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Research Article

Development of validated RP-HPLC method for antidiabetic drugs in pharmaceutical dosage form

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Abstract

A RP-HPLC method was developed using Comosil RP-C18 (4.6 x 150mm, 5µm) as stationary phase with younglin (S.K.) isocratic system UV detector in a gradient mode with mobile phase comprising of Acetonitrie : Pott. Dihydrogen Phosphate (adjusted pH-2.5 using OPA). 0.7ml/min flow rate and monitoring of effluent were done at 254.0 nm for MET (Metformin) and PIOG(Pioglitazone) estimation in combined dosage form. 2.1 min for MET and 7.53 min for PIOG retention times were found. The dynamic range of linearity 50.0-250.0 µg/mL for METand 3.0- 15.0 µg/mL for PIOG were exhibited for the assay. The linear calibration curves were found over the entire range linearity (r2 = 0.996for MET and r2 = 0.995for PIOG) and99.81%for MET and 99.33% for PIOG mean % recovery was found with % RSD was NMT 2.0 for both estimations which fully agrees by system suitability in good agreement with labeled claimed of formulation. The % RSD for Intra & Inter-Day Precision was NMT than 2.0 for both drugs. The developed method was accurate, precise, rugged and linear as per ICH guidelines

Keywords: MET, PIOG, RP-HPLC, Method Validation, Assay method.

1. Introduction

HPLC is a physical separation technique carried out in the liquid phase in which a sample is separated into its constituent components (or analytes) by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). An online detector monitors the concentration of each separated component in the column effluent and generates a chromatogram. HPLC is the most widely used analytical technique for the quantitative analysis of pharmaceuticals, biomolecules, polymers, and other organic compounds. Thus developed performance separation technique over classical chromatographic techniques. Under high pressure liquid moving phase is pumped into the column containing porous stationary phase. The technique is developed on basis small particle size of stationary phase in column which required high pressure for the easy flow of moving phase without any resistance.[1, 5, 7] Development of analytical method, degradation profile, stability indicating assay methode, validation features and certain other quality attributes are the key elements of any pharmaceutical development program to provide certain quality products oh high purity and identity in behalf of public interest and for its own benefit for any manufacturing organization, research institute through quality assurance department. HPLC technique is used to obtain qualitative and quantitative information of different organic inorganic compounds. Technically the process involves the step which shortly focus on the validation and method development which proves its acceptance for intended purpose to a particular drug products. Method validation parameters as defined by ICH (International Conference on Harmonization) guidelines are Accuracy, Precision, Selectivity/Specificity, Limit ofQuantitation, Limit of Detection, Linearity, Range, Ruggedness and Robustness[5-12].On the basis of literature review[14-20] it has been found that only two to three analytical methods for above combination have been reported. Hence the attempt is made to develop accurate, precise, rugged, rapid and economical RP-HPLC method for estimation of Metformin (MET) and Pioglitazone (PIOG) in combine dosage form. Metformin [Fig. 1] chemically is *N, N*-dimethylimidodicarbonimidic diamide hydrochloride. It is a white to off-white crystalline compound used as antidiabetic having solubility in methanol and freely in water, sparingly soluble in ethanol. While Pioglitazone [Fig. 2] chemically is (dl)-5- 4-2-(5-ethyl-2-pyridinyl) ethyl] phenyl-2, 4- thiazolidinedione

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monohydrochloride.[3, 25, 26] It is white powder and practically odorless. Used as antidiabetic having solubility in methanol and 1-propanol insoluble in water, slightly soluble in ethanol.

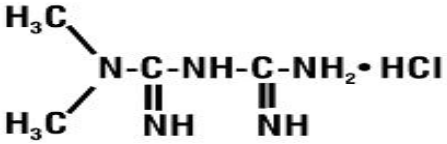

Fig. 1 Chemical Structure of Metformin HCl.

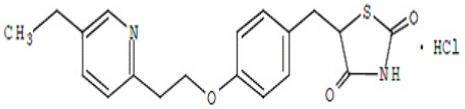

Fig. 2 Chemical Structure of Pioglitazone HCl.

2. Experimental

2.1 Reagents & Chemicals

Standard samples of MET&PIOG were obtained as gift samples from Madras Pharmaceuticals/Maral lab (Chennai) India. The marketed formulation Pioz-MF30(USV LIMITED, B.S.D. Marg Govandi, Mumbai-400088) was purchased from the local market containing MET 500.0 mg and PIOG 30.0 mg and all the chemicals were used are of analytical grade.

2.2 Instruments

HPLC System of Younglin Quaternary pump with UV- VIS detector (190-990 nm) Software Autochro-3000. Ultrasonicator servewell instruments model RC-SYSTEM MU-1700 used for sonication purpose.Analytical balance of citizen model CY 104 (micro analytical balance) was used for weighing purpose.

2.3 Preparation of Stock solution for MET& PIOG

An accurately weighed quantity of MET working standard about 500.0 mg and PIOG working standard about 30.0 mg were transferred separately into 50.0 mL volumetric flask. About 40.0 mL of methanol (HPLC Grade) was added to the volumetric flask and sonicated to dissolve the drug. The solution was cooled to the room temperature and made up to the mark with methanol (HPLC Grade) which gave the final concentrations of 10000.0 µg /mL and 600.0 µg /mL for MET and PIOG respectively.

2.4 Preparation of Working Standard Solution A

Take 1.0 mL from stock solution of PIOG and MET respectively in a 10.0 mL volumetric flask and make up the volume up to the mark with mobile phase to get 30.0 µg/mL PIOG & 500.0 µg/mL MET.

2.5 Preparation of Sample Stock Solution

Take the powder weight of tablet equivalent to 500 mg of MET in 50.0 mL of volumetric flask and add sufficient mobile phase and sonicate it for 15 min. Make up the volume up to the mark with mobile phase and filtered it with 0.24µ to get 10000.0 µg/mL and 600.0 µg/mL of MET and PIOG respectively.

Working Sample Solution (B) Take 1.0 mL from above solution of PIOG and MET respectively in a 10.0 mL volumetric flask and make up the volume up to the mark with mobile phase to get 30.0 µg/mL PIOG & 500.0 µg/mL METas final concentration and sonicated for 10.0 min in ultrasonicator.

2.6 Optimization of Mobile Phase and Chromatographic Conditions

Procedure

The chromatographic conditions were set as per the optimized parameters. The mobile phase was allowed to equilibrate with stationary phase as was indicated by a steady baseline. Solution (A)was injected in theRheodyne injector (20.0 µl) and the respective chromatograms were recorded.Various mobile phases were tried by permutations and combinations and also by varying column, flow rate, column temperature and type of buffers with varying pH and solvents. The various mobile phases tried are as follows.

- **Trial** -1 MEOH: KH2PO4 (30:70%, v/v), pH 2.5 with 0.05% TEA.
- **Trial** -2 ACN: KH2PO4 (50:50%, v/v), pH 2.5 with 0.05% TEA.
- **Trial** -3 ACN: KH2PO4 (50:50%, v/v), pH 2.5 with 0.05% OPA.
- **Trial** -4 ACN: KH2PO4 (50:50%, v/v), pH 2.5 with 0.05% OPA.
- **Trial** -5 ACN: KH2PO4 (20:80%, v/v), pH 2.5 with 0.05% OPA.

- **Trial** -6 ACN: KH2PO4 (40:60%, v/v), pH 2.5 with 0.05% OPA.
- **Trial** -7 ACN: KH2PO4 (30:70%, v/v), pH 2.5 with 0.05% OPA.

Above mentioned various mobile phases were tried. The mobile phase containing Acetonitrile : KH2PO4(30.0: 70.0) at pH 2.5, injection volume 20.0 µL flow rate of 0.7mL/min was selected, due to its high resolving power, sensitivity and system suitability, for the determination of MET and PIOG. The chromatogram is shown in **Figure1**. Hence the following optimized chromatographic parameters were selected to carry out further experimentation.

- **Column** : Comosil RP-C18 (4.6 x 150mm, 5µm)
- **Flow Rate** : 0.7mL/min
- **Wavelength** : 254.0 nm
- **Injection Volume** : 20.0 µL
- **Column Temperature** : Ambient
- **Run Time** : 10.0 min
- **Mobile Phase** : Acetonitrile: Pott. Dihydrogen Phosphate (30.0:70.0 V/V)
- **pH** : 2.5 (Using OPA)

2.7 System Suitability Studies

This studies are the pharmacopoeial requirement and is used to verify, whether thereproducibility andresolution of the chromatographic system for analysis to be carried outare adequate or not. To ensure that the system is readto deliver results with acceptable accuracy and precision andoperating properly this studies are performed. From five replicate injections of standard solutions the tests were performed to collecting data.

Procedure

Mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline and the chromatographic conditions were set as per optimized parameters. The chromatograms were recorded for both drugs by five replicate injections of mixed working standard solution (A) were injected in to the system, and results are shown in **Table1&2.**

2.8 Analysis of Standard Laboratory Mixtures

Preparation of Standard Laboratory Mixtures (Standard)

MET Standard Stock Solution (A) Accurately weighed quantity of MET (500.0 mg) was transferred to 50.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of (10000.0 µg/mL of MET). The resultant solution was then sonicated for 10.0 -15.0 min in ultrasonicator.

PIOG Standard Stock Solution (B) Accurately weighed quantity of PIOG (30.0 mg) was transferred to 50.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of (600.0 µg/mL of PIOG). The resultant solution was then sonicated for 10.0 – 15.0 min in ultrasonicator.

Mixed Standard Solutions 1.0 mL of solution (A) and 1.0mL of solution (B) was then transferred to 10.0mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (500.0 µg/mL of MET & 30.0 µg/mL of PIOG) respectively.

2.9 Preparation of Standard Laboratory Mixtures (Sample)

Accurately weighed 500.0 mg of MET and 30.0 mg of PIOG (as per labeled requirement of marketed formulation) was transferred to 50.0mL volumetric flask and dissolved in sufficient quantity of methanol. Then the volume was made up to the mark with methanol. The resultant solution was then sonicated in ultrasonicator for 10.0 min. then aliquots portions of 0.05mL and 0.15 mL was then transferred to two separate 10.0mL volumetric flask and then volume was made up to the mark with mobile phase to get final concentrationsof (50.0 µg/mL& 3.0 µg/mL, 100µg/mL & 6.0 µg/mL of MET and PIOG) respectively. The peak area of standard laboratory mixture and sample laboratory mixture was compared to obtain the concentration. The amount of each drug estimated in laboratory mixture was calculated using following formula –

$$\% \text{ Estimation} \quad \frac{At}{As} \times \frac{Ds}{Dt} \times \frac{Ws}{Wt} \times 100$$

Where,

At = Area count for sample solution
As = Area count for standard solution

Ds = Dilution factor for standard
Dt = Dilution factor for sample
Ws = Weight of standard (mg)
Wt = Weight of sample (mg)

The results are shown in **Table 3.**

2.10 Analysis of Marketed Formulation

Preparation of Standard Solutions

Prepared as per the methodology adopted for laboratory mixtures

Preparation of Sample Solutions

Ten Tablets were weighed accurately and ground to fine powder. An accurately weighed quantity of Tablet powder equivalent to (500 mg of MET & 30 mg of PIOG) were transferred to 50.0 mL of volumetric flask and dissolved in sufficient amount of methanol. Then the volume was made up to the mark with methanol. The resultant solution was then filtered through whatmann filter paper (no. 41). The filtered solution was then sonicated in ultrasonicator for 10.0-15.0 min. then aliquot portions of 0.05 mL and 0.10 mLwas then transferred to the three separate 10.0 mL volumetric flask and then the volume was mad up to the mark with mobile phase to get final concentration of (50.0 µg/mL, 100.0µg/mLand 3.0 µg/mL, 6.0µg/mL of MET and PIOG) respectively.

Procedure

Equal volume (20.0 µL) 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 amount of drug in a Tablet was calculated using following formula

$$\text{mg/Tablet} = \frac{AT1 \times WS1 \times D0s \times P1}{AS1 \times WT \times Dt} \times \text{Avg. wt}$$

Where,

AT1 = Average area of MET/PIOG peaks in Test chromatogram
AS1 = Average area of MET/PIOG peaks in Standard chromatogram
Ds = Dilution factor for standard
Dt = Dilution factor for test
P1 = Potency of working standards of MET/PIOG peaks of % w/w basis
Avg. wt = Average weight of 10 Tablets

Further calculate the amount of MET/PIOG peaks present in % of Label claim using following formula

$$\% \text{ Label Claim} = \frac{\text{Assay (mg/Tablet)} \times 100}{\text{Label claim of MET/PIOG peaks}}$$

The results are shown in **Table 4**, while chromatogram is shown in **Figure4.**

2.11 Method Validation

2.11.1. Linearity

Preparation of Standard Solutions

MET Standard Stock Solution (A)

Accurately weighed quantity of MET (500.0 mg) was transferred to 50.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of (10000.0 µg/mL of MET). The resultant solution was then sonicated for 10.0 – 15.0 min in ultrasonicator.

PIOG Standard Stock Solution (B)

Accurately weighed quantity of PIOG (30.0 mg) was transferred to 50.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of (600 µg/mL of PIOG). The resultant solution was then sonicated for 10.0-15.0 min in ultrasonicator.

Mixed Standard Solutions aliquots portions of 0.05 to 0.25 mL from the standard stock solutions (A & B) were transferred to five 10.0 mL volumetric flasks and then volume was made up to the mark with mobile phase to get 5 different mixed standard solutions having concentrations of (50.0:3.0, 100.0:6.0, 150.0:9.0, 200.0:12.0, 250.0:15.0 µg/mL of MET & PIOG) respectively. The resultant solutions was then sonicated in ultrasonicator for 10.0 – 15.0 min

Procedure

Equal volumes (20.0 µL) of 5 mixed standard solutions were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. Then calibration curve (Peak area *vs* concentration) was plotted and it is shown in **Figure5&6**. The observations are shown in **Table5.**

2.11.2. Accuracy

Preparation of Standard Solutions

Standard solutions of (MET & PIOG) were prepared at the level of 80 %, 100.0 %, 120 %.

Preparation of Sample Solution

To the preanalysed sample solution (100.0 µg/mL of MET & 6.0 µg/mL of PIOG) a known amount of standard solutions of pure drugs (MET& PIOG) were added in different levels i.e. 80%, 100.0 %, 120%. The results of recovery studies shown in **Table6 (a) & (b)**. The percent recovery was then calculated by using formula;

$$\% \text{ Recovery} = \frac{E_w - B}{C} \times 100$$

Where,

E_w = Total drug estimated (mg)

B= Amount of drug contributed by preanalyzed Tablet powder (mg)

C= Weight of pure drug added (mg)

2.11.3. Precision

It was determined by analyzing the 3 different solutions having concentration **(**100.0 µg/mL of MET & 6.0 µg/mL of PIOG). Results are shown in **Table 7.**

2.11.4. Ruggedness

2.11.4.1 Intra-Day Studies for MET

It was determined by analyzing the 3 different solutions having concentration **(**100.0 µg/mL of MET & 6.0 µg/mL of PIOG) at 3 different times over a period of day.

2.11.4.2 Inter-Day Studies for PIOG

It was determined by analyzing the 3 different solutions having concentration **(**100.0 µg/mL of MET& 6.0 µg/mL of PIOG) at 3 days over a period of week.

Procedure

Equal volumes (20.0 µL) of these solutions were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak areas, retention time of major peaks were measured. The results are shown in **Table8&9.**

2.11.5. Robustness

Preparation of Sample Solution

Sample solution of marketed formulation was prepared as per the methodology adopted for marketed formulation analysis.

Procedure

Equal volume (20.0 µL) of sample solution was injected separately after equilibrium of stationary phase. Then deliberate variation in method parameters such as flow rate (>0.1mL/min), change in detection wavelength (>1.0 nm) was carried out. The chromatograms were recorded and the response i.e. peak area, retention time of the major peaks were measured. The results are shown in **Table 10 (a) & (b)** chromatograms are shown in **Figure 12&13.**

3. Results and discussion

Optimization of Mobile Phase and Chromatographic Conditions

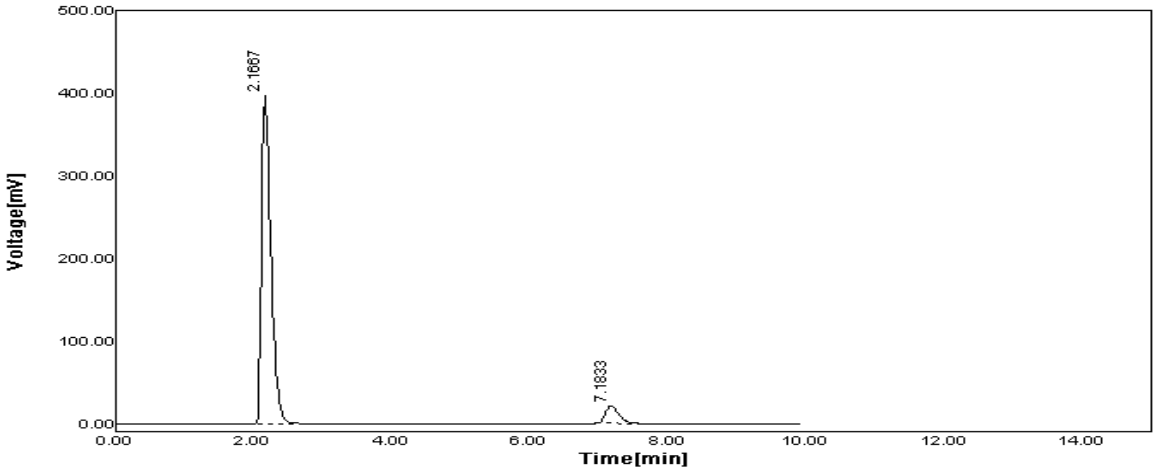

Fig. 3 Optimized Chromatogram of MET & PIOG

Observation

Good resolution with minimized tailing also proper peak shape and system suitability was observed within the limits. Hence the above chromatographic parameters are finalized.

System Suitability Studies

Table 1 Result of System Suitability Studies for (MET)

System Suitability Test (MET)					
Sr. No	**Area Reproducibility**	**Retention Time**	**Tailing Factor**	**Resolution**	**Theoretical Plates**
1	3777.33	2.1667	1.7295	0	3453
2	3777.31	2.1652	1.7289	0	3462
3	3775.24	2.1658	1.7288	0	3448
4	3776.38	2.1649	1.7291	0	3464
5	3775.36	2.1660	1.7293	0	3470
Mean	3776.324	2.16572	1.72912	0	3458.2
%RSD	0.025	0.779	1.024	0	1.723
Limit	**NMT 2%**	**NMT 1%**	**< 2**	**> 2**	**> 2000**

Observation

All the parameters of system suitability are observed within the limits for MET.

Table 2: Results of System Suitability Studies for (PIOG)

System Suitability Test (PIOG)					
Sr. No	**Area Reproducibility**	**Retention Time**	**Tailing Factor**	**Resolution**	**Theoretical plates**
1	275.5991	7.1833	1.2599	19.00	4380
2	275.4384	7.1582	1.2601	19.01	4365
3	274.6804	7.1764	1.2498	19.05	4410
4	275.7506	7.1832	1.2607	18.99	4398
5	274.8702	7.1799	1.2589	19.00	4387
Mean	275.26774	7.1762	1.25788	19.01	4388.2
%RSD	0.163	0.412	1.234	0.573	0.048
Limit	**NMT 2%**	**NMT 1%**	**< 2**	**> 2**	**> 2000**

Observation

All the parameters of system suitability are observed within the limits for PIOG.

Analysis of Standard Laboratory Mixtures

Table 3 Results of Analysis of Standard Laboratory Mixtures

Average Wt.=914mg								
Std weight(mg)		Sample weight (mg)	Area of Std		Area of Sample		% Labeled Claim	
MET	PIOG		MET	PIOG	MET	PIOG	MET	PIOG
500.0	30.0	914	3733.9841	274.1824	3729.87	270.14	99.88	98.52
		920			3727.98	271.01	99.83	98.84
		918			3731.89	272.08	99.94	99.23

Analysis of Marketed Formulation

Table 4: Results of Marketed Formulation Analysis

Sr. No.	MET		PIOG	
	Assay (mg)	Assay (%)	Assay (mg)	Assay (%)
1	498.90	99.78	29.80	99.3
2	499.25	99.85	29.90	99.6
3	499.12	99.82	29.80	99.3
Mean	499.09	99.81666	29.83333	99.33333
SD	0.1855	0.034129	0.014275	0.512101
% RSD	0.0372	0.034188	0.514598	0.7165978

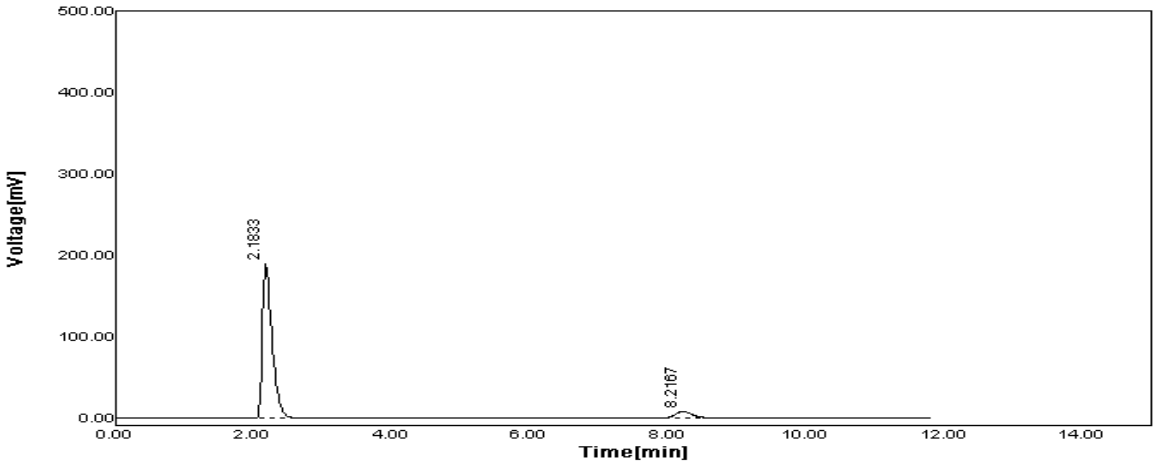

Fig. 4 Chromatogram of Marketed Formulation

The proposed method was applied to the determination of MET&PIOG in marketed formulation. The **mean % amount** found was **99.81 (MET) &99.33 (PIOG)** with **% RSD** values was **NMT 2.0%** indicates the developed method was successfully applied for analysis of marketed formulation. All the results found were in good agreement with the label content of marketed formulation.

Method Validation

1. Linearity

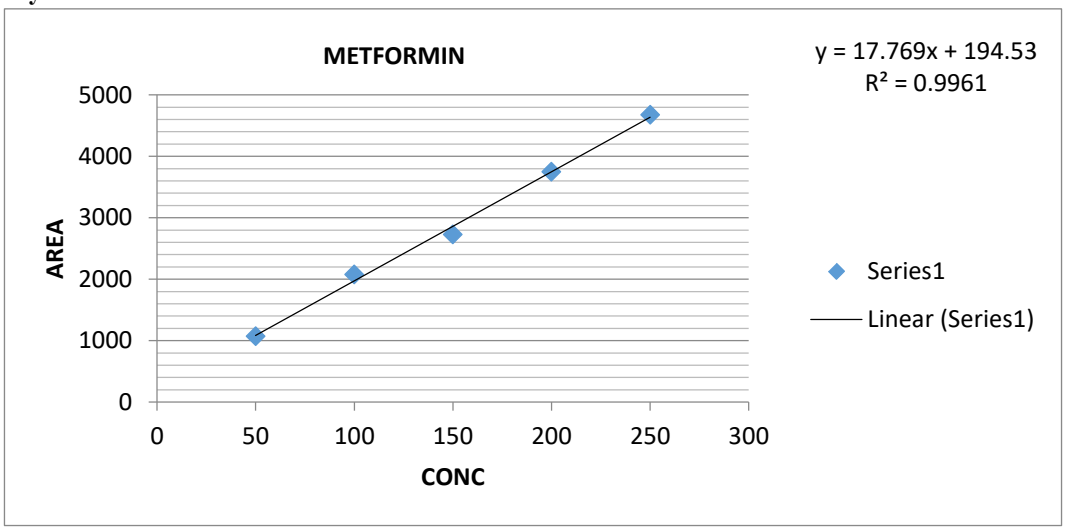

Fig. 5 Calibration Curve of MET

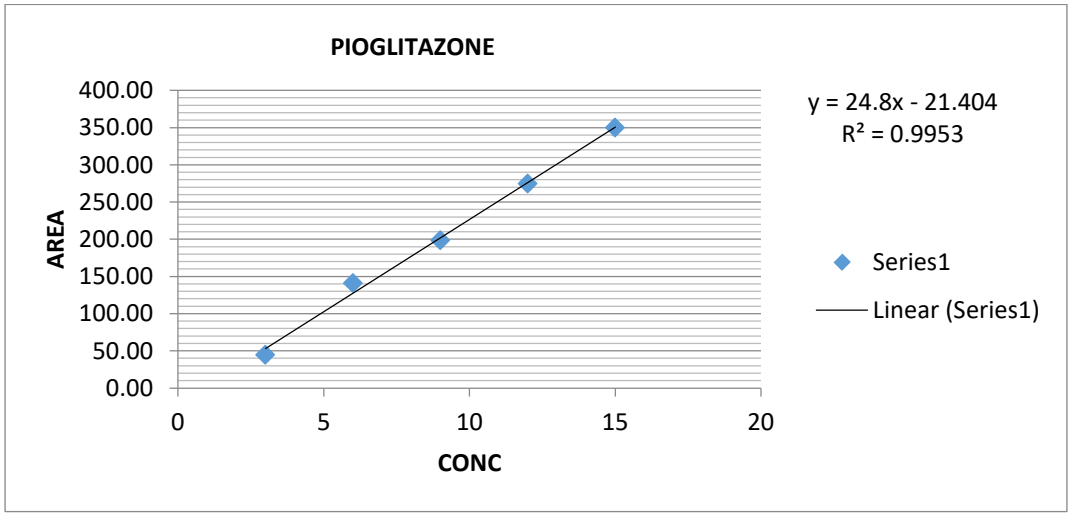

Fig. 6 Calibration Curve of PIOG

Table 5 Linearity Studies of MET&PIOG

Concentration (µg/mL)		Peak Area	
MET	**PIOG**	**MET**	**PIOG**
50	3	1069.522	44.82
100	6	2078.46	140.95
150	9	2726.968	198.58
200	12	3746.876	274.67
250	15	4677.567	349.96
	Mean	2859.8786	201.796
	SD	24.026	2.596
	%RSD	0.648	1.39

In both calibration curves the $\mathbf{r^2}$ value was found to be **0.996** for **MET** and **0.995** for **PIOG** which nearly equals to unity. The regression equation for MET was **y = 17.769x + 194.53** while for PIOG it was **y = 24.8x – 21.404**. It indicates the capability of developed method to estimate both the drugs over the desired concentration range.

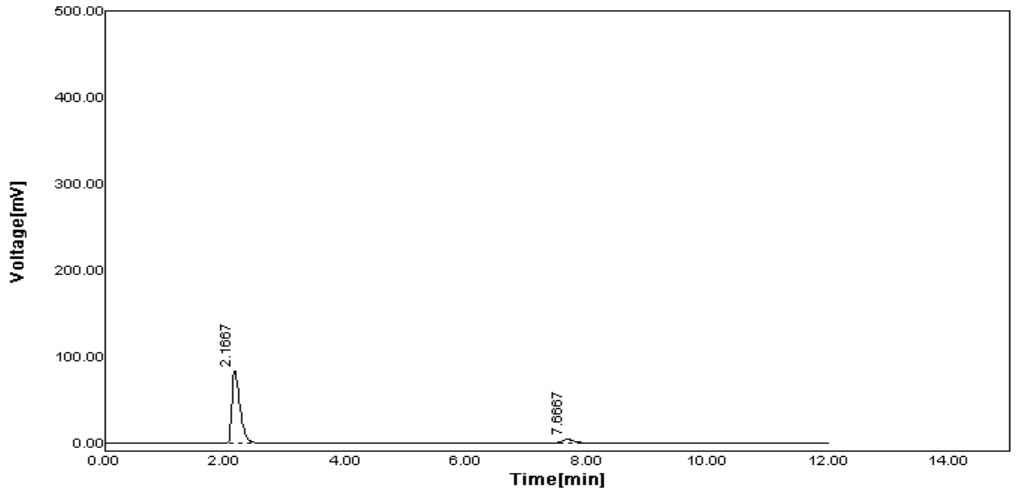

Fig. 7 Linearity Chromatogram for (50.0 µg/mL of MET & 3.0 µg/mL of PIOG)

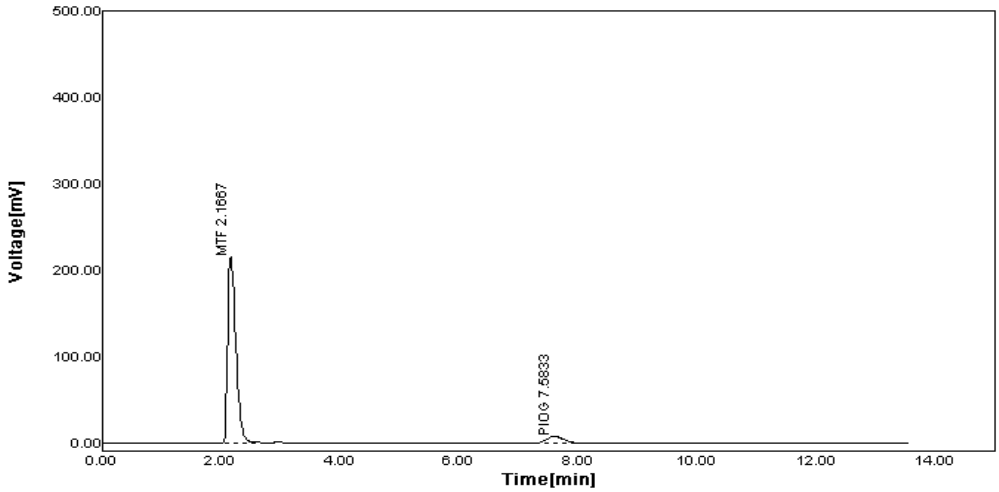

Fig. 8 Linearity Chromatogram for (100.0 µg/mL of MET & 6.0 µg/mL of PIOG)

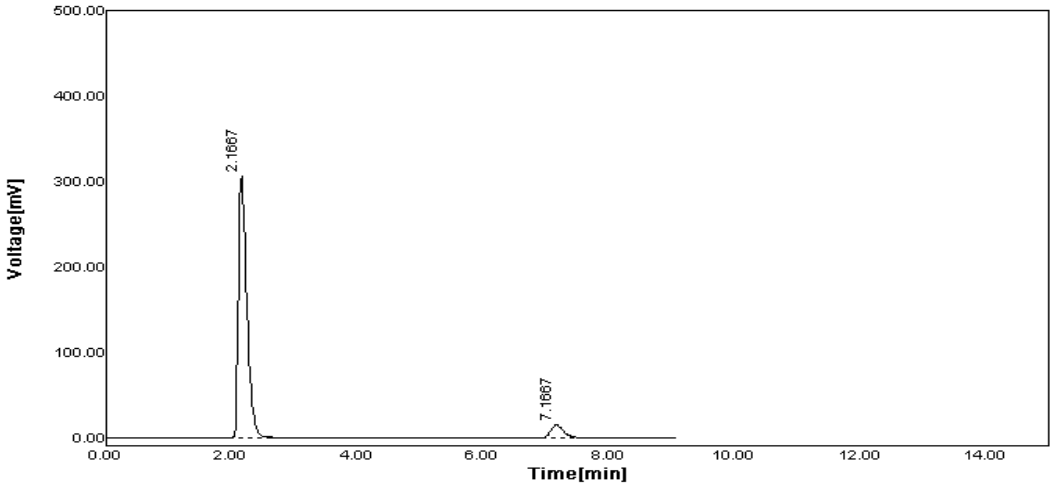

Fig. 9 Linearity Chromatogram for (150.0 µg/mL of MET & 9.0 µg/mL of PIOG)

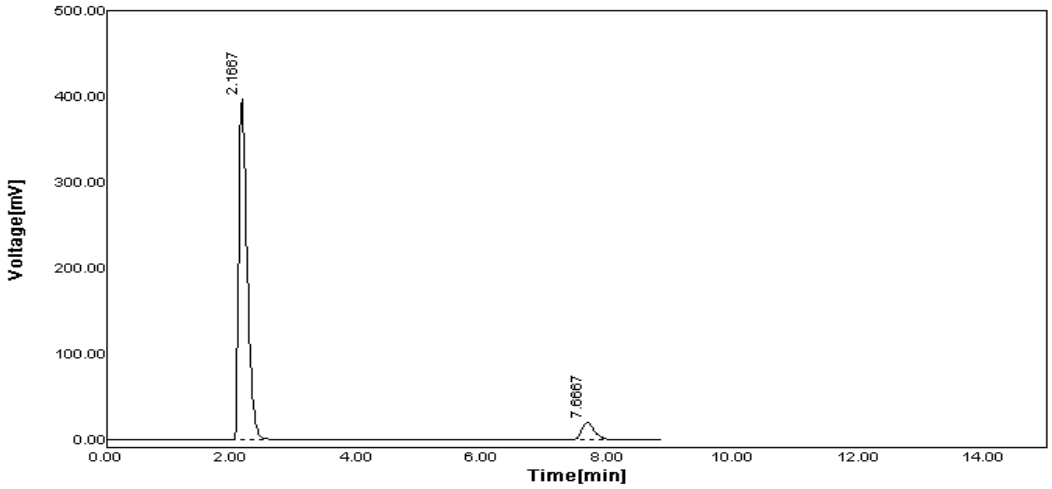

Fig. 10 Linearity Chromatogram for (200.0 µg/mL of MET & 12.0 µg/mL of PIOG)

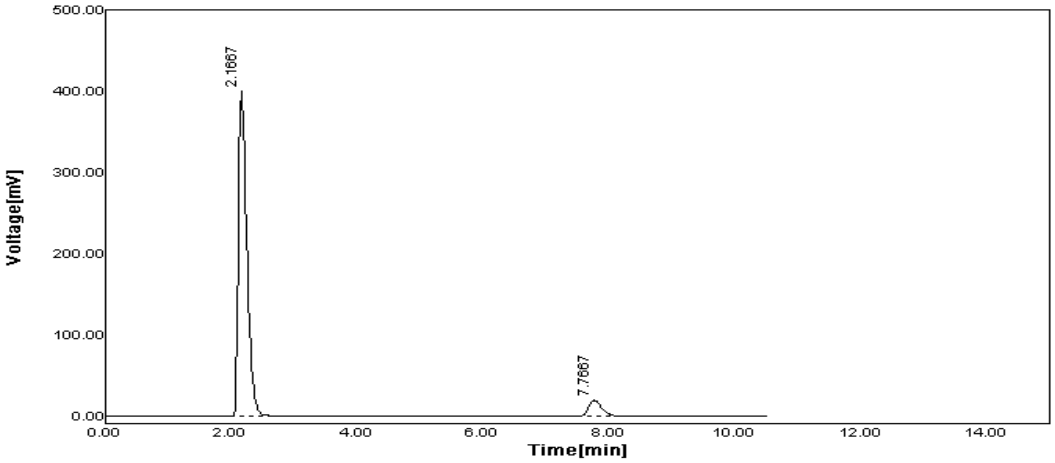

Fig. 11 Linearity Chromatogram for (250.0 µg/mL of MET & 15.0 µg/mL of PIOG)

2. Accuracy

This is performed on the basis of recovery studies by standard addition method.Standard solutions of pure drugs (MET & PIOG) were added in different levels i.e. 80%, 100 %, 120%.

Table 6 (a) Results of Recovery Studies

Level of % Recovery	Amount present (mg/tab)		Amount taken (µg/ml)		Amount of Std. Drug Added(µg/ml)		Total Amount Recovered (µg/ml)		%Recovery	
	MET	PIOG	MET	PIOG	MET	PIOG	MET	PIOG	MET	PIOG
80%	500	30	100	6	80	4.8	2971.97	196.3	99.66	101.32
	500	30	100	6	80	4.8	2963.92	196.5	99.62	102.22
	500	30	100	6	80	4.8	2898.96	196.3	99.64	101.33
100%	500	30	100	6	100	6.0	3198.66	225.7	101.0	100.69
	500	30	100	6	100	6.0	3199.59	226.5	100.7	100.89
	500	30	100	6	100	6.0	3193.66	226.4	100.5	100.48
120%	500	30	100	6	120	7.2	3498.28	245.7	101.2	101.23
	500	30	100	6	120	7.2	3488.25	246.7	101.0	101.67
	500	30	100	6	120	7.2	3467.28	245.7	100.7	101.23

Table 6(b) Statistical Validation Data for Accuracy

Level of % Recovery	MET			PIOG		
	Mean*	±SD	%RSD	Mean*	±SD	%RSD
80%	99.64	0.132	0.02	101.62	0.51	0.50
100%	100.77	3.19	0.25	100.69	0.218	0.20
120%	101.05	0.32	0.051	101.38	0.238	0.25

% mean recoveries were found with **% RSD** for **MET& PIOG** which fully agrees with system suitability. This showed that, the proposed HPLC method for the determination of MET and PIOG in a tablet was found to be sufficiently **accurate**.

3. Precision

Table 7 Result of precision study using tablet

Sr.No.	MET		PIOG	
	Peak Area Sample	%Assay	Peak Area Sample	%Assay
1	4891.2998	99.77	358.60	99.75
2	4882.2468	99.58	351.48	99.68
3	4893.2113	99.86	360.01	99.79
4	4891.2284	99.72	353.37	99.71
5	4880.3102	99.63	357.46	99.73
6	4892.2136	99.89	358.55	99.75
	Mean	99.74166666	**Mean**	99.733333
	SD	0.072159863	**SD**	0.0128984
	%RSD	0.015865822	**%RSD**	1.3459337

Precision study was determined by peak area. Peak area was found with **%RSD (NMT than 2%)**which was in agreement with system suitability. Therefore, the proposed HPLC method for the determination of METand PIOG in a tablet was found to be sufficiently **precise.**

4. Ruggedness

4.1 Intra andInter Day studies for MET

Table 8 Results of Intra- Inter Day Precision Studies for MET

Sr. No	Observations	% Drug estimation		
		Intra-day	Inter-day	Different Analyst
1	I	99.52	98.92	98.92
2	II	99.52	99.37	99.60
3	III	99.62	99.54	99.86
Mean		99.58	99.24	99.42
±S.D.		0.050	0.39	0.43
%R.S.D.		0.053	0.32	0.45

4.2 Intra andInter Day studies for PIOG

Table 9 Results of Intra- Day and Inter – Day Studiesfor **PIOG**

Sr. No	Observations	% Drug estimation		
		Intra-day	Inter-day	Different Analyst
1	I	99.62	98.72	98.92
2	II	98.72	99.67	99.62
3	III	99.52	99.82	99.86
Mean		99.22	99.37	99.42
±S.D.		0.56	0.51	0.44
%R.S.D		0.50	0.55	0.46

Ruggedness was determined as Intra-day, Inter-day & Different Analyst. % amount of drugs were found with **%RSD (NMT than 2%)** which was in agreement with system suitability. Therefore, proposed HPLC method for determination of METand PIOG in a tablet was found to be sufficiently **rugged.**

5. Robustness

Table 10(a) Results of Robustness Studies for MET

Condition	Mean	± SD n=3	%RSD
Change in flow rate (± 0.1 ml)	3257.665	2.02	0.062
Change in detection wavelength (± 1 nm)	5320.575	0.05	0.035

Table 10 (b) Results of Robustness Studies for PIOG

Condition	Mean	± SD n=3	%RSD
Change in flow rate (± 0.1 ml)	230.32	0.10	0.334
Change in detection wavelength (± 1 nm)	185.25	1.48	0.034

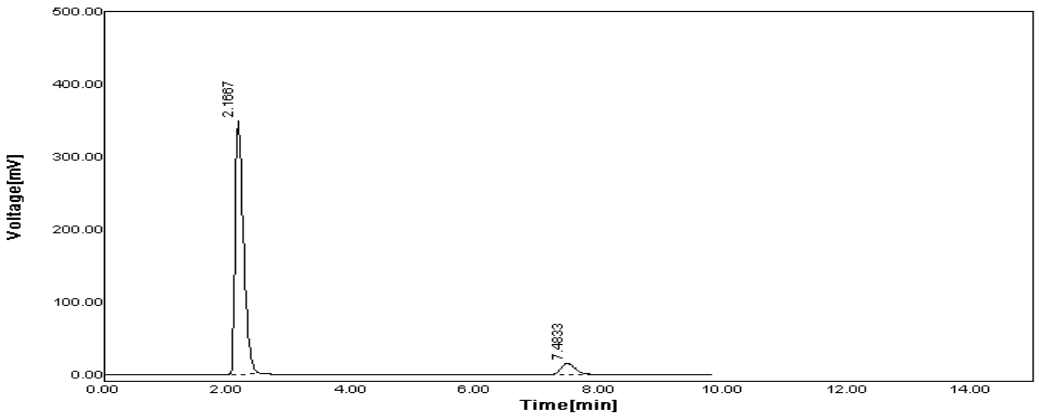

Fig. 14 Chromatogram of Robustness (>1.0 nm)

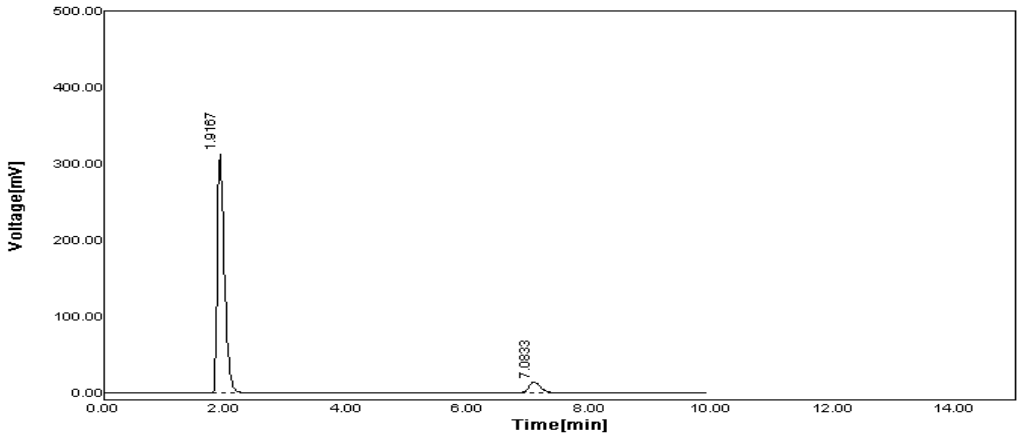

Fig. 15 Chromatogram of Robustness (>1.0 mL/min)

The results of assay of test solution were not affected by varying the conditions. They fully agree with the results obtained under original conditions. The **% RSD** for **(Retention time, Peak area and % Amount Found)** is not more than **2%** for both (MET & PIOG) which is in agreement with system suitability. Hence the proposed HPLC method for the determination of METand PIOG in a tablet was found to be **robust.**

4. Conclusion

The developed RP-HPLC method was found to be simple, accurate, sensitive, precise, rugged, robust, economical and rapid. The developed RP-HPLC method shows the good resolution between MET and PIOG within the run time of 10 min. The developed RP-HPLC method was found to be linear over wider concentration range. Therefore the developed RP-HPLC method can be applied for routine quantitative and qualitative analysis of MET and PIOG in bulk and pharmaceutical formulations like tablets. The developed RP-HPLC method was validated as per the ICH guidelines. The developed RP-HPLC method has a stability indicating nature hence the proposed method could be employed for the stability studies on pharmaceutical preparations within pharmaceutical industry.

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