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Method Development, Validation and Forced Degradation Studies of Voglibose and Metformin in Pure and Pharmaceutical Dosage Form by RP-HPLC

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Abstract

Objective: The objective of the present research work was to develop an innovative, simple, and economic method for estimation of Voglibose and Metformin in bulk and dosage form by RP-HPLC. **Methods:** The chromatographic conditions were performed on Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm i.d.as stationary phase and mobile phase was prepared with a mixture of Phosphate Buffer (pH- 6.5): Acetonitrile = (65: 35) flow 1.0 ml/min, with Injection Volume 10µl, at detection wavelength 251 nm and run time at 6.0 mins. **Results:** The analytical method is valid for estimation of Voglibose and Metformin over a range of 10 µg/ml–60 µg/ml and 05 µg/ml–40 µg/ml. The results of system suitability test, linearity, precision and accuracy, robustness, specificity, LOD and LOQ and stabilities presented in this report are within the acceptance range. **Conclusion:** A specific, sensitive, economic method estimation of Voglibose and Metformin has been developed based on ICH Guidelines with bulk and dosage forms.

Keywords
Voglibose and Metformin, HPLC, Method Development, ICH, Validation, Accuracy, Precision.

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1. INTRODUCTION:

Voglibose (INN and USAN, trade name Voglib, marketed by Mascot Health Series) is an alpha-glucosidase inhibitor used for lowering post-prandial blood glucose levels in people with diabetes mellitus.[1-3] Voglibose delays the absorption of glucose thereby reducing the risk of macro vascular complications. Voglibose is a research product of Takeda Pharmaceutical Company, Japan's largest pharmaceutical company. Voglibose was first launched in 1994, under the trade name BASEN, to improve postprandial hyperglycemia in diabetes mellitus.[4-7] Postprandial hyperglycemia (PPHG) is primarily due to first phase insulin secretion. Alpha glucosidase inhibitors delay glucose absorption at the intestine level and thereby prevent sudden surge

of glucose after a meal. [8-10] Voglibose is an alpha-glucosidase inhibitor used for lowering post-prandial blood glucose levels in people with diabetes mellitus. It is made in India by Ranbaxy Labs and sold under the trade name Volix. Voglibose, an alpha-glucosidase inhibitor, is a synthetic compound with potent and enduring therapeutic efficacies against disorders of sensory, motor and autonomic nerve systems due to diabetes mellitus. [11-13] The drug was approved in Japan in 1994 for the treatment of diabetes, and it is under further investigation by Takeda for the treatment of impaired glucose tolerance.

The IUPAC Name of Voglibose is (1S, 2S, 3R, 4S, 5S)-5-[(1,3-dihydroxypropan-2-yl)amino]-1-(hydroxymethyl) cyclohexane - 1, 2, 3, 4-tetrol. [14] Metformin, marketed under the trade name Glucophage among others, is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It is also used in the treatment of polycystic ovary syndrome. It is not associated with weight gain. It is taken by mouth. [5] Metformin is generally well tolerated. [15-17] Common side effects include diarrhea, nausea, and abdominal pain. [5] It has a low risk of causing low blood sugar. High blood lactic acid level is a concern if the medication is prescribed inappropriately or in overly large doses. [18] It should not be used in those with significant liver disease or kidney problems. While no clear harm comes from use during pregnancy, insulin is generally preferred for gestational diabetes. Metformin is a biguanide antihyperglycemic agent. [19] It works by decreasing glucose production by the liver and increasing the insulin sensitivity of body tissues. [20]

The IUPAC Name of Metformin is 1-carbamimidamido-N, N-dimethylmethanimidamide. [21]

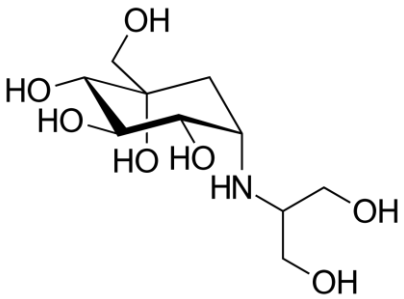


Fig-1: Structure of Voglibose

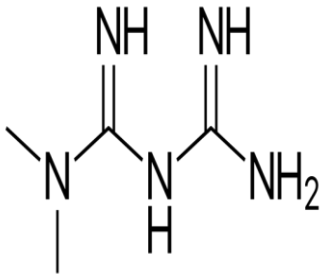


Fig-2: Structure of Metformin

A survey of literature reveals that good analytical methods are not available for Voglibose and Metformin. The present research manuscript describes innovative, simple, economical, accurate, specific, robust, rugged and rapid RP-HPLC method developed [22] in selected solvent system (Mobile Phase) and validated in accordance with International Conference on Harmonization (ICH) Guidelines Q2 (R1), for the estimation of Voglibose and Metformin in bulk drug and in its dosage forms. [23]

2. EXPERIMENTAL:

2.1 Materials and Methods:

Pharmaceutical grade working standard Voglibose and Metformin were obtained from Syncorp Pvt. Laboratories, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt Ltd, Mumbai, India.

2.2 Instrumentation:

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system EMPOWER2 software, UV-Visible double beam spectrophotometer (T-60 LABINDIA), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Waters ODS (C18) RP Column (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

2.3 Sample & Standard Preparation for the Analysis

25 mg of Voglibose standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

25 mg of Metformin standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

2.4 Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. While scanning the Voglibose and Metformin solution we observed the maxima at 259 nm and 239 nm. The isobestic point for the drugs was found at 251nm.

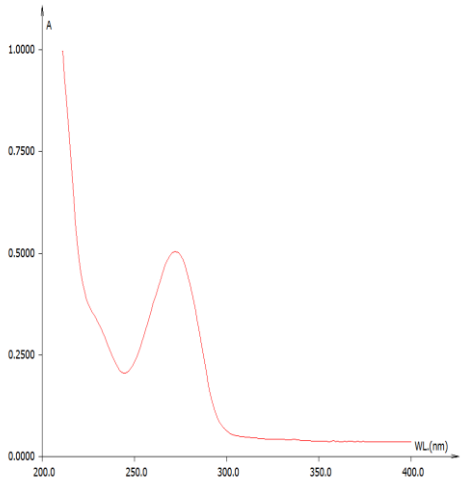


Fig – 3. UV Spectrum for Voglibose

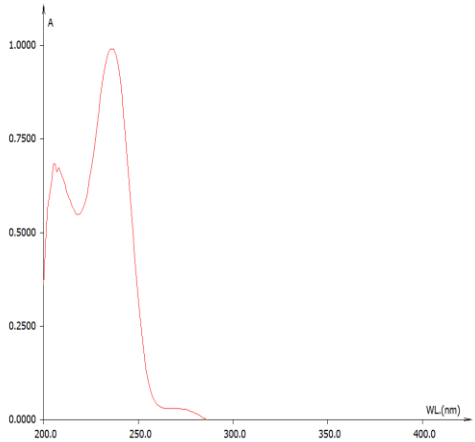


Fig-4. UV Spectrum for Metformin

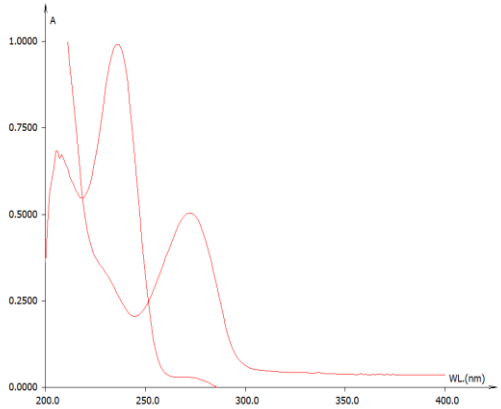


Fig -5. Isobestic Point for Voglibose and Metformin

2.5 Method Development

2.5.1 Preparation of 0.02M Phosphate Buffer Solution:

About 2.72168 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. The pH was adjusted to 6.5 with diluted Orthophosphoric acid.

2.5.2 Preparation of Mobile Phase:

The mobile phase was prepared with the combination of Phosphate Buffer (pH- 6.5) and Acetonitrile at the volume of 1000ml. 650ml of Phosphate Buffer and 350ml of Acetonitrile were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

2.5.3 Summary of Optimized Chromatographic Conditions:

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-1: Summary of Optimized Chromatographic Conditions

Mobile phase	Phosphate Buffer (pH- 6.5) : Acetonitrile = 65 : 35
Column	Waters ODS (C18) RP Column
Column Temperature	Ambient
Detection Wavelength	251 nm
Flow rate	1.0 ml/ min.
Run time	06 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic

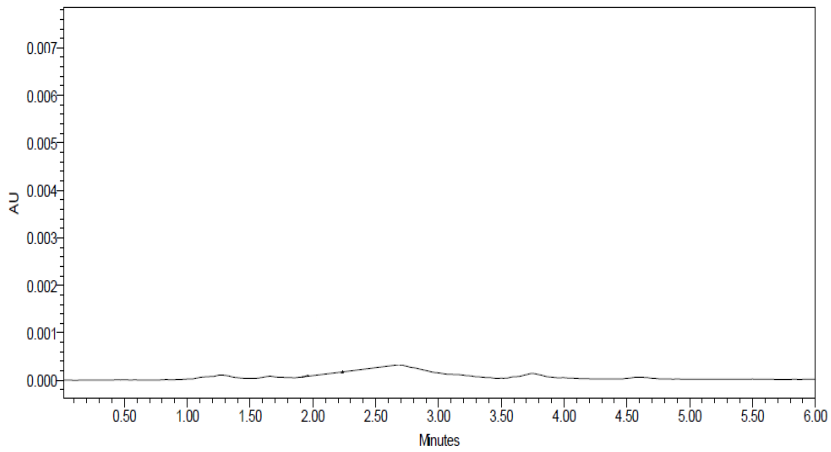
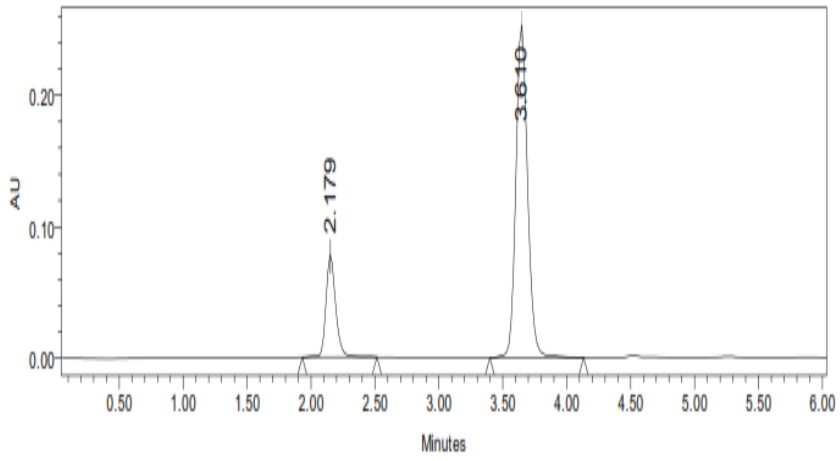


Fig-6: Chromatogram for Blank Preparation



Fig–7: Chromatogram of Voglibose and Metformin in Optimized Condition

2.6 Method validation:

2.6.1 Linearity & Range:

Calibration standards at five levels were prepared by appropriately mixed and further diluted standard stock solutions in the concentration ranges from 10-60 µg/ml and 5-40 µg/ml for Voglibose and Metformin. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the linearity graphs. Chromatograms of each solution were recorded.

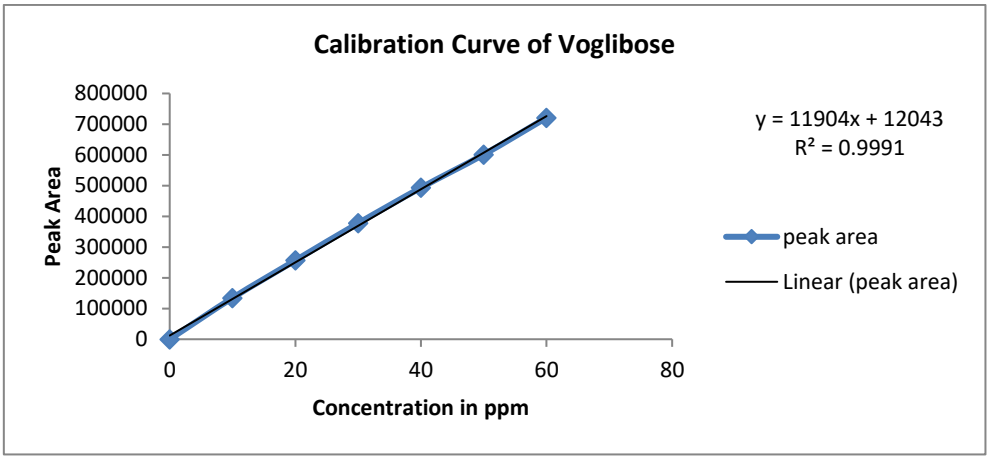


Fig-8: Standard curve for Voglibose

Table-2: Linearity Readings for Voglibose

CONC. (µg/ml)	AUC (n=6)
0	0
10	134528
20	256580
30	377574
40	493125
50	601256
60	721010

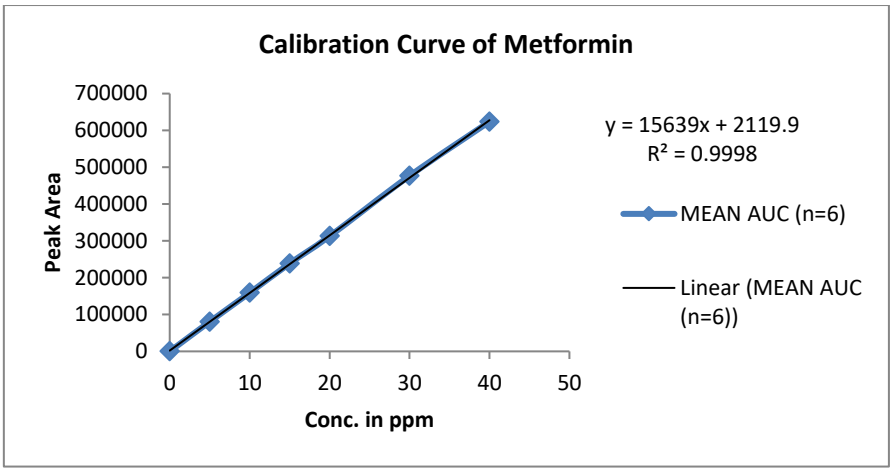


Fig-9: Standard curve for Metformin

Table-3: Linearity Readings for Metformin

CONC. (µg/ml)	MEAN AUC (n=6)
0	0
5	80586
10	158963
15	238722
20	312830
30	476594
40	623852

2.6.2. Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100% and 120%) of pure drug of Voglibose and Metformin were taken and added to the pre-analyzed formulation of concentration 50 µg/ml and 40 µg/ml. From that percentage recovery values were calculated. The results were shown in table - 4 and 5.

Table-4: Accuracy Readings of Voglibose

Sample ID	Concentration (µg/ml)			% Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	40	39.947	487574	99.867	Mean= 100.4113%
S_2 : 80 %	40	40.255	491241	100.637	S.D. = 0.473694
S_3 : 80 %	40	40.292	491685	100.73	% R.S.D.= 0.471754
S_4 : 100 %	50	49.705	603735	99.41	Mean= 100.6647%
S_5 : 100 %	50	50.434	612421	100.868	S.D. = 1.166369% R.S.D.= 1.158668
S_6 : 100 %	50	50.858	617459	101.716	
S_7 : 120 %	60	59.927	725421	99.878	Mean= 100.4637%
S_8 : 120 %	60	60.414	731214	100.69	S.D. = 0.511543
S_9 : 120 %	60	60.494	732165	100.823	% R.S.D. = 0.509182

Table-5: Accuracy Readings of Metformin

Sample ID	Concentration (µg/ml)			% Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	32	32.195	505624	100.609	Mean= 100.7527%
S_2 : 80 %	32	31.915	501243	99.734	S.D. = 1.097575
S_3 : 80 %	32	32.613	512164	101.915	% R.S.D.= 1.089375
S_4 : 100 %	40	40.668	638137	101.67	Mean= 100.5967%
S_5 : 100 %	40	39.738	623584	99.345	S.D. = 1.172714
S_6 : 100 %	40	40.310	632541	100.775	% R.S.D.= 1.165758
S_7 : 120 %	48	48.181	755635	100.377	Mean= 100.0547%
S_8 : 120 %	48	48.085	754124	100.177	S.D. = 0.397865
S_9 : 120 %	48	47.813	749878	99.610	% R.S.D. = 0.397647

2.6.3. Precision:

2.6.3.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Voglibose and Metformin (API). The percent relative standard deviation was calculated for Voglibose and Metformin are presented in the Table - 6.

Table-6: Repeatability Readings of Voglibose and Metformin

HPLC Injection Replicates	AUC for Voglibose	AUC for Metformin
Replicate – 1	613568	645214
Replicate – 2	613241	635241
Replicate – 3	625408	635424
Replicate – 4	617412	635987
Replicate – 5	612541	635216
Average	616434	637416.4
Standard Deviation	5363.157	4370.055
% RSD	0.870029	0.685589

2.6.3.2. Intermediate precision:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Voglibose and Metformin revealed that the proposed method is precise.

Table-7: Results of Intra-Assay & Inter-Assay for Voglibose

Conc. Of Voglibose (API) (µg/ml)	Observed Conc. of Voglibose (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
40	40.02	0.36	40.06	0.86
50	49.87	0.45	50.26	0.37
60	59.13	0.65	59.62	0.76

Table-8: Results of Intra-Assay & Inter-Assay for Metformin

Conc. Of Metformin (API) (µg/ml)	Observed Conc. of Metformin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
32	31.44	1.08	32.01	0.29
40	40.07	0.35	40.052	0.45
48	48.89	0.75	47.97	0.18

2.6.4. Method Robustness:

Influence of little changes in optimized chromatographic conditions like changes in flow rate (± 0.1 ml/min), mobile phase ratio (±2 %), Wavelength of detection (±2 nm) and organic phase (±5 %) studied to measure the robustness of the method are also in favour of (Table - 9, % RSD < 2 %) the developed RP-HPLC method for the analysis of Voglibose and Metformin (API).

Table-9: Results of Method Robustness Test for Voglibose

Change in parameter	% RSD
Flow (0.8 ml/min)	0.78
Flow (1.2 ml/min)	0.62
More Organic	0.76
Less Organic	0.52
Wavelength of Detection (261 nm)	0.86
Wavelength of detection (257 nm)	0.54

Table-10: Results of Method Robustness Test for Metformin

Change in parameter	% RSD
Flow (0.8 ml/min)	1.03
Flow (1.2 ml/min)	0.28
More Organic	0.71
Less Organic	0.65
Wavelength of Detection (233 nm)	1.04
Wavelength of detection (229 nm)	0.96

2.6.5. LOD & LOQ:

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3 (SD/S).

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The LOD was found to be 0.06 µg/ml and 0.08 µg/ml for Voglibose and Metformin respectively. The LOQ was found to be 0.18 µg/ml and 0.24 µg/ml for Voglibose and Metformin respectively.

2.6.6. System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

Following system suitability test parameters were established. The data are shown in Table - 11.

Table-11: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	3.87
2	Asymmetry	T ≤ 2	Voglibose = 0.22 Metformin = 0.32
3	Theoretical plate	N > 2000	Voglibose = 2956 Metformin= 3028

2.6.6 Estimation of Voglibose and Metformin in Tablet Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Finally, the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra sonicator, Then the final volume was make up to the mark with the mobile phase. The final solution was filtered through a selected membrane filter (0.45 µm) and in order to sonicated to degas the mobile phase (Solvent system). From this above stock solution (1 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system (Mobile phase). The prepared solutions were injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection (Blank Solution) of the standard solution also injected into the HPLC system and the chromatograms and peak areas were recorded and calculated. The obtained data are shown in Table - 12.

Table-12: Assay of VOGLIBOSE AND METFORMIN Tablets

Brand name of Tablets	Labelled amount of Drug (mg) Voglibose / Metformin	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
(Vogipax M Tablet (Vidakem Lifesciences Pvt Ltd)	0.2/500	0.199 (±0.28)/ 499.86 (±0.11)	99.5(±0.34) / 99.15(±0.12)

2.6.7 Stability studies:

The API (Voglibose and Metformin) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/ or after administration to body. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation, photolytic degradation and oxidative degradation.

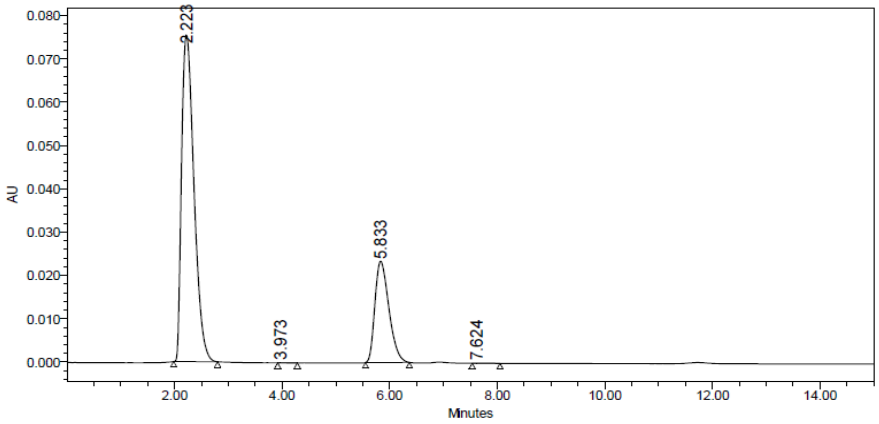


Fig-8: Chromatogram for Acid Degradation

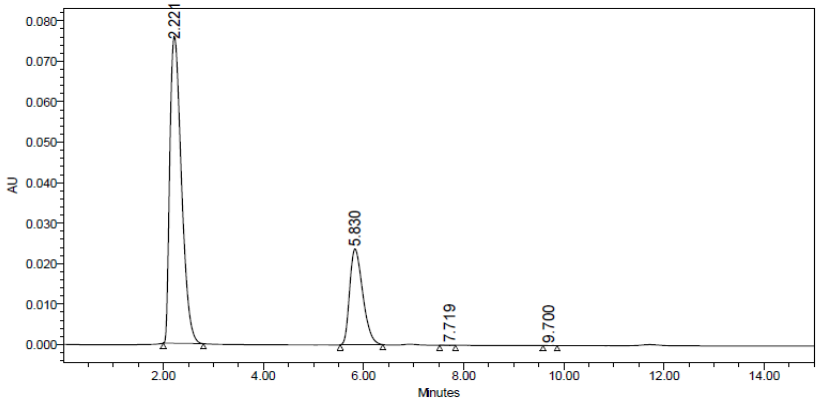


Fig-9: Chromatogram for Basic Degradation

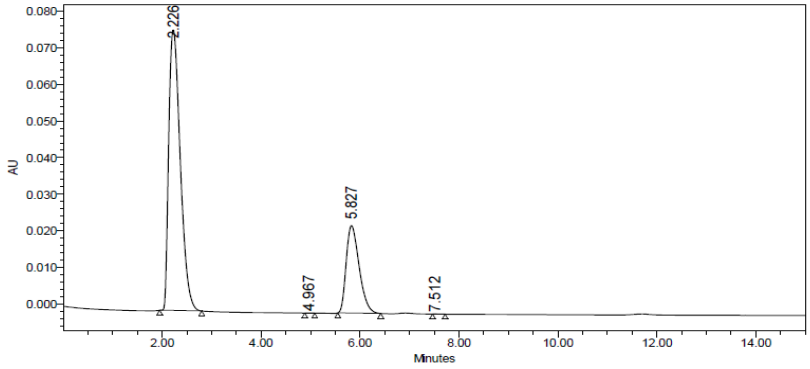


Fig-10: Chromatogram for Thermal Degradation

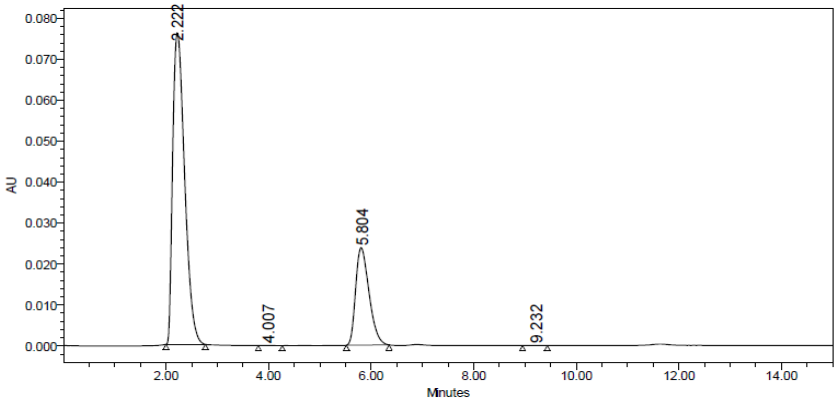


Fig-11: Chromatogram for Photolytic Degradation

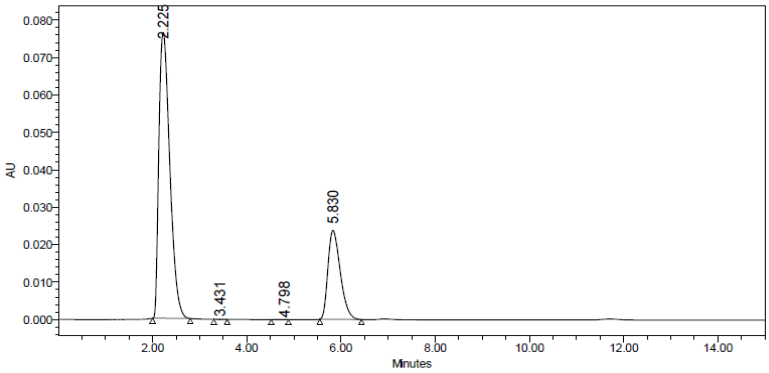


Fig-12: Chromatogram for Oxidation with 3% H_2O_2 Degradation

Table-13: forced degradation studies of Voglibose and Metformin API.

Stress condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	67.43	32.57	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	80.63	19.37	100.00
Thermal Degradation (50 ^{0}C)	24Hrs.	99.07	0.93	100.00
UV (254nm)	24Hrs.	99.21	0.79	100.00
3% Hydrogen peroxide	24Hrs.	69.64	30.36	100.00

3. RESULTS:

The optimized chromatographic conditions were Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm i.d.as stationary phase and mobile phase was prepared with a mixture of Phosphate Buffer (pH-6.5): Acetonitrile = (65: 35), flow 1.0 ml/min, with Injection Volume 10 µl, at detection wavelength 251 nm and run time at 6.0 min. In these chromatographic conditions the peak was pure, sharp, symmetric and found a greater number of theoretical plates.

The results obtained in method validation were:

Linearity & Range: The calibration curve showed good linearity in the range of 10-60 µg/ml and 5-40 µg/ml, for Voglibose and Metformin (API) with correlation coefficient (r^2) of 0.999 and 0.999. A typical calibration curve has the regression equation of y = 11904x + 12043 and y = 15639x + 2119 for Voglibose and Metformin.

Accuracy: The mean recoveries were found to be 100.4113, 101.647, 100.4637% for Voglibose and 100.7527, 100.5967, 100.0547% Metformin. The limit for mean % recovery is 98-102 % and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Repeatability: The repeatability study which was conducted on the solution having the concentration of about 50 µg/ml and 40 µg/ml for Voglibose and Metformin showed % RSD of 0.870029 % and 0.685589 %. It was concluded that the analytical technique showed good repeatability.

LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) are 0.06 µg/ml and 0.08 µg/ml for Voglibose and Metformin. The quantified (LOQ) were found to be 0.18 µg/ml and 0.24 µg/ml respectively.

Assay: The assay in Vogipax M Tablet containing Voglibose and Metformin was found to be 99.5 % and 99.15 %.

Degradation studies: The results of the stress studies indicated the specificity of the method that has been developed. Voglibose and Metformin were stable only in acidic, basic and thermal stress conditions and photolytic stress conditions.

4. DISCUSSION:

To develop a precise, linear, specific RP-HPLC method for analysis of Voglibose and Metformin, different chromatographic conditions were applied & the results observed were compared with the methods available in literatures.

Kuna Mangamma, et al. achieved separation by using acetonitrile: buffer pH- 6.5 in the ratio of (62:38) v/v as mobile phase.[24] Shubhangi C. Daswadkar, et al developed method by using a mobile phase in combination of acetonitrile: water in a ratio (20:80 v/v) but we have used Phosphate Buffer (pH- 6.5) : Acetonitrile = (65 : 35).[25] As per P. Jitendrakumar, et al. used Zodiac C18 column(250 mm × 4.6 mm × 5 µ particle size).[26] N.Mallikarjuna Rao, et al. used RP-18e, Hibar RT column (250 × 4.6 mm), with a mobile phase composed of 0.025M potassium dihydrogen phosphate pH 2.5 : acetonitrile : methanol (40:55:5) in isocratic mode, maintained at ambient temperature, is used as stationary phase applied for pharmaceutical dosage form.[27]

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Voglibose and Metformin in formulations.

5. CONCLUSION:

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Voglibose and Metformin API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Voglibose and Metformin indicated that the developed method is specific for the estimation of Voglibose and Metformin. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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