Research Article

Stability Indicating RP-HPLC Method Validation for Assay of Metformin and Pioglitazone in Pharmaceutical Dosage Form as per USP/ICH Guidelines

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ABSTRACT

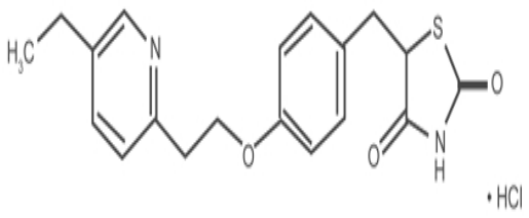
A simple, specific, accurate stability indicating RP-HPLC method was developed for simultaneous determination of metformin and pioglitazone in pure and tablet dosage form using Zorbax Eclipse XDB, C18, 150 X 4.6 mm, 5µm column, and mobile phase composing of phosphate buffer (pH 7.1) ,Acetonitrile & Triethylamine in proportion of 660:340:1 (v/v). The flow rate was 1.0 ml/min and the effluents were monitored at 225 nm. The retention time of metformin, pioglitazone were 3.7min, 7.2 ±0.2min respectively. Drugs were subjected to acidic, alkali, neutral hydrolysis, oxidation, photolytic and UV degradation. The degradation studies indicated the susceptibility of drugs to various degradations. The method was statistically validated for accuracy, precision, linearity and forced degradation. Quantitative and recovery studies of the dosage form were also carried out NLT 98%and NMT 102.0% ,the % RSD was found to be not more than 2.0% , regression and correlation coefficients for both the drugs NLT 0.999, Tailing factor NMT 2, Theoretical plates for metformin and Pioglitazone NLT 1500 and 3000 respectively. The developed method is simple, rapid and accurate and hence can be used for routine quality control analysis.

Keywords: Metformin, Pioglitazone, RP-HPLC, Stability indicating.

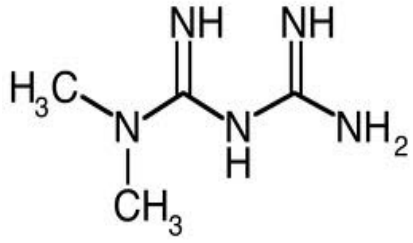
INTRODUCTION

Stability is defined as the capacity of a drug substance to remain within established specifications to maintain its identity, strength, quality, and purity throughout the re-test or expiration dating periods1. Stability testing of an active substance or finished product provides evidence on how the quality of a drug product varies with time. It is influenced by a variety of environmental factors such as temperature, humidity, acid, alkali and light. Knowledge from stability studies enables understanding of the long-term effects of environment on drugs. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and excipients in the drug product. The results are applied in developing manufacturing processes and selecting proper packaging, storage conditions, product's shelf life and expiration dates. Because the distribution environment is highly variable, products must be distributed in a manner that ensures product quality will not be adversely affected. The effect of possible temperature and humidity fluctuations, outside of labelled storage conditions, during transportation of drug products can be evaluated on the basis of the stability analysis of the drug.

Metformin (1, 1-Dimethylbiguanid Hydrochloride), improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis6. Pioglitazone is a thiazolidinedione derivative. Chemically it is [(±)-5-[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl]-methyl]-2, 4-thiazolidinedionemonohydro-chloride, widely used in patients with type-2 diabetes (non insulin dependent diabetes) 7. 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 in order to achieve optimal glycemic control.



Pioglitazone hydrochloride



Metformin

MATERIALS AND METHODS

Pure metformin, pioglitazone samples was received as gift from Smruthi Organics, Solapur and Kenvista Pvt. Ltd., Hyderabad, India. Metformin& Pioglitazone 850 mg /15mg and 500 mg/15mg tablets were purchased from local drug market. HPLC grade methanol and water was purchased from RFCL Ltd, New Delhi, India. Buffer materials, Acetonitrile, Triethylamine and all other chemicals were of analytical-reagent grade.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

HPLC system Agilentseries-1200consists of autosampler G1329A,Degasser G1322A, Diode array detector G1315C Chromatographic separations were achieved by using Zorbax Eclipse C18 (150 X 4.6 mm), 5µm column . Injections of sample were made by auto injector. Mobile phase was buffer, Acetonitrile and pH was adjusted to 7.1 using ortho ph0sphoric acid. Other parameter like injection volume was set to 20 µl for a run time of 15 min with flow rate 1.0 ml/min. The column was maintained at ambient temperature and the eluent was detected at 225 nm. Thermal study was conducted by using thermo lab-humidity chamber, Andheri, Mumbai, India. The UV study was conducted by using thermocon-instruments (p) LTD, precision incubator, Bangalore, India.

Diluent preparation

0.1N Hydrochloric acid

Transferred 8.8mL of Hydrochloric acid to 1000mL of water and mix well.

Diluent-1

Mixed 0.1N HCl and Methanol in the ratio 70:30 (v/v).

Diluent-2

Mobile phase used as diluent-2.

Blank Preparation

i. Mix Diluent-1 and Diluent-2 in the ratio 20:80 (v/v)
ii. Filter a portion of blank through Pall life sciences Ultrapore N66, 0.45µm Nylon 66,13mm membrane syringe filter. Discard 5ml of the initial sample and fill the HPLC vial with next sample.

Standard Stock Preparation

For Metformin

a. Accurately weigh about 85 mg of Metformin HCl working standard into a 100mL volumetric flask.
b. Add 70mL of diluent -1, sonicate to dissolve the material completely, dilute to volume with diluent-1 and mix well.

For pioglitazone

a. Accurately weigh about 65 mg of Pioglitazone HCl working standard into a 200 mL volumetric flask.
b. Add 140mL of diluents -1, sonicate to dissolve the material completely, dilute to volume with diluent-1 and mix well**.**

Standard preparation

a. 5mL of Metformin standard stock solution and 5mL of Pioglitazone standard stock solution transfer into a 50 mL volumetric flask and make up to the volume with diluent-2.
b. Filter through 0.45µm Pall life sciences filter.
c. Discard initial 5mL of sample and collect clear solution.

Test Preparation

Direct Dropping Method (850/15mg):

a. Weigh and transfer 5 tablets into 500mLvolumetric flask.
b. Add 350mL of diluents-1 and sonicate for 40 minutes with intermediate shaking and make up to the volume with diluents-1 and mix well.
c. A portion of the above solution Centrifuge at 3500 RPM for 10 minutes.

For Metformin

a. 5 mL of clear test solution is transfer into a 50 mL volumetric flask and make up to the volume with diluent-2.(stock-1)
b. 5 mL of clear test solution (stock-1) is transfer into a 50 mL volumetric flask and make up to the volume with diluent-2.

c. Filter a portion of solution through Pall Life Science Ultipor N 66, 0.45µm Nylon 66,13mm membrane syringe filter.
d. Discard initial 5mL of sample and collect clear solution into HPLC vial.

For Pioglitazone

a. 5mL of clear test solution is transfer into a 25 mL volumetric flask and make up to the volume with diluent-2.
b. Filter a portion of solution through Pall Life Science Ultipor N 66, 0.45µm Nylon 66,13mm membrane syringe filter.
c. Discard initial 5mL of sample and collect clear solution into HPLC vial.

Direct Dropping Method (500/15mg)

i. Weigh and transfer 8 tablets into 500mLvolumetric flask.
ii. Add 350mL of diluents-1 and sonicate for 40 minutes with intermediate shaking and make up to the volume with diluents-1 and mix well.
iii. A portion of the above solution Centrifuge at 3500 RPM for 10 minutes.

For Metformin

i. 5 mL of clear test solution is transfer into a 50 mL volumetric flask and make up to the volume with diluent-2.(stock-1)
ii. 5 mL of clear test solution (stock-1) is transfer into a 50 mL volumetric flask and make up to the volume with diluent-2.
iii. Filter a portion of solution through Pall Life Science Ultipor N 66, 0.45µm Nylon 66,13mm membrane syringe filter.
iv. Discard initial 5mL of sample and collect clear solution into HPLC vial.

For Pioglitazone

i. 3 mL of clear test solution is transfer into a 25 mL volumetric flask and make up to the volume with diluent-2.
ii. Filter a portion of solution through Pall Life Science Ultipor N 66, 0.45µm Nylon 66,13mm membrane syringe filter.
iii. Discard initial 5mL of sample and collect clear solution into HPLC vial.

FORCED DEGRADATION STUDIES (stress testing)

Sampling was carried out every day to study its degradation behaviour. For all the stability study, the formation of degradable product was conformed by comparing to chromatogram of the degradable mixture with the blank solvent stored under normal condition and the drug solution kept under normal condition. All stressed samples were analyzed by developed HPLC method.

RESULTS AND DISCUSSION

Development and optimization of the stability-indicating HPLC method

In all HPLC runs, the mobile phase was filtered through 0.45µm nylon membrane under vaccum and degassed before use. The injection volume was 20 µL and the mobile phase flow rate was 1 ml/min, the analytical wavelength selected was 225 nm. HPLC studies were carried out on all reaction solution individually. Mobile phase optimization was performed using various solvents at different compositions. Solutions were adjusted with varying pH values between 3 to 8. Mobile phase composed of potassium dihydrogen phosphate buffer: Acetonitriler: Triethylamine in the ratio of 660:340:1 (v/v) at pH 7.1with orthophosphoric acid was found to give chromatograms with satisfactory resolution.

Acid stressed sample (5N HCl)

For Test Sample

851.47 mg of Metformin API and 16.605 mg of Pioglitazone API and 228.26 mg Placebo blend is transferred into 250mL Round bottom flask, added about 5mL of cosolvent and then added 45 mL of 5 N Hcl Refluxed at 90^0C for 5Hrs . Centrifuged a portion of solution at 3500 RPM for 10 minutes.

For metformin

Neutralised 5mLof above clear sample to 10mL with 5N NaOH. Diluted 5mL of above solution to 50mL with diluents-2. Diluted 5mL of above solution to 50mL with diluents-2.

For pioglitazone

Neutralised 5mLof above clear sample to 10mL with 5N NaOH. Diluted 5mL of above solution to 25mL with diluents-2

Alkali stressed sample (1 N NaOH)

Metformin Test Sample

1280.1 mg of Metformin API is transferred into 50mL Round bottom flask, added about 5mL of cosolvent and then added 45 mL of 1 N NaOH. Refluxed at 90^0C for 15mins for metformin . Centrifuged a portion of solution at 3500 RPM for 10 minutes. Neutralised the above clear sample to 50mL with 1N Hcl. Diluted 5mL of above 5ml of solution to 10mL

with diluents-2. Diluted 2mL of above solution to 25mL with diluents-2.

Pioglitazone Test Sample

1280.1 mg of Pioglitazone API is transferred into 50mL Round bottom flask, added about 5mL of cosolvent and then added 45 mL of 1 N NaOH. Refluxed at 90^{0}C for 15mins for metformin. Centrifuged a portion of solution at 3500 RPM for 10 minutes .Neutralised the above clear sample to 50mL with 1N Hcl. Diluted 5mL of above 5ml of solution to 10mL with diluents-2. Diluted 3mL of above solution to 10mL with diluents-2.

UV light Exposed Sample

For Test Sample

5463.75 mg of Metformin, Pioglitazone and Placebo mix and transferred into 500.0 mL volumetric flask, added about 350 mL of diluen-1, sonicated for 40 minutes with intermittent shaking and diluted to volume with diluents-1. Centrifuged a portion of solution at 3500 RPM for 10 minutes and 5.0 mL of clear centrifugate is diluted to 50 mL with diluents-2 and taken 5.0 ml of above solution and made upto 50 ml with Diluent-2 for metformin. and 5.0 mL of the above solution is diluted to 25 mL with diluents-2.

Humidity Exposed Sample

For Test Sample

5381.83mg of Metformin, Pioglitazone and Placebo mix and transferred into 500.0 mL volumetric flask, added about 350 mL of diluen-1, sonicated for 40 minutes with intermediate shaking and diluted to volume with diluents-1. Centrifuged a portion of solution at 3500 RPM for 10 minutes and 5.0 mL of clear centrifugate is diluted to 50 mL with diluents-2 and taken 5.0 ml of above solution and made upto 50 ml with Diluent-2 for metformin. 5.0 mL of the centrifugate solution is diluted to 25 mL with diluents-2 for pioglitazone.

Neutral Stressed sample

For Test Sample

850.42 mg of Metformin and 17.599mg of Pioglitazone API and 228.12 mg Placebo blend is transferred into 250 mL Round bottom flask, added about 5 mL of cosolvent, refluxed the solution at 90°C for 24 Hrs and Centrifuged a portion of solution at 3500 RPM about 10 min. 5 mL of clear solution is diluted to 50 mL with diluents-2. 5 mL of clear solution is diluted to 50 mL with diluents-2 for metformin. 5 mL of clear solution is diluted to 25 mL with diluents-2 for pioglitazone.

Peroxide stressed Sample

For Test Sample(metformin)

850.55 mg of Metformin and 17.383mg Pioglitazone API and 228.23 mg Placebo blend is transferred into 250 mL round bottom flask, added about 5 mL of cosolvent and dissolved then added 45mL of 10% H_2O_2 Refluxed at 90^{0}C For 1 Hr. Centrifuged a portion of solution at 3500 RPM for 10 minutes . Diluted 5mlof above sample to 100mL with diluents-2 for metformin. Diluted 5mlof above sample to 50mL with diluents-2.

For Test Sample (Pioglitazone)

17.086 mg of Pioglitazone API and 228.91 mg of placebo blend and 849.62mg of metformin API is transferred into 250 mL round bottom flask, added about 5 mL of cosolvent and dissolved then added 45mL of 10% H_2O_2 Refluxed at 90^{0}C For 1 Hr. Centrifuged a portion of solution at 3500 RPM for 10 minutes . Diluted 5mlof above sample to 50mL with diluents-2.

Acceptance criteria

- Metformin and Pioglitazone peak should be pure.
- Degradation products should be well separated from the main peak.
- The peaks of Diluent, Placebo and impurities should not interfere with Metformin and Pioglitazone peak.

For API

For Metformin

Conditions	Neutral	Dry heat	UV Light	Humidity 75 % RH	5 N HCl	1 N NaOH	H2O2 10%	Aqueous Hydrolysis	Sample as is
Purity**	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)
%Assay	91.9	100.7	99.5	100.2	95.9	97.8	94.0	98.9	NA
%Degradation	0.02	0.0	0.02	0.0	1.66	2.45	8.28	0.8	NA

For Test Sample

Conditions	Neutral	Dry heat	UV Light	Humidity 90% RH	5 N HCl	1 N NaOH	H2O2 10%	Aqueous Hydrolysis	Sample as is
Purity**	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)
% Assay	94.1	98.8	97.4	98.0	95.5	93.5	92.4	95.9	NA
%Degradation	1.43	1.6	0.02	0.71	4.31	2.75	0.85	0.2	NA

For API
Pioglitazone

Conditions	Neutral	Dry heat	UV Light	Humidity 75 % RH	5 N HCl	1 N NaOH	H2O2 10%	Aqueous Hydrolysis	Sample as is
Purity**	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)
%Assay	97.05	100.3	97.5	97.1	96.9	96.5	91.8	99.4	NA
%Degradation	0	0.0	0.0	0.0	7.95	1.26	12.72	1.25	NA

For Test Sample

Conditions	Neutral	Dry heat	UV Light	Humidity 90% RH	5 N HCl	1 N NaOH	H2O2 10%	Sample as is
Purity**	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)	1.00(P)
%Assay	92.8	99.3	97.6	96.9	99.0	98.4	92.8	NA
%Degradation	0.02	0.0	0.01	0.03	0.91	2.0	8.39	NA

** Peak purity: 'P' indicates Metformin and Pioglitazone peak is pure which is confirmed by Diode array detector (DAD) using Agilent Ezchrome Software.

RESULTS

- Degradation products are well separated from the main peak.
- The peaks of diluent, Placebo and impurities are not interfering with Metformin and Pioglitazone peak.

Data Interpretation

From the above results it is observed that the degradation is observed under all stressed conditions and the degradation products are well separated from Metformin, diluent, placebo and impurity peaks. Hence, the Assay method is considered as stability indicating method.

METHOD VALIDATION

Precision

The precision of an analytical method is the degree of agreement among individual test results, when the method is applied repeatedly to multiple samplings of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurements.

System Precision

The system precision is checked by using standard solution to ensure that the analytical system is working properly. The retention times, areas of six determinations are measured and % relative standard deviation for retention times and areas is calculated. Injected standard solution six times into the chromatograph and recorded the chromatograph and calculated the %relative standard deviation.

System precision

Injection No.	Metformin		Pioglitazone	
	RT	Peak Area	RT	Peak Area
1	3.45	12830160	7.54	3484287
2	3.46	12828410	7.53	3484194
3	3.46	12826845	7.53	3484055
4	3.46	12829490	7.53	3486234
5	3.46	1282592	7.53	3485666
6	3.46	12829937	7.53	3486048
Mean	3.46	12828906	7.53	3485081
%RSD	0.1	0.0	0.1	0.0

Method precision

In method precision, a homogenous sample of a single batch is analyzed six times. This indicates whether a method is giving consistent results or not for a single batch.

Method precision for 500/15 mg Tablets

For Metformin

Solution Name	% Assay
Precision solution-1	98.7%
Precision solution-2	98.4%
Precision solution-3	98.6%
Precision solution-4	98.5%
Precision solution-5	98.6%
Precision solution-6	98.2%
Mean	98.5%
RSD	0.2%

For Pioglitazone

Solution Name	% Assay
Precision solution-1	98.4%
Precision solution-2	100.0%
Precision solution-3	98.4%
Precision solution-4	99.0%
Precision solution-5	101.7%
Precision solution-6	100.6%
Mean	99.7%
RSD	1.3%

For 850/15 mg Table
For Metformin

Solution Name	% Assay
Precision solution-1	102.0%
Precision solution-2	100.2%
Precision solution-3	100.2%
Precision solution-4	100.0%
Precision solution-5	99.9%
Precision solution-6	99.9%
Mean	100.4%
RSD	0.8 %

For Pioglitazone

Solution Name	% Assay
Precision solution-1	98.5%
Precision solution-2	98.1 %
Precision solution-3	98.7 %
Precision solution-4	98.1 %
Precision solution-5	98.1 %
Precision solution-6	99.0 %
Mean	98.4 %
RSD	0.4%

RESULTS

- The RSD calculated for assay values of Metformin and Pioglitazone 500/15mg tablets from 6 determinations is 0.2% and 1.3% respectively.
- The RSD calculated for assay values of Metformin and Pioglitazone 850/15mg tablets from 6 determinations is 0.8% and 0.4% respectively.

Intermediate Precision

The intermediate precision is carried out to ensure that the analytical results will remain unaffected by change in instrument and analyst.

The method precision set is repeated by different analyst using different column and different instrument and calculated %RSD for 12 determinations (Method precision and Intermediate precision).

Intermediate precision for 850mg/15 mg Tablets

For Metformin

Solution Name	% Assay
Intermediate precision solution-1	102.0%
Intermediate precision solution -2	100.5%
Intermediate precision solution -3	100.0%
Intermediate precision solution -4	99.7%
Intermediate precision solution -5	99.8%
Intermediate precision solution -6	99.6%
Mean	100.3%
%RSD	0.9%

For Pioglitazone

Solution Name	% Assay
Intermediate precision solution-1	98.5%
Intermediate precision solution -2	98.1%
Intermediate precision solution -3	99.3%
Intermediate precision solution -4	98.3%
Intermediate precision solution -5	98.1%
Intermediate precision solution -6	99.9%
Mean	98.7%
%RSD	0.8%

Comparison between method precision and intermediate precision for 850mg/15mg Tablets

Parameter	% Assay of Metformin	% Assay of Pioglitazone
Method Precision	102.0%	98.5%
	100.2%	98.1 %
	100.2%	98.7 %
	100.0%	98.1 %
	99.9%	98.1 %
	99.9%	99.0 %
Intermediate Precision	102.0%	98.5%
	100.5%	98.1%
	100.0%	99.3%
	99.7%	98.3%
	99.8%	98.1%
	99.6%	99.9%
Mean	100.3%	98.6%
%RSD	0.8%	0.6%

Intermediate precision for 500mg/15 mg Tablet
For Metformin

Solution Name	% Assay
Intermediate precision solution-1	98.6
Intermediate precision solution -2	98.7
Intermediate precision solution -3	98.5
Intermediate precision solution -4	98.6
Intermediate precision solution -5	98.3
Intermediate precision solution -6	98.2
Mean	98.5
%RSD	0.2

For Pioglitazone

Solution Name	% Assay
Intermediate precision solution-1	99.0
Intermediate precision solution -2	98.7
Intermediate precision solution -3	98.9
Intermediate precision solution -4	99.0
Intermediate precision solution -5	99.1
Intermediate precision solution -6	99.1
Mean	99.0
%RSD	0.1

Comparison between method precision and intermediate precision for 500mg/15mg Tablets

Parameter	% Assay of Metformin	%Assay of Pioglitazone
Method Precision	98.7	98.4
	98.4	100.0
	98.6	98.4
	98.5	99.0
	98.6	101.7
	98.2	100.6
Intermediate Precision	98.6	99.0
	98.7	98.7
	98.5	98.9
	98.6	99.0
	98.3	99.1
	98.2	99.1
Mean	98.5	99.3%
%RSD	0.2%	1.0%

RESULTS

- The RSD calculated for assay values of Metformin and Pioglitazone 850/15mg tablets from 6 determinations is 0.9%&0.8% respectively.
- The RSD calculated for assay values of Metformin and Pioglitazone 500/15mg tablets from 6 determinations is 0.2%&0.1% respectively.
- The RSD calculated for assay values of Metformin and Pioglitazone 850/15mg tablets from 12determinations (Method precision and Intermediate precision) is 0.8%&0.6% respectively.
- The RSD calculated for assay values of Metformin and Pioglitazone 500/15mg tablets from 12 determinations (Method precision and Intermediate precision) is 0.2%&1.0% respectively.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation,

proportional to the concentration of analyte in samples within a given range.

Linearity stock preparation

For Pioglitazone

66.81mg of Pioglitazone is transferred in to 100.0 mL volumetric flask, added about 70ml of diluents-1 and sonicated it for 5 minutes, diluted to volume with diluents-1.

For Metformin

85.62 mg of Metformin is transferred in to 100.0 mL volumetric flask and added about 70 of diluents-1 and sonicated it for 5 minutes and diluted to volume with diluent.

Preparation of Linearity solutions

Name of the solution	Vol. of Metformin stock solution taken (mL)	Vol. of Pioglitazone stock solution taken (mL)	Made up to volume with diluent-1
Linearity solution-1	1.0	1.0	100
Linearity solution-2	5.0	2.5	100
Linearity solution-3	5.0	2.5	50
Linearity solution-4	2.5	2.0	20
Linearity solution-5	3.0	2.5	20
Linearity solution-6	4.0	3.0	20

Acceptance Criteria

The Correlation coefficient and Regression coefficient (R square) should be NLT 0.999 for Metformin and Pioglitazone

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value (Standard value).

Procedure

Carried out Recovery of Metformin and Pioglitazone in presence of Excipients with concentrations ranging from 5% to 200% of Target concentration for Metformin and 10% to 300% for Pioglitazone by spiking drug substance into placebo blend and tabulated the results in the tables given below.

% Recovery for Metformin at different levels

S.No	Level	Area	mg added	Mg founded	%Recovery	Mean % recovery
1	10%-01	5496953	0.0043	0.004345	99.1	99.7
2	10%-02	5522291	0.0043	0.004365	99.6	
3	10%-03	5553106	0.0043	0.004390	100.2	
4	10%-04	5504432	0.0043	0.004351	99.3	
5	10%-05	5545223	0.0043	0.004384	100.0	
6	10%-06	5548023	0.0043	0.004386	100.1	
7	50%-01	54137801	0.0428	0.04280	99.9	100.5
8	50%-02	55096338	0.0428	0.04355	101.7	
9	50%-03	54045135	0.0428	0.04272	99.8	
10	100%-01	105769692	0.0847	0.08361	98.8	99.0
11	100%-02	106105603	0.0847	0.08388	99.1	
12	100%-03	106275765	0.0847	0.08401	99.2	
13	150%-01	162104628	0.1270	0.1281	100.9	99.7
14	150%-02	158857215	0.1270	0.1256	98.9	
15	150%-03	159522795	0.1270	0.1261	99.3	
16	200%-01	210337229	0.1693	0.1663	98.2	98.5
17	200%-02	210413321	0.1693	0.1663	98.2	
18	200%-03	212096229	0.1693	0.1677	99.0	
19	200%-04	209470739	0.1694	0.1656	98.0	
20	200%-05	209468819	0.1694	0.1656	98.0	
21	200%-06	212992779	0.1694	0.1684	99.7	

RESULTS

- Mean % recovery for Metformin from 5 levels is **99.4 %.**
- %RSD obtained for 21 determinations is **0.9%**.

% Recovery for Pioglitazone at different levels

S.No	Level	Area	mg added	Mg founded	%Recovery	Mean % ecovery
1	10%-01	2910223	0.00300	0.003014	100.3	99.8
2	10%-02	2881042	0.00300	0.002983	99.3	
3	10%-03	2890612	0.00300	0.002993	99.6	
4	10%-04	2914121	0.00299	0.003018	100.4	
5	10%-05	2880017	0.00300	0.002982	99.4	
6	10%-06	2900775	0.00300	0.003004	100.0	
7	50%-01	13484505	0.014077	0.013963	99.2	99.1
8	50%-02	13340913	0.014044	0.013815	98.4	
9	50%-03	13753882	0.014295	0.014242	99.6	
10	100%-01	27965829	0.029470	0.028959	98.3	99.4
11	100%-02	29767792	0.303085	0.030825	101.4	
12	100%-03	28165344	0.029619	0.029166	98.5	
13	150%-01	5958635	0.047106	0.047591	101.0	101.1
14	150%-02	46035933	0.047124	0.047671	101.2	
15	150%-03	46061460	0.047167	0.047698	101.1	
16	300%-01	83909344	0.088632	0.086890	98.0	98.4
17	300%-02	84466275	0.088617	0.087466	98.7	
18	300%-03	83867972	0.088588	0.086847	98.0	
19	300%-04	84586934	0.088588	0.087591	98.9	
20	300%-05	84108400	0.088625	0.087096	98.3	
21	300%-06	84395079	0.088632	0.087393	98.6	

RESULTS

- Mean % recovery for Pioglitazone from 5 levels is 99.6 %.
- %RSD from 21 determinations is 1.1 %.

% Assay of Metformin for 850/15 mg Tablets

Sample No.	System-1 /Analyst-1 /Column-1	System-2 /Analyst-2 /Column-2
1	102.0%	102.0%
2	100.5%	100.5%
3	100.0%	100.0%
4	99.7%	99.7%
5	99.8%	99.8%
6	99.6%	99.6%
Mean	100.3%	100.3%
%RSD	0.9%	0.9%

% Assay of Pioglitazone for 850/15 mg Tablets

Sample No.	System-1 /Analyst-1 /Column-1	System-2 /Analyst-2 /Column-2
1	98.5%	98.5%
2	98.1 %	98.1%
3	98.7 %	99.3%
4	98.1 %	98.3%
5	98.1 %	98.1%
6	99.0 %	99.9%
Mean	98.4 %	98.7%
%RSD	0.4%	0.8%

% Assay of Metformin for 500/15 mg Tablets

Sample No.	System-1 /Analyst-1 / Column-1	System-2 /Analyst-2 / Column-2
1	98.7%	98.6
2	98.4%	98.7
3	98.6%	98.5
4	98.5%	98.6
5	98.6%	98.3
6	98.2%	98.2
Mean	98.5%	98.5
%RSD	0.2%	0.2

% Assay of Pioglitazone for 500/15 mg Tablets

Sample No.	System-1 /Analyst-1 / Column-1	System-2 /Analyst-2 / Column-2
1	98.4%	99.0
2	100.0%	98.7
3	98.4%	98.9
4	99.0%	99.0
5	101.7%	99.1
6	100.6%	99.1
Mean	99.7%	99.0
%RSD	1.3%	0.1

Acceptance Criteria

- The % Assay values obtained should be with in 98.0% to 102.0%.
- Relative Standard deviation of % assay results should be not more than 2.0% by both analysts.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Acceptance Criteria

- The system suitability parameters should pass for all the conditions.
- The %Assay difference from initial value should be NMT 2.0%.

Data Interpretation

From the above data, it is observed that the tailing factor, %RSD and Theoretical plates obtained for Metformin and Pioglitazone at flow rate (0.8 to 1.2mL/min.), at pH (6.90 to 7.30), column temperature (40°C to 50°C),% Organic (90 to 110) and wavelength (220 to 230 nm) are within the acceptance criteria hence the method is robust.

CONCLUSION

The proposed HPLC method for estimation of Assay of Metformin and Pioglitazone Tablets is carried out as per USP/ICH guidelines. The method is found to be specific for the quantitation of Met & Pio. The method is also stability indicating as evident by degradation studies. This method is found to be linear in the specified range. Accuracy of the method is also established for the drug product is found to be precise and robust. LOD and LOQ are also established by this method. Hence, this validated method stands for usage of routine and stability sample analysis. Statistical analysis proved that method was accurate, precise, and repeatable. The developed method was found to be simple, sensitive and selective for analysis of MET and PIO in combination without any interference from the excipients.

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