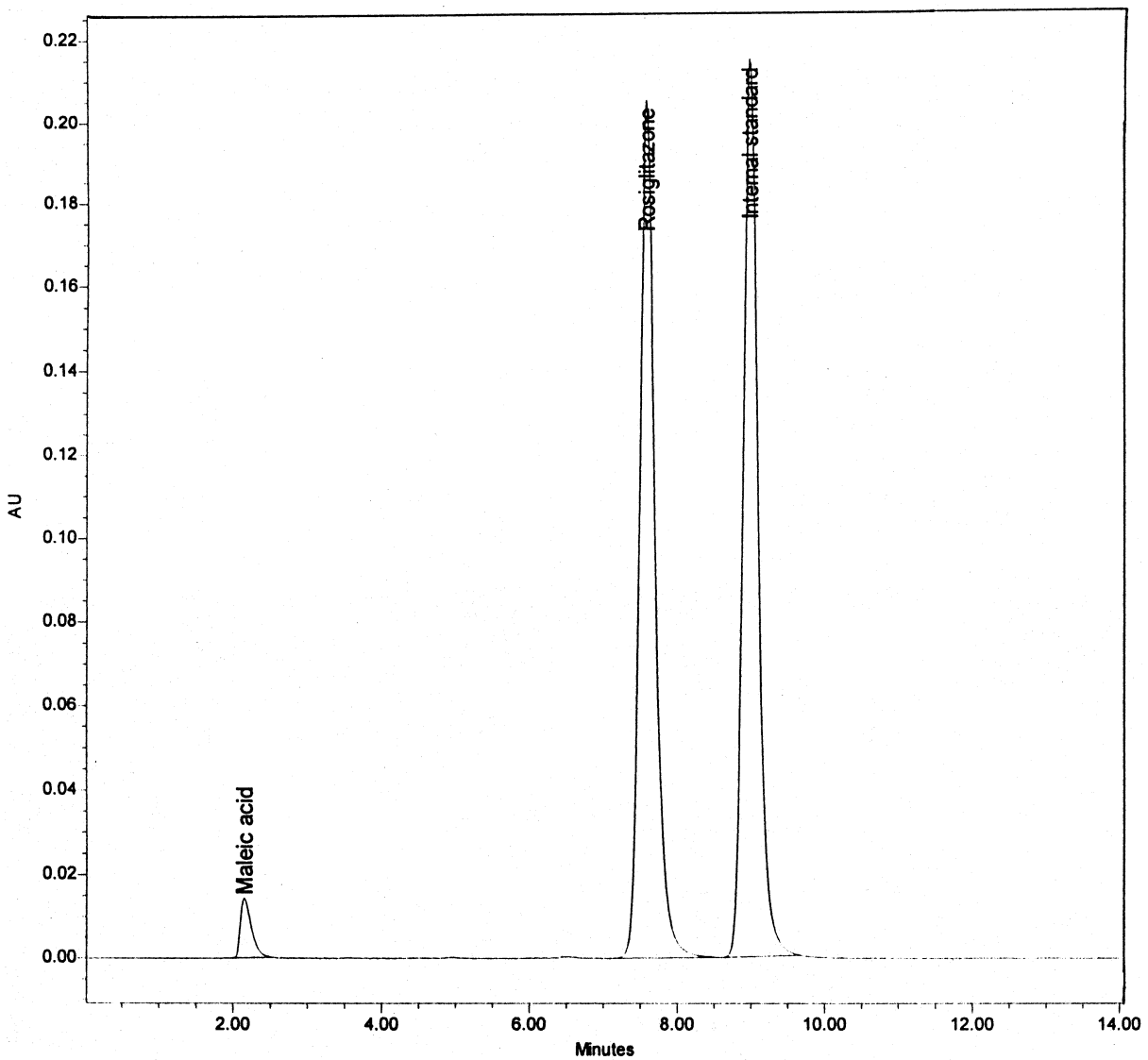


Fig. 4. Chromatogram of formulated rosiglitazone (Avandia 8 mg) with internal standard.

tion and resolution. The method development was started with a C18 column using a mobilephase of water–acetonitrile in the ratio of 1:1. In the above conditions elution was very broad for rosiglitazone and compound III. Early elution with a little separation was observed for all compounds with the mobile phase consisting of aqueous 10 mM potassium dihydrogen phosphate–acetonitrile (50:50, v/v), pH adjusted to 3.5. Interestingly, the same mobile phase with pH 6.0, gave reasonable separation between all compounds but rosiglitazone resulted in a broad peak shape with small plate count and therefore needs further optimization. Finally, the mobile phase consisting of aqueous 0.025 M sodium di hydrogen with a pH 6.2 and acetonitrile in the ratio of 50:50 (v/v) was found to be appropriate, allowing good separation and symmetrical peaks at a flow rate of 1.0 ml/min using Symmetry C18, 250 mm column. In the above conditions compound III eluted prior

to rosiglitazone. The chromatogram of crude rosiglitazone sample spiked with the related substances recorded using the proposed method is shown in Fig. 2. In the presented method the selectivity was found to be more than 1.2 with a resolution more than 3.1 for all the compounds. System suitability results of the method are presented in Table 1. Rosiglitazone and its related compounds show significant UV absorbance at wavelength 245 nm. Hence this wavelength has been chosen for detection in the analysis of rosiglitazone.

3.1.2. Quantification of impurities

The relative response factors (RRF) of impurities **II** and **III** with respect to rosiglitazone were found to be 1.38 and 1.61, respectively at the detection wavelength of 245 nm. The weight percentage of the impurity present in rosiglitazone sample was calculated using its RRF value and peak response [7].

3.1.3. Column selection

Based on the retention and separation of the compounds, Hichrom RPB, 5μ, 4.6×250 mm column was selected as an alternative for Symmetry C18 column.

3.2. Method validation

The developed LC method has been extensively validated for assay and related impurities of rosiglitazone using the following parameters. Indole was used as internal standard for the purpose of quantification of rosiglitazone.

3.2.1. Specificity

The specificity of the method was demonstrated by adding all the possible known impurities discussed above to pure rosiglitazone sample and analyzing the mixture for assay and the results were compared with pure sample results. Reproducibility was observed in both the cases (RSD < 2.0).

Accelerated degradation studies were performed to demonstrate the validity of the method.

The sample exposed to UV light did not give any degradation products. The samples, which are refluxed with 0.1 N HCl or subjected to high temperature, gave several small degradation products. But the samples refluxed with 0.1 NaOH or 3% H2O2 were mostly converted to degraded products, eluted at around 2.2 and 2.4 min. Fig. 3 shows the separation of the rosiglitazone from its degraded products.

Photodiode array detection was also used as evidence of the specificity of the method, and to evaluate the homogeneity of the peak. Chromatographic peak purity was determined using wavelength comparison (230 nm versus 245 nm) [8]. The samples exposed to acidic, basic, oxidative, thermal and UV stress conditions were subjected to photodiode array analysis for peak purity of rosiglitazone. The plots with flat tops in all instances showed that rosiglitazone peak had no detectable impurity peaks embedded in and are free of co-eluting degradation compounds. From above results, it is clear that the method can be used for determining the stability of rosiglitazone as bulk and pharmaceutical formulations.

3.2.2. Linearity

Standard solutions at seven different concentration levels ranging from 180 to 910 μg/ml were prepared to demonstrate the linearity. The equation for calibration curve is $y = 2.66x + 0.004$ with a correlation coefficient more than 0.999.

3.2.3. Accuracy

Accuracy of the method for assay determination of rosiglitazone was demonstrated at three concentration levels i.e. at 150, 300, and 450 μg, each in triplicate for 3 days. The calculated RME and TE [9] values presented in Table 2 are below ± 2.0 for the intra-day assay experiments.

Standard addition and recovery experiments were also conducted to determine the accuracy of the present method for the quantification of impurities. As per ICH guidelines, the range of addition levels of impurities used in this study is 0.35–1.0% of the concentration (0.75 mg/ml) of the rosiglitazone and the recovery of impurities added to the rosiglitazone sample was calculated from the slope and intercept of the calibration graph drawn in the concentration range 0.45–10 μg (0.05–2%) of each impurity. The values of slope, intercept and % recovery of each impurity are shown in Table 3. The correlation coefficient

was found to be more than 0.992 for the two impurities. The mean recoveries of all the impurities were found to be in the range of 95–102%.

3.2.4. Precision

The precision of the method for the determination of assay and impurities of rosiglitazone was studied using the parameters, repeatability and intermediate precision.

Repeatability is the intra-day variations in assay or in recovery of impurities of rosiglitazone obtained at different concentration levels are expressed in terms of RSD values calculated from the data of each day for 3 days. RSD values of assay and recovery of impurities were found to be below 1.0% (Table 4) and 3.6%, respectively.

The intermediate precision, which is the inter-day variation at the same concentration level, was determined on successive days. The intermediate precision for assay and recovery of impurities II and III of rosiglitazone were found to be 1.0% RSD (Table 4) and 3.9% RSD, respectively.

3.2.5. LOD and LOQ of impurities

The limit of detection and limit of quantification for impurities **II** and **III** were found to be 0.16, 0.42 and 0.13, 0.35 μg, respectively.

3.3. Assay determination of rosiglitazone from Avandia tablets

The assay results of the extracted samples from tablets at five different concentrations were comparable with claimed values (Table 5). The excipient peaks did not interfere with rosiglitazone and internal standard in the formulation sample. A typical LC chromatogram is shown in Fig. 4.

4. Conclusion

Analytical HPLC method was developed and validated for the determination of insulin-sensitizing agent, rosiglitazone in bulk in presence of related impurities and in pharmaceutical dosage form has been described. The developed method was found to be selective, sensitive and precise for the determination of selected drug and their process related impurities. This method can be used for the routine determinations in pharmaceutical quality control.

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