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Research Article

A NEW AND RAPID ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METFORMIN, PIOGLITAZONE AND GLIMEPRIDE IN TABLET DOSAGE FORM BY USING UPLC

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ABSTRACT
The present work was undertaken with the aim to develop and validate a rapid and consistent UPLC method in which the peaks will be appear with short period of time as per ICH Guidelines. The proposed method was simple, fast, accurate and precise method for the Quantification of drug in the dosage form, bulk drug as well as for routine analysis in Quality control. UPLC method was developed and validated for simultaneous estimation of Metformin, Pioglitazone and Glimepiride in bulk drug and in combined dosage forms. The UPLC separation was achieved on a Symmetry C_{18} (2.1 x 100 mm, 1.7 µm, Make: BEH) or equivalent in an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (25 %) whose pH was adjusted to 4.3 by using Ortho Phosphoric Acid and Methanol (75 %) [UPLC Grade] The flow rate was monitored at 0.25 ml per min. The wavelength was selected for the detection was 258 nm. The run time was 5 minutes. The retention time found for the drugs Metformin, Pioglitazone and Glimepiride were 0.002 minutes, 1.773 minutes and 2.409 minutes respectively. The % recovery was found to be 99.7 % - 100.9 % for the drug Metformin. The % recovery was found to be 98.4 % - 100.9 % for the drug Pioglitazone. The % recovery was found to be 99.9 % - 101.2 % for the drug Glimepiride. The linearity was established in the range of 400 to 600 ppm for the drug Metformin and 12 to18 ppm for the drug Pioglitazone and 0.8 to 1.2 ppm for the drug Glimepiride. The LOD for the drugs Metformin, Pioglitazone and Glimepiride were found to be 0.12 µg/ml**,** 0.002 µg/ml and 0.002 µg/ml respectively. The LOQ for the drugs Metformin, Pioglitazone and Glimepiride were found to be 0.45 µg/ml**,** 0.15 µg/ml and 0.08 µg/ml respectively. The proposed method was adequate sensitive, reproducible, and specific for the determination of Metformin, Pioglitazone and Glimepiride in bulk as well as in Tablet dosage form. The validation of method was carried out utilizing ICH-guidelines. The described UPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form. Overall the proposed method was found to be suitable and accurate for the Quantitative determination of the drug in Tablet dosage form. The method was simple, precise, accurate and sensitive and applicable for the simultaneous determination of Metformin, Pioglitazone and Glimepiride in bulk drug and in combined dosage forms.
Keywords: Metformin, Pioglitazone and Glimepiride, ICH Guideline, UPLC, LOD, LOQ.

INTRODUCTION

Diabetes is a lifelong (chronic) disease in which there are high levels of sugar in the blood. The diabetes is classified into three major types namely, type I, II, and gestational diabetes. Type II diabetes constitutes 90 % of the diabetic population. The combinational therapy for type II diabetes[1-2] is frequently prescribed when mono therapy fails. The combination of Metformin (MET), Pioglitazone (PIO), and Glimepiride (GLIMP) is approved by FDA for treatment of type II diabetes[3]. Metformin is chemically, 1, 1-dimethyl biguanide hydrochloride. It is the first line drug of choice for the treatment of type2 diabetes. Metformin hydrochloride is a white crystalline powder. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. Bio analytical, HPLC, HPTLC and UV-visible spectrophotometry methods have been reported for its individual determination of Metformin and in combination with other drugs[4-8]. Glimepiride (is chemically 2-(3-ethyl-4-methyl-2-oxo-3 pyrroline-1-carboxamido) ethyl-phenylsulfonyl-3-(trans-4-methylcyclohexyl) urea. It is a medium to long acting sulphonyl urea anti-diabetic drug. Several Spectrophotometric methods, HPLC, HPTLC have been reported for estimation of Glimepiride[5-7]. Glimepiride is a white to yellowish-white, crystalline, odorless to practically odorless powder and is practically insoluble in water. Pioglitazone is one of the PPAR-alpha agonist, insulin sensitizer used to reduce the insulin resistance. Pioglitazone hydrochloride is an odorless white crystalline powder. It is soluble in N, N-dimethyl formamide, slightly soluble in anhydrous ethanol, very slightly soluble in acetone and acetonitrile, practically insoluble in water, and insoluble in ether. It is a thiazolidine Dione derivative and chemically (RS)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxy] benzyl) thiazolidine-2, 4-dione. HPLC and UV-visible spectrophotometry methods have been reported for its individual determination of Pioglitazone and in combination with other drugs[8-15]. As per the literature, various methods are available for the estimation of these three drugs individually or in combination of two drugs in a pharmaceutical dosage form and also from biological samples. Very few methods are available for simultaneous estimation of all the three drugs together in a tablet dosage form. This paper describes a simple, precise, and accurate UPLC method for simultaneous estimation of MET, PIO, and GLIMP. Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows about 9-fold decreases in analysis time as compared to the conventional HPLC system using 5 µm particle size analytical columns, and about 3-fold

decrease in analysis time in comparison with 3 µm particle size analytical columns without compromise on overall separation. The chemical structures for the drug were represented in Figure 1, 2 and 3.

MATERIALS AND METHOD

Chemicals and Reagents Used

The following chemicals were procured for the process: Water [UPLC Grade], Methanol [UPLC Grade], DMSO [UPLC Grade], Metformin, Pioglitazone and Glimepiride [Working standards], Orthophosphoric Acid and Potassium Dihydrogen Phosphate all the chemicals were procured from Standard Solutions and the tablets were collected from the Local market[16-20].

Apparatus and Chromatographic Conditions

The proposed method was performed on Ultra performance liquid chromatography equipped with Auto Sampler and DAD or UV detector. Chromatographic separation was achieved at ambient temperature on column Symmetry C_{18} (2.1 x 100 mm, 1.7 µm, Make: BEH) or equivalent. The flow rate and run time was set to 0.25 ml/min and 5 minutes respectively. Analytical balance Afcoset ER-200A and pH meter Adwa – AD 1020 were used. The wavelength selected was 258 nm. The injection volume was 3 µl.

Preparation of Phosphate buffer and Mobile Phase

The buffer solution was prepared by dissolving accurately weighed 7.0 grams of Potassium Dihydrogen Phosphate and transferred into a clean and dry 1000 ml volumetric flask, dissolved and diluted with 1000 ml water [UPLC Grade]. The final pH of the buffer was adjusted to 4.3 by using Ortho Phosphoric Acid. The Mobile Phase was prepared by mixing 250 ml (25 %) of the above buffer and 750 ml of Methanol [UPLC Grade] (75 %) and degassed in an ultrasonic water bath for 10 minutes. Then the resultant solution was filtered through 0.45 µ filter under vacuum filtration. The Mobile phase was used as Diluent.

Preparation of the Metformin, Pioglitazone and Glimepiride Standard and Sample Solution

Preparation of Stock solution

The stock solution was prepared by weighing accurately 500 mg Metformin, 15 mg Pioglitazone and 1 mg Glimepiride and transferred into a clean and dry 100 ml volumetric flask. About 70 ml of diluent was added and sonicated. The volume was made up to the mark with the same diluent. From the above prepared Stock solution pipette out 1.0 ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added up to the mark to get final concentration.

Preparation of Sample Solution

The sample solution was prepared by weighing equivalently 1423.5 mg of Metformin, Pioglitazone and Glimepiride and transferred into a 100 ml clean and dry volumetric flask and about 70 ml of diluent was added and sonicated to dissolve it completely and the volume made up to the mark with the same solvent. From above prepared stock solution pipette out 1.0 ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added up to the mark to get final concentration. The standard and sample solutions were injected five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

System Suitability

The Tailing factor for the peaks due to Metformin, Pioglitazone and Glimepiride in Standard solution should not be more than 2.0. The Theoretical plates for the Metformin, Pioglitazone and Glimepiride peaks in Standard solution should not be less than 2000. The system suitability of the method was checked by injecting five different preparations of the Metformin, Pioglitazone and Glimepiride standard. The parameters of system suitability were checked.

Assay calculation for Metformin, Pioglitazone and Glimepiride

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt.}{Label\ Claim} \times 100$$

Where, AT = average area counts of sample preparation, AS = average area counts of standard preparation, WS = Weight of working standard taken in mg, WT = Weight of test taken in mg, DS = Dilution of standard solution, DT = Dilution of sample solution, P = Percentage purity of working standard

System Suitability Results for Metformin

- The Tailing factor obtained from the standard injection was 1.66.
- The Theoretical Plates obtained from the standard injection was 2374.

Assay Result for Metformin

$$\frac{1615825}{1614639} \times \frac{500}{100} \times \frac{1}{10} \times \frac{100}{1423.5} \times \frac{10}{1} \times \frac{99.9}{100} \times \frac{1423.5}{500} \times 100 = 99.9\%$$

System Suitability Results for Pioglitazone

- The Tailing factor obtained from the standard injection was 1.41.
- The Theoretical Plates obtained from the standard injection was 6012.

Assay Result for Pioglitazone

$$\frac{186450.3}{189225.7} \times \frac{15}{100} \times \frac{1}{10} \times \frac{100}{1423.5} \times \frac{10}{1} \times \frac{99.8}{100} \times \frac{1423.5}{15} \times 100 = 98.3\%$$

System Suitability Results for Glimepiride

- The Tailing factor obtained from the standard injection was 1.36.
- The Theoretical Plates obtained from the standard injection was 7825.0.

Assay Result for Glimepiride

$$\frac{26326.6}{26455.3} \times \frac{1}{100} \times \frac{1}{10} \times \frac{100}{1423.5} \times \frac{10}{1} \times \frac{99.6}{100} \times \frac{1423.5}{1} \times 100 = 99.1\%$$

Table 1: Precision result for the drug Metformin

Injection	Area
Injection-1	1646797
Injection-2	1589406
Injection-3	1629092
Injection-4	1624144
Injection-5	1628717
Average	1623631
Standard Deviation	20999.81
%RSD	1.29

Table 2: Precision result for the drug Pioglitazone

Injection	Area
Injection-1	188501
Injection-2	188463
Injection-3	188247
Injection-4	188368
Injection-5	189551
Average	188626
Standard Deviation	526.31
%RSD	0.27

Table 3: Precision result for the drug Glimepiride

Injection	Area
Injection-1	26420
Injection-2	26531
Injection-3	26908
Injection-4	25714
Injection-5	26063
Average	26327.2
Standard Deviation	456.42
%RSD	1.73

Table 4: Ruggedness result for the drug Metformin

Injection	Area
Injection-1	1581944
Injection-2	1604180
Injection-3	1628861
Injection-4	1630246
Injection-5	1631640
Average	1615374
Standard Deviation	21854.8
%RSD	1.35

Table 5: Ruggedness result for the drug Pioglitazone

Injection	Area
Injection-1	187658
Injection-2	184345
Injection-3	189163
Injection-4	191303
Injection-5	191727
Average	188839.2
Standard Deviation	3004.481
%RSD	1.59

Table 6: Ruggedness result for the drug Glimepiride

Injection	Area
Injection-1	26645
Injection-2	26084
Injection-3	26556
Injection-4	26912
Injection-5	26600
Average	26559.4
Standard Deviation	299.71
%RSD	1.12

Table 7: Accuracy result for the drug Metformin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50 %	1195607	250.0	250.2	100.1 %	100.2 %
100 %	2383653	500.0	498.9	99.7 %	
150 %	3376292	700.0	706.7	100.9 %	

Table 8: Accuracy result for the drug Pioglitazone

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50 %	145291	7.50	7.57	100.9 %	99.7 %
100 %	287213.3	15.0	14.9	99.8 %	
150 %	424912.7	22.5	22.1	98.4 %	

Table 9: Accuracy result for the drug Glimepiride

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50 %	20418.3	0.5	0.5	100.9 %	100.7 %
100 %	40985.3	1.0	1.01	101.2 %	
150 %	60682.6	1.5	1.49	99.9 %	

Table 10: Linearity Curve for the drug Metformin

S. No.	Linearity Level	Concentration	Area
1	I	400 ppm	1140127
2	II	450 ppm	1354078
3	III	500 ppm	1621076
4	IV	550 ppm	1820616
5	V	600 ppm	2058383
Correlation Coefficient			0.999

Table 11: Linearity Curve for the drug Pioglitazone

S. No.	Linearity Level	Concentration	Area
1	I	12 ppm	128658
2	II	13.5 ppm	157561
3	III	15 ppm	189193
4	IV	16.5 ppm	212302
5	V	18 ppm	246300
Correlation Coefficient			0.999

Table 12: Linearity Curve for the drug Glimepiride

S. No.	Linearity Level	Concentration	Area
1	I	0.8 ppm	19728
2	II	0.9 ppm	22783
3	III	1.0 ppm	27091
4	IV	1.1 ppm	30545
5	V	1.2 ppm	34700
Correlation Coefficient			0.999

Table 13: Result for effect of variation in flow rate for the drug Metformin

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.20	2205.0	1.59
2	0.25	2374.0	1.66
3	0.30	2119.0	1.58

Table 14: Result for effect of variation in flow rate for the drug Pioglitazone

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.20	5480.0	1.31
2	0.25	6012.0	1.41
3	0.30	2201.0	1.21

Table 15: Result for effect of variation in flow rate for the drug Glimepiride

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.20	6679.0	1.07
2	0.25	7825.0	1.36
3	0.30	3422.0	1.05

Table 16: Result for effect of variation in mobile phase composition for the Drug Metformin (Organic Phase)

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2563.0	1.58
2	Actual	2374.0	1.66
3	10% more	2419.0	1.81

Table 17: Result for effect of variation in mobile phase composition for the Drug Pioglitazone (Organic Phase)

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	5893.0	1.31
2	Actual	6012.0	1.41
3	10% more	5701.0	1.21

Table 18: Result for effect of variation in mobile phase composition for the Drug Glimepiride (Organic Phase)

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6873.0	1.11
2	Actual	7825.0	1.36
3	10% more	6631.0	1.12

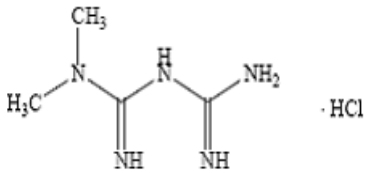

Figure 1: Chemical Structure of Metformin HCL

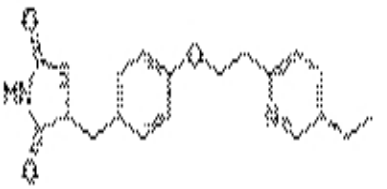

Figure 2: Chemical Structure of Pioglitazone

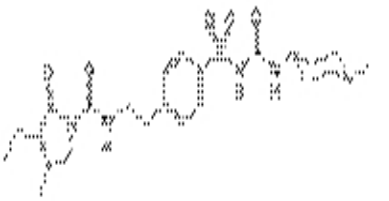

Figure 3: Chemical Structure of Glimepiride

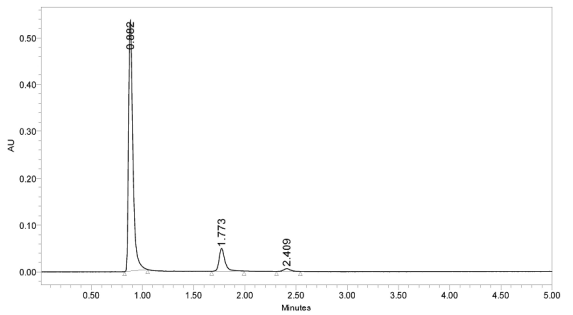

Figure 4: Chromatogram for the Optimized Method Development

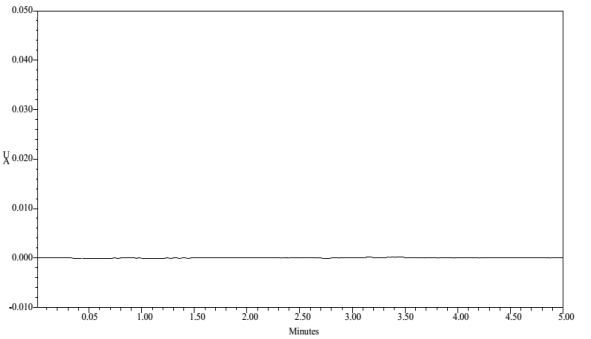

Figure 5: Chromatogram for the Blank

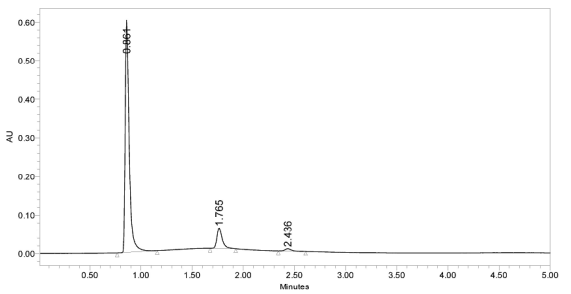

Figure 6: Chromatogram for the Standard Drug

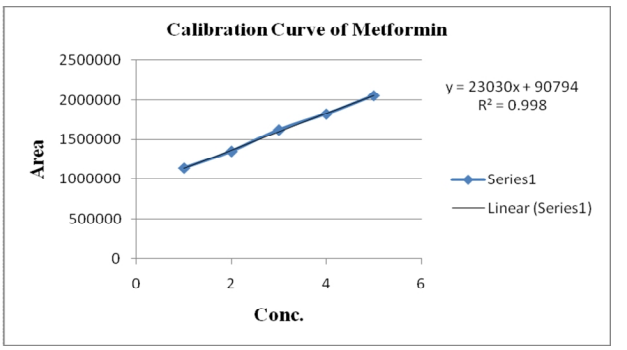

Figure 7: Linearity Curve for the drug Metformin

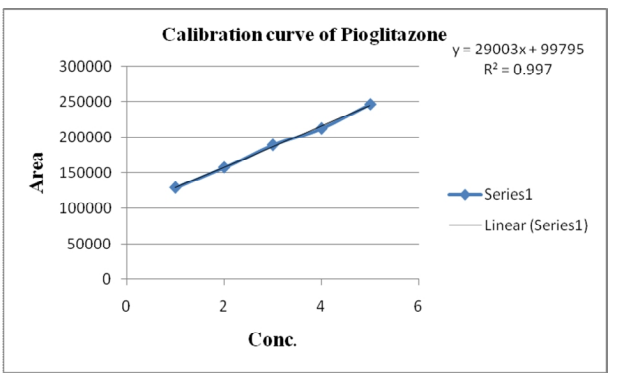

Figure 8: Linearity Curve for the drug Pioglitazone

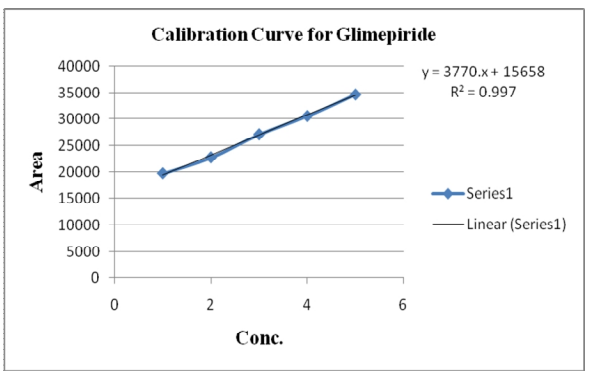

Figure 9: Linearity Curve for the drug Glimepiride

RESULTS AND DISCUSSION

In this UPLC method, chromatographic conditions were optimized to obtain best peak shape and resolution. Mobile phase selection of is based on the peak parameters like symmetry, theoretical plates, capacity factor tailing factor and ease of preparation and cost. Chromatograms of Standard and Formulation are given below in Figure 4 and Figure 6 respectively. The optimum wavelength for detection and quantification was set at 262 nm. The Ultra performance liquid chromatography (UPLC) methods was developed and validated for simultaneous estimation of Metformin, Pioglitazone and Glimepiride in bulk drug and in combined dosage forms. The UPLC separation was achieved on a Symmetry C_{18} (2.1 x 100 mm, 1.7 µm, Make: BEH) or equivalent in an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (25 %) whose pH was adjusted to 4. 3 by using Ortho Phosphoric Acid and Methanol (75 %) [UPLC Grade] The flow rate was monitored at 3.0 ml per min. The wavelength was selected for the detection was 258 nm. The run time was 5 minutes. The retention time found for the drugs Metformin, Pioglitazone and Glimepiride were 0.002 minutes, 1.773 minutes and 2.409 minutes respectively. It was represented in Figure 4.

Method Validation

Method was validated according to ICH guidelines for validation of analytical procedures[21-23].

Calibration Curve and Linearity

In order to test the linearity of the method, five dilutions of the working standard solutions for the drugs Metformin, Pioglitazone and Glimepiride were prepared. The linearity was established in the range of 400 to 600 ppm for the drug Metformin and 12 to18 ppm for the drug Pioglitazone and 0.8 to1.2 ppm for the drug Glimepiride. The data were represented in Table 10, 11 and 12. Each of the dilution was injected into the column and the Linearity Curve was represented in Figure 7, 8 and 9. The Correlation coefficient (R^2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which was in the acceptance limit.

Precision

The Precision data for the drugs Metformin, Pioglitazone and Glimepiride were represented in Table 1, 2 and 3. The % RSD for sample should be NMT 2. The % RSD for the standard solution was found to be 1.29, 0.27 and 1.73 for the drugs Metformin, Pioglitazone and Glimepiride respectively, which is within the limits hence the method was precise.

Intermediate Precision

When the drugs Metformin, Pioglitazone and Glimepiride were analyzed by the proposed method in the intra and inter-day (Ruggedness) variation, a low coefficient of variation was observed it was represented in Table 4, 5 and 6 which shows that the developed UPLC method was highly precise. The % RSD was found to be 1.35, 1.59 and 1.12 for the drugs Metformin, Pioglitazone and Glimepiride respectively, which is within the limits.

Accuracy

The standard solution with Accuracy -80 %, Accuracy -100 % and Accuracy -120 % were injected into chromatographic system and calculated the amount found and amount added for Metformin, Pioglitazone and Glimepiride and further calculated the individual recovery and mean recovery values (Table 7, 8 and 9). The % recovery was found to be 99.7 % - 100.9 % for the drug Metformin. The % recovery was found to be 98.4 % - 100.9 % for the drug Pioglitazone. The % recovery was found to be 99.9 % - 101.2 % for the drug Glimepiride.

Limit of Detection and Limit of Quantification

The Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The LOD for the drugs Metformin, Pioglitazone and Glimepiride were found to be 0.12 µg/ml, 0.002 µg/ml and 0.002 µg/ml respectively. The LOQ for the drugs Metformin, Pioglitazone and Glimepiride were found to be 0.45 µg/ml, 0.15 µg/ml and 0.08 µg/ml respectively. The Signal to noise ratio should be 3 for LOD. The results obtained were within the limit. The Signal to noise ratio should be 10 for LOQ solution. The results obtained were within the limit.

Robustness

The Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in Phosphate Buffer and Methanol [UPLC Grade] in the mobile phase. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The system suitability parameters were within the limits and shown in Table 13, 14, 15, 16, 17 and 18.

CONCLUSION

It was concluded that the proposed new UPLC method developed for the quantitative determination of Metformin, Pioglitazone and Glimepiride in bulk as well as in its formulations was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phases were simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopted as an alternative method to report routine determination of Metformin, Pioglitazone and Glimepiride depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies for the drug Metformin, Pioglitazone and Glimepiride. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. The proposed method can be use in future for the clinical, biological and pharmacokinetic studies of Metformin, Pioglitazone and Glimepiride.

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