International Journal of Pharmaceutical Chemistry
ISSN: 2249-734X (Online)
Journal DOI: 10.7439/ijpc
CODEN: IJPCH3 (American Chemical Society)
Research Article

Development of validated analytical method for estimation of Metformin and Pioglitazone in pharmaceutical dosage form

Manoj Charde*[1], SK Jafar[1] and Rita Chakole[2]

[1]*Government College of Pharmacy, Kathora Naka, Amravati – 444604 Maharashtra, India*
[2]*Department of Pharmacy, Government Polytechnic, Gadge Nagar, Amravati – 444603 Maharashtra, India*

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 50-250 µg/mL for MET and 3- 15 µg/mL for PIOG were exhibited for the assay. The linear calibration curves were found over the entire range linearity (r^2 = 0.996 for MET and r^2 = 0.995 for PIOG) and 99.81 % for MET and 99.33 % for PIOG mean % recovery was found with % RSD was NMT 2 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 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 methode, 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 particular drug products. Method validation parameters as defined by ICH (International Conference on Harmonization) guidelines are Accuracy, Precision, Selectivity/Specificity, Limit of Quantitation, 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 [Figure 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

* Correspondence Info
Dr. Manoj Charde.
Government College of Pharmacy,
Kathora Naka, Amravati – 444604
Maharashtra, India
E mail: manojudps@rediffmail.com

Pioglitazone [Figure 2] chemically is (dl)-5-{[[4-2-(5-ethyl-2-pyridinyl) ethyl] phenyl}-2, 4- thiazolidinedione 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.

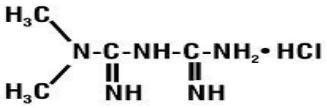


Figure 1: Chemical Structure of Metformin HCl.

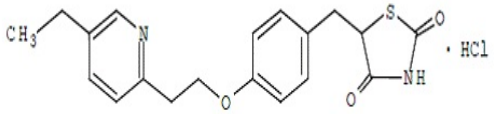


Figure 2: Chemical Structure of Pioglitazone HCl.

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 mg and PIOG 30 mg and all the chemicals were used are of analytical grade.

2.32 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 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 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.

2.6 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 µg/mL PIOG & 500.0 µg/mL MET as final concentration and sonicated for 10.0 min in ultrasonicator.

2.7 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 the Rheodyne 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: 70) 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 **Figure 1**. Hence the following optimized chromatographic parameters were selected to carry out further experimentation.

- **Column** : Comosil RP-C18 (4.6 x 150mm, 5µm)
- **Flow Rate** : 0.7 mL/min
- **Wavelength** : 254.0 nm
- **Injection Volume** : 20.0 µL
- **Column Temperature** : Ambient
- **Run Time** : 10.0 min
- **Mobile Phase** : Acetonitrile: Pott. Dihydrogen Phosphate (30:70)
- **pH** : 2.5 (Using OPA)

2.8 System Suitability Studies

This studies are the pharmacopoeial requirement and is used to verify, whether the reproducibility and resolution of the chromatographic system for analysis to be carried out are adequate or not. To ensure that the system is read to deliver results with acceptable accuracy and precision and operating 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 **Table 1 & 2.**

2.9 Analysis of Standard Laboratory Mixtures

2.9.1 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 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: 1.0 mL of solution (A) and 1.0 mL of solution (B) was then transferred to 10.0 mL 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.

2.9.2 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 50.0 mL 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 0.05 mL and 0.15 mL was then transferred to two separate 10.0 mL volumetric flask and then volume was made up to the mark with mobile phase to get final concentrations of (50.0 µg/mL & 3.0 µg/mL, 100 µg/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.**

2.9.3 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 mL was 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, 100.0 µg/mL and 3.0 µg/mL, 6.0 µg/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 **Figure 4.**

2.10 Method Validation

2.10.1 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 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 **Figure 5 & 6**. The observations are shown in **Table 5.**

2.10.2 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 µg/mL of MET & 6 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 showed in **Table 6 (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 pre analyzed Tablet powder (mg)

C= Weight of pure drug added (mg)

2.10.3 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.**

2.10.4 Ruggedness

2.10.4.1 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.

2.10.4.2 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 **Table 8 & 9.**

2.10.5 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 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

3.1 Optimization of Mobile Phase and Chromatographic Conditions

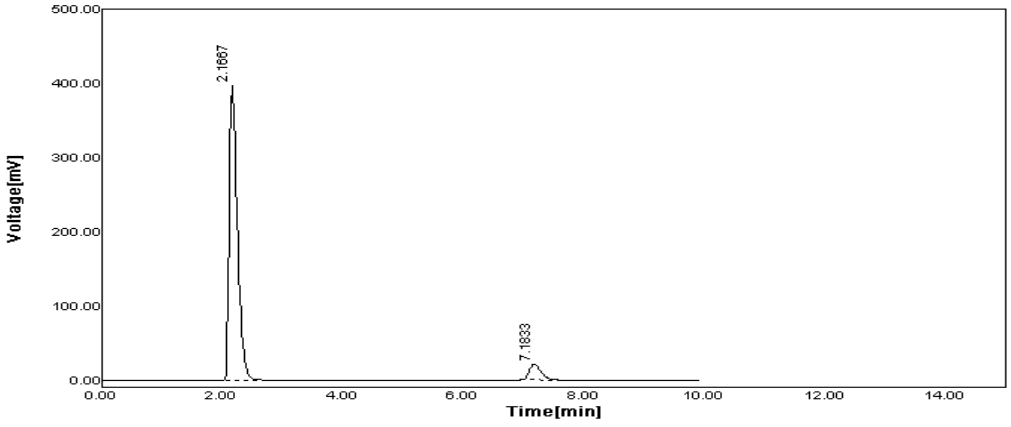


Figure 3: Optimized Chromatogram of MET & PIOG

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

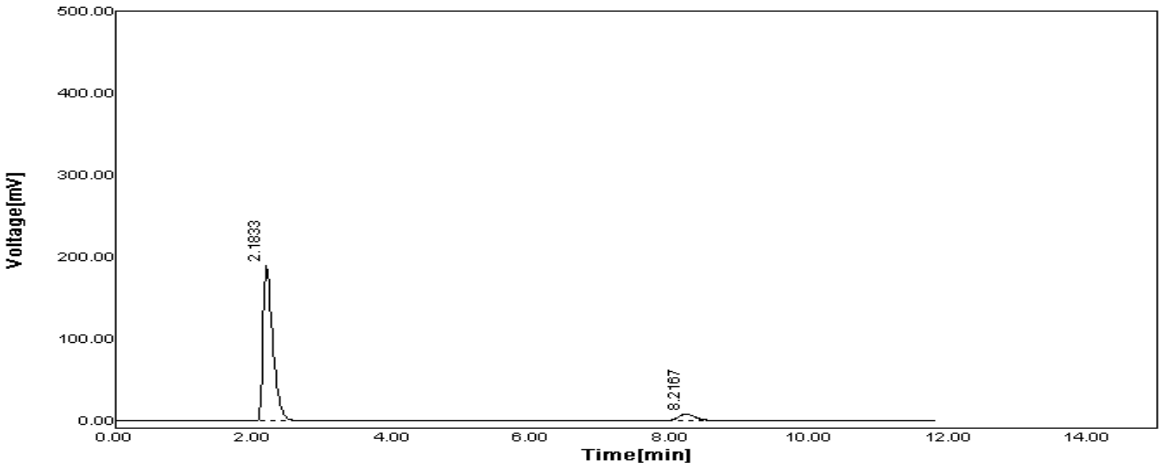


Figure 4: Chromatogram of Marketed Formulation

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

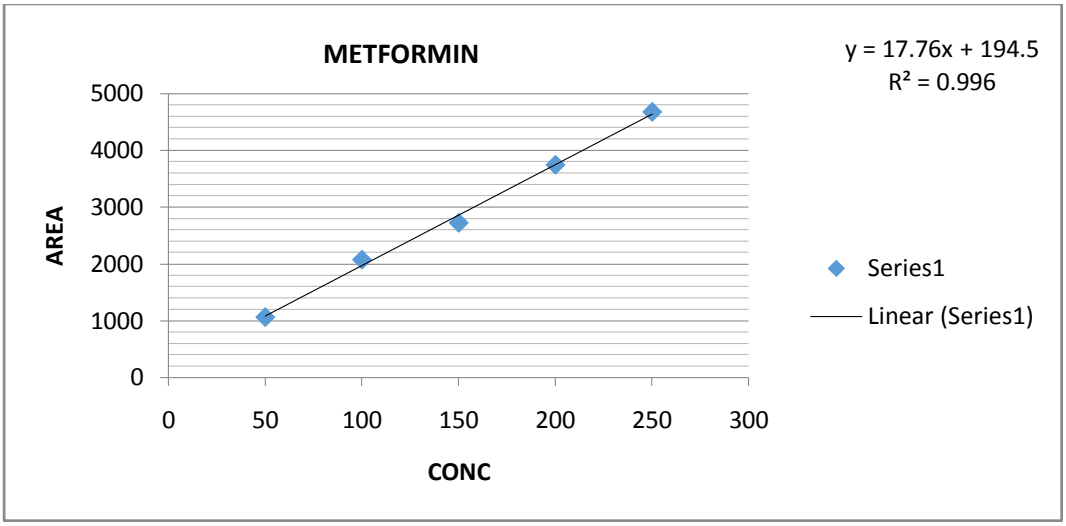


Figure 5: Calibration Curve of MET

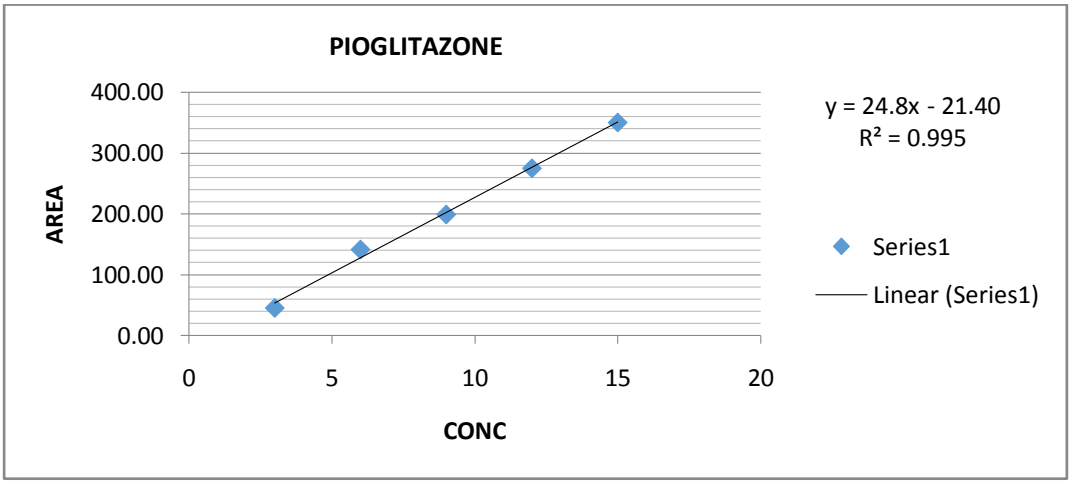


Figure 6 Calibration Curve of PIOG

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.

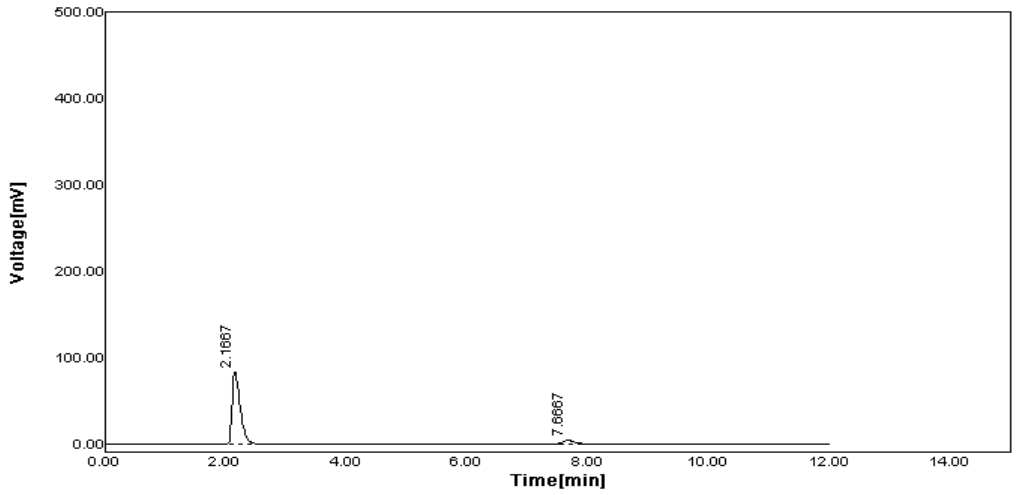


Figure 7: Linearity Chromatogram for (50 µg/mL of MET & 3 µg/mL of PIOG)

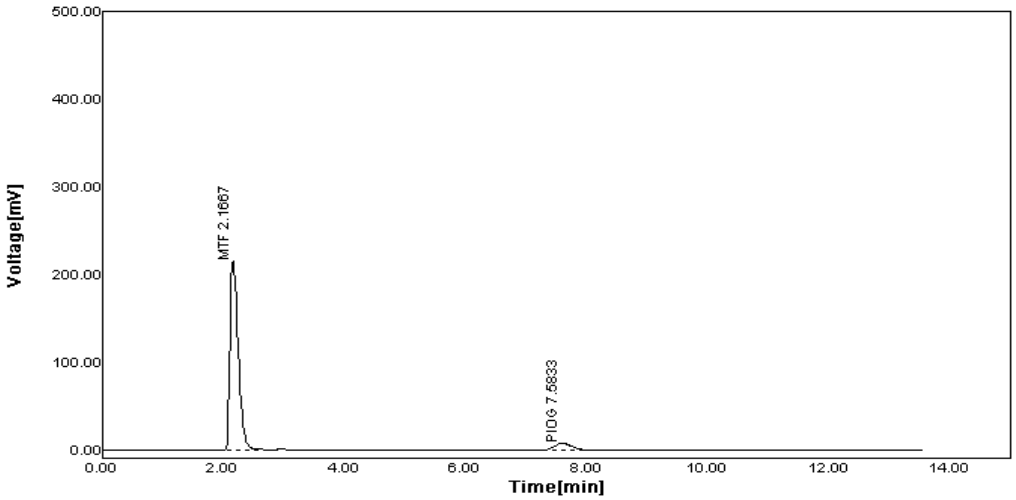


Figure 8: Linearity Chromatogram for (100 µg/mL of MET & 6 µg/mL of PIOG)

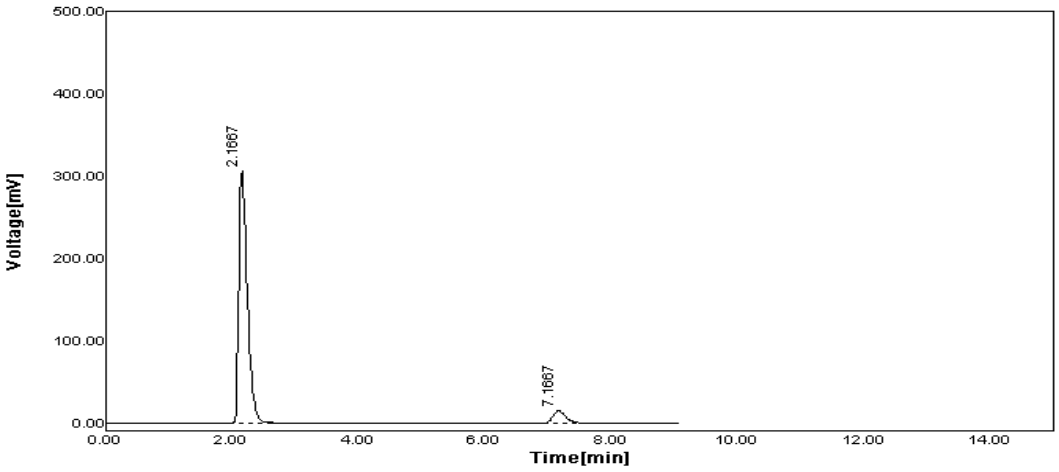


Figure 9: Linearity Chromatogram for (150 µg/mL of MET & 9 µg/mL of PIOG)

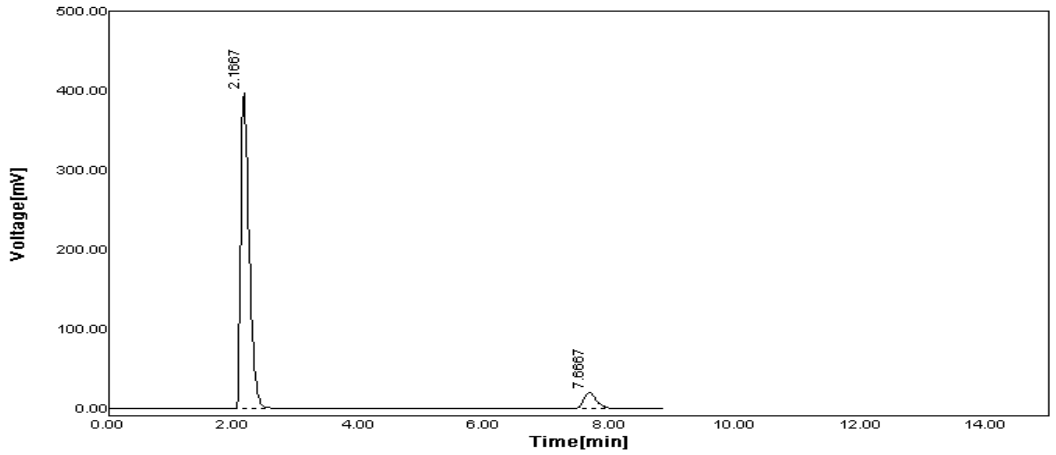


Figure 10: Linearity Chromatogram for (200 µg/mL of MET & 12 µg/mL of PIOG)

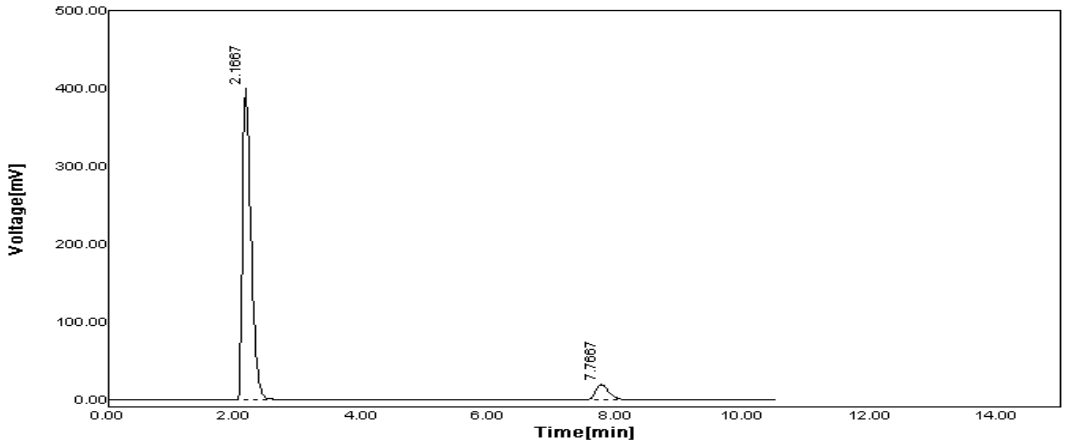


Figure 11: Linearity Chromatogram for (250 µg/mL of MET & 15 µg/mL of PIOG)

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 MET and PIOG in a tablet was found to be sufficiently **precise.**

4. Ruggedness

4.1 Intra and Inter 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 and Inter Day studies for PIOG

Table 9 Results of Intra- Day and Inter – Day Studies for **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 MET and PIOG in a tablet was found to be sufficiently **rugged.**

5. Robustness

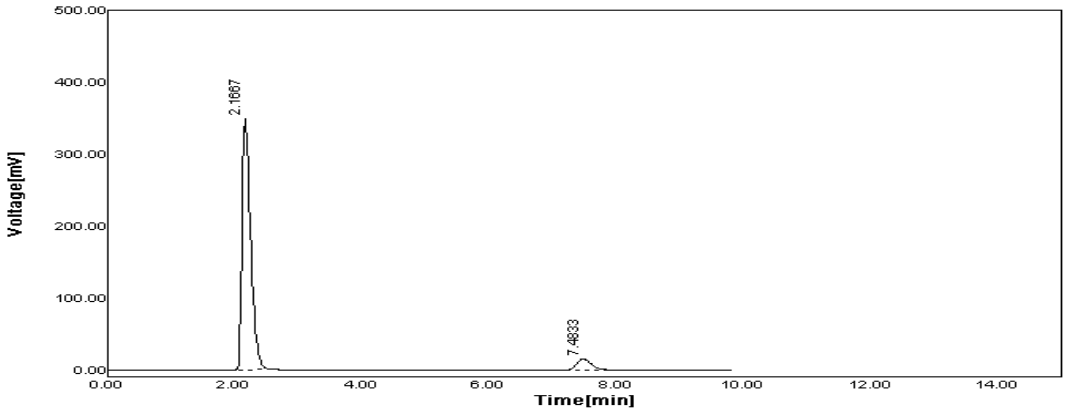
Table 10 (a) Results of Robustness Studies for MET

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

Figure 14: Chromatogram of Robustness (>1.0 nm)



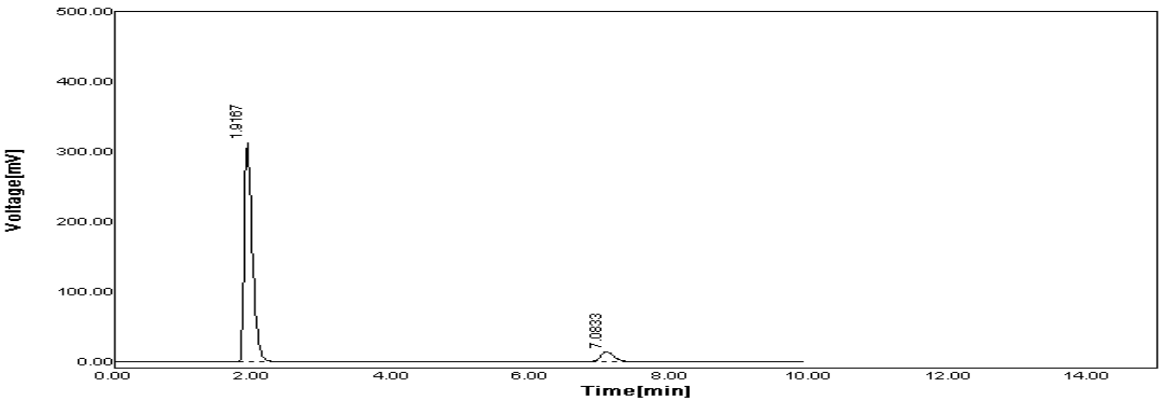


Figure 15: Chromatogram of Robustness (>1.0 mL/min)

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 MET and 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.

References

[1] Beckett A.H., Stenlake J.B., Practical Pharmaceutical Chemistry, 4th ed., CBS Publishers and Distributors, New Delhi, Part-2, 2002.
[2] Sharma B.K., "Instrumental methods of chemical analysis", 23rd ed. Goal publishing House, Meerut, 2004.
[3] Christian G.D., Analytical Chemistry. 5th ed., John Wiley and Sons, Inc., 2003.
[4] Beckett A. H, Stenlake J B, Practical Pharmaceutical Chemistry, 4th edition, part II, CBS Publisher and Distributor, New Delhi, 1997: pg.no.281-288.
[5] Katz E., Quantitative Analysis Using Chromatographic Techniques, Wiley India Pvt. Ltd.: 2009, pp. 193 -211.
[6] Skoog D. A., Holler F. J. and Nieman T.A., Principles of Instrumental Analysis, 5th edn, Thomson Brook/cole, 2005, pp. 674-696.
[7] Connors K. A., Liquid Chromatography- A Textbook of Pharmaceutical Analysis, 3rd edn., Willey Interscience, New York, 1999, pp. 373-438.
[8] Beckett H., Stenlake J. B., Practical Pharmaceutical Chemistry, 4thedn., Part II, CBS Publications and Distributors, New Delhi, 1997, pp. 1, 275-300.
[9] Heftman E., Chromatography- Fundamentals & applications of Chromatography and Related differential migration methods, 6thedn, Elsevier, Amsterdam, Vol. 69A, 2004, pp. 253-291.
[10] Lough W.J., I.W. Wainer, "HPLC fundamental principles and practices," (1991), Blackie Academic and professional, 52-67.
[11] Ahuja S. and M. W. Dong, "Handbook of Pharmaceutical Analysis by HPLC", (2005), Ist Ed., 56.
[12] Sethi P. D., "HPLC-Quantitative Analysis of Pharmaceutical Formulations", CBS publishers and distributors, New Delhi, Ist Ed., (2001), 1-19.
[13] Dong M. W., "Modern HPLC for Practicing Scientist", A John wiley & Sons, Inc. Publication, (2006), 86-87.
[14] Chandanam Sreedhar. *et al*., "New analytical methode development and validation of some oral hypoglycemic drugs",*RJBPCS*(2012), vol-3(4), page no: 20
[15] Subhashini Edla and Syama Sundhar "New analytical method development and validation for the simultaneous estimation of metformin and glibenclamide in bulk and tablet dosage form using RP-HPLC", *RJCABP* (2014), vol-7, page no: 55-63.
[16] Patel Deepa R., Laxmabhai J. Patel and Madhabhai M. Patel, "For Stability-indicating reversed phase high performance liquid chromatographic method the simultaneous determination of Repaglinide and Metformin hydrochloride", *Asian journal of Research in Chemistry* (2011)4,500-505.
[17] Inamdar. *et al*., "Revised RP-HPLC for simultaneous determination of vildagliptin and Pioglitazone HCL application in commercially available drug products", *IJPSR* (2013),vol-4(2), page no: 847-855.
[18] Gumieniczek A. *et al*., "Stability-indicating validated HPLC method for simultaneous determination of oral antidiabetic drugs from thiazolidinedione and sulfonylurea groups in combined dosage forms", *JAOAC Int*. 2010 Jul-Aug; 93(4):1086-92.
[19] Shantikumar S. *et al*., "Simulteneous estimation of Sitagliptin and Pioglitazone by U.V.spectroscopic methode & study of interference of various excipients on this combination of drugs", *IJCPR* (2012), vol-4.

[20] Pathan Habibulla *et al*., "Analytical method development & validation for some oral hypoglycemic drugs", *DHR-IJPS* (2014), vol-5(1).

[21] Sakala Bhargavi. Gopisetty Suryasagar, Dantu Krishna Sowmya, Kota Ashok, Sree Kanth Nama "UV spectrophotometric methode for determination of glimepiride in pharmaceutical dosage form", *IJPSR* (2013), vol-21(2), page no: 131-133.

[22] Ayyappan J., Umapathi.P, Darlin Quine S. , "Validated HPLC methode for fast & sensitive determination of few antidiabetic drugs residues in support of cleaning validation in formulation area", *IJPPS* (2011), vol-3(5).

[23] Jitendra Kumar *et al*. Recent Approaches for Impurity Profiling of Pharmaceuticals. *International Journal of Advances in Pharmaceutics* 2013; 2(3): 25-34.

[24] Abhijeet Welankiwar *et al*. Photostability Testing of Pharmaceutical Products. *Int Res J Pharm* 2013; 4(9): 11-15.

[25] www.drugbank.ca/DB00335/Metformin.

[26] www.drugbank.ca/DB00310/Pioglitazone.