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TO DEVELOP A VALIDATED ANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN, GLICLAZIDE AND VOGLIBOSE BY RP-HPLC

B.Nishanth, V.Kiran Kumar

Mother Teresa College of Pharmacy, Ghatkesar, NFC Nagar, Hyderabad, Telangana, India

Abstract:

Another technique was built up for concurrent estimation of Metformin and Gliclazide and Voglibose RP-HPLC strategy. Metformin and Gliclazide was unreservedly solvent in water and liquor. Voglibose was uninhibitedly solvent in liquor and sparingly dissolvable in water. Methanol and potassium dihydrogen ortho phosphate (pH 3) was picked as the versatile stage. The run season of the HPLC strategy was 5 minutes. The strategy was approved for framework appropriateness, linearity, exactness, precision, explicitness, roughness vigor, LOD and LOQ. The framework appropriateness boundaries were inside breaking point, subsequently it was presumed that the framework was reasonable to play out the examine. The strategy shows linearity between the focus scope of 10-100 µg/ml. The % recuperation of Metformin, Gliclazide and Voglibose were seen as in the scope of 99.23 % - 98.11 %. As there was no impedance due to excipients and portable stage, the technique was seen as explicit. The technique was powerful and rough as seen from immaterial variety in the consequences of examination by changes in Flow rate and Mobile stage sythesis independently and investigation being performed by various investigators.

Keywords: *Metformin, Gliclazide and Voglibose, RP-HPLC, Simultaneous estimation.*

Corresponding author:

B.Nishanth,

M. Pharmacy,

Mother Teresa College of Pharmacy, Department of pharmaceutical Analysis, Ghatkesar, NFC Nagar, Hyderabad, Telangana, India

E-mail: karne.rahul98@gmail.com

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INTRODUCTION:

Metformin is an antihyperglycemic agent of the biguanide class, used for the management of type II diabetes. Metformin is considered an antihyperglycemic drug because it lowers blood glucose concentrations in type II diabetes without causing hypoglycemia. Metformin is commonly described as an *insulin sensitizer* leading to a decrease in insulin resistance and a clinically significant reduction of plasma fasting insulin levels [1]. Metformin's mechanisms of action are unique from other classes of oral antihyperglycemic drugs. Metformin decreases blood glucose levels by decreasing hepatic glucose production (gluconeogenesis), decreasing the intestinal absorption of glucose, and increasing insulin sensitivity by increasing peripheral glucose uptake and utilization. It is well established that metformin inhibits mitochondrial complex I activity, and it has since been generally postulated that its potent antidiabetic effects occur through this mechanism. The above processes lead to a decrease in blood glucose, managing type II diabetes and exerting positive effects on glycemic control [2-3]. IUPAC name 3-(diaminomethylidene)-1,1-dimethylguanidine.
Molecular weight is 129.16. Molecular formula is $C_4H11N5.HCl$.

Gliclazide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It has been classified differently according to its drug properties in which based on its chemical structure, gliclazide is considered a first-generation sulfonylurea due to the structural presence of a sulfonamide group able to release a proton and the presence of one aromatic group.[4] On the other hand, based on the pharmacological efficacy, gliclazide is considered a second-generation sulfonylurea which presents a higher potency and a shorter half-life. Gliclazide belongs to the sulfonylurea class of insulin secretagogues, which act by stimulating β cells of the pancreas to release insulin. Sulfonylureas increase both basal insulin secretion and meal-stimulated insulin release. Medications in this class differ in their dose, rate of absorption, duration of action, route of elimination and binding site on their target pancreatic β cell receptor. Sulfonylureas also increase peripheral glucose utilization, decrease hepatic gluconeogenesis and may increase the number and sensitivity of insulin receptors.[5] IUPAC name 3-[(3aR,6aS)-octahydrocyclopenta[c]pyrrol-2-yl]-1-(4-methylbenzenesulfonyl) urea. Molecular Weight is 323.4. Molecular formula is $C_{15}H_{21}N_3O_3S$.

Voglibose is for the treatment of diabetes. It is specifically used for lowering post-prandial blood glucose levels thereby reducing the risk of macrovascular complications. 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.[5] The membrane-bound intestinal alpha-glucosidases hydrolyze oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the small intestine. Acarbose also blocks pancreatic alpha-amylase in addition to inhibiting membrane-bound alpha-glucosidases.[6] Pancreatic alpha-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of complex carbohydrates.[7] IUPAC name is (1S,2S,3R,4S,5S)-5-[(1,3-dihydroxypropan-2-yl) amino]-1-(hydroxymethyl) cyclohexane-1,2,3,4-tetrol. Molecular Weight is 267. Molecular formula is $C_{10}H_{21}NO_7$.

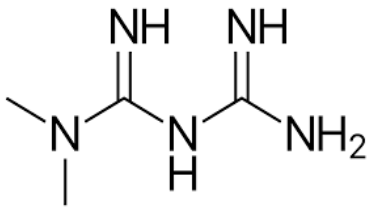


Fig no: 1 Structure of Metformin

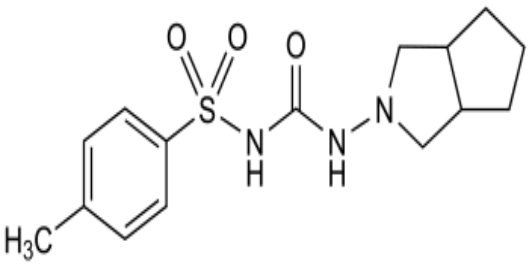


Fig no: 2 Structure of Gliclazide

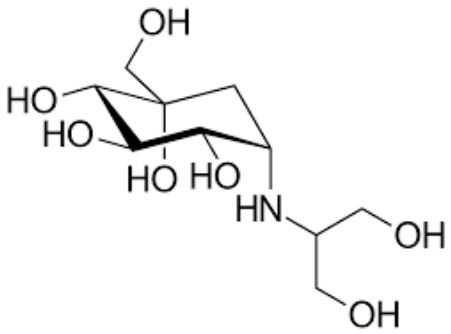


Fig no: 3 Structure of Voglibose

The literature survey revealed that There are very few methods reported in the literature for analysis of Metformin, Gliclazide and Voglibose alone or in combination with other drugs in the pure form and pharmaceuticals formulations. There was some HPLC methods,[8-20] which have been reported for analysis of these drugs alone or combination with other drugs. Hence, an attempt was made to develop RP-HPLC method for simultaneous estimation of metformin, Gliclazide and Voglibose in pharmaceutical dosage form. It can be adopted in regular quality control test in industries and laboratories.

MATERIALS AND METHODS:

Chemicals and Reagents: Metformin, Gliclazide, Voglibose were supplied by KP labs, Hyderabad. Dosage form was purchased by Local Pharmacy.Ortho phosphoric acid was analytical grade supplied by Finer chemical LTD, Mumbai, Water and Methanol for HPLC LICHROSOLV (MERCK)

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 240 nm with an Agilent C_{18} (4.6 x 150mm, 1.7µm) dimensions at ambient temperature. The optimized mobile phase consists of Methanol: phosphate buffer PH 3.5 (65:35). Flow rate was maintained at 1 ml/min and run time for 10 min.

Selection of wavelength:
An answer of 10 µg/ml of Metformin, Gliclazide and Voglibose were set up in milli Q water. The subsequent arrangements were examined exclusively on HPLC PDA finder from 190 to 400 nm and furthermore in UV-Visible spectrophotometer. The ideal reaction for three of them was acquired at 240 nm. Henceforth the total strategy was handled at the frequency of 240 nm.

Preparation of solutions:

Preparation of standard:
10 mg of Metformin,320 mg of Gliclazide and 25 mg of Voglibose were precisely gauged and moved into a 100 ml clean dry volumetric cup, around 70 ml of diluent was included, sonicated to break down it totally and the volume was made sufficient with a similar dissolvable to give a convergence of 100 µg/ml,3200 µg/ml,250 µg/ml. (Stock arrangement) Further 1 ml was pipetted out from the above stock arrangement into a 10 ml volumetric carafe and weakened sufficient with diluent to give a grouping of 10µg/ml, 320µg/ml and 25 µg/ml individually.

Preparation of Sample:
10 Tablets of substance were gauged and triturated in glass mortar. The amount of powder equal to 10 mg of Metformin present in 10 tablets (1754.5mg) was moved into a 100 ml clean dry volumetric carafe, 70 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for around 30 minutes by shaking at time frames minutes each and was weakened sufficient with diluent to give a convergence of 100 µg/ml,3200 µg/ml,250 µg/ml, permitted to remain until the buildup settles before taking an aliquot for additional weakening (stock arrangement). 1 ml of upper clear arrangement was moved to a 10 ml volumetric flagon and weakened with diluent sufficient to give the separate focuses as standard with standard arrangement. The arrangement was separated through 0.45 □m channel before infusing into HPLC framework.

Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Metformin, Gliclazide and Voglibose peaks and calculate the %Assay by using the formulae.

Preparation of mobile phase :(for Optimized Conditions)
Take 2.5 gm of potassium dihydrogen ortho phosphate into 1000ml volumetric flask dissolved in hplc grated water and adjust ph upto 3 with ortho phosphoric acid. From the above prepared buffer take 350 ml (35%) and 650ml of Methanol HPLC (65%) were mixed and degassed in ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtration.

METHOD:
System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1ml/min for 10 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Agilent C_{18} (4.6 x 150mm, 1.7µm), the mobile phase of composition Methanol: phosphate buffer P^H 3.5 (65:35) was allowed to flow through the column at a flow rate of 1ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Metformin, Gliclazide and Voglibose in their tablet dosage form. The result obtained for Metformin, Gliclazide and Voglibose was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity and Range: Stock solution was prepared by dissolving the appropriate amount of Metformin, Gliclazide and Voglibose in 100 ml of diluent and further diluted to the required concentrations with diluent. The solution was prepared at five concentration levels ranging from 600 µg/ml to 1800 µg/ml of Metformin, 02 µg/ml to 10µg/ml of Gliclazide, 1 µg/ml to 5 µg/ml of Voglibose. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3,4,5.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Metformin, Gliclazide and Voglibose and calculate the individual recovery and mean recovery values. The results are shown in table 6,7,8.

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all five Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 9.

Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all five injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 10.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The results are shown in table 11-16.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines.

RESULTS AND DISCUSSION:

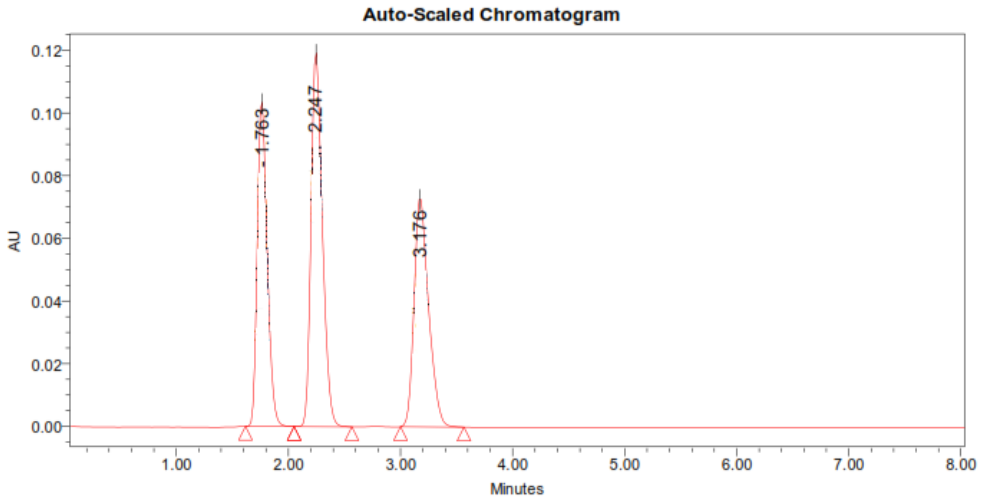


Figure 5: Standard chromatogram

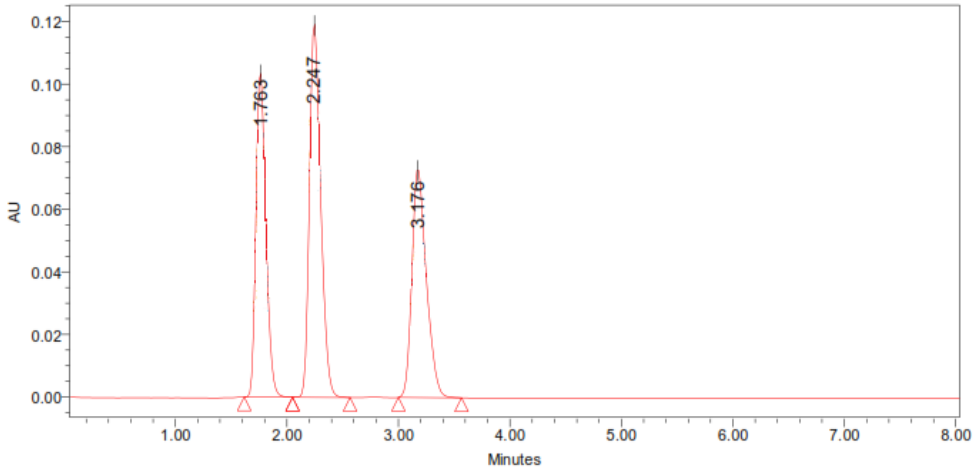


Figure 6: Sample chromatogram

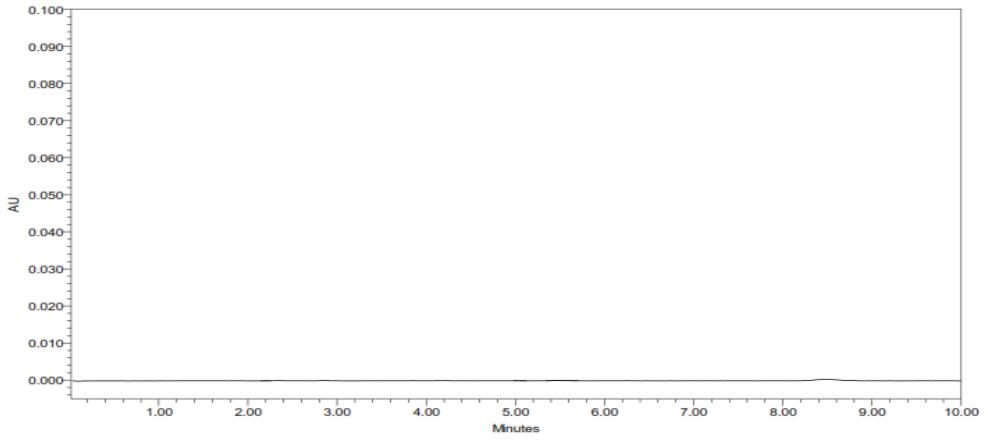


Figure 7: Blank chromatogram

Table 1: System suitability parameters for Metformin, Gliclazide, Voglibose

Parameters	Metformin	Gliclazide	Voglibose
Retention time	1.76	2.247	3.176
USP Plate count	2496	11451	2281
USP Tailing	1.00	1.07	1.51
USP Resolution	--	12.24	3.57

Table 2: Assay results for Metformin, Gliclazide, Voglibose

	Label Claim (mg)	% Assay
Metformin	10	99.07
Gliclazide	320	98.89
Voglibose	25	99.83

Table 3: Linearity results for Metformin

S.No	Linearity Level	Concentration	Area
1	I	5 ppm	631737
2	II	10 ppm	753615
3	III	15 ppm	899796
4	IV	20 ppm	1035191
5	V	25 ppm	1194356
Correlation Coefficient			0.99908

Table 4: Linearity results for Voglibose

S.No	Linearity Level	Concentration	Area
1	I	12.5 ppm	626221
2	II	25ppm	778750
3	III	37.5ppm	931447
4	IV	50ppm	1070162
5	V	62.5ppm	1196060
Correlation Coefficient			0.99916

Table 5: Linearity results for Gliclazide

S.No	Linearity Level	Concentration	Area
1	I	160 ppm	839286
2	II	320ppm	1067774
3	III	480 ppm	1246474
4	IV	640 ppm	1439994
5	V	800 ppm	1639065
Correlation Coefficient			0.99932

Table 6: Accuracy results for Metformin

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.31%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.89%
		15	14.86	99.0%	
		15	14.99	99.79%	

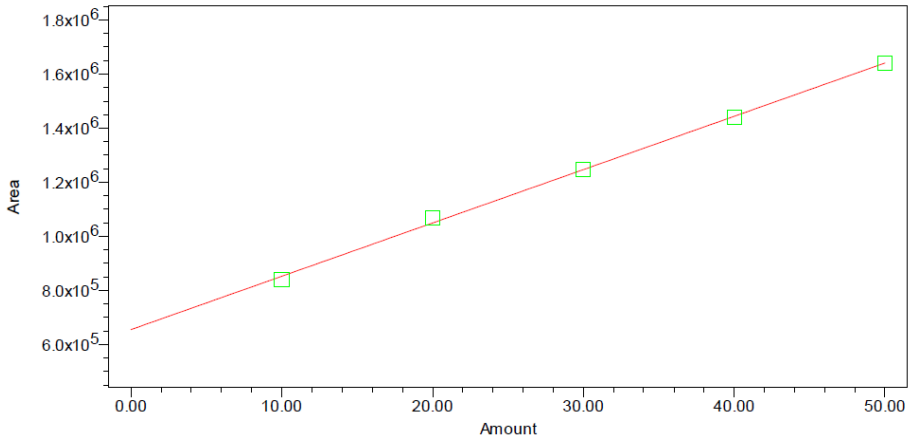


Fig: 8: Calibration curve of Voglibose

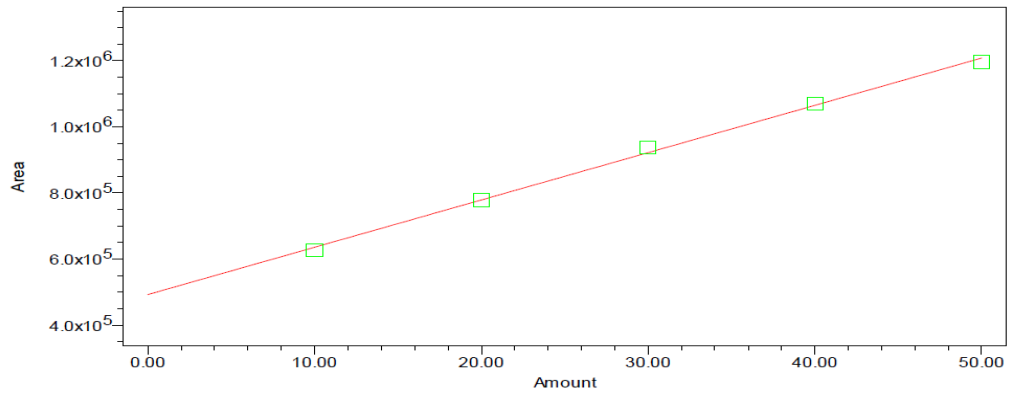
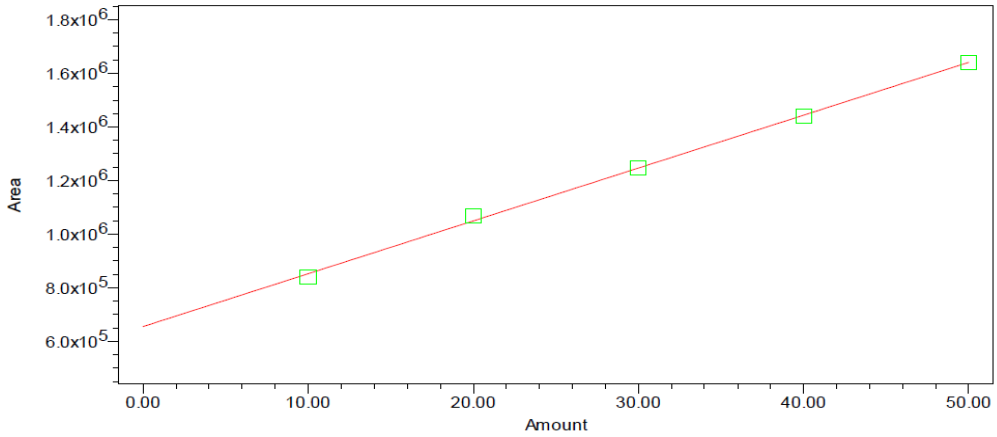


Fig: 9 Calibration curve of Gliclazide



Calibration curve of Metformin

Table 7: Accuracy results for Gliclazide

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	10	9.8	98%	100%
		10	10.2	102%	
		10	10	100%	
2	100 %	20	19.8	99%	100%
		20	20.2	101%	

		20	20	100%	
3	150 %	30	29.6	98.66%	99.33%
		30	30	100%	
		30	29.8	99.33%	

Table 8: Accuracy results for Voglibose

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.69%
		15	14.86	99.0%	
		15	14.82	99.79%	

Table 9: Precision results for Metformin, Gliclazide, Voglibose

Injection	Area for Metformin	Area for Gliclazide	Area for Voglibose
Injection-1	1247256	935035	954854
Injection-2	1248579	929353	937615
Injection-3	1243273	930459	950694
Injection-4	1243262	932389	940252
Injection-5	1249574	922057	922057
Average	1246389	929858.6	945423.4
Standard Deviation	2965.62	4865.16	7200.575
%RSD	0.23793	0.5232	0.761

Table 10: Ruggedness results for Metformin, Gliclazide, Voglibose

Injection	Area for Metformin	Area for Gliclazide	Area for Voglibose
Injection-1	1231404	912412	914922
Injection-2	1233196	913062	909335
Injection-3	1231008	909642	913266
Injection-4	1238575	916881	909418
Injection-5	1232407	914005	911496
Average	1233318	913200.4	911687.4
Standard Deviation	3061.06	2621.886	2432.859
%RSD	0.2481	0.287	0.2668

Robustness Results:

Table 11: Results for variation in flow for Metformin

S.No	Drug	Flow Rate ml/min		
		0.8ml/min	**1.0ml/min**	**1.2ml /min**
1	**Metformin**	2.098	1.620	1.587
USP Plate count		2511	2490	2484
USP Tailing		1.65	1.62	1.67

Table 12: Results for variation in flow for Gliclazide

S.No	Drug	Flow Rate ml/min		
		0.8 ml/min	1.0ml/min	1.2m l/min
1	Gliclazide		2.501	2.207
USP Plate count		2279	2268	2185
USP Tailing		1.47	1.48	1.48

Table 13: Results for variation in flow for Voglibose

S.No	Drug	Flow Rate ml/min		
		0.8ml/min	**1.0ml/min**	**1.2m l/min**
1	**Voglibose**	7.583	6.547	5.841
USP Plate count		2346	2556	2096
USP Tailing		1.28	1.24	1.27

*Results for actual flow (1.0ml/min) have been considered from Assay standard.

Table 14: Results for variation in mobile phase composition for Metformin

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	9160	1.03
2	*Actual	8586.2	1.02
3	10% more	8548	1.02

Table 15: Results for variation in mobile phase composition for Gliclazide

S.No	Drug	Mobile phase		
		Less organic	Normal	ore organic
1	**Gliclazide**	2.639	2.562	2.404
USP Plate count		2432	2262	2223
USP Tailing		1.35	1.48	1.5

Table 16: Results for variation in mobile phase composition for Voglibose

S.No	Drug	Mobile phase		
		Less organic	Normal	More organic
1	**Voglibose**	7.733	6.654	5.521
USP Plate count		2482	2556	2030
USP Tailing		1.20	1.24	1.31

*****Results for actual Mobile phase composition have been considered from Accuracy standard.

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Metformin, Gliclazide and Voglibose in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Metformin, Gliclazide and Voglibose in pure and its pharmaceutical dosage forms.

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