DEVELOPMENT AND VALIDATION OF A RP- HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PIOGLITAZONE AND METFORMIN IN BULK AND TABLET DOSAGE FORM

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Abstract
A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of pioglitazone and metformin in bulk and tablet dosage form. Chromatographic analysis was performed on a C_{18} column (250x 4.6 mm, 5µm) with a mixture of Methanol:Phosphate buffer in in the ratio 68:32 as mobile phase, at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 260 nm. The method was validated for accuracy,precision,specificity,linearity, and sensitivity. The retention times of pioglitazone and metformin were 7.24±0.051 and 2.54±0.038 min respectively. Calibration plots were linear over the concentration ranges 10–35 µg mL^{-1} and 15–40 µg mL^{-1} for pioiglitazone and metformin respectively. The Limit of detection was 0.382 and 0.131 µg/ml and the quantification limit was 1.27 µg/ml and 0.436 µg/ml for metformin and pioglitazone respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98.65% to 98.90%. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of rosiglitazone and metformin in bulk and tablet dosage form.

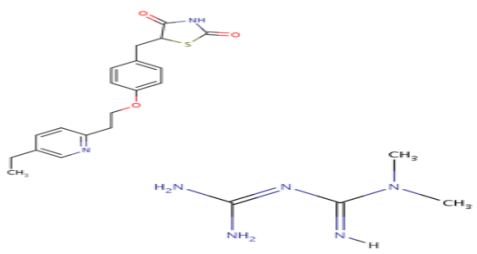
Keywords: Pioglitazone, Metformin, RP-HPLC, ICH guidelines

1. Introduction
Pioglitazone is a thiazolidinedione derivative and it is used for the treatment of type 2 diabetes mellitus, chemically it is 5-[[4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl] methyl]-1, 3-thiazolidine-2, 4-Dione. Pioglitazone is an oral antidiabetic agent and acts as an agonist at PPAR gamm receptors have acts primarily by reducing insulin resistance.Metformin is an antihyperglycemic agent, which improves glucose tolerance in patients, chemically it is 3-(diaminomethylidene)-1,1-dimethylguanidine. Metformin is used for the treatment of with type 2 diabetes, lowering both basal and postprandial plasma glucose. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.

A literature survey reveals that various analytical methods like pioglitazone by rapid determination of metformin in human plasma using ion-pair HPLC[4]Simultaneous estimation of metformin, pioglitazone, and Glimepiride by RP-HPLC[2], Simultaneous spectrophotometric estimation of three component tablet formulation Containing pioglitazone, metformin and Glibenclamide[3], Simultaneous Estimation of metformin in combination with rosiglitazone by RP-HPLC[6] & in human plasma by liquid chromatography/tandem mass spectrometry with electrospray ionization[5] , liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma[7]

But these methods are sophisticated,expensive and time consuming when compared to simple HPLC method. There is need for a interest to develop simple,accurate,specific,sensitive, precise and reproduciable simultaneous HPLC method for the estimation of rosiglitazone and metformin in bulk and its formulation.

Fig. 1: Chemical structure of Pioglitazone and Metformin



2. Experimental

2.1 Materials and Methods: Pure standard of pioglitazone and metformin (Assigned purity 99.98%) was obtained as a gift sample from Micro labs Pvt. Ltd, Badi, India. The gift samples were used as standard without further purification. . HPLC grade water, methanol (Qualigens), potassium dihydrogen phosphate, di sodium hydrogen phosphate, orthophosphoric acid (S.D. fine chemicals, Mumbai, India), were used throughout the experiment. Commercial pharmaceutical preparation (Pioglar) which was claimed to contain 500mg of metformin and 15mg of pioglitazone is used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies.

2.2 Instrumentation and chromatographic conditions: High performance liquid chromatograph, Shimadzu pumpLC-10AT VP equipped with universal injector (Hamilton 25 µL) SPD10A, UV-VIS detector SPD10A-10A VP (Shimadzu) was used. Isocratic elution of mobile phase comprising of Methanol: Phosphate buffer in in the ratio 75:25 at flow rate of 1.0 ml min^{-1} was performed on C_{18} column (250x 4.6 mm, 5µm). The effluent was detected at 260 nm. The retention time's pioglitazone and metformin were 7.24±0.051 and 2.54±0.038 min. The column temperature was maintained at ambient and the volume of injection was 20 µl. Prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase.

2.3 Preparation of mobile phase: The HPLC grade solvents were used for the preparation of mobile phase, isocratic elution of mobile phase comprising of of Methanol:Phosphate buffer in in the ratio 68:32 [(Solvent A), Phosphate Buffer: Dissolve 5.04 gm of disodium hydrogen phosphate and 3.01 gm potassium dihydrogen phosphate in 1000 ml of water, adjust the pH to 4.0 with glacial acetic acid. (solvent A), Methanol]. The contents of the mobile phase were filtered before use through a 0.45µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 ml/min.

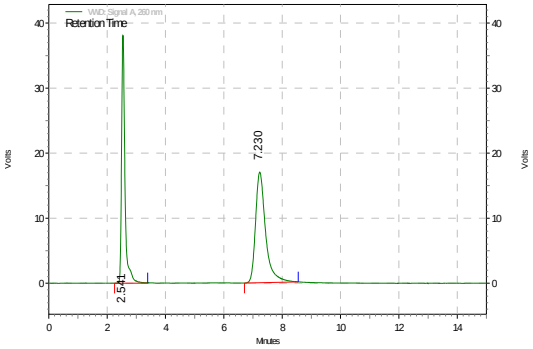
2.4 Standard solution: Standard stock solutions 1 mg mL^{-1} of pioglitazone and metformin were prepared in methanol and further diluted in mobile phase. The working standard solutions were prepared in mobile phase to contain mixture of rosiglitazone and metformin in over the linearity range from 10 –35 µg/ml and 15 - 40 µg/ml.

2.5 Assay in formulation: Twenty tablets each containing and their average weight was calculated. The tablet were crushed to furnish a homogenious powder and a quantity equivalent to one tablet were weighed in to a 100 ml volumetric flask, dissolve in methanol, sonicated for about 15 min and then made up to volume with mobile phase. The solution was stirred for 10 min using a magnetic stirrer and filtered into a 100 ml volumetric flask through 0.45 µm membrane filter. The residue was washed 3 times with 10 ml of mobile phase, and then the volume was completed to 100 ml with the same solvent. Further add mobile phase to obtain an expected concentration. All determinations were conducted in triplicate.

3. Results and Discussions

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of pioglitazone and metformin were shown in (Fig No.1). There was clear resolution between pioglitazone and metformin with retention time of pioglitazone and metformin were 7.24±0.051 and 2.54±0.038 min.

Fig No. 2.Typical chromatogram showing metformin and pioglitazone



3.1. Linearity: The response was determined to be linear over the range of 10 µg/ml to 35 µg/ml (10,15,20,25,30,35) for pioglitazone and 15- 40µg/ml (15,20,25,30,35,40) for metformin. The solutions were injected into HPLC system. Each of the concentration was injected in triplicate to get reproduciable response. The run time was 15 min and the peak areas were measured (Table No1 &2). The calibration curve was plotted as

concentration of the respective drug versus the response at each level. The purposed method was evaluated by its correlation coefficient and intercept value calculated by statistical study. They were represented by the linear regression equation. (Fig 2& 3 calibration curve)

$Y_{Pioglitazone}$ = 420622.8x + 2441190
Coefficient of correlation (r^2) value = 0.9992
$Y_{Metformin}$ = 280170x +1050546
Coefficient of correlation (r^2) value =0.9982

Table No.1: For Peak Area of Pioglitazone

Conc. In µg/mL	10	15	20	25	30	35
Replicate 1	6723239	8688240	10576263	12834146	14966312	17213634
Replicate 2	6703172	8692867	10691545	13086680	15051996	17263586
Replicate 3	6769190	8688341	10783184	12918115	15095696	17317457
Avg	6731867	8689816	10683664	12946314	15038001	17264892
SD	33844.14	2642.726	103685.4	128606.9	65817.5	51923.83
RSD	0.502745	0.030412	0.970504	0.993386	0.437674	0.300748

Table No.2.Peak area of Metformin

Conc. In µg/mL	15	20	25	30	35	40
Replicate 1	5173264	6611955	8203268	9397049	10822391	12249738
Replicate 2	5159701	6673335	8204150	9469368	10829188	12179936
Replicate 3	5158865	6711727	8225142	9439304	10832142	12253317
Avg	5163943	6665672	8210853	9435240	10827907	12227664
SD	8082.75	50325.44	12382.2	36330.35	5000.122	41372.09
RSD	0.156523	0.754994	0.150803	0.38505	0.046178	0.338348

Figure 3: Calibration curve for Pioglitazone

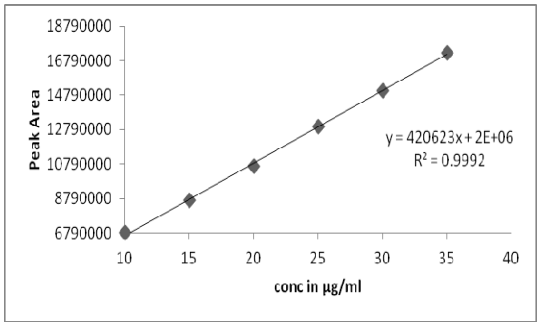
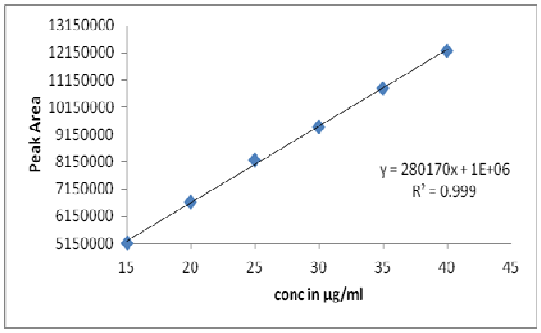


Figure 4: Calibration curve for Metformin



3.2. Accuracy: The accuracy is the closeness of the measured value to the true value for the sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 1ml, 2ml and 4 ml of sample drug solution were pipetted into each of three volumetric flasks. To this 0.8 ml of rosiglitazone standard drug solution of 100 µg/ml was added to each volumetric flask respectively. To this 1 ml of metformin standard drug solution of 100µg/ml was added to each volumetric flask respectively. The volume was made up to 10 ml with mobile phase. 20 µl of each solution was injected and chromatograms were recorded. The range was found between 97.72 to 100.43 % respectively. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients. (Table No.3)

Table No. 3. Result of recovery studies

S.No.		Conc. taken in (µg/ml)	Std addition in (µg/ml)	Total Conc. found in (µg/ml)*	% recover ± SD
1	PIO	10	5	14.79	98.65±0.721
	MET	15	5	19.76	98.84±0.824
2	PIO	10	15	24.83	99.34±0.421
	MET	15	15	29.57	98.59±0.646
3	PIO	10	25	34.80	99.45±0.227
	MET	15	25	39.56	98.90±0.554
Mean ± SD		ROSI	99.48±0.157		
		MET	98.77±0.164		

3.3. Limit of Detection and Quantification: Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S) Where, SD = Standard deviation of the response

L.O.Q. = 10(SD/S) S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The Limit of detection was 0.382 and 0.131 µg/ml and the quantification limit was 1.27 µg/ml and 0.436 µg/ml for metformin and pioglitazone respectively.which represents that sensitivity of the method is high.

3.4. Precision: Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories, and different batch of reagent, different analysts, and different equipments. The repeatability study which was conducted on the solution having the concentration of about 25 µg/ml for pioglitazone and 30 µg/ml for metformin (n =5) showed a RSD of 0.350% for rosiglitazone and 0.756% for metformin. It was concluded that the analytical technique showed good repeatability. (TableNo.4)

Table No.4.Results of repeatability analysis

S.No.		**Conc (µg/ml)**	**Peak Area (µV*sec)**	**Mean±SD**	**%RSD**
1	PIO	PIO 25+ MET 30	9288627	**PIO**	**PIO**
	MET		13187803	9337605.2 ± 32741.36953	0.350
2	PIO		9319014		
	MET		13271884		
3	PIO		9359975		
	MET		13370954	**MET**	**MET**
4	PIO		9356920	13333264 ± 100872.2	0.756
	MET		13422748		
5	PIO		9363490		
	MET		13412929		

3.5. Reproducibility and Ruggedness: The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay was performed in different condition, different analyst, and different dates. **(**Table N0.5)

Table N0.5. Results of reproducibility

Parameter	Result observed	
	PIO	MET
Average Percentage Recovery	100.14%	100.03%
SD between set of analysis on same date	0.395	0.589
SD between set of analysis on different date	0.704	0.729
RSD between set of analysis on same date	0.588%	0.395%
RSD between set of analysis on different date	0.731%	0.704%

3.6. Robustness: The robustness of the method was determined by delibrate changes in the method like alteration in pH of the mobile phase, percentage organic content, changes in the wavelength. The robustness of the method shows that there were no marked changes in the chromatographic parameters, which demonstrates that the method developed is robust.

3.7. Specificity: The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed that there are no peaks of diluents and placebo at main peak's. Hence, the chromatographic system used for the estimation of pioglitazone and metformin is very selective and specific .Specificity studies indicating that the excipients did not interfere with the analysis. For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Fig No.5)

3.8. System Suitability: A binary solution of 10 µg $mL^{-1}$of pioglitazone and 15 µg mL^{-1} of metformin (in triplicate) was prepared and same was injected, then the system suitability parameters were calculated from the following chromatogram. (Table No 6)

Table No 6. Results of system suitability parameters

Parameters	Data obtained	
	PIO	MET
Number of theortical plates	1320	678
Symmetry factor/ Tailing factor	1.41	1.39
Resolution	3.94	

Conclusion:

The proposed RP-HPLC method is found to be simple,accurate, precise, linear, and specific, and, for quantitative estimation of pioglitazone and metformin in bulk and its formulation.

Acknowledgement

The authors thank Micro labs Pvt. Ltd, Badi, India, for providing a sample of pioglitazone and metformin as a gift.

References

1. www.drugbank.com
2. Kolte BL. J Chrom Science. July 2008; 46(6): 501-504
3. Chaturvedi PK et al**.,** Analytical Letters. August 2008; 41(12): 2133 –42.
4. Zarghi A,Foroutan SM,Shafaati A,Khoddam A(2003). J Pharm Biomed Anal. 31:197-200.
5. Zhang Lu et al J Chrom B. 2007; 854: 91–98
6. Kolte B L,Raut BB,Deo AA,Bagool MA, Shinde DB(2004). J Chrom Science. 42: 70-73.
7. AbuRuz S. J Chrom B. 2005; 817 (2): 277-286
8. International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text and Methodology Q2 (R1), November