Iranian Journal of Pharmaceutical Sciences
2022: 18 (4): 308- 315
www.ijps.ir

Original Article

Development and Validation of High Performance Thin Layer Chromatographic Method for the Estimation of Voglibose in Bulk and Tablet Dosage Forms

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Abstract

A simple, fast, specific, precise, and accurate High-Performance Thin Layer Chromatographic method (HPTLC) was developed and validated for the estimation of Voglibose in bulk and pharmaceutical formulations. The chromatographic separation was carried out on precoated silica gel 60F254 aluminum plates using a mixture of acetonitrile: methanol: ammonia (15:4:0.1 % V/V/V) as mobile phase and densitometric evaluation of spots was carried out at 284 nm using Camag TLC scanner III with CATS 1.3.4 version software. The experimental parameters like band size of the spot application, chamber saturation time, solvent front migration, slit width, etc. were critically studied and optimum conditions were evolved. The drug was satisfactorily resolved with an Rf value of 0.66±0.03. The accuracy and repeatability of the proposed method were ascertained by evaluating various validation parameters like linearity (100 to 450 ng/spot), precision (intra-day % RSD 0.17 to 0.69, inter-day % RSD 0.20 to 0.29), accuracy (96.24±0.20), and specificity according to ICH guidelines. The limits of detection and quantification were 40ng/spot and 100 ng/spot respectively. HPTLC method provides a faster and cost-effective quantitative control for routine analysis of Voglibose in its formulation.

Keywords: Voglibose; HPTLC; Densitometric estimation; Method development; Validation.

1. Introduction

Voglibose (**Fig.1**), 3,4-Dideoxy-4-[2-hydroxy-1-(hydroxymethyl)ethyl]amino-2-c-(hydroxymethyl)-D-epiinositol, has attracted considerable interest 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

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Cite this article as: Mallikharjuna Rao N, Ravikumar K., Development and Validation of High Performance Thin Layer Chromatographic Method for The Estimation of Voglibose in Bulk and Tablet Dosage Forms, Iran. J. Pharm. Sci., 2022, 18 (4): 308- 315

delays glucose absorption and thus, reduces the post-prandial blood glucose peaks [1-3]. Voglibose obtained from organic synthesis processes is similar to structurally related carbohydrates found naturally [4, 5] and has the empirical formula $C_{10}H_{21}NO_7$.

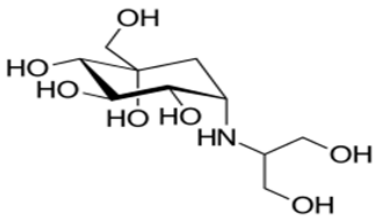

Figure 1. Structure of Voglibose

Since most carbohydrates lack chromophore and/or fluorophore groups, their analysis by liquid chromatography (LC) often requires derivatization procedures [6]. Since Voglibose only absorbs UV in the low-wavelength region, it cannot be directly detected with high sensitivity [7]. Therefore, the derivatization method with taurine and sodium periodate was preferred. A literature survey revealed that no HPTLC method was developed for the estimation of Voglibose in tablet formulation. It was felt that a reliable and rapid method for the estimation of Voglibose was needed. Hence an attempt was made to develop and validate an HPTLC method for the rapid determination of the drug. This study was designed to develop a simple, rapid, precise, accurate, economical, and reproducible method for the determination of Voglibose by HPTLC in bulk and tablet dosage forms and to validate it as per ICH guidelines.

2. Materials and Methods

Voglibose as raw material was a gift sample from Ranbaxy research laboratories, Gurgaon. All other chemicals and reagents used were of analytical grade and purchased from Merck Chemicals Corporation Ltd. Mumbai, India. Deionized and ultra-pure water was obtained from Milli – Q system (Millipore). Silica gel $60F_{254}$ TLC plates (20×10 cm & 10×10 cm, layer thickness 0.2 mm, Merck, Germany) were used as stationary phase.

2.1. Equipment

The instrument used in the present study was the Camag HPTLC system comprising Camag Linomat V automatic sample applicator, Hamilton syringe (100 µL), and Camag TLC scanner III with Wincats software. The HPTLC system was equipped with a Linomat V auto sprayer connected to a nitrogen cylinder, a twin trough glass chamber (10×10 cm), saturated with filter paper for ten minutes.

2.2. HPTLC method and chromatographic conditions

2.2.1. Preparation of standard stock solution:

Voglibose (10 mg) was weighed accurately and transferred into a 100 mL volumetric flask and dissolved in methanol and the volume was made up to the mark with methanol to obtain a standard stock solution of 100µg/mL. It was derivatized using taurine and sodium periodate.

2.3. Prewashing of plates

HPTLC was performed on 10×10 cm precoated silica gel $60F_{254}$ precoated plates from E. Merck. The adsorbent has a very large surface area; it may absorb air and other impurities from the atmosphere, particularly volatile impurities after the pack has been opened. The non-volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid

possible interference from such impurities in quantitative analysis, plates were prewashed with methanol, dried, and activated for 30 minutes at 110 °C with the plates being placed between two sheets of glasses to prevent deformation of the aluminum during heating.

2.4. Sample application

The standard and formulation samples of Voglibose were spotted on precoated TLC plates in the form of narrow bands of lengths 6mm, with 10mm from the bottom and left margin and with a 9 mm distance between the two bands. Samples were applied under a continuous drying stream of nitrogen gas at a constant application rate of 150 nLs^{-1}.

2.5. Mobile phase and migration

Various solvent systems like a mixture of a) methanol: ethanol: water, b) Ammonia: formic acid, c) methanol: ammonia: acetonitrile: water, d) acetonitrile: chloroform: ammonia, e) methanol: chloroform: ammonia in different compositions were tried to separate and resolve the spot of Voglibose from its excipients of the formulation. The mixture of acetonitrile: methanol: ammonia (15:4:0.1% V/V/V) could resolve Voglibose with a better peak shape (**Fig. 2**). The drug was satisfactorily resolved with R_f value 0.66±0.03. Pre-saturation of the TLC chamber with mobile phase for 30 minutes assured better reproducibility in the migration of Voglibose with better resolution.

2.5.1. Method Validation

The developed HPTLC method was validated for Linearity, Accuracy, Precision, Limit of Detection, Limit of Quantification, Repeatability, Specificity, and Robustness by ICH Q2 (R1) guideline [8-10].

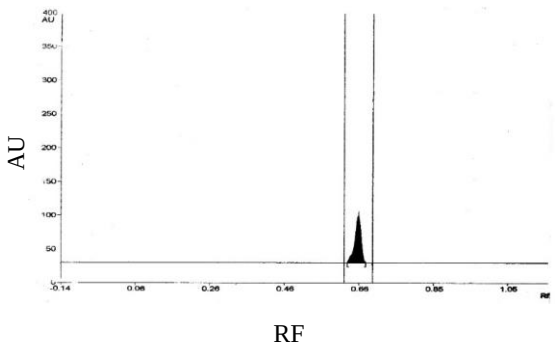

Figure 2. Chromatogram of Voglibose Formulation (200 ng/spot)

2.6. Linearity

The linearity of the method was evaluated by constructing a calibration curve at eight concentration levels. Aliquots of Voglibose working standard solution were applied on the plate to obtain a concentration in the range of 100 to 450 ng/spot. The calibration curve was plotted by plotting peak areas versus the corresponding concentrations with the help of Win-CATS software. Chromatogram was developed in a twin trough glass chamber; using 20 minutes' chamber saturation time. The length of the chromatogram run was 80 mm. The developed plates were air-dried. Scanning was performed in UV mode at 284 nm. The slit dimension was kept at 6×0.45 mm at a scanning speed of 100 nm/s. The completion of scanning peak areas was then noted. The calibration curve was plotted with peak areas against corresponding concentrations and evaluated using linear regression analysis.

2.7. Precision

To evaluate intra-day precision, samples at three different concentrations (2 μL, 4 μL, and

6 µL) were analyzed in triplicate on the same day. The inter-day precision was studied by comparing assays performed on three different days. The precision of an analytical method expresses the degree of scatter between a series of measurements obtained from multiple samples of the same homogeneous sample under prescribed conditions.

2.8. Repeatability

The repeatability of measurement of peak area was determined by spotting 4 µL of standard solution on the TLC plate. After development, the plate with separated spots of Voglibose was scanned six times without changing the position of the plate. The repeatability of sample application was determined by spotting 4 µL of standard drug solution six times on a TLC plate by an automatic spotter. The % RSD was calculated from the obtained peak areas.

2.9. Accuracy

Recovery of the Voglibose was carried out for determining the accuracy parameter. A mixed known quantity of Voglibose standard with the analyzed sample formulation and the contents were reanalyzed by the proposed method. Recovery studies were carried out at 50 %, 100 %, and 150 % levels. The percentage recovery and its %RSD were calculated.

2.10. Limit of Detection and Limit of Quantification

The detection limit (LOD) is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. LOD was calculated using the following formula

LOD = 3.3× Standard Deviation of the Y-intercept / The slope of the calibration curve

The quantification limit (LOQ) is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOQ was calculated using the following formula,

LOQ = 10 × Standard Deviation of the Y-intercept / The slope of the calibration curve

2.11. Specificity

To confirm the specificity of the developed method, Voglibose was spotted on the TLC plate and scanned as described earlier. The UV spectrum of the standard was compared with the spectrum of the formulation. The peak purity of Voglibose was assessed by comparing their respective spectra at the peak start, peak apex, and peak end positions of the spot.

2.12. Robustness

The parameters selected for the robustness study were mobile phase composition, chamber saturation time, and solvent migration distance their effects on response were observed.

2.13. Stability studies

When the developed chromatographic plate was exposed to the atmosphere, the analyte was likely to decompose. Hence it was necessary to conduct stability studies. The stability of the analyte on the plate was studied at different time intervals and peak areas were compared against the peak area of the freshly scanned plate.

3. Results and Discussion

3.1. Linearity

A representative calibration curve was plotted for peak areas versus corresponding concentration over the range of 100 to 450 ng/spot. The slope, intercept, and correlation coefficient values were found to be 3.339, 112.275, and 0.9996 respectively (**Table 1**). It showed that there is a good correlation between the regression coefficient and the concentration of the drug (**Fig. 3**).

Table 1: Linearity and range.

Parameter	Results
Range (ng/spot)	100-450
Regression coefficient (r^2)	0.9996
Slope	3.339
Intercept	112.275

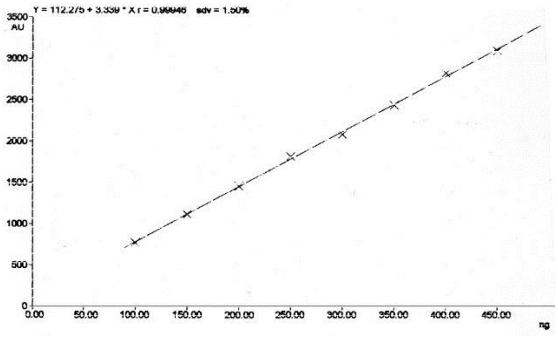

Figure 3. Calibration graph of Voglibose (100-450 ng/spot).

3.2. Precision

The % RSD for intra-day and inter-day was found in the range of 0.17 to 0.69 % and 0.20 to 0.29 %, respectively. The lower % RSD values of intra-day and inter-day variation in the analysis indicated that the developed method was precise (**Tables 2 & 3**).

Table 2: Intra-day precision.

Volume applied (µL)	Peak Area	% RSD
2	1267.6 1257.1 1271.4	0.5853
4	1615.6 1613.2 1610.1	0.1709
6	1573.8 1565.4 1552.3	0.6928

Table 3: Inter-day precision.

Volume applied (µL)	Peak Area	% RSD
2	1267.6 1265.2 1270.3	0.2012
4	1615.6 1608.9 1618.1	0.2947
6	1573.8 1570.9 1578.3	0.2368

3.4. Repeatability

The % RSD for measurement of peak areas was found to be 0.31 %. In the repeatability of the sample application, the % RSD for the peak area values was calculated and found to be 0.34 %. The % RSD values for measurement of peak area and sample application were found below the instrumental specifications (i.e.1 %); proving the proper functioning of the HPTLC system (**Tables 4 & 5**).

Table 4: Repeatability of the sample application.

Volume applied (µL)	Peak Area	% RSD
4	1615.6 1605.5 1620.2 1618.0 1612.2 1609.1	0.3446

Table 5: Repeatability of measurement.

Volume applied (µL)	Peak Area	% RSD
4	1615.6 1612.9 1608.3 1605.2 1614.2 1618.1	0.3117

3.5. Accuracy

The % recovery of Voglibose was found to be 101.5, 100.4, and 99.6 (at 50, 100 & 150 % levels, respectively). The results proved that the proposed method was accurate for the estimation of drugs in tablet dosage form (**Table 6**).

Table 6: Accuracy of Voglibose.

Level (%)	% Recovery*	% RSD*
50	101.5	0.1352
100	100.4	0.1163
150	99.6	0.1254

*n=3, number of times the procedure repeated

3.6. LOD and *LOQ*

The limit of detection was found to be 40 ng/spot (**Fig. 4**), while the limit of quantification was found to be 100 ng/spot (**Fig. 5**), respectively, thereby indicating the developed method's sensitivity.

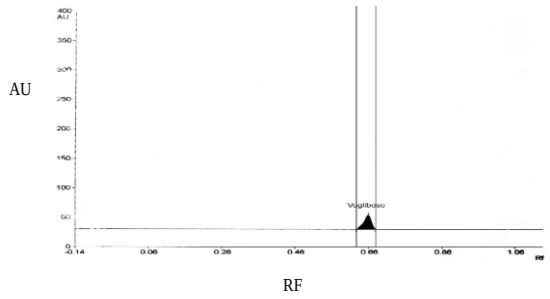

Figure 4. LOD of Voglibose (40 ng/spot).

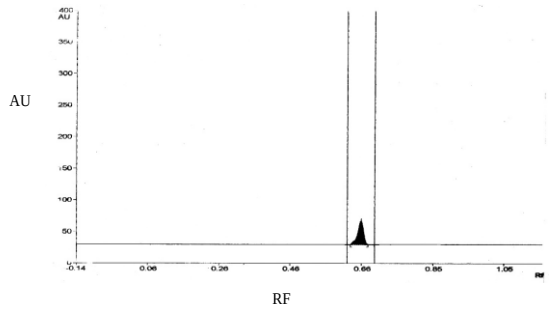

Figure 5. LOQ of Voglibose (100 ng /spot).

3.7. Analysis of formulation

Analysis of formulation was performed using Voglibose 0.2 mg tablets (Ranbaxy) and the content of the drug was calculated. The % assay was found to be 98.24 (**Table 7**).

Table 7: Results for Analysis of Formulation.

Drug	Amount (mg/ tablet) (n=3)*		% Assay	%RSD
	Labeled	Found		
Voglibose	0.2	0.193	98.24	0.2263

*n= no of times procedure repeated

3.8. Specificity

Good correlation of spectra acquired at the start (s), apex (m), and end (e) of the peaks indicates the peak purity of Voglibose [correlation r (s, m) = 0.99910, r (m, e) = 0.99920]. Hence, it can be concluded that no impurities or degradation products migrate with the peaks obtained from

standard solutions of the drug (**Fig. 6**). It was observed that excipients present in the formulation have no significant interference with the analyte (R_f, 0.66±0.03). The UV spectrum of Voglibose extracted from the tablet, showed a good correlation (between what?). The proposed HPTLC method was found to be specific.

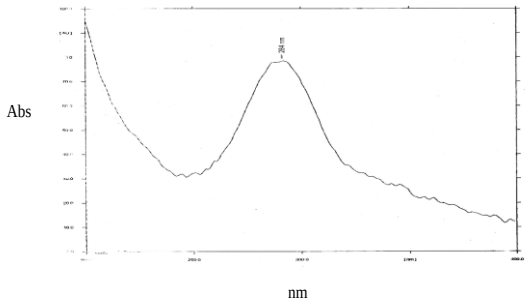

Figure 6. UV spectrum of standard Voglibose on TLC plate.

3.9. Robustness

Robustness tests examine the effect of the operational parameters on the analysis results. It was determined by introducing small changes in mobile phase composition, chamber saturation time, and solvent migration distance. The % RSD was found below 2 %. The results indicated that the method was robust.

3.9.1. Stability studies

The developed plate was found to be stable for up to 17 hours at room temperature and 24 hours at refrigerator conditions. The observed results were within the specified limits (**Table 8**).

Table 8: Stability of the analyte on the plate.

Concentration (µg/mL)	The peak area of the Drug (scale?)			
	Time hrs.	At room temperature	Time hrs.	Refrigeration
50	0	1615.6	0	1615.6
	3	1567.13	5	1534.82
	5	1534.82	8	1486.35
	7	1502.50	11	1437.88
	11	1437.88	14	1357.10
	14	1389.41	19	1324.79
	17	1340.94	24	1276.32

4. Conclusion

The developed HPTLC technique for the determination of Voglibose is simple, precise, specific, accurate, selective, sensitive robust, and reproducible. The amounts found in formulations well agreed with the labeled claim. Statistical analysis proved that the method is suitable for the analysis of Voglibose as a bulk drug and as a formulation without interference from its excipients. Thus, the reported method is of considerable importance and has great industrial applicability for quality control and analysis of Voglibose from bulk drug and formulation. It may be extended for the quantitative estimation of the said drug in plasma and other biological fluids.

Acknowledgments

The authors are thankful to Dr. Mahesh D. Burande, Bilcare Research Academy, and Mr. Vinod Arora, Vice President, R&D, Ranbaxy for providing the gift sample of voglibose. I would also extend my thanks to Mr. Nagaraj, Medical Representative, Ranbaxy, Coimbatore for supplying the formulation on time.

Conflict of interest

The authors declare to have no conflict of interest.

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