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

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ABSTRACT : The objective of the present research work was to develop a innovative, simple, and economic method for estimation of Alogliptin in bulk and dosage form by RP-HPLC.The chromatographic conditions were performed on Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d.as stationary phase and mobile phase was prepared with a mixture of Phosphate Buffer: Acetonitrile = 60:40 (pH-2.9) flow 1.0 ml/min, with Injection Volume 20µl, at detection wavelength 237 nm and run time at 6.0 min.The analytical method is valid for estimation of Alogliptin over a range of 10µg/ml–50 µg/ml. The results of system suitability test, linearity, precision and accuracy, robustness, specificity, LOD and LOQ and stabilities presented in this report are within the acceptance range.A specific, sensitive, economic method estimation of Alogliptin has been developed based on ICH Guidelines with bulk and dosage forms.
Key Words: Alogliptin, HPLC, Method Development, ICH, Validation, Accuracy, Precision.

I.INTRODUCTION

Alogliptin is a selective, orally - bioavailable inhibitor of enzymatic activity of di peptidyl peptidase-4 (DPP-4). Alogliptin chemically prepared as a salt of benzoate and exists predominantly as the R-enantiomer (>99%). It undergoes little or no chiral conversion in vivo to the (S)-enantiomer. FDA approved January 25, 2013. Alogliptin inhibits dipeptidyl peptidase 4 (DPP-4), which normally degrades the incretins glucose-dependent insulin tropic polypeptide (GIP) and glucagon like peptide-1 (GLP-1). The inhibition of DPP-4 increases the amount of active plasma incretins 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[1-8].
The chemical name of Alogliptin is 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl}methyl)benzonitrile[9-12].

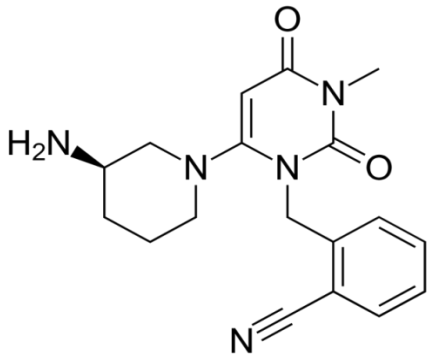


Fig-1: Structure of Alogliptin

A survey of literature reveals that good analytical methods are not available for Alogliptin. The present research manuscript describes innovative, simple, economical, accurate, specific, robust, rugged and rapid RP-HPLC method developed in selected solvent system (Mobile Phase) and validated in accordance with International Conference on Harmonization (ICH) Guidelines Q2 (R1), for the estimation of Alogliptin in bulk drug and in its dosage forms[13-16].

II.EXPERIMENTAL

2.1 Materials and Methods:

Pharmaceutical grade working standard Alogliptin were obtained from Syncorp Pvt. Laboratories, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt Ltd, Mumbai, India.

2.2 Instrumentation:

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system EMPOWER2 software, UV-Visible double beam spectrophotometer (ELICO SL-159), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

2.3 Sample & Standard Preparation for the Analysis

25 mg of Alogliptin standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

2.4 Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. While scanning the Alogliptin solution we observed the maxima at 237 nm.

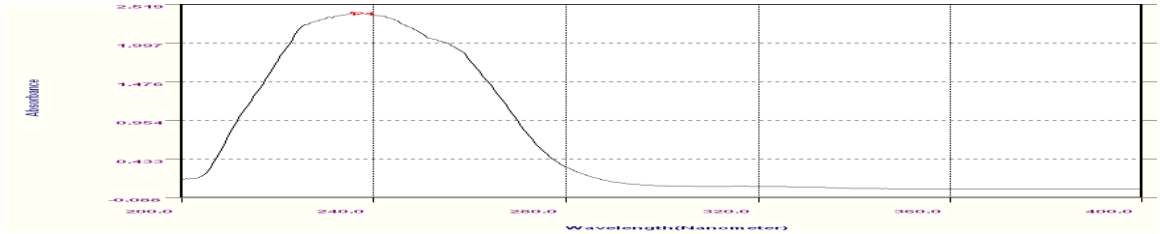


Fig-2: UV Spectrum for Alogliptin

2.5 Method Development

2.5.1 Preparation of Phosphate buffer (pH-2.9) Solution:

About 2.72168 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. The pH was adjusted to 2.9 with diluted Orthophosphoric acid.

2.5.2 Preparation of Mobile Phase:

The mobile phase used in this analysis consists of a mixture of Phosphate Buffer (pH adjusted to 2.9 with ortho phosphoric acid) and Acetonitrile in a ratio of 60:40. 600mL (60%) of Phosphate Buffer (pH adjusted to 2.9 with ortho phosphoric acid) and 400mL of Acetonitrile (40%) of above prepared phosphate buffer were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

2.5.3 Summary of Optimized Chromatographic Conditions:

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-1: Summary of Optimised Chromatographic Conditions

Mobile phase	Phosphate Buffer: Acetonitrile = 60:40 (pH-2.9)
Column	Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.
Flow rate	1.0 ml/ min.
Wavelength	237nm
Sampling System	Automatic
Temp. of Auto sampler	Ambient
Volume of injection	20µl
Run time	06min.
Mode of Separation	Isocratic

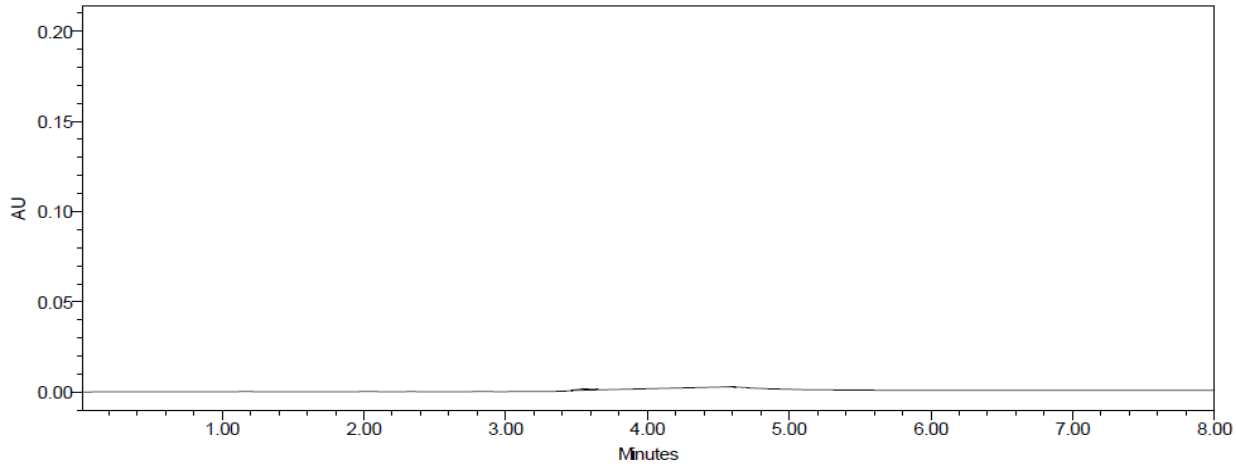
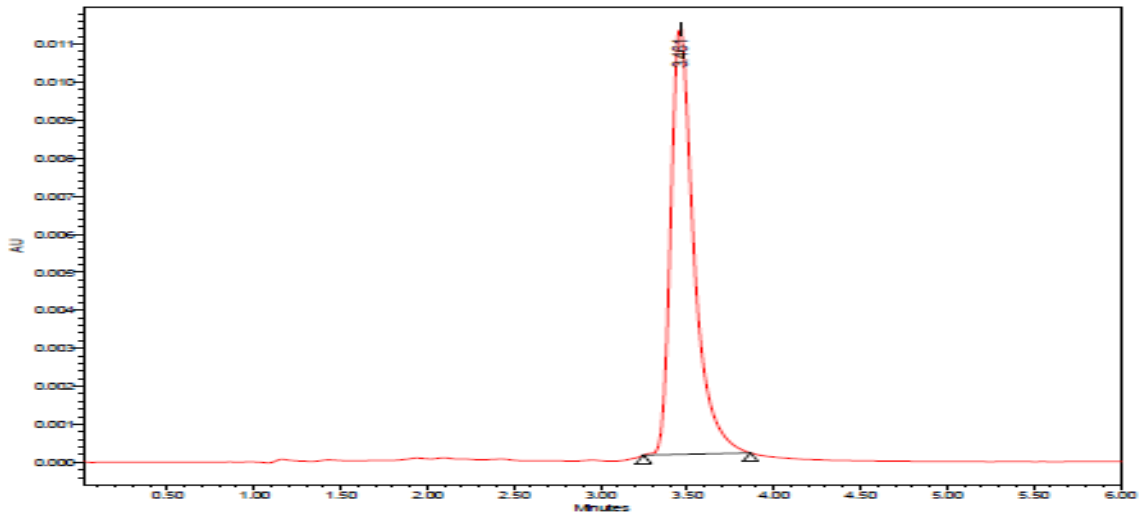


Fig-3: Chromatogram for Blank Preparation



Fig–4: Chromatogram of Alogliptin in Optimized Condition

2.6 Method validation:

2.6.1 Linearity & Range:

Calibration standards at five levels were prepared by appropriately mixed and further diluted standard stock solutions in the concentration ranges from 10-50µg/mL for Alogliptin. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the linearity graphs. Chromatograms of each solution were recorded.

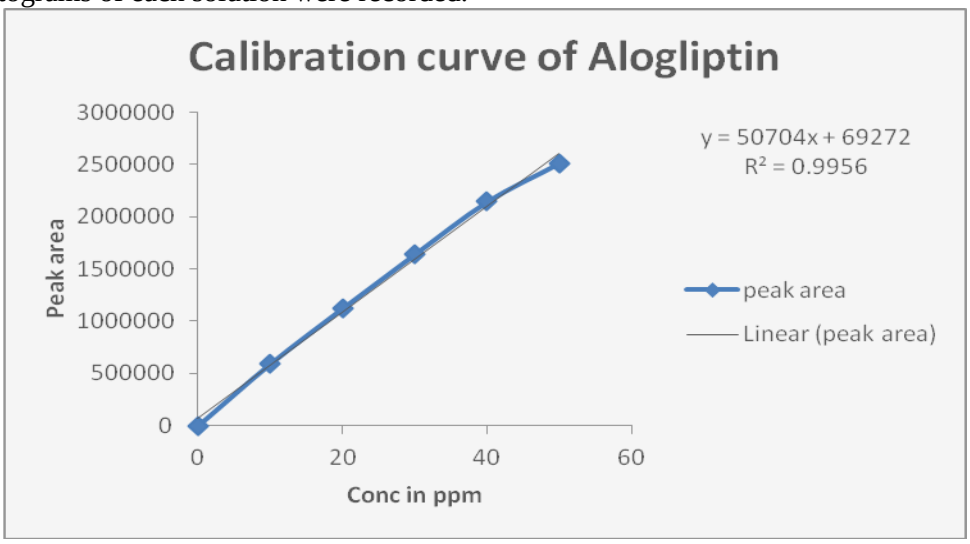


Fig-5: Calibration Curve of Alogliptin (API)

Table-2: Concentration of Alogliptin

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
10	594015
20	1124587
30	1642145
40	2145838
50	2514698

2.6.2. Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Alogliptin were taken and added to the pre-analyzed formulation of concentration 100µg/ml. From that percentage recovery values were calculated. The results were shown in table-3.

Table-3: Accuracy Readings of Alogliptin

Accuracy level	Concentration (µg/ml)		Area	% Recovery of Pure drug	Statistical Analysis
	Amount added	Amount found			
80 %	8	7.8	468679	98.46536	Mean= 98.9595% S.D. = 1.2581 % R.S.D.= 1.27%
80 %	8	8.0	476485	100.3898	
80 %	8	7.8	466887	98.02358	
100 %	10	10.0	578767	100.4842	Mean= 100.7166% S.D. = 0.811511 % R.S.D.= 0.805%
100 %	10	10.1	584521	101.619	
100 %	10	10.0	576549	100.0467	
120 %	12	11.8	668476	98.48072	Mean= 100.40737% S.D. =1.670669 %R.S.D.= 1.663%
120 %	12	12.1	685546	101.2862	
120 %	12	12.1	686574	101.4552	

2.6.3. Precision:

2.6.3.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual

determination of six replicates of a fixed amount of drug. Alogliptin (API). The percent relative standard deviation was calculated for Alogliptin are presented in the Table-4.

Table-4: Repeatability Readings of Alogliptin

HPLC Injection Replicates of Alogliptin	Retention Time	Area
Replicate – 1	3.464	1014845
Replicate – 2	3.463	1034241
Replicate – 3	3.464	1065823
Replicate – 4	3.463	1021645
Replicate – 5	3.462	1045472
Replicate – 6	3.461	1026245
Average	3.46283	1034711
Standard Deviation	0.001169	18552.7
% RSD	0.033756	1.79296

2.6.3.2. Intermediate precision:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Alogliptin revealed that the proposed method is precise.

Table-5: Results of Intra-Assay & Inter-Assay

Conc. Of Alogliptin (API) (µg/ml)	Observed Conc. Of Alogliptin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	8.12	0.79	8.12	0.72
10	10.01	0.12	10.05	0.99
12	11.62	0.40	12.12	0.79

2.6.4. Method Robustness:

Influence of little changes in optimized chromatographic conditions like changes in flow rate (± 0.1ml/min), mobile phase ratio (±2%), Wavelength of detection (±2nm) and Acetonitrile content in mobile phase (±2%) studied to measure the robustness of the method are also in favour of (Table-36, % RSD < 2%) the developed RP-HPLC method for the analysis of Alogliptin (API).

Table-6: Results of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.62
Flow (0.9 ml/min)	0.54
More Organic	0.36
Less Organic	0.27
Wavelength of Detection (239 nm)	0.86
Wavelength of detection (235 nm)	0.91

2.6.5. LOD & LOQ:

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

2.6.6. System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-7.

Table-7: Data of System Suitability Parameter

S. No.	Parameter	Limit	Result
1	Resolution	Rs > 2	3.68
2	Asymmetry	T ≤ 2	Alogliptin=0.89
3	Theoretical plate	N > 2000	Alogliptin =5694

2.6.7 Estimation of Alogliptin in Tablet Dosage Form

Twenty tablets were taken and the I.P. method was followed to calculate the average weight. Above weighed tablets were finally powdered and triturated well. Some quantity of powder which is equivalent to 25 mg of drug was transferred to a clean and dry 25 ml volumetric flask, make and solution was sonicated for fifteen minutes. Then the volume was made up to 25 ml with the same Mobile Phase. Then 10 ml of the prepared above solution was diluted to 100 ml with the help of mobile phase. The resulted solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The final solution prepared was injected in 5 replicates into the HPLC system and the s are record the observations. Two duplicate injections of the standard solution were also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-8.

Table-8: Assay of ALOGLIPTIN Tablets

Brand name of tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	% Purity
Nesina (Takeda Pharmaceuticals America, Inc.)	25mg	24.81 (±0.09)	99.24% (±0.48)

III.RESULTS

The optimized chromatographic conditions were Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d.as stationary phase and mobile phase was prepared with a mixture of Phosphate Buffer: Acetonitrile = 60:40 (pH-2.9) flow 1.0 ml/min, with Injection Volume 20µl, at detection wavelength 237 nm and run time at 6.0 min. In these chromatographic conditions the peak was pure, sharp, symmetric and found a greater number of theoretical plates.

The results obtained in method validation were

Linearity & Range: The calibration curve showed good linearity in the range of 10-50 µg/ml, for Alogliptin (API) with correlation coefficient (r^2) of 0.999 (Fig-5). A typical calibration curve has the regression equation of y = 50704x + 69272 for Alogliptin.

Accuracy: The mean recoveries were found to be 98.9595, 100.7166 and 100.40737 for Alogliptin. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed

method was accurate.
Repeatability: The repeatability study which was conducted on the solution having the concentration of about 10 µg/ml for Alogliptin (n =6) showed %RSD of 1.79296%. It was concluded that the analytical technique showed good repeatability.
LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.02 & 0.06 µg/ml respectively.
Assay: The assay in Nesina tablets containing Alogliptin was found to be 99.24 %.

IV.DISCUSSION

To develop a precise, linear, specific RP-HPLC method for analysis of Alogliptin, different chromatographic conditions were applied & the results observed were compared with the methods available in literatures.
***Subrata Sarkar[17] et al.,**A Simple, precise, rapid, robust and accurate reverse phase-high performance liquid chromatographic method for the determination of Alogliptin in bulk and pharmaceutical dosage form. The chromatographic analysis was performed on a Symmetry C18, 25cm x 4.6mm (i.d.), 5µm, particle size column in isocratic mode, the mobile phase consisted of methanol and 0.01M phosphate buffer (adjusted to pH 3.20 with ortho-phosphoric acid) at a ratio of 65:35 v/v and a flow rate of 1.0 ml/min and UV detector is used. The eluents were monitored at 236nm. **Shubhangi C. Daswadkar*[18] et al.,** A simple, economic, selective, precise, and stability-indicating HPLC method has been developed and validated for estimation of Alogliptin benzoate in bulk drug and tablet dosage form. The drug was separated using a mobile phase acetonitrile: water, (40:60 v/v) on an Agilent, TC C18 (250 × 4.6 mm) 5 µm column at flow rate of 1.0 mL min-1 at ambient temperature and detection was performed at 277 nm. The detector linearity was established in concentrations ranging from 5-50 µg mL−1, the regression coefficient was 0.9997. **A. Madhukar*[19] et al.,** 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 C18 (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. **G. Satya Sri*[20] et al.,**Approach: Metformin is a biguanide antihyperglycemic agent used for treating non-insulin- dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Alogliptin is a selective, orally-bioavailable inhibitor of enzymatic activity of dipeptidyl peptidase-4. A new reversed-phase High Pressure Liquid Chromatographic (RP-HPLC) method was developed for the determination of Metformin & Alogliptin (ALG) based on isocratic elution using a mobile phase consisting of potassium dihydrogen phosphate buffer [pH 4.0] and Acetonitrile [HPLC Grade] (70:30, v/v) at a flow rate of 1 mL min−1with UV detection at 235nm. **Pavan kumar H.K[21] et al.,** The objective of the current study was to develop a simple, accurate, precise and rapid RP-HPLC method with subsequently validate as per ICH guidelines for the determination of Alogliptin benzoate (ALO) and Metformin hydrochloride (MET) using mobile phase [mixture of Phosphate buffer- pH-3.6 and acetonitrile in the ratio of 65:35] as the solvent. The proposed method involves the measurement of Retention time at selected analytical wavelength. 235.0 nm was selected as the analytical wavelength. The retention time of ALO and MET was found to be 5.055 and 2.838 respectively.

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Alogliptin in formulations.

V.CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Alogliptin API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Alogliptin indicated that the developed method is specific for the estimation of Alogliptin. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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