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Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR SIMULTANEOUS ESTIMATION OF PIOGLITAZONE AND GLIMEPIRIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC METHOD

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A B S T R A C T

Pioglitazone and glimepiride are the anti-diabetic agents used in the treatment of type-2 diabetes. There are very few methods to analyze Pioglitazone and glimepiride individually or in combination form. The present work describes a Reverse Phase High Performance Liquid Chromatography for simultaneous estimation of pioglitazone and glimepiride in bulk and pharmaceutical dosage form on RP C-18 Column (15 cm x 4.6 mm, 5 µm) using methanol and water (80:20 v/v) as mobile phase at a flow rate of 1.0 ml/min and the detection wavelength was 251 nm. The retention time of pioglitazone and glimepiride was found to be 1.9 min. and 6.6 min. respectively. Linearity was observed in the concentration range of 15-75 µg/mL for pioglitazone and 2-10 µg/ml for glimepiride. Percent recoveries obtained for pioglitazone and glimepiride were 99.62 and 101 respectively.

INTRODUCTION

Pioglitazone is a thiazolidinedione derivative and it is used in patient with NIDDM type of antidiabetic, chemically it is 5-(4-[2-(5-ethypyridin-2-yl] benzyl) thiazolidine-2, 4-dione. The molecular formula is $C_{19}H_{20}N_2O_3S$. Glimepiride is a sulfonylurea derivative chemically 3-ethyl-2,5-dihydro-4-methyl-N- [2-[4-s(trans-4-methyl cyclohexyl) amino] carbonyl]amino] sulfonyl] phenyl]ethyl]-2-oxo-1Hpyrrole- 1-carboxamide1, widely used in patient with type 2 diabetic mellitus. Its molecular formula is $C_{24}H_{34}N_4O_5S$. There are very few methods to analyze Pioglitazone and glimepiride individually or in combination form. In present study the method for analyzing Pioglitazone and glimepiride was developed using Reverse Phase High Performance Liquid Chromatography. Many methods have been described in the literature for the determination of pioglitazone and glimepiride individually and in combination with other drug[2-11]. Our present plan is to develop new, simple, precise, & accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated. The present work described a Reverse Phase High Performance Liquid Chromatography for simultaneous estimation of pioglitazone and glimepiride in bulk and pharmaceutical dosage form on RP C-18 Column (15 cm x 4.6 mm, 5 µm) using methanol and water (80:20 v/v) as mobile phase at a flow rate of 1.0 ml/min and the detection wavelength was 251 nm. The proposed methods are precise, accurate, selective and rapid for the simultaneous determination of pioglitazone and glimepiride. The method was validated according to the ICH (Q2A 1995) guidelines.

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MATERIALS AND METHODS[3-4]

Pioglitazone and glimepiride were obtained as gift samples from Lupin Pharmaceuticals Pune.

Selection of solvent

HPLC grade methanol and Water of analytical reagent grade in the ratio of 80:20 v/v was selected as common solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility of the drug in different solvents.

Preparation of standard solutions

Standard stock solution was prepared by dissolving 2.0 mg of glimepiride and 15.0 mg of pioglitazone in 10 ml methanol 200 ugm/ml glimepiride and 1500 ugm/ml pioglitazone – stock solution.

Procedure

The mobile phase was allowed to equilibrate with stationary phase until steady baseline as obtained the standard solution containing glimepiride and pioglitazone was run and same individual solvents as well as combination of solvents have

been tried to get a good separation and stable peak. Each mobile phase as filtered through whatmann filter paper no.41 Based on sample solubility and stability, various mobile phase composition were evaluated to achieve acceptable separation using selected chromatographic conditions. From various mobile phases tried, mobile phase containing methanol and water 80:20 was selected since it gives sharp reproducible retention time for pioglitazone and glimepiride.

System Suitability Parameters: [5]

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from three replicate injections of standard solutions.

Preparation of standard drug solution

Standard stock solution was prepared by dissolving 2.0 mg of Glimepiride and 15.0 mg of Pioglitazone in 10 ml Methanol. that give concentration 200 and 1500 µg/ml for Glimepiride and Pioglitazone respectively.

Procedure

The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Take 0.1 ml from standard drug solution a and make vol. with mobile phase 10 ml to produce 2.0 µg/ml Glimepiride & 15 µg/ml Pioglitazone. Three replicate injections were made separately and the chromatograms were recorded. In the chromatogram obtained with Standard preparation:

1. The relative standard deviation of for Glimepiride and Pioglitazone peak area response for three replicates injections were not more than 2.0%.
2. USP tailing factor for Glimepiride and Pioglitazone for peaks was not more than 2.0.
3. The column efficiency for the Glimepiride and Pioglitazone peak was not less than 2000 theoretical plates.

Validation Procedure: [6-12]

Standard Stock solution for Glimepiride and Pioglitazone

Standard stock solution was prepared by dissolving 2.0 mg of Glimepiride and 15.0 mg of Pioglitazone in 10 ml Methanol that give concentration 200 and 1500 µg/ml for Glimepiride and Pioglitazone respectively.

Preparation for different concentrations of solution of Glimepiride and Pioglitazone

Preparation for different concentration of solution of Glimepiride and Pioglitazone is as follow:

Table No.1 Preparation of different concentrations of Glimepiride and Pioglitazone

Quantity of standard stock solution	Quantity of Mobile phase	Concentration of Glimepiride in ug/ml	Concentration of Pioglitazone ug/ml
0.1 ml	10 ml	2	15
0.2 ml	10 ml	4	30
0.3ml	10 ml	6	45
0.4 ml	10 ml	8	60
0.5 ml	10 ml	10	75

Linearity

From the standard stock solution of Glimepiride and Pioglitazone 0.1 ml were taken in 10 ml volumetric flask diluted up to the with Methanol such that final concentration of Glimepiride and Pioglitazone in the range 2-10 µg/ml of Glimepiride and 15-75 µg/ml of Pioglitazone was produce. Volume of 20 µl of each sample was injected with the help of Hamilton Syringe. All measurements were repeated two times for each concentration and calibration curve was constructed by plotting the peak area versus the drug concentration.

Acceptance criteria

The correlation coefficient shall be NLT 0.999.

Precision

Repeatability

Prepare the standard solution as per test method and inject into the HPLC system in two replicates. Evaluate the % RSD for the area responses and record the observations into the table Glimepiride. 4ug/ml and Pioglitazone30 ug/ml was injected into the HPLC system in two replicates.

Acceptance criteria

The % RSD six replicate shall be NMT 2.0%.

Method precision

Intraday precision

Three sample preparation of drug were prepared and estimated for parameter as per methodology for all strength of sample in one day. Glimepiride 2, 4, 6 µg/ml and of Pioglitazone 15, 30, 45 µg/ml was injected into the HPLC after 1 Hour interval.

Acceptance criteria

The % RSD Two replicates shall be NMT 2.0%.

Accuracy

It was ascertained on the basis of recovery studies performed by standard addition method. The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte. The accuracy of an analytical method was determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy was calculated from the test results as the percentage of analyte recovered by the assay.

The results of recovery studies and statistical data are recorded in Table.

Preparation of standard solution

Take 2 mg Glimipiride and 15 mg Pioglitazone in 10 ml of Methanol to produce 200 ug/ml and 1500 ug/ml solution of Glimepiride and Pioglitazone respectively. Take 0.1ml from standard solution of Glimepiride and Pioglitazone and volume make up to 10 ml with addition of Methanol then 2 ug/ml conc. of Glimepiride and 15 ug/ml conc. of Pioglitazone is produced. Prepare the standard solution by taking stock solution equivalent to 80%, 100%, and 120% of the standard each in duplicate. Injecte each preparation into the HPLC system.

Table No 2 Dilution table for Accuracy

Sample	Accuracy stock solution-Transfer (ml)	Final volume (ml)	Concentration of Glimipiride (ug/ml)	Concentration of Pioglitazone (ug/ml)
Accuracy- 80%	0.08	10	1.6	12
Accuracy- 80%	0.08	10	1.6	12
Accuracy- 100%	0.1	10	2	15
Accuracy- 100%	0.1	10	2	15
Accuracy- 120%	0.12	10	2.4	18
Accuracy- 120%	0.12	10	2.4	18

Acceptance criteria

Mean recovery should be in the range of 98-102%. The % RSD should be NMT 2.0%

Limit of Detection

Limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantities, under the stated experimental conditions. There are various approaches for determining LOD depending on whether the procedure is non instrumental or instrumental.

Calculation formulae

$$\text{LOD} = \frac{3.3a}{S}$$

Where, a = STD deviation of the response of S1 (lowest standard in the calibration curve)

S= the slope of the calibration curve

Limit of Quantification

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Calculation formula

$$\text{LOQ} = \frac{10a}{S}$$

Where, a = STD deviation of the response of S1 (lowest standard in the calibration curve)

S= the slope of the calibration curve

Robustness

It is measure of capacity of the method to remain unaffected by small but deliberate variation in method parameter and provide an indication of its reliability under normal usage. Robustness of method was studied by deliberately changing the Chromatographic Parameter such as flow rate, % organic phase & wavelengths.

Preparation of standard solution

Standard stock solution was prepared by dissolving 2.0 mg of Glimepiride and 15.0 mg of Pioglitazone in 10 ml Methanol. That gives concentration 200 and 1500 µg/ml for Glimepiride and Pioglitazone respectively. Take 0.1 ml from standard solution of Pioglitazone and Glimepiride and volume make up to 10 ml with addition of Methanol then 200 µg/ml conc. Of Glimepiride and 15000 µg/ml Pioglitazone conc. is produced.

Effect of variation in flow rate of mobile phase by +/- 1%

Standard solution of Glimepiride and Pioglitazone was prepared & injected by varying flow by ±1 % i.e.at (0.90 ml/min) and (1.1 ml/min) chromatogram were recorded & as shown in figure.

Procedure

Standard solution of Glimepiride and Pioglitazone were Injected in chromatograph conditions having varying flow rate of (0.90 ml/min) and (1.1 ml/min) after each injection chromatogram were recorded and system suitability parameter were recorded.

Effect of variation in mobile phase composition by ±1 % (Methanol)

Standard solution of Glimepiride and Pioglitazone were injected in developed method which was varied for ±1 % conc.of mobile phase used i.e. methanol Concentration of methanol was varied by ±1 % hence the mobile phase composed methanol & water in ratio 79:21 & 81:19.v/v.

Procedure

Standard solution of Glimepiride and Pioglitazone was Injected in chromatograph conditions having variation in % mobile phase by ±1 % i.e. con of mobile phase used is methanol & water in ratio 79:21 & 81:19 v/v after each injection chromatogram were recorded and system suitability parameter were recorded.

Effect of variation in wavelength

The effect of wavelength was studied. Standard solution of Glimepiride and Pioglitazone was injected & chromatograms were recorded and system suitability parameter were recorded & reported.

Procedure

Standard solution of Glimepiride and Pioglitazone were Injected in chromatograph conditions having variation in wavelength i.e. 250 nm and 252 nm, after each injection chromatogram was recorded and system suitability parameter were reported.

Application of proposed method for estimation of Glimepiride and Pioglitazone on marketed formulation

Standard Stock solution

Standard stock solution was prepared by dissolving 2 mg of Glimepiride and 15 mg of Pioglitazone in 10.0 ml was Methanol that give concentration 200 ug/ml and 1500 µg/ml for Glimepiride and Pioglitazone respectively. From the standard stock solution, take 0.1 ml standard stock solution then using Methanol volume make up to 10 ml to produce 2 µg/ml of Glimepiride and 15 µg/ml of Pioglitazone.

Sample solution preparation

Ten tablets were weighed and the average weight determined. It was finely powdered and mixed thoroughly. Accurately weighed tablet powder equivalent to 2 mg Glimepiride and 15 mg of Pioglitazone was transferred in a 100 ml volumetric flask and methanol was added. It was sonicated for 10 to 15 minutes. Later the volume was made up to mark with methanol. The solution was filtered through 0-0.45 µm filter

paper.

Total weight of 10 tab wt. = 1.20 gm
Average Weight = 0.120 gm /Tab
Equivalent weight for 30 mg = 15 X 120 /15 = 120 mg

Sample solution preparation

Take 120 mg of powdered tablet sample, added in 10 ml Methanol to produce concentration 200 µg/ml. Glimepiride and 1500 µg/ml of Pioglitazone.

Procedure

Equal volume of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content of Glimepiride and Pioglitazone was calculated by comparing a sample peak with that of standard.

RESULT AND DISCUSSION [09-15]

Mobile Phase Selection

From various mobile phases tried, mobile phase containing Methanol: water (80:20) was selected, since it gives sharp reproducible retention time for Glimipiride and Pioglitazone.

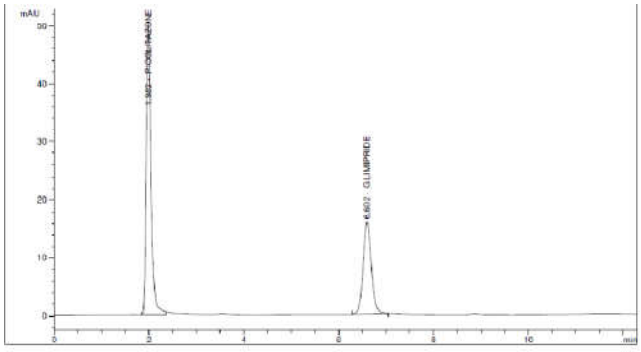

Figure No 1 Chromatogram of Pioglitazone and Glimipiride with mobile phase-Methanol: Water (80:20), wavelength-251 nm

Validation

Linearity

The linearity range was found to be 2-10 ug/ml for Glimepiride and 15-75 ug/ml for Pioglitazone with correlation coefficient value of 0.999. Linearity study was obtained and mentioned as follows.

Table No.3 Results for linearity study of Glimepiride

Sr No.	Conc.(µg/ml)	Area I	Area II	Mean	SD	%RSD
1	2	44.28	44.53	44.4	0.18	0.40
2	4	90.83	89.61	90.22	0.86	0.96
3	6	134.62	134.61	134.615	0.01	0.01
4	8	177.96	178.17	178.065	0.15	0.08
5	10	222.04	221.66	221.85	0.27	0.12

Average Standard Deviation = 0.29
Slope = 22.13, Intercept = 1.006, Correlation Coefficient = 0.999

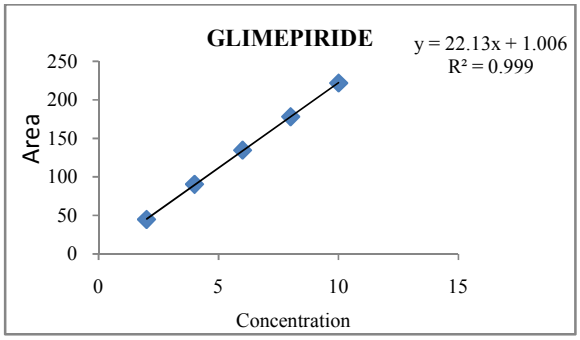

Figure No 2 Calibration curve of Glimepiride

Table No4 Results for linearity study of Pioglitazone

Sr No.	Conc.(µg/ml)	Area I	Area II	Mean	SD	%RSD
1	15	211.32	212.2	211.76	0.62	0.29
2	30	433.11	435.93	434.52	1.99	0.46
3	45	636.57	637.51	637.04	0.66	0.10
4	60	840.04	839.55	839.795	0.35	0.04
5	75	1048.96	1049.54	1049.25	0.41	0.04

Average S.D.= 0.81
Slope = 13.86, Intercept= 10.39, Correlation Coefficient=0.999

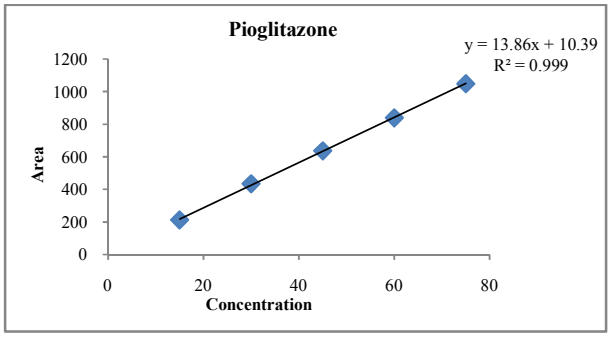

Figure No.3 Calibration curve of pioglitazone

Precision

The % RSD in all two replicate was not more than 2.0% hence the method was found to be precise. The obtained results were mentioned as follows.

Table No 5 Precision studies for Glimepiride

Sr. No.	Conc. µg/ml	Measured area (µg/ml) ± S.D, RSD (%)	
		Repeatability (n=2)	Intermediate Precision (n=2)
1	4	91.03 ± 0.22, 0.24	90.32 ± 0.96, 1.06
2	6	133.32 ± 0.27,0.20	132.72 ± 0.81, 0.61
3	8	177.24 ± 0.02, 0.01	177.98 ± 0.05, 0.03

Table No 6 Precision studies for Pioglitazone

Sr. No.	Conc. µg/ml	Measured area (µg/ml) ± S.D, RSD (%)	
		Repeatability (n=2)	Intermediate Precision (n=2)
1	30	437.19 ± 4.45, 1.02	437.66 ± 3.10, 0.71
2	45	629.66 ± 0.20, 0.03	628.89 ± 6.12, 0.97
3	60	837.41 ± 0.16, 0.02	840.74 ± 2.76, 0.33

Accuracy

Table No.7 Results for accuracy study (80%) of Glimepiride

Sr.no.	Conc.	Amt. Added	Area	Amt. Found	Amt. Recovery	%Recovery
1	2	1.6	80.61	3.59	1.59	99.37
2	2	1.6	80.28	3.58	1.58	98.88
	Mean			3.59	1.59	99.13
	S.D.			0.01	0.01	0.35
	%R.S.D.			0.20	0.45	0.35

Table No 8 Results for accuracy study (100%) of Glimepiride

Sr.no.	Conc.	Amt.Added	Area	Amt. Found	Amt. Recovery	%Recovery
1	2	2	89.5	3.99	1.99	99.94
2	2	2	89.6	4.00	2.00	100.00
		Mean		4.00	20.58	99.97
		S.D.		0.01	0.01	0.04
		%R.S.D.		0.18	0.03	0.04

Table No 9 Results for accuracy study (120%) of Glimepiride

Sr.no.	Conc.	Amt.Added	Area	Amt. Found	Amt. Recovery	%Recovery
1	2	2.4	98.9	4.42	2.42	100.98
2	2	2.4	98.73	4.41	2.41	100.66
		Mean		4.42	2.42	101.58
		S.D.		0.01	0.01	0.23
		%R.S.D.		0.16	0.29	0.22

Table No.10 Results for accuracy study (80%) Pioglitazone

Sr.no.	Conc.	Amt.Added	Area	Amt. Found	Amt. Recovery	%Recovery
1	15	12	384.62	27.00	12.00	100.00
2	15	12	385.36	27.06	12.06	100.57
		Mean		27.03	12.03	100.29
		S.D.		0.04	0.04	0.40
		%R.S.D.		0.16	0.35	0.40

Table No.11 Results for accuracy study (100%) Pioglitazone

Sr.no.	Conc.	Amt.Added	Area	Amt. Found	Amt. Recovery	%Recovery
1	15	15	430.13	30.28	15.28	101.89
2	15	15	428.21	30.14	15.14	100.97
		Mean		30.21	20.58	101.43
		S.D.		0.10	0.10	0.65
		%R.S.D.		0.33	0.48	0.64

Table No.12 Results for accuracy study (120%) Pioglitazone

Sr.no.	Conc.	Amt.Added	Area	Amt. Found	Amt. Recovery	%Recovery
1	15	18	470.94	33.22	18.22	101.27
2	15	18	468.08	33.02	18.02	100.12
		Mean		33.12	18.12	96.23
		S.D.		0.14	0.14	0.81
		%R.S.D.		0.43	0.78	0.85

Mean recovery was found to be in the range of 99-101% for Glimipiride and 96-101% for Pioglitazone respectively. The % RSD was not more than 2.0%. The % RSD was NMT 2.0%. Hence method was found to be accurate.

Limit of Detection

The LOD value of Glimepiride and Pioglitazone was found to be 0.04 and 0.19 respectively.

Limit of Quantification

The LOQ value of Glimepiride and Pioglitazone was found to be 0.13 and 0.58 respectively.

Robustness

Effect of variation in Flow rate of mobile phase by ±1%

Standard solution of Glimepiride and Pioglitazone was prepared & injected by varying flow by ±1% i.e.at (0.90 ml/min) and (1.1 ml/min) chromatograms were recorded & The following results were obtained:

Result for robustness study for change in flow rate of mobile phase

Table No.13 Result for robustness study for change in flow rate of Glimepiride

Sr. no.	Flow rate	Conc.ug/ml	Area	Mean	S.D.	% R.S.D.
1	0.9	4	103.45	103.18	0.38	0.37
2		4	102.91			
3	1.1	4	79.04	79.28	0.34	0.43
4		4	79.52			

Table No14 Result for robustness study for change in flow rate of Pioglitazone

Sr. no.	Flow rate	Conc.ug/ml	Area	Mean	S.D.	% R.S.D.
1	0.9	30	491.83	490.65	1.67	0.34
2		30	489.47			
3	1.1	30	384.59	387.41	3.99	1.03
4		30	390.23			

All the system suitability parameters were passed and method was found to be robust.

Effect of variation in mobile phase composition by±1 % v/v (Methanol)

The chromatographs were obtained as given below:

Result for robustness study for change in mobile phase composition

Table No.15 Result for change in organic phase variation ±1 % v/v Glimepiride

Sr. no.	Mobile phase composition	Conc.ug/ml	Area	Mean	S.D.	% R.S.D.
1	M:W (79:21)	4	82.23	82.0	0.3	0.41
2		4	81.76		3	
3	M:W (81:19)	4	81.92	82.6	1.0	1.27
4		4	83.41	7	5	

Result for robustness study for change in mobile phase composition

Table No.16 Result for change in organic phase variation ±1 % v/v Pioglitazone

Sr. no.	Mobile phase composition	Conc.ug/ml	Area	Mean	S.D.	% R.S.D.
1	M:W (79:21)	30	457.12	454.6	3.53	0.78
2		30	452.13			
3	M:W (81:19)	30	446.96	449.58	3.71	0.82
4		30	452.2			

All the system suitability parameters were passed and method was found to be robust.

Effect of variation in wavelength

Result for robustness study for change in wavelength

Table No 17 Result of change in wavelength of Glimipiride

Sr. no.	Wavelenth change(nm)	Conc.ug/ml	Area	Mean	S.D.	% R.S.D.
1		4	92.63			
2	250	4	9.51	92.6	0.08	0.09
3		4	75.3			
4	252	4	74.87	75.07	0.30	0.40

Table No.18 Result of change in wavelength of Pioglitazone

Sr. no.	Wavelenth change(nm)	Conc.ug/ml	Area	Mean	S.D.	% R.S.D.
1		30	379.67	379.8	0.21	0.05
2	250	30	379.96			
3		30	405.96	405.59	0.53	0.13
4	252	30	405.21			

All the system suitability parameters were passed and method was found to be robust.

Application of proposed method for estimation of Glimepiride from marketed formulation

Table No.19 Results and statistical data for estimation of Glimipiride in marketed formulation

Sr.no.	Conc.	Area	Amount found	%label claim
1	2	89.5	1.99	99.94
2	2	89.6	2.00	100.00
Mean		4.00	20.58	99.97
S.D.		0.01	0.01	0.04
%R.S.D.		0.18	0.03	0.04

Table No.20 Results and statistical data for estimation of pioglitazone in marketed formulation

Sr.no.	Conc.	Area	Amount found	%label claim
1	15	30.28	15.28	101.89
2	15	30.11	15.14	100.97
Mean		30.21	20.58	101.43
S.D.		0.10	0.10	0.65
%R.S.D.		0.33	0.48	0.64

The 99.97% label claim of Glimipiride and pioglitazone as found to be104.43% and 0.04% respectively with 0.64 % RSD not more than 2

CONCLUSION

The method employed is simple, specific and easy to perform and requires short time to analyze the samples. Low limit of quantification and limit of detection makes this method suitable for use in quality control. The mobile phase was optimized to get well seperated peaks of pure samples of Pioglitazone and Glimipiride. From various mobile phases tried, mobile phase containing Methanol: water (80:20) since it gaves sharp reproducible, symmetric peaks for Pioglitazone and Glimipiride in reasonable period of time. Validation is normally done to assure the reliability of the proposed method and was performed as per the ICH guidelines for the following parameters. The developed method was validated by means of Accuracy, Precision, Linearity and Range, LOD, LOQ as per the guidelines prescribed by ICH. The developed HPLC method was found to be stability indicating, accurate, precise, specific and robust which can be used for routine analysis of Pioglitazone and Glimipiride.

References

1. Jane Kelly.CDC. Diabetes. Web sites: http://www.cdc.gov/diabetes/index.htm
2. Tripathi KD. Chapter 19: Insulin, Oral Hypoglycaemic Drugs and Glucagon. Essentials of medical pharmacology.17th Ed.New Delhi: Jaypee; 2013:701-05.
3. Indian pharmacopoeia monograph2014.Vol-1.Pg:1864-1866.
   Website: https://www.pmda.go.jp/files/000214416.pdf
4. Indian pharmacopoeia monograph 2014. Vol-1.Pg: 2498-2500.
   Website: https://www.pmda.go.jp/files/000214416.pdf
5. Bose A.HPLC calibration process parameters in terms of system suitability Test. Austin Chromatogr. 2014;1(2) :4.
6. ICH, Q2 (R1), Harmonized Tripartite Guideline, Validation of Analytical Procedure Methodology, IFPMA, Proceedings of the International Conference on Harmonization Geneva, November 2005; 3-10.
7. US FDA, General principles of validation, Rockville, MD, Center for Drug Evaluation and Research (CDER), May 1987;14.
8. US FDA, Guidelines for submitting samples and analytical data for method validation, Rockville, MD, Center for Drugs and Biologics Department of Health and Human Services, Feb. 1987.5-10.
9. Hossain *et al*.A Validated RP-HPLC Method for Simultaneous Estimation of Antidiabetic Drugs Pioglitazone HCl and Glimepiride. *Bangladesh Pharmaceutical Journal* 16(1), 2013: 69-75.
10. D.Boopathy *et al*. Method development and validation of Simultaneous determination of pioglitazone And Glimepiride in pharmaceutical dosage Form by rp-hplc. *Int.J. ChemTech Res*.2010,2(1): 50-53.
11. Kunithala VK, Vemula SK, Kunithala VB. A new validated rp-hplc method for simultaneous determination of pioglitazone and Glimepiride in the combined tablet dosage form. IJPBS, Volume 2 (2); 2012:191-197.
12. Nirupa Gand *et. al*. "RP-HPLC Analytical Method Development and Validation for Simultaneous Estimation of Three Drugs: Glimepiride, Pioglitazone, and Metformin and Its Pharmaceutical Dosage Forms", Hindawi Publishing Corporation, *Journal of Chemistry*. Volume 2013:1-8.
13. M. S. V. Sakuntala *et al*. "A RP- HPLC method development and validation for the simultaneous estimation of glimepiride and pioglitazone HCl in tablet dosage forms", *Journal of Chemical and Pharmaceutical Research*, 2012, 4(1):154-159.
14. Khohinur Hossain *et al*. "A Validated RP-HPLC Method for Simultaneous Estimation of Antidiabetic Drugs Pioglitazone HCl and Glimepiride" *Bangladesh Pharmaceutical Journal* 16(1): 69-75, 2013.
15. M Suchitra *et al*. "method development and validation of metformin, glimepirideand pioglitazonein tablet dosage form by RP-HPLC" Int. Res. J. Pharm. 2013, 4 (8), 250-254.