Spectrophotometric Method for Simultaneous Estimation of Metformin, Voglibose and Pioglitazone in Triple Fixed Combined Pharmaceutical Dosage Form

M. A. Shende[1], A. A. Sayyad[2]
[1]*Government College of Pharmacy, Vidyanagar, Karad-415124, (MS), India*
[2]*Government College of Pharmacy, Kathora Naka, Amravati- 444604, (MS), India*

Abstract **- The purpose of the present study was to develop a rapid, sensitive, simple and precise spectrophotometric method for the simultaneous estimation of metformin hydrochloride, Voglibose and pioglitazone from single tablet triple fixed dose combinations of oral hypoglycemic agents. The simultaneous equation method involves the measurement of absorbance at three wavelengths 236, 256 and 268 nm (λmax) for Metformin, Voglibose and Pioglitazone respectively using methanol as diluent. Validation of method was carried out according to International Conference on Harmonization (ICH) guidelines. The linearity lies between 5-25µg/ml, 0.2-1 µg/ml, and 2-10 µg/ml for Metformin hydrochloride, Voglibose and Pioglitazone respectively and r^2 values were above 0.998 for all drugs. The accuracy and precision of the methods were determined and validated statistically. The developed method exhibited good reproducibility 100.21, 98.70 and 101.28% for Metformin hydrochloride, Voglibose and Pioglitazone respectively and recovery with a relative standard deviation of <2%. The developed spectrophotometric method was found to be rapid, sensitive, selective, and reproducible and can be successfully applied for the routine analysis of Metformin hydrochloride, Voglibose and Pioglitazone in a combined solid dosage form.**

Index Terms - UV-Spectrophotometry, Simultaneous equation method, Metformin hydrochloride (MET), Voglibose (VOG), Pioglitazone (PIO)

INTRODUCTION

The assurance of quality is achieved through analysis of drug product. Marketed survey is revealed day by day new drugs and their combination with another drugs are being introduced in market as they have more patient compliance than a single drug. Analytical methods are necessary to assure the identity, strength, quality, purity, bioavailability and stability of drug as well as pharmaceutical dosage forms. Metformin hydrochloride is a biguanide hypoglycemic agent used in the treatment of non-insulin-dependent diabetes mellitus and chemically it is 3-(diaminomethylidene)-1, 1-dimethylguanidine. Metformin is used to control glycemic condition by improving insulin sensitivity and decreasing intestinal absorption of glucose. Voglibose is chemically valiolamine derivative (1S, 2S, 3R, 4S, 5S)-5-(1, 3-dihydroxypropan-2-ylamino)-1-(hydroxymethyl) cyclohexane-1, 2, 3, 4-tetrol and inhibitor of α-glucosidase with antihyperglycemic activity. Voglibose inhibits an enteric enzyme α-glucosidase which found in the brush border of the small intestines that hydrolyzes oligosaccharides and disaccharides into glucose and other monosaccharides.

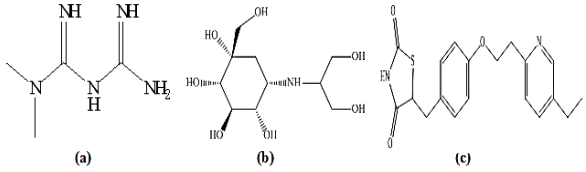

Fig.1: Chemical structure of Metformin (a), Voglibose (b) and Pioglitazone (c)

Pioglitazone is 5-[[4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl] methyl]-1, 3-thiazolidine-2, 4-dione and it is selective agonists for the nuclear peroxisome proliferator-activated γ-receptor (PPARγ) which enhances the transcription of several insulin responsive genes (Fig.1). The combination of Metformin (MET), Voglibose (VOG), and Pioglitazone (PIO) is widely available in the market for the treatment of diabetes mellitus and particularly demonstrated significant decrease in glucose level in diabetes. In general, these kinds of multicomponent dosage forms are useful for effective therapy and augment patient compliance.

Method developed can be conveniently used for quality control and routine determination of drug in pharmaceutical preparation in pharmaceutical industry. Ultraviolet visible spectrophotometry is most frequently employed techniques in pharmaceutical analysis. The estimations by photometric methods are based on the Bouger-Lambert-Beer's law, which establishes the absorbance of a solution is directly proportional to analyte concentration and path length in the solution. It involves measurement of the amount of ultraviolet (190-380 nm) or visible (380-800 nm) radiation absorbed by a substance in a solution. Instrument, which measure the ratio, or a function of the ratio of the intensity of two beams of light in the ultraviolet visible regions are called ultraviolet visible spectrophotometer. A range of analytical techniques, such as RP-HPLC, HPLC and UV spectrophotometric methods have been reported for this combination in individually or in combination of listed two drugs. From literature survey, there is need to develop accurate method for the concurrent determination of Voglibose, Pioglitazone and Metformin in combined dosage forms. Therefore, it was aimed to develop a simple, accurate, sensitive and reproducible method for combined Voglibose, Pioglitazone and Metformin in combined dosage forms by UV spectrophotometry using Simultaneous equation method.

EXPERIMENTAL

Materials

Active pharmaceutical ingredients of Metformin, Pioglitazone and Voglibose were received as gift sample from Macloids Pharmaceuticals Ltd. Gujarat, India. Methanol of HPLC grade was procured from Merck limited, India. The commercial sample containing Voglibose (0.2 mg), Metformin (500 mg) and Pioglitazone (7.5 mg) was purchased from local market of D-Bose MP275 brand of Sinsan Pharmaceuticals Pvt. Ltd. Pune, India. All the chemicals and reagents were used of analytical grade.

Instrumentation

Spectrophotometric analysis was performed on a double beam UV/Visible spectrophotometer, Shimadzu (UV 1800), Software UV Probe V2.42. Ultrasonicator, Spectra lab (UCB30) was used for sonication. Standard and sample drugs were weighed by using Contech (model 1473) digital analytical balance.

Selection of Solvent

Solubility studies were done by dissolving drugs in solvents like water and methanol. It was observed that Metformin (MET) was freely soluble in water and methanol but Voglibose (VOG) and Pioglitazone (PIO) were sparingly soluble in water forms turbidity and freely soluble in methanol therefore methanol was selected as a common solvent.

Preparation of standard solution for MET, VOG and PIO

Standard stock solution of MET, VOG and PIO was prepared by dissolving 10 mg of each drug separately in 100 ml volumetric flask using methanol as solvent up to 100 ml and each sample sonicated up to 15 min. The resulting solution contains 100 µg/ml of the drug. From these stock solutions, working stock solutions of concentration were prepared by appropriate dilutions to obtain final concentrations.

Preparation of calibration curve

1 µg/ml solution of MET, VOG and PIO was subjected to a UV spectrophotometric scanning (200-400 nm) to determine the λ_{max} using methanol as blank. The scanning spectra of 1 µg/ml solution of MET, VOG and PIO showed clear peaks at 236, 256 and 268 nm respectively. The overlay spectra of MET, VOG and PIO were also recorded. From the overlay spectra, isoabsorptive point of MET, VOG and PIO was calculated.

Standard dilutions of each drug were prepared separately having concentration of 5-25µg/ml, 0.2-1µg/ml and 2-10µg/ml for MET, VOG and PIO respectively. The absorbance of these standard solutions were measured at 236, 256 and 268 nm. The Calibration curves were constructed by plotting the Absorbance versus concentration and subjected to least square linear regression analysis.

Preparation of standard solution for bulk drugs

Accurately weighed and transferred 500 mg of MET, 0.2 mg of VOG and 7.5 mg of PIO standards into a 100 ml clean dry volumetric flask. Then a small amount of diluent was added and the flask was sonicated for 30 min and diluted up the mark with diluent to obtained final concentrations of MET, PIO and VOG were 5000, 2 and 75µg/ml, respectively. From the above stock solutions, 1 ml was pipette out to a 10ml volumetric flask and the final volume was

made up with diluent to obtain final concentrations of 500µg/ml MET, 0.2µg/ml VOG and 7.5 µg/ml of PIO.

Analysis of tablet formulation

20 tablets of D-Bose MP275 (500/0.2/7.5 mg Metformin hydrochloride, Voglibose and Pioglitazone) were weighed and triturated using mortar and pestle to a fine powder. A quantity equivalent to 500 mg of Metformin hydrochloride 0.2 mg of Voglibose and 7.5 mg of Pioglitazone was transferred into a 100 ml volumetric flask containing 50 ml of methanol and sonicated for 15 min. The final volume was made up to the mark and filtered through Whatman filter paper (12.5 cm). 5 ml of the resulting solution was diluted with distilled water to 100 ml making the solution of 100µg/ml. The resulting solution was again transferred to volumetric flask diluted with methanol and volume was adjusted up to mark to get concentration is 500/0.2/7.5 µg/ml (MET/VOG/PIO). The absorbance was taken at 236 nm, 256nm and 268 nm against methanol blank. The concentration of Metformin hydrochloride, Voglibose and Pioglitazone was calculated by using the simultaneous equation method equation.

Simultaneous Equation Method/Vierodt's Method

A multi-component system consisting of three components X, Y and Z, each of which absorbs at the λ_{max} of the other, λ_1 (236 nm) being the wavelength of maximum absorbance of X (Metformin), λ_2 (256 nm) being the wavelength of maximum absorbance of Y (Voglibose) and λ_3 (268 nm) being the wavelength of maximum absorbance of Z (Pioglitazone).

Determination of absorptivity value

The absorptivity value of Metformin hydrochloride, Voglibose and Pioglitazone from each solution was calculated at selected wavelengths using the following formula (Eq.1);

$$E\ (1\%1cm) = \frac{\text{Absorbance}}{\text{concentration}} \qquad (1)$$

For measurements in 1cm cell, b= 1, therefore, the concentration of Cx, Cy and Cz of MET, VOG and PIO respectively in sample solution can be calculated by using following equations (Eq.2-4);

$$C_x = \frac{A_1(ay_2az_3 - ay_3az_2) - A_2(ay_1az_3 - ay_3az_1) + A_3(ay_1az_2 - ay_2az_1)}{ax_1\ (ay_2az_3) - ax_2\ (ay_1az_3 - ay_3az_1)\ + ax_3\ (ay_1az_2 - ay_2az_1)} \quad (2)$$

$$C_y = \frac{A_1(ax_2az_3 - ax_3az_2) - A_2\ (ax_1az_3 - ax_3az_1) + A_3(ax_1az_2 - ax_2az_1)}{ay_1(ax_2az_3) - ay_2(ax_1az_3 - \ ax_3az_1)\ + ay_3(ax_1az_2 - ax_2az_1)} \quad (3)$$

$$C_z = \frac{A_1(ax_2ay_3 - ax_3ay_2) - A_2(ax_1ay_3 - ax_3ay_1) + A_3(ax_1ay_2 - ax_2ay_1)}{az_1(ax_2ay_3) - az_2(ax_1ay_3 - ax_3ay_1) + az_3(ax_1ay_2 - ax_2ay_1)} \quad (4)$$

Where, A_1, A_2 and A_3 are the absorbance values of mixture/tablet solution, ax_1, ax_2, ax_3 are absorptivities of MET at 236, 256 and 268 nm respectively, ay_1, ay_2 and ay_3 are absorptivities of VOG at 236, 256 and 268nm respectively, and az_1, az_2 and az_3 are absorptivities of PIO at 236, 256 and 268 nm respectively; and Cx, Cy and Cz are concentration of MET, VOG and PIO respectively.

Method Validation

The analytical method was validated as per ICH guidelines with respect to parameters such as linearity, accuracy, precision, assay, ruggedness, and robustness, limit of detection and limit of quantification.

RESULTS AND DISCUSSION

For selection of common solvent as a diluent, all three combined MET, VOG and PIO were added to water and methanol. Metformin was freely soluble in water and methanol. But other two drugs, Voglibose and Pioglitazone were sparingly soluble in water and obtained turbid solution. Therefore, methanol was selected as a common solvent due to freely soluble three combined drugs for proposed method. The working standard solutions of MET, VOG and PIO were scanned in the entire UV range of 400-200 nm to get absorbance spectrum and overlay spectra of MET, VOG and PIO (Fig.2). The absorption maxima of Metformin hydrochloride, Voglibose and Pioglitazone were found to be 236, 256 and 268 nm respectively. From the absorbance spectra, three wavelengths 236 nm (λ_{max} of MET), 256 nm (λ_{max} of VOG) and 268 nm (λ_{max} of PIO) were selected for estimation of these drugs using simultaneous equation method. An absorptivity value of these drugs at selected wavelengths is given in Table 1. When method development and optimization is complete, it is necessary to accomplish method validation. For validation of analytical method, the guidelines of the international conference on the harmonization of technical requirements for the registration of pharmaceuticals for human use has recommended

validation characteristics including system suitability, accuracy (% recovery), linearity, precision (% RSD) were investigated.

Table 1. Absorptivity values of drugs at selected wavelengths

Absorptivity value	Absorbance maxima (nm)		
	(λ_1-236)	(λ_2-256)	(λ_3-268)
MET (ax_1)	0.03836	-	-
MET (ax_2)	-	0.000270	-
MET (ax_3)	-	-	0.000031
VOG (ay_1)	0.00136	-	-
VOG (ay_2)	-	0.48000	-
VOG (ay_3)	-	-	0.000320
PIO (az_1)	0.00110	-	-
PIO (az_2)	-	0.00154	-
PIO (az_3)	-	-	0.033100

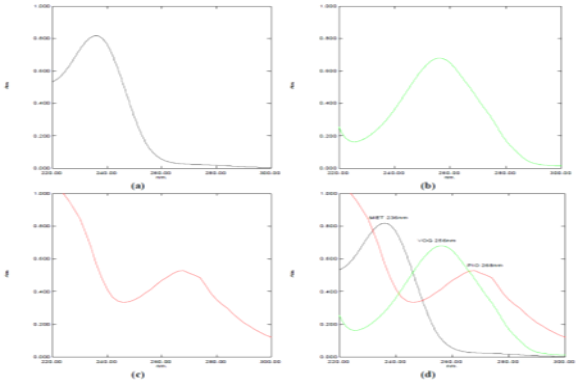

Fig.2: UV-Visible spectrum of A) Metformin, B) Voglibose, C) Pioglitazone, and D) Overlay spectra

Linearity is the ability of the method to elicit test results that are proportional to the concentration of the analyte in the sample. The proposed method was found to be linear over concentration range of 5-25 µg/ml, 0.2-1 µg/ml and 2-10 µg/ml for MET, VOG and PIO respectively. The standard curves for MET, VOG and PIO were linear and exhibited good correlation coefficient (r^2=0.998, 0.999 and 0.999) respectively (Fig. 3). The result shows an excellent correlation between the absorbance and the concentrations of drugs in the selected range (Table 2).

Accuracy is the closeness of the best result obtained by the method to the true value (Table 3). The concentration recovered should be within ±2% to the true value. Accuracy of the developed method was confirmed by recovery study as per ICH norms at three different concentration levels of 80%, 100% and 120%. Amount of the drug recovered was calculated using simultaneous equation method for accuracy. The percentage of the standard added to the pre analyzed sample was calculated and it was found to be 100.04-100.48% for MET, 98.70-99.70% for VOG and 98.67-100.27% for PIO indicates good accuracy of the method. The results of recovery studies with statistical validation are shown in Table 4. The precision of analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under prescribed conditions. Precision is under the same operating conditions over a short interval of time. It was carried out by measuring three different samples of the same concentration at different intervals of time. Intermediate precision expresses precision within laboratory variations: different days, different analysts, different equipment, etc. It was carried out by different analysts.

Table 2. Standard curve data of Metformin, Voglibose, and Pioglitazone

S. No.	Concentration (µg/ml)			Absorbance (nm)		
	MET	VOG	PIO	MET	VOG	PIO
1	5	0.2	2	0.21	0.15	0.06
2	10	0.4	4	0.41	0.27	0.13
3	15	0.6	6	0.59	0.41	0.19
4	20	0.8	8	0.81	0.54	0.25
5	25	1	10	0.98	0.68	0.31

In determination of precision, % RSD were not more than 0.25%, 0.87% and 0.51% for MET, VOG and PIO respectively indicated that the method was precise (Table 5). The % RSD found were within 2.0% which indicates that the system was precise to analyze the sample. Repeatability was determined by the analyzing MET (25µg/ml), VOG (1µg/ml) and PIO (10µg/ml) of drug solution for three replicates (Table 6). The repeatability showed the closeness of the observed results that enhance the reliability of the above method. LOD for MET, VOG and PIO were found to be 0.42, 0.02 and 0.25µg/ml respectively. LOQ for MET, VOG and PIO were found to be 1.27, 0.06 and 0.75µg/ml respectively. The mean standard deviation is 0.005, 0.004 and 0.002 and slope was 0.038, 0.665 and 0.031 for MET, VOG and PIO respectively. Ruggedness is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories or different analysts. Ruggedness of the proposed method was studied by two different analysts using the same experimental and environmental conditions; the results are given in

Table 7. The % RSD was found to be 0.32-0.90% for MET, 0.38-0.46% for VOG and 0.34-0.76% for PIO respectively. A new UV spectrophotometric method was developed for assay of MET, PIO and VOG in solid dosage form. The validated method was applied for the determination of MET, VOG and PIO in commercially available D-Bose MP275 tablets. The results of assay (n=3) undertaken yielded 100.21 % (% RSD=0.29) of MET, 98.70 % (% RSD=0.44) of PIO and 101.28 % (% RSD=1.3%) of VOG (Table 8). All the results found were in good agreement with the label content of marketed formulation. The result of analysis of tablets formulation and recovery studies obtained by spectrophotometric method was statistically validated and high percentage of recovery studies suggest that the developed method was free from interferences of excipients generally used in tablet formulation. The developed method was statically validated in terms of linearity, accuracy, precision and reproducibility. Method developed can be conveniently used for quality control and routine determination of Metformin, Voglibose and Pioglitazone in bulk and commercial tablets formulation in pharmaceutical industry.

The developed UV-spectrophotometry method based on the results is new, simple, rapid, robust, precise, accurate and sensitive. Many samples of Metformin, Voglibose and Pioglitazone can be suitably analyzed by this method. Hence, developed and validated method can be conveniently used for quality control and for routine analysis of Metformin, Voglibose and Pioglitazone in pharmaceutical dosage forms.

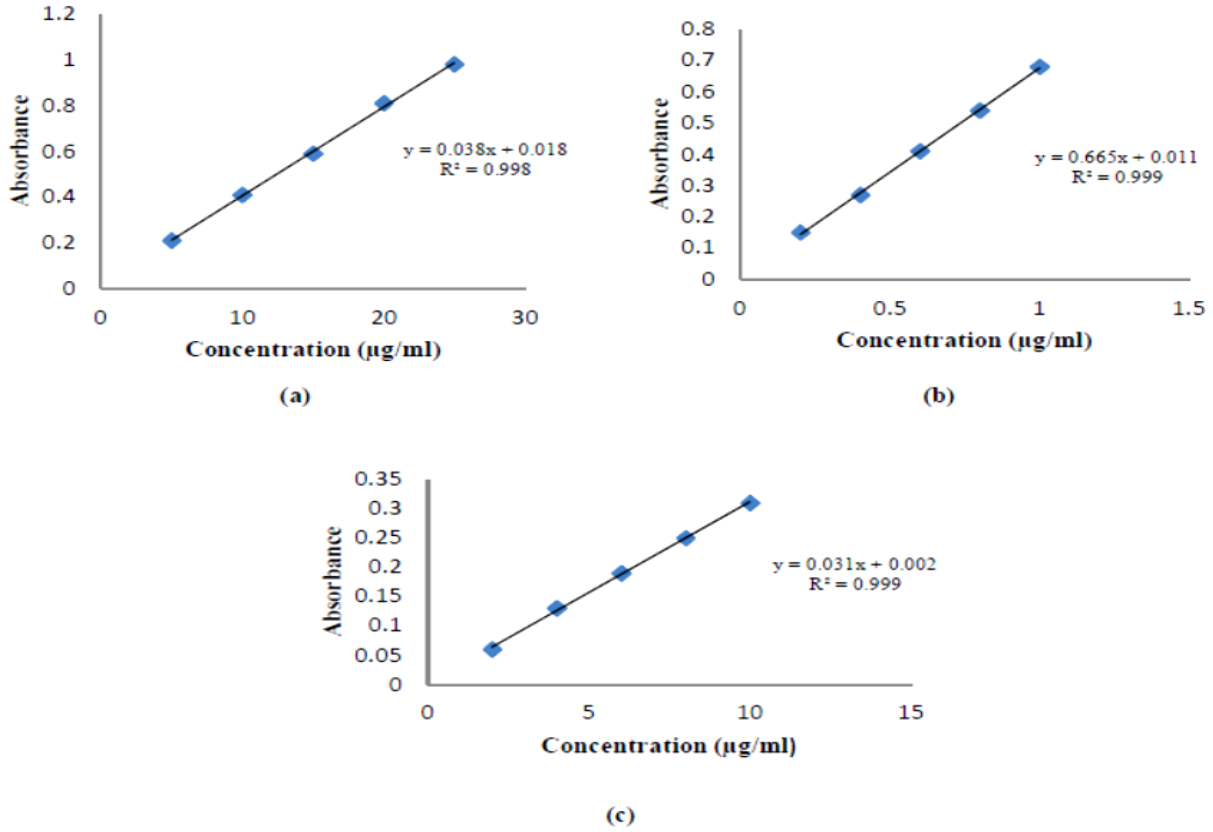

Fig.3: Standard calibration curve of (a) MET, (b) VOG and (c) PIO

Table 3. Accuracy studies for MET, VOG and PIO

Level % recovery	Drug	80%		100%		120%	
Amount present (mg/tab)	MET	500	500	500	500	500	500
	VOG	0.2	0.2	0.2	0.2	0.2	0.2
	PIO	7.5	7.5	7.5	7.5	7.5	7.5
Amount taken (µg/ml)	MET	25	25	25	25	25	25
	VOG	0.01	0.01	0.01	0.01	0.01	0.01
	PIO	0.375	0.375	0.375	0.375	0.375	0.375
	MET	20	20	25	25	30	30

Amount of std. drug added (µg/ml)	VOG	0.008	0.008	0.01	0.01	0.012	0.012
	PIO	0.3	0.3	0.375	0.375	0.45	0.45
Total amount recovered (µg/ml)	MET	20.01	20.12	25.04	25.03	30.04	30.12
	VOG	0.008	0.008	0.01	0.01	0.012	0.012
	PIO	0.299	0.295	0.373	0.376	0.45	0.449
% Recovery	MET	100.04	100.48	100.16	100.12	100.16	100.48
	VOG	99.3	98.9	98.7	98.9	99.2	99.7
	PIO	99.73	98.67	99.47	100.27	100	99.73

Table 4. Statistical validation for accuracy for Metformin, Voglibose, and Pioglitazone

Recovery	MET		VOG		PIO	
	Mean	% RSD	Mean	% RSD	Mean	% RSD
80%	100.26±0.311	0.31	99.10±0.283	0.29	99.20±0.754	0.76
100%	100.14±0.028	0.03	98.80±0.141	0.14	99.87±0.566	0.57
120%	100.32±0.226	0.23	99.45±0.354	0.36	99.87±0.189	0.19

All values represents mean ± SD (n=3)

Table 5. Precision data for MET, VOG and PIO

Drug	Amount taken (µg/ml)	Intra-day		Inter-day	
		Amount found (µg/ml)	% RSD	Amount found (µg/ml)	% RSD
MET	15	14.99	0.12	15.03	0.14
	20	19.99	0.18	20.01	0.12
	25	25.00	0.57	25.04	0.25
VOG	0.6	0.59	1.17	0.60	0.87
	0.8	0.80	0.54	0.80	0.74
	1	0.99	0.81	1.00	0.77
PIO	6	5.97	0.35	6.01	0.45
	8	7.95	0.52	7.97	0.51
	10	9.99	0.18	10.00	0.32

Table 6. Repeatability for MET, VOG and PIO

Repeatability for MET			
Conc. (µg/ml)	Absorbance	Amount found	% Amount found
25	0.9676	24.99	99.96
25	0.9682	25.01	100.02
25	0.9688	25.02	100.08
Mean	0.9682	25.01	100.02
SD	0.001	0.016	0.063
% RSD	0.062	0.063	0.001
Repeatability for VOG			
1	0.6754	0.9991	99.91
1	0.6754	0.9991	99.91
1	0.6759	0.9998	99.98
Mean	0.6756	0.9993	99.93
SD	0.0003	0.0004	0.0434
% RSD	0.0427	0.0434	0.0434
Repeatability for PIO			
10	0.3122	10.01	100.06
10	0.3125	10.02	100.16
10	0.3139	10.06	100.61
Mean	0.3129	10.03	100.28
SD	0.001	0.029	0.293
% RSD	0.290	0.292	0.292

Table 7. Results of ruggedness

Analyst	Amount found (%)			% RSD		
	MET	VOG	PIO	MET	VOG	PIO

Analyst-1	100.20	99.60	100.19	0.32	0.46	0.76
Analyst-2	101.00	100.31	100.37	0.90	0.38	0.34

Table 8. Assay Data for estimation of MET, PIO and VOG in tablet dosage form

Sr. No.	MET		VOG		PIO	
	Assay (µg)	Assay (%)	Assay (µg)	Assay (%)	Assay (µg)	Assay (%)
1	50.03	100.06	0.0197	98.45	0.7548	100.64
2	50.01	100.02	0.0197	98.45	0.7709	102.79
3	50.27	100.54	0.0198	99.20	0.7530	100.40
Mean	50.10	100.21	0.0197	98.70	0.76	101.28
SD	0.145	0.289	0.0001	0.433	0.010	1.314
% RSD	0.29	0.29	0.44	0.44	1.30	1.30

ACKNOWLEDGMENT

This research was supported by Government College of Pharmacy, Amravati providing necessary facilities to carry out this work. Authors are thankful to Macloids Pharmaceuticals Ltd. Gujarat for providing gift samples of Metformin, Voglibose and Pioglitazone.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Jain D, Jain S and Amin M. (2008). Simultaneous estimation of metformin hydrochloride, pioglitazone hydrochloride and glimepiride by RP-HPLC in tablet formulation. Journal of Chromatographic Science, 46 (6), 501-504.

[2] Kadam VN, Yadav PJ, Mohite SK and Magdum CS. (2014). Development and validation of analytical methods for simultaneous estimation of Voglibose, Glimepiride and Metformin hydrochloride in bulk and tablet dosage form by HPLC. IJPPR. Human, 1(2), 10-21.

[3] Tripathi KD. (2008). Essentials of medical pharmacology. In: Insulin, oral hypoglycemic drugs and glucagon. Jaypee brother's medical publisher's ltd. New Delhi, 6th edition, 266-270.

[4] Cannors KA. (1982). A textbook of pharmaceutical analysis. In: Absorption spectroscopy. John Wiley and Sons, New York, 3rd edition, 173-247.

[5] Lakshmi KS, Rajesh T and Sharma S. (2009). Simultaneous determination of metformin and pioglitazone by reversed phase HPLC in pharmaceutical dosage forms. International Journal of Pharmaceutical Sciences, 1(2), 162-166.

[6] Sonia K and Babu PK. (2013). RP-HPLC analysis of Metformin hydrochloride and Voglibose and study of its different analytical parameter. International Journal of Pharmaceutical Sciences and Research, 1(4), 1469-1474.

[7] Sujana K, Swathi Rani G, Bhanu Prasad M and Reddy MS. (2010). Simultaneous estimation of Pioglitazone hydrochloride and Metformin hydrochloride using UV spectroscopic method. Journal of Biomedical Science and Research, 2(2), 110-115.

[8] Neha R, Manjula B, Prachi K and Ritu K. (2011). Simultaneous quantification of Voglibose and Metformin by validated analytical method in tablet dosage form. International Journal of Pharmacy and Technology, 3(2), 53-56.

[9] International conference on harmonization (ICH) of technical requirements for the registration of pharmaceuticals for human use, Validation of analytical procedures methodology. ICH- Q2 (R1), Geneva; 1996, 1-8.

[10] Pallavi P, Sonali R and Praveen C. (2012). Development and validation of UV derivative spectrophotometric methods for the determination of glimepiride, metformin HCl and pioglitazone HCl in bulk and marketed formulation. Journal of Pharmaceutical and Scientific Innovation, 1, 58-62.