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Method Development and Validation for Determination of Voglibose in Tablet Formulation Using LC-MS/MS

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Abstract: Voglibose is a potent α glucosidase inhibitor, used for the treatment of diabetes mellitus. For quantitative determination of voglibose in pharmaceutical formulations of low doses, simple, sensitive, accurate and precise LC-MS/MS method using electrospray ionization in positive mode was developed and validated. The method was found linear in the concentration range of 25.0-1200 ηg/mL with a correlation coefficient of 0.9998. The limit of detection (LOD) of the method was found to be 1.5 ηg/mL and limit of quantitation (LOQ) was achieved at 3.0 ηg/mL. The recoveries of voglibose from spiked samples at different concentration levels were found in the range of 98-102%. The proposed method was found suitable for quantitation of voglibose and for the determination of uniformity of content of the dosage units of the tablet formulations.

Keywords: Voglibose, LC-MS/MS, α-Glucosidase inhibitor, Multiple reaction monitoring

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, mitglitol & voglibose[5]. Voglibose is the safest and most effective of them all. It is most commonly available in the form of tablets with the dosages of 0.2 mg to 0.3 mg per tablet.

Structure of voglibose is similar to that of carbohydrate[6]. Inspite of the fact that voglibose has already been established as an important pharmaceutical active, the already published work on voglibose, describes this molecule in different manner[7-9]. A number of ways in which structure of voglibose has been described till now is shown in Figure 1. The authors of this paper propose the description of the structure of voglibose as per the IUPAC system of nomenclature *i.e.* [1S, 2S, 3R, 4S, 5S]-5-[1,3-dihydroxypropan-2-ylamino]-1-[hydroxymethyl] cyclohexane–1,2,3,4-tetraol[8] (Figure 1, corresponding with structure A). For determining the content of voglibose in the formulations, not many analytical methods have been reported[10-13].

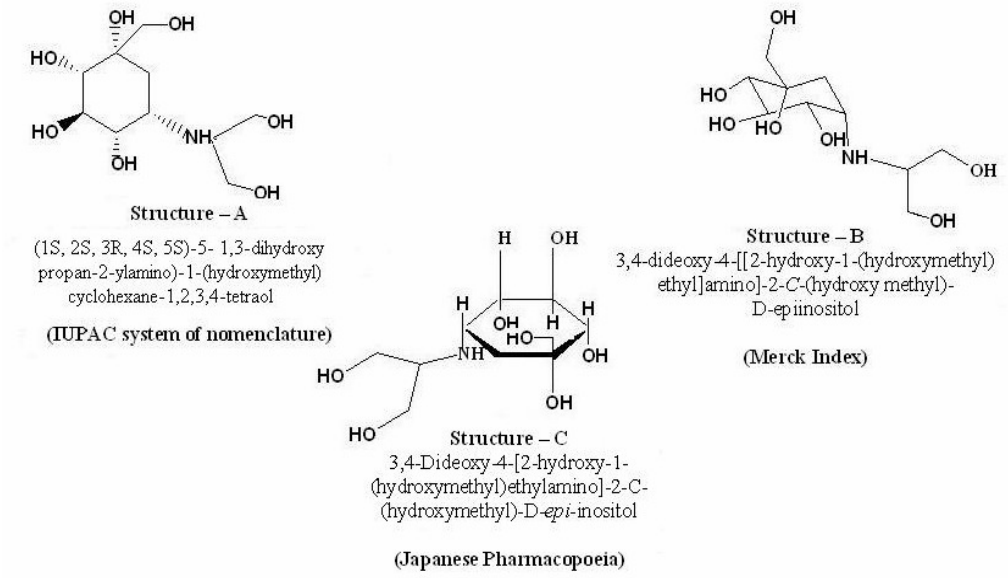

Figure 1. Chemical structures of voglibose as per different publications

Since the molecule of voglibose neither has a chromophore nor any fluorescent groups in its structure, its detection using the available HPLC detectors has always been a challenge. All these reported methods suffer from the fact that they are not only cumbersome but they are also not reproducible. The LC-MS methods reported, though validated and sensitive up to concentration levels of 5 ηg/mL[12], they are based on only a single ion recording of the parent ion [SIR, *m/z* 268.1] using electron spray ionization in positive mode. Therefore, the existing methods need to be improved to take care of the deficiencies. The present paper deals with the development and validation of an analytical method using liquid chromatography-tandem mass spectrometry [LC-MS/MS] for the quantitative determination of voglibose in its formulations. An attempt has been made to develop the method using isocratic system of elution without use of any buffers in the mobile phase system.

Experimental

Reference standard of voglibose (purity of 99.2%) was procured from Sigma-Aldrich. Methanol and water both of liquid chromatographic grade were procured from Merck, India. Formic acid, AR grade was procured from s.d. fine chemicals Ltd., India. Tablets from six

different batches of voglibose, three coded as A, B and C with dosage of 0.2 mg per tablet and the other three coded as D, E and F with dosage of 0.3 mg per tablet manufactured by different pharmaceutical companies were procured from the local market.

LC-MS/MS system

Waters 2695 series alliance quaternary liquid chromatographic system [Waters, USA] with a triple quadrupole mass spectrometer, quattro micro API [Micromass, UK] equipped with an electrospray interface and masslynx 4.1 software [Micromass, UK] for data acquisition and processing was used. The instrument was provided with a 120 vial capacity sample management system.

Chromatographic column

Separation and analysis of the analyte was carried out on Waters X Terra MS C-18, 100 mmx2.1 id, 5 µm column

Preparation of mobile phase

The mobile phase was prepared by mixing two solutions *i.e*. solution A (1 mL of formic acid in 1000 mL of water) and solution B (1 mL of formic acid in 1000 mL of methanol) in the ratio of 50:50 and filtered through 0.45 micron filter membrane using the Millipore filtration unit.

Preparation of standard stock solution [S] 10 µg/mL

Approximately, 1.00±0.05 mg of reference standard of voglibose was accurately weighed into a 100 mL volumetric flask. About 60 mL of methanol was added and the solution was sonicated in the sonicator for 30 minutes. After sonication the volume was made to mark with methanol. This gave a standard stock solution having concentration of 10 µg/mL (solution S). The solution was stored at 2-8 °C. From the standard stock solution S, a series of standard calibration solutions of 25 ηg/mL, 50 ηg/mL, 150 ηg/mL, 300 ηg/mL, 600 ηg/mL, 900 ηg/mL and 1200 ηg/mL were prepared. All the calibration standards were injected into LC-MS/MS for preparation of the calibration curve.

Preparation of sample solution for determination of voglibose content in formulations

20 Tablets of voglibose were taken, weighed accurately and crushed to obtain sample powder. From the sample powder, amount equivalent to 0.3 mg of voglibose was accurately weighed and transferred to 100 mL volumetric flask. About 60 mL of methanol was added and the sample solution was sonicated for about 30 minutes and then made to volume using methanol. This solution was further shaken thoroughly and filtered through syringe filter. 2.0 mL of this filtrate was diluted to 10 mL to obtain a solution having final concentration of about 600 ηg/mL. This solution was injected into LC-MS/MS for the determination of content of voglibose in the formulations. The sample solutions for all the six tablet formulations were prepared in the similar way.

Preparation of sample solution for determination of uniformity of content in the individual dosage units of the formulation

Uniformity of content indicates the consistency of active content within individual units with little variation[14] (allowed variation ±15% of the claim value) among tablets within a batch. For determination of active content of the individual dosage units, 10 tablets from a same batch of a formulation were selected randomly and transferred to 10 individual 100 mL

volumetric flasks. Approximately 60 mL of methanol was added to each flask and the contents were sonicated for 30 minutes. The solution in all the flasks was then made to the mark using methanol. From each sample solution, an aliquot of about 5 mL was filtered through syringe filter and 2 mL of the filtrate was diluted to 10 mL with methanol so as to give a solution having concentration of 600 ηg/mL. This way ten different solutions obtained were injected individually into LC-MS/MS to determine the content of the 10 individual dosage units. Uniformity of content was determined for all the 6 tablet formulations (A to F) as per this method.

LC condition

Analysis of voglibose in pharmaceutical formulation was carried out on Waters X Terra MS C 18, (100 mmx2.1 mm id, 5 µm) column maintained at 30 °C using the mobile phase comprising of mixture of two solutions *i.e* A and B (50:50) as described above. Mobile phase was delivered at flow rate of 0.2 mL/minutes. The chromatographic injection volume was 20 µL. Under these conditions, the conditions, the retention time of voglbose was about two minutes.

MS conditions

The MS acquisition parameters were as follows: Ion polarity: positive ion mode; ion source type: atmospheric pressure electrospray ionisation; capillary voltage (kV): 3.00; cone voltage(V), 30.0; source temperature (°C): 120; desolvation gas flow (l/h) : 700; RF lens: 0.4; desolvation gas temperature (°C): 350. A mass spectrum was over a scan range of *m/z* from 50 Da to 500 Da for MS/MS mode. Product ion scan mass spectra of protonated molecule of voglibose was acquired in the mass range of 50Da-500Da. Two different characteristic fragments *i.e.* 268.1>73.86 were monitored for voglibose in the multiple reaction monitoring mode [MRM] using a dwell time of 100 milli second and collision energy of 30 v.

Results and Discussion

A simple, sensitive, accurate and precise LC-MS/MS method using electrospray ionization in positive ion mode was developed for the quantitative determination of voglibose in pharmaceutical tablet formulations at very low concentrations voglibose.

Analysis of formulation for voglibose content

Figure 2, 3 and 4 show the LC-MS/MS chromatograms for the solvent blank, standard solution (600 ηg/mL) and the sample solutions for the six tablet formulations with dosages of 0.2 mg per tablet (A, B and C) and 0.3 mg per tablet (D, E and F). The chromatograms for both *i.e.* the standard (Figure 3) and the various samples *i.e.* A to F (Figure 4) show a well resolved symmetric peak for voglibose (RT: 1.06) with no interferences from the matrix. Voglibose content was calculated in each of the formulations [analyzed in triplicate] and the results are tabulated in Table 1. The results were found to be within the range of 1.1% to 1.4% for the six formulations and in agreement with the claim value of 0.2 mg and 0.3 mg respectively within acceptable limits of pharmacopoeial requirements of ±10%. The % RSD for the assay determination for different tablets varied between 1.1% to 1.4%, which is well within the acceptable norms.

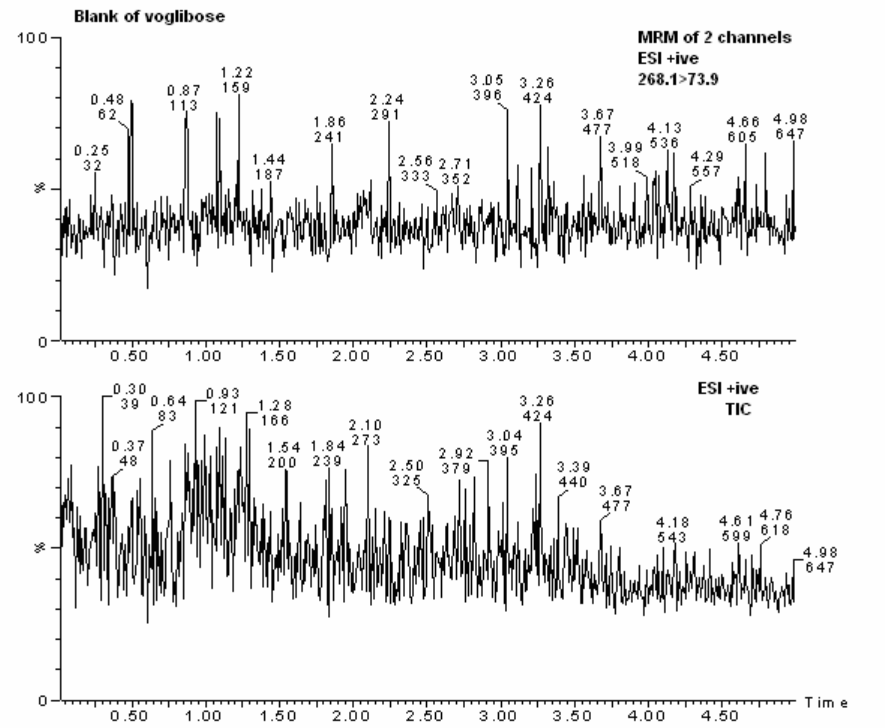

Figure 2. LC-MS/MS chromatogram of voglibose blank

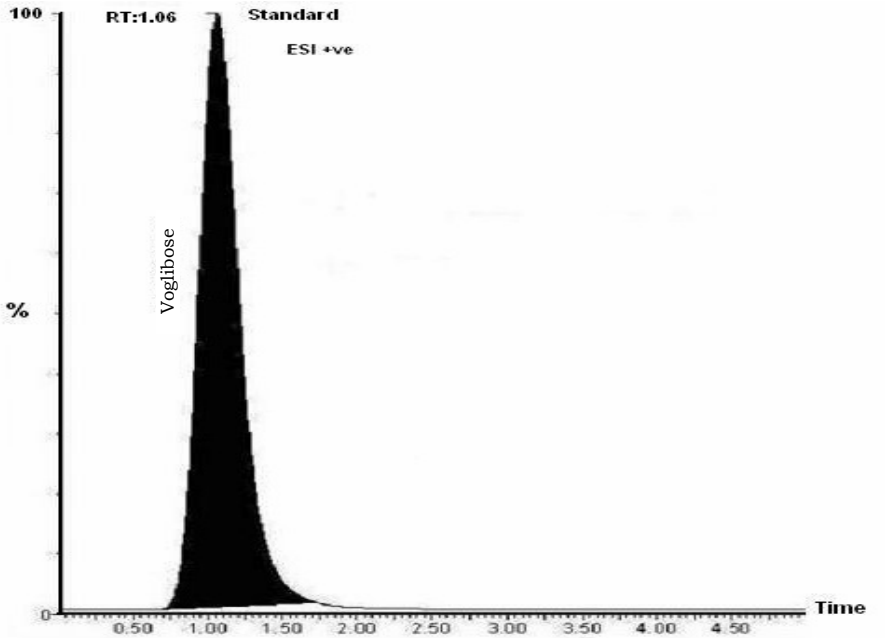

Figure 3. LC-MS/MS chromatogram of standard solution

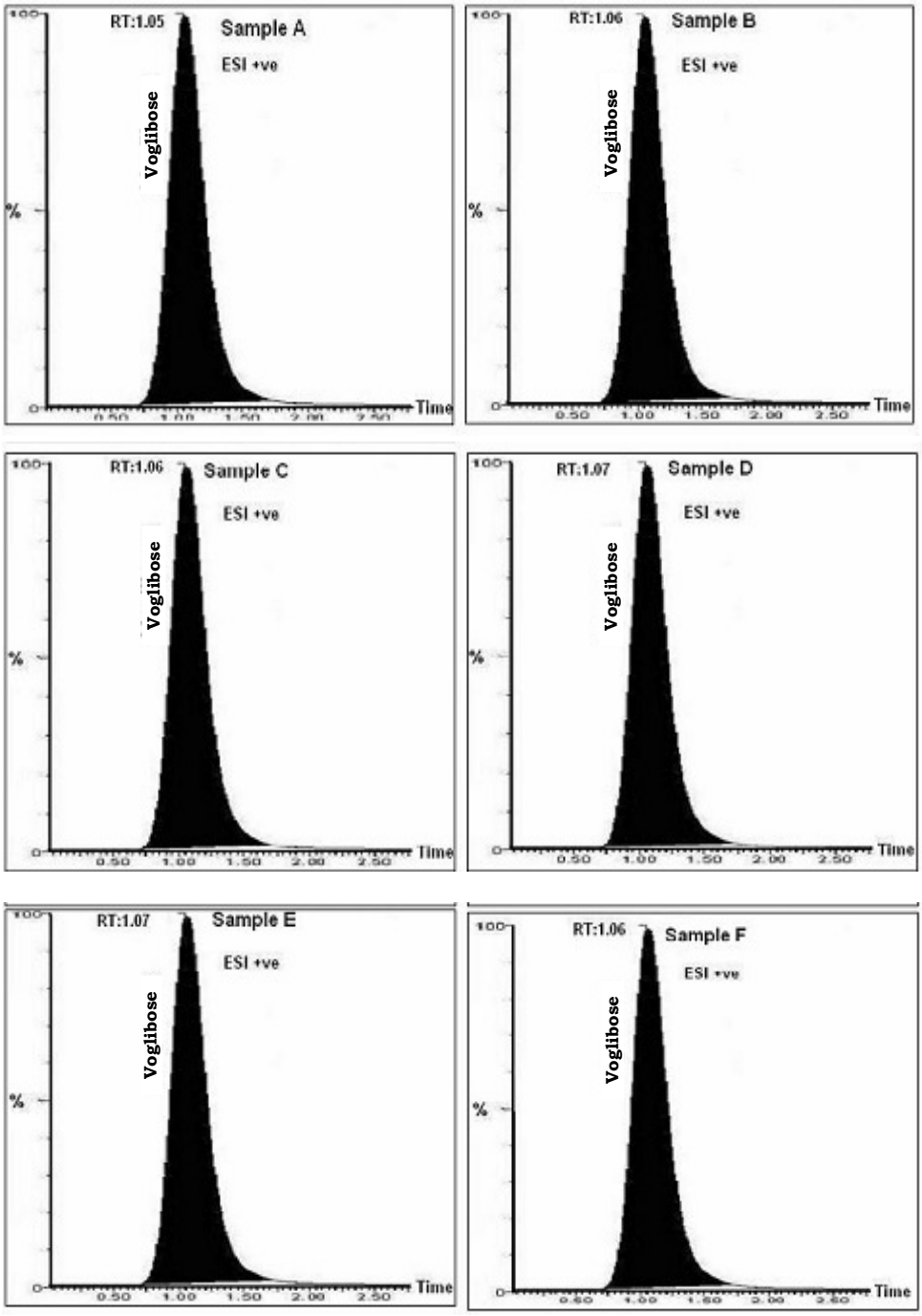

Figure 4. LC-MS/MS chromatogram of six formulations of voglibose (A to F)

Table 1. Results obtained for voglibose assay for the six formulations using LC-MS/MS

Sample number	Label claim, mg	Observed average content, mg (n=3)	%RSD
A	0.200	0.199	1.1
B	0.200	0.201	1.2
C	0.200	0.201	1.1
D	0.300	0.301	1.2
E	0.300	0.301	1.4
F	0.300	0.300	1.1

Determination of uniformity of content

On analysis of ten individual dosage units from the same batch for determination of uniformity of content of voglibose, the results were found to be in the range of 0.198 to 0.201 mg for the 0.2 mg tablet and 0.298 to 0.301 for the 0.3 mg tablet, which is well within the acceptable criteria of the pharmacopoeial requirement of ±15%. The results are shown in Table 2. Thereby suggesting that the tablets were of good quality.

Table 2. Results obtained for uniformity of content for the six formulations using LC-MS/MS

Sample number	Label claim, mg	Observed uniformity of content, mg (n=10)	Allowed variation from label claim, mg (as per USP)	%RSD
A	0.200	0.198 - 0.201		1.0
B	0.200	0.198 -0.200	±15% *i.e.* 0.170-0.230	1.1
C	0.200	0.199- 0.201		0.9
D	0.300	0.298 - 0.300		1.0
E	0.300	0.299 - 0.301	±15% *i.e.* 0.255-0.345	1.1
F	0.300	0.298 – 0.301		1.2

Method development

The liquid chromatographic separation and quantitation was achieved using isocratic system of elution with no salts used in the mobile phase. A well resolved peak for voglibose was obtained within two minutes of the injection. Other chromatographic conditions, especially the composition of the mobile phase were optimized through several trials to achieve good resolution with symmetric peak shapes and a linear response for concentration. Different volumes of methanol - water and acetonitrile-water were tried as the mobile phase, along with additives like acetic acid and formic acid of varying concentrations in order to get a good peak shape. It was observed that a mixture of methanol with 0.1% formic acid and water with 0.1% formic acid in the ratio of 50:50 was the most suitable mobile phase, Figure 5. The use of X-Terra MS C-18 column [100 mmx2.1 mm i.d., 5 µm particle size] helped in separation of voglibose with no interferences from the matrix of the formulations and elution of voglibose at 1.06 minutes. A flow rate of 0.2 mL/ minute produced good peak shape and enabled completion of run within 4 minutes.

The inherent selectivity of MS/MS detection was also expected to be beneficial in developing a selective and sensitive method. The present study was conducted using electrospray ionization (ESI) because it gave high intensity for voglibose and good linearity for the regression curve. Initially the parent and product ions were optimized by infusing a 600 ηg/mL standard solution of voglibose in both the positive and the negative polarity modes. The intensity was much higher in the positive mode due to protonation. Also the use of formic acid in the mobile phase (Figure 6), further augmented the response of the protonated precursor $[M+H]^+$ ions at *m/z* 268.1 in the Q1 MS full scan spectra given in Figure 7.

The most abundant product ion at *m/z* 73.9 was found by applying collision energy of 30 eV. The MRM parameters were suitably optimized to obtain a consistent and adequate response for the analyte. Quantitation was performed using multiple reaction monitoring mode to study parent-to-product ion transition for voglibose [*m/z* 268.1> 73.9].

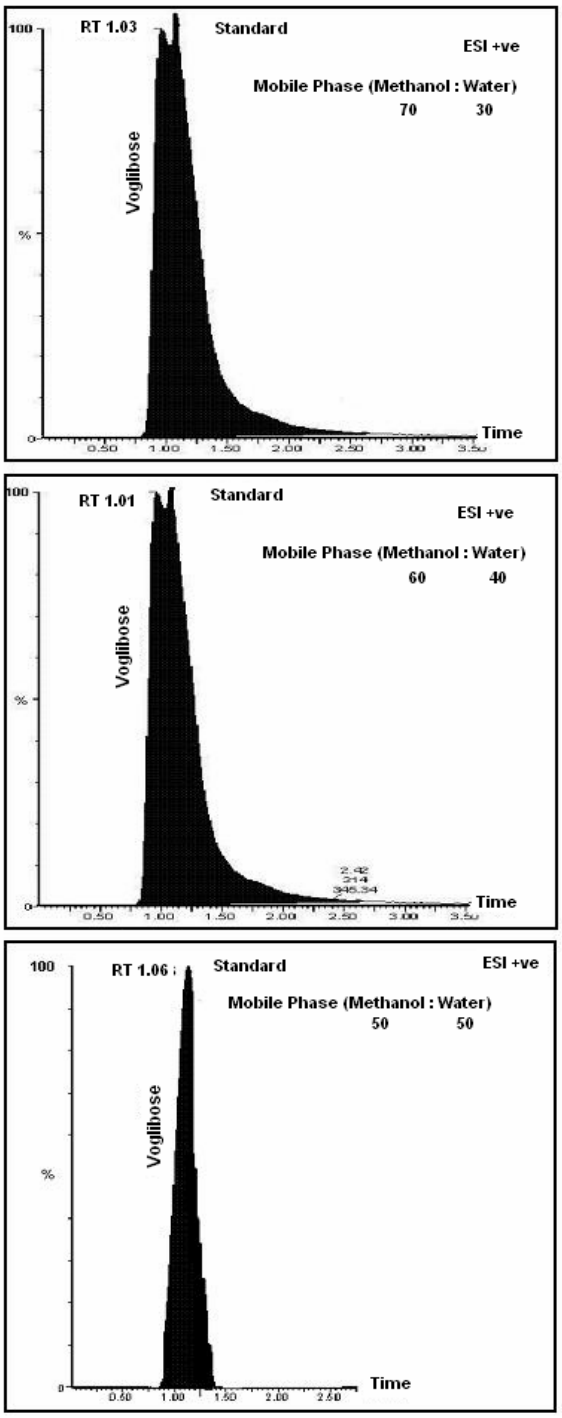

Figure 5. Optimization of mobile phase for determination of voglibose by LC-MS/MS phases

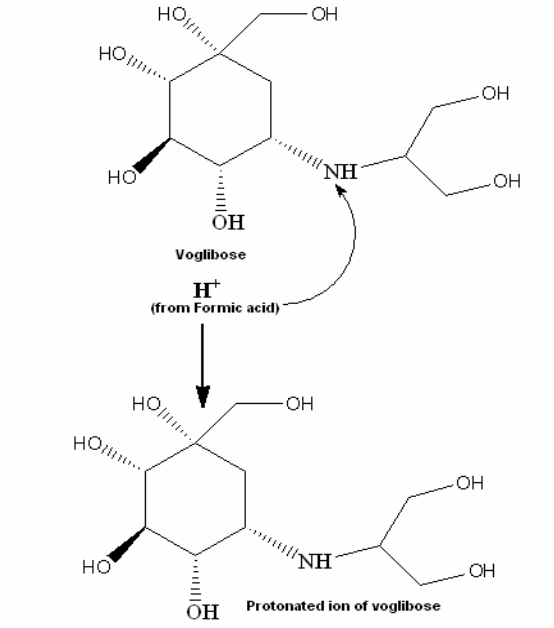

Figure 6. Role played by formic acid in protonating the molecule

Representative mass spectra of parent (A) and product ion (B) for voglibose are given in Figure 7 and 8 respectively. Figure 9 explains the fragmentation of voglibose parent ion into the first and the subsequent daughter ions, thereby accounting for identification of all the ion fragments which are seen in the mass spectra of the product ion, Figure 8.

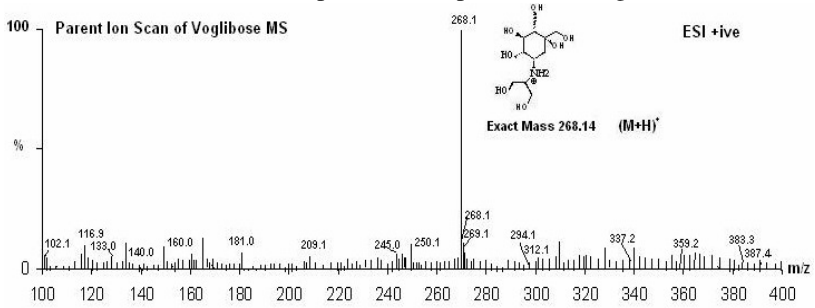

Figure 7. Mass spectra of parent ion for voglibose

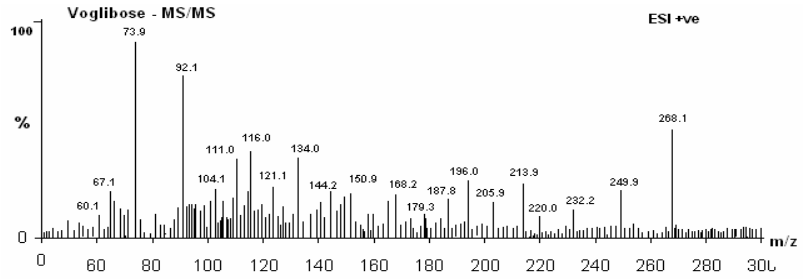

Figure 8. Mass spectra of product ion for voglibose

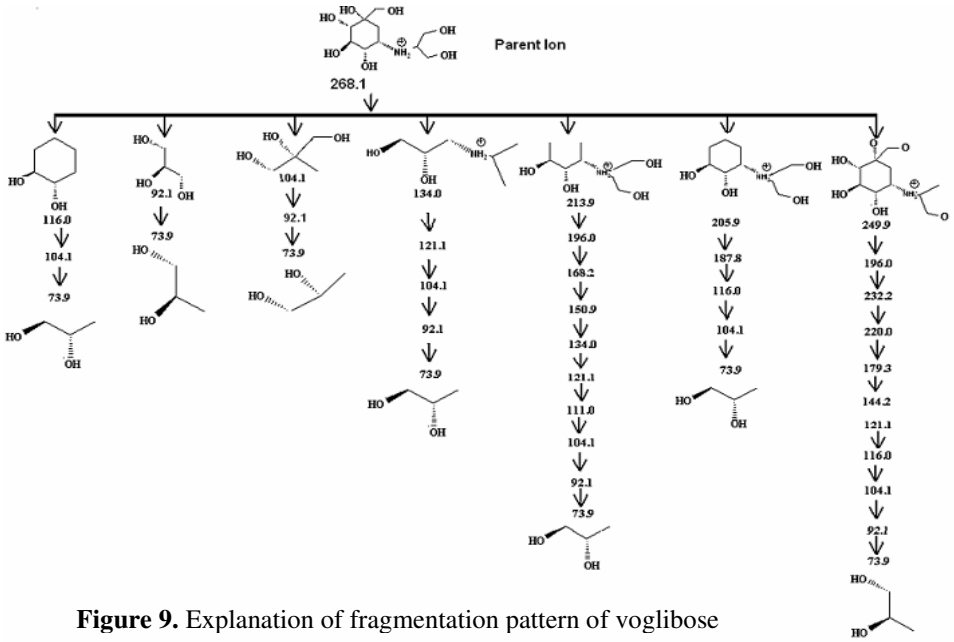

Figure 9. Explanation of fragmentation pattern of voglibose

Method performance characteristics

The method was validated for linearity, precision, accuracy, sensitivity, selectivity and robustness as per the guidelines of International conference on harmonization [ICH][15].

Linearity

The seven point calibration curve, Figure 10 plotted for voglibose concentration *vs* peak area response was linear over the concentration range of 25.0 ηg/mL to 1200.0 ηg/mL. All the calibration standards were injected in triplicate. The mean correlation coefficient [r^2] was calculated as 0.9998 indicating excellent linearity and the mean calibration equation was Y = 121.49x+1959.3.

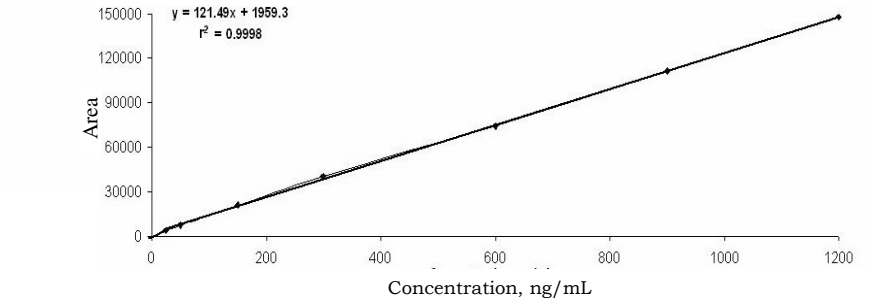

Figure 10. Calibration curve for voglibose (25 ηg/mL to 1200 ηg/mL)

Precision

The precision, evaluated as the repeatability of the method was studied on sample D (containing 0.3 mg voglibose) by calculating the percent relative standard deviation (%RSD) for six determinations each for four different solutions performed on the same day (Intra-day) and on the three different days (inter-day or intermediate precision) and by different analysts. The data for the precision studies for inter-day, intra-day are given in Table 3 while the between-analyst precision data is given in Table 4.

Table 3. Inter-day and intra-day precision studies for the determination of voglibose in sample D (containing 0.301 mg voglibose) using LC-MS/MS

Sample solution concentration, ηg/mL	Intra-day precision		Inter-day precision			
	Mean observed concentration (n=6)	% RSD	Days	Mean observed concentration (n=6)	Mean observed concentration (3 days)	% RSD
25.08	24.3	3.27	Day 1	24.8	25.2	3.15
			Day 2	25.7		
			Day 3	25.1		
150.5	147.3	2.31	Day 1	151.1	149.8	2.21
			Day 2	149.7		
			Day 3	148.5		
602	603.2	1.85	Day 1	598.2	600.7	1.68
			Day 2	602.5		
			Day 3	601.3		
1204	1210.2	1.22	Day 1	1208.9	1206.1	1.02
			Day 2	1207.5		
			Day 3	1201.9		

Table 4. Between-analysts precision data for the determination of voglibose in samples using LC-MS/MS

Sample solution concentration, ηg/mL	Analyst	Mean observed concentration (n=6)	Mean observed concentration for 3 days	%RSD
25	A	24.1	25.3	3.15
	B	26.1		
	C	25.6		
150	A	151.8	151.0	2.51
	B	148.6		
	C	152.5		
600	A	598.6	601.4	2.05
	B	603.5		
	C	602.1		
1200	A	1208.9	1205.1	1.05
	B	1205.2		
	C	1201.3		

The percent RSD value (<5%) obtained for inter-day, intra-day and between-analysts at four different concentration levels are within the acceptable norms, indicating that the proposed method is precise and can be adopted for analysis.

Accuracy /recovery

The accuracy of the method was evaluated by spiking different known concentrations of voglibose standard solution into the sample solution of two different preanalyzed tablet formulations of voglibose, A and D consisting of different dosages (0.2 mg and 0.3 mg tablets respectively). The sample solutions for both the formulations containing approximately 600 ηg/mL of voglibose were spiked with varying concentrations of voglibose standard solution *i.e.* 100 ηg/mL, 200 ηg/mL and 400 ηg/mL [each in triplicate],

so as to give a total concentration of 700 ηg/mL, 800 ηg/mL and 1000 ηg/mL of voglibose. The theoretical concentration level of the spiked solutions was compared with the observed concentration levels of the spiked solutions and the percent recoveries were calculated for each spiked level, Table 5. The percent recoveries obtained for different spiked levels ranged between 98% to 102% with %RSD not more than 4%, which is well within the acceptable range indicating the accuracy of the method.

Limit of quantitation [LOQ] and limit of detection [LOD]

The LOQ was taken as the lowest concentration of analyte in the sample that could be determined with acceptable precision and accuracy and the LOD was taken as the lowest absolute concentration of analyte in a sample that could be detected but not necessarily quantified. LOD was determined by considering signal to noise ratio (S/N) of 3: 1 for the strongest mass transition with respect to the background noise obtained from the blank sample, whereas LOQ was determined similarly by considering signal to noise ratio [S/N] of 6:1. Based upon the mean noise level for the six injections, lowest detection limit of the instrument was calculated as 1.5 ηg/mL. Limit of quantitation (LOQ) was obtained as 3.0 ηg/mL evaluated on the basis of minimum concentration for which a reproducible signal was obtained with %RSD less than 5 for five replicates.

Table 5. Recovery studies for the determination of voglibose using LC-MS/MS (Spiked in triplicate at each concentration levels)

Formulations	Original concentration, ηg/mL	Spiked conc., ηg/mL	Total conc., ηg/mL	Observed conc., ηg/mL	% Recovery	% RSD
A (0.2 mg voglibose tablet)	600	100	700	698	98	2.3
				702	102	
	600	200	800	698	98	2.5
				795	98	
				796	98	
				804	102	
	600	400	1000	1008	102	2.3
				1001	100	
				990	98	
B (0.3 mg voglibose tablet)	600	100	700	701	101	1.7
				698	98	
	600	200	800	698	98	2.1
				804	102	
				796	98	
				798	99	
	600	400	1000	1006	102	1.9
				998	100	
				991	98	

Selectivity

Selectivity of the method was determined in the presence of different ingredients used as excipients for formulations of voglibose tablets. The different excipients[16] *i.e.* lactose, corn starch, crystalline cellulose, low substituted hydroxypropyl cellulose and magnesium stearate were spiked one by one into the drug, homogenized thoroughly, dissolved and

brought into the solution form and analyzed using LC-MS/MS. No endogenous interferences were found at the elution time of voglibose (1.07 minute) thereby demonstrating that the method is highly selective and free from interferences due to the matrix components.

Robustness

Robustness of the method was determined by analyzing all the six formulations with change in some of the parameters: such as same column chemistry from different manufacturers, different analysts, different injection volumes, change in flow rate and change in the column temperature. Under all these conditions, the analytical values obtained for the voglibose content by the developed method remained the same within the acceptable limits (Table 6) with slight shift in the retention time.

Uniqueness of method

The LC-MS/MS method presented in this paper has certain edge over the previously reported methods *i.e.* the HPLC method with pre and post column derivatization technique and the LC-MS method.

It was observed that when HPLC method with either of the pre column or the post column derivatization techniques were tried, the results obtained were not highly reproducible. Percent recoveries ranging between 75- 110% were obtained.

Table 6. Data for the robustness studies for voglibose content in formulation by LC-MS/MS (sample analyzed in triplicate under changed conditions)

Parameters	Formulations, mg					
	0.2 mg voglibose tablet			0.3 mg voglibose tablet		
	1	2	3	4	5	6
Developed method	0.199	0.200	0.199	0.301	0.299	0.298
Change in operational parameters						
Column (Varian C18 (100 mm x2.1 mm i.d., 5 µ)	0.199	0.198	0.199	0.300	0.299	0.299
Column temperature (35 °C)	0.198	0.199	0.200	0.301	0.300	0.299
Analyst II	0.199	0.199	0.200	0.301	0.300	0.300
Flow rate (0.1 mL/ minute)	0.199	0.199	0.198	0.300	0.300	0.300
Injection volume, 25 µL	0.199	0.198	0.200	0.300	0.299	0.299
%RSD	0.21	0.38	0.41	0.18	0.18	0.25

Also the desired sensitivity of the method to undertake the test for uniformity of content of the dosage units could not be achieved since this particular test requires each dosage unit to be dissolved in 100 mL which results into low concentration of the final solution. The LC-MS method reported although is definitely much more sensitive as compared to the HPLC methods but the method uses buffer such as ammonium acetate in the solvent system. Presence of salts in the solvent system usually creates problems in both LC systems as well as LC columns unless proper precautions and use of adequate washing solvents is not carried out. Moreover, only the parent ion [*m/z* 268.1] is monitored which can be misleading at times if there are impurities having similar molecular weight are present or excipients having same molecular weight are used. Therefore all these factors led the authors to develop method using LC-MS/MS.

The uniqueness and advantages of the present LC/MS/MS method are in terms of simplicity of the method with no requirements of elaborate sample preparation, high sensitivity that can always be used for determining even the lowest concentrations present in the sample. The monitoring of parent to product ion transitions for voglibose [*m/z* 268.1 to 78.86] using triple quadrupole in the MRM mode further enables confirmation of the identity of the compound present at very low concentrations.

Therefore, unlike the earlier reported method based on LC-MS method where only the parent ion at *m/z* 268.1 is observed, in the present method the further confirmation by the daughter ion makes the method suitable for measurement of voglibose at lower detection limits besides improved reproducibility and reliability of results specially when results are calculated for residual amount of voglibose. Further more, the confirmation by daughter ion has made this method suitable for complex matrices like body fluids *etc*.

Conclusion

The developed LC-MS/MS method for the determination of voglibose in tablet formulations is simple, rapid, sensitive, accurate and precise and can easily be adopted for routine measurements. The method, besides being applicable for the assay of voglibose has also been found applicable for determining the uniformity of content of the dosage units. The method being highly sensitive can also be used for determination of voglibose in clinical samples such as blood plasma or urine for the purpose of bioavailability studies although the extraction procedure of voglibose from the clinical samples would need to be standardized

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