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Original Research Paper

STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF VOGLIBOSE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, economic, selective, precise, and stability-indicating HPLC method has been developed and validated for analysis of voglibose both in bulk drug and tablet dosage form. The drug was separated using a mobile phase acetonitrile: water, (20:80 v/*v*) on an Agilent, TC C_{18} (250 × 4.6 mm) 5µm column at flow rate of 1.0 ml/min at ambient temperature and detection was performed at 272 nm. The retention time was 3.17 ± 0.01 min for voglibose. The detector linearity was established in concentrations ranging from 10-70µg/ml, the regression coefficient was 0.9992. For stability study, the drug was exposed to the stress conditions such as acid, base, oxidation, neutral and sunlight as per the recommendations of ICH guidelines. The results of the analysis were validated in terms of specificity, limit of detection, limit of quantification, linearity, precision and accuracy As per ICH guidelines and were found to be satisfactory. The high recovery and low relative standard deviation confirm the suitability of these methods can be employed for the routine analysis of tablet containing voglibose.

Keywords: Voglibose, RP-HPLC, Acetonitrile, Stability indicating, ICH, Validation.

INTRODUCTION

Voglibose (figure 1), 3, 4-Dideoxy-4-[2-hydroxy-1-(hydroxyl methyl) ethyl] amino-2-c (hydroxyl methyl)-D-epiinositol, a new potent α-glucosidase inhibitor used for type- 2 diabetes, has shown strong anti-obesity and anti-diabetic activity. Alpha-glucosidase inhibitors are agents that delay the glucose absorption at the intestinal level and thereby prevent sudden surge of glucose after a meal. Voglibose is the safest and most effective drug of its class.[1-3] Voglibose obtained from organic synthesis processes is similar to structurally related carbohydrates found naturally.[4,5] Literature survey revealed spectrofluorimetric[6], high-performance liquid chromatographic [7-13], high-performance thin-layer chromatographic [14,] Spectroscopic[15,16] and UPLC-ELSD[17] methods are reported for the estimation of voglibose alone or in combination with other antidiabetic agents. Literature survey revealed that no stability indicating RP-HPLC method have been found to be reported for determination of voglibose in bulk drug and tablet dosage form. The main objective of the proposed work was to develop a simple, accurate, precise and sensitive

RP-HPLC method for the estimation of voglibose in bulk drug and tablet. The method was further optimized and validated in accordance with guidelines suggested by International Conference on Harmonization (ICH).[18,19]

MATERIALS AND METHODS

Authenticate voglibose sample was a kind gift from Ranbaxy Research Laboratory, Gurgaon, India. HPLC grade water and acetonitrile (Merck Ltd, Mumbai, India) was used as solvent. All the aqueous reagents were prepared using carbon dioxide free distilled water.

Instrumentation

The HPLC system, Agilent 1120 compact with manual Rheodyne injector facility operates at 20 µL capacity per injection was used. The column used was Agilent TC C_{18} (250 X 4.6 mm) 5µm and the detector consisted of UV/VIS operated at 272 nm. The instrumentation was controlled by use of EZChrom Elite Compact software.

Chromatographic Conditions

Optimization of chromatographic conditions was carried out using water: acetonitrile (80:20 v/v) as mobile phase. Prior to deliver into the system, mobile phase was filtered through 0.45 µm filter and sonicate for 10 min. The samples were introduced by injector with a 20 µL sample loop. The analysis was carried out under gradient conditions using flow rate 1.0 mL/min at 18 ^{0}C and chromatograms were recorded at 272 nm.

Standard Solutions

Preparation of standard stock solution

Weighed accurately 10 mg of voglibose and transferred to 100 ml volumetric flask, add 25 mL of mobile phase and sonicate for 15 min and volume was made up to mark with mobile phase (100 µg/mL).

Preparation of standard solution

From the standard stock solution 1mL solution was pipetted out in 10 mL volumetric flask and volume was made up to the mark with mobile phase to get a final concentration 10 µg/mL.

Preparation of Sample Solutions

Twenty tablets of voglibose were weighed, triturated, mixed thoroughly and average weight of tablet was calculated. Accurately weighed quantity of tablet powder equivalent to 0.3 mg of voglibose (label claim) was transferred to 10 mL volumetric flask, added 5 mL of mobile phase and sonicate for 10 min. The resultant solution was filtered through 0.45µ membrane filter, diluted to volume with mobile phase and injected to HPLC system. The amount of drug present in the sample solutions were determined using calibrated curves of standard voglibose (table 1).

Method Validation

The developed method was validated according to ICH guidelines. System suitability parameters were evaluated from retention times, asymmetry, capacity factor and theoretical plates of standard chromatograms. Different standard solutions were prepared by diluting standard stock solution with mobile phase in the concentration range 10-70 µg/mL. Diluted samples were injected and chromatograms were taken under standard chromatographic conditions. The peak area was plotted against corresponding concentrations to obtain the calibration graph. Specificity is the ability of a method to discriminate between the analyte of interest and other components that may present in the sample. The specificity of the method was evaluated to ensure separation of voglibose. Precision of analytical method was expressed in relative standard deviation (RSD) of a series of measurements. The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses (i.e. three concentrations/ three replicates each) of the sample solution on the same day and on three different days respectively. The signal-to-noise ratio (*S/N*) method was adopted for the determination of limit of detection and limit of quantification. The limit of detection was estimated as three times the *S/N* ratio and the limit of quantification was estimated as ten times the *S/N* ratio. To check the accuracy of the proposed method, recovery studies were carried out by standard addition method. A known amount of standard voglibose corresponding to 80, 100 and 120% of the label claim was added to preanalysed sample of tablet. The recovery studies were carried out in triplicate at each level. Robustness is

a measure of the performance of a method when small and deliberate changes are made to the conditions of method. Robustness was determined by changing the mobile phase flow rate to 0.8 and 1.2 mL min^{-1} and the concentration of acetonitrile in the mobile phase to 18 and 22%.

Force Degradation Studies

Specificity is the ability of method to measure the analyte response in the presence of degradation products. Forced degradation studies were performed on for voglibose bulk drugs. The specificity of the method was determined by exposing a solution of the sample to acidic (0.1 M HCl), basic (0.1 M NaOH), oxidizing (3% H_2O_2), UV light and dry heat stress conditions to determine the ability of the proposed method to separate voglibose from degradation products generated during forced decomposition studies. The resulting solutions were then analyzed and the analyte peak was evaluated both for peak purity and for resolution from the nearest eluting peak. For UV studies, study period was 7 days where as for acid, base, dry heat and oxidation it was 24 hrs. Peak purity test was carried out on the stressed samples by using PDA.

RESULTS AND DISCUSSIONS

Optimization of Chromatographic Method

Figure 2 shows an LC elution of voglibose. During the development phase, the mobile phase containing methanol water resulted in broad and asymmetric peak with a greater tailing factor (>2). The successful use of acetonitrile and water resulted in drastic reduction of peak tailing, which was found to be within the acceptable limit (1.11) resulting good peak symmetry and resolution. The mobile phase optimized contained water and acetonitrile (80: 20) at 1.0 mL/min flow rate. The retention time was found to be 3.17 min for voglibose. There were no interferences at drug retention time. Voglibose was determined using high performance liquid chromatography with UV detector and found to be well suited technique for the analysis.

Method Validation

The retention time of voglibose was 3.17±0.1 min., asymmetry 1.11, Capacity factor 3.881 and theoretical plates 7159. The mobile phase is water: acetonitrile (80:20) was selected as optimized mobile phase, because of the high purity, asymmetry, proper tailing, high area and low Rt value at same concentration as compared to other trail mobile phase. Voglibose showed a good linear relationship over the concentrations level ranging from 10-70 µg/mL as summarized in (figure 3) and (table 2) The limit of detection (LOD) and the limit of quantification (LOQ) of voglibose were found to be 0.037 and 0.114 µg/ml indicates that method is sensitive (table 2) by scanning the solution of voglibose having different lower concentrations. Inter-day as well as intra-day replicates of voglibose, gave an R.S.D. below 0.770 revealed that the proposed method is highly precise. Accuracy and precision calculated during the intra- and inter-day run are given in (table 3). Extraction efficiency was performed to verify the effectiveness of the extraction step and the accuracy of the proposed method. The extraction efficiency of voglibose from formulation samples was satisfactorily ranged from 98.89 to 100.30 % (R.S.D. was less than 1.13) at all three concentration levels, which confirm no interference effects due to formulation excipients (table 4). The method was found to be robust with respect to flow rate and change in mobile phase composition without any changes in system suitability parameters (table 5). The proposed RP-HPLC method was accurate, precise, sensitive and rapid. The method also can be extended for the routine analysis of voglibose in tablet dosage form.

Force Degradation Study

The specificity of the method was determined by exposing a solution of voglibose to stress conditions, i.e. 0.1 M HCl, 0.1 M NaOH, 3% H_2O_2, UV light and dry heat. There was no degradation of VGB in the presence of 3% H_2O_2, UV light and dry heat and no significant change in peak area and retention time of voglibose. In the presence of 0.1 M HCl or 0.1 M NaOH it was found there was a substantial change in the peak area of voglibose, but not in the retention time. The results from these tests are listed in (table 6) and a chromatogram obtained from voglibose after

treatment with 0.1 M HCl and 0.1 M NaOH is shown in (figure 4 and figure 5). A degradation products (peak 1 of figure 4) eluted with a retention time of 1.59 ± 0.03 min and (peak 2 of figure 5) eluted with a retention time of 3.95 ± 0.06 min. Undegraded voglibose (peak 1, R*t* 3.17 min in figure 4 and peak 2 figure 5) were compared and found to be similar with regard to appearance. This indicated the specificity of the method. The results from stress testing, including separation of the degradation product and quantification of voglibose after exposure to stress conditions show the method is stability-indicating.

CONCLUSION

The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters tested. As the method able to separate the parent drug from degradation products it can be employed as a stability indicating method for voglibose.

Table 1: Assay data of marketed formulation

Formulation	Amount present	Amount found (mg)	%Amount found	%RSD	Average %RSD
Volix	0.3	0.301	100.33	0.66	0.65
	0.3	0.299	99.67	0.57	
	0.3	0.303	101.00	0.73	

Table 2: Statistical parameters of voglibose

Parameters	Values
Correlation coefficient (r^2)	0.9993
Regression equation	y=114086x+1614981
Intercept (a)	1614981
Slope (b)	114086
Limit of detection (LOD µg/mL)	0.037
Limit of quantification(LOQ µg/ml)	0.114
Linearity	10–70 µg/mL
Retention time	3.17±0.1
Asymmetry	1.11±0.0048
Capacity	3.881±0.0047
Theoretical Plates	7159±7.772

Note: r^2 is correlation coefficient, LOD is the limit of detection and LOQ is limit of quantification

Table 3: Precision and accuracy data for voglibose

Parameters	% Estimated	S.D.	% RSD
Intra-day*	100.20	0.031	0.309
	100.19	0.060	0.085
	100.01	0.077	0.770
Inter-day*	99.90	0.046	0.46
	100.15	0.073	0.182
	100.28	0.054	0.076

* indicates mean of three replicates, SD is standard deviation. *RSD is Relative standard deviation*

Table 4: Recovery study data

Level of standard addition (%)	Amount of tablet powder (mg)	Amount of pure drug added (mg)	Amount of pure drug recovered (mg)	% Recovery	% RSD
80	0.3	0.24	0.534	98.89	1.1236%
100	0.3	0.3	0.601	100.16	0.8486%
120	0.3	0.36	0.662	100.30	0.4531%

Table 5: Robustness data for Voglibose

Parameters	% Recovery	S.D.	% RSD
Change in flow rate (10 ± 0.2 mL/ min)	99.87	0.009	0.09
Mobile Phase Composition (80:20) ±2 of Each Phase	100.03	0.013	0.13

Table 6: Summary of forced degradation of Voglibose

Stress	Voglibose	Degradants	Voglibose remaining	Amount recovered (%)
0.1 M HCl	3.17	1.59	8.38	83.8
0.1 M NaOH	3.17	3.95	7.10	71.0
3% H_2O_2	3.17	---	10.02	100.2
UV Light	3.17	---	9.95	99.50
Dry Heat	3.17	---	10.05	100.50

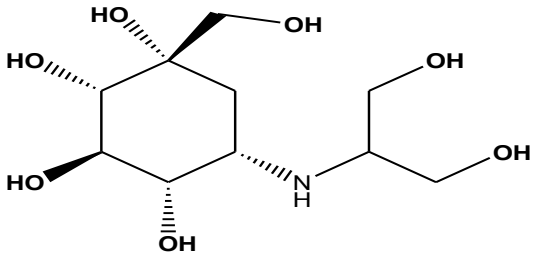

Figure 1: Structure of voglibose

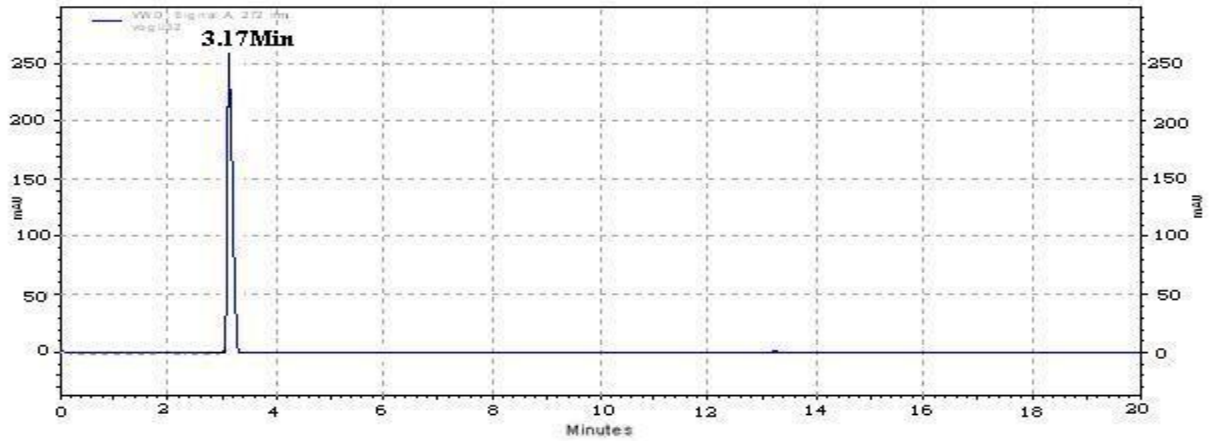

Figure 2: Chromatogram of Voglibose

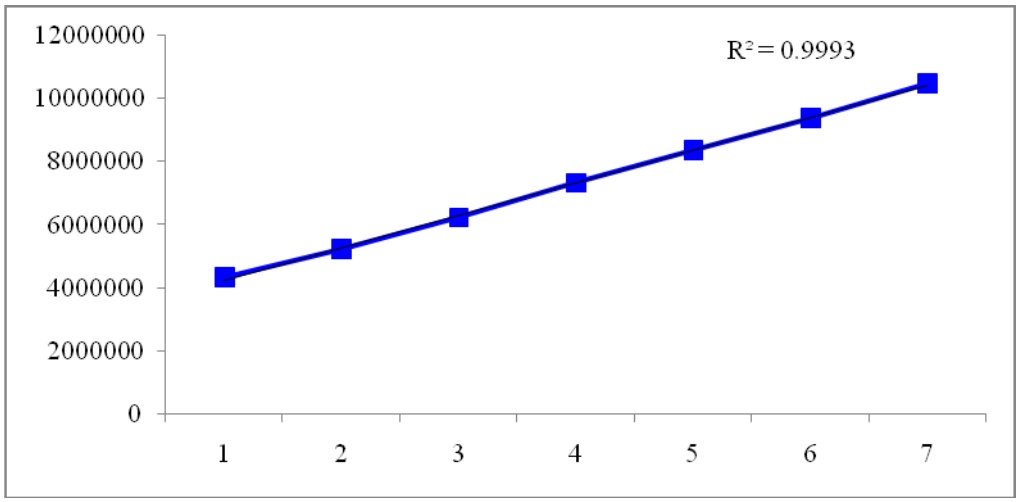

Figure 3: Calibration Curve of Voglibose

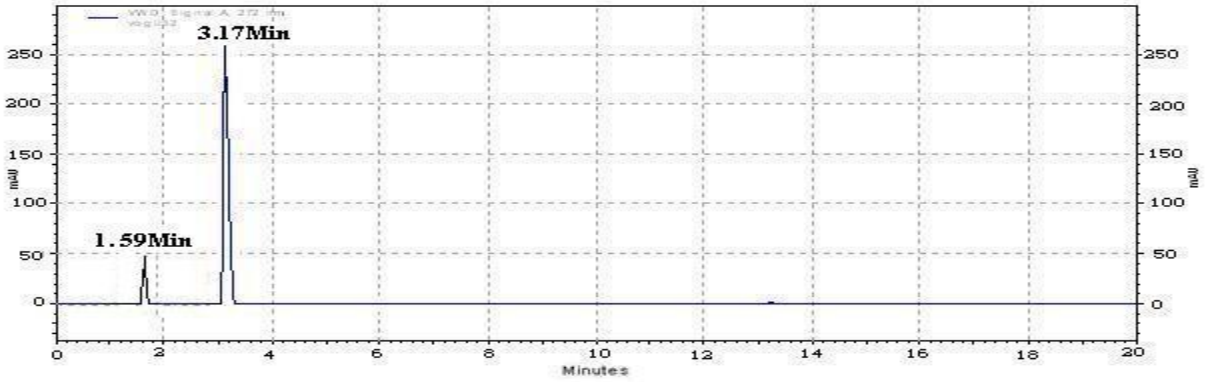

Figure 4: Chromatogram obtained in the presence of 0.1M HCl (peak 1, *Rt* 1.59min; peak 2, *Rt*3.17min

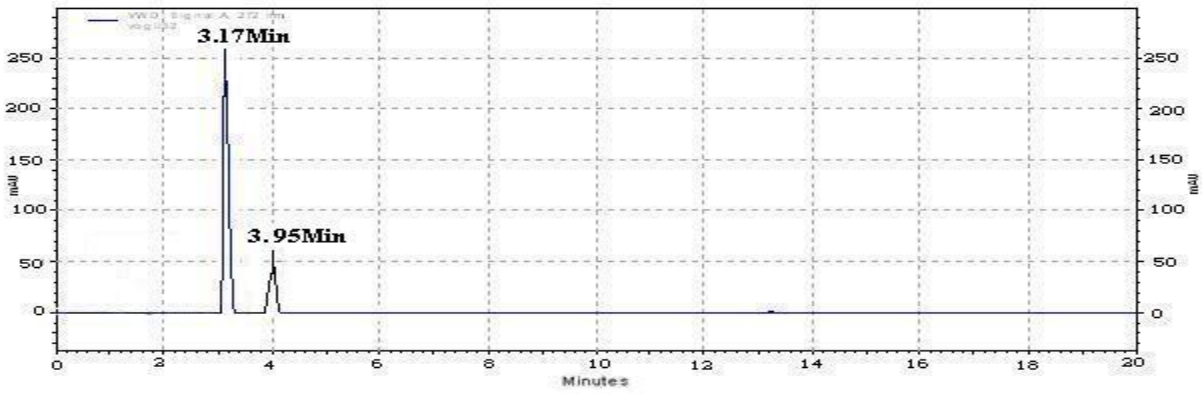

Figure 5: Chromatogram obtained in the presence of 0.1M NaOH (peak 1, *Rt*3.17min; peak 2, *Rt*3.95min)

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