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Development and validation of high performance thin layer chromatographic method for the determination of voglibose in bulk and their formulations

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ABSTRACT

To develop a simple, fast, specific, precise and accurate High Performance Thin Layer Chromatographic method (HPTLC) for the determination of Voglibose in bulk and their dosage forms. The chromatographic separation was achieved on precoated silica gel $60F_{254}$ aluminum plates using a combination 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 chamber saturation time, spot application, slit width, solvent front migration, etc. were studied critically and evolved optimized conditions. The drug was well resolved satisfactorily with R_f value 0.66±0.03. The repeatability and accuracy of the optimized method were ascertained by evaluating various validation parameters like linearity (100 to 450 ng/spot), precision (intra-day % RSD 0.21 to 0.74, inter-day % RSD 0.21 to 0.29), accuracy (99.8% to 101.2%, % RSD below 1%), and specificity according to ICH guidelines. The limits of detection and quantification were 40 ng/spot and 100 ng/spot respectively.

The developed HPTLC method was faster and cost effective quantitative control for routine analysis of Voglibose in bulk and its formulations.

Keywords: *Voglibose; HPTLC; Densitometry estimation; Method development; Validation; Formulations.*

1. INTRODUCTION

Voglibose(Fig.1),3,4-Dideoxy-4-[2-hydroxy-1-(hydroxylmethyl)ethyl]amino-2-c-(hydroxymethyl)-D-epiinositol, has attracted considerable interest due to its broader range of pharmacological and therapeutic properties, including its inhibitory activity against α-glucosidase and hyperglycemia as well as various disorders caused by hyperglycemia. Voglibose, a new potent glucosidase inhibitor used to cure type 2 diabetes, has shown strong anti-obesity and anti-diabetic activity. As a glucosidase inhibitor, the compound was highly active within the gastrointestinal tract of humans. The drug delays glucose absorption and thus, reduces the post-prandial blood glucose peaks [1-3]. Voglibose is similar to structurally related carbohydrates found naturally [4-5] and has the empirical formula $C_{10}H_{21}NO_7$.

From the literature survey, it was evident that several methods like HPLC, [6-8] Spectroflurometry method [9] and UV spectrophotometric method [10] were reported for estimation of Voglibose. A literature survey revealed that one HPTLC method was reported for the estimation of Voglibose in bulk and its tablet formulations [11]. 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 a HPTLC method for the rapid determination of the drug. This study was designed to develop a simple, rapid, precise, accurate, economical and reproducible for the determination of Voglibose by HPTLC in bulk and tablet dosage forms and to validate as per ICH guidelines.

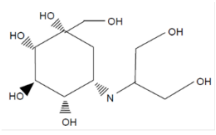

Figure 1. Chemical structure of Voglibose.

2. MATERIALS AND METHODS

Voglibose pure drug was obtained as a gift sample from Dr.Reddys research laboratories, Hyderabad. All other chemicals and reagents used were of analytical grade and purchased from Merck Chemicals Corporation ltd. Mumbai, India. Ultra-pure and deionized 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. Instrumentation.

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

3. RESULTS

3.1. Methodology.

3.1.1. Statistical analysis.

The analytical parameters of the developed HPTLC method were validated to ensure the suitability of the analytical requirements and reliability of the test results. The statistical one way variance analysis treatment was performed with the statistical software GraphPad InStat.

3.2. Preparation of standard stock solution.

Accurately weighed and transferred 10 mg of Voglibose into a 100 mL volumetric flask and dissolved in and diluted the volume up to the mark with methanol to obtain a standard stock solution of 100 µg/mL.

3.3. Prewashing of plates.

HPTLC was performed on 10×10 cm precoated silica gel $60F_{254}$ precoated plates from E.Merck. Due to a very large surface area of the adsorbent it may absorb air particularly volatile impurities and other impurities from the atmosphere, 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 these impurities in quantitative analysis, plates were prewashed with methanol, dried and activated at 110 °C for 30 minutes with the plates being placed between two sheets of glass to prevent deformation of the aluminum during heating.

3.4. 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 maintained 9 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at a constant application rate of 150 nLs^{-1}.

3.5. Mobile phase and migration.

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

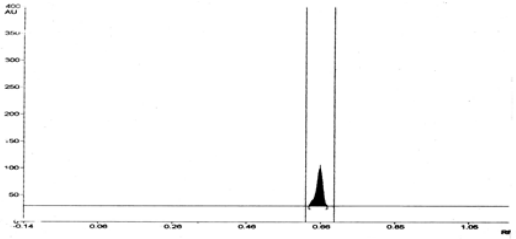

Figure 2. Chromatogram of Voglibose Formulation (200 ng/spot).

3.6. Method validation.

The developed HPTLC method was validated for Specificity, Linearity, Accuracy, Precision, Limit of Detection, Limit of Quantification, Repeatability, and Robustness in accordance with ICH Q2 (R1) guideline [12-13].

3.6.1. Specificity.

Specificity is the ability of the method to detect the analyte of interest in the presence of the other components. Voglibose was spotted on TLC plate and scanned. UV spectrum of the standard was compared with spectrum of the formulation. The peak purity of Voglibose was assessed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot.

3.6.2. Linearity.

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

3.6.3. 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.

3.6.4. Repeatability.

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

3.6.5. Accuracy/Recovery.

Recovery provides an indication of the error. It was carried out for determining accuracy parameter. Mixed known quantity of Voglibose standard with the analyzed sample formulation and the contents were reanalyzed by the proposed method. Recovery studies carried out at three different levels i.e. 50 %, 100 % and 150 % levels. The % recovery and its %RSD were calculated.

3.6.6. Limit of Detection and Limit of Quantification.

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

$$LOD = \frac{3.3\times \text{Standard Deviation of the Y- intercept}}{\text{Slope of the calibration curve}}$$

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,

$$LOQ = \frac{10\times \text{Standard Deviation of the Y- intercept}}{\text{Slope of the calibration curve}}$$

3.6.7. Robustness.

The parameters selected for the robustness study were mobile phase composition, solvent migration distance and chamber

saturation time were made and their effect on response was observed.

3.6.8. Stability studies.

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

3.7. Discussion.

3.7.1. Specificity.

Good correlation of spectra acquired at 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 was proved that no impurities or degradation products migrated with the peaks obtained from standard solutions of the drug (Figure 3). It was observed that there was no significant interference of the excipients present in formulation with the analyte (R_f , 0.66±0.03). The UV spectrum of Voglibose extracted from formulation, which showed a good correlation. The developed HPTLC method was found to be specific.

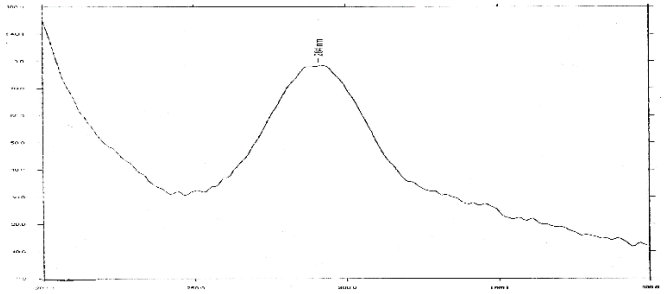

Figure 3. UV spectrum of standard Voglibose on TLC plate.

3.7.2. Linearity.

A representative calibration curve was plotted between peak areas versus corresponding concentration over the range of 100 to 450 ng/spot. The slope, intercept and correlation co-efficient values were found to be 3.338, 112.276 and 0.9995 respectively (Table 1). It showed that a good correlation between regression coefficient and concentration of the drug (Figure 4).

Linearity and range	Voglibose
Range (ng/spot)	100-450
Regression coefficient (r^2)	0.9995
Slope	3.338
Intercept	112.276

Table 1. Linearity values.

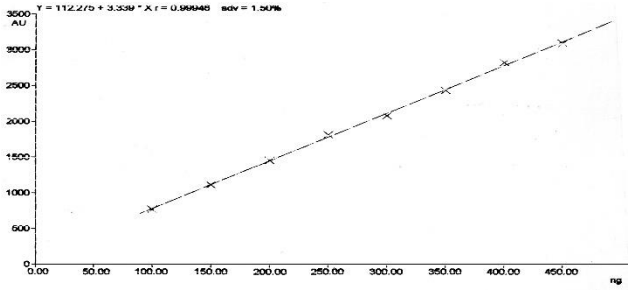

Figure 4. Calibration graph of Voglibose (100-450 ng/spot).

3.7.3. Precision.

The % RSD values for intra and inter-day was found in the range of 0.21 to 0.74 % and 0.21 to 0.29 % respectively. The lower % RSD values of intra-day and inter-day variation in the analysis indicates that the developed method was precise (Table 2-3).

Table 2. Intraday precision

Volume applied (µL)	Peak Area	% RSD
2	1261.5	0.63
	1258.3	
	1273.4	
4	1618.8	0.21
	1614.3	
	1612.3	
6	1574.9	0.74
	1566.2	
	1552.1	

Table 3. Inter day precision

Volume applied (µL)	Peak Area	% RSD
2	1265.7	0.29
	1264.3	
	1271.2	
4	1614.3	0.21
	1618.8	
	1612.3	
6	1572.6	0.24
	1569.8	
	1577.2	

3.7.4. Repeatability.

The % RSD for the peak areas in repeatability of sample application was found to be 0.28 %. In the repeatability for measurement % RSD for the peak area values were calculated and found to be 0.43 %. The % RSD values were found below the instrumental specifications (i.e.1.0 %); for measurement of peak area and sample application and proved that proper functioning of HPTLC system (Table 4-5).

3.7.5. Accuracy.

The % recovery of Voglibose was found to be 101.6, 100.3 and 99.8 (at 50, 100 & 150 % levels respectively). The results were proved that the proposed method was accurate for the estimation of drug in tablet dosage form (Table 6).

Table 4. Repeatability of sample application

Volume applied (µL)	Peak Area	% RSD
4	1614.8	0.28
	1609.6	
	1619.5	
	1620.1	
	1613.5	
	1609.6	

Table 5. Repeatability of measurement.

Volume applied (µL)	Peak Area	% RSD
4	1611.3	0.43
	1624.8	
	1607.4	
	1608.7	
	1605.9	
	1613.2	

Table 6. Accuracy of Voglibose.

Level (%)	% Recovery*	% RSD*
50	101.2	0.48
100	100.3	0.79
150	99.8	0.42

*n=3, number of times procedure repeated

3.7.6. LOD & LOQ.

The limit of detection was found to be 40 ng/spot (Figure 5), and the limit of quantification was found to be 100 ng/spot (Figure 6) respectively it indicates the sensitivity of the developed method.

3.7.7. Analysis of formulation.

Analysis of formulation was performed using Voglibose 0.2 mg tablets and the content of drug was calculated. The % assay was found to be 98.2 (Table 7).

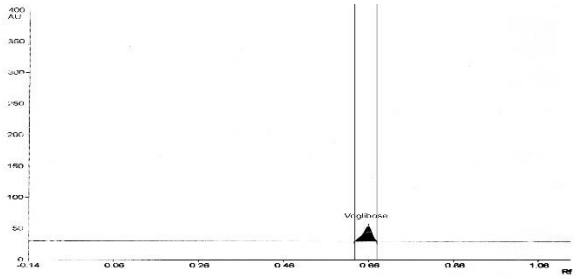

Figure 5. LOD of Voglibose (40 ng/spot)

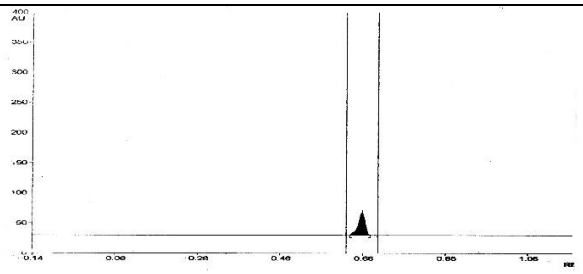

Figure 6. LOQ of Voglibose (100 ng /spot)

Table 7. Results for Analysis of formulation.

Drug	Amount (mg/ tablet) (n=3)*		% Assay	%RSD
	Labeled	Found		
Voglibose	0.2	0.193	98.2	0.52

*n= no of times procedure repeated

3.7.8. Robustness.

To verify the effect of the operational parameters on the analysis results robustness was performed. It was determined by introducing small deliberate changes in the optimized chromatographic conditions like mobile phase composition, solvent migration distance and chamber saturation time. The % RSD values were found below 2.0 %. The results indicate that the method was robust.

3.7.9. Stability studies.

The developed plate was found to be stable up to 18 hours and 24 hours at room temperature and at refrigerator condition. The observed results were within the specified limits (Table 8).

Table 8. Stability of the analyte on plate.

Concentration (µg/mL)	Peak area of the Drug			
	Time in hrs.	At room temperature	Time in hrs.	Refrigeration
50	0	1614.52	0	1614.58
	3	1566.12	5	1535.79
	5	1535.75	8	1485.27
	7	1503.62	11	1436.91
	11	1439.92	14	1358.09
	14	1379.39	19	1325.25
	18	1324.32	24	1277.18

4. CONCLUSIONS

The proposed HPTLC method for the estimation of Voglibose is simple, precise, specific, accurate, sensitive, robust and reproducible. The amounts found in formulations are well agreed with the label claim. Statistical analysis proves that the method was suitable for the analysis of voglibose as bulk and its formulation without any interference from its excipients. Hence the reported method is of considerable importance and has great industrial applicability for quality control for the analysis of Voglibose in bulk and its formulation.

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