Method Development and Validation of Rp-Hplc Method for The Determination of Metformin Hydro Chloride And Pioglitazone in Tablet Dosage Forms

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ABSTRACT

The main objective of the present work is to develop a simple, precise, specific and stability method indicating reverse phase high performance liquid chromatography method for the estimation of Metformin hydrochloride and Pioglitazone in tablet dosage form. The analysis was carried out by using a mobile phase consisting of 0.02M dipotassium hydrogen phosphate and acetonitrile in the ratio 55:45.the detection was carried out by using UV – Visible SPD 20 A at 240 nm.The column was phenominex Gemini C18 (250×4.6mm×5µ).The flow rate was selected as 1ml/min. The retention time of Metformin HCL and Pioglitazone was found to be 4.285 and 7.485 respectively. It was observed that Metformin HCL and Pioglitazone was linear in the range of 80% to 120% for the target concentration .The linearity range of 10-50mg/ml for Metformin HCL and Pioglitazone were found to obey linearity with a correlation coefficient of 0.999 and 0.999 respectively. The forced degradation studies were performed as per the guidelines of the The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use under acidic, alkaline, oxidative, thermal, photostability, and neutral conditions. This method was successfully validated for all the parameters and could detect the correct amounts of active drug substance in formulations that are available in the market. This developed method in the present study could be successfully employed for the simultaneous estimation of Metformin hydrochloride and Pioglitazone in pharmaceutical dosage form.

Key words: Metformin, Pioglitazone, RP-HPLC, validation, force degradation, stability

INTRODUCTION

Metformin (MET) is chemically 1-carbamimidamidoN,N-dimethylmethanimidamide. It belongs to the biguanide class of antidiabetic drugs. It is the first line drug of choice for the treatment of type-2 diabetes. It activates adenosine monophosphate activated protein kinase, a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and metabolism of glucose and fats.[3-5] Pioglitazone, (±)-5-[p-[2-(5-ethyl-2-pyridyl)- ethoxy] benzyl]-2,4-thiazolidinedione1 are used in the treatment of type 2 diabetes. Pioglitazone hydrochloride has been shown to affect abnormal glucose and lipid metabolism associated with insulin resistance by enhancing insulin action on peripheral tissues. Many patients suffering from type 2 diabetes require treatment with more than one antihyperglycemic drug to achieve optimal glycemic control..

A literature survey reveals a good number of analytical methods for the estimation of metformin hydrochloride and Pioglitazone individually or in combination with other drugs using ultraviolet (UV) spectrophotometry,[6] high performance liquid chromatography (HPLC),[7] HPTLC,[8] and LC-MS/MS[9] Hence, we tried to develop a simple stability indicating HPLC method for the estimation of the selected drugs. The developed method has been validated as per the guidelines of the ICH.[10] To establish the stability indicating nature of the method forced degradation studies were planned for the proposed method under acidic, alkaline, oxidative, thermal, photostability, and neutral conditions.

The combination of Metformin HCL and Pioglitazone was selected for the present study. According to the literature survey conducted, it was observed that no method was reported in RP-HPLC for the estimation of individual drug carried out. Hence present study aims to develop an accurate, precise, specific, linear, simple, rapid, validated and cost effective analytical method for Metformin HCL and Pioglitazone in tablet dosage form by RP-HPLC method.

METHODS AND MATERIAL

Chemicals and Reagents: Pure Metformin and Pioglitazone were obtained as gift samples from Glenmark Pharmaceutical Ltd. Sinnar, Dist. Nasik. HPLC grade Acetonitrile and

Methanol from Merck, AR grade ortho phosphoric acid from Qualigens, and AR grade Potassium Dihydrogen Orthophosphate from Emplura, UV-1100 Shimadzu, HPLC, Sonicator from PCI.

Instrumentation

Separation was performed with Shimadzu HPLC equipped with a pump 2695, auto sampler and UV detector. HPLC workstation software was applied for data collecting and processing. UV – Visible SPD 20 A at 240nm.The column was phenominex Gemini C18 (250×4.6mm×5µ).The flow rate was selected as 1ml/min. The injection volume was 20 µL and all the experiments were performed at temperature 300C. The run time was set at 10.20 min. Mixture of Methanol and Water in the ratio of 10:30% v/v is used as a solvent which is sonicated to degas. HPLC grade water was obtained from a Milli – Q water purification system.

Methodology

Preparation of buffer pH 4.5

Prepare about 0.02M dipotassium hydrogen phosphate in a suitable conical flask and adjust the pH to 4.5 with orthophosphoric acid.(0.02M of di potassiumhydrogen phosphate is prepared by taking 1.3602mg of dipotassiumhydrogen phosphate in a volumetric flask , and make up to 1L with water).

Preparation of mobile phase:

Prepare a mixture of buffer 4.5 pH, acetonitrile and methanol in the ratio 65:25:10 filter through 0.45µ membrane filter and degas it.

Mobile phase: Prepare a mixture of Buffer and Acetonitrile in the ratio of (70:30). Filter and degas.

Chromatographic condition Use suitable High Performance Liquid Chromatography equipped with UV-visible detector.

Column : Phenominex Gemini C18 (250×4.6mm×5µ).

Wavelength : 229 nm Injection Volume : 10µL

Column Temperature : Ambient

Flow rate : 1.0 mL/min.

Retention time of Metformin hydrochloride is about 3.0-4.0 min and Sitagliptin is about 5.0-7.0min.

Preparation of Diluent: Used mobile phase as diluents.

Preparation of Standard Stock Solution:

Standard stock solution of Metformin and Pioglitazone was prepared by dissolving 100 mg of Metformin and 10 mg of Pioglitazone respectively in 100 mL mobile phase. The solutions are sonicated to dissolve the drugs. Further these solutions were diluted to prepare concentrations of 500 µg/ml and 2 µg/ml of Metformin and Pioglitazone respectively.

Preparation of Test Solution: Test stock solution of Metformin and Pioglitazone was prepared by dissolving about 2.0 g of test sample (Brand Name: Glimestar M) (which is equivalent to 100 mg Metformin and 10 mg of Pioglitazone) into 100 ml volumetric flask. The solutions are sonicated to dissolve the drugs. Further these solutions were diluted to prepare concentrations of 500 µg/ml and 2 µg/ml of Metformin and Pioglitazone respectively. Preparation of Potassium Dihydrogen Phosphate Buffer: Weighed about 272.1 mg of Potassium Dihydrogen phosphate and dissolved in 100 ml of water and pH was adjusted to 3.0 with Phosphoric acid and then filtered through 0.45 µ nylon membrane filter.

Method Validation

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. The other component may include excipients, impurities, degradation product etc. Peak purity test may be useful to show that the analyte chromatographic peak is not contributed by more than one component (e.g. .diode array, mass, spectroscopy).

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line. Linearity should be evaluated by visual inspection of a plot

of signal as a function of analyte concentration. The correlation coefficient, y- intercept, slope of the regression line and the residual sum of squares should be calculated.

System precision

The system precision was evaluated by measuring the peak response of Metformin HCL and Pioglitazone, WS solution prepared as per the proposed method and chromatograms were recorded.

Accuracy

To document accuracy, the ICH guideline on methodology recommends collecting data from a minimum of nine determinations over a minimum of three concentration levels covering the specified range.

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters.

The robustness of a method is evaluated by varying method parameters such as percent organic solvent, pH, ionic strength or temperature and determining the effect on the results of the method. Robustness tests were generally introduced to avoid problems in linear laboratory studies and toidentify the potentially responsible factors.

Ruggedness

To determine the degree of reproducibility of the results by this method involved the studies of the analyst to analyst and day to day; that is to carry out precision study in six replicate of an assay of a single batch sample bytwo different analysts on two different days.

LOD and LOQ

ICH has recommended some method for determining the limit of detection. The method may be either instrumental or non-instrumental. . It is calculated using formula

LOD = 3.3 σ/S; where, σ = S.D; S =Slope

Limit of Quantitation (LOQ) is also based on standard deviation of the response and the slope of calibration curve.

LOD = 3.3s / S

Where, s = Standard deviation of the response S = Slope of calibration curve

Force Degradation Studies

Performed the forced degradation of test method to demonstrate the noninterference of impurities, degradation products in quantification of analyte by various stress conditions like acid, base peroxide and thermal.

RESULT AND DISCUSSION

The separation method was carried out by using a mobile phase consisting of 0.02M potassium hydrogen phosphate and acetonitrile in the ratio 55:45.the detection was carried out by using UV -Visible SPD 20 A at 240nm.The column was phenominex Gemini C18 (250×4.6mm×5µ).The flow rate was selected as 1ml/min.

The retention time of Metformin HCL and Pioglitazone was found to be 4.287 and 7.473 respectively. The asymmetry factor or tailing 1.008 and 1.011 respectively, which indicates symmetrical nature of the peak. The number of theoretical plates of Metformin HCL and Pioglitazone was found to be 8840 and 12044 respectively, which indicates the efficiency performance of the column.

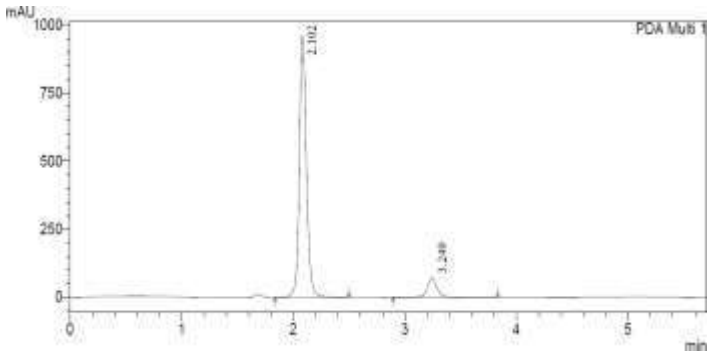

Figure: 1 Standard chromatogram of Metformin hydrochloride & Pioglitazone

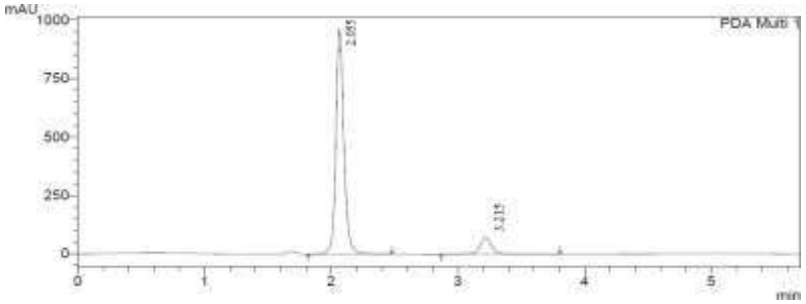

Figure: 2 Sample chromatogram of Metformin hydrochloride & Pioglitazone

Table 1. Percent purity of Metformin hydrochloride and Pioglitazone in combined dosage form.

Drug	Label claim	% Drug found ± SD	% RSD
Metformin hydrochloride	500 mg	99.8	0.15
Pioglitazone	1 mg	99.9	0.20

System Suitability: To verify whether the analytical system is working properly or it can give accurate and precise results, the system suitability parameters are to be set.
Inject separately 20 µ L each of the following solutions into the HPLC.

Table: 2 System suitability parameters.

System Suitability Parameter	Metformin HCL	Glimeperide
Tailing factor	1.056	1.000
No. of Theoretical Plates	9226	11340
Resolution	13.861	

From the linearity studies, specified concentration levels were determined .It was observed that Metformin HCL and Pioglitazone was linear in the range of 80% to 120% for the target concentration .The linearity range of 10-50mg/ml for Metformin HCL and Pioglitazone were found to obey linearity with a correlation coefficient of 0.999 and 0.999 respectively.
The validation of proposed method was verified by recovery studies. The percentage recovery range was found to be satisfied which represent in results. The robustness studies were performed by changing the pH and wavelength. The ruggedness study was also performed.
The analytical method validation was carried as per ICH guidelines and given below are the tables are the summary of the result.

Linearity: Metformin HCL & Pioglitazone were found to be linear in the range of 80 to 120 ugl/ml. The correlation coefficient of metformin HCL. & Pioglitazone were found to be 0.999 and 0.999 respectively. The linearity range of metformin HCL and Pioglitazone were shown in table number 3 the calibration curves were plotted as peak area vs concentration of the standard solution. The calibration graph show linear response over the range 80 to 120 ug/ml.

Accuracy: The accuracy of the method was determined by recovery experiment; a known quantity of the pure drug was added to the pre-analyzed sample formulation at 80%, 100% and 120%. The recovery studies were carried out three times of each level and the percentage recovery and percentage relative standard deviation were calculated.

Precision: Precision was measured in terms of repeatability of application and measurement. Study was carried out by injecting six replicates of the standard at a concentration of 500µg/mL for Metformin hydrochloride and 5µg/mL for Pioglitazone. And the RSD calculated from replicates of assay values NMT 2.0%.

Table: 3 Precision study parameters.

Drug	Concentration added, µg mL	Intra-day precision		Inter-day precision	
		Mean amount found, µg/mL	% RSD (n = 6)	Mean amount found, µg/mL	% RSD (n = 6)
Metformin hydrochloride	500	499.21±0.23	0.48	499.01±0.31	0.42
Pioglitazone	1	4.94 ±0.52	0.35	4.88±0.21	0.35

The percentage recovery of metformin HCL and Pioglitazone were found to be in the range 99.78, 99.24, 101.18 and 99.92, 99.05, 100.03 respectively.

Limit of Detection (LOD): The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, though not necessarily quantitated. It is a limit test that specifies whether or not an analyte is above or below a certain value. ICH has recommended some methods for determining the limit of detection. The methodmay be either instrumental or non-instrumental.

Table 4. Robustness Study for Metformin HCL and Pioglitazone

LOD	Metformin HCL:(ug)	Glimeperide(ug)
1.	1.05	7.12

Limit of Quantitation (LOQ): The limit of Quantitation (LOQ) is defined as the lowest concentration of the analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Limit of Quantitation (LOQ) is also based on standard deviation of the response and the slope of the calibration curve.

Table 5 Robustness Study for Metformin HCL and Pioglitazone

LOQ	Metformin HCL (ug)	Pioglitazone (ug)
1.	5.6	3.5

Robustness: Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. The robustness of a method is evaluated by varying method parameters such as percent organic solvent, pH, ionic strength or temperature and determining the effect on the results of the method. Robustness tests were generally introduced to avoid problems in linear laboratory studies and to identify the potentially responsible factors.

Table 6. Robustness Study for Metformin HCL and Pioglitazone

Robustness Criteria	RT of Metformin	RT of Pioglitazone
Change in flow +0.2	3.707	6.100
Change in flow -0.2	4.790	7.560
Change in Wavelength by -PH	4.27	7.44
Change in Wavelength by +PH	4.28	7.48

Ruggedness: Ruggedness of analytical method is the degree of reproducibility of the results obtained by the analysis of the same samples under a variety of test conditions such as different laboratories, analysts, instruments, temperature, different days etc.

Table 7. Ruggedness analysis study

Sample No.	% Assay of Metformin HCL	% Assay of Pioglitazone
Analyst- 1	98.9	102.0

CONCLUSION

The present work involved the development of accurate, precise, simple and suitable RPHPLC method for estimation of the drugs in multicomponent formulations. Hence the present study was undertaken with an objective of developing suitable, sensitive and simple analytical method like RP-HPLC method for simultaneous estimation of both drugs in their combined dosage form. The proposed method is found to be accurate, precise, linear, specific and robust.

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