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Original Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF VOGLIBOSE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: This paper describes a new, simple, precise, accurate and specific HPTLC method for estimation of voglibose as a bulk drug and in tablet dosage forms.

Methods: Chromatographic separation of the drug was performed on aluminum plates pre-coated with silica gel 60 F_{254} as the stationary phase and a mobile phase comprising of toluene: ethyl acetate: methanol: 30% ammonia 5:4:1.5:0.2 (v/v/v/v). Densitometric quantification of voglibose was carried out at 292 nm. Voglibose was detected satisfactorily with a R_f value 0.26.

Results: The accuracy and reliability of the method was assessed by evaluation of linearity (0.2-1.2 µg/spot), precision (intra-day RSD 0.6-0.9% and inter-day RSD 0.20-0.25%), accuracy (97.32-102.46%) and specificity according to ICH guidelines.

Conclusion: The proposed HPTLC method is less expensive, simpler, rapid and more flexible than the reported RP-HPLC method for routine analysis of voglibose in bulk and tablet dosage forms.

Keywords: Voglibose, HPTLC, Densitometric estimation, Method development, 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, miglitol and voglibose. Voglibose is the safest and most effective of them all. It is most commonly available in the form of tablets with the dose of 0.2 mg to 0.3 mg per tablet. Structure of voglibose is similar to that of a carbohydrate. Chemically voglibose (fig. 1) is [5-(1, 3-dihydroxypropane-2-yl-amino)-1-(hydroxymethyl) cyclohexane-1, 2, 3, 4-tetrol]. From the literature survey it was evident that several HPLC [5-7] and UV spectrophotometric methods [8, 9] were reported for estimation of voglibose HPLC method for estimation of voglibose in combined dosage form was also found in literature [10]. But we could not find any HPTLC method for the estimation of voglibose in bulk and tablet formulation. Therefore it was felt that a reliable and rapid method for the estimation of voglibose was needed. The primary goal was to develop and validate a HPTLC method for the rapid quantitation of the drug. The present study illustrates development and validation of simple, accurate, economical and reproducible method for determination of voglibose by HPTLC as bulk and tablet dosage forms. The proposed method was validated as per ICH guidelines which could be used effectively in industry for routine analysis of bulk drug and formulations.

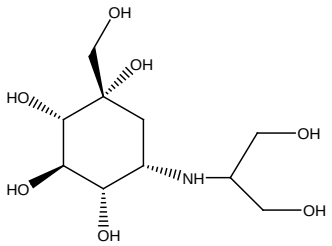

Fig. 1: Structure of voglibose

MATERIALS AND METHODS

Reagents and chemicals

Voglibose was procured as a gift sample from Zim Laboratories Ltd. Kalmeshwar, Nagpur, India. All other reagents and chemicals used were of analytical grade and purchased from Merck Chemicals Corporation Ltd. Mumbai, India. Silica gel 60F_{254} TLC plates (20×10 cm and 10×10 cm, layer thickness 0.2 mm, Merck, Germany) were used as stationary phase.

Instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag 100 µl samples (Hamilton, Bonaduz, Switzerland) syringe, on silica gel pre-coated aluminum plate 60F_{254} plates (10×10 cm) with 250 µm thickness; (E. Merk, Darmstadt, Germany), supplied by Anchrom technologist, Mumbai using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110 °C for 5 min prior to chromatography. A constant application rate of 0.1 µl/s was used and the space between the two bands was 6 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm/s. The monochromatic band width was set at 20 nm and 320 cut off filter; each track was scanned three times and baseline correction was used. The mobile phase consisted of toluene: ethyl acetate: methanol: 30% ammonia 5:4:1.5:0.2 (v/v/v/v) and 10.7 mL of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm × 10 cm twin through glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature (25 °C±2) at relative humidity of 60%±5. Each chromatogram was developed over a distance of 8 cm. Following the development, the TLC plates were dried in a stream of air with the help of hair dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 292 nm and operated by Wincats software (v 3.15, Camag). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample analysis.

HPTLC method and chromatographic conditions

Preparation of standard stock solution

Voglibose (100 mg) was weighed accurately and transferred to a 10 mL volumetric flask and diluted up to the mark with DMSO (10 mg/ml). This stock solution (0.1 ml) was diluted to 10 mL with methanol (0.1 mg/ml).

Method of sample preparation

Twenty tablets (content-0.2 mg of voglibose) were weighed (average weight 75.88 mg) and powdered using mortar and pestle. Quantity of powder equivalent to 0.1 mg of voglibose was transferred to 10 mL volumetric flask. The content was dissolved in DMSO, was sonicated for 15 min and then filtered using Whatmann filter paper. The volume was made upto 10 mL with DMSO (0.01 mg/ml). From this standard stock solution, aliquot of 5 ml was taken and diluted to 10 mL (5µg/ml).

Prewashing of plates

Densitometric estimation was carried out on 10×10 cm pre-coated silica gel $60F_{254}$ pre-coated plates from E. Merck. The plates were pre-washed with methanol, dried and activated for 30 min at 110 °C.

Sample application

The standard and formulation samples of voglibose were spotted on pre-coated TLC plates in the form of narrow bands of width 6 mm, with 10 mm from the bottom and left margin and 10 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at a constant application rate of 150nL/s.

Optimization of mobile phase

Various solvent systems like mixture of a) methanol: ethanol: 30% ammonia 3:2:0.1 (v/v/v) b) acetonitrile: methanol: 30% ammonia 3:3:0.1 (v/v/v) c) toluene: ethyl acetate: methanol 5:4:3 (v/v/v) and d) toluene: ethyl acetate: methanol: formic acid 3:4:3:0.1 (v/v/v/v) were tried to separate and resolve spot of voglibose from its impurities and other excipients of formulation. The mixture of toluene: ethyl acetate: methanol 5:4:1.5 (v/v/v) could resolve voglibose but there was tailing in the peaks. To improve peak shape 30% ammonia was added. Finally, the mixture of toluene: ethyl acetate: methanol: 30% ammonia 5:4:1.5:0.2 (v/v/v/v) showed well resolved peak with better peak shape. The drug was satisfactorily resolved with R_f value 0.24±0.03. Pre-saturation of TLC chamber with the mobile phase for 30 min assured better reproducibility in migration of voglibose and better resolution (fig. 2).

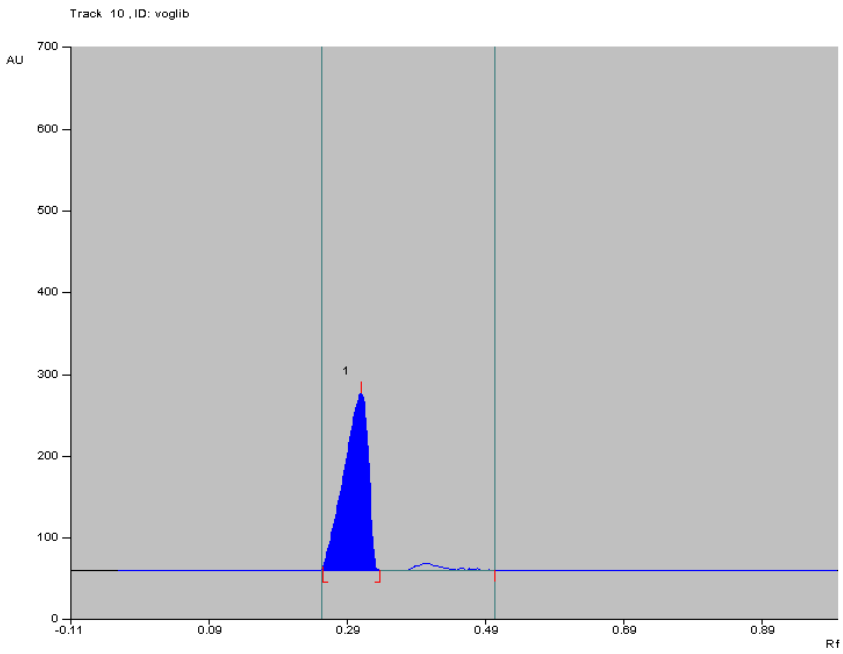

Fig. 2: Densitogram of voglibose formulation (0.8 µg/spot)

Method validation

The developed HPTLC method was validated as per the ICH guidelines Q2 9(R1) [11-13] for linearity, accuracy, precision, limit of detection, limit of quantification, repeatability, specificity and robustness.

Linearity and calibration curve

Linearity of the method was evaluated by constructing calibration curves at six concentration levels. Aliquots of standard working solution of voglibose (2, 4, 6, 8, 10 and 12 µl) were applied to the plate, to obtain concentration 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 µg/spot in the range of 0.2 to 1.2µg/spot. The calibration curves were developed by plotting peak area *vs* concentration with the help of Win-CATS software. The plate was developed in a twin through glass chamber, using 20 min chamber saturation time. The length of the run was 80 mm. The developed plates were air-dried. Scanning was performed in UV mode at 292 nm. The slit dimension was kept at 5 × 0.45 mm at scanning speed of 100 nm/s. After completion of scanning, peak areas were noted. Peak areas were plotted against the corresponding concentration and least square regression analysis was performed to generate the calibration equation.

Precision

To evaluate intra-day precision, three samples at three different concentrations were analyzed 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.

Repeatability

Repeatability of sample application was assessed by spotting 0.4 µg/spot of standard drug solution six times on a TLC plate at different times on the same day by sample applicator, followed by development of plate and recording of the peak areas for six spots.

Accuracy

Recovery studies of the drug were carried out for determining accuracy of the developed method. It was done by mixing known quantity of standard drug with the sample formulation and the contents were analyzed by the proposed method. Recovery studies were carried out at 80-120% levels. The percentage recovery and percentage RSD were calculated.

Limit of detection and limit of quantitation

To estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted six times. Spotting for LOD was done by taking different concentrations as 0.02, 0.03, 0.04, 0.05, 0.06µg/spot. No spot was detected up to concentration 0.04 µg/spot. The peak was detected at 0.05µg/spot with a signal-to-noise ratio of 3:1. The LOQ was done by taking different concentrations as 0.2, 0.3, 0.4, 0.5, 0.6 µg/spot. The peak was detected with quantifiable area at 0.5 µg/spot with a signal-to-noise ratio of 10:1.

Specificity

To confirm the specificity of the proposed method, voglibose was spotted on TLC plate, developed and scanned as described earlier. The UV spectrum of standard voglibose was also compared with spectrum of voglibose extracted from tablet. The peak purity of voglibose was assessed by comparing their respective spectra at peak start, peak apex and peak end position of the spot.

Robustness

The parameter selected for the robustness study were mobile phase composition, chamber saturation time and solvent migration distance. By introducing small changes, in these parameters the effect on the results was examined.

RESULTS

Linearity

A representative calibration curve was obtained by plotting peak area of the compound against the concentration over the range of 0.2 to 1.2 µg/spot. The slope, intercept and correlation co-efficient value is given in table 1. It showed good correlation between regression coefficient and concentration of the drug (fig. 3).

Table 1: Linearity and range

Linearity and range	Voglibose
Range (µg/spot)	0.2-1.2
Regression coefficient (r^2)	0.999
Linearity equation	y = 2013x+952.4

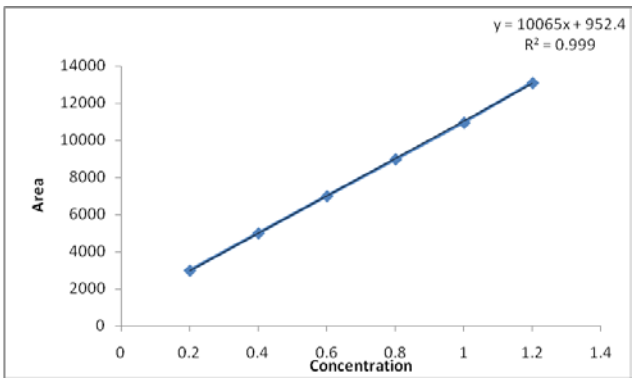

Fig. 3: Calibration curve of voglibose (0.2-1.2 µg/spot)

Precision

The intra-day and inter-day relative standard derivations were found in the range 0.20-0.25 % respectively. The smaller values of intra-day and inter-day variation in the analysis indicate that the method is precise (table 2 and 3).

Table 2: Intra-day precision study

Volume applied (µl)	Peak area	% RSD
	2071	
2	2035	0.9589
	2067	
	2545	
4	2513	0.7822
	2509	
	3076	
6	3050	0.6082
	3040	

Table 3: Inter-day precision study

Volume applied (µl)	Day	Peak area	%RSD
	1	2057	
2	2	2060	0.2489
	3	2067	
	1	2515	
4	2	2508	0.2041
	3	2518	
	1	3071	
6	2	3070	0.2547
	3	3057	

Repeatability

In repeatability of sample application, the % RSD for the peak area was found to be 0.22 %. The RSD values for measurement of peak area and sample application, were both below the instrumental specifications (i.e.1%); ensuring proper functioning of the system (table 4).

Accuracy

The % recovery of voglibose was 97.32-102.46% (at 80%, 100% and 120% respectively), which was found to be satisfactory. The result of recovery studies indicated that the proposed method was accurate for estimation of drug in a tablet dosage form (table 5).

Table 4: Repeatability study

Volume applied (µl)	Peak area	%RSD
4	2515 2505 2520 2518 2512 2509	0.2242

Table 5: Recovery studies of voglibose tablet

%Level	Concentration of drug added µg/spot	Concentration of drug found µg/spot	% Recovery	% RSD
80	0.72	0.7007	97.32	0.71
100	0.8	0.7969	99.61	0.45
120	0.88	0.9017	102.46	1.42

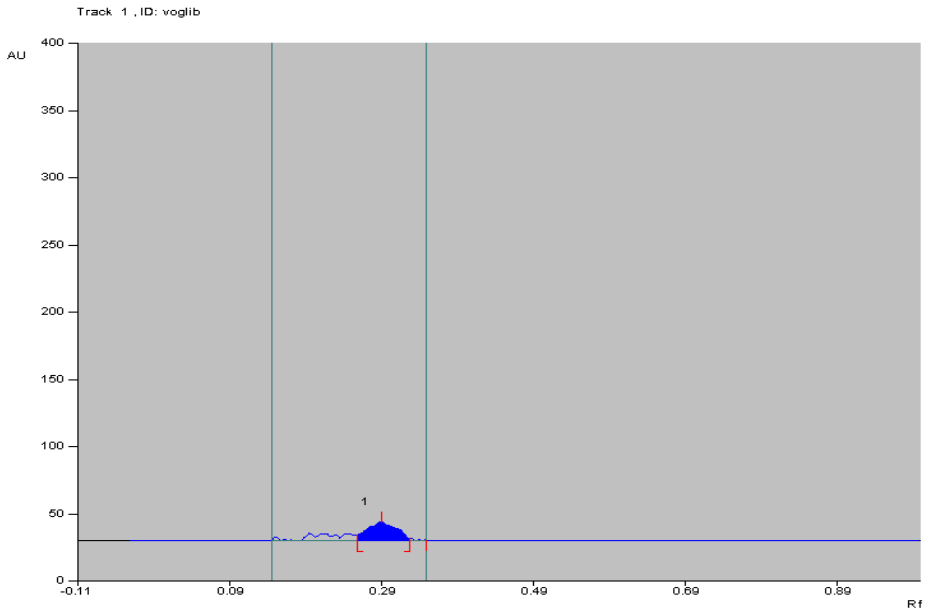

Fig. 4: LOD of voglibose (0.05µg/spot)

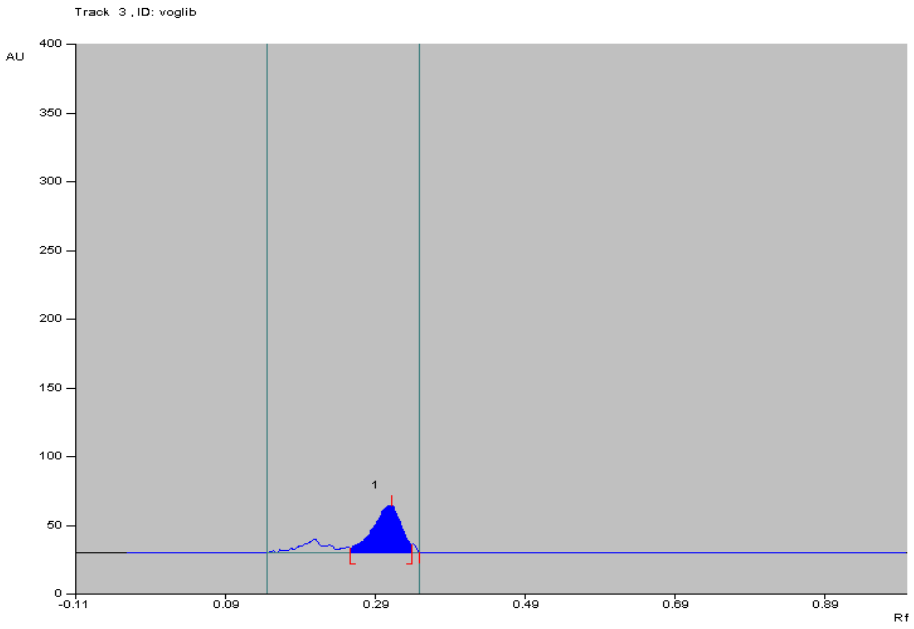

Fig. 5: LOQ of voglibose (0.5 µg/spot)

LOD and LOQ

The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3:1). The LOD for voglibose found to be 0.05 µg/spot (fig. 4). The LOQ is the smallest concentration of the analyte, which gives the response that can be accurately quantified (signal to noise ratio of 10:1). The LOQ was 0.5 µg/spot for voglibose (fig. 5). It was concluded that the developed method was sensitive.

Analysis of formulation

The content of the voglibose in the tablet dosage form (Sun Pharma Laboratories Ltd., Samba, Jammu and Kashmir) was calculated from the peak area recorded. Analysis was the performed using voglibose 0.2 mg tablets and the % label claim was found to be 99.62% (table 6), (fig. 6).

Specificity

A good correlation among spectra acquired at start (s), apex (m), and end (e) of the peaks indicates the peak purity of voglibose [correlation r (s, m) = 0.20, 0.26, r (m, e) =0.26, 0.31]. Hence it can be concluded that no impurities or degradation products migrated with the peaks obtained from standard solutions of the drug. It was observed that excipients present in the formulation did not interfere with peak of drug (R_f 0.26).

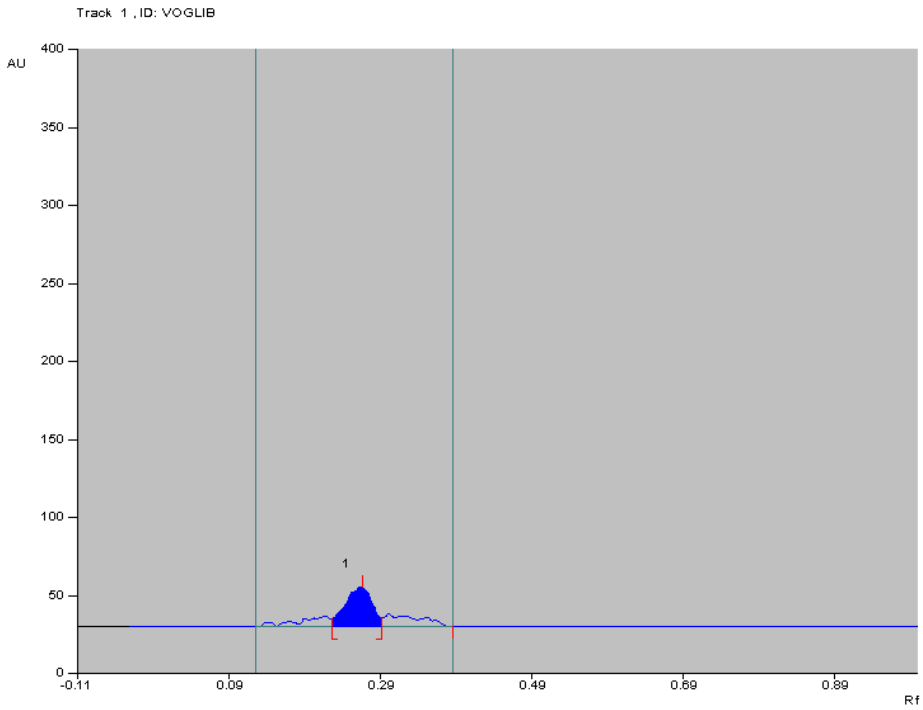

Fig. 6: Densitogram of voglibose tablet sample

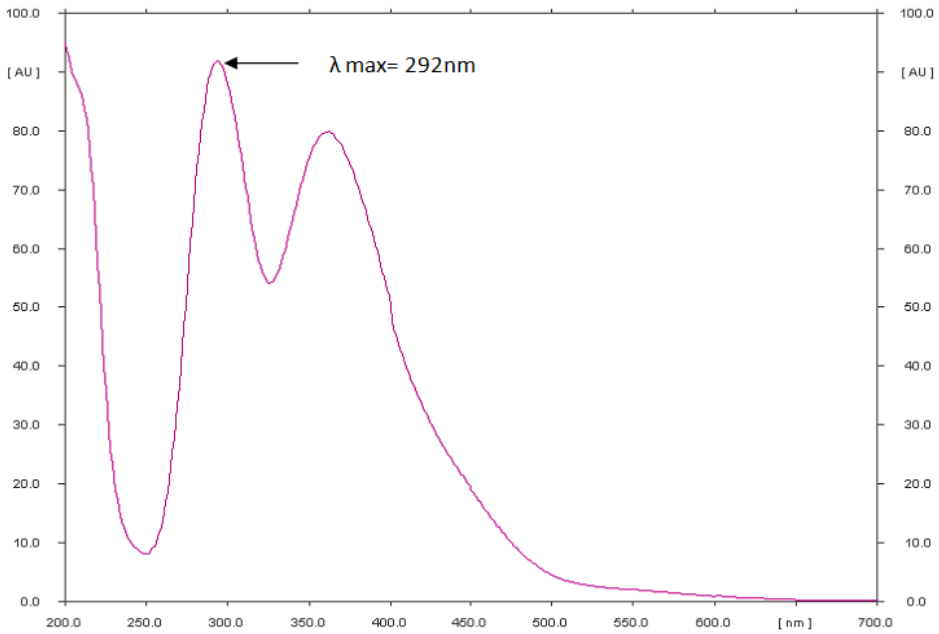

Fig. 7: UV spectrum of standard voglibose on TLC

Table 6: Analysis of formulation

Drug	Amount (µg/spot)(n*=3)		% Label claim	% RSD
	Added	Found		
Voglibose	0.600	0.597	99.62	0.14

*n= no of times procedure repeated

Robustness

Robustness examines the effect of the operational parameters on the analysis results. Small changes in mobile phase composition were introduced. The deviation obtained by deliberate changes in the aforementioned parameter was below 2% RSD which confirmed the robustness of the method.

DISCUSSION

The chromatographic method was validated according to ICH guidelines. Linearity study indicated that area was directly proportional to concentration ($r^2$0.999) and that the developed method was linear. Quantitation was achieved with linear calibration curves at concentration range of 0.2-1.2 µg/spot indicating that the method is sensitive. % RSD for repeatability and reproducibility study was less than 1.5 showing that the method was precise. In roubustness study, % RSD was found to be less than 1.5 indicating that small changes in process parameters, such as time from development to scanning and mobile phase ratio did not show any major changes in results. The LOD, LOQ were found to be at 0.05µg/spot and 0.5 µg/spot respectively. Recovery study was carried out at concentration level of 0.72, 0.80, 0.88 µg/spot. Mean % recovery was found to be 99.4. In specificity study, the UV spectrum of voglibose showed good correlation. The proposed HPTLC method was found to be specific.

Results of validation were compared with the reported HPLC and UV methods. The proposed HPTLC method was more sensitive, less time consuming, required less amount of solvents than HPLC for analysis hence more economic.

CONCLUSION

The developed HPTLC method is simple, precise, specific, accurate, sensitive, selective and reproducible. The amounts of concentration of voglibose found in formulation were in agreement with label claim. Thus, the reported method is of considerable importance and has sound industrial applicability for quality control and analysis of voglibose from bulk drug and formulations.

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CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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