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Validated RP-HPLC Method for the Quatitation of Alogliptin In Bulk and Tablet Dosage Form

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ABSTRACT

A simple, specific, accurate, precise and sensitive RP- HPLC method has been developed for the rapid estimation of Alogliptin in bulk and its formulations. The chromatographic separation was carried on Phenomenex Gemini-NX-5 µm C18(2) 110 Å, LC Column 250 x 4.6 mm, using Acetonitrile:1-octasulphonoic acid (0.005mM) at pH-5 [60:40] (v/v) as mobile phase, at a flow rate of 1.0 ml/min. The detection was carried out at 220 nm and drug eluted with a retention time of 3.48 min. Beer's law was obeyed in the concentration range of 2-10µg/ml with correlation coefficient 0.9995. The method has been validated according to ICH guidelines for specificity, linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ. The method was found to be specific, accurate, and precise, robust, rugged and sensitive. The developed method was good linearity, novel, rapid for the estimation of Alogliptin in bulk and tablets dosage form. Thus it can be employed for the routine analysis.

Keywords: Alogliptin, RP-HPLC, Validation.

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INTRODUCTION

Alogliptin[1-2] is chemically 2-({6-[(3R)-3-amino pipiperidin-1-yl]-3-methyl-2-4-dioxo-3,4-dihydropyrimidin-1(2H)-y1} benzo nitrile. Alogliptin inhibits dipeptidyl peptidase 4 (DPP-4), which normally degrades the incretions glucose-dependent insulin tropic polypeptide (GIP) and glucagon like peptide 1(GLP-1). The inhibition of DPP-4 increases the amount of active plasma increments, which helps with glycemic control. GIP and GLP-1 stimulate glucose dependent secretion of insulin in pancreatic beta cells. GLP-1 has the additional effects of suppressing glucose dependent glucagon secretion, inducing satiety, reducing food intake, and reducing gastric emptying. Alogliptin is a white powder and soluble in water, methanol, ethanol, and dimethyl sulphoxide. Its molecular weight 339.39g/mol.

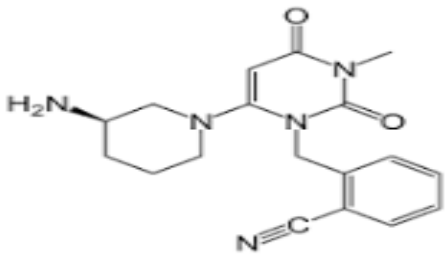

Figure 1: Chemical Structure of Alogliptin.

Literature survey reveals that UV spectrometric[3-7], RP-HPLC[8-14] and HPTLC[15-16] methods reported for Alogliptin. The aim of present work was to develop and validate a novel, rapid, simple, precise, sensitive and specific RP-HPLC method for estimation of Alogliptin in its bulk and tablets dosage form.

MATERIALS AND METHOD

Instrument:

Chromatographic separation was performed on a Shimadzu LC-10ATVP HPLC system comprising a Shimadzu spd10A uv-vis detector, Shimadzu LC-10ATVP pump and enable Phenomenex Gemini-NX-5 µm C18(2) 110 Å, LC Column 250 x 4.6 mm). A manually operating Rheodyne injector 50µl (20µl injection valve) was used for injecting sample and standard solution Baseline Chromatography Data system N2000 software was used to collect and process the data.

Chemicals and Reagents:

Alogliptin pure form was obtained as gifted sample from pharma industry and its pharmaceutical dosage form Nesina 25 tablets labelled claim 25 mg were purchased from local pharmacy manufactured by Takeda LTD. Acetonitrile of HPLC grade (Merck India), Water of HPLC grade (Merck India) and 1-octanesulphonoic acid of Analytical grade (SD Fine Chemicals) were used.

Selection of mobile phase:

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases, Acetonitrile:1-octasulphonoic acid[0.005mM] at pH 5[60:40] and pH adjusted by orthophosphoric acid. was chosen with detection wavelength 220 nm, since it gave sharp peak with good symmetry within limits.

Preparation of mobile phase

Mobile phase was prepared by mixing 60% of HPLC grade Acetonitrile and 40% of 0.005mM 1-octanesulphonoic acid pH 5. This solution was filtered using a 0.45 micron Millipore filter paper and was sonicated for 10mins.

Diluent: Mobile phase

Chromatographic condition:

The optimized chromatographic conditions of the developed method as shown in table no-1.

Table 1: Optimized chromatographic condition

Mobile phase	Acetonitrile: 0.005mM 1-octasulphonoic acid pH5 (60:40)
Stationary phase	Phenomenex Gemini-NX-5 µm C18(2) 110 A, LC Column 250 x 4.6 mm
Wavelength	220 nm
Run time	10min
Injector	Rheodyne 20µl
Flow rate	1.0 ml per min
Injection volume	20 µl
Temperature	Ambient
Mode of operation	Isocratic elution

Preparation of standard stock solution

Weigh accurately about 100mg of Alogliptin pure drug and then transferred into 100ml volumetric flask and diluted with diluent up to the mark and sonicated for 5 min to dissolve it completely (stock solution-1). From the above solution pipette out 10ml into 100ml volumetric flask and made up to the mark with diluent (stock solution-2), from this solution pipette out 0.2, 0.4, 0.6, 0.8 and 1ml into 10ml individual volumetric flask and add diluent up to the mark, this gives 2, 4, 6, 8, and 10 µg/ml concentrations.

Preparation of sample solution:

Ten tablets were weighed and powdered, the tablets powder equivalent to 100mg of Alogliptin was transferred into100ml volumetric flask then it was diluted with diluent and made up to mark and the solution was filtered through Millipore filter 0.4 micron. From this pipette out 10 ml in a 100ml

volumetric flask and make up the volume up to the mark with diluent. From this solution pipette out 0.2ml in 10ml volumetric flask and make up the volume with diluent, this gives 2µg/ml concentrations.

Flow rate selection:

Different flow rates in between 0.98 to 1.2 ml /min were studied. A flow rate of 1.0 ml /min gave an optimal signal to noise ratio with a reasonable separation time.

Results and Discussion:

System suitability

20µl of the standard solution was injected under optimized chromatographic conditions to evaluate the suitability of system. Parameters such as number of theoretical plates (N), tailing factor, retention time (RT) were determined. The values of system suitability parameters were shown in Table- 2, it indicates good performance of system (Figure-2).

Table 2: System suitability parameters

System suitability parameters	Results
Retention time	3.48
Area	162416.80
Number of Theoretical plate	8030.41
Tailing factor	1.24

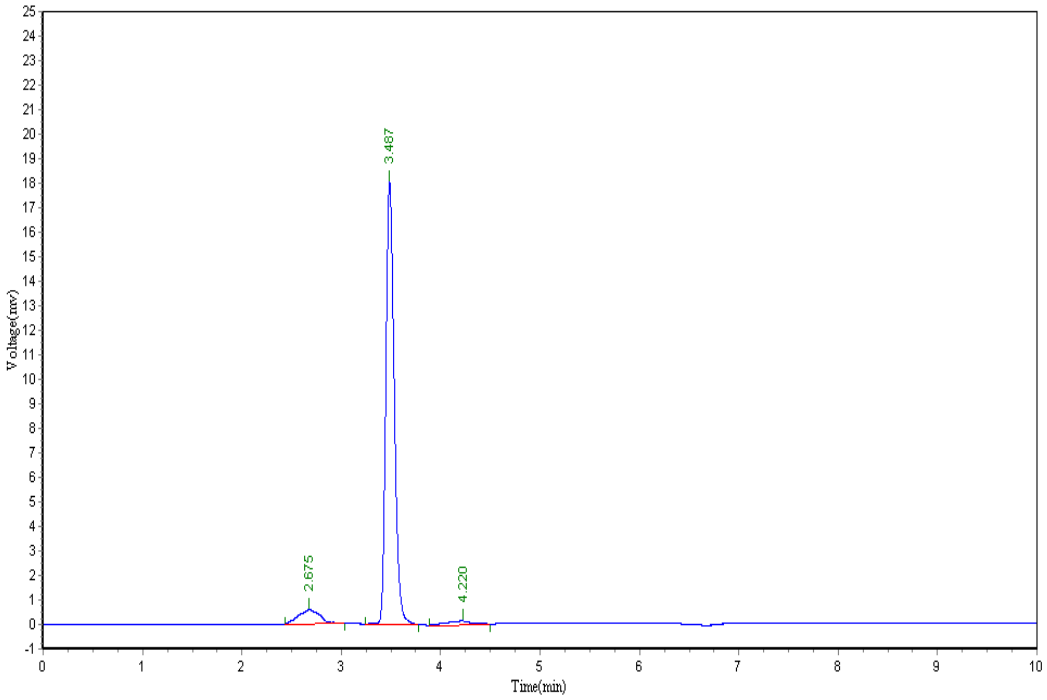

Figure-2: Chromatogram of Alogliptin

Method validation

The method is validated according to the ICH guidelines [17-20].

Specificity

Specificity was checked for the interference of excipients in the analysis of sample solution and was determined by injecting sample solution with added excipients under optimized chromatographic conditions to demonstrate separation of Alogliptin from excipients. There is no interference of excipient peak on the peak of Alogliptin indicating the high specificity of method.

Linearity and Range

Calibration curve was plotted for different concentrations of working standards prepared from standard drug solution of pure drug, shown in Fig-3 and showed linearity over a concentration range of 2-10µg/ml shown in Table-3, along with regression parameters in Table-4. Each calibration was injected three times. The calibration curve was performed in triplicate

Table 3: Linearity data for Alogliptin

Sl.no	Concentration(mcg/ml)	Peak Area
1	0	0
2	2	54360.250
3	4	106700.229
4	6	163387.599
5	8	221313.515
6	10	280782.271

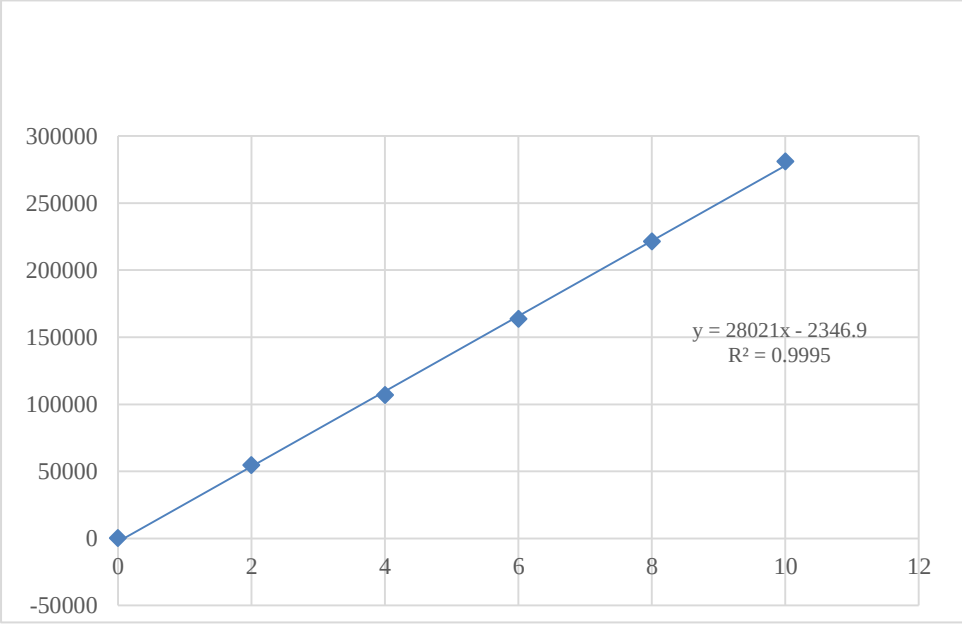

Figure-3: Calibration curve for Alogliptin

Table -4: Regression parameters table for Alogliptin

Regression	Parameter Alogliptin
Regression Equation*	Y=28021x-2346.9
Slope (b)	28021
Intercept (a)	2346.9
Correlation Coefficient (r2)	0.9995

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. Intra-day precision was determined by analyzing Alogliptin for six times in the same day at wavelength range 220 nm. Inter-day precision was determined by analyzing the drug daily once for six days at wavelength range 220 nm. The results were shown in terms of %RSD were within the limits, shown in Table 5.

Table -5: Results of Precision studies

Sl.No.	Concentration (µg/ml)	Intraday precision (Area)	Interday precision (Area)
1.	6	158320.218	163926.641
2.	6	164241.721	160022.094
3.	6	159455.08	158323.011
4.	6	162881.203	159987.281
5.	6	163030.932	163926.641
6.	6	162416.810	162416.797
Mean		161724.3	160935.21
SD		2306.5	2330.542
%RSD		1.42	1.44

Each determination is average of six replicates, RSD indicates relative standard deviation

Accuracy

Accuracy (recovery) is the closeness of the test results obtained by the method to the true value. The was obtained by spiking 80, 100 and 120% of Alogliptin working standard concentrations, in which the amount of marketed formulation was kept constant and the amount of pure drug was varied. Solutions were prepared in triplicates and accuracy was indicated by % recovery which was between 99.32 to 101.42%. The results were shown in Table-6.

Table 6:-Results of Accuracy studies

% spiked levels	Amount of formulated drug added (µg/ml)	Amount of pure drug added (µg/ml)	Amount found (µg/ml)	% Recovery ± Standard deviation	%RSD
80	3	1.8	4.82	101.11 ± 0.57	0.57
100	3	3	5.98	99.32± 0.96	0.96
120	3	4.8	7.26	101.42 ± 1.25	1.25

Robustness

Robustness was carried by varying two parameters deliberately from the optimized chromatographic conditions like pH, temperature, and flowrate The %RSD was found to be <2, shown in Table- 7.

Table- 7:- Robustness results for Alogliptin

Changed condition		RT	Mean area	Standard deviation	%RSD
pH	4.5	3.48	158840.0	172.88	0.1088
	5.0	3.48	162582.40	2967.45	1.82
	5.5	3.48	159674.95	899.88	0.0563
Temperature	29	3.48	162480.25	1625.75	1.005
	3.0	3.48	162022.54	39.226	0.0242
	31	3.48	158661.40	642.04	0.4046
Flow rate	0.98	3.41	168208.140	607.206	0.360
	1.0	3.48	163892.67	2783.99	1.698
	1.02	3.55	15589134	2911.122	1.83

Ruggedness

The Ruggedness was determined by using the data obtained by the analysis performed by two between different analysts. The value of %RSD was found to be <2, showed ruggedness of developed analytical method. The values were shown in Table-8.

Table 8:-Ruggedness results for Alogliptin

Analyst	Analyst-1	Analyst-2
Mean area	162840.48	163437.96
Standard deviation	748.13	2906.12
%RSD	0.4594	0.17781

RSD indicates relative standard deviation

Limit of detection and Limit of quantitation

The LOD and LOQ were calculated by using the slope and SD of response (intercept). LOD and LOQ values of Alogliptin were found to be 0.0169µg/ml and 0.0512µg/ml.

CONCLUSION:

Thus, the developed method was found to novel, simple, accurate, precise, selective and sensitive for the routine estimation of Alogliptin in bulk and tablet dosage form.

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