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Research article

RP-HPLC method for the quantification of Metformin and Rosiglitazone in bulk and combined tablet dosage form: method development and validation

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Key words: Metformin, rosiglitazone, **RP-HPLC**, simultaneous quantification, combined tablet form.

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Abstract

A rapid, simple and selective reverse phase high performance liquid chromatographic method for simultaneous estimation of metformin and rosiglitazone in bulk and combined tablet dosage form was developed and validated. The method employed Phenomenex (150 mm x 4.6 mm, 5-µm particle size) C8 analytical column as the stationary phase. The solvent system consisted of orthophosphoric acid (0.1%) and methanol in the ratio of 60:40 (*v/v*). The detection and quantification of metformin and rosiglitazone was carried out with PDA detector set at 220 nm. Retention time for metformin and rosiglitazone were 2.176 and 2.919 min, respectively. Linear regression analysis data for the calibration curves showed good linear relationship with R^2 = 0.9995 with respect to peak area in the concentration range 500-1500 µg/ml for metformin and R^2 = 0.9999 with respect to peak area in the concentration range 1-30 µg/ml for rosiglitazone. The method was validated for sensitivity, precision, accuracy, selectivity, recovery and robustness. The limits of detection & quantitation were 7.151 & 23.838 µg/ml for metformin and 0.012 & 0.043 µg/ml for rosiglitazone, respectively. Statistical validation analysis proved that the method is selective, precise, accurate and robust for the estimation of metformin and rosiglitazone.

Introduction

Rosiglitazone, an anti-diabetic drug, belongs to the thiazolidinedione class of drugs. Chemically it is known as (*RS*)-5-[4-(2-[methyl(pyridin-2-yl)amino] ethoxy)benzyl] thiazolidine-2,4-dione (Figure 1). Rosiglitazone is given as an adjunct to diet and exercise to improve control of blood glucose level in adults with type 2 diabetes mellitus [1,2]. Rosiglitazone enhances tissue sensitivity to insulin by acting as a potent and selective agonist at peroxisome proliferator activated gamma receptor in adipose tissue, skeletal muscle and liver [3].

Metformin is an oral hypoglycemic agent belonging to the biguanides class of compounds. Chemically, metformin is described as 3-(diaminomethylidene)-1,1-dimethylguanidine (Figure 1), Metformin is prescribed for the treatment of non insulin dependent diabetes mellitus [4,5]. The hypoglycemic effect of metformin is mediated by the activation of AMP-activated protein kinase [6,7].

The combination of rosiglitazone and metformin has been approved by FDA in 2002 [8]. This combination is used to improve blood sugar control in type 2 diabetes patients who are not sufficiently controlled on metformin only [8]. In this combination, rosiglitazone acts as insulin sensitizing agents and enhances peripheral glucose utilization, whereas metformin acts by decreasing hepatic glucose production endogenously.

There are many methods that are employed for the simultaneous determination of metformin and rosiglitazone in bulk, pharmaceutical formulations and/or biological samples. They include zero-crossing first-derivative spectrophotometry [9,10], visible spectrophotometry [10], simultaneous equation spectrophotometry [11], HPTLC [12,13], LC-MS [14-16] and HPLC [17-21] methods. Though the reported UV spectrophotometric methods [9-11] are simple, they are less selective as it involves absorbance in the UV region where the interference from tablet excipients is more. The reported visible spectrophotometric methods [10] are critically dependent on pH, expensive chromogenic reagents, requires extraction step and the reaction products are less stable. Although the HPTLC [12,13] and LC-MS [14-16] procedures are sensitive and specific, the methods are

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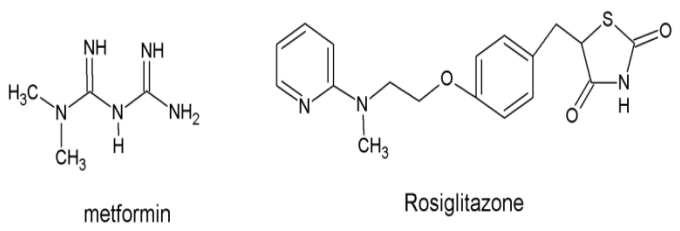


Figure 1. Chemical structure of the selected drugs

time consuming, require multistage extraction procedures, sophisticated instrumentation and expertise operational personnel. Furthermore, the LC-MS methods [14-16] are applied only for human plasma and dog plasma samples.

Though the reported HPLC methods [17-21] are sensitive, they suffer from one or more disadvantages like narrow range of linearity [17,19-21], lack of precision & accuracy [17-19,21] and not fully validated [17,18]. The total run time of all the reported HPLC methods is greater than five minutes [17-21]. The increased runtime may increase the time taken for the single analysis, utilization of solvents and cost per analysis.

The present study reports the development of a RP-HPLC method for the simultaneous estimation of metformin and rosiglitazone in bulk and combined tablet dosage forms. The method was fully validated according to International Conference on Harmonization guidelines [22]. The developed and validated RP-HPLC method was demonstrated to be rapid, simple, selective, precise, accurate and cost-effective compared to many reported methods.

Experimental

Apparatus

Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector and Waters Empower2 software was used.

Phenomenex 150 mm x 4.6 mm, 5 µm particle size, C8 analytical column was used for separation and simultaneous analysis of metformin and rosiglitazone.

Electronic balance ELB 300 was used for weighing the materials.

Digisun pH meter was used for all pH measurements.

Chemicals and solvents

All the chemicals and solvents used were of analytical reagent and HPLC grade, respectively.

Orthophosphoric acid was obtained from Sd Fine Chemicals Ltd., Mumbai, India.

Methanol was from Merck India Ltd., Mumbai, India.

Reference drugs and tablet dosage forms

Reference standards of metformin and rosiglitazone were supplied by Lara Drugs Private Limited, Hyderabad, India.

Avandamet tablets, labeled to contain 2 mg of rosiglitazone 1000 mg of metformin (product of GlaxoSmith Kline, Research Triangle Park, NC) was purchased from the local pharmacy.

Chromatographic conditions

Mobile Phase: 0.1% orthophosphoric acid: methanol (40:60 *v/v*)

Flow Rate: 1.0 ml/min

Column temperature: 30˚C

Volume: 10 µl

Detection wavelength: 220 nm

Runtime: 5 min

Standard solutions

Stock solution equivalent to 10000 µg/ml of metformin and 20 µg/ml of rosiglitazone was prepared by dissolving 1000 mg and 2 mg of metformin and rosiglitazone, respectively in 100 ml mobile phase in a volumetric flask. Working standard solutions equivalent to 500, 750, 1000, 1250 and 1500 µg/ml of metformin and 1, 1.5, 2, 2.5 and 3 µg/ml of rosiglitazone was prepared by apt dilution of the stock solution with the same solvent.

Calibration graphs

Working standard solutions in the concentration range 500-1500 µg/ml of metformin and 1-3 µg/ml of rosiglitazone were prepared from the stock standard solution with mobile phase. 10 µl of each working standard solution is injected into the HPLC system (n=3) under the chromatographic conditions described. The chromatograms and peak areas were recorded. The mean peak area was then plotted against the final drug concentration (µg/ml) to get the calibration graphs of metformin and rosiglitazone. The corresponding regression equation was derived.

Assay of metformin and rosiglitazone in combined tablet dosage

The average weight of ten tablets was determined. The tablets were finely powdered using mortar and pestle. An accurately weighed amount of tablet powder equivalent to 1000 mg and 2 mg of metformin and rosiglitazone, respectively was transferred into a clean dry 100 ml beaker and about 50 ml of mobile phase was added. The content of the beaker was sonicated for 15 min. The contents were quantitatively transferred into 100 ml volumetric flask, completed to the mark with the same solvent and filtered through 0.45 mm membrane filter. This solution was suitably diluted with mobile phase to give a final concentration of 1000 µg/ml metformin and 2 µg/ml rosiglitazone for analysis. The procedure described under "Calibration graphs" was followed. The nominal content of the metformin and rosiglitazone in the combined tablet dosage form was determined either from the corresponding calibration graph or from the corresponding regression equation.

Results and Discussion

Method development

RP-HPLC procedure was optimized to develop a rapid, precise and accurate method that can be used for quality control analysis of metformin and rosiglitazone simultaneously in laboratories. During method optimization, two different analytical columns, Symmetry C8 (250 x 4.6 mm, 5 µm) and Phenomenex C8 (250 x 4.6 mm, 5 µm) were tried. For the separation and analysis of metformin and rosiglitazone, Phenomenex C8 (250 x 4.6 mm, 5µm) analytical column maintained at a temperature of 30°C was found to be efficient.

0.1% orthophosphoric acid and methanol in different ratios and flow rates were tested to select the best mobile phase composition and flow rate. Finally, a mobile phase composed of 0.1% orthophosphoric acid and methanol in the ratio of 60:40 v/v with a flow rate of 1.0 ml/min was chosen for analysis that showed proper separation of drug peaks, good peak shape and resolution. For the detection and quantification of metformin and rosiglitazone, 220 nm was selected as the optimum detection wavelength. Both the selected drugs showed good response at 220 nm. Under optimized chromatographic conditions, the peaks of metformin and rosiglitazone were shaped well and free from tailing. The retention times were 2.176 and 2.919 min for metformin and rosiglitazone, respectively (Figure 2).

HPLC method validation

Method validation was done in accordance with ICH recommendation [22].

System suitability

Chromatographic parameters associated to the developed method must pass the system suitability limits before the analysis of sample. The relative standard deviation of peak area, theoretical plates, resolution and tailing factor for metformin and rosiglitazone peaks were evaluated using a solution containing 1000 µg/ml of metformin and 2 µg/ml of rosiglitazone. All the results (Table 1) assure the satisfactoriness of the proposed method for routine analysis of metformin and rosiglitazone simultaneously

Selectivity

The selectivity study was assessed to make sure the absence of interference by the excipients commonly found the pharmaceutical formulations and components of mobile phase. For this study, standard solution (1000 µg/ml - metformin: 2 µg/ml - rosiglitazone), tablet sample solution (1000 µg/ml - metformin: 2 µg/ml - rosiglitazone), placebo blank and mobile phase blank solution were injected into the chromatographic system. The chromatograms were recorded and are shown in Figures 3a-3d. The chromatogram demonstrated the selectivity of the proposed method, since there were no peaks at the retention time of selected drugs in the chromatograms of placebo blank & mobile phase blank. The retention times of the selected drugs were same in the chromatograms of standard solution & tablet sample solution.

Linearity

Linearity was investigated via replicate analysis of five standard concentrations: metformin-500, 750, 1000, 1250 and 1500 µg/ml; rosiglitazone-1, 1.5, 2, 2.5 and 3 µg/ml. Calibration graphs of metformin and rosiglitazone were constructed by plotting the mean peak area against the drug concentration (Figures 4a and 4b). The results of the regression equations and correlation coefficients were presented in Table 2. Good linearity for metformin and rosiglitazone was achieved in the range of 500-1500 µg/ml and 1-3 µg/ml for metformin and rosiglitazone as indicated by higher value of correlation coefficients (>0.999).

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ values determine the sensitivity of the method. Both were calculated as signal-to-noise ratio of 3:1 (LOD) and 10:1 (LOQ). The LOD and LOQ values of metformin and rosiglitazone were calculated and are presented in Table 2. The values indicate the adequate sensitivity of the method.

Precision and Accuracy

The precision and accuracy of the method was established using the standard solution with a concentration of 1000 µg/ml of metformin and 2 µg/ml of rosiglitazone. Six injections of the standard solution were made into the HPLC system. Peak areas, percentage recovery and their relative standard deviation were calculated. Small values of the relative standard deviation and good percent recoveries gave a good indication for the high precision and accuracy of the proposed method, respectively (Table 3).

Table 1. System suitability results

Parameters	Metformin	Rosiglitazone	Recommended limits
Peak area	3536621.4 (%RSD – 0.284)	5040258.4 (%RSD – 0.323)	RSD ≤2
USP resolution	-	5.05	> 1.5
USP plate count	4713	5741	> 2000
USP tailing factor	1.63	1.49	≤ 2

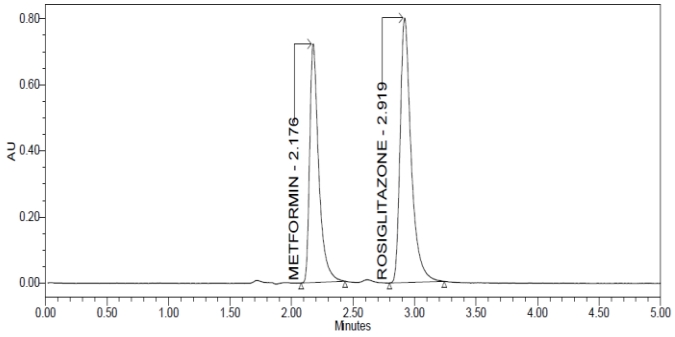


Figure 2. Chromatogram of metformin and rosiglitazone under optimized HPLC conditions

Table 2. Linearity, LOD and LOQ values of selected drugs

Parameters	Metformin	Rosiglitazone
Linearity (µg/ml)	500-1500	1-3
Regression equation (y= mx + c)	y = 3577 x - 29148	y = 25060x + 7254
Slope (m)	3577	25060
Intercept (c)	-29148	7254
Regression coefficient (R^2)	0.9995	0.9999
LOD(µg/ml)	7.151	0.0129
LOQ(µg/ml)	23.838	0.0430

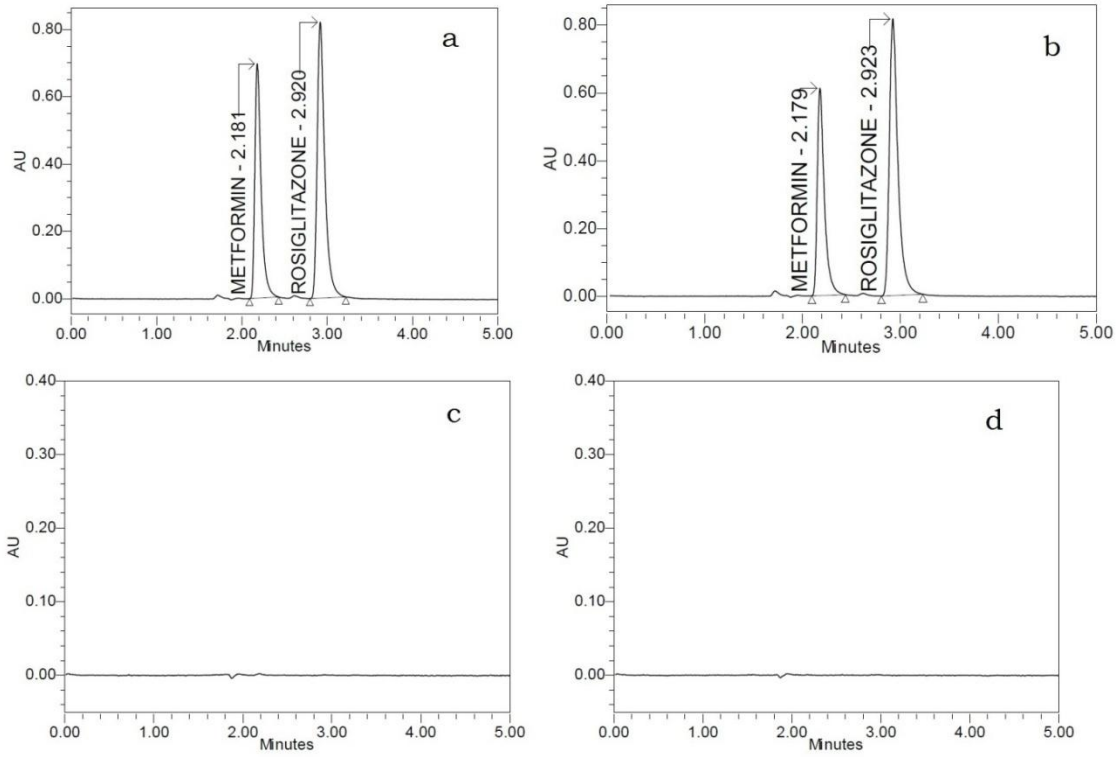


Figure 3. Chromatogram of [a] standard solution [b] tablet sample solution [c] Placebo blank [d] Mobile phase blank

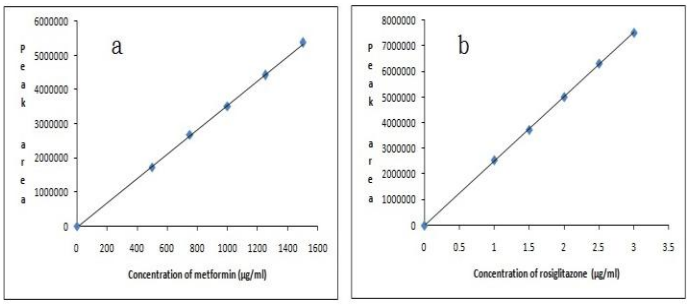


Figure 4. Calibration curve of [a] Metformin [b] Rosiglitazone

Table 3. Precision and accuracy of the method

Injection	Peak area	Recovery (%)	Peak area	Recovery (%)
1	3538360	99.35	5034538	99.09
2	3589889	100.80	5027237	98.94
3	3578487	100.48	5057055	99.53
4	3578152	100.47	5002249	98.45
5	3531453	99.15	5013392	98.67
6	3531602	99.16	5098763	100.35
Mean	3557991	99.90	5038872	99.17
RSD (%)	0.757	0.760	0.691	0.691

Recovery study

The recovery study was done to establish further the accuracy of the method. This was performed by adding known amounts of metformin and rosiglitazone to a known concentration of the tablet sample solution at three different concentration levels. The percentage recoveries for three replicates were calculated. According to the results revealed in Tables 4, good accuracy was observed for the proposed method. There is no interference observed from the tablet excipients.

Table 4. Recovery study of metformin and rosiglitazone

Spiked Level (%)	Concentration of drug (µg/ml)		Recovery (%)	Mean (%)
	Added	Found		
Metformin				
50	500.000	490.94	99.19	99.30
	500.000	490.94	99.19	
	500.000	487.60	99.52	
100	1000.000	1003.49	100.35	100.09
	1000.000	991.24	99.12	
	1000.000	1007.88	100.79	
150	1500.000	1515.62	101.04	100.45
	1500.000	1489.78	99.32	
	1500.000	1514.64	100.98	
Rosiglitazone				
50	0.990	0.99	99.84	100.27
	0.990	0.99	99.55	
	0.990	1.00	101.42	
100	1.980	1.99	100.73	100.64
	1.980	1.99	100.56	
	1.980	1.99	100.63	
150	2.970	2.97	100.15	100.06
	2.970	2.96	99.51	
	2.970	2.99	100.53	

Table 5. Robustness of the method

Parameter varied	Retention time	Peak area	USP plate count	USP Tailing	USP resolution
Metformin					
Column temperature: 29°C	2.188	3476114	4415	1.66	-
Column temperature: 31°C	2.184	3470996	4528	1.66	-
Flow rate: 0.9 ml/min	2.897	4707619	4010	1.64	-
Flow rate: 1.1 ml/min	2.748	4682483	4912	1.51	-
Rosiglitazone					
Column temperature: 29°C	2.932	4988765	5492	1.50	4.96
Column temperature: 31°C	2.926	5033913	5499	1.52	4.96
Flow rate: 0.9 ml/min	2.894	5755980	5148	1.50	4.81
Flow rate: 1.1 ml/min	2.343	5889072	5779	1.42	5.16

Robustness

The robustness of the proposed method was investigated via an analysis of working standard sample under a variety of experimental conditions, such as small changes in column temperature (30±1 °C) or changing the flow rate of mobile phase (1.0±0.1 ml/min). The effect on retention time, peak area, USP plate count, USP tailing and USP resolution was studied. The results are summarized in Table 5. It was found that the method was robust when the column temperature and the mobile phase flow rate were varied.

Conclusion

A rapid and simple RP-HPLC method was explored for the simultaneous quantification of metformin and rosigitazone in bulk and in commercially available tablet dosage forms. The method was validated as per ICH guidelines. The method proved that the linearity, selectivity, precision, accuracy, robustness. The simple mobile phase used provides simple and economic applications. Therefore, the developed and validated RP-HPLC method was found to be suitable for the routine quality control analysis of metformin and rosiglitazone simultaneously in laboratories with no interference from the common excipients.

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