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HUMAN

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR SIMULTANEOUS ESTIMATION OF VOGLIBOSE, GLIMEPIRIDE AND METFORMIN HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM BY HPLC

Kadam V. N.[*], Yadav P. J., Mohite, S. K., Magdum C. S.

Rajarambapu College of Pharmacy, Kasegaon;

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ABSTRACT

Fast, economical, accurate, precise and reproducible RP–HPLC method was developed for the determination of Voglibose (VGB), Glimepiride (GLM) and Metformin HCl (MET). RP–HPLC method was developed on Jasco 2075 HPLC systems with Fine pack ODS C18 column (250mm) and using a mobile phase mixture containing mixed acetonitrile: phosphate buffer in the ratio of 85:15 (pH 4).The flow rate was 1 ml/min and the effluent was monitored at 223nm. The retention time of Voglibose, Glimepiride and Metformin HCl were 2.3, 3.8 and 5.1 min respectively. The method was validated in terms of linearity, precision, accuracy, specificity and system suitability parameters. The proposed method's results were found to be satisfactory and are suitable for simultaneous determination of Voglibose, Glimepiride and Metformin HCl for routine quality control of drugs in bulk drug and formulation.

Correspondence to Author:
Kadam V. N.
Tal- Walwa; Dist- Sangli; 415 404
Maharashtra, India
Email id - varshakadam60@rediffmail.com

INTRODUCTION

Glimepiride is a Sulfonyl Urea derivative. Chemically it is [[p-[2-(3ethyl-4-methyl-2-oxo-3-Pyrroline-1-Oxamide) ethyl] phenyl] sulfonyl] 3-(Trans 4-methyl cyclohexyl) urea. It is widely used in type-2 diabetes. It is an oral AntiDiabetic with prolonged effect and it maintains a more physiological regulation of insulin secretion during physical exercise, which suggests that there may be less risk of hypoglycemia [1].

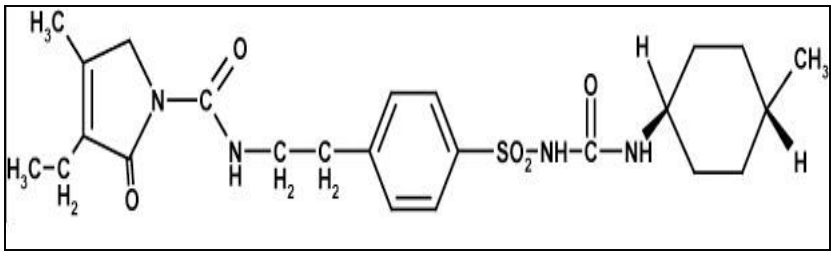

Fig 1: Structure of Glimepiride

Voglibose3,4-Dideoxy-4-[2-hydroxy-1-(hydroxyl methyl) ethyl]amino-2-c-(hydroxymethyl)-Depiinositol, has attracted considerable interests due to its wide range of therapeutic and pharmacological properties, including its excellent inhibitory activity against α-glucosidase and its action against hyperglycemia and various disorders caused by hyperglycemia. Voglibose, a new potent glucosidase inhibitor used for type 2 diabetes, has shown strong anti-obesity and antidiabetic activity[2].

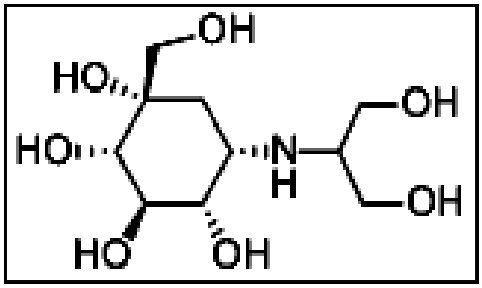

Fig 2: Structure of Voglibose

Chemically Metformin (MET) is 3-(diaminomethylidene)-1, 1-dimethylguanidine, an oral antidiabetic drug, used for the treatment of type 2 diabetes, particularly in overweight and obese people; also used in the treatment of polycystic ovary syndrome and has been investigated for other diseases where insulin resistance may be an important factor [2].

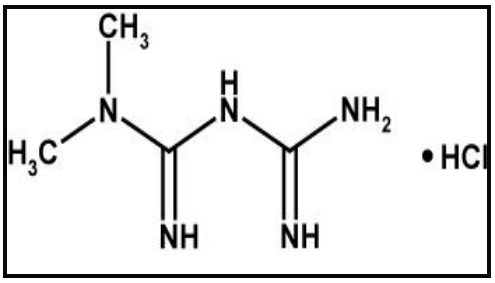

Fig 3: Structure of Metformin HCl

INSTRUMENTS AND MATERIALS

Instrument used were JASCO 2075 HPLC systems. Voglibose pure drug was obtained from Micro labs. Ltd. Bangalore, Glimepiride from Ipca laboratories, Mumbai and Metformin HCl from Sohan Health Care Pvt. Ltd, Pune as gift sample and was used without further purification. All chemicals and reagents used were of analytical grade. Tablets were purchased from market.

EXPERIMENTAL

A. Preparation of Standard Stock Solutions:

Standard stock solutions 100 µg/ml of Voglibose, Glimepiride and Metformin HCl were prepared separately in methanol. And further dilutions were made in selected mobile phase.

B. Selection of analytical wavelength:

Standard stock solutions of Voglibose, Glimepiride and Metformin HCl were prepared in methanoland from the standard stock solution further dilutions were done using mobile phase. Each solution was scanned over the range of 200- 400 nm and their spectra were overlaid. It was observed that 226 nm is the λ max for VGB, 229 nm is the λ max for GLM & 232 nm is λ max of MET. 223 nm was selected as isobestic wavelength for analysis of VGB, GLM and MET which is selected from overlay spectra of three drugs shown in Fig. no. 4.

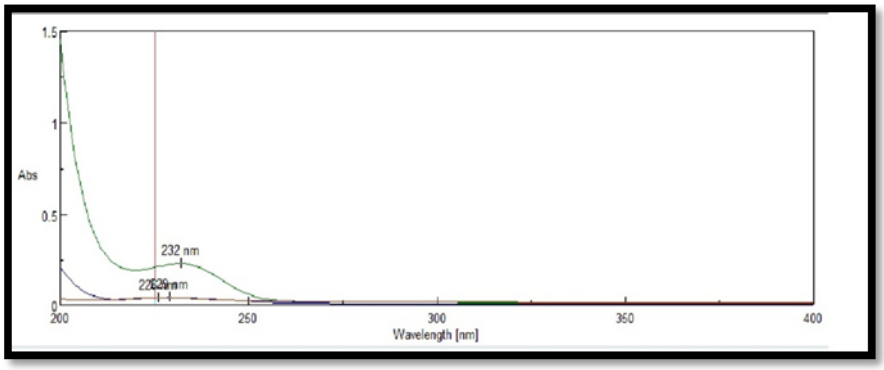

Fig. no.4: Overlain spectra of Voglibose, Glimepiride and Metformin HCl

C. Selection of mobile phase:

Different mobile phases like Water: Methanol, Methanol: Potassium dihydrogen phosphate buffer, Water: Acetonitrile, Acetonitrile: Potassium dihydrogen phosphate buffer were tried in order to find the optimum conditions for the separation of Voglibose, Glimepiride and Metformin HCl. Finally, Acetonitrile: Potassium dihydrogen Phosphate buffer (0.01 M, pH 4), (85:15 v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for VGB, GLM and MET.

D. Preparation of mobile phase:

Acetonitrile: Potassium dihydrogen Phosphate buffer (0.01 M, pH 4), in the ratio (85:15 v/v) was prepared. pH of mobile phase was adjusted to 4 by using ortho-phosphoric acid.

E. Preparation of Standard drug solutions:

An accurately weighed quantity of about 10 mg of VGB, 10 mg GLM and 10 mg of MET were taken in 100 ml three separate volumetric flask dissolved in sufficient quantity of methanol then sonicated for 15 min and diluted to 100 ml with the same solvent so as to get the concentration of 100 µg/ml of VGB, GLM and MET each. From standard stock solution of each drug, appropriate dilution was done using the mobile phase to get mixed standard solutions. The chromatogram obtained is shown in Fig. no. 5.

F. Sample Preparation:

Twenty Tablets were weighed and powdered. Powder equivalent to 500 mg of MET, 2 mg of GLM and 0.2 mg of VGB was taken in 100 ml flask, 80 ml of methanol was added, derivatization of Voglibose was done by taurine and sodium periodate and sonicated for 15 min and solution was filtered through Whatmann paper no. 42 into a 100 ml volumetric flask. Filter paper was washed with same solvent, adding washings to the volumetric flask and volume was made up to the mark with the solvent to get a stock solution containing 5000 µg/ml of MET, 20 µg/ml of GLM and 2 µg/ml of VGB. The solution was suitably diluted with mobile phase to have 50 µg/ml of MET, 0.2 µg/ml of GLM and 0.02 µg/ml of VGB. The chromatogram obtained is shown in Fig. no. 6. The results obtained are shown in Table no. 1[3,4].

RESULTS AND DISCUSSION

The objective of the present work is to develop precise and reliable HPLC method for the analysis of Voglibose, Glimepiride & Metformin HCl in bulk and pharmaceutical dosage form. This is achieved by using the most commonly employed column C18 with U.V. detection at 223 nm. The representative chromatogram indicating combination of Voglibose, Glimepiride & Metformin HCl is shown in fig. 6.

Table 1: Analysis of marketed formulation

Marketed Formulation	Drug	Label Claim (mg)	Amount taken (µg/ml)	Amount Found (µg/ml)	% Label claim± SD*
TRIVOLIB 2	Voglibose	0.2	0.02	0.0198	99.00
	Glimepiride	2	0.2	0.196	98.73
	Metformin HCl	500	50	49.93	99.86

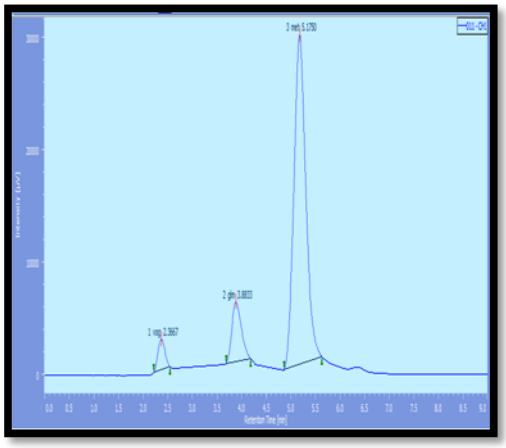

Fig. no. 5: Chromatogram of Standard formulation of VGB, GLM & MET

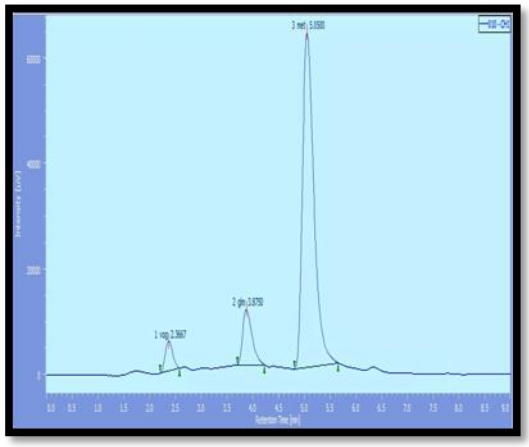

Fig. no.6: Chromatogram of Marketed mixture of VGB, GLM & MET

VALIDATION OF THE PROPOSED METHOD[5]

As an integral part of analytical method development is validation. The proposed method was validated as per ICH guidelines**.**

a) Linearity and Range

From the standard stock solutions, suitable dilutions using mobile phase were made containing 100 µg/ml of VGB, 100 µg/ml of GLM and 100 µg/ml of MET to prepare range of standard solutions containing six different concentrations of analytes. The linearity of the relationship between peak area and concentration was determined by analyzing six working standard solutions over the concentration

range of 1 µg/ml – 6 µg/ml for VGB, 2 - 12 µg/ml of GLM and 10 µg/ml – 60 µg/ml for MET as per ICH guidelines. The results obtained are shown in Table no. 2 & 3.

b) Precision Studies

The precision of the method was evaluated by intraday and inter-day variation studies. In intraday studies, solution of standard and sample were analyzed for assay determination thrice in a day and % RSD was calculated. The result obtained for intraday and inter-days variations are shown in Table no. 4. For Inter-days variations studies, same concentrations of the mixed standard were analyzed on three consecutive days.

c) Accuracy

To check the accuracy of the method, recovery studies were carried out at three different levels 50%, 100% and 150% according to ICH guidelines. From the tablet powder blend, weight equivalent 0.02 mg VGB, 0.2 mg GLM and 50 mg of MET was weighed and transferred to 100 ml volumetric flask. To three flasks, appropriate quantity of working standard of VGB, GLM and MET was added equal to 50%, 100% and 150% of the equivalent weight of Tablet taken and volume was made up with double distilled water. The solutions are then filtered through Whatmann filter paper No. 42. From these stock solutions further dilutions were prepared using mobile phase. The results obtained are shown in Table no. 5.

d) Limit of Detection & Limit of Quantitation

The Limit of Detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value and Limit of Quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOD & LOQ of Voglibose, Glimepiride and Metformin HCl are shown in table no.6.

e) Ruggedness

Ruggedness of the methods was assessed by carrying out assay six times with two different analysts by using same equipment. The results of the same are presented in Table no. 7.

f) System suitability

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed or The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Resolution (R), Asymmetry, LOD (µg·mL-1) and LOQ (µg·mL-1) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of Voglibose, Glimepiride and Metformin HCl in bulk and marketed tablet dosage form was validated or not. The results of the same are presented in Table no. 8.

Table No. 2: Linearity study for VGB, GLM & MET HCl

Sr. No.	Voglibose		Glimepiride		Metformin HCl	
	Conc. (µg/ml)	Absorbance	Conc. (µg/ml)	Absorbance	Conc. (µg/ml)	Absorbance
1	0	000000	0	000000	0.00	000000
2	1	111647	2	235054	10	1129677
3	2	222141	4	471363	20	2256136
4	3	331254	6	718100	30	3284579
5	4	440395	8	943363	40	4517206
6	5	550175	10	1157928	50	5652034
7	6	668682	12	1415180	60	6762202

Table 3: Linear regression analysis of calibration curves with their respective Absorptivity values

Parameters	Voglibose	Glimepiride	Metformin HCl
Slope	11037	11750	0.0702
Intercept	1681.85	2646	13208.35
Correlation coefficient (R^2)	0.9991	0.9997	0.9981
Range	1-6µg/ml	2-12 µg /ml	10-60 µg /ml

Table 4: Results of Precision Studies (Intra-day and Inter-day)

Analyte	Concentrations Of sample solution (µg/ml)	Intra-day precision % RSD (n=3)	Inter-day precision % RSD (n=3)
VGB	0.02	0.071	0.0595
GLM	0.2	0.044	0.037
MET	50	0.076	0.077

Table 5: Results of Recovery Studies

Level of % Recovery	% Mean Recovery			SD*			% RSD*		
	VGB	GLM	MET	VGB	GLM	MET	VGB	GLM	MET
50	98.32	98.76	99.43	0.0966	0.0101	0.7583	0.1268	0.0441	0.7810
100	98.55	98.66	98.56	0.295	0.6072	0.6450	0.5308	0.6800	0.7058
150	99.08	99.44	98.50	0.2532	0.6354	0.5357	0.2552	0.6446	0.5438

Table 6: Results of LOD & LOQ

Parameter	VGB	GLM	MET
LOD (µg /ml)	0.5854	0.0637	0.6447
LOQ (µg /ml)	0.7176	0.1931	0.8100

Table no. 7: Results of Ruggedness studies

	Drugs	Label claim(mg)	AmountFound (µg /ml)	% Label claim ± SD*
Analyst 1	**Voglibose**	0.04	0.0398	99.50±0.0884
	Glimepiride	0.4	0.3961	99.25±0.0665
	Metformin HCl	50	50.02	100.04±0.0965
Analyst 2	**Voglibose**	0.04	0.0395	99.66±0.2134
	Glimepiride	0.4	0.3997	99.92±0.0365
	Metformin HCl	50	49.994	99.98 ±0.0985

*****Average of six determinations.

Table no. 8: System suitability parameters

Parameters	**Obtained Values**		
	Voglibose	Glimepiride	Metformin HCl
Theoretical plates (N)	3157	2219	2157
Resolution (R)	9.72		
Asymmetry	1.007	1.084	1.74
LOD (µg·mL^{-1})	0.5854 µg /ml	0.0637µg /ml	0.6447µg /ml
LOQ (µg·mL^{-1})	0.71760 µg /ml	0.1931µg /ml	0.8100µg /ml

CONCLUSION

RP-HPLC method was developed and validated as per ICH and USP guidelines by using Acetonitrile: 0.01 M Potassium dihydrogen phosphate buffer, as mobile phase (85:15), pH was adjusted 4 with ortho phosphoric acid. The wavelength for detection used was 223 nm and flow rate was 1 ml/min. Retention times of Voglibose, Glimepiride and Metformin hydrochloride were found to be 2.3 min, 3.8 min and 5.1 min respectively. Retention time was much less and resolution was more than reported method. The method was linear over the concentration range of 1-6 µg/ml for Voglibose, 2-12 µg/ml for Glimepiride and 10-60 µg/ml for Metformin hydrochloride. The validation of method

carried out as per ICH guidelines. The methods were found to be accurate, precise, economical and reproducible.

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