A NEW STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF VOGLIBOSE IN BULK AND TABLET DOSAGE FORM USING AN ION PAIRING AGENT

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Abstract

The analysis of voglibose by direct HPLC method is difficult due to its ability to show UV absorption at lower wavelength region. Here we have developed and validated a simple, sensitive, precise and specific reverse phase high performance liquid chromatography (RP-HPLC) method with ion pairing reagent, 1-hexane sulfonic acid for estimation of voglibose in bulk and its pharmaceutical dosage form (Tablets) The novelty of this method lies in the addition of the ion-paring salt directly. The proposed method was found to be more accurate, precise and easier compared to other reported methods where voglibose was needed to be derivatized to increase the sensitivity. The excipients in the tablet dosage forms did not interfere in the quantification of active drug by the proposed method. The HPLC separation was carried out by reverse phase chromatography on Cosmosil C18 (250mm x 4.6mm× 5µm) with a mobile phase composed of Methanol: Water (90:10) at pH 3 and flow rate was 1 mL/min. The detection was monitored at 221 nm. The calibration curve of voglibose was linear from 10 to 50µg/mL. The intraday and interday precision was found to be within the limit. The % recoveries for voglibose obtained in the accuracy study was 98.83-100.70%. LOD and LOQ for voglibose were found to be 0.4µg/mL and 1.22 µg/mL. Voglibose was also subjected to various stress condition like acid and alkali, hydrolysis, oxidation, photolysis and thermal degradation. The developed method is successfully applied for estimation of voglibose from bulk and tablet dosage form.

Keywords: Voglibose, RP-HPLC, Methanol, Forced degradation, ICH, 1-hexane sulfonic acid.

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1 Introduction

Voglibose is chemically known as 3,4- Dideoxy-4-[2-hydroxy-1-(hydroxyl methyl) ethyl]amino-2-c-(hydroxymethyl)-D-epinositol). It is an oral antidiabetic drug. Voglibose belongs to the class of competitive α glucosidase inhibitors (α-GIs) [1]. It binds to and inhibits alpha-glucosidase, an enteric enzyme found in the brush border of the small intestines that hydrolyses oligosaccharides and disaccharides into glucose and other monosaccharide [2]. It is used for the treatment of diabetes mellitus by lowering the blood sugar levels post prandial [3]. Voglibose delays the absorption as well as digestion of dietary polysaccharides by reversibly inhibiting carbohydrate digestive enzymes like sucrose, maltose, isomaltase, etc.[4]. Voglibose obtained from organic synthesis processes is similar to structurally related carbohydrates found naturally 4, 5 and has the empirical formula $C_{10}H_{21}NO_7$ [5].

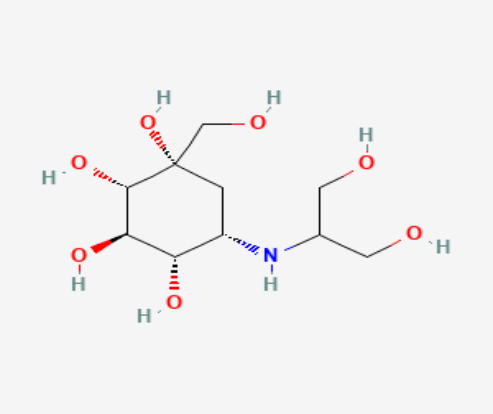
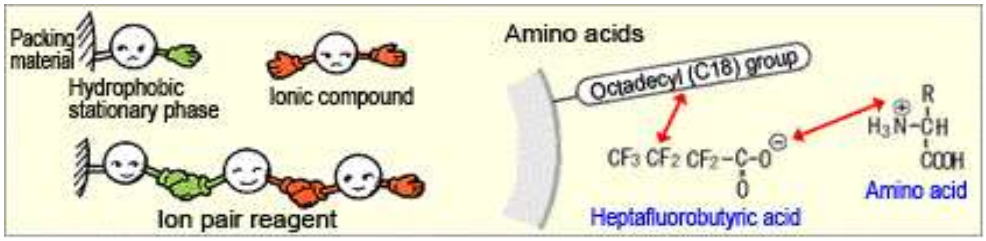


Fig.1 The chemical structure of voglibose

1.1 Introduction to Ion Pair Chromatography

Ion pair chromatography (IPC) is an effective reversed-phase liquid chromatographic (RPLC) technique for separation of organic ions and partly ionized organic analytes. The technique utilizes the same types of stationary phases and mobile phases as RPLC; The main characteristic for IPC is that an ion pair reagent is added to the mobile phase. The ion pair reagent is usually an alkylsulfonate, an alkylsulfate or an alkylammonium salt. The high efficiency of RPC columns compared with columns used in ion exchange or ion chromatography also makes IPC a valuable alternative to these techniques [6].



The purpose of adding an ion pair reagent to the mobile phase is usually to change the retention time of ionic analytes. By varying the mobile phase concentration of the ion pair reagent, the retention factor for an oppositely mono-charged analyte can be continuously increased by a factor of 10–20 compared to the value with no added ion pair reagent. Correspondingly, it is possible to continuously reduce the retention factor for a similarly mono-charged analyte by a factor of 10–20. The retention factor for non-charged analytes is usually more or less unaffected by the presence of the ion pair reagent [7]. The perfluorinated homologous series of acids represents a useful series of anionic ion-pairing reagents used for peptide separations, with TFA the most commonly employed, but pentafluoropropionic acid (PFPA) and hexanesulfonicacid (HSA) have also been used occasionallyThe negatively charged reagents like trifluoroacetate (TFA−), pentafluoropropionate (PFPA−) or heptafluorobutyrate (HFBA−) anion will interact (ion-pair) with positively charged peptide residues of amino acids [8]. Such hydrophobic anions will not only neutralize the positively charged groups, thereby decreasing peptide hydrophilicity, but will increase further the affinity of the peptides for the reversed-phase stationary phase [9]. In addition, more hydrophilic anionic ion-pairing reagents such as phosphoric acid (producing the

negatively charged phosphate anion) have also seen use for specific peptide applications in RP-HPLC [10], permitting a significant decrease in the concentration of organic solvent in the mobile phase, thus reducing the possibility of denaturation or precipitation [11].

Voglibose cannot be directly detected with high sensitivity in the HPLC due to its ability to show the UV absorption in the lower wavelength region. In order to increase the sensitivity of voglibose, people have started using derivatization method. For example, derivatization of voglibose with taurine and sodium periodate [12]. In the proposed RP-HPLC method, N-hexane sulfonic acid (HSA) has been used as an ion pairing reagent as there is a limited research available on the use of ion pair reagents to determine the voglibose in the RP-HPLC method.

2 Experimental

2.1Instruments

HPLC binary gradient system Model No HPLC 3000 Series, Analytical technologies Ltd. with a P-3000-M Reciprocating (40MPa) pump, UV-3000-M detector with HPLC workstation software. The Cosmosil C18 (250mm x 4.6mm× 5µm) column was used. UV-Visible Double Beam Spectrophotometer (Model 2012) wavelength scanning range 190nm-1100nm with 0.1nm variability with UV-VIS Analyst software. The Wenser High Precision Balance (PGB100) and Wenser Ultra Sonicator (WUC-4L) were used.

2.2Materials

Pure voglibose was obtained as a gift sample from Hetero Labs Ltd. Vishakhapatnam. voglibose tablets were purchased from retailer shop, India. HPLC grade water and methanol were used as a solvent from RAP analytical Lab.

2.3Chromatographic Conditions

Optimization of chromatographic conditions was carried out using Methanol: Water (90:10 v/v) as mobile phase. pH of the mobile phase was 3, (adjusted by O-phosphoric acid). The flow rate was 1 ml/min and run time was 7.97 minutes.

2.4Preparation of standard stock solution

A standard stock solution of 1000 µg/mL was prepared by dissolving 10 mg of voglibose in 10 mL of mobile phase. The working standard solution was prepared by diluting 0.1 mL of standard solution up to 10 mL mobile phase. The resulting solution was of 10 µg/mL.

2.5Preparation of sample solution

A sample stock solution of 1000 µg/mL was prepared by dissolving 3013.33 mg of tablet powder in 10 mL of mobile phase. The working stock solution was prepared using diluting 0.1 mL of sample solution up to 10 mL of mobile phase. The resulting solution was of 10 µg/mL.

2.6Selection of analytical wavelength

The HPLC analytical wavelength was determined from UV spectra of voglibose solution (20 µg/mL) prepared in methanol and water (90:10 %). The solution was scanned in the UV range between 200-400nm against methanol: water as blank. The working wavelength obtained was 221nm.

3 Method Validation

The optimized method for determination of voglibose has been validated as per International Conference of Harmonisation (ICH) guidelines Q2 (R1) for evaluating system suitability, specificity, precision, accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness [13].

3.1System suitability

System suitability tests are integral part of liquid chromatographic methods. It was determined by six replicates of standard solution (10 µg/mL). The % RSD of peak area, retention time (RT), theoretical plates (N) and asymmetry factor were determined.

3.2Linearity

The capacity of a method to produce test results that are directly proportional to the concentration of an analyte in a sample is known as linearity. Linearity was estimated by diluting standard stock solution (1000 µg/mL) in concentration range between 10-50 µg/mL. The calibration curve was obtained by plotting peak area vs concentration and R^2 was determined.

3.3Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Method of precision was accessed on two levels, intraday and inter day precision.

3.4Accuracy

The closeness of agreement between the value acknowledged as a conventional true value or an

approved reference value and the value found is expressed by the accuracy of an analytical method. The accuracy of voglibose was determined by spiking known concentration of voglibose at different level. Standard solution of drug at 20µg/mL was accurately prepared from stock solution and was further spiked at 50%, 100% and 150% by sample solution. The percentage recovery was calculated.

3.5Limit of Detection and Limit of Quantification

The LOD and LOQ were calculated by the use of signal to noise ratio. The three times the noise level gives LOD while ten times the noise value gives the LOQ. The values of LOD and LOQ were found to be 0.4 µg/mL and 1.22 µg/mL, respectively.

3.6Robustness

Robustness of the developed method was carried out at two different levels i.e. change in wavelength and change in flow rate. From the result it was demonstrated that there was insignificant change in the retention time, peak area, theoretical plates and asymmetry factor. The % RSD of change in flow rate and change in wavelength was found to be 0.523% and 0.263% respectively.

3.7Assay

Twenty tablets were weighed and finely powdered; quantity of powder equivalent to 10 mg was taken and transferred to a 10ml volumetric flask. The contents were mixed thoroughly, sonicated for 15 minutes and filtered through a 0.45 µ filter. Aliquot of this solution was further diluted to achieve a final concentration of 30µg/mL.

Forced degradation studies

Forced degradation studies were carried out as per ICH guidelines to demonstrate the stability-indicating ability and specificity of the develop method [14]. Standard samples were prepared at a concentration of 100 µg/mL and exposed to different stress conditions. All samples were then diluted accordingly to give a final concentration of 50 µg/mL. The samples were neutralized if required and filtered prior to injection. Control samples of 50 µg/mL were also prepared during analysis

3.8Acid degradation studies

10 mg drug was weighed and transferred it to 100 ml volumetric flask. About 20 ml of methanol was added and sonicated for 10 min, 10 ml of 0.1N HCl was added and sample was stored at 60^0C for 1 hr. The solution was neutralized with 0.1N NaOH and volume was made up to the mark with methanol. The sample was then diluted to give final concentration of 50 µg/mL.

3.9Base degradation studies

10 mg of drug was weighed and transferred it to 100 ml volumetric flask. About 20 ml of methanol was added and sonicated for 10 min, 10 ml of 0.1N NaOH was added and sample was stored at 60^0C for 1 hr. The solution was made up to the mark with methanol. The sample was then diluted to give final concentration of 50 µg/mL.

3.10 Oxidative degradation studies

10 mg of drug was weighed and transferred it to 100 ml volumetric flask. About 20 ml of methanol was added and sonicated for 10 min, 3% of H_2O_2 was added and sample was stored at room temperature for 24 hrs. The volume was made up to the mark with methanol and the sample was then diluted to give final concentration of 50 µg/ml.

3.11 Photolytic degradation studies

10 mg of drug was weighed and transferred it to 100 ml volumetric flask. About 30 ml of methanol was added and sonicated for 10 min. The volume was made up to the mark with methanol. And then the sample was kept under UV light with 200-400 nm for 24 hrs. The sample was then diluted to give final concentration of 50 µg/mL.

3.12 Thermal degradation studies

10 mg of drug was weighed and transferred to 100 ml volumetric flask. About 30 ml methanol was added and sonicated for 10 min. The volume was made up to the mark with methanol. The sample was then kept in hot air oven at 60^0C for 24 hrs. The sample was then diluted to give final concentration of 50 µg/ml

4 RESULTS AND DISCUSSION

4.1Method development and optimization

Suitable column and solvent system for the analysis of voglibose were selected based on the peak parameters after screening. For the mobile phase selection Methanol: Water in different ratios such as 80:20 %v/v, 50:50 %v/v, 60:40 %v/v, 90:10 %v/v, were selected for the screening study on C18 columns at pH 3.0 (adjusted with o-phosphoric acid). The flow rate and wavelength were optimised using different screening combinations of 0.8mL/min at 244nm and 206nm,

0.9mL/min at 206nm and 1.0mL/min at 250nm and 221nm.

Based on the result obtained from screening of various mobile phase compositions, columns, flow rate and wavelength, the Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron) column and Methnol:Water at 90:10 %v/v at pH 3 as mobile phase with ion pairing reagent (1-hexane sulfonic acid) and 1.0 mL/min flow rate at 221nm wavelength with 20µL of injection volume were selected for the analysis of voglibose

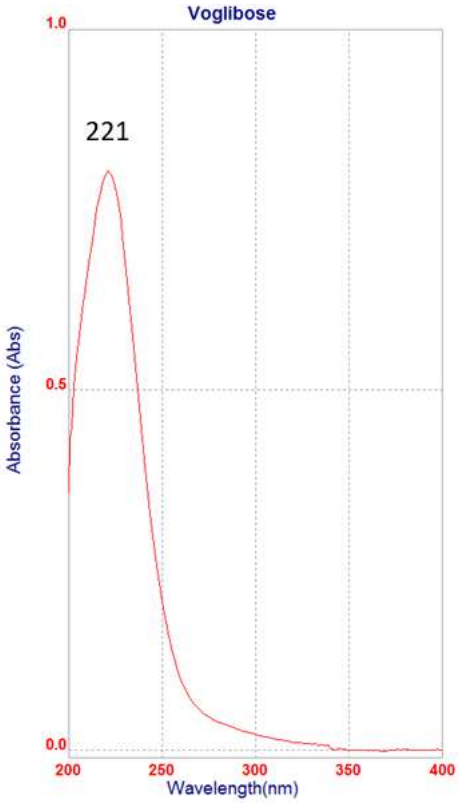


Fig. 2 UV spectra of voglibose (scanned for 200-400nm).

4.2Method validation

System suitability

System suitability was achieved by checking various parameters and found within the ICH Q2 (R1) limit.

Table 1 System suitability parameters

Parameter	Voglibose
Calibration range	10-50µg/mL
Theoretical plate	8267
Tailing Factor	1.09
LOD	0.4µg/mL
LOQ	1.22µg/mL

Linearity

Voglibose shows linearity in the range of 10-50µg/mL, and the regression co-efficient was found to be 0.9996.

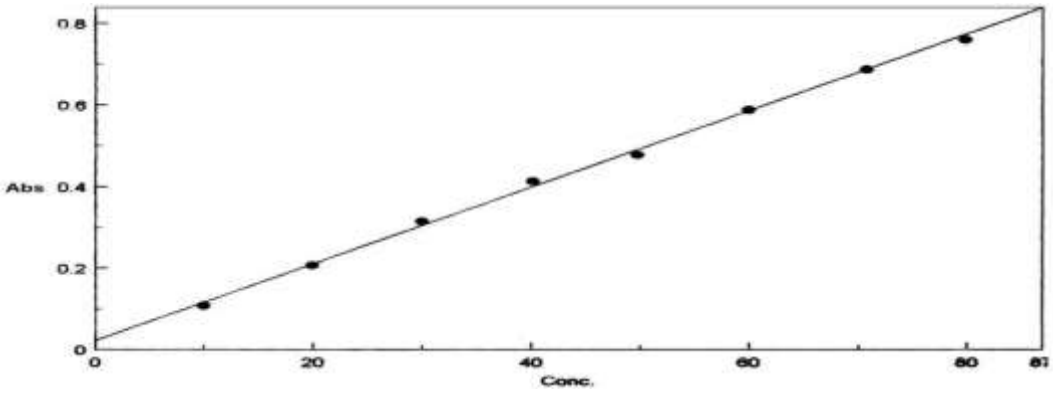


Fig. 3 Linearity of voglibose

Table 2 Validation Parameters

Parameters	Voglibose
Linearity range	10-50µg/mL
Correlation coefficient	0.9996
Slope	37840
Y-intercept	220288

Recovery

Recovery studies were carried out at 50%, 100% and 150% levels and the results were found to be within the limits mentioned as per ICH guidelines.

Table 3 Recovery studies

Level	% Recovery	%RSD
50%	98.83	0.75
100%	98.76	0.90
150%	100.71	0.64

Precision

The intraday and interday precisions were performed for the concentration of 30µg/mL and the % RSD values were found to be within 1%.

Table 4 Interday precision results

Interday	Peak area	Mean	SD	%RSD
	1337621			
Day 1	1313954	1324756	11968	0.90
	1322694			
	1344475			
Day 2	1317528	1327151	15034	1.13
	1319450			

Table 5 Intraday precision results

Day	Peak area	Mean	SD	%RSD
	1337621			
Morning	1313954	1324756	11968	0.90
	1322694			
	1327352			
Evening	1306815	1316214	10378	0.79
	1314474			

Formulation analysis

Analysis of formulation was performed for voglibose tablet 0.3 mg, and the percent assay (label claim) was found to be 99.

Table 6 Analysis of formulation

Amount of drug (mg/tab)		%Label claim
Labelled	Estimated	
0.3 mg	0.2975 mg	99.1731

4.3Forced degradation study

Standard samples of voglibose were prepared at a concentration of 50µg/mL and exposed to the acid, base, oxidative, photolytic and thermal degradations. The % assay was performed to determine the amount of pure drug remained after the degradations.

In acidic stress condition, treatment of voglibose solution with 0.1N HCl at 60^0C for 1 hour showed about 7.85% of actual degradation and % assay was about 92.14. In basic stress condition, treatment of voglibose solution with 0.1N NaOH at 60^0C for 1 hour showed about 9.85 % of actual degradation and %assay was about 90.15 and one degradation product was observed. In oxidative degradation, treatment of voglibose solution with 3% v/v hydrogen peroxide at room temperature for 24 hours. The drug showed 1.75% actual degradation and % assay was found about 98.25. In photolytic degradation, after exposure of voglibose solution to UV light at 200-400nm wavelength, 1.23% of actual degradation was found. The % assay was found to be 98.76. No degradation product was observed. In thermal stress condition, the treatment of voglibose solution kept at 60^0C for 24 hours showed 1.50% of actual degradation and % assay was about 98.50. No degradation of drug was observed. The results after each stress condition are shown in the table below.

Table 7 Forced degradation study of voglibose

Sr. No.	Degradation	Area of Standard	Area of Degraded Sample	Degraded up to %	Actual % Degradation
1	Acid Degradation	2123562	1956754	92.14	7.86
2	Base Degradation	2123562	1914469	90.15	9.85
3	H_2O_2 Degradation	2123562	2086402	98.25	1.75
4	Photolytic Degradation	2123562	2097365	98.76	1.24
5	Thermal Degradation	2123562	2091678	98.50	1.50

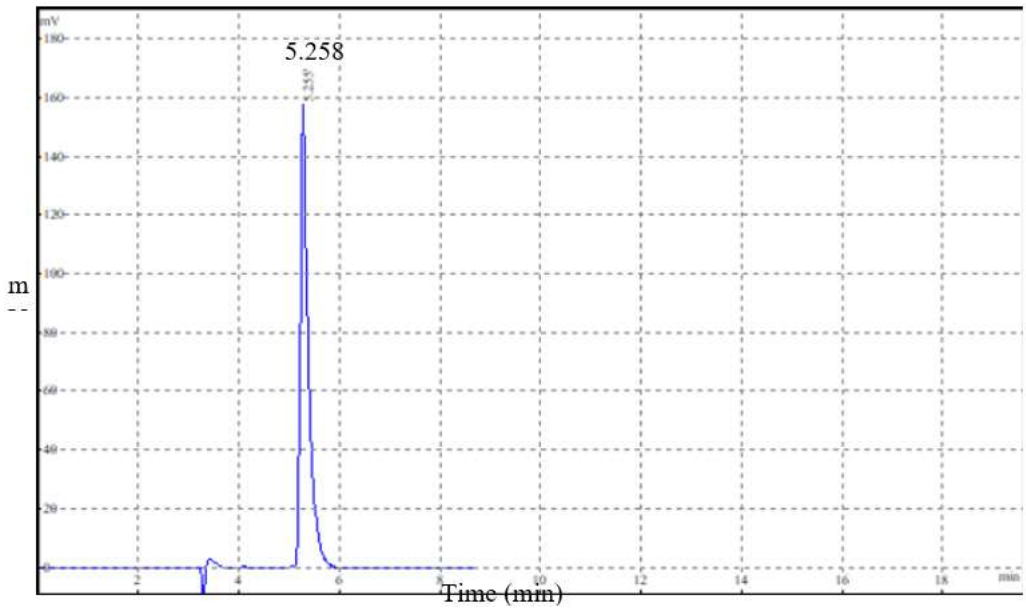


Fig. 4 A typical chromatogram of untreated standard solution of voglibose (50µg/mL)

Acid degradation

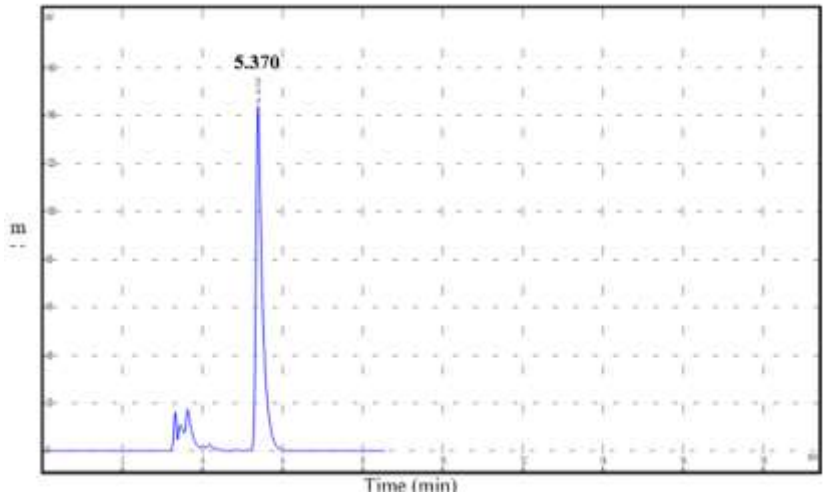


Fig. 5 A typical chromatogram of voglibose solution treated with 0.1N HCl at 60ºC for 1 hr**.**

Base degradation

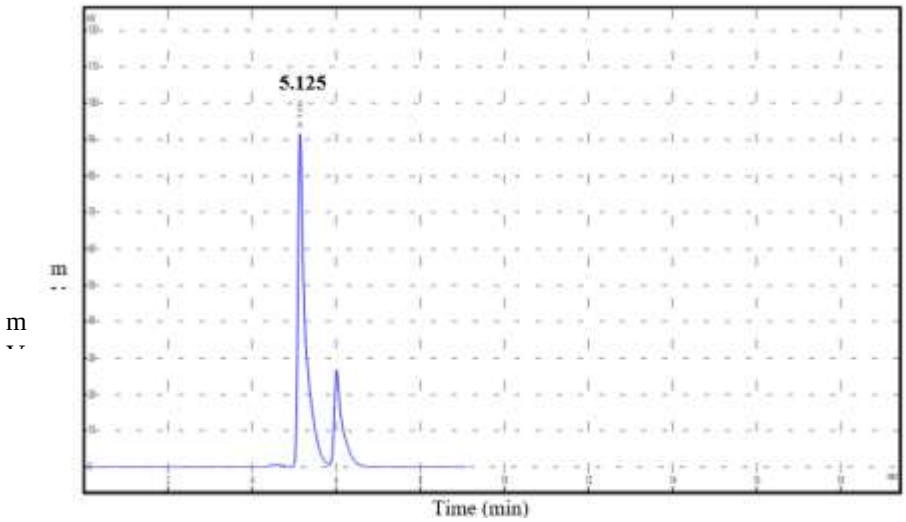


Fig. 6 A typical chromatogram of voglibose solution treated with 0.1N NaOH at 60ºC for 1 hr.

Oxidative degradation

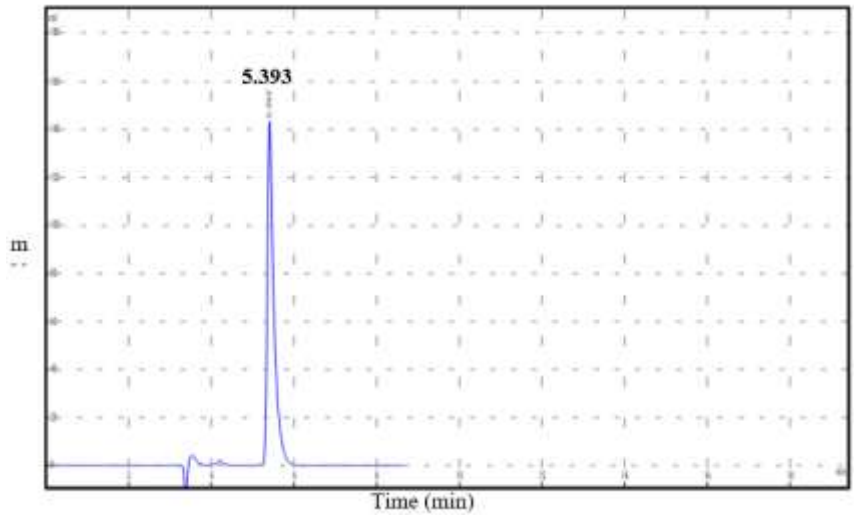


Fig. 7 A typical chromatogram of voglibose solution treated with 3% H_2O_2 at RT for 24 hr.

Photolytic degradation

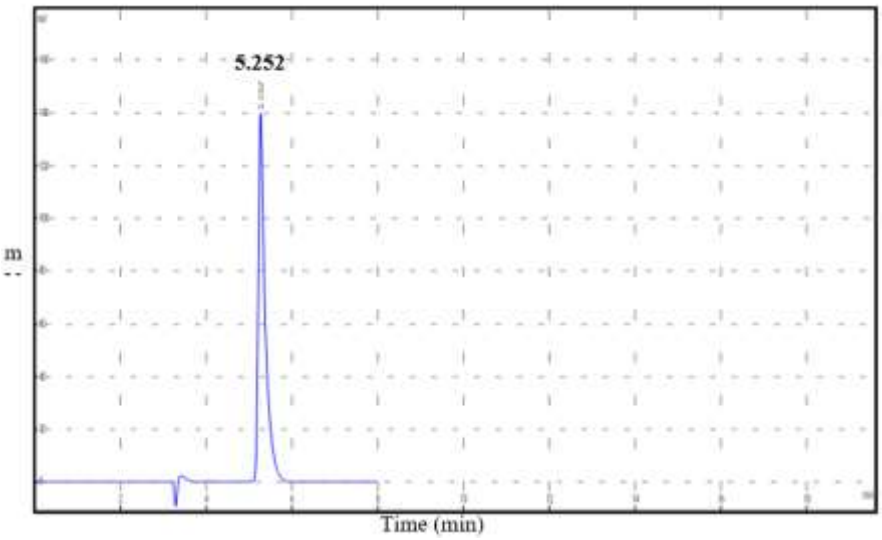


Fig. 8 A typical chromatogram of voglibose solution exposed for 24 hrs.

Thermal degradation

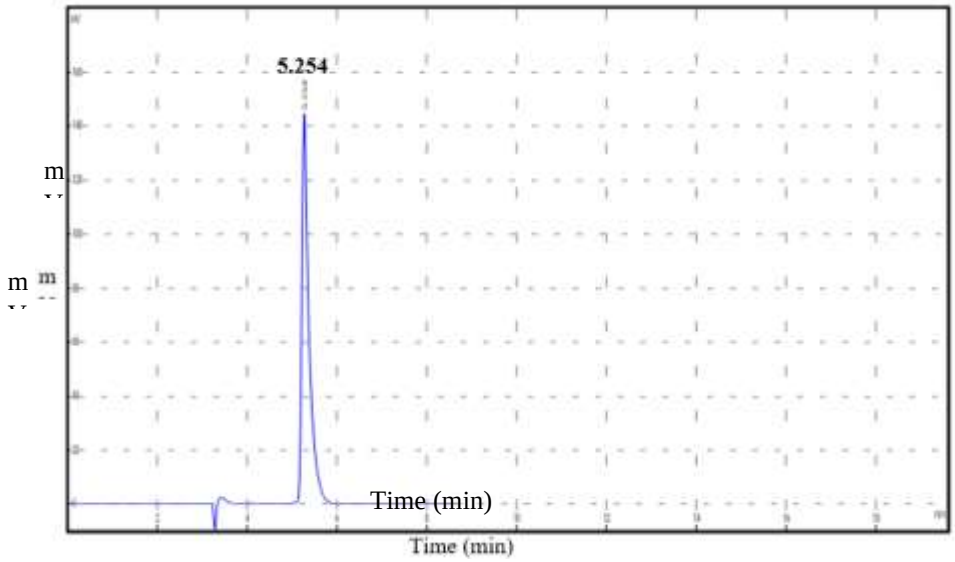


Fig. 9 A typical chromatogram of voglibose solution exposed at 60ºCfor 24 hrs.

5 Conclusion

The analysis of voglibose by direct HPLC method is difficult due to its ability to show UV absorption at lower wavelength region. The proposed RP-HPLC method with ion pairing reagent, 1-hexane sulfonic acid for estimation of voglibose was found to be accurate, precise and easier compared to other reported methods where voglibose was needed to be derivatized to increase the HPLC sensitivity. The method was completely validated showing satisfactory data for all the method validation parameters tested. As the method was able to separate the parent drug from the degradation products, wherever formed, it can be used as a stability indicating method for voglibose.

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