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Spectrophotometric Method Development for Estimation of Multicomponent Antidiabetic Drugs in Bulk and Solid Dosage Form

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Abstract: The aim of the present study was the development and validation of a simple, precise and accurate UV-spectrophotometric method for the estimation of metformin (MET), voglibose (VOG) and pioglitazone (PIO) in bulk and tablet dosage form using methanol as solvent. The method was proposed in the present work, the maximum absorbance was shown at 236nm for MET, 256nm for VOG and 268nm for PIO. The concentration range was 5-25, 0.2-1 and 2-10µg/mL with correlation coefficient 0.0998, 0.999 and 0.999 for MET, VOG and PIO respectively. The various parameters, such as linearity, system suitability, accuracy, precision, ruggedness, limit of detection and limit of quantification were studied as per ICH guidelines. Accuracy of the method was verified by performing recovery studies using simultaneous equation method and 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. Excellent mean recovery studies for precision, repeatability, ruggedness and sensitivity results showed that the method has been validated successfully, the results are also in accordance with the % RSD values obtained within specified limits. The proposed method was applied to the determination of MET, VOG and PIO, the mean % amount was found to be 100.21 (MET), 98.70 (VOG) & 101.28 (PIO) with % RSD values NMT 2.0% indicates the developed method was successfully applied for analysis of marketed formulation. The developed spectrophotometric method can be employed for routine analysis of MET, VOG and PIO in bulk and tablet formulation.

Keywords: UV-spectrophotometry, Metformin (MET), Voglibose (VOG), Pioglitazone (PIO), Methanol.

I. INTRODUCTION

Ultraviolet visible spectrophotometry is most frequently employed techniques in pharmaceutical analysis. The photometric methods of estimations are based on the Bouguer-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 [1]. A compound or drug possess a functional group, absorbs UV radiation at a specific wavelength and this character of the drug is specific for a fixed solvent system. The wavelength at which maximum absorption occurs is called as λ_{max} . It is independent of concentration. With the help of Beer-Lambert's law any molecule present as a single component system or multiple component system could be quantified effectively by UV spectroscopic method. For a drug to be measured by the ultraviolet analytical method it should follow the Beer-Lambert's law [2], which is represented as

$$A = abc \text{ ----- (1)}$$

Where, A- absorbance, a-absorptivity, b- path length and c- concentration Metformin (MET) (Fig.1a) 3-(diaminomethylidene)-1,1-dimethylguanidine is a biguanide hypoglycemic agent used in the treatment of non-insulin-dependent diabetes mellitus not responding to dietary modification. Metformin improves glycemic control by improving insulin sensitivity and decreasing intestinal absorption of glucose [3]. Voglibose (VOG) (Fig.1b) (1S,2S,3R,4S,5S)-5-(1,3-dihydroxypropan-2-ylamino)-1-(hydroxymethyl)cyclohexane-1,2,3,4-tetrol is a valiolamine derivative and inhibitor of α -glucosidase with antihyperglycemic activity. Voglibose binds to and inhibits α -glucosidase, an enteric enzyme found in the brush border of the small intestines that hydrolyzes oligosaccharides and disaccharides into glucose and other monosaccharides. This prevents the breakdown of larger carbohydrates into glucose and decreases the rise in postprandial blood glucose levels [4]. Pioglitazone (PIO) (Fig.1c) 5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione is a thiazolidinedione and it is selective agonists for the nuclear peroxisome proliferator-activated γ -receptor (PPAR γ) which enhances the transcription of several insulin responsive genes.

They tend to reverse insulin resistance by stimulating GLUT4 expression and translocation therefore entry of glucose into muscle and fat is improved. Hepatic gluconeogenesis is also suppressed. Activation of genes regulating fatty acid metabolism and lipogenesis in adipose tissue contributes to the insulin sensitizing action^[5]. The literature survey reveals that various methods are present for the determination of metformin, voglibose and pioglitazone, individually or in combination of listed two drugs. The methods, UV-spectroscopy and HPLC are already developed in combination for metformin and voglibose and for metformin and pioglitazone. There are no any analytical method reported previously for the simultaneous estimation of voglibose, pioglitazone and metformin in multi component dosage form. Therefore, it is aimed to develop a simple, accurate, sensitive and reproducible method for combined voglibose, pioglitazone and metformin in bulk and tablet dosage form by UV-spectrophotometry.

II. MATERIALS AND METHODS

A. Materials

Metformin, Pioglitazone and Voglibose were received as gift sample from Macloids Pharmaceuticals Ltd. Gujrat, India. Methanol of HPLC grade was procured. The D-Bose MP275 tablet as a marketed formulation used which contains 0.2mg Voglibose, 500mg Metformin and 7.5mg Pioglitazone, marketed by Sinsan Pharmaceuticals PVT. LTD. Pune, India.

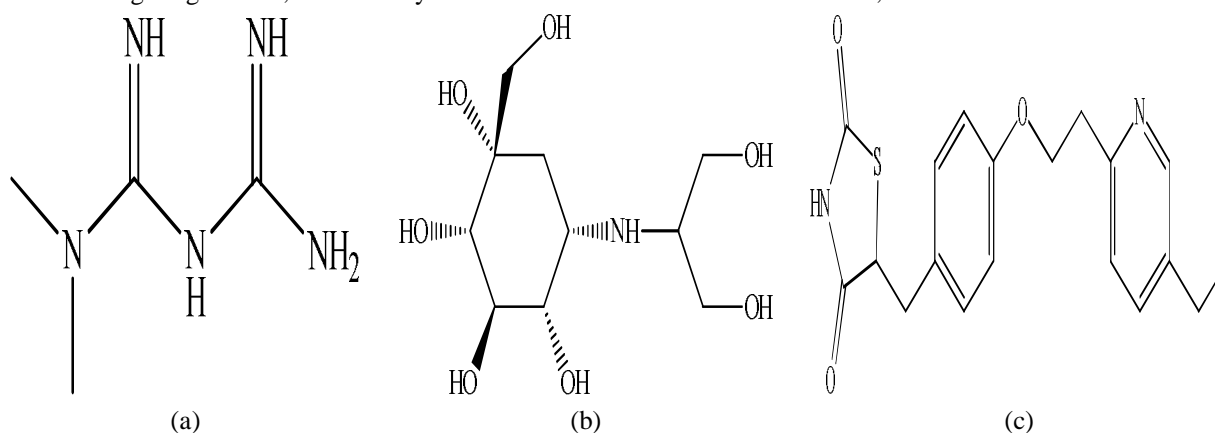


Fig. 1: Chemical structures of (a) Metformin (MET), (b) Voglibose (VOG) and (c) Pioglitazone (PIO)

B. Methods

1) Instrumentation

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

2) 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.

3) Preparation of standard solution for MET, VOG and PIO

Standard stock solution of MET, VOG and PIO was prepared by dissolving 10mg of each drug separately in 100ml volumetric flask using methanol as solvent up to 100ml and each sample sonicate up to 15min. Stock solution of 100 μ g/mL were obtained. From these stock solutions, working stock solutions of concentration were prepared by appropriate dilutions.

4) Selection of wavelength

The working standard solutions of MET, VOG and PIO were scanned in the entire UV range of 400-200nm to determine λ_{max} . The λ_{max} of MET, VOG and PIO were found to be 236nm, 256nm and 268nm respectively.

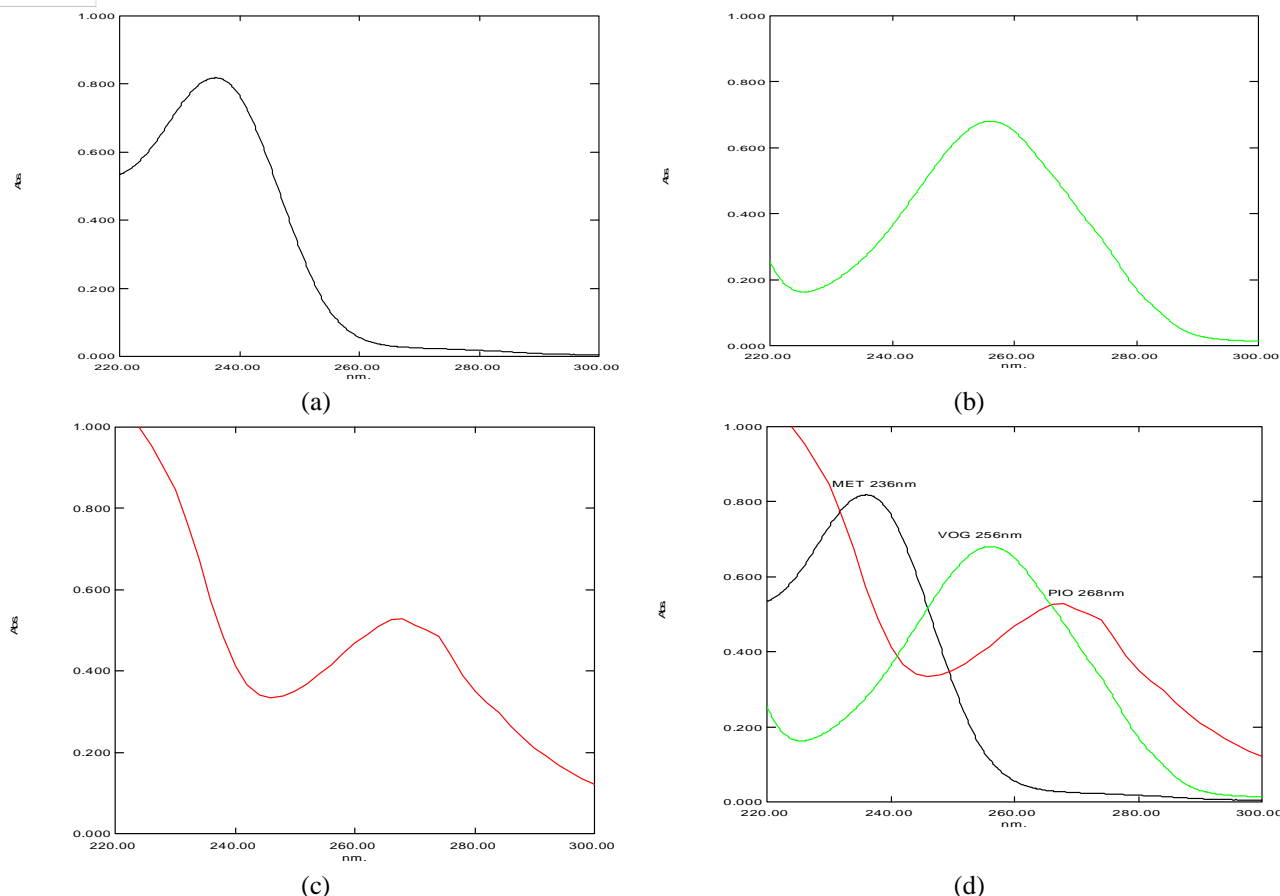


Fig.2: UV- Visible spectrum and overlay spectra (d) of MET (a), VOG (b) and PIO (c)

Determination of E (1% 1cm) of drugs at selected wavelengths

Absorptivity values of drugs at selected wavelengths measured by using following formula

$$E (1\%1cm) = \frac{\text{Absorbance}}{\text{concentration}} \dots\dots (2)$$

Where,

A- Absorbance,

a- Molar absorptivity,

c- Concentration

Concentration of C_x , C_y and C_z of MET, VOG and PIO respectively in sample solution can be calculated by using following equations ^[15],

$$C_x = \frac{A_1 (ay_2 az_3 - ay_3 az_2) - A_2 (ay_1 az_3 - ay_3 az_1) + A_3 (ay_1 az_2 - ay_2 az_1)}{ax_1 (ay_2 az_3) - ax_2 (ay_1 az_3 - ay_3 az_1) + ax_3 (ay_1 az_2 - ay_2 az_1)} \dots\dots (3)$$

$$C_y = \frac{A_1 (ax_2 az_3 - ax_3 az_2) - A_2 (ax_1 az_3 - ax_3 az_1) + A_3 (ax_1 az_2 - ax_2 az_1)}{ay_1 (ax_2 az_3) - ay_2 (ax_1 az_3 - ax_3 az_1) + ay_3 (ax_1 az_2 - ax_2 az_1)} \dots\dots (4)$$

$$C_z = \frac{A_1 (ax_2 ay_3 - ax_3 ay_2) - A_2 (ax_1 ay_3 - ax_3 ay_1) + A_3 (ax_1 ay_2 - ax_2 ay_1)}{az_1 (ax_2 ay_3) - az_2 (ax_1 ay_3 - ax_3 ay_1) + az_3 (ax_1 ay_2 - ax_2 ay_1)} \dots\dots (5)$$

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 236nm, 256nm and 268nm respectively.

ay_1 , ay_2 and ay_3 are absorptivities of VOG at 236nm, 256nm and 268nm respectively.

az_1 , az_2 and az_3 are absorptivities of PIO at 236nm, 256nm and 268nm respectively.

C_x , C_y and C_z are concentration of MET, VOG and PIO respectively.

Table 1: Absorptivity values of drugs at selected wavelengths

Absorptivity value	Wavelength in nm		
	236	256	268
ax ₁	0.03836	-	-
ax ₂	-	0.000270	-
ax ₃	-	-	0.000031
ay ₁	0.00136	-	-
ay ₂	-	0.48000	-
ay ₃	-	-	0.000320
az ₁	0.00110	-	-
az ₂	-	0.00154	-
az ₃	-	-	0.033100

Where,

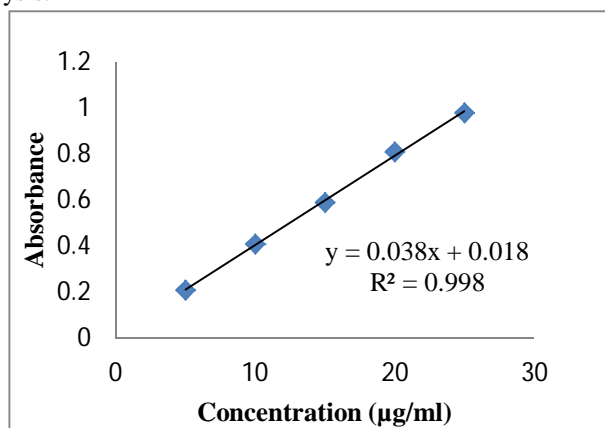
ax₁, ax₂ and ax₃=Absorptivity of MET

ay₁, ay₂ and ay₃=Absorptivity of VOG

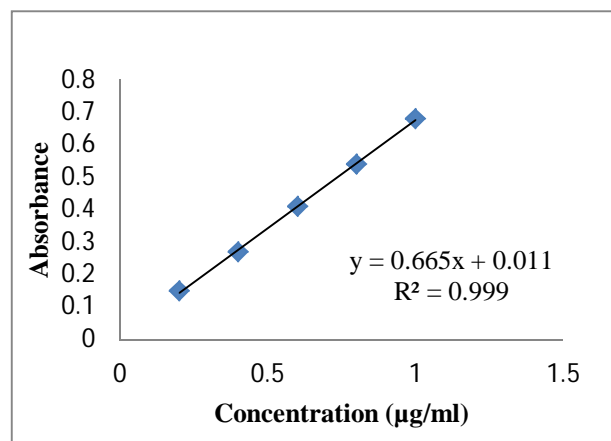
az₁, az₂ and az₃=Absorptivity of PIO

Procedure for calibration curve

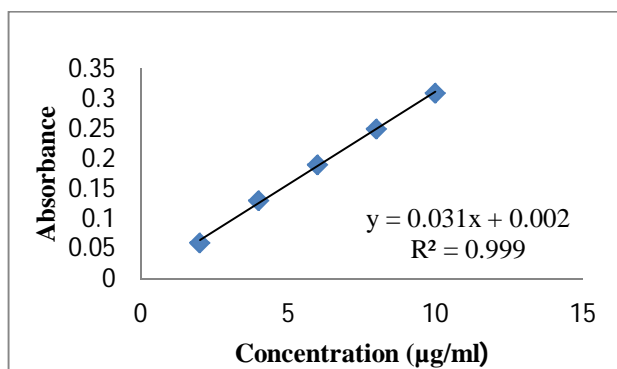
Standard dilutions of each drug were prepared separately having concentration of 5-25µg/mL, 0.2-1µg/mL and 2-10µg/mL for MET, VOG and PIO respectively. The absorbances of these standard solutions were measured at 236nm, 256nm and 268nm. The Calibration curves were constructed by plotting the Absorbance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



(a)



(b)



(c)

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

Table 2: Calibration of MET, VOG and PIO

Sr. No.	Concentration in µg/mL			Absorbance		
	MET	VOG	PIO	MET(236nm)	VOG(256nm)	PIO(268nm)
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
			Slope	0.038	0.665	0.031
			Intercept	0.018	0.011	0.002
			Correlation Coefficient	0.998	0.999	0.999

Procedure for analysis of tablet formulation

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 100mg of MET was transferred to a 100ml volumetric flask, dissolved in 100ml methanol, sonicate for 15min and then filtered through Whatman filter paper no. 41. From the above 5ml of solution was taken and diluted to 100ml with methanol to get final concentration of 50µg/mL of MET, 0.02µg/ml of VOG and 0.75µg/ml of PIO respectively. The absorbance of sample solution was recorded at selected wavelength. The results of estimation of MET, VOG and PIO are shown in Table 3.1, 3.2, 3.3 and 3.4. The proposed method was applied to the determination of MET, VOG and PIO in tablet formulation. The mean % amount found were 100.21 (MET), 98.70 (VOG) & 101.28 (PIO) with % RSD values were NMT 2.0% indicates the developed method was successfully applied for analysis of marketed formulation.

Table 3.1: Results for estimation of MET in Tablet formulation

Sr. No.	Label claim (mg)	Amount taken (µg/mL)	Amount found (µg/mL)	% Drug Estimation
1	500	50	50.03	100.06
2	500	50	50.01	100.02
3	500	50	50.27	100.54
		Mean	50.10	100.21
		SD	0.145	0.289
		%RSD	0.29	0.29

Table 3.2: Results for estimation of VOG in Tablet formulation

Sr. No.	Label claim (mg)	Amount taken (µg/mL)	Amount found (µg/mL)	% Drug Estimation
1	0.2	0.02	0.0197	98.45
2	0.2	0.02	0.0197	98.45
3	0.2	0.02	0.0198	99.20
		Mean	0.0197	98.70
		SD	0.0001	0.433
		%RSD	0.44	0.44

Table 3.3: Results for estimation of PIO in Tablet formulation

Sr. No.	Label claim (mg)	Amount taken ($\mu\text{g/mL}$)	Amount found ($\mu\text{g/mL}$)	% Drug Estimation
1	7.5	0.75	0.7548	100.64
2	7.5	0.75	0.7709	102.79
3	7.5	0.75	0.7530	100.40
		Mean	0.76	101.28
		SD	0.010	1.314
		%RSD	1.30	1.30

Table 3.4: Statistical data for estimation of MET, PIO and VOG in Tablet formulation

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

III. METHOD VALIDATION

For validation of analytical method, the guidelines of the ICH of Technical Requirements for the Registration of Pharmaceuticals for human use has recommended validation characteristics including linearity, accuracy (%recovery), precision (%RSD), repeatability and ruggedness were investigated.

IV. RESULTS

A. Linearity

The linearity of proposed method was evaluated by linear regression analysis, which was calculated by least square method. These drugs found were linear in the concentration range of 5-25 $\mu\text{g/mL}$, 0.2-1 $\mu\text{g/mL}$ and 2- 10 $\mu\text{g/mL}$ for MET, VOG and PIO respectively. The correlation coefficients calculated from calibration curve were 0.998, 0.999 and 0.999 for MET, VOG and PIO respectively (Table 2). The result shows an excellent correlation between the absorbance and the concentrations of drugs in the selected range. The regression equations of calibration curves were $y = 0.038x + 0.018$ ($r^2 = 0.998$) at 236nm for MET, $y = 0.665x + 0.011$ ($r^2 = 0.999$) for VOG at 256nm and $y = 0.031x + 0.002$ ($r^2 = 0.999$) for PIO at 268nm. From the data obtained standard deviation (SD) and % RSD were calculated.

B. Accuracy

Accuracy of the developed method was confirmed by recovery study as per ICH norms at three different concentration levels of 80 %, 100 %, and 120 %. Here to a preanalysed sample solution, standard drug solutions were added and then percentage drug content was calculated.

Table 4.1: Accuracy studies for MET, VOG and PIO

Level of % recovery	Amount present (mg/tab)			Amount taken (µg/mL)			Amount of Std. Drug Added (µg/mL)		
	MET	VOG	PIO	MET	VOG	PIO	MET	VOG	PIO
80%	500	0.2	7.5	25	0.01	0.375	20	0.008	0.300
	500	0.2	7.5	25	0.01	0.375	20	0.008	0.300
100%	500	0.2	7.5	25	0.01	0.375	25	0.010	0.375
	500	0.2	7.5	25	0.01	0.375	25	0.010	0.375
120%	500	0.2	7.5	25	0.01	0.375	30	0.012	0.450
	500	0.2	7.5	25	0.01	0.375	30	0.012	0.450

Table 4.2: Accuracy studies for MET, VOG and PIO

Level of % recovery	Amount of Std. Drug Added (µg/ml)			Total Amount Recovered(µg/ml)			% Recovery		
	MET	VOG	PIO	MET	VOG	PIO	MET	VOG	PIO
80%	20	0.008	0.300	20.01	0.008	0.299	100.04	99.3	99.73
	20	0.008	0.300	20.12	0.008	0.295	100.48	98.9	98.67
100%	25	0.010	0.375	25.04	0.010	0.373	100.16	98.7	99.47
	25	0.010	0.375	25.03	0.010	0.376	100.12	98.9	100.27
120%	30	0.012	0.450	30.04	0.012	0.450	100.16	99.2	100.00
	30	0.012	0.450	30.12	0.012	0.449	100.48	99.7	99.73

Table 4.3: Statistical Validation Data for Accuracy studies for MET, VOG and PIO

Level of % recovery	MET			VOG			PIO		
	Mean*	±SD	%RSD	Mean*	±SD	%RSD	Mean*	±SD	%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

C. Precision

Precision of method was evaluated by intraday and interday variation studies. Intraday precision were carried out by analyzing the 15, 20, 25µg/ml, 0.6, 0.8, 1µg/ml and 6, 8, 10µg/ml of MET, VOG and PIO respectively for three times in a day and %RSD was calculated. Inter-day precision was determined by analyzing the same conc. as for intraday on three consecutive days and %RSD was calculated.

Table 5: Results of precision study

Drug	Amount taken (µg/ml)	Intra-day(n=3)		Inter-day(n=3)	
		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

D. Repeatability

The test was performed by collecting data from three replicates of standard solutions of concentration 25 µg/mL, 1µg/mL and 10µg/mL for MET, VOG and PIO respectively.

Table 6.1: Result of Repeatability study for MET

Sr. No.	Conc.(µg/ml)	Absorbance	Amount found	% Amount found
1	25	0.9676	24.99	99.96
2	25	0.9682	25.01	100.02
3	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

Table 6.2: Result of Repeatability study for VOG

Sr. No.	Conc.(µg/ml)	Absorbance	Amount found	% Amount found
1	1	0.6754	0.9991	99.91
2	1	0.6754	0.9991	99.91
3	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

Table 6.3: Result of Repeatability study for PIO

Sr. No.	Conc.(µg/ml)	Absorbance	Amount found	% Amount found
1	10	0.3122	10.01	100.06
2	10	0.3125	10.02	100.16
3	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

Limit of detection and Limit of quantification

The LOD and LOQ are both are calculated based on mean standard deviation and slope of the calibration curve at the levels approaching the LOD and LOQ according the following formula:

$$LOD = 3.3 \frac{SD}{S}$$

$$LOQ = 10 \frac{SD}{S}$$

Where, S = slope of calibration curve, SD = standard deviation of the response.

E. Ruggedness

Ruggedness of the proposed method was studied by two different analysts using the same experimental and environmental conditions.

Table 7: Result of ruggedness study

Sr. No.	No. of Analyst	Amount found (%)			%RSD		
		MET	VOG	PIO	MET	VOG	PIO
1	Analyst-1	100.20	99.60	100.19	0.32	0.46	0.76
2	Analyst-2	101.00	100.31	100.37	0.90	0.38	0.34

V. DISCUSSION

An attempt was made to develop UV spectrophotometric method for the estimation of Metformin, Pioglitazone and Voglibose in bulk and tablet dosage form. 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.

The working standard solutions of MET, VOG and PIO were scanned in the entire UV range of 400-200nm to get absorbance spectrum. UV- Visible spectra and overlay spectra of MET, VOG and PIO are shown in Fig.2. From the absorbance spectra, three wavelengths 236nm (λ_{max} of MET), 256nm (λ_{max} of VOG) and 268nm (λ_{max} of PIO) were selected for estimation of these drugs using Simultaneous Equation Method (SEM). The % RSD found were within 2.0%, which indicates that the system is precise to analyze the sample. Accuracy is the closeness of the best result obtained by the method to the true value. The concentration recovered should be within $\pm 2\%$ 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 %. Here to a preanalysed sample solution, standard drug solutions were added and then percentage drug content was calculated. 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 (Table 4.1, 4.2). The recovery study results with statistical validation have shown in Table 4.3. In determination of precision, the %RSD, were not more than 0.25%, 0.87% and 0.51% for MET, VOG and PIO respectively, indicating the method was precise and results are shown in Table 5. Repeatability was determined by the analyzing MET (25 μ g/mL), VOG (1 μ g/mL) and PIO (10 μ g/mL) of drug solution for three replicates and results are shown in Table 6.1, 6.2 and 6.3. The repeatability again shows 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 0.25 μ g/mL respectively. LOQ for MET, VOG and PIO were found to be 1.27, 0.06 and 0.75 μ g/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 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.

VI. CONCLUSION

The developed spectrophotometric method was found linear over wider concentration range. Therefore the developed spectrophotometric method can be applied for routine quantitative and qualitative analysis of MET, VOG and PIO in bulk and pharmaceutical formulations.

The proposed method based on the UV-spectrophotometry is suitable for determination of MET, VOG and PIO in tablet formulation. Method developed can be conveniently used for quality control and routine determination of drug in pharmaceutical dosage forms in pharmaceutical industry.

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