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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF ALOGLIPTIN BULK AND TABLET DOSAGE FORM

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Abstract:

This present study reports, a simple, specific, accurate and validated Reverse Phase-Rapid Resolution Liquid Chromatography for identification of Alogliptin. The method employed Inertsil Extend C_{18} (250 × 4.6 mm, packed with 5 µm) and mobile phase Water and Acetonitrile (70:30), at flow rate of 1.0 ml/min and UV-detection at 252 nm and it was detected with run time (RT) of 2.766 mins. No other co-eluting, interfering peak from excipients, impurities, or degradation products due to variable stress conditions was found, and method is specific for the identification of the Alogliptin. The method was validated in terms of linearity, precision, accuracy. Present work also describes the development and validation of HPLC method for the identification of Alogliptin. The method employed same chromatographic condition as above and validated in terms of linearity, accuracy, precision and system suitability.

Keywords: *RP-HPLC, Alogliptin, Method Development and Validation.*

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INTRODUCTION:

Alogliptin [1, 2] is an oral anti-diabetic drug in the DPP-4 inhibitor (gliptin) class [3]. Alogliptin does not decrease the risk of heart attack and stroke. Like other members of the gliptin class, it causes little or no weight gain, exhibits relatively little risk of hypoglycemia, and has relatively modest glucose-lowering activity. Alogliptin and other gliptins are commonly used in combination with metformin in people whose diabetes cannot adequately be controlled with metformin alone [4]. The chemical name is 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl}methyl)benzonitrile. Chemical Formula of Metformin Hcl and is C18H21N5O2. The molecular weight is 339.3916 g/mol.

The literature reveals that various methods for the determination of Alogliptin and pharmaceutical validations among these HPLC methods for Alogliptin are reported [5-8].

Pharmaceutical validations among these methods undergo the world "Validation" means "Assessment" of validity or action of providing effectiveness [9, 10], Australian GMP validation [11, 12] and validation as per ICH guidelines [13].

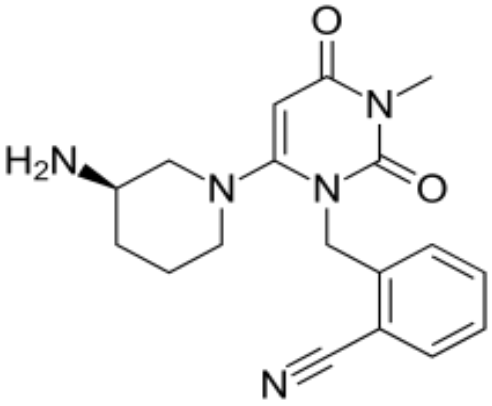

Fig. 1: Chemical Structure of Alogliptin

MATERIALS AND METHOD:

Apparatus:

The analysis was performed by using the analytical balance G285 (Mettler Toledo), pH meter (Labindia), the HPLC used is of Younglin with UV-detector. Column used in HPLC is of Inertsil Extend C_{18} (250 × 4.6 mm, packed with 5 µm) with the mobile phase Water and Acetonitrile (70:30), at flow rate of 1.0 ml/min and UV-detection at 252 nm.

Reagents and solutions:

Pure samples of Alogliptin of 100mg and other ingredients such as Acetonitrile and water used were of HPLC grade. All other all chemicals used were AR grade. Optimized chromatographic conditions are listed in **Table. 1**.

Diluent Preparation:

1L of diluent was prepared by mixing 700 ml of Water and 300 ml of Acetonitrile.

Preparation of stock solution:

Accurately weighed 10 mg of the Alogliptin was transferred to 10 ml clean and dry volumetric flask. Then the volume was made up to the mark with the Acetonitrile and mixed well. This yielded a stock solution with concentration 1000 ppm of Alogliptin.

Preparation of standard solution:

Accurately amount of 1 ml of the Alogliptin stock was transferred to 100 ml clean and dry volumetric flask. Then the volume was made up to the mark with the diluent and mixed well. This yielded a standard stock solution with concentrations of 10 ppm Alogliptin.

Preparation of sample solutions:

Twenty tablets of Alogliptin were weighed, triturated, mixed thoroughly and average weight of tablet was calculated. Accurately weighed quantity of tablet powder equivalent to 12.5 mg of Alogliptin (label claim) was transferred to 10 mL volumetric flask, added 5 mL of mobile phase and sonicate for 10 min. The resultant solution was filtered through 0.45 µ membrane filter, diluted to volume with mobile phase. 0.04 mL of resultant solution further diluted to 10 mL and injected to HPLC system.

Validation experiments were performed to demonstrate System suitability, precision, linearity, Accuracy study of analytical solution and robustness.

Linearity & Range: The Linearity of detector response is established by plotting a graph to concentration versus area of Alogliptin standards and determining the correlation coefficient. A series of solution of Alogliptin standard solutions in the concentration ranging from about 1 to 25 µg/ml levels of the target concentrations were prepared and injected into the HPLC system.

Accuracy: Accuracy for the assay of Alogliptin is determined by applying the method in triplicate samples to which known amount of Alogliptin standards are added at different levels (50%, 100%, and 150%).

Precision: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample.

RESULTS AND DISCUSSION:

Alogliptin standards having concentrations 10µg/ml were scanned in UV- region between 200-400nm.λ_{max} of Alogliptin was found to be at 252nm. Alogliptin Retention time was found to be 2.766 min.

The estimation of Alogliptin was carried out by RP-HPLC using Mobile phase having a composition of 700 volumes of water, 300 volumes of Acetonitrile. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes. The column used was Inertsil, C_{18} (250 x 4.6 mm, 5µ is suitable). Flow rate of Mobile phase was 1.0ml/min. And all the Optimized chromatographic conditions are listed in **Table. 1**.

Table No. 1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil C_{18} (250 × 4.6 mm, packed with 5 µm)
Mobile Phase	70:30 (Water : Acetonitrile)
Flow rate (ml/min)	1.0
Run time (minutes)	8.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	252
Drugs RT (min)	2.766

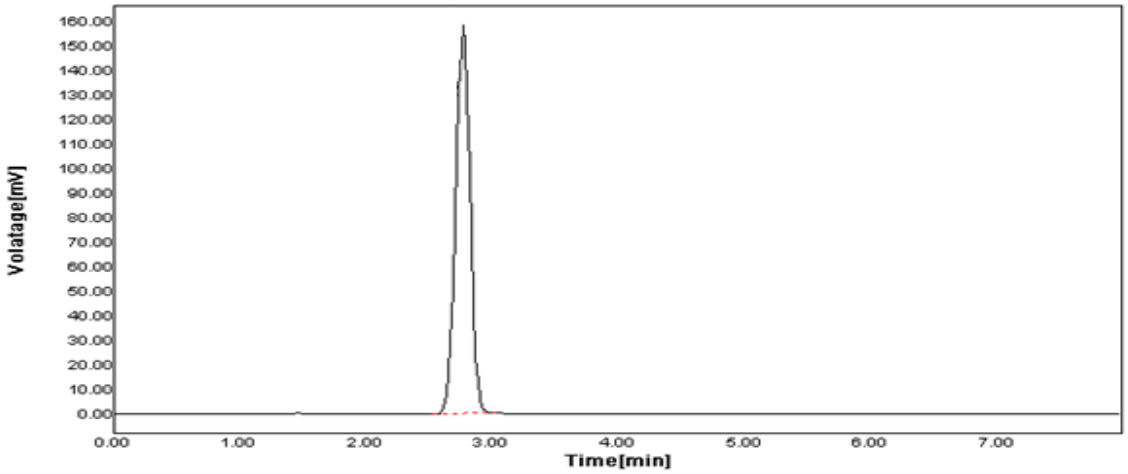

Fig. 2: Standard Chromatogram of Alogliptin

System suitability parameters such as RSD for six replicate injections was found to be less than 2%, theoretical plates – 6387.6, and tailing factor - 1.0. The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show System Suitability 0.167 which shows that the method is repeatable and they are listed in **Table. 2**.

Table No. 2: System Suitability for Alogliptin

Conc. of Alogliptin	Injection	Area of Alogliptin	RT
10 ppm	**Inj-1**	1273926	2.766
	Inj-2	1277798	2.742
	Inj-3	1271245	2.753
	Inj-4	1273846	2.757
	Inj-5	1274947	2.76
	Inj-6	1273542	2.756
Statistical Analysis	**Mean**	1274217	2.755667
	SD	2138.56	0.008017
	% RSD	0.167833	0.290915
	Tailing Factor	1.00	
	Plate Count	6387.6	

The acceptance criteria of Method Precision and injection Precision %RSD should be not more than 2.0% and the method show Method Precision 0.312% and injection Precision 0.23% of Alogliptin which shows that the method is precise and they are listed in **Table. 3, 4**.

Table No.3: Summary of results of Method Precision parameter for Alogliptin

	inj-1	inj-2	Avg	MEAN	SD	% RSD
MP-1	1268093	1268825	1268459	1273084.6	3976.1159	0.31232142
MP-2	1279459	1276947	1278203			
MP-3	1272256	1268245	1270250.5			
MP-4	1272199	1269542	1270870.5			
MP-5	1272879	1273846	1273362.5			
MP-6	1276798	1277926	1277362			

Table No.4: Summary of results of Injection Precision parameter for Alogliptin

S. No.	Alogliptin
I.P-1	1273926
I.P-2	1267798
I.P-3	1269245
I.P-4	1273846
I.P-5	1274947
I.P-6	1273542
Mean	1272217
SD	2937.454
% RSD	0.230892

The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response were found to be linear from 1 - 25µg/ml of target concentration for Alogliptin standard with a correlation coefficient value is greater than 0.999. The correlation coefficient of (r^2) = 0.999 of Alogliptin which shows that the method is capable of producing good response in UV-detector and they are listed in **Table. 5**.

Table No. 5: Summary of results of Linearity parameter for Alogliptin

Alogliptin Conc. (ppm)	Average
1	372215
5	684365
10	1273021
15	1947635
20	2486107
25	3640216

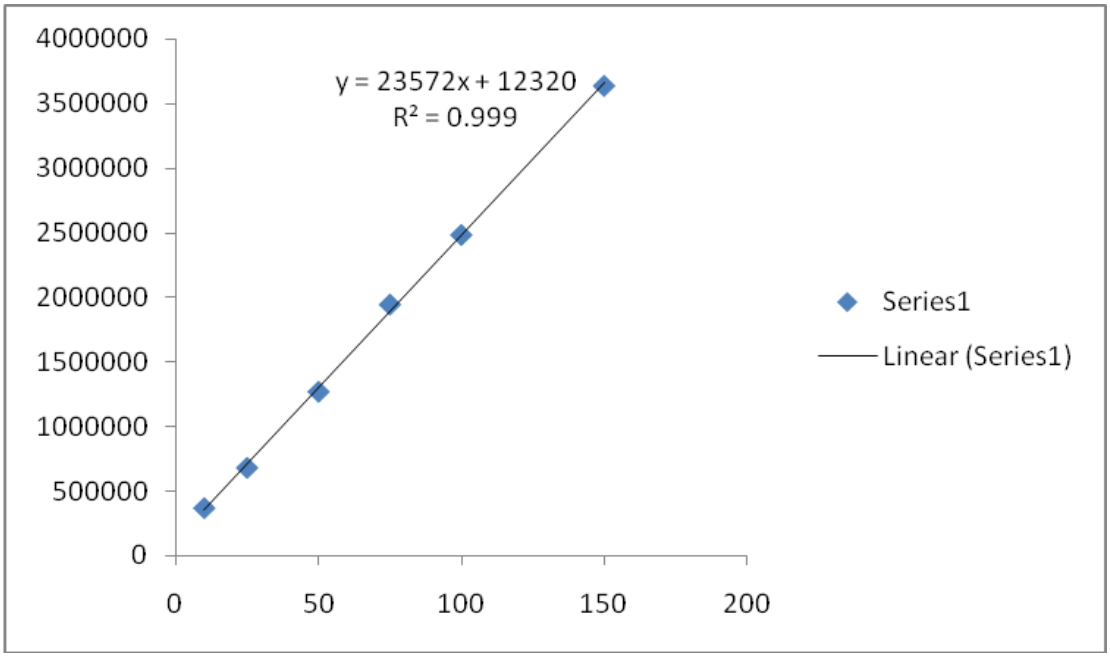

Fig. 3: Linearity Curve of Alogliptin

The Accuracy limit is the % recoveries were found be in the range of 98.59-101.43%. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy. And the results of all System suitability parameters are listed in **Table. 6**.

The values of LOD and LOQ are given in **Table. 7**. The different concentration ranging from 0.001 to 0.02ppm of Alogliptin were injected.

The low levels of LOD and LOQ indicate that the method is quite sensitive for the determination of LOD was 0.0052µg/ml & LOQ was 0.017µg/ml.

Table No. 6: Summary of results of Accuracy parameter for Alogliptin

Conc.		inj-1	inj-2	inj-3	Mean	% Recovery	STD	% RSD
5 ppm	50%	628462	627543	626643	627549.3	98.59214	909.5165	0.144931
10 ppm	100%	1271937	1272670	1272803	1272470	99.95672	466.3572	0.03665
15 ppm	150%	1925734	1935573	1949463	1936923	101.4345	11921.99	0.615512

Table No.7: Summary of results of LOQ for Alogliptin

Injection	Area of Alogliptin
Inj-1	17374
Inj-2	17573
Inj-3	17605
Inj-4	17481
Inj-5	17773
Inj-6	17617
Mean	17570.5
SD	134.8981
% RSD	0.767753

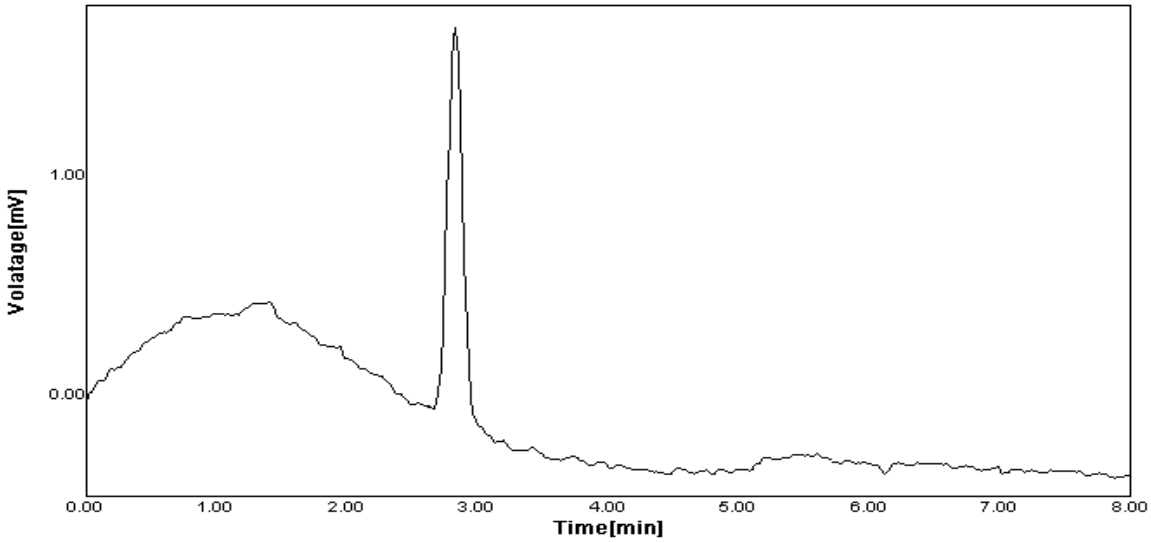

Fig. 4: Chromatogram of LOD for Alogliptin

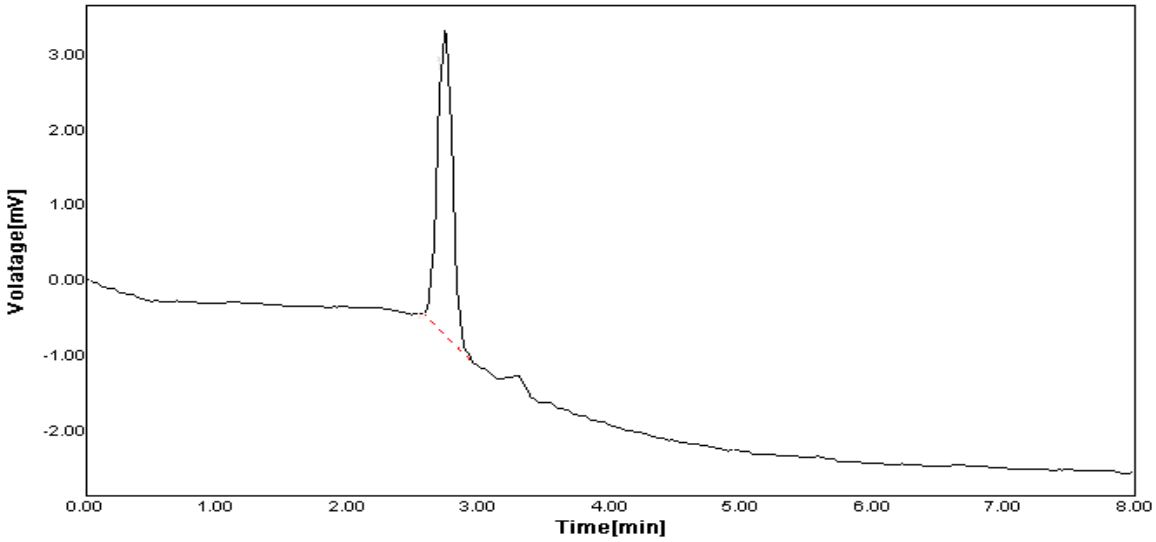

Fig. 5: Chromatogram of LOQ for Alogliptin

The Robustness % RSD was found for 0.9 and 1.1 ml/min flow rate were 0.58 and 0.5 % respectively. And the % RSD was found for the mobile phase composition with 65:35 and 75:25 are 0.22 and 0.31 % respectively. The Ruggedness % RSD was found for 0.16%. And the results of Robustness and Ruggedness are listed in **Table. 8 & 9**.

Table No. 8: Summary of results of Robustness parameter for Alogliptin

Parameters	Adjusted TO	Avg. Area[a]	RT	SD	% RSD
Flow Rate As per method 1.0ml/min	**0.9 ml/min**	1635126.7	3.17	9505.82	0.58
	As it is	1276041.5	2.75	1998.77	0.16
	1.1ml/min	862340.33	2.38	4341.03	0.50
Mobilephase comp[n] (70:30v/v, Buffer: Acetonitrile**)**	**65:35**	1419049.83	3.26	3096.83	0.22
	As it is	1276041.5	2.75	1998.77	0.16
	75:25	1083250.00	2.25	3340.39	0.31

[a]Avg. Area = Six Repeatable injections.

Table No. 9: Summary of results of Ruggedness parameter for Alogliptin

Inj. No.	Alogliptin
I.P-1	1283678
I.P-2	1279764
I.P-3	1277853
I.P-4	1280982
I.P-5	1282351
I.P-6	1279026
Mean	1280609
SD	2163.696097
% RSD	0.16895837

CONCLUSION:

The developed RP-HPLC method was developed and validated for the determination of Alogliptin in bulk and tablet dosage forms. The method was found to be simple, precise and rapid. The assay result obtained by this method is in fair agreement. This method can be use for the determination of Alogliptin in commercial formulations.

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