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METHOD DEVELOPMENT AND VALIDATION OF ALOGLIPTIN BY UV SPECTROSCOPIC METHOD

Dr. CH. V. Suresh*, E. Poojitha, K. Umesh, A. Vamshi, E. Hemanth, Dr. K.N.V. Rao, Santhosh Illendula

Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Charlapally, Nalgonda, Telangana-508001.

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***Corresponding Author**
Dr. CH. V. Suresh
Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Charlapally, Nalgonda, Telangana-508001.

ABSTRACT

A new simple, accurate, rapid, precise, reproducible and cost-effective spectrophotometric method for the quantitative estimation of Alogliptin. The developed UV spectrophotometric method for the quantitative estimation of Alogliptin is based on measurement of absorption at maximum wavelength 277 nm using Methanol & Water as a solvent. The stock solution of Alogliptin was prepared and subsequent suitable dilution was prepared in distilled water to obtained standard curve. The standard solution of Alogliptin shows absorption maxima at 277 nm. The drug obeyed beer lambert's law in the concentration range of 5 - 25 µg/ml with regression 1 at 277 nm. The overall % recovery was found to be 99.4% which reflects that the method was free from the interference of the impurities and other excipients used in the marketed dosage form. The low value of % RSD was indicative of accuracy and reproducibility of the method. The % RSD for inter-day and intra-day precision was found to be 0.5454 & 0.8564 respectively which is <2% hence proved that method is precise. The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. The developed method can be adopted in routine analysis of Alogliptin in bulk and tablet dosage form.

KEYWORDS: Alogliptin, UV Visible Spectrophotometry, Method development, Validation, ICH guidelines, Accuracy &s Precision.

INTRODUCTION

DRUG PROFILE

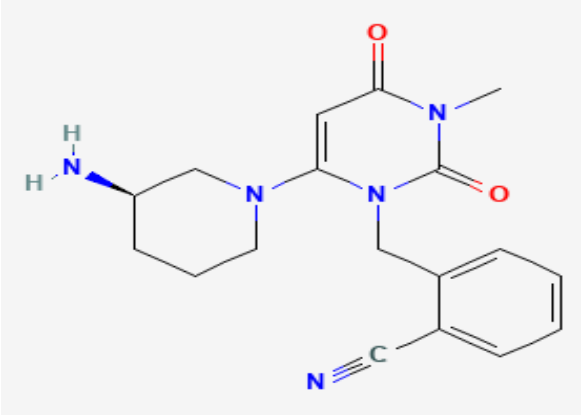
Drug name: Alogliptin

Common trade name: Kazano, Nesina Oseni, Incresync

Molecular formula: $C_{18}H_{21}N_5O_2$

Molecular weight: 339.39g/mol

Structure



IUPAC Name: **2-({6-[(3R)-3-Aminopiperidin-1-yl]-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl}methyl)benzonitrile**

Approved use: To treat hyperglycemia in patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

Chemicals and Reagents: Methanol, 0.1N HCl, Acetonitrile, Water.

Instruments

SHIMADZU UV-1601 UV – Vis spectrophotometer, Electronic Balance **(CITIZEN BALANCE BL-220H),** Ultra Sonicator **(ANALYTICAL),** and P^H Analyzer **(ELICO),** Distillation unit **(BOROSIL),** Vaccum filteration unit**(BOROSIL).**

Reagents and Solutions

Diluent preparation: In a 100ml volumetric flask take 50:50 v/v methanol and water.

Preparation of standard Stock Solution of Alogliptin: Accurately weighed 100mg of alogliptin was weighed accurately and transferred into 100ml volumetric flask. About 10 ml of diluent was added and sonicated to dissolve. The volume was made up to the mark with

same solvent. The final solution contained about 100µg/ml of alogliptin. Working standard solution of Alogliptin containing 10µg/ml for method. Finally add those above solutions and prepare the final solution is about 10µg/ml.

Preparation of Sample Solutions

Take 20 Tablets average weight and crush in a mortar by using pestle and weight powder 100 mg equivalent weight of Alogliptin sample into a 100ml clean dry volumetric flask, dissolve and make up to volume with diluent. Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with diluent.

Determination of wavelength of maximum absorbance for Alogliptin

The absorbance of the final solution scanned in the UV spectrum in the range of 200 to 400nm against solvent mixture as blank.

Optimization of selection of Solvent

It is well known that the solvents do exerts a profound effect on the quality and the shape of the peak. The choices of solvents for UV method development are: Methanol, 0.1NHCl, Acetonitrile, First optimize the different solvents. From that solvents methanol satisfied the all the optimized conditions.

5.4. Wavelength Selection

The standard solutions are prepared by transferring the standard drug in a selected solvent and finally diluting with the same solvent or Diluent. That prepared solution is scanned in the UV visible wavelength range of 200-400nm. This has been performed to know the maxima of Alogliptin. While scanning the Alogliptin solution we observed the maxima at 277 nm. The visible spectrum has been recorded on (SHIMADZU UV-1601 make UV – Vis spectrophotometer model UV-1601. The scanned visible spectrum is attached in the following page. The λ_{max} of the Alogliptin was found to be 277 nm in diluents as solvent system.

METHOD VALIDATION

1. Accuracy: ***Recovery study:*** 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-analysed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in Table-1.

2. Precision

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 deviations were calculated for Alogliptin is presented in the Table-2.

Intermediate Precision

Intra-assay & inter-assay

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. The results were shown in Table-3.

3. Linearity & Range

The calibration curve showed good linearity in the range of 5-25µg/ml, for Alogliptin (API) with correlation coefficient (r^2) of 1 (Fig-2). A typical calibration curve has the regression equation of y = 0.0617x + 0.0038 for Alogliptin.

Standard solutions of Alogliptin in the concentration range of 5 µg/ml to 25 µg/ml were obtained by transferring (5,10,15 and 20,25 ml) of Alogliptin stock solution (100ppm) to the series of clean & dry 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbances of the solutions were measured at 277 nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law is linear in concentration range of 5 to 25 µg/ml at 277 nm for Alogliptin. The results were shown in Table-4.

4. Method Robustness

Robustness of the method was determined by carrying out the analysis under different Wavelength i.e. at 275nm, 277nm and 279nm. The respective absorbances of 10µg/mlwere noted SD < 2%) the developed UV-Spectroscopic method for the analysis of Alogliptin (API). The results were shown in Table-5.

5. LOD & LOQ

The LOD and LOQ were calculated using the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.00070 & 002148 µg/ml respectively.

6. ASSAY OF ALOGLIPTIN IN DOSAGE FORM

ALOGLIPTIN 20mg.

Assay of marketed tablet formulation Brands

Alogliptin was procured from the local market as tablets of strength having 25mg, marketed with brand names of Nesina. When referring to the generic drug name alogliptin.

Weighed accurately about twenty tablets and calculate the weights of individual tablets and finally calculate the average weight. They were triturated to fine powder by using a mortar and pestle. The powdered tablet equivalent to 5mg of Alogliptin was dissolved in 15ml of diluent with the help of sonication process and the final volume was made up to the mark with the diluent in 25 ml volumetric flask. The resulted solution was filtered using Whatman filter paper (0.45µm). This final solution was further diluted to obtain 10µg/ml concentration of the solution by using diluents used as a solvent and observed by UV analysis. This procedure was repeated in triplicate.

$$\text{Amount Present} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times \frac{\text{Standard Dilution}}{\text{Sample Dilution}} \times \frac{\text{Potency}}{100} \times \text{Average weight}$$

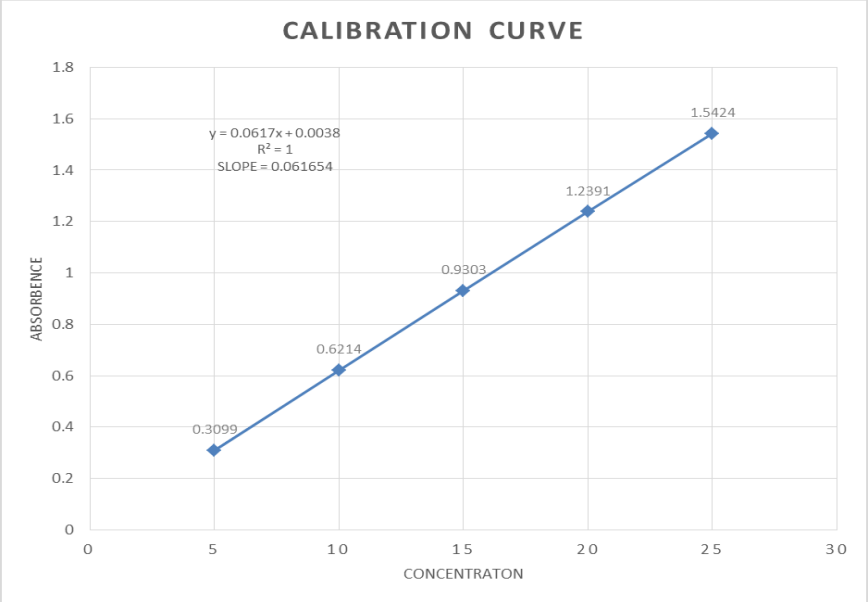
$$\%\text{Content} = \frac{\text{Amount Present}}{\text{Label Claim}} \times 100.$$

RESULTS AND DISCUSSION

The standard solutions of Alogliptin in methanol and Water (10µg/ml) subjected to a scan individually at the series of wavelengths of 200 nm to 400 nm. Absorption maximum of Alogliptin was found to be at 277 nm. Therefore, 277 nm was selected as λ_{max} of Alogliptin for the present study. The calibration curve of Alogliptin was found to be linear in the range of 5-25 µg/ml at 277 nm. Therefore, it was clear that Alogliptin can be determined without interference of any irrelevant substance in single component pharmaceutical products. The used technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated.

The % recovery was carried out at 3 levels, 80%, 100% and 120% of Alogliptin standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly developed method was validated according to the ICH guidelines and the method validation parameters.

The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness, and ruggedness etc.



Calibration curve of Alogliptin (API)

5. Accuracy

Table 1: Results of accuracy.

Level of Recovery	Sample Conc. (µg/ml)	Absorbance	% Recovery	Mean % Recovery
80%	8	0.4961	99.9	99.4
80%	8	0.4898	98..9	
80%	8	0.4994	99.3	
100%	10	0.6191	99.7	99.6
100%	10	0.6238	98.4	
100%	10	0.6209	99.1	
120%	12	0.7450	99.8	99.3
120%	12	0.7392	99.5	
120%	12	0.7389	99.6	

Acceptance criteria: correlation coefficient should not be less than 0.999.

6. Precision

Table 2: Results of Repeatability.

S.No.	Conc. (µg/ml)	Wavelength (nm)	Absorbance
1	10	277	0.6193
2	10	277	0.6211
3	10	277	0.6124
4	10	277	0.6175
5	10	277	0.6129
6	10	277	0.6144
Mean ± S.D.			0.616267
Standard Deviation			0.003174
% RSD			0.57988

Table 3: Results of intra-Day & inter-Day.

Conc. taken (µg/mL)	Observed Conc. Of Alogliptin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Absorbance	Statistical Analysis	Con. found (µg/mL)	Statistical Analysis
10	0.6291	Mean = 0.6229 SD = 0.005335 %RSD = 0.8564	0.6154	Mean = 0.6161 SD = 0.003361 %RSD = 0.545427
10	0.6204		0.6132	
10	0.6194		0.6198	

Table 4: Results of Linear Curve.

Concentration (µg/ml)	Absorbance
5	0.3099
10	0.6214
15	0.9303
20	1.2391
25	1.5424

Acceptance criteria: correlation coefficient should not be less than 0.999.

Table 5: Result of Method Robustness Test Change in Wavelength.

Concentration (µg/ml)	Wavelength	Absorbance	Statistical Analysis
10	275	0.6104	Mean =0.6152 SD =0.003685 % RSD = 0.599009
10		0.6178	
10		0.6143	
10	279	0.6214	
10		0.6168	
10		0.6135	

Table 6: Assay Results of Marketed Formulations.

Formulations	Actual concentration of Alogliptin [Label Claim] (µg/ml)	Amount obtained of Alogliptin (µg/ml)	% Alogliptin
A	25	24.8	99.32

CONCLUSION

From the experimental studies it can be concluded that best UV-Spectroscopic method is developed for Alogliptin in bulk and marketed pharmaceutical dosage form. The developed method for the drug (Alogliptin) was found to be accurate and precise.

The great features of spectrophotometric methods are their simplicity, economical and rapidity. The results of method validation showing that the developed analytical procedure is suitable for its intended purpose and meets the Guidelines given by the ICH.

The developed method was successfully applied for the routine analysis of Alogliptin in bulk and pharmaceutical dosage form in the future.

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