THE DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OFVOGLIBOSE AND METFORMIN HCL IN BULK AND TABLET DOSAGE FORM WITH THE PRE-COLUMN DERIVATIZATION OFVOGLIBOSE.

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ABSTRACT

Simultaneous determination of Voglibose and Metformin HCl has been accomplished using a high performance liquid chromatographic method with UV detection on C\textsubscript{18} columns. Separation was achieved on an Inertsil ODS (150mm×4.6 mm) and The column was equilibrated with mobile phases consisted of acetonitrile: 0.01M phosphate buffer pH 3 (85:15, v/v) and The flow rate was 0.8 ml/min. and the total elution time was 10min. The selected chromatographic conditions were found to effectively separate Voglibose and Metformin HCl with retention time 2.11 ± 0.015 and 4.62 ± 0.020 min. Voglibose was derivatized by using Taurine and Sodium Periodate. This method was applied to combination of standard bulk drug and marketed formulations. The linearity range was found to be 0.01-0.06µg/ml and 10-60µg/ml for Voglibose and Metformin HCl respectively. The proposed method was found to be accurate, precise, reproducible and specific and it can also be used for routine quality-control analysis of these drugs in combination tablets.

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INTRODUCTION

Voglibose is chemically 4(+)-V[1(OH),2,4,5/3]-5-[2-hydroxy-1-(hydroxymethyl)ethyl] amino-1-C-(hydroxymethyl)-1,2,3,4-cyclohexanetetral. Voglibose is a new potent alpha-glycosidase inhibitor used for type 2 diabetes, has shown strong anti-obesity and anti diabetic activity and the drug delays glucose absorption and thus, reduces the post-prandial blood glucose level[1-3]. Voglibose has a structural relation with natural carbohydrates [4,5]. Since most carbohydrates lack chromophore and/or flurophore groups, their analysis by UV often requires derivatization procedures. Since Voglibose only absorbs UV in the low wavelength region, it cannot be detected with high sensitivity. So special detection methods are necessary for analysis of Voglibose. Voglibose shown effective results for various analytical instruments only in the combination of Taurine and Sodium periodate. Drug solution was derivatized using Taurine and Sodium periodate in water.

Chemical structure of Voglibose  
Chemical structure of Metformin HCl

Metformin hydrochloride is chemically, 1, 1-dimethyl biguanide hydrochloride, used as oral hypoglycemic drug from the biguanide class and it is also used in the management of type 2 diabetes mellitus. It improves hyperglycemia primarily through its suppression of hepatic glucose production (hepatic gluconeogenesis) and activates AMP-activated protein kinase which is required for the inhibitory effect for the production of glucose by liver cells [6-8]

A survey of literature revealed that Several spectrophotometric9, UV-Visible spectrophotometry10, capillary electrophoresis[11] methods have been used for the qualitative and quantitative determination of Metformin, Uv – spectroscopic[12], spectrofluorimetric[13] methods for estimation of Voglibose individually have been developed. Also there is one Uv Spectrophotometric method for simultaneous estimation of Voglibose and Metformin HCl by multicomponent mode of analysis [14].

So, our aim is to develop RP-HPLC method for simultaneous estimation of Voglibose and Metformin HCl in bulk and tablet dosage form.

EXPERIMENTAL

Instrumentation

The liquid chromatographic system, used in the present study, consisted of an Agilent technologies 1120 series instrument equipped with a binary solvent delivery system with UV detector. A sample injector with a 20 µl sample loop was used for the injection of analytes. Chromatographic data were collected and processed using EZ Chrome software. The separation was performed at ambient temperature, on Inertsil ODS column (150mm×4.6 mm). All experiments were employed in the isocratic mode.

Materials and reagents

Voglibose (VGB) and Metformin hydrochloride (MET) were kindly provided by Micro Labs ltd., Bangalore, (Karnataka, India) and Sohan Health Care Pvt. Ltd., Pune (Maharashtra, India) Respectively. Methanol and acetonitrile were of HPLC grade and Ortho-phosphoric acid (85%) was of analytical grade and purchased from Merck Company (Mumbai, India). Taurine and Sodium Peri-iodate were of AR grade and were purchased from Research Labs Fine Chem. Industries, Mumbai. Doubly distilled water was used for preparing mobile phase and other solutions. Pharmaceutical finished dosage forms utilized in the present work include: VOGLIBO M 0.3 tablet claimed to contain 0.3mg of VGB and 500 mg of MET.

Chromatographic conditions

The mobile phase was prepared by mixing acetonitrile and potassium phosphate buffer of 0.01M in varying proportions. Before mixing with organic solvents, the final pH of buffer solution (0.01M) was adjusted to the desired value (pH 3) with orthophosphoric acid. The optimum mobile phase which was used in the validation studies consisted of acetonitrile: 0.01M Phosphate buffer (85:15 v/v). This phase was filtered through a 0.45µm membrane and degassed by ultrasonication, prior to use. Solvent delivery was employed at a flow rate of 0.8 ml/min. Detection of the analytes was carried out at 226 nm for binary mixtures. Injection volume of the analytes was set to a constant volume of 20 µl using a sample loop.

Preparation of standard stock solution

The standard stock solutions were prepared by dissolving 10 mg of VGB and MET separately in 100 ml of double distilled water to get a concentration of 100µg/ml each. The stock solution of Voglibose is then derivatized by pre column derivatization with Taurine and Sodium Periodate. They were further diluted with mobile phase to get working standard solutions of VGB and MET, having concentrations 0.03µg/ml and of 50 µg/ml of VGB and MET respectively.
Preparation of calibration curve
Aliquots of working standard solution of VGB (100 µg/ml) and MET (100 µg/ml) were transferred in separate 10 ml volumetric flasks. From each working standard solutions of VGB and MET the amount was pipette out to prepare solution having concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 µg/ml of VGB and 10, 20, 30, 40, 50, 60 µg/ml of MET was injected and the chromatograms were recorded. The respective calibration curves were plotted of the response factor against the concentration of drug.

Sample preparations
Twenty VOG LIBO M 0.3 tablets were accurately weighed, their mean weight was determined, and they were then finely powdered. An amount equivalent to one tablet was transferred into a 100ml volumetric flask, added 80 ml of double distilled water, sonicated for 30 min. 0.625gm Taurine and 0.256 gm sodium periodate was added for the derivatization of Voglibose and sonicated again for 10 min. then diluted to 100 ml with same solvent. This solution was filtered by 0.42 m filter.

Method validation [15]

Linearity
The linearity of the method was evaluated by analyzing different concentration of the drugs. According to ICH recommendations, In this study six concentrations were chosen, in the ranges 0.001-0.006 µg/ ml and 10-50 µg/ ml for VGB and MET, respectively.

Recovery studies
For carrying out the accuracy of the proposed method recovery studies were employed by the standard addition method. This was carried out by adding known amounts of standard combination of VGB and MET at three different levels of 80%, 100%, and 120% to the sample.

Precision
The precision of the method was assessed by studying intra-day and inter-day variation. In the intra-day studies, standard and sample solutions were analyzed in triplicate on the same day and percentage RSD was calculated. In the inter-day studies, standard and sample solutions were analyzed in triplicate on three consecutive days and percentage RSD were calculated.

Limits of detection (LOD) and limit quantitation (LOQ)
In accordance with ICH recommendations, the approach based on the standard deviation of the response and the slope of the calibration plots was used to determine detection and quantification limits. LOD and LOQ values were estimated as [(standard deviation of repeatability)/(Slope of the regression equation)] by multiplying with 3.3 and 10 respectively.

Specificity and selectivity
The selectivity of the method was evaluated by assessing whether excipients present in the pharmaceutical formulations interfered with the analysis. A placebo for each tablet was prepared by mixing the respective excipients, and solutions were prepared by following the procedure described in the section on sample preparation. The commonly used tablet excipients did not interfere with the method.

Robustness
The Robustness of the developed method was determined by the small but deliberate changes in chromatographic conditions such as the flow rate (± 0.02 mL/min), wavelength (± 1 nm), and mobile phase composition (± 2%). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

For development and validation of an efficient method for analysis of these drugs in pharmaceutical formulations, preliminary tests were performed with the objective of selecting optimum chromatographic conditions. The separation was tried using columns Nucleosil C18, 250/4.6mm and Chromosil C18,250/4.6mm.,the main problems encountered during these investigations was tailing effect of Metformin HCl. So the column Inertsil ODS (150/4.6mm) was used with which the problem of tailing effect was solved. The best resolution and peak shape, without excessive tailing, were obtained by use of the C18 column. The effect of mobile phase composition, flow rate and pH were also studied. Mobile phase containing Acetonitrile: 0.01M potassium dihydrogen phosphate buffer pH 3.0 adjusted with orthophosphoric acid (85:15) with 0.8 ml/min flow rate was selected which shows best resolution with reasonable retention time. The specificity of the method is illustrated in (Figs. 3) which indicates separation of the compounds was complete. Average retention times ± standard deviation for MET and VGB were 2.11 ± 0.015 min and 4.62 ± 0.020 min, respectively, for six replicate analyses. In determination of accuracy and precision, recovery was 100 ± 2%, which indicates the method is accurate, and intraday and inter-day variation, as RSD, were no more than 2%, indicating the method is precise. For study of robustness of the method, slight variation of mobile phase pH, amount of buffer in the mobile phase, and detector wavelength had no significant effect on chromatographic resolution.

Method validation
System suitability
The RSD values of peak area and retention time for both the drugs are within 2% indicating the suitability of the system (Table 1).
Linearity
The calibration curves were prepared by plotting the peak areas of the drug against the concentration which were linear in the range of 0.01-0.06 and 10-60mg/ml for VGB and MET respectively. The correlation coefficients were found to be 0.9995 and 0.9994 for Voglibose and Metformin HCl respectively. The result shows that there is an excellent correlation between the peak area ratios and the concentrations of drugs in the range tested. The calibration curves for both drugs are shown in Fig.1 and Fig.2. Linear regression data is given in Table 2.

Recovery
Recovery study was carried out by adding known amounts of standard combination of VGB and MET at three different levels of 80%, 100%, and 120% to the sample. Results of recovery studies are given in Table 3.

Precision
Intra-day precision was performed by relative standard deviation of five repeated assays of samples at the three concentration levels. Inter-day precision was determined by analyzing the same set of samples of five different days. The mean RSD values were found to be 0.07155% for VGB and, 0.31065% for MET, indicating good precision (Table 4).

LOD and LOQ
The LOD was found to be 0.00054µg/ml for VGB and 0.14645µg/ml for MET at a signal to noise ratio of 3.1. The limit of quantification was found to be 0.00165µg/ml for VGB and 0.44381µg/ml for MET at a signal to noise ratio of 10:1. Results are given in Table 1.

Specificity.
The specificity of the RP-HPLC method was determined by the complete separation of VGB and MET as shown in (Fig.3) with parameters like retention time (tR), resolution (Rs) and tailing factor (T). The peaks obtained for both drugs are sharp and have a clear baseline separation.

Robustness
It is necessary to study the minor changes in the experimental conditions to ensure the insensitivity of the HPLC method, which it is important to demonstrate robustness of the method. None of the modifications caused a significant change in the resolution between the two drugs, peak area, USP tailing factor, peak width or theoretical plates. Results of robustness are given in Table 5 and 6.

![Calibration Curve of VGB](Figure1.png)

**Figure 1:** Calibration Curve of VGB

![Calibration Curve of MET](Figure2.png)

**Figure 2:** Calibration Curve of MET
Figure 3: Chromatogram of combination of standard MET and VGB.

Table 1: System suitability parameters for RP-HPLC method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Obtained Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Voglibose</td>
</tr>
<tr>
<td>Theoretical plates (N)</td>
<td>4735</td>
</tr>
<tr>
<td>Resolution (R)</td>
<td></td>
</tr>
<tr>
<td>Asymmetry (Tailing factor)</td>
<td>1.33</td>
</tr>
<tr>
<td>LOD µg/ml</td>
<td>0.00054</td>
</tr>
<tr>
<td>LOQ µg/ml</td>
<td>0.00165</td>
</tr>
</tbody>
</table>

Table 2: Linear regression and validation data for VGB and MET

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Voglibose</th>
<th>Metformin HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear regression range</td>
<td>0.01-0.06 µg/ml</td>
<td>10-60 µg/ml</td>
</tr>
<tr>
<td>Linear regression equation</td>
<td>y = 1131066986 x + 1675933.67</td>
<td>y = 2231208x - 62036</td>
</tr>
<tr>
<td>Slope ±SD</td>
<td>1131066986±44046.04</td>
<td>2231208±94332.81</td>
</tr>
<tr>
<td>Intercept</td>
<td>1675933.67</td>
<td>62036</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9995</td>
<td>0.9994</td>
</tr>
</tbody>
</table>

Table 3: Results of recovery studies of VGB and MET

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Accuracy level (%)</th>
<th>Amount Added (µg/ml)</th>
<th>Total amount found (µg/ml)*</th>
<th>% Recovery + SD*</th>
<th>%RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voglibose</td>
<td>80</td>
<td>0.024</td>
<td>0.0241</td>
<td>99.96 ± 0.1222</td>
<td>0.1224</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.030</td>
<td>0.0299</td>
<td>99.94+ 0.9865</td>
<td>0.9870</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.036</td>
<td>0.0361</td>
<td>100.52+ 0.9290</td>
<td>0.9241</td>
</tr>
<tr>
<td>Metformin HCl</td>
<td>80</td>
<td>40.00</td>
<td>39.97</td>
<td>100.05± 0.2309</td>
<td>0.2307</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>50.00</td>
<td>50.09</td>
<td>99.83+ 0.0611</td>
<td>0.0612</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>60.00</td>
<td>59.48</td>
<td>100.36+ 0.6148</td>
<td>0.6125</td>
</tr>
</tbody>
</table>

*Denotes average of three estimations at each level of recovery.

Table 4: Data for Intra-day and Inter-day precision of VGB and MET

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Intra-day precision</th>
<th>Inter-day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%Amount Found ± SD*</td>
<td>% RSD</td>
</tr>
<tr>
<td>Voglibose</td>
<td>100.11± 0.0423</td>
<td>0.0422</td>
</tr>
<tr>
<td>Metformin HCl</td>
<td>99.98 ± 0.1923</td>
<td>0.1923</td>
</tr>
</tbody>
</table>

*Denotes average of three estimations at each level.
Table 5: Results of robustness study of VGB

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Flow rate (ml/min)</th>
<th>Mobile phase pH</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>± SD</td>
<td>0.6</td>
<td>1.0</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>0.285715</td>
<td>0.223681</td>
<td>0.131149</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.286242</td>
<td>0.224138</td>
<td>0.132233</td>
</tr>
</tbody>
</table>

Table 6: Results of robustness study of MET

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Flow rate (ml/min)</th>
<th>Mobile phase pH</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.7</td>
<td>0.9</td>
<td>3.3</td>
</tr>
<tr>
<td>± SD</td>
<td>0.39598</td>
<td>0.13576</td>
<td>0.21031</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.39692</td>
<td>0.13567</td>
<td>0.20989</td>
</tr>
</tbody>
</table>

CONCLUSION
The developed method is a suitable and valid method for the simultaneous determination of a combination of Voglibose and Metformin hydrochloride in pure and marketed tablet dosage form and will be conveniently adopted for the routine quality control analysis from its pharmaceutical formulations and bulk drug.

ACKNOWLEDGEMENT
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REFERENCE