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Research Article

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## RP-HPLC Method Development and Validation for estimation of Metformin, Voglibose, Glimepiride in Bulk and Combined Tablet Dosage Forms

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### ABSTRACT

**Background:** A simple, sensitive, linear, precise, and accurate method by gradient reversed-phase-high performance liquid chromatography for the simultaneous estimation of metformin (MET), voglibose (VOG) and glimepiride (GLI) in bulk and in their combined tablet dosage form was developed and validated. **Materials and Methods:** The separation of the three drugs was based on the use of Inertsil ODS 3V (150 × 4.6 mm, i.e. 5 μm) column in a gradient mode. Mobile phase consisted of 0.02 M Phosphate buffer adjusted to pH 2.5 using dilute orthophosphoric acid (solvent A) and acetonitrile (solvent B) was set with gradient programming for 18 min and was delivered at 1 ml/min flow rate and effluents are achieved with variable wavelength: Photodiode array detector at 230 nm. **Results:** The retention times of MET, VOG and GLI were found to be 2.423, 8.191, and 11.708, respectively. The percentage assay of MET, VOG and GLI was found to be 99.92%, 99.32, and 99.72%, respectively. Calibration curves were linear for MET, VOG and GLI at concentration ranges of 200-600 μg/ml, 0.08-0.24 μg/ml, and 0.8-2.4 μg/ml with the regression coefficient of 0.999 for all the three drugs and precise with (% relative standard deviation <2). The limit of detection for MET, VOG and GLI was found to be 0.05 μg/ml, 0.004 μg/ml, 0.002 μg/ml, and limit of quantitation for MET, VOG and GLI was found to be 1.5 μg/ml, 0.012 μg/ml, and 0.006 μg/ml, respectively. **Conclusion:** The method was validated by determining its linearity, accuracy, precision, system suitability and can be employed for routine quality control analysis as per International Conference on Harmonization guidelines.

**Keywords:** Cytosine, Biosensor, Amperometry, Polymer, Glassy carbon electrode

### ARTICLE INFO

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## 1. Introduction

Metformin (MET), is chemically known as 1-carbamimidamido-N, N-dimethylmethanimidamide1 is an oral anti-diabetic drug in the class of biguanides. It is used for the treatment of noninsulin-dependent diabetes mellitus as a first-line drug. It act by improving glycemic control by decreasing glucose absorption, decreasing hepatic glucose production, and increasing the insulin-mediated uptake of glucose. MET is the only antidiabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. Its use in gestational diabetes has been limited by safety concerns. It is also used in the treatment of polycystic ovary syndrome and also has been investigated for other diseases where insulin resistance may be an important factor. It helps reducing low-density lipoprotein cholesterol and triglyceride levels and is not at all associated with weight gain.<sup>2</sup>

Voglibose (VOG) chemically known as (1S,2S,3R,4S,5S)-5-[(1,3-dihydroxypropan-2-yl) amino]-1-(hydroxymethyl) cyclohexane-1,2,3,4-tetrol is an alpha-glucosidase inhibitor, which is prescribed for lowering post-prandial blood glucose levels in persons suffering from diabetes mellitus.<sup>3</sup> It act by delaying the absorption of glucose at the intestine level thereby preventing sudden surge of glucose after a meal and also lowers the risk of micro vascular complications. It is also indicated for the management of post-prandial hyperglycemia, which is mainly due to the first phase insulin secretion.<sup>4</sup>

Glimepiride (GLI) is chemically known as 3-ethyl-4-methyl-N-{2-[4-({[(4-ethylcyclohexyl)carbamoyl] amino} sulfonyl) phenyl]ethyl}-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide is a third generation sulfonyl urea, which is very potent with long duration of action. GLI is used with diet to decrease blood glucose by increasing the sensitivity of peripheral tissues in pancreas to insulin<sup>5</sup> thereby increasing the secretion of insulin from pancreas.

Detailed survey of the literature revealed, several methods are reported such as Spectrophotometry,<sup>6</sup> reversed-phase-high performance liquid chromatography (RP-HPLC),<sup>7-13</sup> high performance thin layer chromatography,<sup>14</sup> gas chromatography,<sup>15</sup> LC in biological fluids <sup>16</sup> for MET alone as well as in combination with other drugs using pharmaceutical formulations. Literature for VOG revealed several methods which include Spectrophotometric method,<sup>17</sup> ultraviolet (UV)-Spectroscopic method,<sup>18</sup> Spectrofluorimetric method,<sup>19</sup> RP-HPLC<sup>20,21</sup> LC<sup>22</sup> method for VOG alone as well as with other drugs in different pharmaceutical formulations. So far, methods are reported for the estimation of GLI alone such as UV spectrophotometric method,<sup>23</sup> in biological fluids<sup>24</sup> using pharmaceutical formulation and methods in combination based on RP-LC,<sup>25</sup> HPLC,<sup>26</sup> High performance thin layer chromatography<sup>27</sup> using pharmaceutical formulation. Until date, as there is no method for the simultaneous estimation

of MET, VOG and GLI by RP-HPLC in bulk and combined tablet dosage forms, our main objective is to develop and validate a simple, accurate, sensitive, precise, and reproducible method for the simultaneous estimation of MET, GLI, and VOG RP-HPLC in bulk and combined tablet dosage form

## 2. Materials and Methods

### Chemicals and reagents

Reference standards of MET, VOG and GLI (Figure 1) were obtained as gift samples from Dr. Reddy's Laboratories, Hyderabad, India. The formulation used was Glucoryl-MV-2 tablets containing (Label claim: 500 mg of MET, 0.2 mg of VOG, and 2 mg of GLI) was procured from the local market. Acetonitrile, methanol, and water used were of HPLC grade.

### Instrumentation

The development and validation was carried out by using HPLC (waters) separation 2996 series, variable wavelength photodiode array (PDA) detector module equipped with auto-sampler with injection volume 20  $\mu$ l, 2693 pump, column used was Inertsil ODS 3V (150  $\times$  4.6 mm, i.d., 5  $\mu$ m) column and data recorded using Empower software.

### Chromatographic conditions

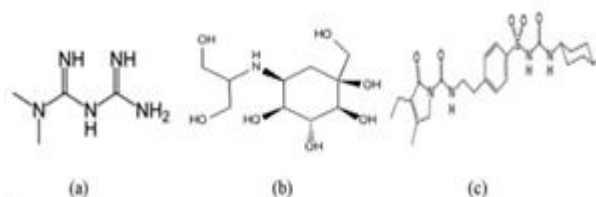
Various combinations of mobile phases were screened and finally, the mobile phase consisting of 0.02 M Phosphate buffer adjusted to pH 2.5 using dilute orthophosphoric acid (solvent A) and acetonitrile (solvent B) was set with gradient programming for 18 min was optimized at a flow rate of 1 ml/min, 230 nm wavelength, injection volume of 20  $\mu$ L and ambient temperature was maintained during the entire process to obtain symmetric peaks of MET, VOG and GLI.

### Mobile phase A:

2.72 g of potassium dihydrogen orthophosphate (0.02M) dissolved in 1000 ml water, adjusted to pH 2.5 using dilute orthophosphoric acid and filtered through 0.45  $\mu$ m membrane filter.

### Mobile phase B: Acetonitrile

Diluent: Water: acetonitrile (50:50).



Time programming of the gradient elution used to determine MET, VOG and GLI is given in Table 1. 2.72 g of potassium dihydrogen orthophosphate (0.02 M) in 1000 ml of water and pH adjusted to 2.5 with dilute orthophosphoric acid and filtered through 0.45  $\mu$ m membrane filter.

### Preparation of standard solution

Standard stock solution was prepared by dissolving separately 500 mg of MET, 0.2 mg of VOG and 2 mg of GLI in 100 ml clean dry volumetric flask. Dissolved and diluted with mobile phase up to the mark and filtered through 0.45  $\mu\text{m}$  membrane filter. From the prepared standard stock solution, 5 ml was transferred to 50 ml volumetric flask and volume made up with the mobile phase to obtain concentration of 500  $\mu\text{g/ml}$  for MET, 0.2  $\mu\text{g/ml}$  for VOG, and 2  $\mu\text{g/ml}$  for GLI respectively.

### Preparation of sample solution

The formulation used was Glucoryl-MV-2 Tablets with (Label claim: 500 mg of MET, 0.2 mg of VOG and 2 mg of GLI). Twenty tablets of combined dosage form of MET, VOG and GLI were weighed and made to a fine powder. 650.2 mg of powdered tablets equivalent to 500 mg of MET, 0.2 mg of VOG and 2 mg of GLI was weighed accurately and transferred into a 100 ml clean dry volumetric flask. Dissolved and diluted with mobile phase up to the mark and filtered through 0.45  $\mu\text{m}$  membrane filter. From the prepared standard stock solution, 5 ml was transferred to 50 ml volumetric flask and the volume made up with the mobile phase to obtain concentration of 500  $\mu\text{g/ml}$ , 0.2  $\mu\text{g/ml}$  and 2  $\mu\text{g/ml}$  for MET, VOG and GLI respectively.

### Method validation

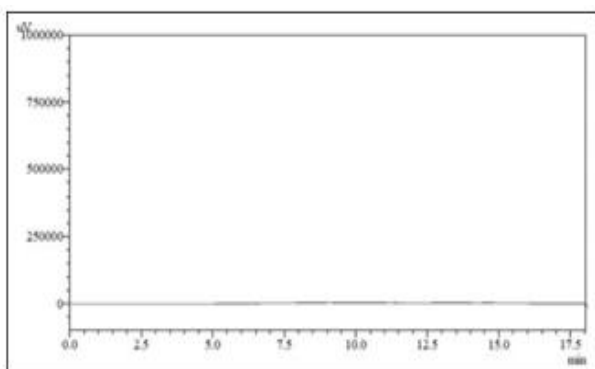
The developed method for simultaneous estimation of MET, VOG and GLI has been validated in accordance with the International Conference on Harmonization guidelines.

### Selectivity

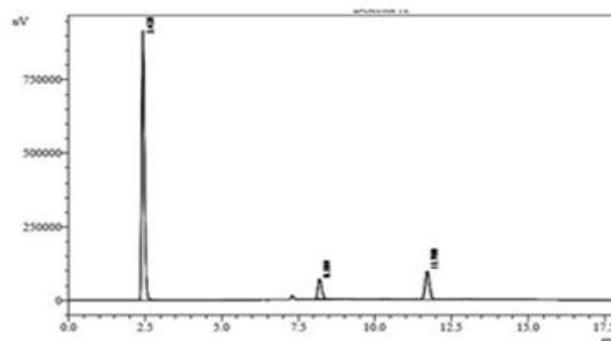
Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard solution of MET, VOG and GLI, commercial product solution and blank solutions were run in the instrument one after another. The results of the tests proved that the components other than the drug did not produce any detectable signal at the retention time of MET, VOG and GLI as shown in Figure 2.

Figure 3 shows the chromatogram of MET, VOG and GLI mobile phase. There were no interfering peaks at retention time of MET, VOG and GLI.

Figure 2 shows the chromatogram of working placebo sample solution.



**Figure 2:** Shows the chromatogram of working placebo sample solution



**Figure 3:** Shows the chromatogram of MET, VOG and GLI mobile phase. There were no interfering peaks at retention time of MET, VOG and GLI.

**Linearity:** Several aliquots of standard stock solution of MET, VOG and GLI were taken in different 10 ml volumetric flask and diluted up to the mark with mobile phase such that their final concentrations were 200-600  $\mu\text{g/ml}$  for MET, 0.08-0.24  $\mu\text{g/ml}$  for VOG, and 0.8-2.4  $\mu\text{g/ml}$  for GLI, respectively. Peak areas were plotted against the corresponding concentrations to obtain the calibration graph for each compound as shown in Table 2. The linearity regression co-efficient ( $R^2$ ) values were found to be 0.999 for MET, VOG and GLI, respectively. Linearity equation obtained for MET, VOG and GLI were  $y = 1E + 06x + 1E + 06$ ,  $y = 12138x + 12618$ , and  $y = 18641x + 18792$ , respectively. Figures 4-6 shows linearity graphs for MET, VOG and GLI, respectively.

### Accuracy

The accuracy of the method for assay determination was achieved at three concentration levels of 80%, 100%, and 120% for MET, VOG and GLI. Known amount of standard drug concentration was added to the sample and peak area was determined. The mean percentage recovery values are shown in Table 3.

### Precision

The precision at 100% concentration was evaluated by carrying out six independent assays of MET, VOG and GLI with the reference standard of the same drugs as shown in Tables 4 and 5.

**Sensitivity** (limit of detection [LOD] and limit of quantitation [LOQ])

The sensitivity of the method was evaluated by determining the LOD and LOQ. The values of LOD and LOQ for MET, VOG and GLI are given in Table 6.

### System suitability

Six replicate of sample containing MET, VOG and GLI were given to evaluate equipment, electronics, and analytical operations and samples suitability. Parameters calculated for system suitability were percentage of relative standard deviation of retention time and area, number of theoretical plates and resolution. Results found are given in Table 7 and within acceptable limits.

### Robustness

Method robustness was evaluated by making slight and deliberate changes made in chromatographic condition like proportion of flow rate, small changes in buffer pH, and use of different columns as illustrated in Table 8.

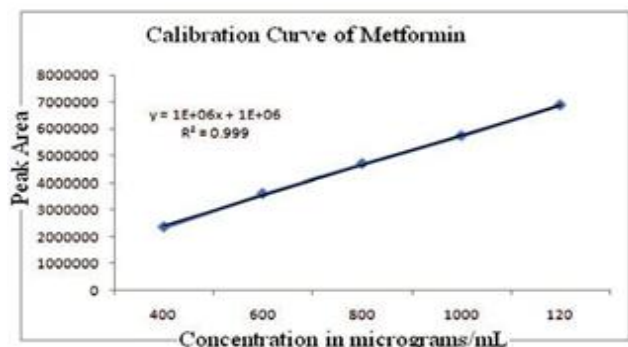


Figure 4: Linearity plot for metformin

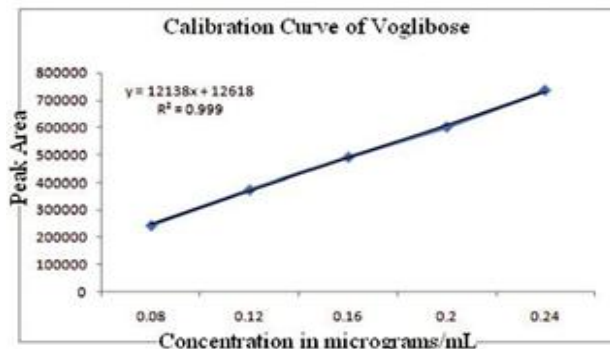


Figure 5: Linearity plot for voglibose

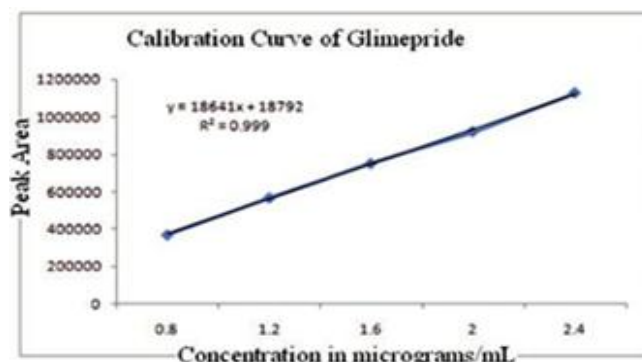


Figure 6: Linearity plot for glimepiride

### 3. Results and Discussion

#### Optimization of chromatographic conditions

A gradient, rapid and simple RP-HPLC method was developed and validated for the simultaneous estimation of MET, VOG and GLI. Mobile phase consisting of 0.02 M phosphate buffer adjusted to pH 2.5 using dilute orthophosphoric acid (solvent A) and acetonitrile (solvent B) was set with gradient programming for 18 min. Chromatographic conditions were optimized for mobile phase using Inertsil ODS 3V 150 × 4.6 mm, i.d., 5 μm) column at a flow rate of 1 ml/min. Effluents were detected at 230 nm by variable wavelength PDA detector. Column compartment temperature was maintained at 25°C. Chromatogram of MET, VOG and GLI at optimized chromatographic condition is shown in Figure 2.

#### Selectivity

Figure 2 shows the chromatogram of working placebo sample solution. Figure 3 shows the chromatogram of MET, VOG and GLI mobile phase. There were no interfering peaks at retention time of MET, VOG and GLI.

#### Linearity and range

The linearity regression co-efficient (R2) values were found to be 0.991 for MET and 0.997 for VOG and 0.009 for GLI. Linearity equation obtained for MET, VOG and GLI were  $y = 1E + 06x + 1E+06$ ,  $y = 12138x + 12618$  and  $y = 18641x + 18792$ , respectively. Figures 4-6 show linearity graphs for MET, VOG and GLI respectively.

#### System suitability

Six replicates of sample containing MET, VOG and GLI were given to evaluate equipment, electronics, and analytical operations and samples suitability. Parameters calculated for system suitability were a number of theoretical plates, tailing factor, resolution, retention time, and area.

#### Assay

The proposed method was applied for the analysis of Glucoryl-MV-2 tablets and the results of the assay of MET, VOG and GLI are shown in Table 9.

Table 1: Time programming of gradient elution

Time	Mobile phase-A (0.02 M potassium Dihydrogen orthophosphate buffer)	Mobile phase-B (acetonitrile)
0	80	20
3	50	50
5	30	70
12	30	70
15	80	20
18	80	20

Table 2: Linearity of Metformin, Voglibose and Glimperide

Metformin Conc. (μg/mL)	Peak area	Voglibose Concentration (μg/mL)	Peak area	Glimperide Concentration (μg/mL)	Peak area
200	2351054	0.08	244375	0.8	370624.5
300	3595069	0.12	374702	1.2	568053
400	4690602.5	0.16	492629	1.6	750589
500	5726290	0.20	602700	2.0	919672
600	6874446	0.24	737298.5	2.4	1126875

**Table 3:** Accuracy results

Drugs	Drugs Conc. (µg/ml)	Amount %	Conc. after spiking(µg/ml)	Amount %	Recovered (µg/ml)	Recovery RSD
	80	60	460	412.06	89.58	0.0
MET	100	60	560	518	92.5	0.1
	120	60	660	777.6	117.83	0.2
	80	0.024	0.184	0.162	88.33	0.1
VOG	100	0.024	0.224	0.198	88.58	0.0
	120	0.024	0.264	0.294	111.91	0.2
	80	0.24	1.84	1.67	91.08	0.1
GLI	100	0.24	2.24	2.03	90.83	0.1
	120	0.24	2.64	2.80	106.16	1.4

MET: Metformin, VOG: Voglibose, GLI: Glimepiride, RSD: Relative standard deviation

**Table 4:** Results for precision of the standard

Injection	Metformin Retention time	Area	Voglibose Retention Time	Area	Glimepiride Retention time	Area
1	2.421	5735858	8.187	622809	11.716	935541
2	2.422	5738972	8.189	606552	11.713	922964
3	2.422	5753564	8.189	605189	11.702	921717
4	2.424	5743597	8.191	605106	11.700	922997
5	2.425	5747941	8.194	605885	11.704	923517
6	2.425	5751030	8.194	606724	11.698	923612
Mean	2.423	5745160.3	8.191	608710.8	11.705	925058.0
%RSD	0.060	0.1	0.033	1.1	0.061	0.6

RSD: Relative standard deviation

**Table 5:** Results for precision of the sample

Injection	Metformin Retention time	Area	Voglibose Retention time	Area	Glimepiride Retention time	Area
1	2.425	5771972	8.194	607055	11.693	926267
2	2.426	5773228	8.195	608105	11.696	927709
3	2.426	5778001	8.196	608723	11.692	928509
4	2.426	5779250	8.200	609552	11.699	929952
5	2.426	5784303	8.198	610453	11.693	930743
6	2.426	5792709	8.193	610354	11.685	931079
Mean	2.426	5779910.5	8.196	609040.3	11.693	929043.2
Average	0.019	7679.2	0.031	1334.2	0.040	1877.9
%RSD	2.425	0.1	8.194	0.2	11.693	0.2

**Table 6:** LOD and LOQ of MET, VOG and GLI

	Metformin	Voglibose	Glimepiride
LOD (µg/ml)	0.05	0.004	0.002
LOQ (µg/ml)	1.5	0.012	0.006

LOD: Limit of detection, LOQ: Limit of quantitation, MET: Metformin, VOG: Voglibose, GLI: Glimepiride

**Table 7 System suitability results for MET, VOG and GLI**

S.No.	Parameters	Metformin	Voglibose	Glimepiride
1	Theoretical plates	3033.879	17953.186	31545.673
2	Tailing factor	1.454	1.399	1.090
3	Resolution	---	27.439	13.884
4	Relative retention time (minutes)	2.423	8.191	11.708

MET: Metformin, VOG: Voglibose, GLI: Glimepiride

**Table 8:** Results of robustness by variations in flow rate, pH and column

Parameter	Modification	Metformin		Voglibose		Glimepiride	
		RT	% RSD	RT	% RSD	RT	% RSD
	0.9 ml/min	2.686	0.065	8.647	0.107	12.528	0.202
Flow-rate	1.0 ml/min	2.423	0.060	8.191	0.033	11.708	0.061
	1.1 ml/min	2.200	0.034	7.783	0.080	10.966	0.072
	2	2.346	0.061	8.126	0.022		

**Table 9 Assay of commercial tablet**

Drug	Label claim (mg)	Drug content (%)	% RSD
MET	500	99.92	0.1
VOG	0.2	99.32	0.2
GLI	2	99.72	0.2

#### 4. Conclusions

The validated HPLC method here proved to be simple, rapid, precise, accurate, robust, and economical and can be used for the routine quality control analysis of MET, VOG and GLI in combined tablet dosage form.

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