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Research Article

RP-HPLC method development and validation for estimation of Voglibose in bulk and tablet dosage forms

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ABSTRACT

A simple, sensitive, precise and specific reverse phase high performance liquid chromatographic method was developed and validated for the determination of voglibose in bulk and tablet dosage forms. It was found that the excipient in the tablet dosage forms does not interfere in the quantification of active drug by proposed method. The HPLC separation was carried out by reverse phase chromatography on RP-18e, Hibar RT column (250×4.6mm) with a mobile phase composed of 0.025M potassium dihydrogen phosphate pH 2.5 : acetonitrile: methanol (40:55:5) in isocratic mode at a flow rate of 1ml/min. As voglibose cannot be directly detected with high sensitivity, it was derivatized with Taurine and sodium periodate. The detection was monitored at 282nm. The calibration curve for voglibose was linear from 100 to 500ng/ml. The interday and intraday precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility and specificity for the determination of voglibose in bulk and its tablet dosage forms. LOD and LOQ for voglibose were found to be 30ng/ml and 100ng/ml. Accuracy (recoveries: 99.8-101.2%) and reproducibility were found to satisfactory.

Keywords: Voglibose; RP-HPLC Method; Reverse phase chromatography; Acetonitrile; Validation.

INTRODUCTION

Voglibose (Fig. No. 1) 3,4-Dideoxy-4-[2-hydroxy-1-(hydroxyl methyl) ethyl]amino-2-c-(hydroxymethyl)-D-epinositol has attracted considerable interests interests due to its wide range of therapeutic and pharmacological properties, including its excellent inhibitory activity against α-glucosidase and its action against hyperglycemia and various disorders caused by hyperglycemia. Voglibose, a new potent glucosidase inhibitor used for type 2 diabetes, has shown strong anti-obesity and anti-diabetic activity. As a glucosidase inhibitor, the compound exerts its activity within the gastrointestinal tract of humans. The drug delays glucose absorption and thus, reduces the post-prandial blood glucose peaks (Y. Yamasaki et al, 2005; K. Watanabe et al, 2004; A. Vichayanrat et al, 2002). Voglibose obtained from organic synthesis processes is similar to structurally related carbohydrates found naturally (H. Zhang et al, 2004; X. Chen et al, 2006) and has the empirical formula $C_{10}H_{21}NO_7$.

The proposed RP-HPLC method was simple, precise, sensitive and accurate method for the determination of voglibose in bulk and it's pharmaceutical dosage forms (Tablets).

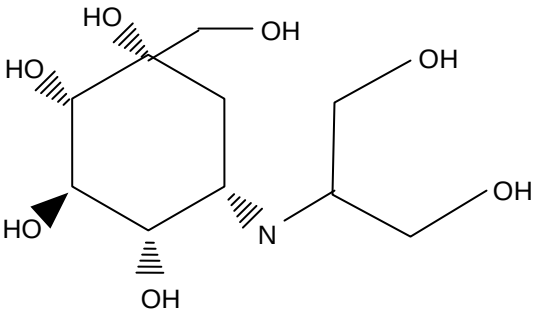

Figure 1: The structure of Voglibose

Apparatus and Chromatographic conditions

Chromatographic separation was performed on a Shimadzu chromatographic system equipped with a LC-20AT pump, variable wavelength programmable UV/Visible detector SPD-20A and Redone (7725 i) with 20µL fixed loop and data analyzed by using spinchrome software. RP-18e, Hibar® RT column (250 × 4.6 mm) was used for separation. Mobile phase consisting of a mixture of 0.025M potassium dihydrogen phosphate

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pH 2.5: acetonitrile: methanol (40: 55: 5 % v/v/v) was delivered at a flow rate of 1ml/min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 15min. Analysis was performed at ambient temperature. Since most carbohydrate lack chromophore and/or fluorophore groups, their analysis by liquid chromatography (LC) often requires derivatization procedures. Since voglibose only absorbs UV in the low wavelength region, it cannot be directly detected with high sensitivity. Taurine and sodium periodate were used for the derivatization of voglibose.

Reagents and Solutions

Pure sample of Voglibose was kindly supplied by the Ranbaxy laboratories, Gurgaon. Methanol, acetonitrile and water used were of HPLC grade. All other reagents used in this study were of AR grade. Optimized chromatographic conditions are listed in Table: 1.

Standard solution

100 µg/ml of voglibose was prepared in methanol. This solution was further diluted with methanol to get a solution of concentration 1 µg/ml and it was derivatized using taurine and sodium periodate.

Sample solution

Thirty tablets, each containing 0.2 mg of voglibose were weighed, average weight was calculated and quantity equivalent to 5 mg of voglibose was weighed accurately and transferred to a 100 ml standard flask, extracted with methanol and later made upto the volume with methanol and it was derivatized using taurine and sodium periodate.

METHOD VALIDATION

Once the HPLC method development was over, the method was validated in terms of parameters like specificity, precision, accuracy, linearity and range, LOD, LOQ, ruggedness, robustness, stability etc. For all the parameters percentage relative standard deviation values were calculated. The proposed HPLC method was validated as per ICH guidelines.(ICH Guidelines; 2006)

Linearity and Range

The linearity of measurement was evaluated by analyzing different concentrations of the standard solutions of the voglibose. The Beer lamberts concentration was found to be 100- 500ng/ml. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed. The results were shown in Table: 2.The slope, intercept and correlation coefficient values were found to be 2.9133, 0.1346 and 0.99967. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above regression graph was shown at Fig: 2.

Precision

Precision was evaluated by carrying out three independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in the sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 1% for within a day and day to day variations, which proves that that method is precise. Results were shown in Table 3-4.

Accuracy

To study the reliability, suitability and accuracy of the method recovery experiments were carried out. A known quantity of the pure drug was added to the pre-analyzed sample formulation at the level of 50% and 100%, dissolved in methanol and made up to 100 ml with same solvent. Further dilutions were made so that each aliquot contained 100ng/ml of Voglibose. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method. The recovery procedure was repeated 10 times and % RSD was calculated by using the following formula. The contents of voglibose per tablet found by proposed method are shown in Table 3; the lower values of %RSD of assay indicate the method is accurate. The mean recoveries were in range of 99.8-101.20 % which shows that there is no interference from excipients. Table: 5.

$$\% \text{ recovery} = \frac{b-a}{c} \times 100$$

Where,

a- The amount of drug found before the addition of standard drug

b- The amount of drug found after the addition of standard drug

c-The amount of standard drug added

Repeatability of solution

A standard solution of drug substance was injected ten times and corresponding peak areas were recorded. The % RSD was found to be less than 1%. Table: 6.

Specificity

Condition of HPLC method like percentage of organic solvent in mobile phase, ionic strength, pH of buffer flow rate etc, was changed. In spite of above changes no additional peaks were found, although there were shift retention times or little changes in peak shapes.

Assay

20 µl of standard and sample solutions were injected into an injector of RP-HPLC, from the peak area of standard amount of drug in sample were computed. The values are given in Table: 7.

Limit of Detection and Limit of Quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Voglibose found to be 30ng/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 100 ng /ml for Voglibose. It was concluded that the developed method is sensitive.

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC and Agilent HPLC by different operators using different columns of similar types. The %RSD values with two different instruments Shimadzu HPLC and Agilent HPLC, analysts and columns were 0.5- 0.5, 0.6- 0.5 and 0.4- 0.3% respectively.

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in mobile phase, flow rate and column temperature. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, is rugged and robust. The robustness limit for mobile phase variation, flow rate variation, and temperature variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria of not more than 2%.

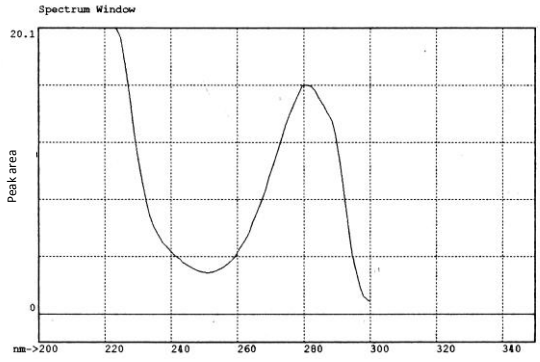

Figure 2: Chromatogram of standard

RESULTS AND DISCUSSION

UV spectrum of Voglibose was recorded from which 282 nm was selected as wavelength. Flow rate of 1ml/min was selected 0.025M potassium Dihydrogen phosphate: Acetonitrile: Methanol was selected as mobile phase. The retention time was found to be 2.6 and Mefenamic acid was used as internal standard. Voglibose shown linearity in the range of 100-500 ng/ml, and the co-efficient was found to be 0.99967. Recovery studies were performed at 50 and 100 % levels. The sensitivity of method LOD and LOQ is shown in Table 2. The stability at room temperature and refrigeration was found to be 3 and 8.5 hrs respectively. Hence the proposed method is simple, accurate, and rapid and can be employed for routine analysis. The low standard deviation and good percentage recovery indicates the reproducibility and accuracy of the method.

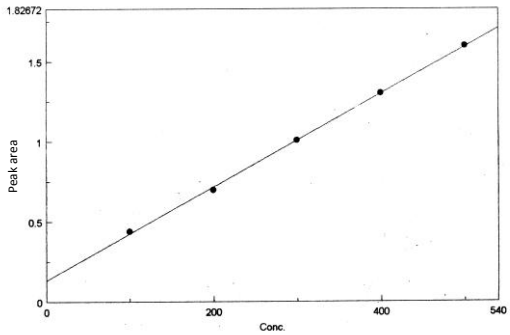

Figure 3: Linearity of Voglibose

Regression analysis of the calibration curve for Voglibose showed a linear relationship between the concentration and peak area with correlation coefficients higher than 0.9967 in all the curves assayed.

Table 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Shimadzu with 2487 PDA)
Column	RP-18e, Hibar® RT column (250 × 4.6 mm)
Mobile Phase*	0.025M potassium dihydrogen phosphate pH 2.5: acetonitrile: methanol (40 : 55 : 5 % v/v/v)
Flow rate	1 ml/min
Detection	282 nm
Injection volume	20µl
column Temperature	Ambient

Table 2: Validation Parameters

Parameters	Voglibose
Linearity range	100 – 500 ng/ml
Correlation coefficient	0.99967
Slope	2.9133
Y Intercept	0.1346

Table 3: Intraday Precession

Conc. (ng/ml)	Area	% RSD
200	3581	0.2284
	3578	
	3571	
400	4956	0.2425
	4939	
	4963	
500	7777	0.2056
	7698	
	7753	

The intraday precision was found to be within 1% RSD for conc. 200, 400 and 500 ng/ml

Table 4: Interday Precision

Concentration (ng/ml)	Day	Area	%RSD
200	1	3581	1.38
	2	3566	
	3	3594	
400	1	4956	1.51
	2	4973	
	3	4960	
500	1	7777	1.77
	2	7681	
	3	7708	

Interday precision was performed for conc. of 200, 400 and 500 ng/ml. for about three days and their peak areas are shown in the table. The %RSD for conc. 200, 400 and 500 ng/ml. was found to be within 2%.

Table 5: Recovery studies

Level	%Recovery	%RSD
50%	99.8	0.05
100%	101.20	0.07

Recovery studies were performed at 50% and 100% levels and the results were found to be within the limits mentioned as per ICH Guidelines.

Table 6: Repeatability of injection

Conc (ng/ml)	Peak area	%RSD
500	7777	0.5617
	7740	
	7683	
	7761	
	7707	
	7783	
	7691	
	7801	
	7782	
	7790	

Repeatability of injection was performed using 500ng/ml sample for10 times and corresponding peak areas were recorded. The %RSD peak was reported.

Table 7: Analysis of formulation

Amount of drug (mg/tab)		% Label claim	%RSD
Labelled	Estimated		
0.2 mg	0.194	97	0.08

Analysis of formulation was performed using Voglibose 0.2 mg tablets, and the % label claim was found to be 97.

Table 8: System suitability parameters

Parameter	Voglibose
Calibration range	100- 500ng/ml.
Theoretical plates	7658
Resolution	-
Tailing factor	1.26
LOD	30 ng/ml
LOQ	100 ng/ml

CONCLUSION

A convenient and rapid RP- HPLC method has been developed for estimation of Voglibose in tablet dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % RSD coupled with excellent recoveries. The proposed method is simple, fast, accurate and precise for the simultaneous quantification of Voglibose in dosage form, bulk drugs as well as for routine analysis in quality control.

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