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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND PIOGLITAZONE BY RP-HPLC IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT

Objective: The day by day new combinations drugs are being introduced in market. Then the multiple therapeutic agents which acts at different sites are used in the management of various diseases and disorders are done. Thus it is necessary to develop methods for analysis with the help of number of analytical techniques which are available for the estimation of the drugs in combinations. An accurate, precise and reproducible RP-HPLC method was developed for the simultaneous quantitative determination of Metformin Hydrochloride (MET) and Pioglitazone (PIO) in tablet dosage forms. Methods: Younglin (S. K.) gradient system UV detector and C18 column with 250 mm x 4.6 mm i. d. and 5µm particle size Acetonitrile: OPA water (80: 20v/v) pH 2.5 was used as the mobile phase for the method. The detection wavelength was 283 nm and flow rate was 0.9 ml/min. Results: In the developed method, the retention time of MET and PIO were found to be 6.366 min and 8.616 min. The developed method was validated according to the ICH guidelines. Conclusion: In this methods linearity, precision, range, robustness were observed. The method was found to be simple, accurate, precise, economic and reproducible. So the proposed methods can be used for the routine quality control analysis of MET and PIO in bulk drug as well as in formulations.

Key Words:-Metformin Hydrochloride, Pioglitazone, Method- Development, Validation, HPLC.

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INTRODUCTION

Pharmaceutical Analysis plays a vital role in quality assurance and quality control of bulk drugs and their formulations. Pharmaceutical analysis is a particular branch of analytical chemistry, which includes isolating, identifying and determining the relative amounts of compounds in a sample matter. It is concerned with chemical characterization of matter both quantitative and qualitative. In recent years many analytical techniques have been developed. Analytical method is a particular utilization of a procedure to solve a problem. Analytical instrumentation assumes an imperative part in the production and evaluation of new products and protection of Consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, medications, water and air.

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an integral part of the quality control system. The second is that current good manufacturing practice regulation requires assay validation.

Metformin Hydrochloride is chemically known as (S)-Isopropyl 2-((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-

methyltetrahydrofuran-2-yl) methoxy)-(phenoxy) phosphorylamino) propanoate . It has a molecular formula of $C_{22}H_{29}FN_3O_9P$ and a molecular weight of 529.45 **(Figure 1).**

Metformin Hydrochloride is a white to off-white powder with a solubility of ≥ 2 mg/ml across the pH range of 2-7.7 at 37°C. The partition coefficient (log P) for Metformin Hydrochloride is 1.62 and the pKa is 9.3 (European Medicines Agency, 2014). Metformin Hydrochloride is a pangenotypic inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is essential for viral replication (Keating GM, 2014).

Pioglitazone (PIO); is chemically known as Methyl [(2S)-1-{(6S)-6-[5-(9,9-difluoro-7- {2-[(1R,3S,4S)-2-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}-2-azabicyclo[2.2.1] hept-3-yl]-1H-benzimidazol-6-yl}-9H-fluoren-2-yl)-1H-imidazol-2-yl]-5-azaspiro[2.4] hept-5-yl}-3-methyl-1-oxobutan-2-yl] carbamate. It has a molecular formula of $C_{49}H_{54}F_2N_8O_6$ and a molecular weight of 889.00 **(Figure 2).** Pioglitazone is a white to tinted (off-white, tan, yellow, orange, or pink), slightly hygroscopic crystalline solid. Pioglitazone is practically insoluble (<0.1 mg/mL) across the pH range of 3.0-7.5 and is slightly soluble below pH 2.3 (1.1 mg/ml). The partition coefficient (log P) for Pioglitazone is 3.8 and the pKa1 is 4.0 and pKa2 is 5.0 (European Medicines Agency, 2014).

Metformin Hydrochloride in human plasma was determined by UPLC-MS/MS method (Rezk MR et al., 2016). Quantification of Metformin Hydrochloride and its metabolite, GS-331007, in human plasma has been determined by UPLC-ESI-MS/MS method (Rezk MR et al, 2015). Simultaneous quantification ofet al., ribavirin, Metformin Hydrochloride and its metabolite in rat plasma by UPLC-MS/MS has been reported (Shi X et al., 2015). MET in pure form (Vikas PM et al., 2016), in bulk and tablet dosage form was determined by RP-HPLC (RavikumarVejendla et al., 2016). Finally, Metformin Hydrochloride (MET) was used as an internal standard (IS) in an UPLC-MS/MS method for the determination of daclatasvir (DAC) in human plasma (Rezk MR et al., 2016). While for PIO, only two methods have been published for its individual determination in bulk drug form by simple UV spectrophotometry (Ranjana S et al., 2016) and by RP-HPLC (Devilal J et al., 2016). Both Metformin Hydrochloride and Pioglitazone in human plasma were determined by UPLC-MS/MS method (Rezk MR et al, 2015) and besides some antiviral agents (Ariaudo A et al., 2016). Pioglitazone, Metformin Hydrochloride and its metabolite in ratplasma were also, determined by UPLC-MS/MS (Pan C et al., 2016).

According to the best of our knowledge, only three HPLC methods (BakhtZaman et al., 2016; Rezk M.R et al., 2016) have been published, during the preparation of the present work for publishing. The present study aimed to develop a simple, sensitive, short retention time and accurate RP-HPLC method for the simultaneous determination of both Metformin Hydrochloride and Pioglitazone together in pure and tablet dosage forms with high sensitivity, selectivity that can be used for the routine analysis of production samples.

MATERIALS AND METHODS

Materials and Reagents

The analysis of the drug was carried out onYoungline (S. K.) Gradient System UV Detector. Equipped with reverse phase (Grace) C_{18} column (4.6mm x 250mm; 5µm), a SP930D pump, a 20µl injection loop and UV730D Absorbance detector and running autochro-3000 software. Metformin Hydrochloride and Pioglitazone were procured from R.S.I.T.C Jalgaon. Orthophopsphoric acid (OPA) (Avantor Performance material India Ltd. Thane, Maharashtra) and methanol, acetonitrile, (HPLC gradeMerck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai.), water, 0.45 µm filter (Millipore, BangPIOre). A combination of Metformin Hydrochloride (400 mg) and Pioglitazone (90 mg) in tablet formulation was procured from Hetero drugs Ltd. Mumbai (PIOfos brand).

Chromatographic Conditions

Column C_{18} (250 mm× 4.6 mm); particle size packing 5µm ; detection wavelength of 283 nm; flow rate 0.9 ml/min; temperature ambient; sample size 20 µl; mobile phase Acetonitrile: water (OPA 0.1% PH 2.5 with TEA) (80:20); run time of 12 mins.

Preparation of standard stock solution

40 mg of Metformin Hydrochloride and 10 mg of Pioglitazone were weighed accurately and transferred to a 10 ml volumetric flask dissolved in methanol and diluted to 10 ml with the mobile phase Acetonitrile + 0.1% OPA water with TEA(80 + 20% v/v) to give a stock solution of 4000 µg/ml Metformin Hydrochloride and 1000 µg/ml Pioglitazone **(Table 1 and Figure . 3).**

Method development and validation

Serial dilutions were done to prepared various concentration stock (Standard solution and diluted to get required concentration for calibration plot and which was injected (ICH, 1996; ICH Harmonised Tripartite Guideline, 2005; ICH, 2002, 2005, 2003, 1996; US DHHS, 2013, 2001; FDA, 1996, 2001; Smith, 2012; Hassan and Bahrani, 2014; Harona et al., 2019).

Assay preparation for commercial formulation

For analysis of the tablet dosage form, weigh 20 Metformin Hydrochloride and Pioglitazone combination tablets and calculated the average weight, accurately weigh and transfer the sample equivalent to 12.2 mg MET and PIO into 10 ml volumetric flask. Add

about 10ml ACN of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm nylon membrane filter. Then volume was made up to the mark with Acetonitrile + 0.1% OPA water with TEA (80 + 20% v/v). The simple chromatogram of test MET and PIO shown in **(Figure 4).** The amounts of MET and PIO per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for % Label claim for % RSD Calculated, Result was shown in **(Table 2).**

RESULTS

Linearity and Range

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 20-100µg/ml for MET and 5-25µg/mL for PIO (**Table 3 and 4)** depict the calibration data of MET and PIO. The respective linear equation for MET was y = 38.01x + 80.60 and PIO equation y = 54.47x +7.385where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of MET and PIO shown (**Figure 5 and 6).**

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To a pre-analysed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed**.**The % recovery was found to be within 98-101%. Statistical validation of recovery studies are shown in **(Table 5, 6 and Figure 7, 8 and 9).**

System suitability parameters

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of MET and PIO system suitability parameters were studied. The result shown **(Figure 10 and Table 7)**

Precision

The method was established by analyzing various standards of MET and PIO. All the solution were analyzed thrice in order to record any intra-day & inter-day variation in the result. The result obtained for interday and intraday variation is shown in the **(Table 8 and Figure 11).**

Robustness

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The results indicate that less variability in retention time and tailing factor were observed **(Table 9 and 10).**

Table 1:Details of chromatogram of standard combination containing MET and PIO

Sr. No.	Name of drug	RT[min]	Area [mV*s]	Area%	TP	TF	Resolution
1	**Metformin Hydrochloride**	6.483	3883.2092	73.98	7934.8	1.333	0.0000
2	**Pioglitazone**	8.7500	1365.7129	26.02	10613.9	1.2273	9.7647
Sum			5248.9219				

Table 2: Analysis of marketed formulation

Assay	Drug	Label Claimed	Amt. Found	% Label claim	SD	%RSD
RP-HPLC Method	**MET**	80	80.31	100.39	0.02	0.01
	PIO	20	20.00	100.00	0.01	0.01
	MET	80	80.28	100.35	0.28	0.01
	PIO	20	19.99	99.95	0.00	0.01

Table 3: Linearity data for Metformin Hydrochloride.

Method	Conc. µg/ml	Peak area (µV.sec) 1	Peak area (µV.sec) 2	Average peak area (µV.sec)	S. D. of Peak Area	% RSD of Peak Area
RP-HPLC Method	20	849.7955	850.6942	850.24	0.64	0.07
	40	1598.8525	1599.3652	1599.109	0.36	0.02
	60	2340.5071	2380.40	2339.454	1.49	0.06
	80	3132.3569	3135.1005	3133.729	1.94	006
	100	3883.2092	3885.1035	3885.156	1.34	0.03
	Equation	y = 38.011x + 80.60				
	R^2	0.999				

Table 4: Linearity data for Pioglitazone

Method	Conc. µg/ml	Peak area (µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	5	276.2312	277.2356	276.7334	0.71	0.26
	10	552.436	552.213	552.3245	0.16	0.03
	15	829.4583	830.2341	829.8462	0.55	0.07
	20	1097.2723	1098.1311	1097.702	0.61	0.06
	25	1365.7129	1365.1014	1365.907	0.27	0.02
	Equation		y = 54.47x + 7.385			
	R^2		0.999			

Table 5: Result of recovery data for Metformin Hydrochloride and Pioglitazone

Method	Drug	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	% Recovery Mean *± S.D.
RP-HPLC Method	MET	80%	20	16	1453.68± 0.06	16.12±0.06	100.77±0.34
		100%	20	20	1604.77± 0.02	20.58±0.02	100.50±0.09
		120%	20	24	1755.25±0.03	24.05±0.03	101.58±0.11
	PIO	80%	5	4	496.06±0.01	3.97±0.01	99.46±0.12
		100%	5	5	547.8± 0.06	4.91±0.06	98.42±1.33
		120%	5	6	604.93±0.01	5.97±0.01	101.58±0.18

***mean of each 3 reading for RP-HPLC method.**

Table 6: Statistical validation of recovery studies Metformin Hydrochloride and Pioglitazone.

Method	Level of Recovery (%)	Drug	Mean % Recovery	S. D.*	% RSD
RP-HPLC Method	80%	MET	100.77	0.34	0.34
		PIO	99.46	0.12	0.12
	100%	MET	100.50	0.09	0.09
		PIO	98.42	1.33	1.35
	120%	MET	101.58	0.11	0.11
		PIO	101.58	0.18	0.18

***Denotes average of three determinations for RP-HPLC**

Table 7: Repeatability studies on RP-HPLC for Metformin Hydrochloride and Pioglitazone.

Method	Conc. of MET and PIO (mg/ml)	Peak area	Amount found (mg)	% Amount found
RP-HPLC Method for MET	60	1419.4238	61.52	102.53
	60	2418.9814		
		Mean	**61.52**	
		SD	**0.31**	
		%RSD	**0.01**	
RP-HPLC Method for PIO	15	828.8961	15.09	100.60
	15	830.4302		
		Mean	**1.08**	
		SD	**0.13**	
		%RSD		

Table 8: Result of Intra day and Inter day Precision studies on RP-HPLC method for MET and PIO.

Method	Drug	Conc.(µg/ml)	Intraday Precision		Intraday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
Rp-HPLC Method	MET	20	849.39±1.52	101.40	846.66±1.93	100.75
		60	2340.47±1.11	99.53	24.08.27±9.80	102.05
		100	3794.04±67.83	97.69	3882.20±1.48	100.01
	PIO	5	278.54±0.81	99.56	279.08±0.96	98.00
		15	828.83±1.01	100.53	830.67±0.50	100.73
		25	1356.12±0.15	99.04	1356.46±0.91	99.04

Table 9: Result of Robustness study of Metformin Hydrochloride

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	%RSD
Chromatogram of flow change 0.8ml	60	2232.99±32.73	1.47
Chromatogram of flow change 1.0 ml	60	2425.59±0.36.82	1.52
Chromatogram of comp change 79ml ACN+21ml water	60	2288.50±10.69	0.47
Chromatogram of comp change 81mlACN +19ml water	60	2401.26±15.20	0.63
Chromatogram of comp change wavelength change 282nm	60	2248.80±7.70	0.34
Chromatogram of comp change wavelength change 284nm	60	2478.70±4.81	0.19

Table 10: Result of Robustness study of Pioglitazone

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	% RSD
Chromatogram of flow change 0.8ml	15	766.25±4.08	0.53
Chromatogram of flow change 1.0 ml	15	829.12±1.83	0.22
Chromatogram of comp change 79ml ACN +21ml water	15	790.40±1.19	0.15
Chromatogram of comp change 81mlACN +19ml water	15	828.50±1.17	0.14
Chromatogram of comp change wavelength change 282nm	15	759.10±0.40	0.05
Chromatogram of comp change wavelength change 284nm	15	860.32±2.68	0.31

Figure 1: Structure of Metformin Hydrochloride

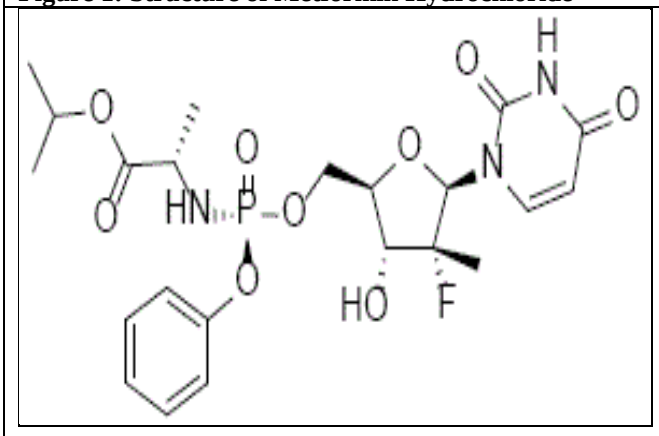


Figure 2: Structure of Pioglitazone

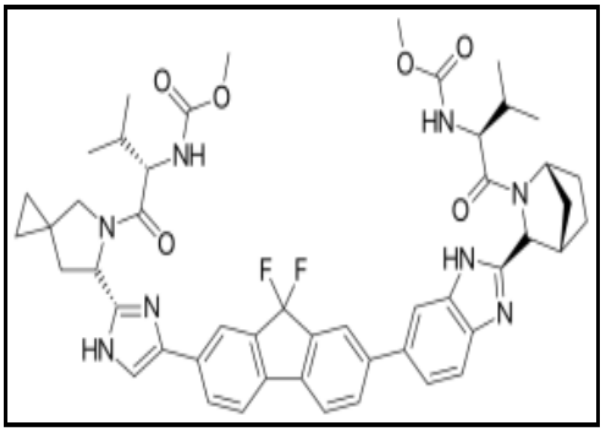


Figure 3: Chromatogram of standard combination of Metformin Hydrochloride and Pioglitazone

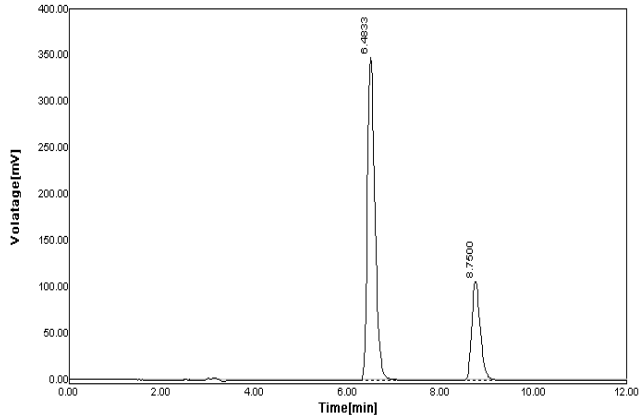


Figure 4: Chromatogram for marketed formulation

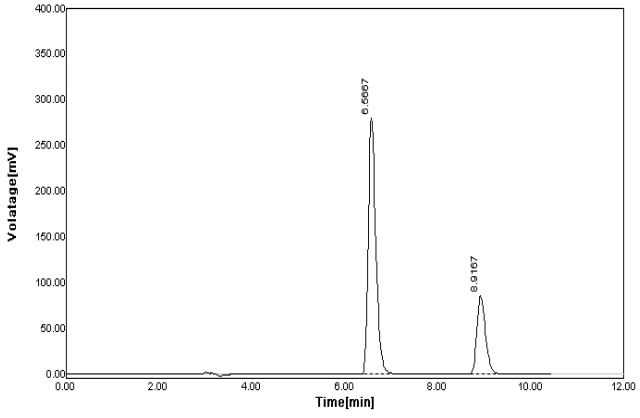


Figure 5: Calibration curve of Metformin Hydrochloride

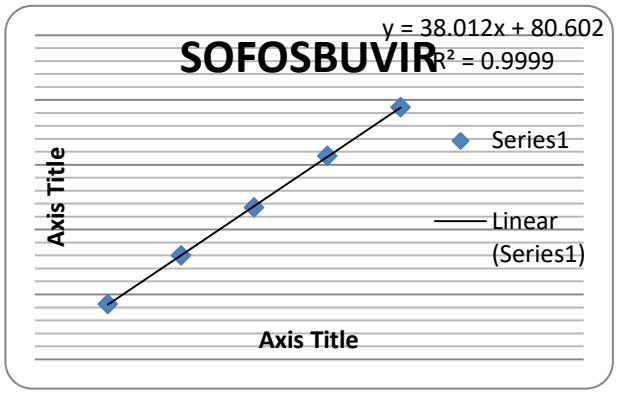


Figure 6: Calibration curve of Pioglitazone

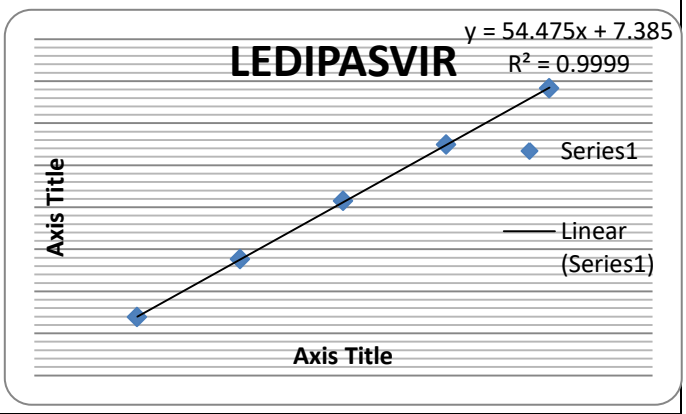


Figure 7: Chromatogram of Accuracy 80%

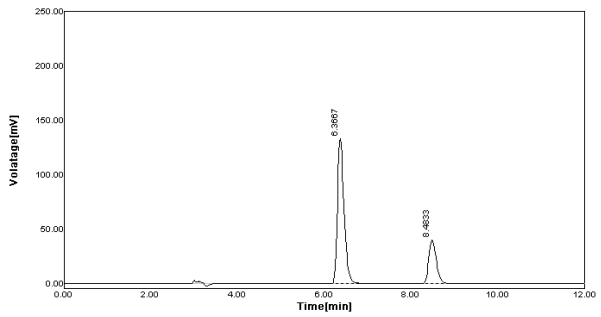


Figure 8: Chromatogram of Accuracy 100%

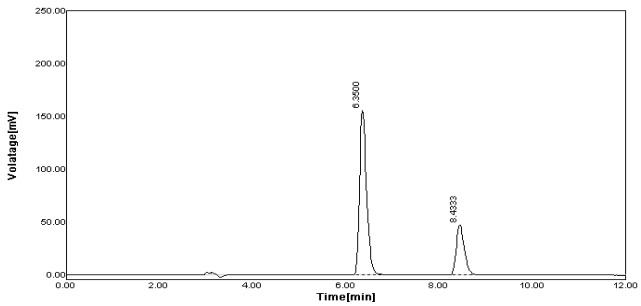


Figure 9: Chromatogram of Accuracy 120%

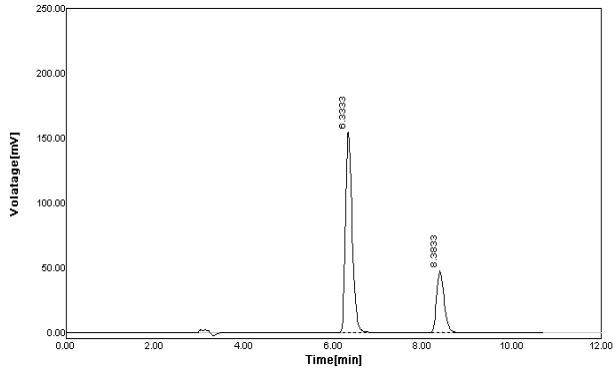


Figure 10: Chromatogram of system suitability -1

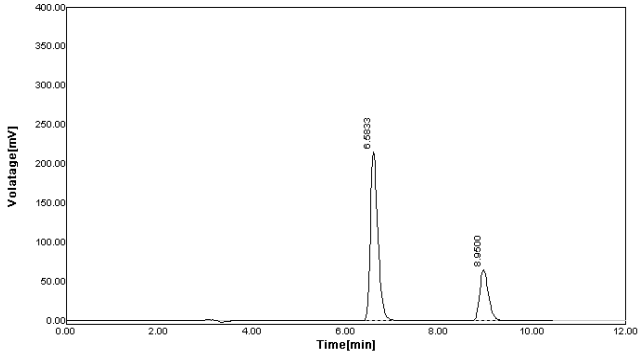
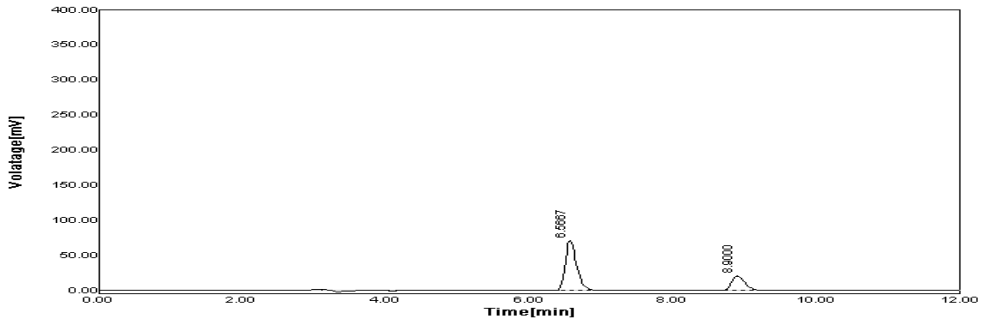


Figure 11: Chromatogram of Precision



DISCUSSION

The proposed methods for simultaneous estimation of MET and PIO in tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration yielded correlation coefficient (r^2) 0.999 for both MET and PIO at all the selected wavelengths. The values of % RSD are within the prescribed limit of 2 %, showing high precision of methods and recovery was close to 100% for both drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for their simultaneous determination with virtually no interference of any additive present in pharmaceutical formulations.Hence, the above methods can be applied successfully for simultaneous estimation of MET and PIO in formulations.

CONCLUSION

The developed HPLC methods in that linearity, precision, range, robustness were found to be more accurate, precise and reproducible. Themethods were found to be simple & time saving. All proposed methods could be applied for routine analysis in quality control laboratories.

Abbreviation used:

HPLC: High performance liquid chromatography; **UV:** Ultraviolet; **ICH:** International Conference on Harmonization; **LOQ:** Limit of quantitation; **LOD:** Limit of detection; **RSD:** Relative standard deviation; **RT:** Retention time; **OPA:**Orthophosphoric acid; **MET:**Metformin Hydrochloride; **PIO: Pioglitazone**;**FDA:** Food and Drug Administration; **SD:** Standard deviation.

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