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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE, VOGLIBOSE AND GLIMEPIRIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

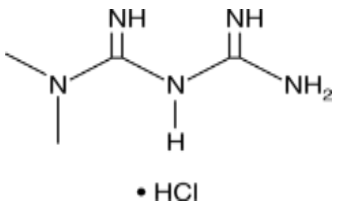
A simple, Precise, Accurate method was developed for the estimation of Metformin HCl, Voglibose and Glimepiride by RP-HPLC technique. Chromatographic conditions used are stationary phase Luna Phenyl Hexyl 250 mm x 4.6 mm, 5µm., Used Buffer as 1ml OPA in 1 lt water: Acetonitrile: Methanol in the ratio of 40:30:30 and flow rate was maintained at 1.0 ml/min, detection wave length was 260 nm, column temperature was set as ambient and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 5% to100 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.2 for repeatability and 1.2 for intermediate precision. LOD and LOQ are 0.10 µg/ml and1.00 µg/ml respectively. By using above method assay of marketed formulation was carried out 100.01% was present. Degradation studies of Metformin HCl, Voglibose and Glimepiride were done, in all conditions purity threshold was more than purity angle and within the acceptable range.

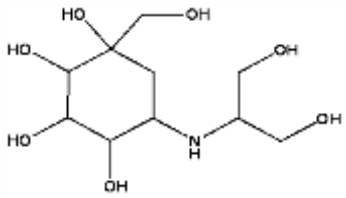
KEYWORDS: HPLC, Metformin HCl, Voglibose and Glimepiride, Method development, ICH Guidelines.

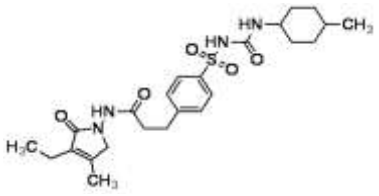
INTRODUCTION

Metformin HCl 3-(diaminomethylidene)-1, 1-dimethylguanidine; hydrochloride, Metformin HCl is an antihyperglycemic agent which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin HCl decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Soluble in water, 95% alcohol. Practically insoluble in ether, chloroform.

Voglibose(1S,2S,3R,4S,5S)-5- [(1,3-dihydroxypropan-2-yl) amino] -1- (hydroxymethyl) cyclohexane-1,2,3,4-tetrol, Voglibose is Alpha-glucosidase inhibitors are saccharides that act as competitive inhibitors of enzymes needed to digest carbohydrates: specifically alpha-glucosidase enzymes in the brush border of the small intestines. Soluble to 100 mM in Water and to75 mM in DMSO Glimepiride 3-ethyl-4-methyl-N-{2-[4- ({[(4-methylcyclohexyl) carbamoyl]amino}sulfonyl) phenyl]ethyl}-2-oxo-2,5-dihydro-1H-pyrrole-1- carboxamide, Glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells, and increasing sensitivity of peripheral tissues to insulin. Soluble in Water.







MATERIALS AND METHODS

Chemicals

Metformin HCl, Voglibose and Glimepiride was purchased from Indian market manufactured by USV, (Mumbai). Commercial Pharmaceutical preparation of Metformin HCl, Voglibose and Glimepiride Hydrochloride tablets which are claimed to contain 500 mg, 0.2 mg, 1 mg were used in analysis.

Instrumentation

Analysis was performed on waters HPLC, 2695 Empower software used separation module equipped with PDA detector, Auto sampler and column compartment with Empower 2

software. Other equipment used in the study was analytical balance (DENVER) and P^H meter (EUTECH instrument). Ultra sonic bath (UNICROME ASSOCIATES: UCA-701).

Chromatographic Conditions

Luna C_{18} column (250 mm x 4.6 mm ID, Particle size 5µm) was used for chromatographic separation using mobile phase composition of 1ml OPA in 1lt water, Acetonitrile, methanol in the ratio (40:30:30 v/v) used as mobile phase and flow rate of 1.0 ml/min.with run time 10min Mobile phase and sample solutions were filtered through a 0.45µm membrane filter and degassed. The detection of three drugs was carried out at 260 nm.

Method Development

Standard stock solutions of 10mg/ml of Metformin HCl, Voglibose and Glimepiride were prepared separately using diluent (Buffer: Acetonitrile - 45:55v/v). The METH stock solution was diluted with diluent to give working standard solution containing 70-650 µg/ml concentration. Similarly the VOG stock solution was diluted with diluent to give working standard solution in the range 0.04-.0.400 µg/ml.GLIM stock solution was diluted with diluent to give working standard solution in the range 0.14-1.560 µg/ml. These solutions were filled into vials and placed in vial holder. The linearity was determined separately for METH, VOG and GLIM by injecting eight concentrations of three drugs prepared in diluent and calibration curves were constructed by plotting area against the respective concentrations

VALIDATION OF METHOD

The HPLC method was validated in accordance with ICH guidelines. The system precision of the method was verified by six replicate injections of standard solution containing Metformin HCl, Voglibose and Glimepiride. The method precision was carried out for the analyte six times using the proposed method. Repeatability was measured by multiple injections of homogenous sample of Metformin HCl, Voglibose and Glimepiride. Accuracy was carried out by percentage recovery studies at three different concentration levels. To the pre-analysed samples solution of Metformin HCl, Voglibose and Glimepiride, a known amount of standard drug powder of Metformin HCl, Voglibose and Glimepiride were added at 50, 100, 150% level. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is a procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantification (LOQ) and was determined using the formulae;

LOD =3.3 **σ** /S and LOQ = 10 **σ** /S, where, **σ** is the standard deviation 0f response and S is the slope of the calibration curve.

Robustness was evaluated by making deliberate variations such as variation of wavelength, flow rate and change in mobile phase composition. The robustness of the method was studied for Metformin HCl, Voglibose and Glimepiride.Ruggedness of the method was performed by two different analysts using same experimental and environmental conditions. It was performed by injecting 400 µg/ml of METH, 0.2 µg/ml of VOG and 1.0 µg/ml solutions of GLIM, respectively.The system suitability parameters such as resolution; number of theoretical plates and tailing factor were studied.

Stability of sample solution was established by the storage of sample solution at 25^{oc} for 12hr and 24hrs. Sample solution was reanalysed after 12 hrs and 24 hrs time intervals and assay was determined for Metformin HCl, Voglibose and Glimepiride and compared against fresh sample.

Forced Degradation Studies we studied different stress conditions like acid degradation, alkali degradation, heat degradation, hydrolysis, peroxide degradation, reduction degradation, thermal degradation and photo degradation. From all the degradation studies the maximum degradation for Metformin HCl is 26.2 at acid, Voglibose is 30 at hydrolysis, and Glimepiride is 29.6 at peroxide Linear correlation was obtained between peak area Vs concentration of Metformin HCl, Voglibose and Glimepiride were within the range. The linearity of the calibration curve was validated by the high value of correlation co-efficient of regression equation.

Analysis of Formulation

To determine the content of METH, VOG and GLIM in injection formulation (METH 500mg, VOG 0.2 mg GLIM 1mg). Weighed 10 tablets and crushed to powdered then 5 tablets equivalent of sample was taken into a 250 ml volumetric flask. Added 200 ml of diluent sonicated to dissolve and diluted to volume with diluent. Further diluted 5 ml to 100 ml with the diluent. Filtered through 0.45µ Nylon syringe filter. From the filtrate a 5ml solution was transferred into 50 ml volumetric flask and volume was made up to the mark with diluent to obtain a concentration of 500 µg/mL of METH, 0.2 µg/mL of VOG and 1µg/mL of GLIM which was then subjected to proposed method and the amounts of MET,VOG and GLIM were determined using calibration curves.

RESULTS

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The objective of this study was to develop a simple, rapid, precise, accurate and sensitive HPLC method for the simultaneous estimation of Metformin HCl, Voglibose, Glimepiride in bulk and its pharmaceutical dosage form.

The developed method for simultaneous estimation of Metformin HCl, Voglibose, Glimepiride was carried out an Luna Phenyl Hexyl (250 mm x 4.6 mm, 5 µm) in an isocratic mode, using mobile phase composition of 1ml OPA in 1lt Water, methanol and acetonitrile in the ratio (40:30:30 v/v) with a flow rate 1.0 ml/min. The effluents were monitored at 260 nm. From the results % assay value of Metformin HCl, Voglibose, Glimepiride were found to be 100.2% and 100.5% respectively.

Linearity was observed over the concentration range of 70 - 650 µg/ml for METH 0.04 - 0.40 µg/ml of VOG and 0.19 – 1.560 µg/ml of GLIM. Correlation coefficient was found to be 0.999 for both drugs which indicates that the concentration had given good linearity. The %RSD values of Metformin HCl, Voglibose, Glimepiride for System precision and Method precision was found to 0.194 & 0.554 and 0.252 & 0.613 and 0.395& 0.502 respectively. As the results are within acceptance limit of less than 2%, indicates that the proposed method has good reproducibility. The results are good for both method precison and system precision.

From the results shown in accuracy, it was found that the mean percentage recovery values of pure drug were found to be 100.2% for Metformin HCl, Voglibose, Glimepiride and as these results are within the acceptance limit of 98%-102% which indicates that the method was accurate. The robustness of the developed method was evaluated by changing the flow rate and mobile phase composition. All the parameters were within the limits at all variable conditions, which indicates that the method was robust. The ruggedness of the proposed method was analyzed by two different analysts. The %RSD was found within the limits i.e., should not be more than 2.0. Hence the proposed method has good repeatability.

Table 1: Precision of Developed Method

S.No	Method precision	System precision

	Metformin HCl		Voglibose		Glimepiride		Metformin HCl		Voglibose		Glimepiride	
	Rt(min)	Area	Rt(min)	Area	Rt(min)	Area	Rt(min)	Area	Rt(min)	Area	Rt(min)	Area
1	2.280	104187	4.453	6485	7.864	61249	2.333	105595	4.454	6155	7.880	60957
2	2.274	104558	4.466	6485	7.910	61180	2.324	106143	4.453	6707	7.879	61381
3	2.272	103200	4.459	6386	7.893	61908	2.321	105965	4.453	6475	7.879	61346
4	2.267	104840	4.453	6476	7.871	61675	2.337	105728	4.459	6633	7.886	61415
5	2.280	104449	4.453	6485	7.864	61249	2.321	105689	4.452	6464	7.873	61419
6	2.274	104558	4.466	6485	7.910	61180	2.317	105714	4.448	6270	7.860	60895
Mean	2.274	104299	4.458	6467	7.885	61407	2.321	105806	4.453	6451	7.876	61236
%RSD		0.5540		0.6130		0.5024		0.1944		0.2524		0.3952

Table 2: Accuracy Data

Spike Level	Area	Amount of sample added (µg/ml)	Amount of API added (µg/ml)	Amount found (µg/ml)	% Recovery	%RSD
			Metformin HCl			
50%	**51781**	662.3	250	913.0	100.1	**Mean 100.1**
	50903	662.9	250	912.9	100.0	**SD 0.09**
	51069	661.3	250	912.7	100.2	**%RSD 0.91**
100%	**104727**	411.2	500	912.9	100.4	**Mean 100.5**
	104945	410.1	500	912.6	100.6	**SD 0.12**
	104816	410.7	500	912.8	100.5	**%RSD 1.10**
150%	**154528**	162.0	750	913.0	100.6	**Mean 100.2**
	154716	162.3	750	912.8	100.3	**SD 0.17**
	154334	163.0	750	912.9	99.9	**%RSD 1.12**
			Voglibose			
50%	**2963**	911.9	0.1	913.0	100.1	**Mean 100.1**
	2928	912.8	0.1	912.9	100.0	**SD 0.10**
	2995	911.1	0.1	913.1	100.2	**% RSD 0.12**
100%	**6908**	913.0	0.2	912.9	99.9	**Mean 100.2**
	6978	910.2	0.2	913.2	100.3	**SD 0.12**
	6926	909.5	0.2	913.4	100.4	**% RSD 0.52**
150%	**9870**	909.8	0.3	912.9	100.3	**Mean 100.4**
	9847	908.3	0.3	913.2	1005	**SD 0.17**
	9782	908.9	0.3	912.8	100.4	**% RSD 0.46**
			Glimepiride			
50%	**28342**	911.5	0.5	913.0	100.1	**Mean 100.1**
	28794	912.3	0.5	912.8	100.0	**SD 0.04**
	28175	910.1	0.5	912.9	100.2	**% RSD 0.12**
100%	**61466**	910.0	1	912.9	100.2	**Mean 100.2**
	61249	910.9	1	913.0	100.1	**SD 0.09**
	61180	908.1	1	912.7	100.4	**% RSD 0.24**
150%	**90646**	907.8	1.5	913.0	100.4	**Mean 100.4**
	89882	908.4	1.5	912.8	100.3	**SD 0.16**
	90171	907.1	1.5	913.2	100.5	**% RSD 0.42**

Table 3: Validation and System Suitability Parameters.

Parameter	Metformin HCl	Voglibose	Glimepiriride

Range (µg/ml)	70-650 µg/ml	0.04- 0.400µg/ml	0.14- 1.560 µg/ml
Slope	234.7	25139	57362
Intercept	234.7+4719	25139+116.3	57362+1772
Correlation coefficient (R^2)	0.999	0.999	0.999
Retention time	2.333	4.454	7.880
Precision (intra and inter day)% RSD	<2	<2	<2
Accuracy	100.1-100.2%,	100.1-100.4%	100.1-100.4%
LOD(µg/ml)	5.001	0.002	0.012
LOQ(µg/ml)	15.003	0.006	0.036
Tailing factor	1.31	1.24	1.05
Theoretical plates	9672	12225	13577
Resolution	---	16.68	15.55

Table 4: influence of flow rate, and mobile phase Composition on analytical parameters.

Parameter	**Metformin HCl**			**Voglibose**			**Glimepiriride**		
	Rt(min)	**Area**	**Tailing**	**Rt(min)**	**Area**	**Tailing**	**Rt(min)**	**Area**	**Tailing**
Flow rate(±0.2ml/min)									
0.8	2.799	131093	1.51	5.566	8024	1.27	9.895	77066	1.06
1.0	1.88	86665	1.35	3.702	5338	1.19	6.503	51600	1.03
1.2	1.883	85341	1.35	4.090	5193	1.30	4.090	50800	1.04
Mobile phase composition (±5:2.5:2.5%v/v)									
45:27.5:27.5	2.184	108696	1.26	4.117	6726	1.09	6.286	61272	1.12
40:30:30	2.333	105595	1.31	4.454	6155	1.24	7.880	60957	1.07
35:32.5:32.5	2.308	104249	1.73	4.860	4404	0.96	10.270	61169	1.06

Table 5: Assay of Commercial Formulation.

Drug	**Label Claim for sample taken (mg)**	**Calculated value (mg±SD)**	**% of Assay**
Metformin HCl	500	500.3	100.06
Voglibose	0.2	0.2	100.0
Glimepiride	1	1.002	100.2

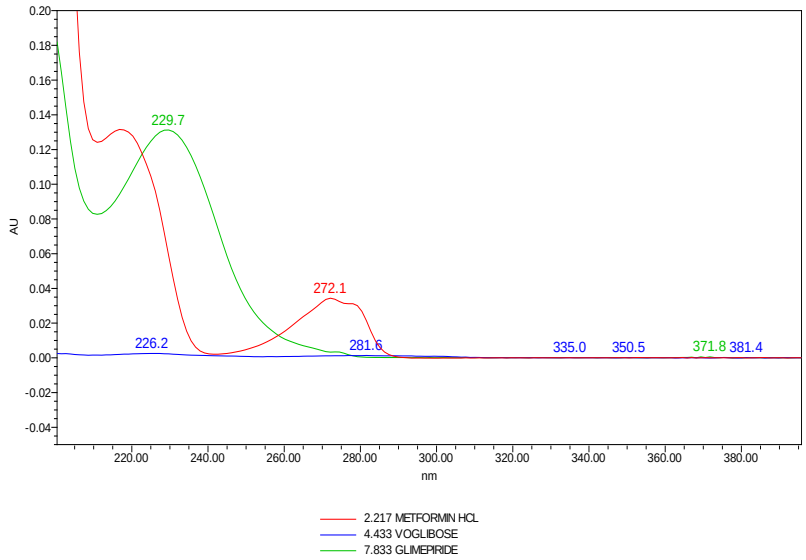


Figure 1: Overlay Spectra of Standard METH, VOG and GLIM

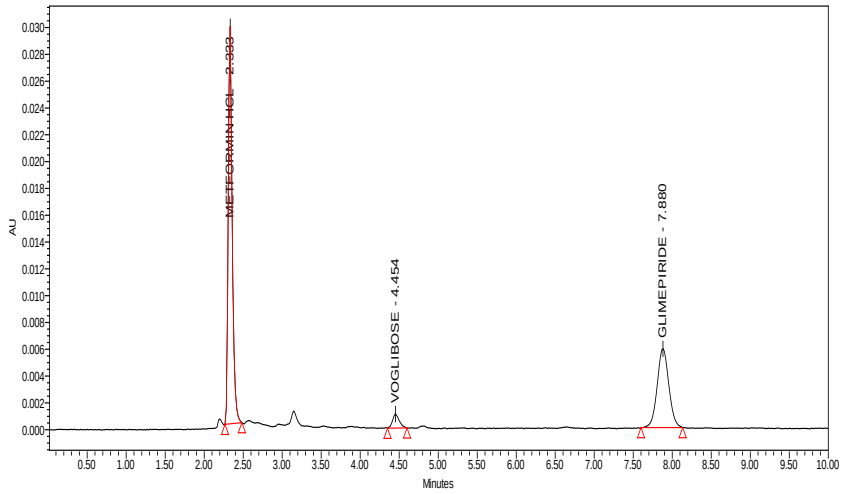
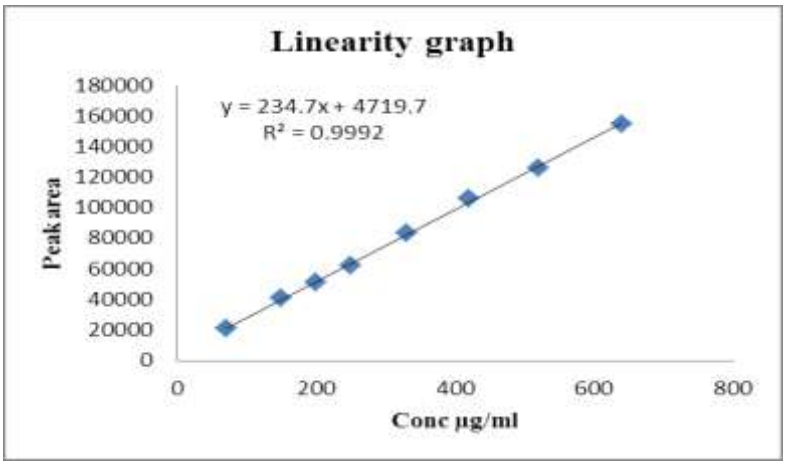
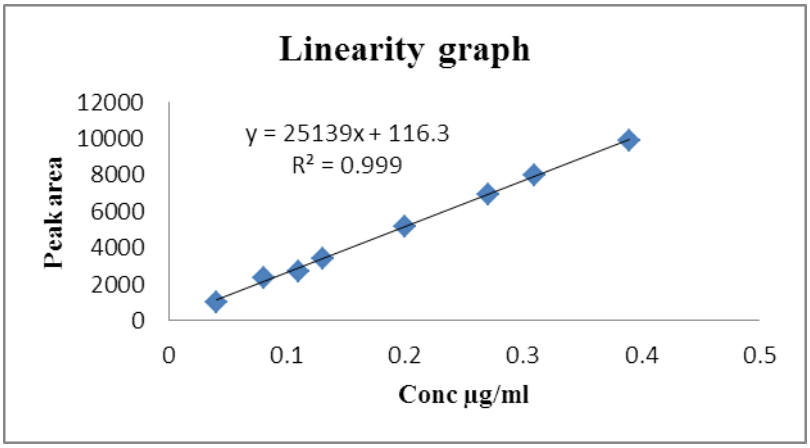


Figure2: Typical HPLC chromatogram ofMETH, VOG and GLIM





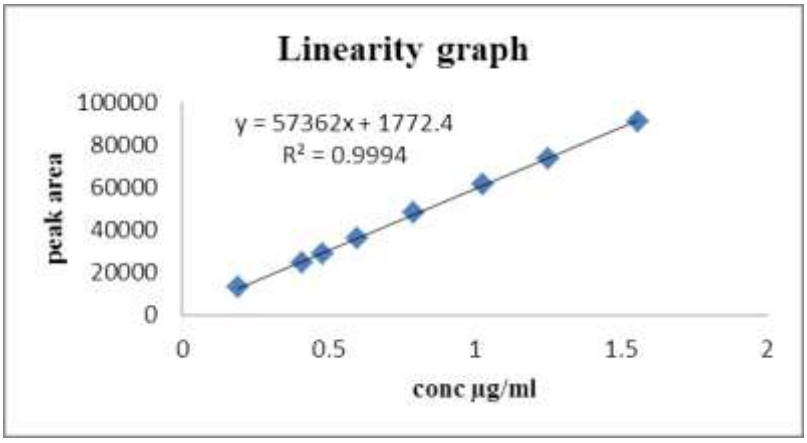


Figure 3: Calibration curves for METH, VOG and GLIM

DISCUSSION

The developed RP-HPLC method was found suitable for simultaneous estimation of METH, VOG and GLIM with good resolution, peak shapes and minimal tailing. The peak areas of the drug were reproducible as indicated by low coefficient of variance indicating the repeatability of the proposed method. High correlation coefficient of 0.999 showed the stable linear detector response in different concentration ranges of both the drugs.

The proposed method was validated as per ICH guidelines. The method exhibited good selectivity and sensitivity. Percent recoveries for METH, VOG and GLIM were 100.1-100.2%, 100.1-100.4% and 100.1-100.4% respectively, indicating the accuracy of the proposed method. Low LOD and LOQ values indicate high sensitivity of the proposed method. The %RSD values of less than 2 for intra and inter day variation studies indicated that the proposed was precise. The developed method was studied for percentage recovery at three concentration levels and %RSD values of less than 2 were found which were in acceptable limits indicates the method was accurate. Low %RSD values of less than 2 in

variation of flow rate and mobile phase ratio indicates the method was robust. When the method was performed by two different analysts under the same experimental and environmental conditions and %RSD was found to be less than 2 indicating the ruggedness of the proposed method. The results from solution stability experiments confirmed that sample was stable up to 24 hr. during assay determination. The sample recoveries of METH, VOG and GLIM from the commercial tablet dosage form were in good agreement with respective label claim indicating that there were no interferences from the commonly used tablet excipients and buffer used in analysis.

CONCLUSION

The low standard deviation and %RSD calculated for the proposed developed method and validation were in conformity with standards. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be employed successfully for the simultaneous estimation of METH, VOG and GLIM in tablet dosage form for routine quality control analysis.

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