



OPTIMIZATION OF RP-HPLC METHOD FOR DETERMINATION OF ALOGLIPTIN BENZOATE IN BULK AND DOSAGE FORM

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ABSTRACT

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. For stability study, the drug was exposed to the stress conditions such as acid, base, oxidation, neutral and sunlight as per the recommendations of ICH guidelines. The results of the analysis were validated in terms of specificity, limit of detection, limit of quantification, linearity, precision and accuracy. As per ICH guidelines results were found to be satisfactory. The method was proved to be robust with respect to changes in flow rate and temperature. The high recovery and low relative standard deviation confirm the suitability of these methods can be employed for the routine analysis of tablet containing Alogliptin.

Key words: Alogliptin benzoate, Stability study, ICH, Validation, Bulk drug.

INTRODUCTION

Recent years, diabetes has been a serious disease affecting the patients around the world. According to International Diabetes Federation (IDF), there are totally 382 million diabetes patients in 2013 and this number will rise to 592 million by 2035¹. Type 2 diabetes

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is a chronic disease and progressive disease usually characterized by resistance of insulin and dysfunction of β -cell²⁻⁴. Dipeptidyl peptidase-4 (DPP-4) is an enzyme, which is widely distributed in living organisms and blood circulation and could inactivate endogenous glucagon-like peptide-1 (GLP-1), an insulintropic hormones playing an important role in promoting insulin secretion, inhibiting glucagon secretion, delaying gastric emptying, enhancing satiety and moderating food intake⁵⁻⁹.

Alogliptin benzoate, 2-[6-[3(R)-Aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydropyrimidin-1-yl methyl] benzonitrile Benzoate, is a novel, potent and highly selective DPP-4 inhibitor¹⁰.

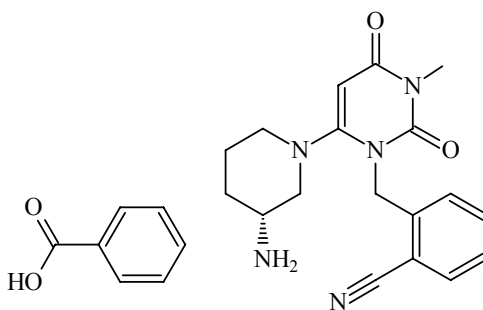


Fig. 1: Chemical structure of alogliptin benzoate

Very few analytical methods have been reported for the analysis of Alogliptin benzoate as single component in formulation¹¹⁻¹⁵. The main objective of the proposed work was to develop a simple, accurate, precise and sensitive RP-HPLC method for the estimation of alogliptin benzoate in bulk drug and tablet dosage form. The method was further optimized and validated in accordance with guidelines suggested by International Conference on Harmonization (ICH)¹⁶.

EXPERIMENTAL

Materials and methods

Authenticate sample of alogliptin benzoate purchased from Swapnroop Drugs Pvt. Ltd., Aurangabad. HPLC grade water and acetonitrile (Merck Ltd., Mumbai, India) was used as solvent. All the aqueous reagents were prepared using carbon dioxide free distilled water.

Instrumentation

The HPLC system, Agilent 1120 compact with manual Rheodyne injector facility operates at 20 μ L capacity per injection was used. The column was used Agilent TC C₁₈

(250 x 4.6 mm) 5 μm and the detector consisted of UV/VIS operated at 277 nm. The data were acquired and processed using EZ Chrom Elite Compact software.

Chromatographic conditions

Optimizations of chromatographic conditions were carried out using water: acetonitrile (60:40 v/v) as mobile phase. Prior to deliver into the system, mobile phase was filtered through 0.45 μm filter and sonicate for 10 min. The samples were introduced by injector with a 20 μL sample loop. The analysis was carried out under gradient conditions using flow rate 1.0 mL min^{-1} at 18°C and chromatograms were recorded at 277 nm.

Preparation of standard stock solution

Weighed accurately 10 mg of alogliptin and transferred to 100 ml volumetric flask, add 25 mL of mobile phase and sonicate for 15 min and volume was made up to mark with mobile phase (100 $\mu\text{g mL}^{-1}$).

Preparation of standard solution

From the standard stock solution 1mL solution was pipetted out in 10 mL volumetric flask and volume was made up to the mark with mobile phase to get a final concentration 10 $\mu\text{g mL}^{-1}$.

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 25 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 μm membrane filter, diluted to volume with mobile phase. 0.04 mL of resultant solution further diluted to 10 mL and injected to HPLC system (Table 1).

Table 1: Assay of marketed formulation

S. No.	Formulation	Taken amount	Amount estimated	% Estimated	% RSD
1		25 mg	25.05 mg	100.2	
2	Nesina	25 mg	24.97 mg	99.88	0.105
3		25 mg	25.01 mg	100.04	
		Mean	25.01 mg	100.04	

RSD is Relative standard deviation

System suitability

System suitability parameters were evaluated from retention times, tailing factor, capacity factor and theoretical plates of standard chromatograms (Table 2).

Validation

Limit of detection (LOD) and limit of quantification (LOQ)

The signal-to-noise ratio (S/N) method was adopted for the determination of limit of detection and limit of quantification. The limit of detection was estimated as three times the S/N ratio and the limit of quantification was estimated as ten times the S/N ratio (Table 2).

Table 2: Statistical parameters of alogliptin

Parameters	Values
Limit of detection ($\mu\text{g mL}^{-1}$)	0.271
Limit of quantification ($\mu\text{g mL}^{-1}$)	0.310
Linearity ($\mu\text{g mL}^{-1}$)	5-50
Regression equation ($y = mx+c$)	$y = 51665 x + 146840$
Correlation coefficient (r^2)	0.9997
Retention time	3.08
Tailing factor	1.05
Capacity	3.52
Theoretical plates	7430

Specificity

Specificity is the ability of a method to discriminate between the analyte of interest and other components that may present in the sample. The specificity of the method was evaluated to ensure separation of Alogliptin.

Linearity

Different standard solutions were prepared by diluting standard stock solution with mobile phase in the concentration range 5-50 $\mu\text{g mL}^{-1}$. Diluted samples were injected and chromatograms (Fig. 1) were taken under standard chromatographic conditions. The peak

area was plotted against corresponding concentrations to obtain the calibration graph (Fig. 2).

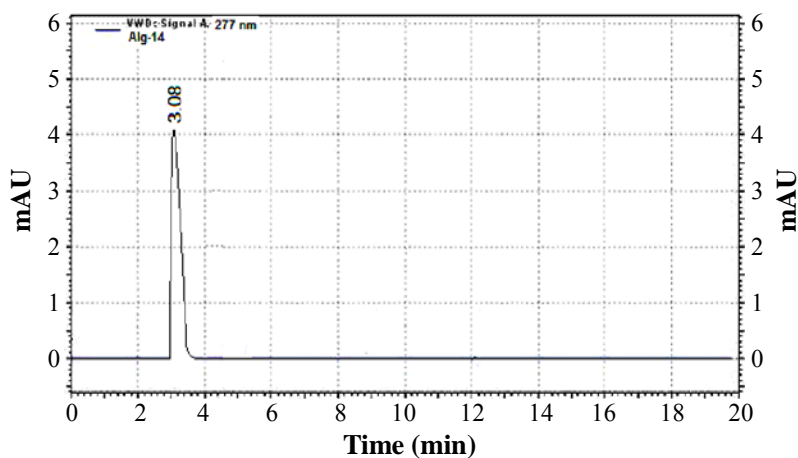


Fig. 2: Chromatogram of standard alogliptin

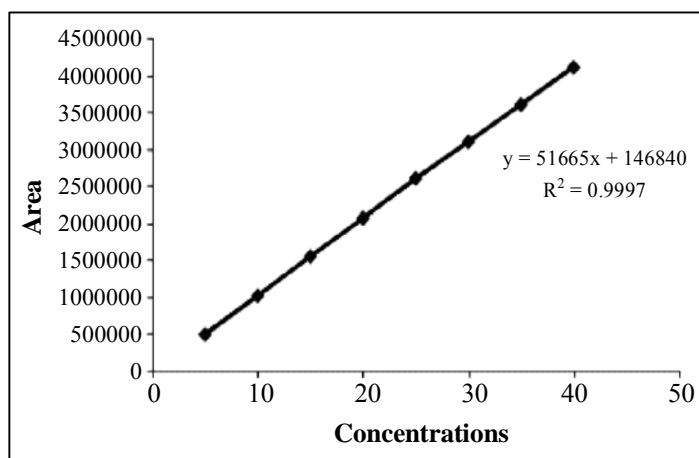


Fig. 3: Calibration plot of alogliptin

Precision

Precision of analytical method was expressed in relative standard deviation (RSD) of a series of measurements. The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses (i.e. three concentrations/three replicates each) of the sample solution on the same day and on three different days, respectively (Table 3).

Table 3: Precision data of alogliptin

Parameters	% Estimated	S.D.	%RSD
Intra-day*	101.10	0.252	0.089
	100.50	0.137	0.028
	100.72	0.155	0.023
Inter-day*	100.15	0.176	0.070
	100.20	0.185	0.038
	99.98	0.184	0.027

Recovery

To check the accuracy of the proposed method, recovery studies were carried out by standard addition method. A known amount of standard alogliptin corresponding to 80, 100 and 120% of the label claim was added to preanalysed sample of tablet. The recovery studies were carried out in triplicate at each level (Table 4).

Table 4: Recovery study data

Level of standard addition (%)	Amount of tablet powder (mg)	Amount of pure drug added (mg)	Amount of pure drug recovered (mg)	% Recovery*	% RSD*
80	25	8	8.03	100.38	0.1540
100	25	10	10.07	100.70	0.1291
120	25	12	12.06	100.50	0.1735

Robustness

Robustness is a measure of the performance of a method when small and deliberate changes are made to the conditions of method. Robustness studies were performed by making slight variations in flow rate and mobile phase composition changes one at a time (Table 5).

Table 5: Robustness data for alogliptin

Parameters	% Recovery	S.D.	% RSD
Change in mobile phase composition (60:40 ± 2)	99.98	0.325	0.06

Cont...

Parameters	% Recovery	S.D.	% RSD
Change in flow rate ($1.0 \pm 0.2 \text{ mL min}^{-1}$)	100.04	0.147	0.11

Forced degradation

Acid and base induced degradation product

To 10 mL of standard stock solution, 10 mL of 0.1 N HCl and 10 mL of 0.01 N NaOH were added separately. These mixtures were reflux separately for 45 min for acid and 10 min for base at 50°C. The forced degradation study in acidic and basic media was performed in the dark in order to leave out the possible degradative effect of light. 0.1 mL of each resultant solution was diluted to 10 mL with the mobile and resultant solution injected into the system.

Hydrogen peroxide induced degradation product

To 10 mL of standard stock solution, 10 mL of hydrogen peroxide (H_2O_2) (3% v/v) was added. This solution was heated in boiling water bath for 10 min to remove completely the excess of hydrogen peroxide and reflux for 20 min at 50°C. 0.1 mL of resultant solution was diluted to 10 mL with the mobile phase and resultant solution injected into the system.

Neutral hydrolysis

10 mL of standard stock solution was mixed with 10 mL water and reflux for 60 min at 60°C. 0.1 mL solution this solution was diluted to 10 mL with the mobile and resultant solution injected into the system.

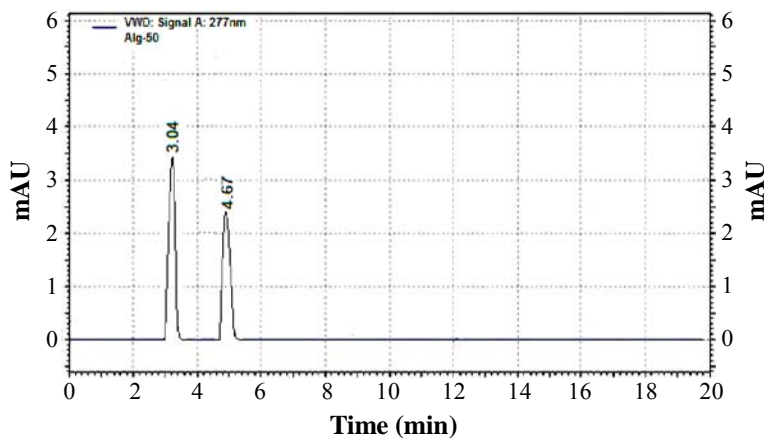
Photolytic induced degradation product

10 mL of standard stock solution was exposed to direct sunlight for 30 min on a wooden plank and kept on terrace. 0.01 mL of resultant exposed solution was transferred to 10 mL volumetric flask, diluted with the mobile phase and solution was injected into the system.

*Indicates mean of three replicates, SD is standard deviation.

Table 6: Summary of force degradation data

Sample stress condition	Stress condition	Alogliptin (R.T.)	Degradants (R.T.)	% Area decreased	Fig. No.
Acid degradation	0.1 N HCl reflux for 45 min	3.04	4.67	34.10	4
Alkaline degradation	0.01 N NaOH reflux for 10 min	3.08	4.83	6.90	5
Oxidative degradation	6% H ₂ O ₂ reflux for 20 min	3.06	4.15	27.52	6
Neutral hydrolysis	Purified water reflux for 1 hr	3.08	4.12	9.46	7
Photolytic degradation	Kept in sunlight for 30 min	3.08	--	--	8

**Fig. 4: Chromatogram of acid [0.1 N HCl (reflux for 45 min at 50°C)] treated**

Sample Peak 1, Alogliptin [$R_t = 3.04$]; Peak 2, degradant [$R_t = 4.67$]

RESULTS AND DISCUSSION

The parameters were focused for improvisation of retention time, separation of degradation products and column life. The Agilent TC C₁₈ column provided good peak shapes and no peak splitting was observed. Alogliptin showed linear responses in concentrations level ranging from 5-50 $\mu\text{g mL}^{-1}$ with correlation co-efficient 0.9996 (Table 2).

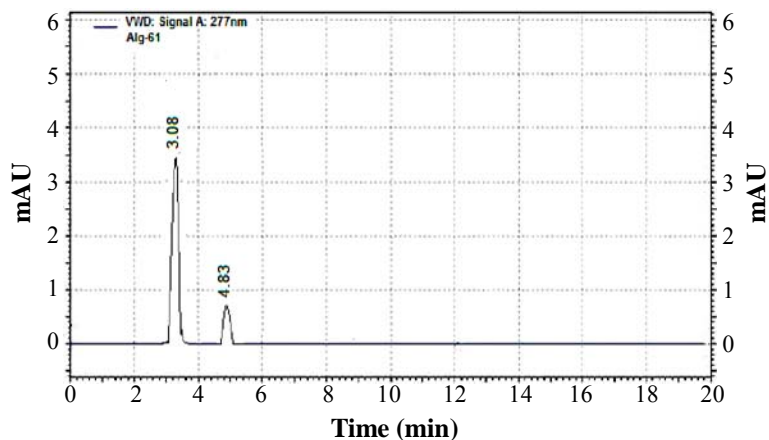


Fig. 5: Chromatogram of base [0.01 N NaOH (reflux for 10 min at 50°C)] treated sample

Peak 1, Alogliptin [$R_t = 3.08$]; Peak 2, degradant [$R_t = 4.83$]

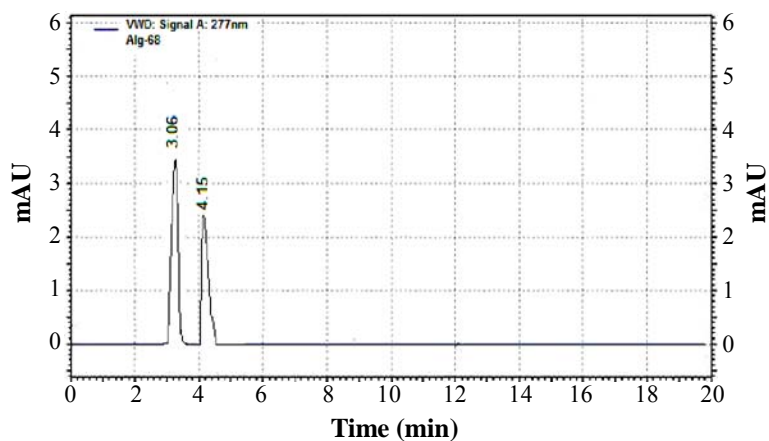


Fig. 6: Chromatogram of hydrogen peroxide [3% H₂O₂ (reflux for 20 min at 50°C)] treated sample

Peak 1, Alogliptin [$R_t = 3.06$]; Peak 2, degradant [$R_t = 4.15$]

The measurement at three different concentration levels showed low value of % R.S.D. (< 2) and low value of S. E. (< 2) for intra- and inter-day variation, which suggested an excellent precision of the method.

The recovery of drug was determined by spiking drug at three different levels and was found to be between 100.38 - 100.70. The method was found to be robust with respect

to flow rate and change in mobile phase composition without any changes in system suitability parameters.

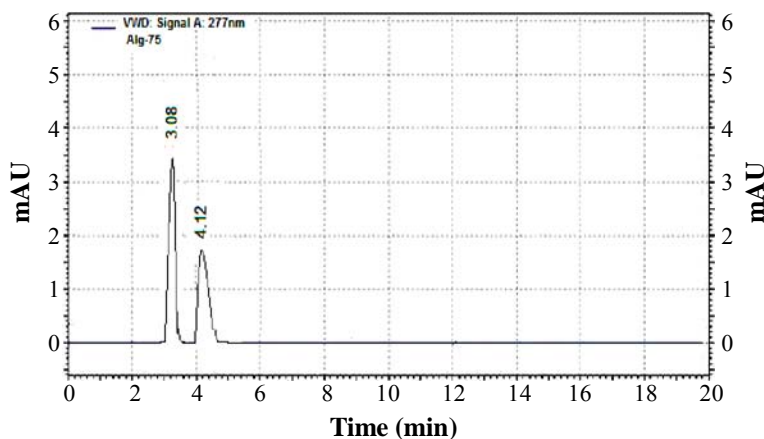


Fig. 7: Chromatogram of neutral hydrolysis (reflux for 60 min at 60°C)]

Peak 1, Alogliptin [Rt = 3.08]; Peak 2, degradant [Rt = 4.12]

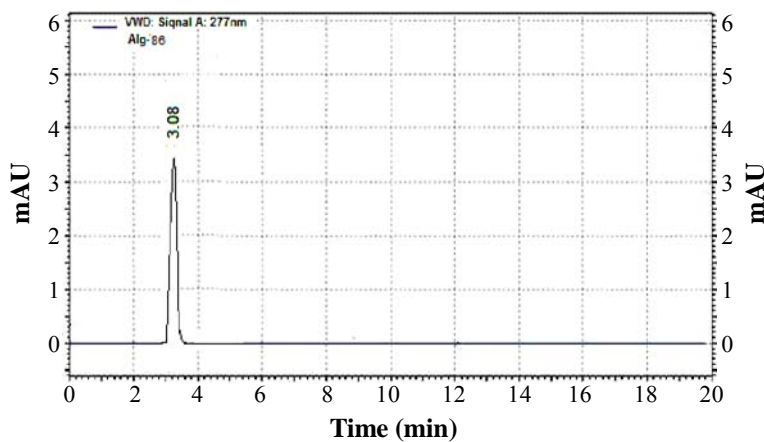


Fig. 8: Chromatogram of sunlight exposed (for 30 min) sample

Peak 1, Alogliptin [Rt = 3.08]

Forced degradation of drug was carried out as per the ICH guidelines (ICH Q2B) by subjecting alogliptin to various stress conditions. The percent area decreased at the level of 6.90-34.10% and additional peaks at retention time different to that of well separated peak of alogliptin indicated that alogliptin undergoes degradation in acidic, basic, oxidative and neutral conditions. Summary of force degradation data are given in Table 6.

CONCLUSION

The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters tested. As the method able to separate the parent drug from degradation products it can be employed as a stability indicating method for alogliptin.

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