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# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF METFORMIN AND VOGLIBOSE IN BULK AND FIXED DOSE COMBINATION (TABLETS) BY RP-HPLC

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**Abstract:**
*A new RP-HPLC method for the quantitative determination of Metformin and Voglibose was developed and validated as per ICH guidelines. The drugs were injected into Hypersil BDS C18 column (250×4.6, 5 µm), maintained at ambient temperature and effluent monitored at 236 nm.The mobile phase consisted of 0.02M $KH_2PO_4$: Acetonitrile (50:50 V/V). The flow rate was maintained at 1.0 ml/min. The calibration curve for Metformin and Voglibose were linear from 50-300µg/ml and 0.3-0.18µg/ml respectively ($r^2$ for Metformin = 0.997,$r^2$ for Voglibose = 0.998).The proposed method was adequate, sensitive, reproducible, accurate and precise for the determination of Metformin and Voglibose in bulk and pharmaceutical dosage forms.*

**Keywords:** *Metformin and Voglibose, Validation.*

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## INTRODUCTION:

Metformin is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It is also used in the treatment of polycystic ovary syndrome. Limited evidence suggests metformin may prevent the cardiovascular disease and cancer complications of diabetes. Metformin is in the biguanide class. It works by decreasing glucose production by the liver and increasing the insulin sensitivity of body tissues. It occurs as a white to almost white powder which is freely soluble in water. Voglibose is (1S,2S,3R,4S,5S)-5-(1,3-dihydroxypropan-2-ylamino)- 1- (hydroxymethyl) cyclohexane-1,2,3,4-tetraol. It is an alpha-glucosidase inhibitor used for lowering post-prandial blood glucose levels in people with diabetes mellitus. It is a white to off-white crystalline powder that is freely soluble in water. A fixed dose combination product of these two drugs is available in the name of Prandial M and Volabay-M in the market [1-7] .Various analytical methods have been reported for the estimation of Metformin and Voglibose, including spectrophotometric methods and HPLC. HPLC is the most widely used technique for the estimation of Metformin and Voglibose in human plasma, saliva, cerebrospinal fluid, and human blood cells, as well as for studying the drug metabolites in the urine. The suggested HPTLC and HPLC methods for assay of Metformin and Voglibose are quite expensive and need complex and sophisticated instrumentation. The present research work describes a HPLC and UV spectrophotometric method for estimation of Metformin and Voglibose in API and its pharmaceutical preparation [8-11]. The present method aims at developing a simple, accurate and precise RP-HPLC method for the estimation of Metformin and Voglibose in bulk and pharmaceutical dosage forms.

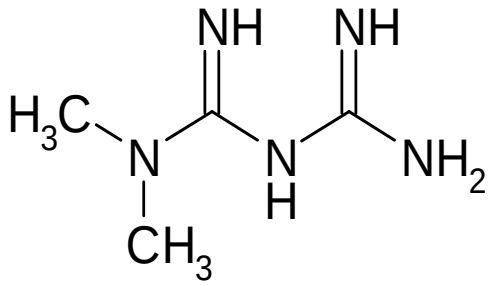


**Fig 1: Chemical structure of Metformin**

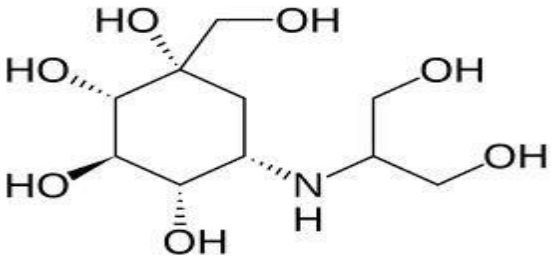


**Fig 2: Chemical structure of Voglibose**

## MATERIALS AND METHODS:

**Chemicals and solvents:** The reference sample of Metformin and Voglibose was obtained as a gift sample from Shreeji Pharma International, India. HPLC grade water (prepared by using 0.45 Millipore Milli –Q) was procured from Standard Reagents, Hyderabad. HPLC grade Acetonitrile was bought from Merck, Mumbai. Buffers were prepared using Potassium dihydrogen ortho phosphate and Di-Potassium hydrogen ortho phosphate of AR grade . These are manufactured by Merck respectively.

**Instrumentation:** A Shimadzu Prominance module LC-20 AT equipped with a UV spectrophotometer for finding out the λmax values of the drugs was used throughout this study. An Hypersil ODS 18(250×4.6, 5 mm) column was employed for the method development. The chromatographic system was monitored by Spinchrom software. Analytes were monitored by UV detection at 236 nm using an isocratic mode with 0.02M $KH_2PO_4$ buffer : Acetonitrile in the ratio 50:50 was used as mobile phase.The flow rate was set at 1.0 ml/min and effluent was monitored at 236 nm.The temperature and run time were maintained at 25°C and 6 min. respectively. Solubility of the compounds was enhanced by sonication on an ultrasonicator (SV scientific).

**Selection of mobile phase:** The objective of this experiment was to optimize the assay method for estimation of Metformin and Voglibose based on the literature survey. Various mobile phases were tested to select the best possible system. The various mobile phases used included Water:Acetonitrile (50:50), Water: Methanol (50:50) , Potassium di hydrogen Ortho Phosphate: methanol (70:30), 0.1M Ammonium Phosphate buffer: ACN (40: 60 ), 0.02M $KH_2PO_4$ , pH 3.0 (adjusted with OPA): ACN (50:50) . Better peak resolution and adequate retention time were obtained with the ratio of 0.02M $KH_2PO_4$ , pH 3.0 (adjusted with OPA): ACN (50:50).

**Preparation of Phosphate buffer:** 1.96 g of Potassium di hydrogen phosphate was weighed and transferred in to a 500ml beaker, dissolved in sufficient HPLC water and the pH was adjusted to 3 with Orthophosphoric acid

**Preparation of Mobile Phase**

The mobile phase was prepared by mixing 550 ml of Phosphate buffer and 450 ml of Acetonitrile in a 1000 ml clean and dry flask. The mobile phase was then degassed using Ultra-Sonicator to remove dissolved gases and the resultant mobile phase was filtered through a 0.45 µm membrane filter under vacuum.

**Preparation of Standard solution**

Standard solution was prepared by accurately weighing 500 mg of Metformin and 0.3 mg Voglibose and transferring them into a 100 ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10 mins. and then made upto volume with the mobile phase. The resultant mobile phase was filtered through a 0.45 µm membrane filter under vacuum. From this 0.1 ml of solution was taken & made upto 10 ml with mobile phase. The solution was sonicated for about 10 mins. and then made upto volume with the mobile phase.

**Preparation of Sample solution**

Sample solution was prepared by accurately weighing 500 mg of Metformin and 0.3 mg Voglibose and transferring them into a 100 ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10 mins. and then made upto volume with the mobile phase. The resultant mobile phase was filtered through a 0.45 µm membrane filter under vacuum. From this 0.1 ml of solution was taken & made upto 10 ml with mobile phase. The solution was sonicated for about 10 mins. and then made upto volume with the mobile phase.

**VALIDATION**

Prior to validation studies blank solution was injected and chromatogram was noted. Optimized conditions maintained where both the drugs were eluted with good retention time and peak area which was shown in the fig 4.

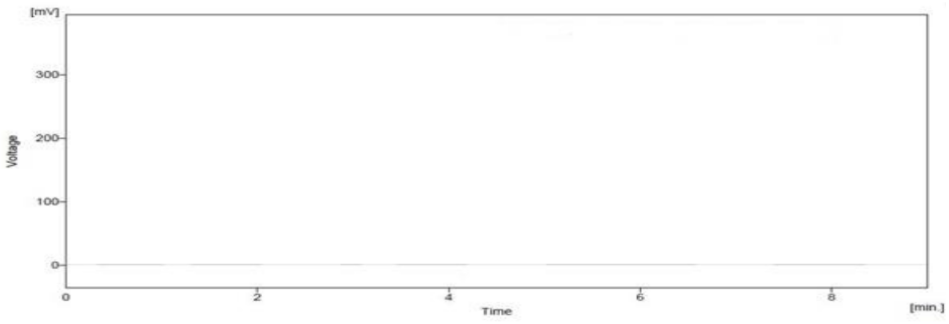


**Fig 3: Blank chromatogram**

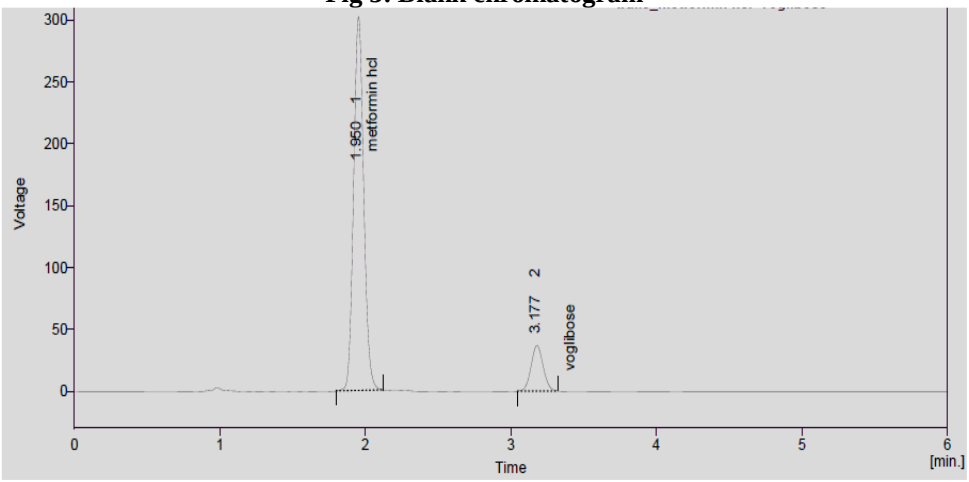


**Fig 4: Optimized Chromatogram**

**Linearity:** The linearity of the method was established by determining the absorbance of different concentrations of Metformin and Voglibose over a range of 50-300µg/ml and 0.3-0.18µg/ml respectively.

**Table 1: Linearity data of Metformin and Voglibose**

| S.No | Concentration of Metformin (mcg/ml) | Peak area | Concentration of Voglibose (mcg/ml) | Peak area |
|---|---|---|---|---|
| 1 | 50 | 343.852 | 0.3 | 47.463 |
| 2 | 100 | 677.667 | 0.6 | 91.438 |
| 3 | 150 | 929.379 | 0.9 | 131.529 |
| 4 | 200 | 1176.314 | 1.2 | 164.746 |
| 5 | 250 | 1437.402 | 1.5 | 200.781 |
| 6 | 300 | 1759.19 | 1.8 | 244.892 |

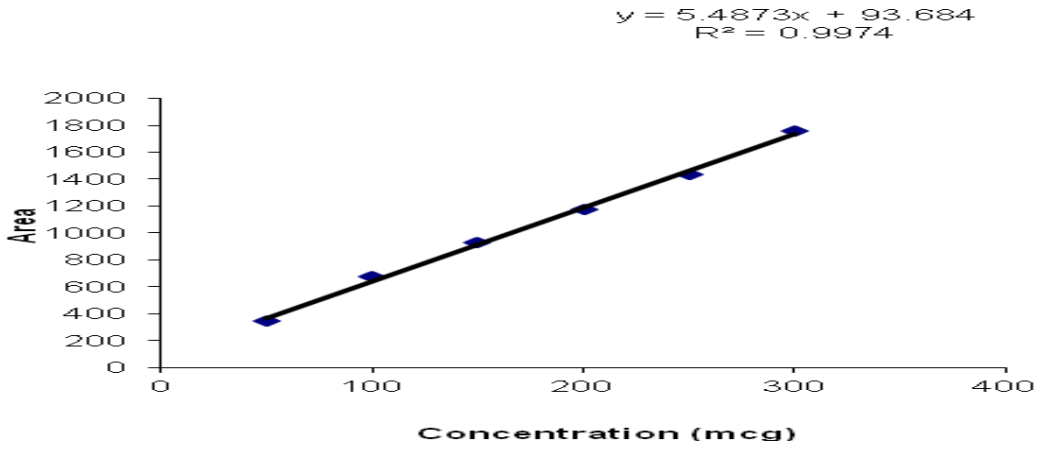


**Fig 5: Calibration curve of Metformin**

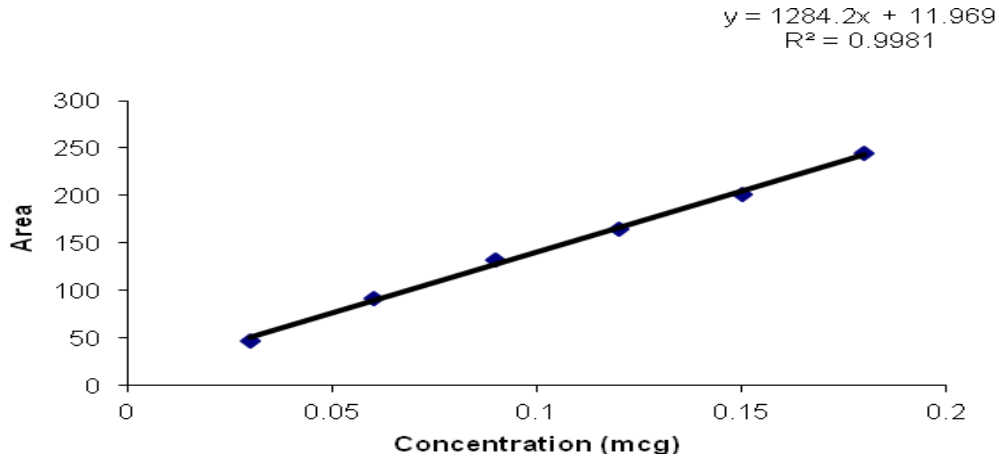


**Fig 6: Calibration curve of Voglibose**

**Accuracy:** To determine the accuracy of the proposed method, recovery studies were carried out by analyzing the samples were carried out by analyzing the measured concentration and the added concentration of the drug. Each sample was injected thrice .The percent recoveries of the drugs were estimated.

**Table 2: Accuracy data of Metformin**

| S. No | Accuracy level | % Recovery | Avg.% Recovery | Standard deviation | % RSD |
|---|---|---|---|---|---|
| 1 | 80% | 99.2 | 99.53 | 3.080287 | 0.260512 |
| 2 | 80% | 99.6 | | | |
| 3 | 80% | 99.8 | | | |
| 4 | 100% | 105.04 | 105.11 | 8.58098 | 0.562715 |
| 5 | 100% | 105.12 | | | |
| 6 | 100% | 105.18 | | | |
| 7 | 120% | 99.26 | 99.28 | 5.88722 | 0.34036 |
| 8 | 120% | 99.30 | | | |
| 9 | 120% | 99.28 | | | |

**Table 3: Accuracy data of Voglibose**

| S. No | Accuracy level | % Recovery | Avg.% Recovery | Standard deviation | % RSD |
|---|---|---|---|---|---|
| 1 | 80% | 100.86 | 100.58 | 1.7878 | 1.0678 |
| 2 | 80% | 100.23 | | | |
| 3 | 80% | 100.65 | | | |
| 4 | 100% | 98.40 | 98.42 | 1.251 | 0.6207 |
| 5 | 100% | 98.53 | | | |
| 6 | 100% | 98.33 | | | |
| 7 | 120% | 100.14 | 100.18 | 1.1245 | 0.461 |
| 8 | 120% | 100.24 | | | |
| 9 | 120% | 100.18 | | | |

**Precision:** Precision is one of the important factors which determine the reliability of an analytical method. The precision of the developed method was tested and was found to be suitable. Both system and method precision were performed and are given in table 4,5.

**Table 4: Method precision data of Metformin and Voglibose**

| S.No | Injection number | Retention time of Metformin | Area of Metformin | Retention time of Voglibose | Area of Voglibose |
|---|---|---|---|---|---|
| 1 | Injection 1 | 1.947 | 1510.152 | 3.060 | 211.519 |
| 2 | Injection 2 | 1.953 | 1495.609 | 3.087 | 207.721 |
| 3 | Injection 3 | 1.960 | 1494.015 | 3.160 | 210.347 |
| 4 | Injection 4 | 1.950 | 1459.619 | 3.177 | 212.684 |
| 5 | Injection 5 | 1.950 | 1473.616 | 3.180 | 209.900 |
| | Average | | 1486.6022 | | 210.4342 |
| | Standard deviation | | 19.92109 | | 1.8636 |
| | % RSD | | 1.340 | | 0.8856 |

**Table 5: System precision data of Metformin and Voglibose**

| S .No | Injection number | Retention Time Of Metformin | Area of Metformin | Retention Time of Voglibose | Area of Voglibose |
|---|---|---|---|---|---|
| 1 | Injection 1 | 1.950 | 1488.889 | 3.187 | 213.035 |
| 2 | Injection 2 | 1.950 | 1487.501 | 3.197 | 210.719 |
| 3 | Injection 3 | 1.947 | 1485.864 | 3.183 | 212.445 |
| 4 | Injection 4 | 1.947 | 1483.832 | 3.180 | 213.045 |
| 5 | Injection 5 | 1.947 | 1481.191 | 3.183 | 213.943 |
| | Average | | 1485.4554 | | 212.637 |
| | Standard deviation | | 3.0398 | | 1.19855 |
| | % RSD | | 0.20463 | | 0.563 |

**Robustness**: The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like volume of injection, wavelength which may differ but the responses were still within the limits of the assay.

**Table 6: Robustness data of Metformin**

| Proposed variations | | Retention time | Theoritical plates | Assymetric factor |
|---|---|---|---|---|
| Variation in flow rate | 0.9 ml | 2.070 | 3572 | 1.143 |
| | 1.1ml | 1.833 | 3572 | 1.153 |
| Variation in wavelength | 227 | 1.943 | 3280 | 1.150 |
| | 234 | 1.947 | 3572 | 1.150 |

**Table 7: Robustness data of Voglibose**

| Proposed variation | | Retention time | Theoritical plates | Assymetric factor |
|---|---|---|---|---|
| Variation in flow rate | 0.9ml | 3.403 | 6485 | 1.160 |
| | 1.1ml | 3.013 | 6960 | 1.174 |
| Variation in wavelength | 227 | 3.183 | 6458 | 1.080 |
| | 234 | 3.193 | 6960 | 1.125 |

**Ruggedness:** Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It was checked that the results were reproducible under different analysts.

**Table 8: Ruggedness data of Metformin and Voglibose**

| | Retention time of metformin | Retention time of voglibose | Area of metformin | Area of voglibose |
|---|---|---|---|---|
| Analyst(1)100% | 1.960 | 3.160 | 1494.015 | 210.347 |
| Analyst(2)100% | 1.960 | 3.160 | 1494.015 | 210.397 |

**Assay:** Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out.

**Table 9: Assay data of Metformin and Voglibose combination marketed formulation**

| Drug | Labelled claim(mg) | Drug found | % Purity |
|---|---|---|---|
| Sample 1 | 500 mg of Metformin & 0.3mg of Voglibose | 494.365mg of Metformin & 0.281mg of Voglibose | 98.33 % |
| Sample 2 | 500 mg of Metformin & 0.3mg of Voglibose | 498.069mg of Metformin & 0.29mg of Voglibose | 99.1 % |

## DISCUSSION:

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and economical RP-HPLC method. It was successfully applied for the determination of Metformin and Voglibose in pharmaceutical dosage forms without the interferences of other constituents in the formulations. Different mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was done based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with phosphate buffer: acetonitrile (50:50) with 1.0 ml/min flow rate was quite robust.

The optimum wavelength for detection was 236 nm at which better detector response for drug was obtained. The average retention time for Metformin and Voglibose were found to be 1.960 and 3.177.The calibration was linear in concentration range of 50-300mcg/ml for Metformin and 0.3-0.18mcg/ml for Voglibose**.** The low values of % RSD indicate the method is precise and accurate.

Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of three days. These results show the accuracy and reproducibility of the assay. Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions; the % RSD. reported was found to be less than 2 %.The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. There was no significant difference in the results achieved by the proposed method.

## CONCLUSION:

The proposed method for the assay of the popular anti- diabetic drugs Metformin and Voglibose in the commercially available tablet formulation is simple, accurate, economical, and rapid. It can be easily adopted for routine quality control for monitoring the assay in the API, in-process samples, and the finished tablet formulation.

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