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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS VOGLIBOSE IN PURE AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A Validated simple, sensitive, specific and precise RP-HPLC method was developed for the determination of Voglibosein pure and pharmaceutical formulations. Method was carried on Zodiac C_{18} column(250mm×4.6mm×5µ particle size) using Methanol: Acetonitrile(75:25) as mobile phase.Detection was carried out by U.V at 215nm. The proposed method obeyed linearity in the range of 20-140 µg/mland met all specifications as per ICH guidelines. Statistical analysis revealed that this method can be used in routine quality control studies of Voglibose in pure and its formulations

Keywords: Voglibose,C_{18} column, Reverse phase,Validation.

INTRODUCTION

Voglibose, a potent α-glucosidase inhibitor is used for the treatment of diabetes mellitus[1-2]. It acts as glucosidase inhibitor, remaining active within the gastrointestinal tract of humans by delaying the glucose absorption thereby preventing the sudden surge of glucose in the human body after meals[3-4]. Most commonly used glucosidase inhibitors include acarbose, mitglitol& voglibose[5]. Voglibose is the safest and most effective of them all. It is most commonly available in the form of tablets with the dosages of 0.2 mg to 0.3 mg per tablet.

Structure of voglibose is similar to that of carbohydrate[6]. Inspite of the fact thatvoglibose has already been established as an important pharmaceutical active, the alreadypublished work on voglibose, describes this molecule in different manner[7-9]. For determining the content of voglibose in the formulations, not many analytical methodshave been reported[10-18].In the present study, a new RP-HPLC method was developed which shown high reproducibility and sensitivity. The developed method was validated as per ICH guidelines.

Instrumentation

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Voglibosean isocratic PEAKHPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. Theinstrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

Standards and chemicals used

Voglibose was provided by Ranbaxylaboratories,Guargon.All the chemicals Acetonitrile, Methanol were HPLC grade, Merck Specialties Private Limited, Mumbai, India. Commercial tablets of Voglibose were purchased from local market.

Preparation of the mobile phase

Into a 1000ml cleaned volumetric flask, HPLC grade methanol 750ml, acetonitrile 250ml (which are filtered through 0.25mm membrane filters by vacuum filtration) were slowly added , mixed well and sonicatedupto 20min. Cool the

solution to room temperature and use for chromatography method.

Preparation of Standard drug solutions

100mg of Voglibose was accurately weighed and is dissolved in few ml of the mobile phase andsonicated for few min to dissolve the drug completely. Then it is filtered through 0.2µ ultipore filter paper and the volume is made upto 100ml with mobile phase to get a concentration of1mg/ml (free base) stock solution. This solution is further diluted with same solvent to obtain required working standard concentrations.

Sample Preparation

20 commercial tablets of Voglibose were finely powdered and the powder equivalent to 0.3mg of Vogliboseaccurately weighed to 50ml volumetric flask and dissolved in few ml of mobile phase. The above solution was subjected to sonication for 15min. After getting clear solution it is filtered through 0.25µm membrane filters and the solution is made upto 50ml with mobile phase resulting in preparation of 1 mg/ml solution. This is further diluted so as to obtain required concentration of Voglibose pharmaceutical dosage form.

Methodology

The HPLC system was stabilized for thirty min. by passing mobile phase, detector was set at 215nm, flow rate of 1.0ml/min to get a stable base line. One blank followed by six replicates of a single standard solution was injected to check the system suitability. Eight replicates of each standard solutions 20, 40, 60, 80, 100, 120,140µg/ml were injected. Calibration graph was plotted by concentration of Voglibose on X-axis and peak area on Y-axis. The amount of drug present in sample was computed in calibration graph.

Pharmaceutical formulations

Prepared dilution of pharmaceutical formulation is injected and the procedure described under bulk samples was followed. The amount of drug present in sample was computed in calibration graph.Chromatographic conditions for estimation of Voglibose were described in
table 1.

RESULTS AND DISCUSSION

The objective of the present work is to develop simple, precise and reliable HPLCmethod for the analysis of Voglibose in bulk and pharmaceutical dosage form. This is achieved by using the most commonly employed column $C_{18}$with U.V. detection at 218snm. The representative chromatogram indicatingVoglibose is shown in fig. 1.

Parameter fixation

In developing this method, a systemic study of effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. The following studies were conducted for this purpose.

Stationary phase characteristics

Based onnature and solubility characteristics of Voglibose, reverse phase mode of HPLC was selected for chromatography. Among different RP-HPLC stationary phases tried C_{18} column was found to be optimum.

Mobile phase characteristics

In order to get sharp peak with base line separation from interfering peaks carried out a number of experiments by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol,waterand aceertonitrile with or without different buffers in different combinations were tested as mobile phase. A mixture of Methanol : ACN (75:25) (v/v) was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

VALIDATION OF THE PROPOSED METHOD

As an integral part of analytical method development is validation. The proposed method was validated as per ICH guidelines.

1. Linearity

It is the ability of the method to elict test results directly propotional to analyate concentration with in a given range[11].Linearity was performed by preparing standard solutions of Vogliboseat different concentration levels, twenty micro liters of each concentration was injected in into the HPLC system. The peak responses were read at 215nm and the corresponding chromatograms were recorded. Linearity plots of concentration over areas were constructed individually. Linearity results wereobtained in the concentration range of 60-200µg/ml .The results were presentedinTable.2.

2. Precision

Precision is the degree of repeatability of an analytical method under normal Operational conditions. Precision of the method was

performed as intraday precision, Inter day precision.

Intraday precision

To study the intraday precision, six replicate standard solutions (60ppm)ofVoglibose was injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.15 which are well within the acceptable criteria of not more than0.53.

Inter Day precision

To study the interday precision, six replicate standard solutions (60ppm) of Voglibosewas injected on third day of sample preparation. The percent relative standard deviation (% RSD) was calculated and it was found to be1.58which are well within the acceptable criteria of not more than 2.0.

3. Specificity

The effect of wide range of excipients and other additives usually present in the formulation of Voglibose in the determinations under optimum conditions were investigated infact, may have no observation at this UV Maximum. Chromatographic parameters maintained are specific for Voglibose.

4. Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC(LC2010 A HT), Aglient HPLC and water's Breeze HPLC By different operators using different columnsof similar type like Hypersil C_{18},PhenomenexyGemini C_{18} and Hichron$_{C18}$and didn't show any significant change.

5. Limit of Detection and Limit of Quantification

A Calibration curve was prepared using concentrations in the range of 20-140 µg/ml (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined and kept in following equation for the determination of Detection limit and Quantitation limit.

The results were reported in table-3.

6. Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed Standard solution. The standard addition method was performed at 50%, 100% and 150% level of40ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table.4.Satisfactory recoveries ranging from 98% to 102% were obtained by the proposed method. This indicates that the proposed method was accurate.

7. Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demon started that the RP-HPLC method developed is robust. The results of robustness were given in table no.5.

Table 1: Optimized chromatographic conditions for estimation of Voglibose

PARAMETER	CONDITION
Mobile phase	Methanol : ACN (75:25) (v/v/)
Pump mode	Isocratic
Ph	4.2
Diluents	Mobile phase
Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	215nm
Injection Volume	20 µl
Flow rate	1.0ml/min
Run time	10 minutes
Retention Time	6.84minits

Table 2: Linearity results of Voglibose

S.NO	Concentration in µg/ml	Area
1	20	180023
2	40	312633
3	60	448575
4	80	568775
5	100	696114
6	120	813811
7	140	953340

Table 3: Limit of Detection and Limit of Quantification for Voglibose

Parameter	Values
Limit of Quantification	0.65µg/ml
Limit of Detection	0.2 µg/ml

Table 4: Recovery Results

% of Recovery	Voglibose				
	Target Conc., (µg/ml)	Spiked conc., (µg/ml)	Final Conc., (µg/ml)	Conc., Obtained	% of Assay
50%	40	20	60	60.27	100.46
	40	20	60	59.5	99.32
	40	20	60	61.1	101.95
100%	40	40	80	80.61	100.77
	40	40	80	79.07	98.84
	40	40	80	81.30	101.63
150%	40	60	100	99.99	99.99
	40	60	100	100.11	100.11
	40	60	100	98.6	98.69

Table 5: Robustness

S.No	Change	Area found	% of Change
1	Standard	448575	.....
2	Mp-1(70:30)	457415	1.9
	Mp-2 (80:20)	443894	1.0
3	WI-1 (210nm)	452183	0.80
	WI-2 (220nm)	456456	1.72
4	Ph-1 (4.0)	442933	1.25
	Ph-2 (4.1)	451724	0.70

Table 6: Formulation

S.NO	Brand name	Available form	Label claim	Concentration	Amount found	% Assay
1	VOLIX	Tablet	0.3mg	60µg/ml	59.49µg/ml	99.15%

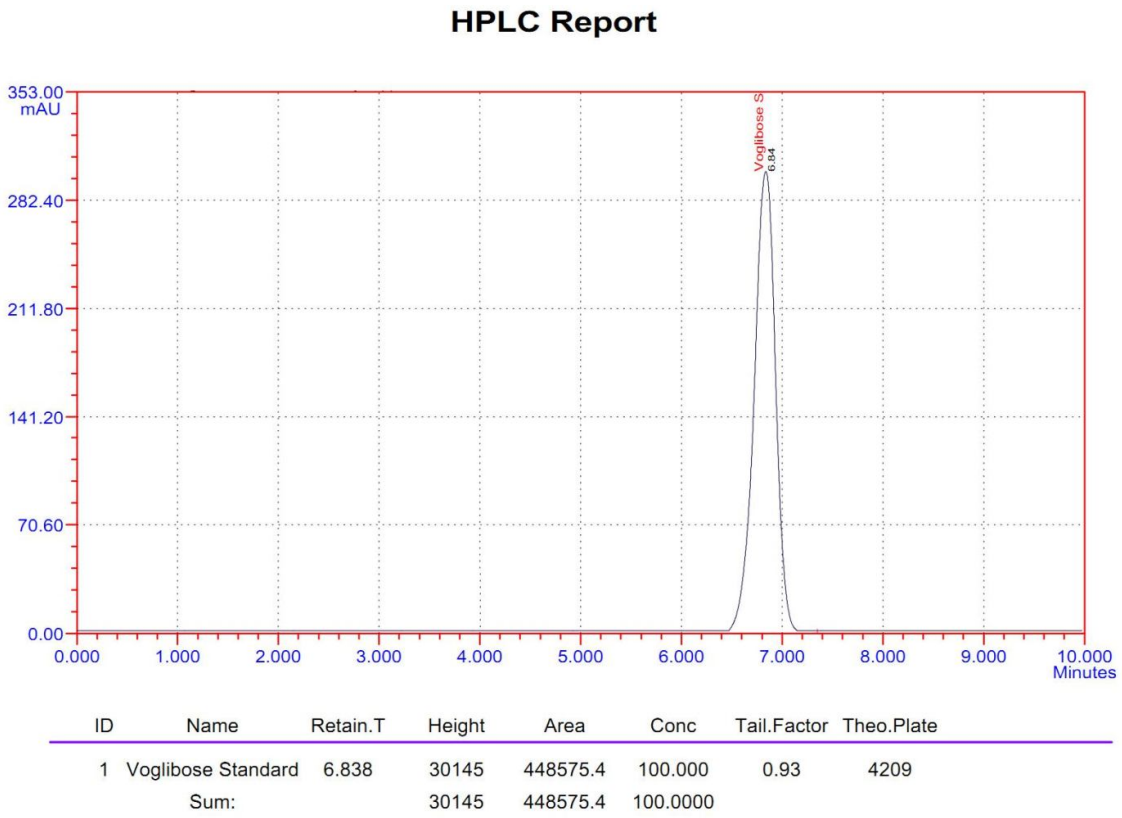

Fig. 1: chromatogram of Voglibose

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