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A novel spectrophotometric method for the determination of voglibose in pharmaceuticals using potassium ferricyanide-Fe (III) as chromogenic agent

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Abstract

The following research presents a simple, fast, accurate and sensitive visible spectrophotometric method for the estimation of voglibose using potassium ferricyanide-Fe(III) as a reagent. It was determined that a reduction to Fe(II) was carried out from Fe(III) using voglibose with the formation of a Prussian blue colored solution as K-Fe (III) [Fe (II) $(CN)_6$] in which the λ_{max} was found at 775 nm. To verify the analytical approach, i.e. simplicity, accuracy, precision, LOD and LOQ , a study was conducted from the parameters established according to the ICH guideline. Drug exhibited distinct λ_{max} in Ferric chloride, Potassium ferricyanide at 775 nm. Linearity was observed with a 1-5 µg/mL range of concentration with correlation coefficient (r^2) 0.999.The estimated amount of drug in tablets is in good-agreement as per the claims written on the label. This technique is statistically validated as well as the recovery percentage of the drug by visible spectrophotometric method was found to be 98.5%-100.7%. The proposed methodology followed to estimate the Voglibose in bulk and dosage form in tablet is economical and sensitive.

Keywords: Voglibose; Ferric chloride; Potassium ferricyanide; Visible-Spectrophotometric method

1. Introduction

An alpha-glucosidase inhibitor called the Voglibose is applied to lower the level of blood sugar post-prandial in patients with the condition of Diabetes Mellitus [1]. Structure of voglibose (Fig.1) is [5-(1, 3-dihydroxypropane-2-yl -amino)-1-(hydroxymethyl) cyclohexane-1, 2, 3, 4-tetrol] similar to that of carbohydrate. It helps in delaying the uptake of glucose, and hence helps in reducing the complication risk of macro vascular conditions. A Japanese company named Takeda Pharma fist researched and released the product Voglibose. PPHG elaborated as the Post-Prandial Hyper-Glycemia is caused primarily by the first stage of insulin secretion. The inhibitors of Alpha Glucosidase delay the uptake of glucose on intestine levels and thus prevents an abrupt rise in glucose post eating. A total of three drugs are included in this class, Voglibose, Acarbose, and Miglitol, among which the most recently researched is Voglibose. Scores of this product is more than the other two drugs in terms of the profile of side-effects [2]. However, in terms of efficiency, Acarbose has an edge over Voglibose.

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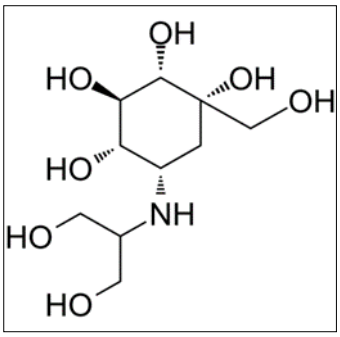


Figure 1 Voglibose Structure

To access, the development of any new drug and its molecular formation, its analysis is highly crucial. An appropriate and valid technique must available in analyzing the drug(s) in bulk, from release dissolution studies and biological models to delivery drug delivery systems. In case one method suitable to its specific requirements is not available, it is necessary that a reproducible, precise, accurate, sensitive, and a simple method is developed to evaluate the sample of a drug. Literature survey reveals various analytical methods reported for estimation of voglibose in the API and pharmaceutical formulations include Ultra-violet spectrophotometric[3,4] , HPLC[5-7], LC-MS[8] and HPTLC[9]. There is no visible spectrophotometry technique followed to estimate the voglibose in pharmaceutical formulation. There were accurate and sensitive analytical methods available in literature for other drugs[10-69]. The main focus of this research is validating the method of visible spectrophotometry as per the guidelines of ICH[70]. Hence, the following research is carried out to validate a reproducible, precise, accurate, sensitive, and a simpler voglibose estimating method.

As a probe reagent, an electro-chemical K_3 [Fe (CN) $_6$] (Potassium Ferricyanide) was used to study the electro-chemical properties of electro active materials, like the reversible process of electrode reactions, absorption surface of an electrode area and the chemical reaction process. Because of the oxidation in potassium ferricyanide it has a highly significant part in chemiluminescence. Our research provides a novel approach to the estimation of voglibose levels with a system to detect the potassium Ferricyanide-Fe (III)(Guoa et al.,2009 , Litao et al.,2011 ,Rao et al.,2015) in pharmaceutical using the method of spectrophotometry. It was determined that a reduction to Fe (II) was carried out from Fe (III) using voglibose while an amount of potassium ferricyanide is present in the form K-Fe-III [Fe (II) $(CN)_6$] as soluble prussian blue is formed by the reaction of Fe (II) between *in situ* reactions with potassium ferricyanide. The method proposed here has higher sensitivity, simple operation, lower detection limitations, and less cost.

2. Materials and method

2.1. Materials

Absorbance measurements were made on LAB INDIA UV-VIS (UVWin5 Software v5.2.0.1104) spectrophotometer with double beam and a matched 1cm width of quartz cells.Ultra sonicator (Citizen Ultra sonicator, India) is used to sonicate the sample preperations. SHIMADZU AUX 220, a Digital weighing balance was used to weight the sample. Voglibose standard gifted from Sun Pharmaceutical Industries Limited, Mumbai, India. A commercial tablet of VOLIBO containing 0.3 mg of voglibose was purchased from local medical store. A Fe (III) solution, that is a 1% w/v $FeCl_3.6H_2O$ (SD Fine Chemicals Limited, Mumbai, India) solution was formed by dissolving 1 grams $FeCl_3.6H_2O$ in 100 mL 0.1N HCl (Merck India). The solution of 0.5% w/v potassium ferricyanide (SD Fine Chemicals Limited, Mumbai, India) was prepared by dissolving 0.5 gm of potassium ferricyanide in 100 mL of distilled water. A 0.1N HCl solution is prepared by diluting 8.5 mL Con. HCl to 1000 mL with distilled water.

2.2. Preparation of standard solution

A standard stock voglibose solution (1000 µg/mL) is formed by dissolving 10 mg of pure voglibose in 10 mL of 0.1N HCl in 10 mL volumetric flask. From this solution pipette out 1 mL and transferred into a 10 mL volumetric flask making up the total quantity up to 10 mL with 0.1N HCl to get (100 µg/mL).

2.3. Calibration Curve Preparation

Different aliquots (0.1, 0.2, 0.3, 0.4,0.5 mL) per 100 µg/mL standard solution of voglibose were transferred into 10 mL series of volumetric flasks. Afterwards, to each volumetric flask added 1 mL of 0.5% (w/v) potassium ferricyanide , 0.1

mL 1% (w/v) $FeCl_3.6H_2O$ and volume made up to the mark with 0.1N HCl. Then the solution was kept aside for 10 minutes at room temperature (27 °C), absorbance was measured at 775 nm using reagents as blank.

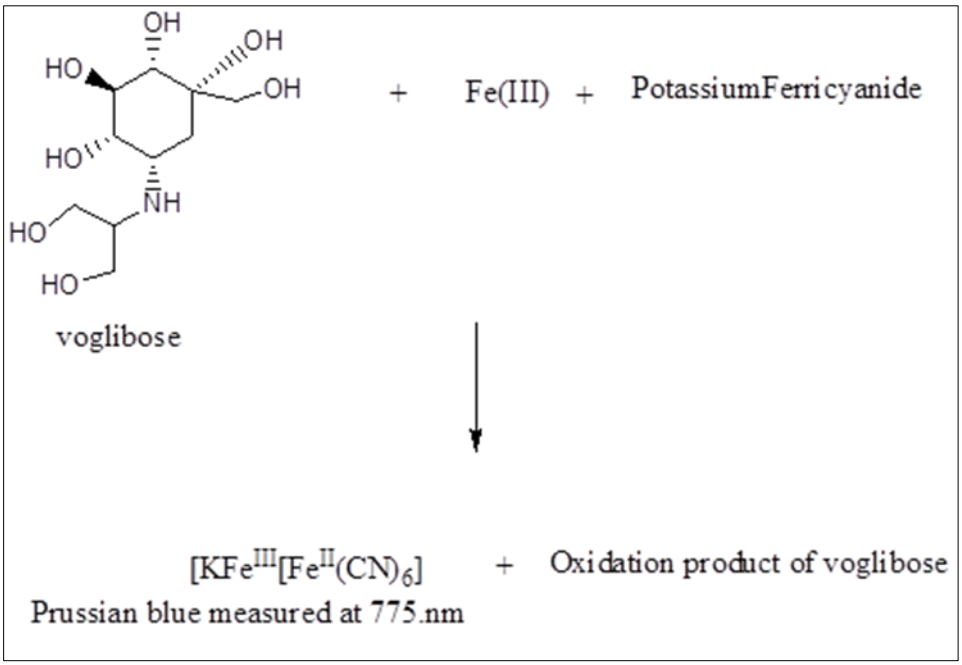
2.4. Procedure for sample preparation

Accurately 20 tablet weight individually and average weight was calculated. The tablets were crushed in motor and pestle to form a fine powder and a tablet, powder weight equivalent to 1.5 mg of voglibose is transferred into a 10 mL volumetric flask. To it little quantity of 0.1N HCl was added and sonicated for 10 min. The solution was then diluted up to mark using 0.1N HCl and are mixed thoroughly, after which the residue which is not soluble was removed using a Whatmann 42 filter paper. From the resulting solution 0.2 mL was pippeted out and added 1 mL of 0.5% (w/v) potassium ferricyanide and 0.1 mL 1% (w/v) $FeCl_3.6H_2O$ and volume was upto 10 mL with 0.1N HCl. Then the solution was kept aside for 10 minutes at room temperature (27 °C), absorbance was measured at 775 nm using reagents as blank.

3. Results and discussion

3.1. Basis of the reaction

The proposed method is based upon the oxidation of voglibose which the hydroxyl group and nitrogen present on it undergo oxidized. Voglibose oxidized by Fe(III) and that the corresponding oxidized and Fe(II) are produced in the reaction. Subsequently, the *in situ* formed Fe(II) reacts with potassium ferricyanide to form soluble prussian blue ($KFeIII[FeII(CN)_6]$). The resulted prussian blue colored complex showed maximum absorbance at 775 nm against the reagent blank. The absorption spectra of the prussian blue colored complex is shown in Fig.2.



Scheme 1 Reaction mechanism

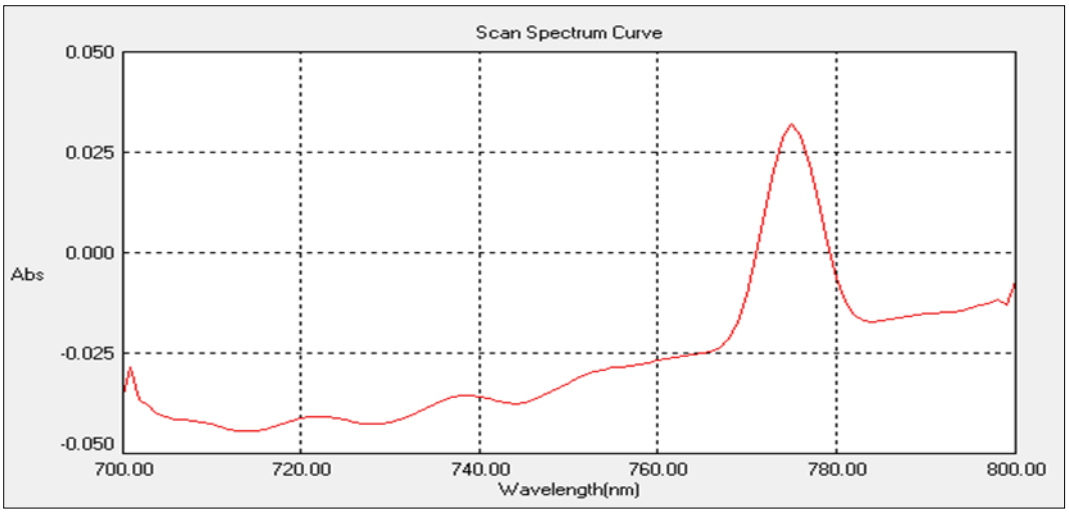


Figure 2 Visible spectra of voglibose using proposed method

3.2. Optimization of the experimental conditions

3.2.1. Influence of ferric chloride and potassium ferricyanide

The amount of ferric chloride ranging from 0.00 to 1.00 mL is presented to the proposed procedure, and the influence of ferric chloride on absorbance is shown in Fig. 4. Absorbance reaches its maximum when the amount of ferric chloride is 0.1 mL, and it does not change with any further by increasing amount of ferric chloride. This clearly indicates that all voglibose are oxidized by Fe(III), and the amount of both Fe(II) and the formed soluble prussian blue reaches their maximum as well.

The influence of the amount of potassium ferricyanide on absorbance has been discussed. From Fig. 4, it is found that then absorbance reaches its maximum when the amount of potassium ferricyanide is 1 mL. Obviously, the absorbance is kept at a constant when the amount of potassium ferricyanide is above 1 mL. This indicates that the amount of the formed soluble prussian blue reaches its maximum.

Consequently, 0.1 mL of 1% w/v ferric chloride and 1 ml of 0.5% w/v. potassium ferricyanide are chosen as the optimum condition.

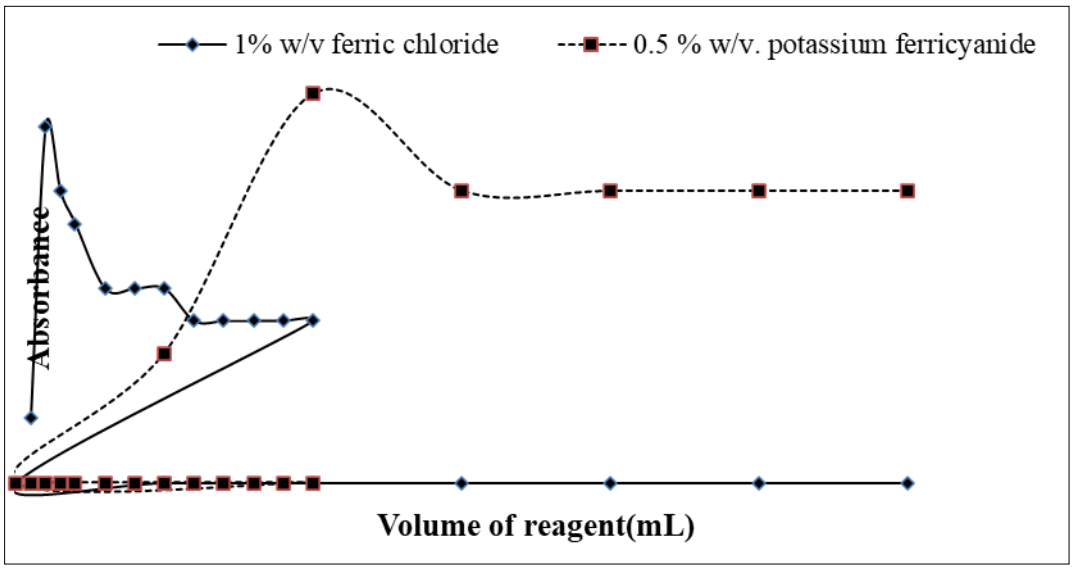


Figure 3 Influence of ferric chloride and potassium ferricyanide

3.2.2. Influence of temperature and standing time

The absorbance decreases with the increase in the temperature above the room temperature(27 °C), so absorbance readings were taken at room temperature. The solution stands for 10 min and to get a stable coloration.

3.2.3. Order of addition

After optimizing all other experimental variables, further experiments were performed to ascertain the influence of sequencing the addition of reactants on the color development by measuring the absorbance based on following three orders of addition for both methods

Order 1 : Voglibose + $FeCl_3$ + $K_3Fe(CN)_6$: A = 0.038

Order 2 : $FeCl_3$ + $K_3Fe(CN)_6$ + Voglibose : A = 0.023

Order 3 : $K_3Fe(CN)_6$ + $FeCl_3$ + Voglibose : A = 0.047

Order 4: Voglibose + $K_3Fe(CN)_6$ + $FeCl_3$: A = 0.154

The order of addition number 4 is recommended.

3.3. Method validation

As per ICH guidelines (ICH 1996;2005) the developed method was validated for linearity, sensitivity, specificity, accuracy, precision, robustness and ruggedness.

3.3.1. Linearity and Sensitivity

The linearity was evaluated by linear regression analysis of the Beer's law data by least-square regression method, which was used to calculate the correlation coefficient, intercept and slope of the regression line. The values are presented in Table 1. The small values of the intercept of the regression equation and the regression coefficient (>0.99) values obtained, specify that there is a good correlation between absorbance values and voglibose concentration in the proposed method. Molar absorptivity, Sandell's sensitivity, limits of detection (LOD) and limit of quantification (LOQ) are calculated as per the current International Conference on Harmonization guidelines to assess the sensitivity of the proposed method. LOD and LOQ were calculated as 3.3 σ/s and 10 σ/s, respectively. Where, σ = standard deviation of six replicate determination values of reagent blank and s = slope of the calibration graph. The results are summarized in Table 1. The high values of molar absorptivity & low values of Sandell's sensitivity, LOD and LOQ point out the good sensitivity of the proposed method.

Table 1 Linearity, regression equation and sensitivity of the proposed method

Parameter	
Linearity range (µg/ mL)	1-5
Regression equation (Y = mx + c)*	Y = 0.003x -0.00017
Regression coefficient (R^2)	0.9999
Slope (m)	0.003
Intercept (c)	-0.00017
Molar absorbtivity (L/mole/cm)	6.42×10^3
Sandell's sensitivity (µg/cm^2/0.001 absorbance units)	0.041
LOD (µg/ mL)	0.27
LOQ (µg/ mL)	0.81
Stability of colored species (hr)	2

* Y = Absorbance ; x = Concentration of voglibose in µg/mL

3.3.2. Stability of colored species

The stability of the colored species formed in the proposed method was monitored by keeping the solution at room temperature (27 ± 1 °C) and then measuring the absorbance of the colored solution at their corresponding analytical wavelength (λ_{max}) at regular intervals of time. The results are presented in Table 1. The increased stability of colored species formed in the proposed methods assisted in proceeding with large batches of samples and their comfortable measurements easily.

3.3.3. Specificity

The specificity of the method was established by observing any interference encountered from the common tablet excipients. A placebo blank containing starch (40 mg), hydroxyl cellulose (35 mg), gum acacia (35 mg), lactose (20 mg), sodium citrate (35 mg), talc (40 mg), sodium alginate (35 mg) and magnesium stearate (35 mg) was prepared by mixing all the components into a homogeneous mixture. A 50 mg of the placebo blank was accurately weighed and transferred to a 100 mL volumetric flask containing 50 mL of 0.1 N HCl. The contents of the flask were shaken for 10 min and filtered using Whatmann no. 1 filter paper.

The filtrate was transferred into another 100 mL volumetric flask and made up to the volume with 0.1 N HCl. The placebo blank solution was then subjected to analysis by following the general assay procedure. The absorbance of the placebo blank solution was negligible. This indicated that these excipients did not interfere with the proposed method.The percentage recovery of voglbose from added excipients was presented in Table 2.

Table 2 Percent recovery of the voglibose in the presence of possible excipients used in tablet formulation

Excipients	**Amount Taken(µg/mL)**	**% Recovery ± RSD (n = 5)**
Lactose	20	99.02 ± 0.87
Talc	40	99.09 ± 0.31
Magnesium Stearate	35	99.13 ± 0.88
Starch	40	99.06±0.49
Hydroxyl cellulose	35	99.27 ±0.37
Gum acacia	35	99.38±1.09
Sodium citrate	35	99.44 ±0.76
Sodium alginate	35	99.13±0.52

3.3.4. Accuracy and precision

The accuracy and precision of the proposed method were determined by intra-day and inter-day analysis. In order to determine the accuracy and precision of the proposed method, solution containing fixed concentration (within the working limits) of the voglibose was prepared at three different concentration levels (2 ,3 ,4 µg/mL).

The above solutions were analyzed in five replicates on the same day by the proposed method to assess the intra-day precision and accuracy. The inter-day precision and accuracy were assessed similarly for five replicate determinations of the same concentration levels on three consecutive days. The precision values are expressed as standard deviation and percent relative standard deviation, while the accuracy values are expressed as standard analytical error and percent recovery. The results are summarized in Table 3. The relative standard deviation indicates the good intra- and inter-day precision of the proposed method.

Regarding the accuracy evaluation, good recoveries were obtained. The percent recovery indicated good accuracy and an agreement between the theoretical value and the real value of concentration.

Table 3 Accuracy and Precision

Drug Concentration Taken(µg/mL)	Intra-day(n=7)			Interday(n=5)		
	Found(µg/mL)±SD	% RSD	% RE	Found(µg/mL)±SD	% RSD	% RE
2	1.97 ± 0.019	1.00	1.50	1.98 ±0.02	1.28	1.00
3	2.98 ± 0.014	0.47	0.66	2.95 ± 0.03	1.13	1.66
4	4.01 ±0.033	0.84	0.25	3.97 ± 0.05	1.42	075

3.3.5. Robustness and ruggedness

To evaluate the robustness of the method, two important experimental variables ,volume of reagent (±0.2 mL) and reaction time (±2 min), were slightly altered and the effect of this change on the absorbance was studied. The results of this study are presented in Table 4 and indicated that the proposed methods are robust. Method ruggedness was evaluated by performing the analysis following the recommended procedures by two different analysts and on two different spectrophotometers by the same analyst. From the % RSD values presented in (Table 4), one can conclude that the proposed methods are rugged.

Table 4 Robustness and Ruggedness

Con. Taken µg/mL	Method robustness		Method ruggedness	
	Parameters altered			
	Reagent Volume, mL % RSD (n=3)	Reaction Time %RSD (n=3)	Inter-analysts RSD%, (n=3)	Inter-cuvettes RSD%, (n=3)
2	0.56	1.21	0.88	0.98
3	0.79	1.87	0.75	0.66
4	0.47	1.35	0.84	1.21

3.4. Application of the method to pharmaceutical formulation

Three-different tablet brands with strength of 0.3 mg was estimated using the method proposed and the outcomes were shown in Table 5. Similar tablets are analyzed following a reference technique for comparison (Kishore et al.,2013). Outcomes showed that there was a close consensus among the methods proposed and obtained results from conventional method. True, the outcomes were found to be closer to claims on the label. When the results were statistically evaluated using the students'"t-test for accuracy and the F-test difference ratio for precision. The calculated student's t-values and F-values did not exceed the theoretical ones at 95% confidence level. Therefore, there is no significant difference between the proposed method and reference method.

Table 5 The analysis Results obtained of tablets by the proposed method and the results of statistical comparison with the reference method

Tablet brand name	Label claim mg/table	Found* (Percent of label claim ±SD)	
		Proposed method	Reference method
Volibo	0.3 mg	99.36 ± 0.75 t = 1.321, F= 1.614	98.6 ± 1.1
Volix	0.3 mg	98.5 ± 1.1 t = 1.414,F= 1.254	99.6 ± 1.3
Volicose	0.3 mg	101.3 ± 0.85 t = 0.983,F = 1.521	100.56 ± 0.94

The theoretical values of t and F at P = 0.05 are 2.31 and 6.39, at four degress of freedom respectively; *Average of five determinations

4. Conclusion

The method of spectrophotometry as used in this research for the estimation of voglibose is found to be simple and there were no significant variables in the experiment as compared with any of the methods reported till now. This technique is more precise and accurate and has demonstrated both ruggedness and robustness.. Such benefits, combined with the use of readily and cheap chemicals available as well as the simple equipment, making the method to be highly suitable to use in estimating voglibose in quality control laboratories in both underdeveloped and developing countries, which can ill-afford HPLC method or other expensive methods.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors attest that they have no conflict of interest in this study.

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