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Determination of voglibose in pharmaceutical formulations by high performance liquid chromatography using refractive index detection

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ABSTRACT

Voglibose is a potent glucosidase inhibitor used for type II diabetes mellitus. A simple and rapid high performance liquid chromatographic method with refractive index detection was developed for the determination of voglibose in pharmaceutical formulations. Development was performed on a C18 (250 x 4.6 mm, 5 µ) column using a mobile phase mixture of acetonitrile and water in the ratio of 50:50 which was fixed at a flow rate of 0.5 mL/min. Polarity of voglibose was found to be positive and elution time was found to be less than 5 min. The method was also validated as per ICH guidelines for its linearity, precision, accuracy and robustness. The limit of detection and quantification (LOD and LOQ) were found to be 2.91 and 9.7 µg/mL. The method could be successfully applied for the quantification of voglibose in pharmaceutical formulations.

1. Introduction

Voglibose (VGB) (Figure 1) a new potent glucosidase inhibitor used for type II diabetes has shown strong anti-obesity and anti-diabetic activity. It is chemically 3,4-dideoxy-4-[[2-hydroxy-1-(hydroxymethyl)ethyl]amino]-2-C-(hydroxyl methyl)-d-epiinositol. It was found that it delays the glucose absorption in the gastrointestinal tract of humans. Thus, reduces the post-prandial blood glucose peaks [1–3]. Voglibose has a structural relation with natural carbohydrates [4,5] and are present without a chromophore and/or fluorophore groups and hence does not show any UV absorbance. The analysis of carbohydrates has an important role in the pharmaceutical and food industry and their analysis by liquid chromatography (LC) often requires derivatization procedures [6]. Quantitative determination of voglibose in pharmaceutical tablets using high-performance liquid chromatography–fluorescence detection with post-column derivatization and mass spectrometric detection has been reported [7].

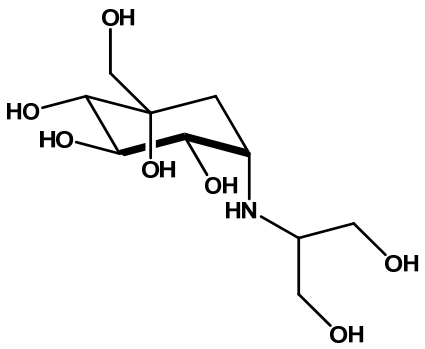

Figure 1. Structure of voglibose.

The purpose of this study was to develop and validate LC–RI method for the analysis of voglibose in pharmaceutical formulations. To our knowledge of literature no LC method with refractive index detection has been published for the analysis of voglibose and could be successfully applied to the analysis of voglibose in commercially available tablets.

2. Experimental

2.1. Reagents and chemicals

Voglibose was obtained from Ranbaxy Research Laboratories, India. Acetonitrile and methanol (HPLC grade) purchased from Rankem Ltd, New Delhi, India. Aqueous solutions were prepared by using Milli Q (Millipore, USA) grade water and all the other reagents used were of analytical reagent grade.

2.2. Chromatography

The HPLC system consisted of Shimadzu CBM-20A Prominence communication bus module with DGU-20A$_5$ prominence degasser and SIL-10Advp auto injector connected to a RID-10A refractive index detector. The data were acquired and processed with LC solution version 1.22 SP1 software. The analytical column was a Waters C$_{18,}$ with 4.6 x 250 mm, 5 µm particle size. The isocratic mobile phase consisted of acetonitrile and water mixture (50:50, v:v) was run at a flow rate of 0.5 mL/min. The cell temperature was maintained at 40 ^{o}C.

2.3. Standard solutions

Stock standard solution of voglibose was prepared by taking 25 mg of the drug in a 25 mL volumetric flask to final

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concentration of 1 mg/mL, and working standard solution of 100 µg/mL was prepared by diluting 1 mL of the stock standard solution with the mobile phase to 10 mL.

2.4. Preparation of the tablet sample

Twenty tablets were finely powdered and quantity equivalent to one tablet was accurately weighed and extracted with 100 mL of the mobile phase. It was sonicated for 30 min with vortex mixing at 10 min intervals to avoid aggregation of the powdered samples. After centrifugation (2000 × g for 10 min), 10 mL of supernatant was collected and diluted in a 50 mL volumetric flask with the mobile phase in order to obtain a final concentrations of 20 and 30 µg/mL. These solutions were then filtered through a 0.22 µm filter and injected into HPLC system.

2.5. Validation

The validation of an analytical method verifies that the characteristics of the method satisfy the requirements of the application domain [8]. The proposed method was validated in the light of ICH Guidelines [9,10] for linearity, intra- and inter-day precision, LOD, LOQ, selectivity and specificity, stability and recovery. Consequently, the following were performed.

2.5.1. Linearity and calibration curve

Calibration curve of peak area as a function of voglibose was constructed over a concentration range of 10-100 µg/mL. For each standard curve, six different concentrations were used. The calibration plot was generated by replicate analysis (n = 6) at all concentration levels and the linear relationship was evaluated using the least square method within Microsoft Excel® program.

2.5.2. Precision and accuracy

Both repeatability (within a day precision) and reproducibility (between days precision) were determined as follows. Solutions containing lowest, intermediate, and highest concentrations of the calibration curve, i.e. 10, 50, 100 µg/mL were prepared. Six injections at each of the specified concentration levels were injected within the same day for repeatability, and over a period of 3 days (6-injections/day) for reproducibility. Mean and relative standard deviation were calculated and used to judge accuracy and precision of the method. Both intra-day and inter-day samples were calibrated with standard curves concurrently prepared on the day of analysis. Accuracy was determined as %bias of VGB amount found.

2.5.3. Limit of detection and limit of quantification

Signal-to-noise ratio was used for the estimation of limit of detection and quantification. The limit of detection (LOD) was defined as the lowest concentration resulting in peak area of three times the baseline noise. The limit if quantification (LOQ) was defined as the lowest concentration resulting in peak area with signal-to-noise ratio higher than 10 [11], with precision (The relative standard deviation, % R.S.D.) and accuracy (% bias) within ±10%.

2.5.4. Specificity and selectivity

The specificity of the method was established through study of resolution factor of the drug peak from the nearest resolving peak, and also among all other peaks.

2.5.5. Stability

Drug solutions prepared with the known amount of voglibose to achieve the concentration of 10, 50, and 100 µg/mL (n = 3) and determined for short-term stability at room temperature for 24 h, long-term stability at -4 °C and checked for up to 1 month by comparing the results with fresh stock prepared on the day of analysis. Further, the freeze–thaw (-20 °C/room temperature) stability of the voglibose samples were determined for three cycles. Samples were considered to be stable, if the assay values were within the acceptable limits of accuracy and precision.

2.6. Recovery of voglibose from formulations

The recovery of an analyte is the extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method. Mobile phase was most efficient in extracting VGB from formulation and had a small variation in extraction recoveries over the concentration range. Samples were prepared in triplicate at three concentrations 10, 50, and 100 µg/mL of VGB and assayed as described above. The extraction efficiency of voglibose was determined by comparing the peak areas measured after analysis of samples from formulation with those found after direct injection of unextracted (pure) samples into the chromatographic system at the same concentration levels.

3. Results and discussion

3.1. Optimization of chromatographic method

Figure 2a 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 (1.5) resulting good peak symmetry and resolution. The mobile phase optimized contained water and acetonitrile (50: 50) at 0.5 mL/min flow rate. The retention time was found to be 3.264 min for VGB. There were no interferences at drug retention time.

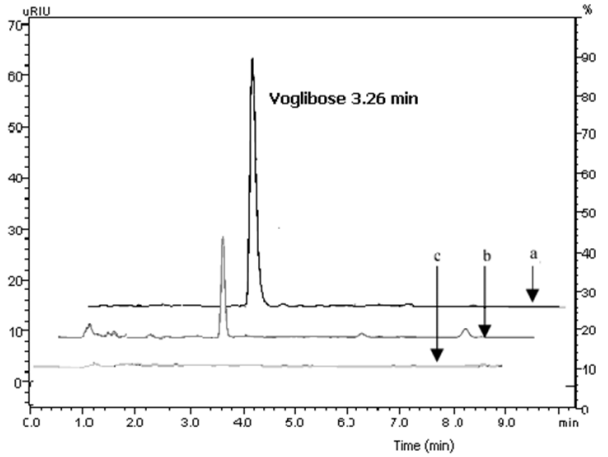

Figure 2. Chromatogram showing (a) voglibose (80 µg/mL) in pure form (b) voglibose (30 µg/mL) in formulations) and (c) blank run devoid of sample.

Voglibose was determined using high performance liquid chromatography with refractive index detector and found to be well suited technique for the analysis of non-UV absorbing compound without derivatization. RI indicator increased the sensitivity of detection.

3.2. Validation of the proposed method

3.2.1. Linearity and Calibration curve

Calibration curve (peak area) was constructed by spiking six different concentrations of VGB. The chromatographic responses were found to be linear over an analytical range of 10-100 µg/mL and found to be quite satisfactory and reproducible with time. The linear regression equation was calculated by the least squares method using Microsoft Excel® program. The correlation coefficient equals 0.9994, indicating a strong linear relationship between the variables. The variance of response variable S^2_{Yx} calculated was 1.86, indicates low variability between the estimated and calculated values. This further confirms negligible scattering of the experimental data points around the line of regression and good sensitivity of the proposed method.

3.2.2. Precision and accuracy

Inter-day as well as intra-day replicates of VGB, gave an R.S.D. below 2.12 revealed that the proposed method is highly precise. Accuracy data (%bias) in the present study ranged from -1.86 to +0.11 and indicated no interference from formulation excipients. Accuracy and precision calculated during the intra- and inter-day run are given in Table 1.

Table 1. Precision and accuracy data for voglibose obtained by the developed method*.

Day	Precision and accuracy data	Nominal concentrations (µg/mL)		
		10	50	100
Day 1	Mean	9.92	50.02	99.25
	S.D.	0.14	1.01	2.11
	% R.S.D	1.41	2.02	2.12
	% Bias	-0.8	+0.04	-0.75
Day 2	Mean	9.89	49.41	100.11
	S.D.	0.13	1.04	1.97
	% R.S.D	1.31	2.10	1.96
	% Bias	-1.1	-1.18	+0.11
Day 3	Mean	9.86	49.07	99.11
	S.D.	0.14	1.04	2.07
	% R.S.D	1.42	2.12	2.09
	% Bias	-1.4	-1.86	-0.89

* Each mean value is the result of triplicate analysis;
% R.S.D = (S.D./Mean) x 100,
% Bias = [(Measured value-True value)/True value] x 100.

3.2.3. LOD and LOQ

The LOD and LOQ were found to be 2.91 and 9.70 µg/mL, respectively. When this method is applied to formulation samples, its sensitivity was found to be adequate for assay of the formulations.

3.2.4. Specificity and selectivity

Any potential interference (overlapping peaks) due to formulation excipients were within 2min only (Figure 2b), later on there was no significant interference from blank (Figure 2c) that affected the response of voglibose.

3.2.5. Stability

The study indicated that samples were stable for 24 h (short-term) at room temperature and samples stored at -4 °C, were injected over a period of 1 month did not suffer any appreciable changes in assay value and meet the criterion mentioned above and the solutions were found to be stable even after three freeze–thaw cycles and the results were found to be with the range of 90-110%.

3.2.6. System suitability

System suitability tests, an integral part of a chromatographic analysis are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis. A system suitability test according to USP was performed on the chromatograms obtained from standard and test solutions to check different above mentioned parameters and the results obtained from six replicate injections of the standard solution are summarized in the Table 2.

Table 2. System suitability parameters.

Parameter	Obtained value*
Retention time, R_t (min)	3.264
Capacity factor (k')	4.834
Theoretical plates (USP)	7928
Tailing factor (T_f)	1.15

* Average of six determination.

3.3. Recovery of voglibose from formulations

Extraction efficiency was performed to verify the effectiveness of the extraction step and the accuracy of the proposed method. The extraction efficiency of VGB from formulation samples was satisfactorily ranged from 98.35 to 100.62 % (R.S.D. was less than 1.55) at all three concentration levels, which confirm no interference effects due to formulation excipients (Table 3).

Table 3. Results of voglibose recovery from formulations.

Formulation	Label claim (mg)	Amount recovered (mg)	Percent recovery	% R.S.D.
Volix (Ranbaxy)	0.3	0.297	99.0	1.37
Volix (Ranbaxy)	0.2	0.199	99.5	1.71
Voglitor (Torrent)	0.3	0.295	98.3	2.06
Vocarb (Glenmark)	0.2	0.201	100.5	2.11

4. Conclusion

In the present work, a new rapid and simple reverse phase high performance liquid chromatographic method has been developed, optimized and validated for the estimation of voglibose in pharmaceutical formulations using refractive index detector and isocratic elution. The short peak retention time of 3.264 min cuts down on overall time of sample analysis and the method was more cost effective as it utilizes very less quantity of mobile phase. Linearity was found in a range of 10-100 µg/mL with LOD and LOQ of 2.91 and 9.70 µg/mL, respectively. The results of t-test applied to accuracy and precision data enabled the conclusion that an excellent accuracy and high precision was achieved. From the extraction efficiency data, the recovery of the active component was found to be quantitative. Selectivity of the method was demonstrated by the absence of any interfering peaks from other coexisting excipient substances at the retention time of the drug. Hence this method can be easily and conveniently used for the routine analysis of the drug in pharmaceutical formulations.

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