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A validated, stability-indicating, LC method for rosiglitazone maleate

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ABSTRACT

A simple, economic, and time-efficient stability-indicating, reversed phase liquid chromatographic (RP-LC) method has been developed for the analysis of rosiglitazone maleate in the presence both of related impurities and of degradation products generated by decomposition. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur in alkali and oxidative stress conditions. The drug was found to be stable to other stress conditions attempted. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.5%. The method was validated with respect to specificity, linearity, accuracy, precision, robustness, limit of detection and quantitation. The method is simple, rapid, selective, accurate and stability indicating, useful in the quality control of bulk manufacturing.

KEYWORDS

Column liquid chromatography;
Rosiglitazone;
Stability indicating;
Impurities;
Validation.

INTRODUCTION

The chemical name of rosiglitazone maleate is 5-[[4-[2-(methyl-2-pyridinylamino)ethoxy] phenyl] methyl]-2,4- thiazolidinedione 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[1]. Rosiglitazone is an anti-diabetic drug in the thiazolidinedione class of drugs. It is marketed by the pharmaceutical company GlaxoSmithKline as a stand-alone drug (AVANDIA) and in combination with metformin (AVANDAMET) or with glimepiride (AVANDARYL).

The different analytical techniques reported so far for the determination of this drug and its metabolites in biological samples as well as in pharmaceuticals formulation include simultaneous determination of rosiglitazone and a combination drugs like gemfibrozil, N-desmethyl rosiglitazone, metformin and glipizide in human plasma are previously published in[2-5]. The determination of rosiglitazone in coated tablets by MEKC and HPLC[6], in human plasma by LC and LC-MS-MS[7-13] and that in rat and dog plasma using fluorescence detection[14,15] were also reported. Most of the reported methods[2-15] are for the quantitation of rosiglitazone in plasma and urine using different techniques and no method is suitable for the separation of

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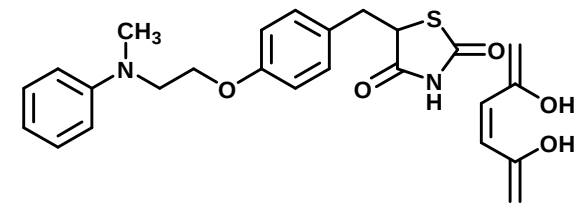
related impurities in bulk drug. However, T. Radhakrishna et al. described a chromatographic method[16] for quantitation of rosiglitazone in bulk and pharmaceutical formulation. The main focus of the study is quantitation of drug in a dosage form, using indole as an internal standard, where in the author considered only two impurities namely compound II and compound III. Moreover, compound III is a starting material and compound II is a precursor of compound III. In the present study, other related impurities namely Imp-A, Imp-B, Imp-C, Imp-D and ROS-III (Starting material) were considered for the validation. Although compound III, described in the publication[16], is a starting material, separation of this impurity with all the others has been achieved.

Organic impurities can arise during the manufacturing process and storage of the drug substance and the criteria for their acceptance up to certain limits are based on regulatory guidelines[17] or known safety data. It is, therefore, essential to isolate and characterize impurities related to the drug sample. During the development of an analytical procedure, the LC method was developed for the determination of in-house synthesized rosiglitazone maleate and the related impurities arising during its manufacturing. In the method, developed, herein, all the related impurities were well resolved and eluted before 20 min. Finally, the method was thoroughly validated as per the ICH guidelines[18].

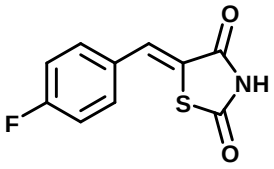
EXPERIMENTAL

Chemicals and reagents

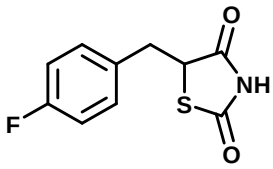
Reference standard of rosiglitazone maleate and five impurities namely Imp-A, Imp-B, Imp-C, Imp-D and ROS-III (Starting material) were synthesized and characterized in M/s SMS Pharma Research Centre, Hyderabad, India. The commercial samples of rosiglitazone maleate are also manufactured by M/s SMS Pharmaceuticals Limited. These related impurities are shown in Figure 1. HPLC grade acetonitrile was obtained from Merck, India. Analytical grade sodium dihydrogen phosphate dihydrate, triethylamine, orthophosphoric acid and maleic acid were purchased from SD Fine chem., India. High purity water was prepared by using Milli-Q Elix and then using Milli-Q Academic purification system (Millipore, USA).



Rosiglitazone maleate



Imp-A



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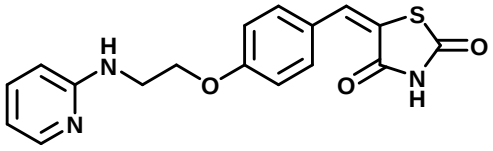
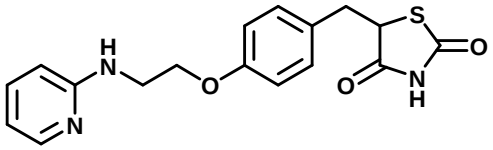
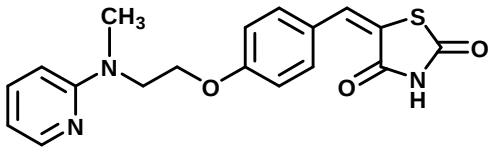


Imp-C



Imp-D



ROS-III (Starting material)

Figure 1 : The chemical structures of rosiglitazone maleate and impurities

Equipment

The LC system was equipped with quaternary gradient pump, auto sampler, and a column oven connected with a photodiode array detector (Prominence, Shimadzu, Japan). The output signal was monitored and integrated using LC-Solutions chromatography manager software.

Preparation of standard solutions

A stock solution of rosiglitazone maleate (5.0 mg/mL) was prepared by dissolving appropriate amount in the mobile phase. Working solutions of 100 and 1000 µg/mL were prepared from the above stock solution for assay and related substances determinations. A stock

solution of impurity mixture (Imp-A, B, C, D and ROS-III) at 1.0 mg/mL was also prepared in mobile phase. As per the approved in-house protocol, different dilutions were made to evaluate specificity, precision, LOD, LOQ, linearity, accuracy and robustness.

Forced degradation samples for specificity study

For acid degradation, rosiglitazone maleate sample was refluxed with 5N HCl at 80°C for 1 hour and the solution was further diluted to required concentration with the mobile phase. For basic degradation, rosiglitazone maleate sample was treated with 1N NaOH at RT for 1 hour and the solution was further diluted to required concentration with mobile phase. For oxidative degradation, rosiglitazone maleate sample was refluxed with 5% hydrogen peroxide solution at 80°C for 60min. and then diluted to required concentration with mobile phase. For photo and thermal degradations, rosiglitazone maleate sample was exposed to ultraviolet light (254 nm) for 24 hours and another sample was kept at 105°C temperature for 24 hours respectively. These samples solutions were prepared to required concentration with mobile phase.

Chromatographic conditions

A Waters XTerra RP-18 analytical column (150 mm × 4.6 mm, 3.5µm) was used. 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 248 nm for the detection. A mixture of aqueous 0.05 M sodium dihydrogen phosphate dihydrate (pH of the buffer adjusted to 6.8±0.1 with triethyl amine) and acetonitrile in the ratio of 70:30, (v/v) was used as mobile phase. It was filtered through a 0.45µm nylon membrane filter prior to use.

Validation of the method

Specificity

The specificity of the developed LC method for rosiglitazone maleate was carried out in the presence of its impurities namely Imp-A, Imp-B, Imp-C, Imp-D and ROS-III. Stress studies were performed for rosiglitazone maleate bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition of photolytic (UV light at 254 nm), heat (at 105°C), acid (5.0 N HCl at 80 °C), alkali (1.0 N NaOH at RT) and oxidation (5% H_2O_2 at 80°C) to evaluate the ability of the proposed method to separate rosiglitazone from its degradation products. For thermal and photolytic studies, the study period was 24 hours, for the acid, alkali and oxidation the study period was 1 hour. Peak purity test was carried out for the rosiglitazone peak by using a PDA detector in stress samples. Assay studies were carried out for stress samples against a qualified rosiglitazone maleate reference standard.

Precision

The precision of the assay method was evaluated by carrying out six independent assays of rosiglitazone maleate test samples against a qualified reference standard and calculated the %RSD of the assay.

The precision of the related substances method was checked by injecting six individual test preparations of rosiglitazone maleate (1.0 mg/ mL) spiked with 0.15% of each impurity with respect to rosiglitazone maleate analyte concentration. The %RSD of the area responses of each impurity was calculated.

Similarly, the intermediate precision of the method was demonstrated by different analyst on different day using different instrument in the same laboratory.

Limit of detection and limit of quantification

The LOD and LOQ concentrations were determined by measuring the magnitude of analytical background. The LOD and LOQ concentrations were calculated from the linearity data using residual standard deviation of the response and slope of the calibration curve for each impurity. The LOD and LOQ values for all five impurities were determined by injecting a series of dilute solutions with known concentrations.

Linearity

Linearity test solutions for the assay method were prepared from rosiglitazone maleate stock solution at six concentration levels from 25 to 150% of the assay analyte concentration (25, 50, 75, 100, 125 and 150 µg/mL). The peak area versus concentration data was treated by least-squares linear regression analysis.

Linearity test solutions for the related substances method were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared from LOQ to 150% of specification level.

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Accuracy

The accuracy study of impurities was carried out in triplicate at four concentration levels i.e. 0.025, 0.05, 0.1 and 0.15% of the rosiglitazone maleate analyte concentration (1000 µg/mL). The %mean recoveries for all the five impurities were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between rosiglitazone and all five impurities were recorded.

The flow rate of the mobile phase was 1.0 mL/min. To study the effect of flow rate on the resolution, flow was changed by ± 0.1 units as 0.9 and 1.1 mL/min. The pH of the buffer was 6.8. To study the effect of pH of the buffer on the resolution, buffer pH was changed by ± 0.2 units as 6.6 and 7.0. The mobile phase composition was buffer:acetonitrile 70:30 (v/v). To study the effect of the mobile phase composition on the resolution, the composition was changed by ± 2.0% absolute, as 68:32 and 72:28 (v/v).

Solution stability

The solution stability of rosiglitazone maleate in the assay method was carried out by leaving both solutions of sample and reference standard in tightly capped volumetric flask at room temperature for 24 hours. The same solutions were assayed for 6 h intervals up to the study period. The %RSD for the assay of rosiglitazone maleate was calculated during solution stability experiments.

The solution stability of rosiglitazone maleate and its impurities in the related substances method was carried out by leaving spiked sample solutions in tightly capped volumetric flasks at room temperature for 48 hours. The content of each impurity was determined for every 6 hours interval up to the study period.

RESULTS AND DISCUSSION

Method development

The main objective of the chromatographic method is to separate rosiglitazone maleate from its related impurities. Different mobile phases and columns were employed to achieve the best separation and resolution. Finally, the mobile phase consisting of aqueous 0.05 M sodium dihydrogen phosphate dihydrate with a pH 6.8 and acetonitrile in the ratio of 70:30 (v/v) at a flow rate of 1.0 mL/min using Waters XTerra RP-18, 150 x 4.6 mm, 3.5 µm column was found to be appropriate, al-

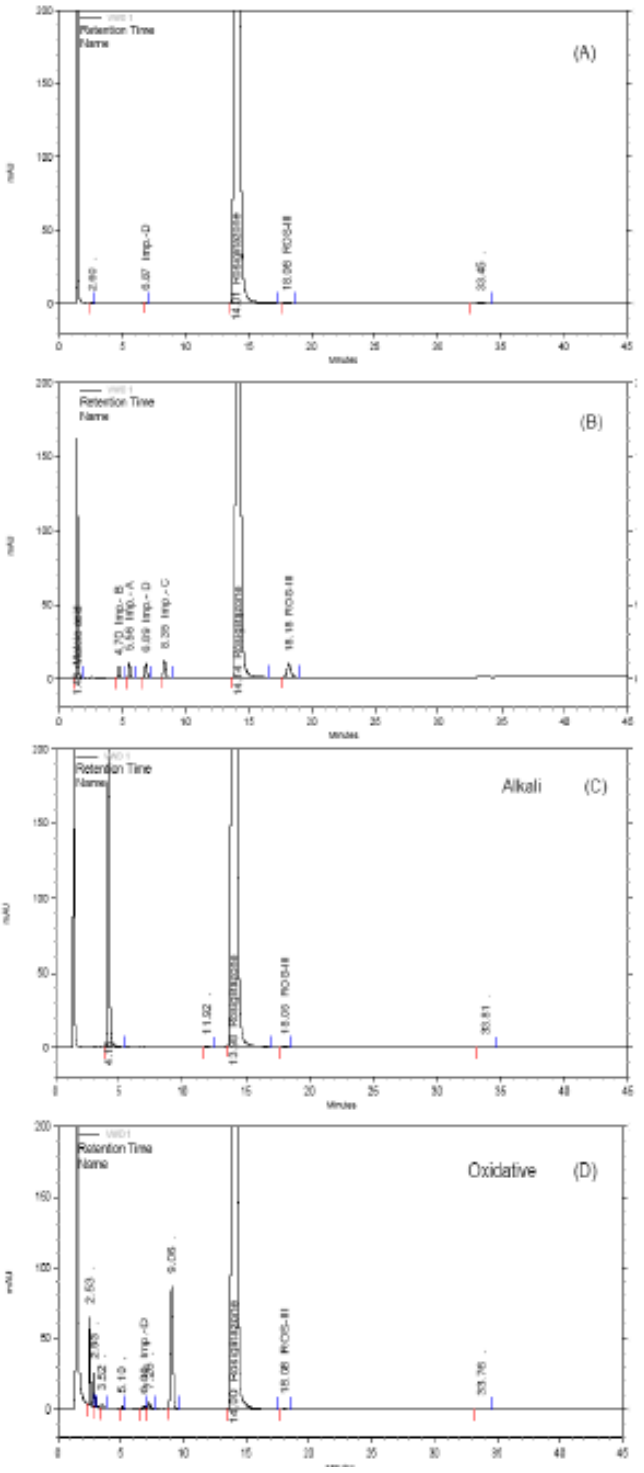

Figure 2 : Chromatograms obtained from (A) Rosiglitazone maleate bulk sample, (B) Bulk sample spiked with all the impurities (0.15%), (C) & (D) Samples obtained from stress studies.

lowing good separation and symmetrical peaks. Rosiglitazone maleate and its process related impurities show significant UV absorbance at wavelength 248 nm. Hence this wavelength has been chosen for detection in the analysis. The peak shape of rosiglitazone was found to be symmetrical. In optimized chromatographic conditions rosiglitazone, maleaic acid, Imp-A, Imp-B, Imp-C, Imp-D and ROS-III were separated with a resolution greater than 2, typical retention times were about 14.14, 1.48, 5.56, 4.70, 8.35, 6.89 and 18.18 min, respectively (Figure 2).

The system suitability results are given in TABLE 2 and the developed LC method was found to be specific for rosiglitazone maleate and its five impurities.

Specificity

No degradation was observed in rosiglitazone maleate samples when subjected to stress conditions like heat, photolytic and acidic medium. Rosiglitazone was degraded under alkali and oxidative media (Figure 2). Peak purity test results obtained by using a PDA detector confirmed that the rosiglitazone peak is homogenous and pure in all the analyzed stress samples. The assay of rosiglitazone maleate is unaffected in the presence of all the five impurities and its degradation products confirm the stability indicating power of the method. The summary of forced degradation studies is given in TABLE 1. The LC chromatograms of bulk sample and the bulk sample spiked with 0.15% of all five impurities are shown in Figure 2.

TABLE 1 : Summary of forced degradation results

Stress condition	Time (hours)	Assay of API (%)	TI (%)	Mass balance (%) (% assay + % impurities)
Real sample	Initial	99.7	0.07	99.77
Thermal (105°C)	24	99.5	0.06	99.56
Photolytic (UV at 254 nm)	24	99.7	0.07	99.77
Acid hydrolysis (5N HCl) at 80°C	1	99.7	0.07	99.77
Alkali hydrolysis (2N NaOH) at RT	1	88.1	10.68	98.78
Oxidation (5% H_2O_2) at 80°C	1	92.6	6.63	99.23

Precision

The %RSD of the assay of rosiglitazone maleate during the precision study was within 0.07% and the %RSD for the area responses of each impurity in the related substances during the precision study was within 1.0%. The overall %RSD of the assay results obtained in the intermediate precision study was within 0.09% and the overall %RSD for the area responses of each impurity was well within 1.2%, conforming good precision of the method. Results are shown in TABLE 2.

TABLE 2 : System suitability report and validation data

System Suitability Report						
#	Name	RT	RRT	Resolution	Tailing Factor	Relative Response Factor
1	Imp-B	4.70	0.33	---	1.2	0.40
2	Imp-A	5.56	0.39	4.1	1.2	0.70
3	Imp-D	6.89	0.48	5.2	1.0	0.87
4	Imp-C	8.35	0.59	4.7	1.1	1.1
5	Rosiglitazone	14.14	1.00	13.6	1.2	1.0
6	ROS-III	18.18	1.29	7.0	1.1	1.5
Validation Data						
Parameter		**Imp-B**	**Imp-A**	**Imp-D**	**Imp-C**	**ROS-III**
Linearity (0.005 - 0.15%)						
Correlation Coefficient		0.9998	0.9999	0.9997	0.9998	0.9996
Slope		10891.7	1898.8	25554.9	27694.3	47940.2
Y-Intercept		-55.5	38.6	-64.2	-304.8	94.9
Limit of detection and quantitation (%)						
LOD		0.0045	0.0029	0.0032	0.0032	0.0034
LOQ		0.0149	0.0096	0.0106	0.0108	0.0112
Accuracy (% recovery)						
0.025 (n = 3)		101.1	96.6	96.4	95.2	93.7
0.050 (n = 3)		94.4	99.5	95.4	92.8	96.0
0.100 (n = 3)		99.4	95.8	99.1	93.2	96.9
0.150 (n = 3)		98.7	96.5	101.6	105.9	99.3
Precision (% RSD)						
Intra-day (n = 6)		0.76	0.81	0.70	0.97	0.55
Intermediate Precision (% RSD)						
Inter-analyst (n = 6)		0.35	0.59	0.53	0.36	0.66
Overall (n = 12)		1.19	0.78	0.62	0.69	0.70
Inter-day (n = 6)		0.66	0.80	0.33	0.64	0.38
Overall (n = 12)		0.96	1.07	0.57	1.00	0.45

Limit of detection and limit of quantification

The limit of detection of a compound is defined as the lowest concentration that can be detected. The limit of quantification is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. A typical S/N ratio of 2-3 and 9-10 are generally considered to be acceptable for LOD and LOQ respectively. The precision at the LOQ concentrations for all the five impurities were below 5.4%. LOD and LOQ values for all the five impurities are shown in TABLE 2.

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Linearity

The linearity calibration plot for the assay method was obtained over the calibration ranges tested, i.e., 25-150 µg/mL and correlation coefficient was 0.9999.

Linear calibration plot for the related substances method was obtained over the calibration ranges tested, i.e., LOQ to 0.15% for each impurity. The correlation coefficients obtained were greater than 0.9996. The result shows that an excellent correlation existed between the peak area and concentration in both the methods. Results are shown in TABLE 2.

Accuracy

The percentage mean recovery (n=3) of the impurities Imp-A, Imp-B, Imp-C, Imp-D and ROS-III in rosiglitazone maleate sample varied from 96-100, 94-101, 93-106, 95-102 and 94-99% respectively. Results are shown in TABLE 2.

Robustness

In all the deliberate varied chromatographic conditions (flow rate, pH of buffer and composition of mobile phase), the resolution between the critical pairs, i.e. Imp-A and imp-B was greater than 2.3, illustrating the robustness of the method. The relative retention of all the impurities with respect to rosiglitazone are shown in TABLE 2.

Solution stability

The %RSD (n=6) of the assay of rosiglitazone maleate during solution stability experiments were within 0.8%. No significant changes were observed in the content of the five impurities during solution stability experiments. The solution stability experiment data confirms that the sample solutions used during assay and the related substances determination were stable for at least 48 h.

CONCLUSION

The RP-LC method developed for assay and related substances determination of rosiglitazone maleate is precise, accurate, rapid and specific. The method was fully validated, stability indicating and can be conveniently used by quality control departments to determine the related substances and assay in production samples as well as stability samples.

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