EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 13
329-337
Year: 2019

EVALUATION AND VALIDATION OF A UPLC METHOD FOR SIMULTANEOUS ESTIMATION OF GLIMEPIRIDE, METFORMIN AND VOGLIBOSE IN ORAL DOSAGE FORM

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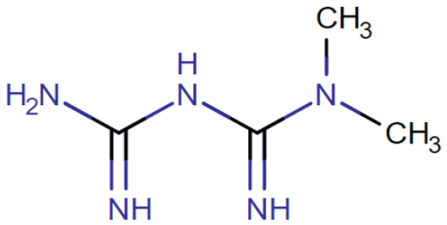
Article Received on 01/10/2019 **Article Revised on 21/10/2019** **Article Accepted on 11/11/2019**

ABSTRACT
A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of Glimepiride + Metformin + Voglibose in bulk drug and market dosage form. The method employed, with Hypersil C18 (100 mm x 2.1 mm, 1.7 µm) in a gradient mode, with mobile phase of Acetonitrile and Methanol in the ratio of 68:32%v/v. The flow rate was 1.0 ml/min and effluent was monitored at 260 nm. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 20- 100 µg/ml respectively. The LOD and LOQ values for were found to be 0.2099 (µg/ml) and 0.6362 (µg/ml) respectively. No chromatographic interference from excipients and degradants were found. The proposed method was successfully used for estimation of Glimepiride + Metformin + Voglibose in market dosage form.

KEYWORDS: Metformin, Glimepiride, Voglibose, oral dosage form, UPLC method.

INTRODUCTION
Glimepiride is indicated to treat type 2 diabetes mellitus; its mode of action is to increase insulin secretion by the pancreas. However, it requires adequate insulin synthesis as prerequisite to treat appropriately. It is not used for type 1 diabetes because in type 1 diabetes the pancreas is not able to produce insulin.



"Fig. 1: "Molecular Structure of Metformin, 1-carbamimidamido-N,N-dimethylmethanimidamide.

Therapeutic category	Antidiabetic drug
CAS Registry number	93479-97-1
Chemical name	*3-ethyl-4-methyl-2-oxo-N-(2-{4-[({[(1r,4r)-4-methylcyclohexyl]-C-hydroxycarbonimidoyl}amino)sulfonyl]phenyl}ethyl)-2,5-dihydro-1H-pyrrole-1-carboximidic acid*
Molecular formula	$C_{24}H_{34}N_4O_5S$
Molecular Weight	490.62
Solubility	Partly miscible in water
pka	2.23
λ_{max}	231 nm
Pharmacology	Glimepiride is indicated for the management of type 2 diabetes in adults as an adjunct to diet and exercise to improve glycemic control as monotherapy. It may also be indicated for use in combination with metformin or insulin to lower blood glucose in patients with type 2 diabetes whose high blood sugar levels cannot be controlled by diet and exercise in conjunction with an oral hypoglycemic (a drug used to lower blood sugar levels) agent alone

Metformin is used with a proper diet and exercise program and possibly with other medications to control high blood sugar. It is used in patients with type 2 diabetes. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, loss of limbs, and sexual function problems.

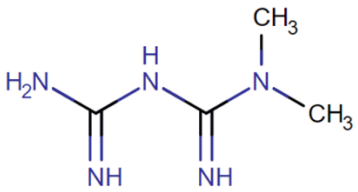


Fig. 2: Molecular Structure of Metformin, 1-carbamimidamido-N,N-dimethylmethanimidamide.

Therapeutic category	Antidiabetic drug
CAS Registry number	657-24-9
Chemical name	1-carbamimidamido-N,N-dimethylmethanimidamide
Molecular formula	$C_4H_{11}N_5$
Molecular Weight	129.163
Solubility	Soluble in 10mL of water
pka	12.4
λ_{max}	230 nm
Pharmacology	Metformin is indicated as an adjunct to diet and exercise to increase glycemic control in adults and pediatric patients 10 years of age and older diagnosed with type 2 diabetes mellitus

Voglibose is an alpha-glucosidase inhibitor used for lowering post-prandial blood glucose levels in people with diabetes mellitus. Voglibose delays the absorption of glucose thereby reducing the risk of macrovascular complications.

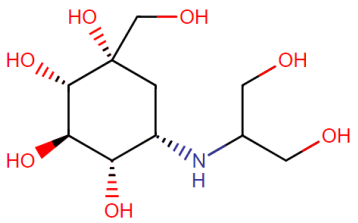


Fig. 3: Molecular Structure of Voglibose, (1S,2S,3R,4S,5S)-5-[(1,3-dihydroxypropan-2-yl)amino]-1-(hydroxymethyl) cyclohexane-1,2,3,4-tetrol.

Therapeutic category	Antidiabetic drug
CAS Registry number	83480-29-9
Chemical name	*(1S,2S,3R,4S,5S)-5-[(1,3-dihydroxypropan-2-yl)amino]-1-(hydroxymethyl)cyclohexane-1,2,3,4-tetrol*
Molecular formula	$C_{10}H_{21}NO_7$
Molecular Weight	267.276
Solubility	190.0 mg/mL
pka	12.46
λ_{max}	242 nm
Pharmacology	For the treatment of diabetes. It is specifically used for lowering post-prandial blood glucose levels thereby reducing the risk of macrovascular complications.

Validation of Analytical Methods (USP/ICH)

Method validation, according to the United States Pharmacopeia (USP), is performed to ensure that an analytical methodology is accurate, specific, reproducible, and rugged over the specified range that an analyte will be analyzed. Regulated laboratories must perform method validation in order to be in compliance with FDA regulations. In a 1987 guideline (Guideline for Submitting Samples and Analytical Data for Methods Validation), the FDA designated the specifications in the current edition of the USP as those legally recognized when determining compliance with the Federal Food, Drug and Cosmetic Act can be referred to as the "eight steps of method validation".

EXPERIMENTAL

MATERIALS

EQUIPMENTS	SOURCE
Ultra Pressure Liquid Chromatography (UPLC)	Acquity UPLC Systems, Waters Laboratories
Electrospray ionization and MS-MS	Mass Spectrometer PE Sciex Model: API 3000
Chromatographic data software	Empower
Column	C18 column (250 ×4.6 mm id)—ACE Generix
Detector	PDA
Injector	Automated
Electronic Balance	Eagle
Sonicator	Band Line Sonerex
p^H Meter	Lab India p^H meter

METHODOLOGY

Method Validation

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc. The described method extensively validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

RESULTS

Preparation of Standard Stock Solution

Preparation of Diluent

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 0.5 mL min-1 shows good resolution. The PDA detector response of Glimepiride + Metformin + Voglibose was studied and the best wavelength was found to be 226 nm showing highest sensitivity.

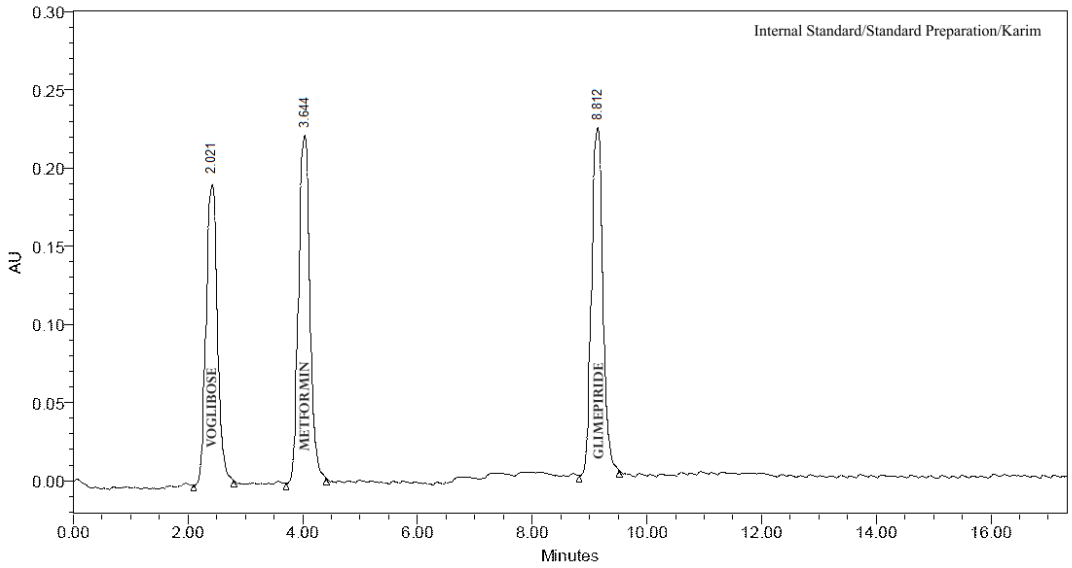
The mixture of two solutions Acetonitrile and Methanol in the ratio of 68:32%v/v with gradient programming was used as mobile phase at 0.5mL/min was found to be an appropriate mobile phase for separation of Glimepiride + Metformin + Voglibose. The column was maintained at ambient temperature.

Preparation of internal standard solution

Weighed accurately about 10 mg of Quinine sulphate working standard and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

Preparation of Glimepiride + Metformin + Voglibose standard solution

Weighed accurately about 10 mg of Glimepiride + Metformin + Voglibose and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.



Chromatogram: Chromatogram of standard preparation of Glimepiride + Metformin + Voglibose.

Accuracy

Glimepiride						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	02.25	02.23	99.11	99.39%	0.24846	0.25%
100	04.50	04.48	99.53			
150	06.75	06.72	99.55			

Table No: 193. Results of Accuracy Study (Glimepiride + Metformin + Voglibose)

Metformin						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	02.45	02.33	95.10	96.49%	0.3432	0.45%
100	04.75	04.54	95.57			
150	06.80	06.72	98.82			

Voglibose						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	02.33	02.21	94.84	97.76%	0.3586	0.35%
100	04.30	04.28	99.53			
150	06.50	06.43	98.92			

System Precision

Procedure

The parameters, retention time (RT), theoretical plates (N), tailing factor (T), peak asymmetry (As) and repeatability were evaluated at a concentration of 4 µg/mL (Glimepiride + Metformin + Voglibose)."

Parameters	**Glimepiride**	**Metformin**	**Voglibose**
Retention time (min) ± % RSD	8.846 ± 0.12	3.587 ± 0.12	2.176 ± 0.12
Theoretical plates ± % RSD	4673.63 ± 0.50	4455.75 ± 0.50	4055.55 ± 0.50
Asymmetry ± % RSD	1.08 ± 0.05	1.05± 0.05	1.10± 0.05
Repeatability (% RSD)	0.32	0.28	0.26

Table:. Results of System Precision (Glimepiride + Metformin + Voglibose)

Method Precision

"***Procedure:*** Precision was investigated using the sample preparation procedure for six consecutive replicates of sample of concentration 4 µg/mL for Glimepiride + Metformin + Voglibose."

Replicate		***Glimepiride + Metformin + Voglibose***			
S.No.	**Concentration Taken (µg/ml)**	**Area *Glimepiride***	**Area *Metformin***	**Area *Voglibose***	**%LC**
1	20.00	363431	358284	273221	99.98%
2		363486	354567	273266	99.97%
3		363393	355246	273213	99.99%
4		363434	357267	273323	99.98%
5		363452	356611	273443	99.98%
6		363391	357332	273656	99.99%
Average					99.98%
Std.Dev					0.00752
% RSD					0.01%
Standard weight					20 mcg
Standard potency					98.60%

Linearity

Glimepiride + Metformin + Voglibose				
Linearity level	**Concentration in µg/mL**	**Area *Glimepiride***	**Area *Metformin***	**Area *Voglibose***
1	20 µg/mL	162728	193814	173214
2	40 µg/mL	263389	294573	263389
3	60 µg/mL	374233	405466	374233
4	80 µg/mL	472884	493373	469884
5	100 µg/mL	562632	593345	562632
Correlation co-efficient		0.9968	0.9974	0.9985
Slope		25465.15	27214.13	24756.25
Intercept		260382.3	290145.5	265136.5

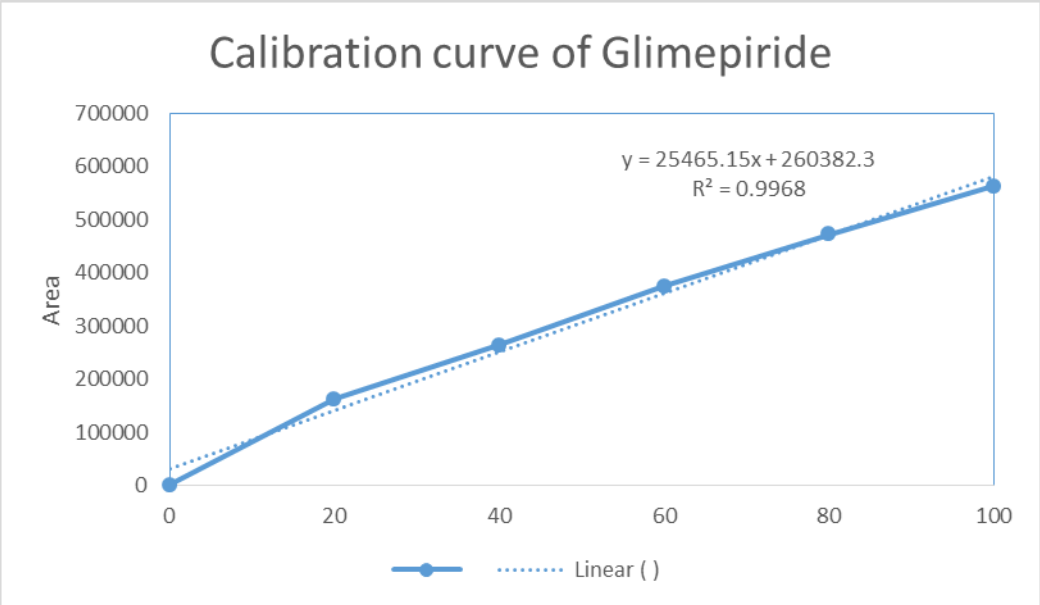


Fig.: Calibration curve of Glimepiride.

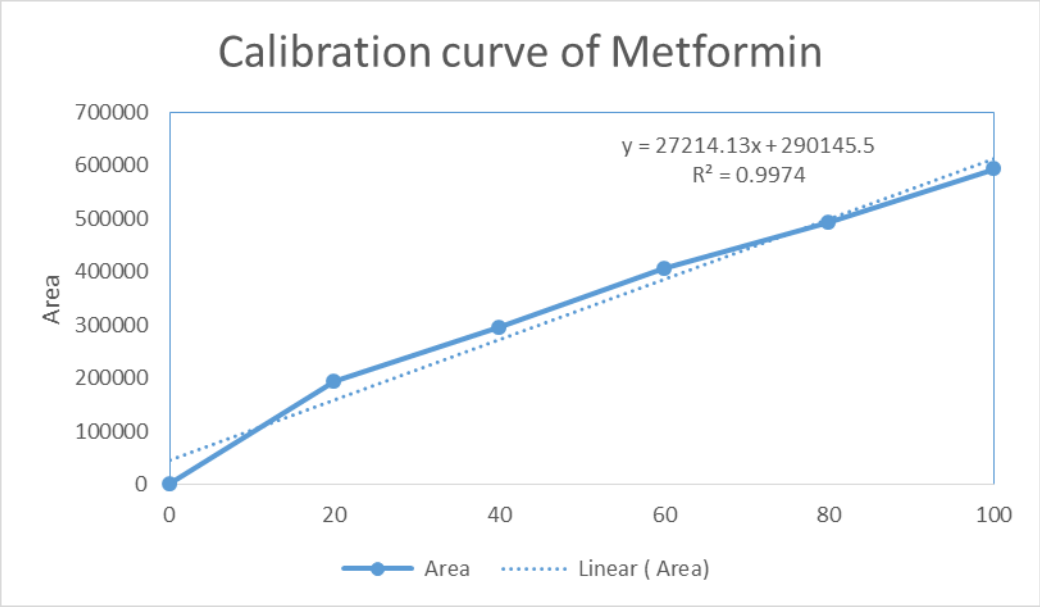


Fig.: Calibration curve of Metformin.

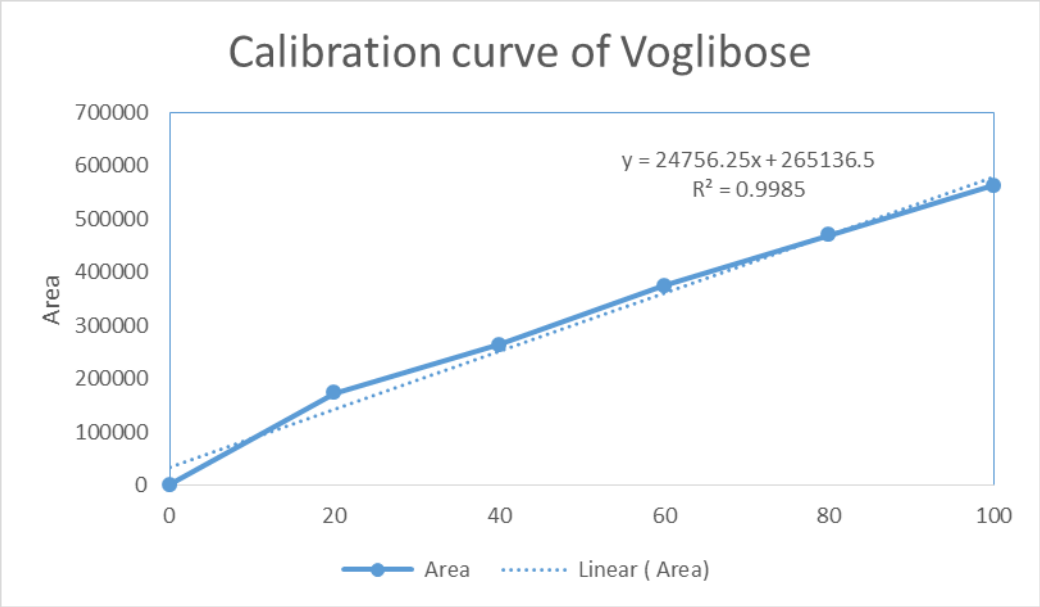


Fig.: Calibration curve of Voglibose.

Robustness

Robustness Studies					
Parameter	**Value**	**Peak Area Glimepiride**	**Peak Area Metformin**	**Peak Area Voglibose**	**% RSD**
Flow Rate	**Low**	364739	358286	273337	0.12%
	Actual	365312	358313	273215	
	Plus	365589	358456	273443	
Temperature	**Low**	364864	358357	273329	0.05%
	Actual	365039	358589	273246	
	Plus	365245	358633	273452	
Wavelength	**Low**	364934	358344	273315	0.14%
	Actual	365477	358441	273182	
	Plus	365973	358538	273461	

Ruggedness

Glimepiride + Metformin + Voglibose					
Ruggedness					
Parameter	**Peak Area Glimepiride**	**Peak Area Metformin**	**Peak Area Voglibose**	**% RSD**	**%LC**
Intraday precision	363489	354286	271337	0.32%	99.36%
	364953	355313	274218		99.76%
	365768	356245	273325		99.99%
Inter day precision	363491	355322	276214	0.30%	99.37%
	364949	356266	274315		99.76%
	365761	356325	275237		99.98%
Instrument:1 Acquity UPLC Waters, 2695H	365288	356241	276338	0.25%	99.86%
	365434	357372	273229		99.90%
	363767	356271	272336		99.44%
Instrument:2 Agilent Technologies, 1290	365289	356315	271224	0.24%	99.86%
	365437	355256	276327		99.90%
	363764	357324	274246		99.44%
Average					**99.71%**
Std.Dev					**0.24431**
%RSD					**0.25%**

LOD and LOQ

Procedure

"The limit of detection and limit of quantification were evaluated by serial dilutions of Glimepiride + Metformin + Voglibose stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ as per ICH guidelines."

Calculations of LOD and LOQ

Slope = a; Intercept = b; The number of tests = N
Standard Error (SE) of Intercept = EXCEL function data analysis → Regression → Table
SD of Intercept = SE of Intercept / Square root of N

LOD

LOD=3.3(SD of intercept/Slope)
Total numbers: 5
SE of Intercept: 3612.82
SD of Intercept: 1620.09
LOD= 3.3*(1620.09/ 25465.15)
LOD= 3.3*(0.06362)
LOD= 0.2099(µg/ml)

LOQ

LOQ=10*(SD/S)
LOQ= 10*(1620.09/ 25465.15)
LOQ= 0.6362(µg/ml)

EVALUATION OF METHODS

➢ **Analysis of Glimepiride + Metformin + Voglibose**

Calculation formula for Glimepiride + Metformin + Voglibose

$$\%\ Assay = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

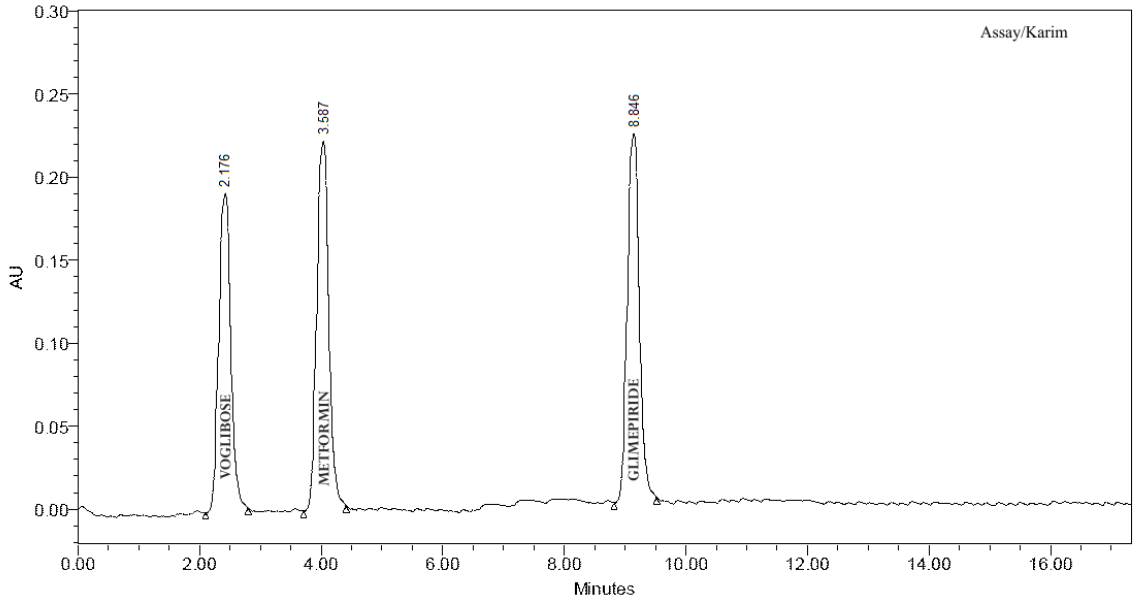
Whereas,"
AT = Average area of test preparation, 363393"
AS = Average area of standard preparation, 365461"
W1 = Weight taken of reference standard (µg), 04.05"
W2 = Weight taken of test sample (µg), 04.06"
AW = Average weight of sample (µg), 1000.60"
LC = Label claim (µg), 1000.50"
P = Potency of reference standard (%), 98.60%"

$$\%\ Assay = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

Sample Control (Glimepiride + Metformin + Voglibose)

$$\%\ Assay = \frac{363393}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \textbf{Error!} \times$$

98.60 = 97.89%



Chromatogram: Assay (API)
Market Sample (VOGLIMAC GM2 HD TABLET)

$$\% \ Assay = \frac{357338}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \textbf{Error!} \times 98.60 = 96.26\%$$

CONCLUSION

A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of Glimepiride + Metformin + Voglibose in bulk drug and market dosage form. The method employed, with Hypersil C18 (100 mm x 2.1 mm, 1.7 µm) in a gradient mode, with mobile phase of Acetonitrile and Methanol in the ratio of 68:32%v/v. The flow rate was 1.0 ml/min and effluent was monitored at 260 nm. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 20- 100 µg/ml respectively. The LOD and LOQ values for were found to be 0.2099 (µg/ml) and 0.6362 (µg/ml) respectively. No chromatographic interference from excipients and degradants were found. The proposed method was successfully used for estimation of Glimepiride + Metformin + Voglibose in market dosage form.

The method provides selective quantification of Glimepiride + Metformin + Voglibose without interference from blank affirming precise method. The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters.

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