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Validated HPTLC method for simultaneous estimation of voglibose and metformin hydrochloride in pharmaceutical dosage forms

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ABSTRACT

The objective of present work was to develop validated HPTLC method for simultaneous estimation of voglibose and metformin hydrochloride in the bulk drug and tablet dosage forms. The chromatographic separation of the drugs was performed on aluminium plates precoated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of ammonium sulphate (0.5%) : n- propanol (2:8;v/v). Densitometric evaluation of separated zones was performed at 248 nm. The two drugs were satisfactorily resolved with R_f values 0.20 ± 0.02 and 0.74 ± 0.02 for voglibose and metformin hydrochloride respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (200-1200 ng/spot for voglibose and 500-5000 ng/spot for metformin hydrochloride), precision (intra-day RSD 0.1379-0.3736% and inter-day RSD 0.4183-0.5706% for voglibose and intra-day RSD 0.2485-0.1355% and inter-day RSD 0.3740 -0.3150% for metformin hydrochloride), accuracy (100.69 ± 1.0% for voglibose and 101.57 ± 0.8% for metformin hydrochloride) and specificity in accordance with ICH guidelines. The proposed HPTLC method is less expensive, simpler, rapid and more precise than reported HPLC method for routine analysis of voglibose and metformin hydrochloride in bulk drug and tablet dosage forms.

Key words: Thin layer chromatography, Densitometry, Analytical method validation, Voglibose, Metformin hydrochloride.

INTRODUCTION

Voglibose (Fig 1) is chemically known as [5-(1, 3-dihydroxypropane-2-yl-amino)-1-(hydroxymethyl) cyclohexane-1, 2, 3, 4-tetrol]. Chemical formula for voglibose is $C_{10}H_{21}NO_7$ with molecular weight 267.28 g mol^{-1}. It is a white to off-white, odourless, crystalline powder, soluble in 100 mM water and 70 mM DMSO [1-7]. Voglibose is used for the treatment of diabetes mellitus. It acts as glucosidase inhibitor, remaining active within the gastrointestinal tract of humans by delaying glucose absorption, thereby preventing sudden surge of glucose in the human body after meals. Voglibose is the safest and most effective glucosidase inhibitor. 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 [8-12].

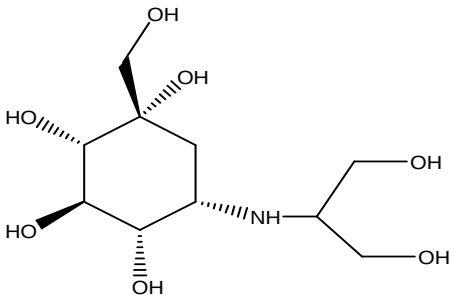

Figure 1: Voglibose

Metformin hydrochloride (Fig 2) is chemically known as [3- (diaminomethylidene) 1, 1-dimethylguanidine]. Metformin hydrochloride is $C_4H_{11}N_5$ with molecular weight 129.16. It is a white to off-white, odourless, crystalline powder, freely soluble in water, slightly soluble in alcohol, insoluble in acetone and methyl chloride. Metformin decreases hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis). The "average" person with type 2 diabetes has three times the normal rate of gluconeogenesis; metformin treatment reduces this by over one-third [1-7].

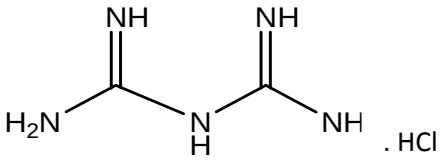

Figure 2: Metformin hydrochloride

From the literature survey it was evident that several HPLC [13-15] and UV spectrophotometric methods [16, 17] were reported for estimation of voglibose. HPLC method for estimation of voglibose in combined dosage form and HPTLC method for estimation of metformin hydrochloride [18] are reported. Several HPLC [19, 20] and UV spectrophotometric methods [21-23] for simultaneous estimation of voglibose and metformin hydrochloride in multicomponent formulation are also reported in literature. But we could not find any HPTLC method for the simultaneous estimation of voglibose and metformin hydrochloride in multicomponent formulation. Therefore it was felt that a reliable method for the simultaneous estimation of voglibose and metformin hydrochloride is needed.

The primary goal was to develop and validate HPTLC method for the rapid quantitation of these two drugs. The present study illustrates development and validation of simple, accurate, economical and reproducible method for estimation of voglibose and metformin hydrochloride by HPTLC in a multi-component formulation.

The proposed method was validated as per ICH guidelines which could be used effectively in industry for routine analysis of bulk drug and formulations.

MATERIALS AND METHODS

2.1. Reagents and chemicals

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. Voglibose and metformin hydrochloride were procured as a gift samples from Zim Laboratories Ltd. Kalmeshwar, Nagpur, India.

2.2. Instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag 100 µl sample (Hamilton, Bonaduz, Switzerland) syringe, on silica gel pre-coated aluminum 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 ammonium sulphate (0.5%): n-propanol (2:8; v/v) and 10 ml of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough 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 248 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.

2.3. HPTLC Method and Chromatographic Conditions

2.3.1. Preparation of standard stock solutions

Metformin hydrochloride (10 mg) was weighed accurately and transferred to a 10 ml volumetric flask and diluted up to the mark with methanol (1mg/ml).

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

2.3.2. Preparation of Sample formulation

Twenty tablets (Content- 0.2mg of voglibose, 500mg- metformin) were weighed (average weight 706.77 mg) and powdered using mortar and pestle. Quantity of powder equivalent to 0.4 mg of voglibose (1000mg of metformin) was transferred to 10 ml volumetric flask. The content was dissolved in DMSO, was sonicated for 15min and then filtered using Whatmann filter paper. The volume was made upto 10 ml with DMSO (0.04mg/mL voglibose and 100mg/mL metformin hydrochloride)

2.3.3. Pre-washing 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.

2.3.4. Sample application

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

2.3.5. Selection of wavelength

Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample analysis at 248 nm using methanol as a blank solution. The selection of detection wavelength is shown in Fig 3.

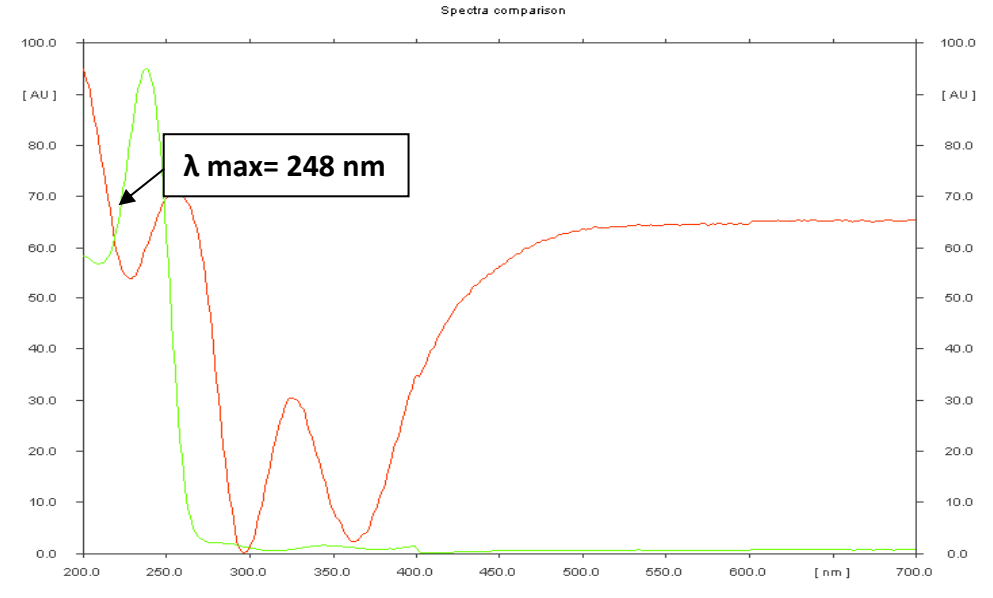

Figure 3: UV overlay spectra of standard voglibose and metformin hydrochloride

2.3.6. 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 spots of voglibose and metformin hydrochloride from their impurities and other excipients of formulation. The mixture of ammonium sulphate (0.5%): methanol: n-propanol (8:1.6:1.6; 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 ammonium sulphate (0.5%): n-propanol (2:8; v/v) showed well resolved peaks with better peak shape. The drugs were satisfactorily resolved with R_f value 0.74 ±0.03 for voglibose and 0.29±0.03 for metformin hydrochloride. Pre-saturation of TLC chamber with mobile phase for 45 min assured better reproducibility in migration of voglibose and metformin hydrochloride with better resolution (Fig 4).

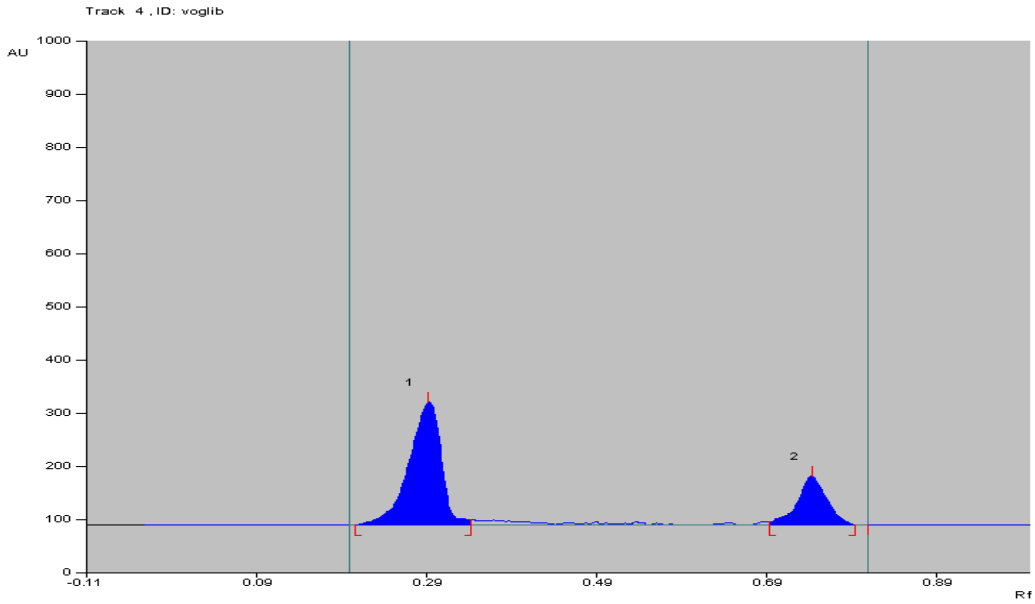

Figure 4: Densitogram of voglibose and metformin hydrochloride formulation (400 ng/spot)

METHOD VALIDATION

The developed HPTLC method was validated as per the ICH guidelines Q2 9(R1) [24-25] for linearity, accuracy, precision, limit of detection, limit of quantification, repeatability, specificity and robustness.

2.4.1. Linearity and calibration curve

Aliquots of standard working solutions of voglibose and metformin hydrochloride were applied on the plate, to obtain concentration in the range 200 to 1200 ng/spot for voglibose and 500-5000 ng/spot for metformin hydrochloride respectively. Linearity of the method was evaluated by constructing calibration curves at six concentration levels. The calibration curves were developed by plotting peak area *vs.* concentration with the help of Win-CATS software. The plates were developed in a twin trough glass chamber, using 45 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 248 nm. The slit dimension was kept at 5 × 0.45 mm at scanning speed of 100nm/s. After completion of scanning, peak areas were noted. Peak areas were plotted against corresponding concentrations and least square regression analysis was performed to generate the calibration equation.

2.4.2. Precision

The inter-day precision was studied by comparing assays performed on three different days. To evaluate intra-day precision, three samples at three different concentrations (300 ng/spot, 400 ng/spot and 500 ng/spot for voglibose and 3000 ng/spot, 4000 ng/spot, and 5000 ng/spot for metformin hydrochloride respectively) were analysed on the same day. 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.

2.4.3. Repeatability

Repeatability of sample application was assessed by spotting 400 ng/spot for voglibose and 5000 ng/spot for metformin hydrochloride from respective of standard drug solutions six times on a TLC plate at different times on same day by sample applicator, followed by development of plate and recording of the peak areas for six spots.

2.4.4. Accuracy

Recovery studies were carried out at 80-120% levels. 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 of the drugs were carried out for determining accuracy of the developed method. The percentage recovery and percentage RSD were calculated.

2.4.5. 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 20, 30, 40, 50, 60 ng/spot for voglibose and metformin hydrochloride. No spot was detected up to concentration 40 ng/spot. The peak was detected at 50 ng/spot with a signal-to-noise ratio of 3:1 for voglibose and metformin hydrochloride. The LOQ were done by taking different concentrations as 200, 300, 400, 500, 600 ng/spot for voglibose and metformin hydrochloride. The peak was detected with quantifiable area at 300 ng/spot for voglibose and 500ng/spot for metformin hydrochloride respectively with a signal-to-noise ratio of 10:1.

2.4.6. Specificity

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

2.4.7. Robustness

The parameters 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 AND DISCUSSION

3.1. Linearity:

The slope, intercept and correlation co-efficient values are given in Table 1. Representative calibration curves were obtained by plotting peak area of compound against the concentration over the range of 200 to 1200 ng/spot for voglibose and 500-5000 ng/spot for metformin hydrochloride. It showed good correlation between regression coefficient and concentration of the drug (Fig 4 and Fig 5).

Table 1 Linearity and range

Linearity and range	Voglibose	Metformin hydrochloride
Range (ng/spot)	200-1200	500-5000
Regression coefficient (r^2)	0.997	0.992
Linearity equation	y = 1.757x + 5765	y = 0.301x + 7827

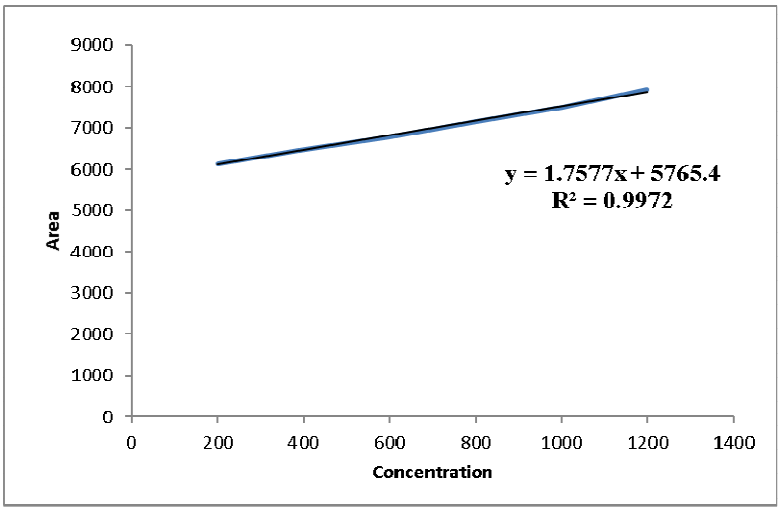

Figure 5: Calibration curve of voglibose (200-1200 ng/spot)

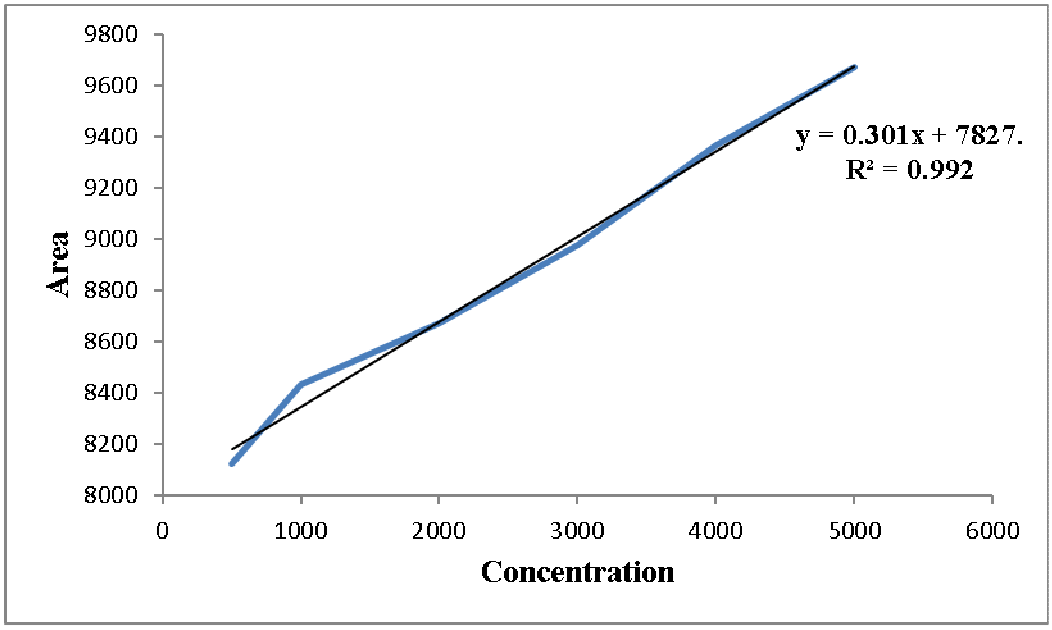

Figure 6: Calibration curve of metformin hydrochloride (500-5000 ng/spot)

3.2. Precision

The inter-day standard deviations were found in range 0.35-0.57% and 0.19- 0.37% for voglibose and metformin hydrochloride respectively. The intra-day and inter-day relative standard derivations were found in the range 0.13 - 0.45 and 0.13-0.51% intra-day precision for voglibose and metformin hydrochloride respectively. The smaller values of intra-day and inter-day variations in the analysis indicated that the method was precise (Table 2-5).

Table 2 Intra-day precision study for voglibose

Volume applied/spot (µl)	Peak area	% RSD
	4031	
3	4035	0.1380
	4042	
	4512	
4	4513	0.4532
	4548	
	5016	
5	5047	0.3736
	5050	

Table 3 Intra-day precision study for metformin hydrochloride

Volume applied/spot (µl)	Peak area	% RSD
	8357	
4	8367	0.2486
	8397	
	8589	
5	8504	0.5115
	8529	
	8863	
6	8850	0.1356
	8874	

Table 4 Inter-day precision study for voglibose

Volume applied/spot (µl)	Peak area	% RSD
	4008	
3	4015	0.4184
	4040	
	4518	
4	4515	0.3524
	4544	
	5070	
5	5028	0.5706
	5015	

Table 5 Inter-day precision study for metformin hydrochloride

Volume applied/spot (µl)	Peak area	% RSD
	8307	
4	8369	
	8344	0.3740
	8559	
5	8526	
	8548	0.1966
	8896	
6	8866	
	8840	0.3150

3.3. Repeatability

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 6 and Table 7). In repeatability of sample application, the %RSD for the peak area was found to be 0.68 % for voglibose and 0.30%for metformin hydrochloride.

Table 6 Repeatability study for voglibose

Volume applied/spot (µl)	Peak area	%RSD
	4441	
	4452	
4	4493	0.6812
	4495	
	4417	
	4454	

Table 7 Repeatability study for metformin hydrochloride

Volume applied/spot (µl)	Peak area	%RSD
	8671	
	8654	
5	8695	0.3033
	8685	
	8631	
	8635	

3.4. Accuracy

The % recovery of voglibose was 98.03-102.44% and metformin hydrochloride was 99.00-101.88% (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 these drugs in a tablet dosage form (Table 8 and Table 9).

Table 8 Recovery studies of voglibose and metformin hydrochloride tablet [Voglejen-ZM®, 0.2 mg, Zenlabs Pharmaceutics Inc. Mumbai]

%Level	Concentration of drug added ng/spot	Concentration of drug found ng/spot	% Recovery ± % RSD
80	200	196.07	98.03 ± 0.43
100	250	253.84	101.53 ± 1.31
120	300	307.34	102.44 ± 0.56

Table 9 Recovery studies of voglibose and metformin hydrochloride tablet [Voglejen-ZM®, 0.2 mg, Zenlabs Pharmaceutics Inc.Mumbai]

%Level	Concentration of drug added ng/spot	Concentration of drug found ng/spot	% Recovery ± % RSD
80	500	495.02	99.00 ± 0.87
100	625	626.24	100.20 ± 0.26
120	750	764.12	101.88 ± 0.72

3.5. 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 and metformin hydrochloride found to be 50 ng/spot (Fig 7). The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10:1). The LOQ was 500 ng/spot for voglibose and 300 ng/spot for metformin hydrochloride (Fig 8). It was concluded that the developed method was sensitive.

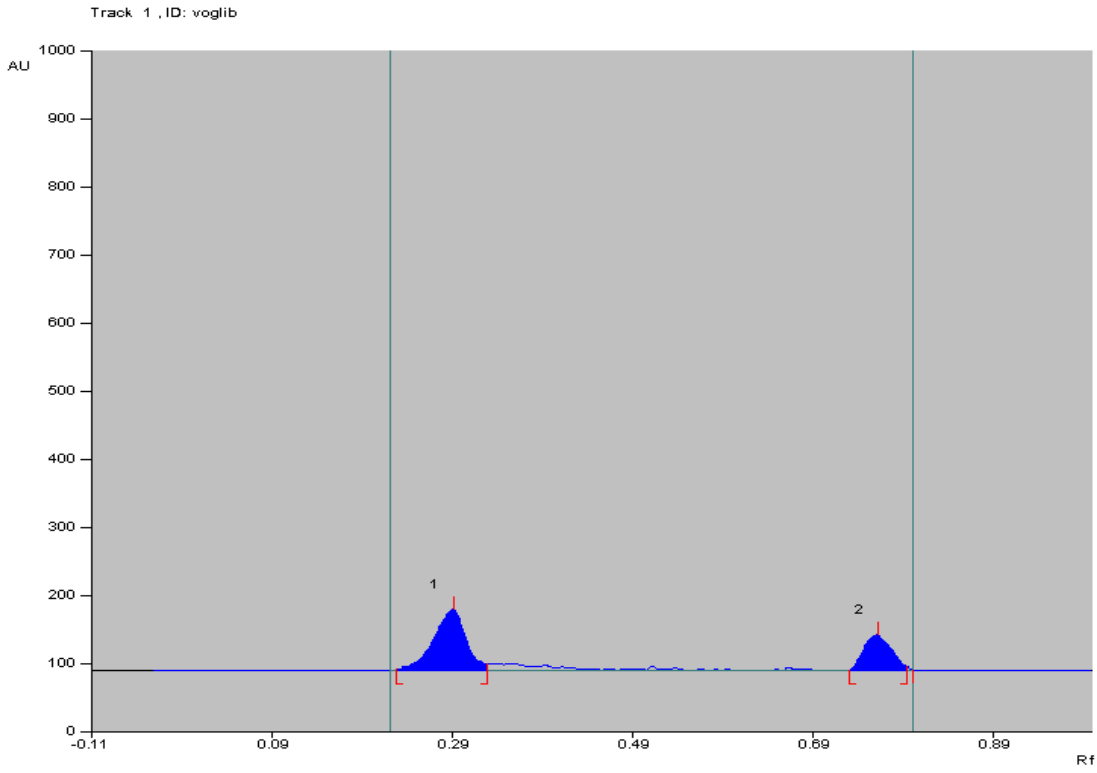

Figure 7: 1: LOD of voglibose (50 ng/spot); 2: metformin hydrochloride (50 ng/spot)

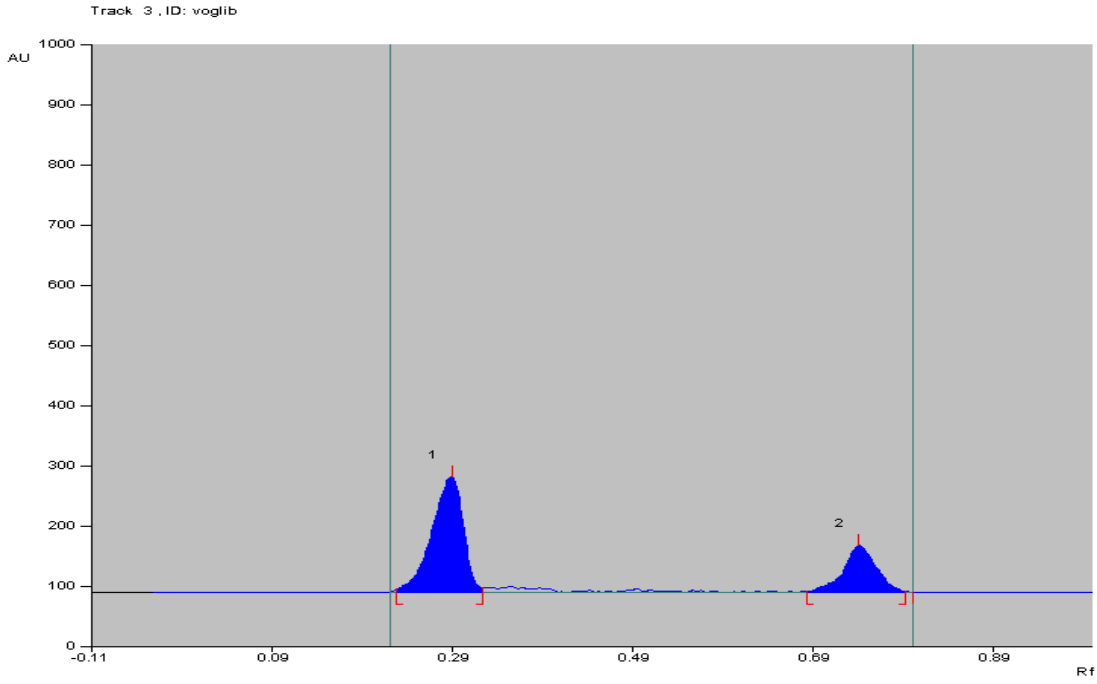

Figure 8: 1: LOQ of voglibose (300 ng/spot); 2: metformin hydrochloride (500ng/spot)

3.6. Analysis of formulation

Analysis was the performed using voglibose 0.2 mg and metformin hydrochloride 500 mg tablets and the % label claim was found to be 101.53% for voglibose and 100.20% for metformin hydrochloride (Table 10), (Fig 9). The content of the voglibose in the tablet dosage form (Zenlabs Pharmaceutics Inc. Mumbai) was calculated from the peak area recorded.

Table10 Analysis of formulation for voglibose and metformin hydrochloride

Drug (mg)	Amount (mg/tablet)(n*=3)		% Label claim ± % RSD
	Added ng/spot	Found ng/spot	
Voglibose	250	253.84	101.53 ± 1.31
Metformin hydrochloride	625	626.24	100.20 ± 0.26

**n= no of times procedure was repeated*

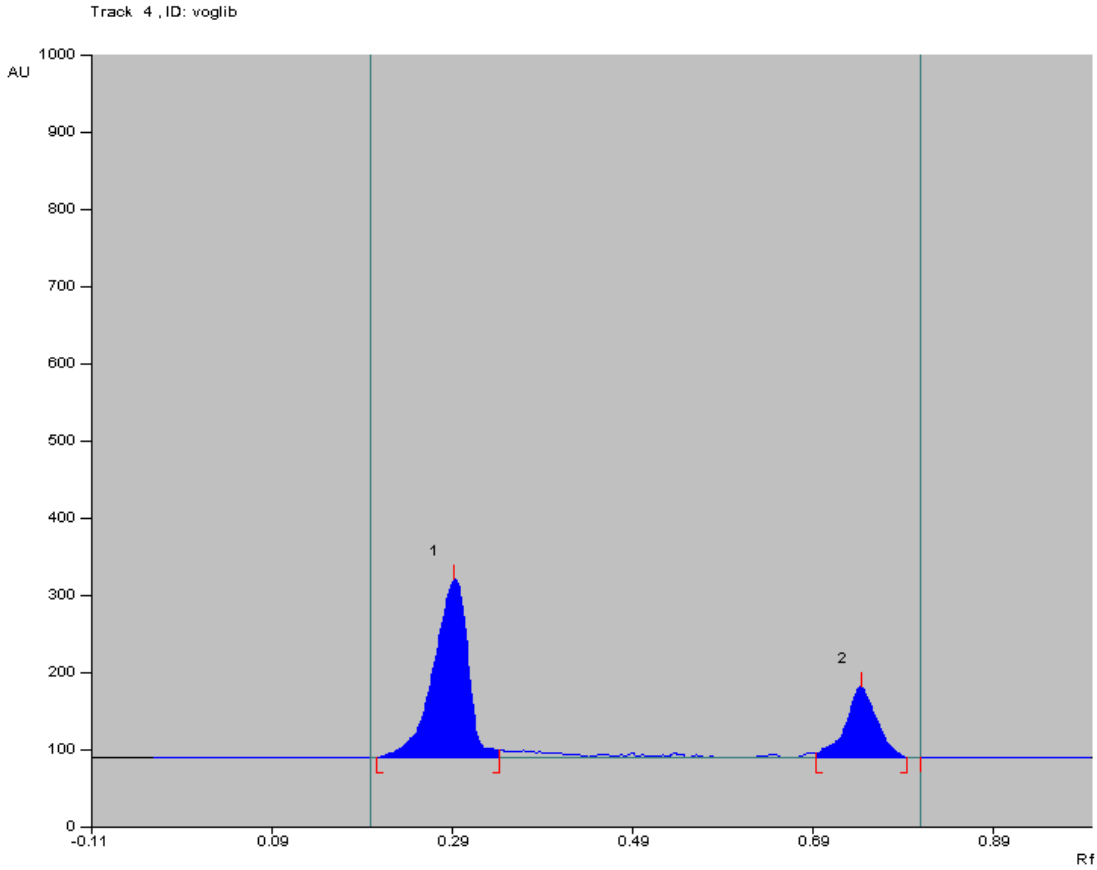

Figure 9: Densitogram of voglibose and metformin hydrochloride tablet sample (1: metformin hydrochloride R_f 0.29; 2: voglibose R_f 0.74)

3.7. Specificity

The good correlation among spectra acquired at start (s), apex (m), and end (e) of the peaks indicated the peak purity of voglibose [correlation r (s, m) = 0.69, 0.74, r (m, e) =0.74, 0.79] and metformin hydrochloride [correlation r (s, m) = 0.24, 0.29, r (m, e) =0.29, 0.35]. Hence it was concluded that no impurities or degradation products migrated with the peaks obtained from standard solutions of the drugs. It was observed that excipients present in formulation did not interfere with peaks of drugs (R_f 0.74 for voglibose and 0.29 for metformin hydrochloride). The proposed HPTLC method was found to be specific.

3.8. Robustness

Robustness examines the effect of the operational parameters on the analysis results. The deviation obtained by deliberate changes in the aforementioned parameters was below 2% RSD which confirmed the robustness of the method (Table 11).

Table 11 Results of robustness testing

S. N	Change process parameters	Name of drug (mg)	Conc. (ng/ml)	Mean area	% RSD
1	Time from development to scanning 15min + 5 min	Voglibose	400	4525.66	0.3523
		Metformin hydrochloride	5000	8544.33	0.1967
2	Mobile phase ratio (Ammonium sulphate (0.5%):n-propanol (2:8; v/v) ±0.1	Voglibose	400	4525	0.3524
		Metformin hydrochloride	5000	8544	0.1966

CONCLUSION

The developed HPTLC method is simple, precise, specific, accurate, sensitive, selective and reproducible. The amounts of concentration of voglibose and metformin hydrochloride 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 and metformin hydrochloride from bulk drug and formulations.

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