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### SIMULTANEOUS DETERMINATION OF METFORMIN AND GLIBENCLAMIDE IN PHARMACEUTICAL PREPARATION BY RP-HPLC

Anitha K<sup>1</sup>, T. M. Kalyankar<sup>2</sup>, V. B. Ingale<sup>2</sup> and Ashok P Gingine<sup>3</sup>

<sup>1</sup>Department of Chemistry, Sri Krishnadevaraya University, Ananthapuram-515003, Andhra Pradesh, India.

<sup>2</sup>School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded (431 606), MS India.

<sup>3</sup>School of Educational Sciences, Swami Ramanand Teerth Marathwada University, Nanded (431 606), MS India.

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#### ABSTRACT

A simple, rapid, precise and accurate reverse phase high performance liquid chromatographic method has been developed for the simultaneous determination of Metformin in combination with Glibenclamide. This method uses a Hypersil ODS C<sub>18</sub> (250mm×4.6mm×5μ particle Size) analytical column, a mobile phase of Acetonitrile and buffer containing 0.05 M Ammonium acetate pH 5.0 in the ratio 60:40 (v/v). The instrumental settings are a flow rate of 0.7 mL/min and PDA detector wavelength at 256 nm. The retention times for Metformin and Glibenclamide are 4.44 min and 7.67, respectively. The method is validated as per ICH guidelines. The linearity range for Metformin and Glibenclamide were 500-1000 & 10-60 μg/mL respectively. The Percentage recovery for Metformin and Glibenclamide are ranged between 99.59–100.00 and 99.98–100.01 respectively. The correlation coefficients of Metformin & Glibenclamide were found to be 0.999, and 0.999, respectively. The relative standard deviation for six replicates was found less than 2%. The method is proved to be suitable for analysis of Metformin and Glibenclamide as a bulk drug and in pharmaceutical formulation without any interference from the excipients.

#### Corresponding author

**Dr. Anitha K.**

Department of Chemistry,

S. K. University, Ananthapuram-515003 (AP)

anitbios@gmail.com

+91 9951406931

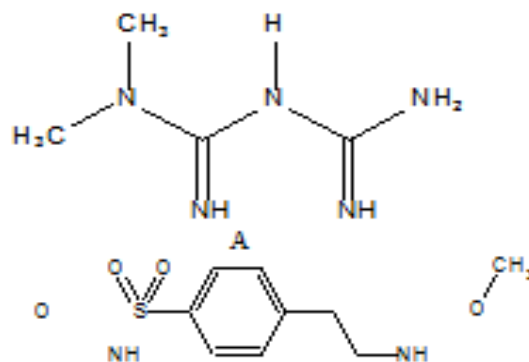
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## INTRODUCTION:

Diabetes is one of the costliest health problems in the world. Globally, diabetes is likely to be the Fourth leading cause of death.[19] Approximately 90% of people with diabetes have type-II diabetes. It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises; the pancreas gradually loses its ability to produce insulin.

Type II diabetes is associated with older age, obesity, family history of gestational diabetes, impaired glucose metabolism, physical inactivity and race ethnicity.[4] If the glycemic target level is not achieved with one oral agent alone, combination oral and/or insulin therapy is recommended [10, 14] Combination oral therapy becomes an obvious choice when glycemic control is not achieved with conventional monotherapy.[5] The advantages of oral dose combinations as compared to their components which are taken alone are lower cost and better patient compliance. [17] Combination therapy has been shown to achieve greater blood glucose lowering than monotherapy because different classes have different and complimentary mechanisms of action. Therefore, it is more logical to add another drug than replace the existing drug. The rapid introduction of combination therapy with two or three complementary oral anti diabetics help in targeting the dual effect and also reduced adverse effects.[16]

Chemically, Metformin (MET) is 1, 1-Dimethylbiguanide and Glabenclamide (GLB) is 5-chloro- N-[2-(4-[[[(cyclohexylcarbonyl)] sulfonyl}phenyl]-2-Methoxybenzamide. Metformin is an anti-diabetic drug from the biguanide class of oral hypoglycemic agents, given orally in the treatment of non-insulin dependent diabetes mellitus.[3] Metformin reduces free fatty acid oxidation because enhanced free fatty acid oxidation in diabetes contributes to increased hepatic glucose production and development of insulin resistance while Glabenclamide is inhibiting ATP-sensitive potassium channels in pancreatic beta cells. This inhibition causes cell membrane depolarization, which causes voltage-dependent calcium channels to open, which causes an increase in intracellular calcium in the beta cell, which stimulates insulin release.[15] Structure of MET and GLB are shown in (Figure 1).



**Figure 1: Structures of Antidiabetic Drugs: A-Metformin, B-Glabenclamide.**

The review of literature reveals that there were analytical methods of two drugs individually or in combinations with other drugs has also been reported in pharmaceutical dosage forms and even in biological samples[1, 2, 6, 7, 11, 12, 13, 18] and no methods has yet been reported for combination of these two drugs. It was essential to develop a chromatographic method for simultaneous estimation of two drugs in a tablet formulation. The method described is rapid, precise, and accurate and can be used for routine analysis of tablets. It was validated as per ICH norm.[8,9]

## MATERIALS AND METHODS:

### Instrumentation

The LC system used for work was Perkin Elmer Quaternary pump Series 200 which comprised of auto sampler injector; and an Intelligence PDA detector connected to the Total Chrome Navigator version 6.3. For controlling the instrumentation as well as processing the data generated.

### Material and reagents

MET and GLB were obtained as gift sample from Macleods Research Laboratory (Mumbai, MS, India) and Cipla, Pvt. Ltd. (Mumbai, India) respectively. Acetonitrile (HPLC grade), Ammonium acetate (AR grade), methanol (HPLC grade), orthophosphoric acid (AR grade) were obtained from Rankem Pvt. Ltd. Delhi, India. The 0.45  $\mu$ m membrane filter was used throughout the experiment. The tablets of MET in combination with GLB (Glucored Forte) were purchased from Local market. HPLC grade water was used throughout the experiment. Other chemicals used in the experiment were of analytical or HPLC grade.

### Chromatographic conditions

The isocratic mobile phase consists of Acetonitrile and Ammonium acetate buffer (pH 5.0) in the ratio of 60:40 (v/v) with a constant flow rate of 0.7 mL/min. A Hypersil ODS C<sub>18</sub> column (250 mm  $\times$  4.6 mm, 5  $\mu$ m) was used as the stationary phase. MET and GLB have different  $\lambda_{\text{max}}$  but considering the chromatographic parameter, sensitivity, and selectivity of the method for these drugs, 256 nm was selected as the detection wavelength for PDA detector. The injection volume for sample was 20  $\mu$ L.

### Mobile phase

The mobile phase consisted of Acetonitrile and Ammonium acetate buffer in the ratio 60:40 (v/v). The pH of the buffer was adjusted to 5.0 with orthophosphoric acid. The buffer used in the mobile phase consisted of 0.05 M Ammonium Phosphate in HPLC grade water. The mobile phase was premixed and filtered through a 0.45- $\mu$ m membrane filter and degassed.

### Standard preparation

**Metformin** - An accurately weighed 100 mg of MET was transferred in a 100 mL volumetric flask, and dissolved in methanol. Appropriate dilutions were done and the final stock solutions was sonicated for 20 min and filtered through 0.45  $\mu$ m membrane filter.

**Glibenclamide**- An accurately weighed 5 mg of GLB was transferred in a 50 mL volumetric flask, and dissolved in methanol. Appropriate dilutions were done and the final stock solutions was sonicated for 20 min & filtered through 0.45  $\mu$ m membrane filter.

### Calibration curve solutions

From the mentioned stock solutions of MET and GLB calibration curve solutions containing 500 - 1000  $\mu$ g/mL of MET, 10-60  $\mu$ g/mL of GLB in each calibration level were prepared.

### Preparation of sample solutions

Twenty tablets were weighed and finely powdered. A quantity equivalent to one tablet containing 500 mg of MET and 5 mg of GLB was transferred in a 50 mL volumetric flask and volume was made with methanol. The contents were sonicated for 20 min with methanol, and filtered through Whatman filter paper.

## RESULTS AND DISCUSSION:

### Optimization of chromatographic conditions

The chromatographic method was optimized after performing different experiments to achieve the adequate retentions and resolution for the peaks of GLB and MET. To set the adequate retentions and resolution, the effects of the mobile phase components, changes in ionic strength were studied, initially methanol and water in different ratios were tried. But MET gave broad peak shape While GLB gave no peak, so water was replaced by potassium dihydrogen buffer (0.2 M), and mixture of methanol and potassium dihydrogen phosphate buffer in different ratios were tried. It was found that both peaks show broad peaks. Finally Acetonitrile: 0.05 M Ammonium acetate buffer pH 5.0 adjusted with OPA in ratio of 60:40 v/v gave acceptable retention time (4.44 min for MET and 7.67 min for GLB) and resolution for MET and GLB was found to be 6.947 at the flow rate of 0.7 mL/min.

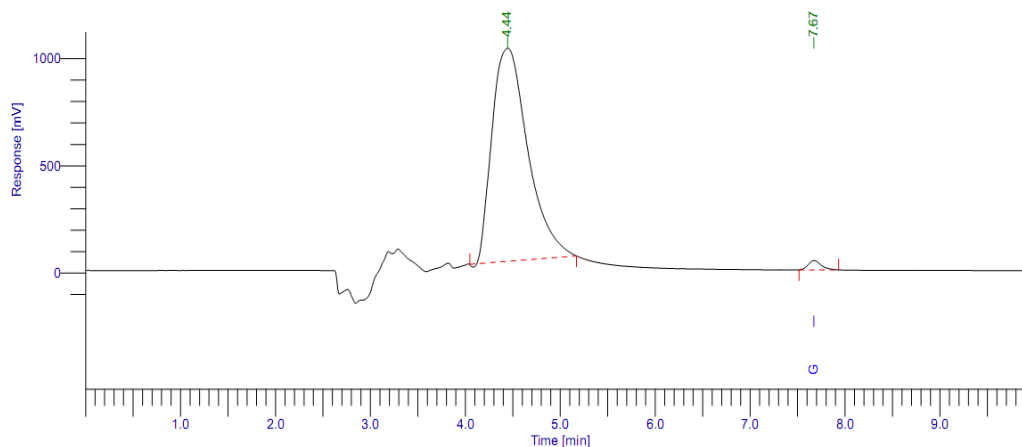
### Determination of active ingredients in tablets

The contents of two drugs in tablets were determined by the proposed method using a calibration curve. The determinations were done in two sets of precision which were repeatability, interday and intraday precision and six samples were prepared for each set. The results of marketed formulation are shown in (Table 1). The chromatogram of the tablet sample is shown in (Figure 2).

**Table 1: Results of marketed formulation.**

Sr. No.	Amount present (mg/tab)		Amount Found (mg/tab)		% of Drug Found	
	GLB	MET	GLB	MET	GLB	MET
1	5.00	500	4.98	500.22	99.60	100.04
2	5.00	500	5.00	499.93	100.00	99.98
3	5.00	500	5.01	499.67	100.20	99.93
4	5.00	500	4.97	499.80	99.40	99.96
5	5.00	500	4.99	500.46	99.80	100.09
6	5.00	500	5.02	500.10	100.40	100.22
				Mean	99.9	100.03
				SD	0.3741	0.1067
				% RSD	0.3744	0.1066

S.D.- Standard Deviation.



**Figure 2: A typical chromatogram of Metformin and Glibenclamide.**

### Validation of method Specificity

The specificity of the method was checked by a peak purity test of the sample preparation done by PDA detector. The result of the peak purity analysis shows that the peaks of the analytes were pure and also the formulation excipients were not interfering with the analyte peaks.

### Calibration and linearity

The standard solutions containing 500 - 1000 µg/mL of MET and 10-60 µg/mL of GLB in each linearity level were prepared. Linearity solutions were injected in triplicate. Calibration graphs were found to be linear for both the analytes in the mentioned concentrations. The coefficient of correlation was found to be 0.999 and 0.999 for MET and GLB, respectively. (Table 2) shows calibration data for Glibenclamide and Metformin.

**Table 2: Calibration data for Glibenclamide and Metformin.**

Sr. No.	Concentration of GLB (µg/ml)	Area	Concentration of MET (µg/ml)	Area
1	10	11951	500	432638
2	20	26510	600	509313
3	30	38230	700	599792
4	40	51253	800	669806
5	50	66249	900	753580
6	60	78579	1000	841026
Slope		1076		24769
Y-intercept		1329		812
Correlation coefficient ( $r^2$ )		0.999		0.999

### Accuracy (recovery test)

The accuracy of the method was done by recovery study. The recovery experiments were performed by adding known amounts of the pure drug to the preanalyzed sample. The recovery was done at three levels: 80%, 100%, and 120% of the label claim. Three samples were prepared for each recovery level. The recovery values for GLB and MET was found to be 99.81 and 99.99 respectively. Both drugs' accuracy test results were shown in (Table 3).

**Table 3: Results of the Recovery Tests for the Drugs.**

Level of % Recovery	Amount present		Amount of standard added		Total amount recovered	% Recovery*		
	(mg/tab)	(mg)	(mg)	(mg)*				
	GLB	MET	GLB	MET	GLB	MET	GLB	MET
80	5	500	4	400	8.96	899.94	99.59	99.99
100	5	500	5	500	10.00	1000.16	100.00	100.01
120	5	500	6	600	10.98	1099.87	99.84	99.98
						Mean	99.81	99.99
						SD	0.2066	0.0152
						% RSD	0.2069	0.0152

\*Each value is the mean of three observations, S.D.- Standard Deviation.

### Precision

The precision of the method was studied by determining the concentrations of each ingredient in the tablet six times for repeatability. In the repeatability study, % relative standard deviation of the MET and GLB were found to be less than 2.0 % indicate that the method is reproducible.

For intraday precision of the method was done by analyzing the six replicates of sample for two times in a day. While for interday precision six sample replicates are analysed once a day for three days. The percentage assay was calculated using the calibration curve. The assay results are shown in (Table 4).

**Table 4: Results of precision study.**

Formulation	Parameter	Repeatability*	Intra-day precision*	Inter-day precision*
GLB	Mean	99.90	100.06	100.16
	SD	0.3741	0.3962	0.5773
	% RSD	0.3744	0.3959	0.5763
MET	Mean	100.03	99.81	99.89
	SD	0.1067	0.2783	0.2990
	% RSD	0.1066	0.2788	0.2993

\*Each value is a mean of six observations, S.D.- Standard Deviation.

### Determination of the limits of detection and Quantitation

For determining the limits of detection (LOD) and quantitation (LOQ), the method based on the relative standard deviation (RSD) of a regression line and slope was adopted. To determine the LOD and LOQ, a specific calibration curve was studied using samples containing the analytes in the range of the detection and quantitation limits. The LOD for GLB and MET were 0.8936 and 0.5160 µg/mL, and the LOQ were 2.994 µg/mL and 1.723 µg/mL respectively.

### System suitability

For system suitability studies, five replicate injections of mixed standard solutions were injected, and the parameters like RSD of peak area ratio, column efficiency, resolution, and tailing factor of the peaks were calculated. Results are reported in (Table 5).

**Table 5: System Suitability Parameters.**

Parameters	MET	GLB
Retention time (min)	4.44	7.67
Tailing Factor	1.36	1.25
Theoretical Plates	13300.17	5018.41
Resolution	6.947	

S.D.- Standard Deviation.

### Robustness

To evaluate robustness of the developed method, few parameters were deliberately varied. These parameters include variation of flow rate, percentage of acetonitrile in the mobile phase, pH of buffer and column temperature. Each factor selected was changed at three levels (-1, 0, +1). One factor was changed at one time to estimate the effect. The results of robustness are reported in (Table 6).

**Table 6: Results of robustness study.**

Factor Level		Retention time*		Tailing Factor*		Area*		% Content*	
Flow Rate									
		GLB	MET	GLB	MET	GLB	MET	GLB	MET
1.3	-1	7.91	4.87	1.32	1.31	12238	842542	99.98	100.47
1.4	0	7.67	4.45	1.25	1.36	11352	842831	99.65	100.08
1.5	1	7.21	4.09	1.26	1.29	11826	854718	99.91	99.97
Mean		7.59	4.47	1.27	1.32	11805	846697	99.84	100.17
± S.D.		±0.35	±0.39	±0.03	±0.03	±443.56	±6947.89	±0.17	±0.26
Mobile Phase									
		GLB	MET	GLB	MET	GLB	MET	GLB	MET
48:52	-1	7.99	4.62	1.29	1.28	11794	837936	99.26	100.56
50:50	0	7.67	4.45	1.25	1.36	11352	842831	99.65	100.08
52:48	1	7.37	4.28	1.27	1.38	12718	838158	100.49	100.97
Mean		7.67	4.45	1.27	1.34	11954	839641.7	99.8	100.53
± S.D.		±0.31	±0.17	±0.02	±0.05	±697.02	±2764.2	±0.62	±0.44

Temp.		GLB	MET	GLB	MET	GLB	MET	GLB	MET
29	-1	7.70	4.52	1.31	1.30	12481	841732	99.50	100.27
30	0	7.67	4.45	1.25	1.36	11352	842831	99.65	100.08
31	1	7.25	4.41	1.23	1.31	12679	836928	100.06	99.83
<b>Mean</b>		<b>7.54</b>	<b>4.46</b>	<b>1.26</b>	<b>1.32</b>	<b>12170</b>	<b>840497</b>	<b>99.73</b>	<b>100.06</b>
<b>± S.D.</b>		<b>±0.25</b>	<b>±0.05</b>	<b>±0.04</b>	<b>±0.03</b>	<b>±715.8</b>	<b>±3139.3</b>	<b>±0.28</b>	<b>±0.22</b>
pH		GLB	MET	GLB	MET	GLB	MET	GLB	MET
6.1	-1	7.69	4.48	1.30	1.34	12673	852914	99.85	100.40
6.2	0	7.67	4.45	1.25	1.36	11352	842831	99.65	100.08
6.3	1	7.49	4.32	1.21	1.27	11673	838136	100.23	99.69
<b>Mean</b>		<b>7.61</b>	<b>4.41</b>	<b>1.25</b>	<b>1.32</b>	<b>1899.33</b>	<b>844627</b>	<b>99.91</b>	<b>100.05</b>
<b>± S.D.</b>		<b>±0.11</b>	<b>±0.08</b>	<b>±0.04</b>	<b>±0.04</b>	<b>±688.9</b>	<b>±7550.92</b>	<b>±0.29</b>	<b>±0.35</b>

\*Each value is the mean of three observations, S.D.- Standard Deviation.

### Ruggedness

Degree of reproducibility of test results obtained by analysing the sample under variety of normal test conditions such as different analysts and days. Such experiments were performed by different analysts. The results of ruggedness are given in (Table 7).

**Table 7: Results of Ruggedness.**

Formulation	Parameter	Different analysts*		Different day*	
		Analyst- I	Analyst- II	Day- I	Day- II
<b>GLB</b>	Mean	99.95	99.82	99.91	99.87
	SD	0.2569	0.4256	0.2315	0.4660
	% RSD	0.2570	0.4263	0.2317	0.4666
<b>MET</b>	Mean	99.98	100.02	100.12	99.71
	SD	0.2581	0.0723	0.2753	0.4823
	% RSD	0.2581	0.0722	0.2749	0.4837

\*Each value is a mean of six observations, GLB- Glibenclamide, MET- Metformin, S.D.- Standard Deviation, R.S.D.- Relative Standard Deviation

### CONCLUSION:

It can be concluded from present study that method can be used for the simultaneous determination of Glibenclamide and Metformin in the pharmaceutical dosage form. The method is validated and shown to be accurate and precise. It can be used in the quality control departments for the analysis of Glibenclamide in combination with Metformin.

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### Conflict of Interest:

The authors declare no conflict of interest.

### Abbreviations:

GLB - Glibenclamide  
MET - Metformin  
S.D. - Standard Deviation  
R.S.D. - Relative Standard Deviation

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