Yadav Priyanka J. et al. Ijppr.Human, 2014; Vol. 1(2): 1-9 **(Research Article)**

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ALOGLIPTIN BENZOATE IN BULK DRUG AND DOSAGE FORM

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ABSTRACT

A reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed for the determination of alogliptin (ALG) based on isocratic elution using a mobile phase consisting of methanol: double distilled water (80:20, *v/v*) at a flow rate of 1 ml/min with UV detection at 222 nm. Chromatographic separation was achieved on a Finepak sil C_{18} column (250 mm × 4.6 mm, 5 µm). Linearity, accuracy and precision were found to be acceptable over the concentration range of 5-30 µg/ml for ALG in bulk. The optimized method was validated and proved to be specific, robust and accurate for the quality control of ALG in pharmaceutical preparations.

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1. INTRODUCTION

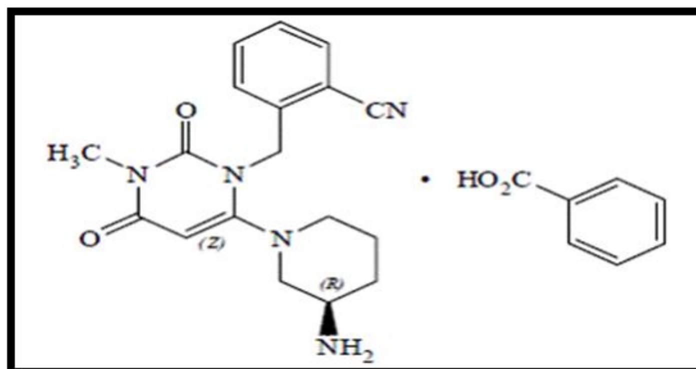

Fig. 1-Structure of Alogliptin benzoate

Alogliptin benzoate:

Alogliptin (ALG), 2-({6-[(3*R*)-3-aminopiperidin- 1-yl]-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)- yl}methyl) benzonitrile (Fig. 1) is a novel hypoglycemic drug that belongs to dipeptidyl-peptidase-4 inhibitor class which stimulates glucose-dependent insulin release (1, 2). DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes that functions to stimulate glucose-dependent insulin release and reduce glucagons levels. This is done through inhibition of the inactivation of incretins, particularly glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycemic control (3). Recently, DPP-4 inhibitors have been recommended in the treatment of diabetes mellitus to improve glycemic control (4) and it is effective in controlling the metabolic syndrome and resulted in significant weight loss, a reversal of insulin resistance, islet and adipocyte hypertrophy, and alleviated hepatic steatosis (5). Thus, the aim of the present work was to develop a RP-HPLC method for the determination of ALG in bulk and pharmaceutical preparation applying UV detection.

2. MATERIALS AND METHODS

Pharmaceutical grade working standards alogliptin purchased from swapnroop drugs pvt. Ltd. Aurangabad. All reagents were of HPLC-grade. All chemicals and reagents were purchased from Merck, Mumbai.

2.1. Instrumentation

The analysis was performed on JASCO Quaternary gradient pump system. It is equipped with four prominence LC Net II pump, and a UV – 2075 UV/Vis detector. Data acquisition was performed by using crome NAV software. Finepak sil, C18 column (250mm x 4.6mm, 5µm.) was used as a stationary phase for analysis. Injections was performed by a manual-injector with 20µl, loop.

2.2. Chromatographic condition

Different mobile phases were tested in order of their polarity to find out the best conditions for separation of ALO. The selected mobile phase containing Methanol: double distilled water (80:20) (pH-6.8) and gave acceptable retention time (RT) at 222nm. The flow rate was maintained at 1.0 ml/min, with run time 10min. The mobile phase was filtered by using 0.45µm Millipore nylon filter paper. Mobile phase was degassed by sonication prior to use. All determinations were performed at ambient temperature and injection volume 10µl.

2.3. Preparation of standard stock solution

10 mg of ALG was dissolved in 100ml double distilled water and sonicated to dissolve. The solutions were filtered followed by serial dilutions to the required concentrations for each experiment.

2.4. Sample preparation

Tablet containing 25mg Alogliptin was dissolved in 100ml volumetric flask containing 30ml methanol sonicate for 10 minutes and volume was made up to 100 with mobile phase. The solutions were filtered followed by serial dilutions to the required concentrations for each experiment.

3. Procedure

3.1. Linearity and repeatability

Accurately measured aliquots of stock solutions equivalent to 5-30 µg ALG were transferred into a series of 10 ml volumetric flasks and then completed to volume with methanol. A volume of 10 µl of each solution was injected into the chromatograph. The conditions including the mobile phase at a

flow rate 1 ml/min, detection at 222 nm and run time program for 10 min were adjusted. A calibration curve was obtained by plotting area under the peak (AUP) against concentration (C). The repeatability of the method was assessed by analyzing 10 μg/ml of ALG (n=6). The precision (%R.S.D) values of peak areas and retention times were shown in Table 2.

3.2. Assay of ALG in bulk and tablet

The procedure mentioned under **Linearity and repeatability** was repeated using concentrations equivalent to 5-30 μg/ ml ALG in bulk. For the determination of ALG in tablets, the sample solution prepared under **Sample preparation** was serially diluted and then injected in triplicates. The concentrations of ALG were calculated using calibration equation.

4. RESULTS AND DISCUSSION

HPLC greatly reduces the analysis time and allows for the determination of many individual components in a mixture using one single procedure. We applied the technique of detection widely applied in routine analysis; namely UV detection.

4.1 Method development

During the optimization cycle, several chromatographic conditions were attempted using Finepak sil C_{18} column (250 mm × 4.6 mm, 5 μm). Various mobile phase compositions containing different ratios of organic and aqueous phases were tried in an isocratic mode. Methanol was found optimum for the elution. Besides, double distilled water in different composition, at different pH values were attempted along with Methanol. Therefore, a mobile phase consisting of Methanol: double distilled water (20:80, *v/v*) pH (6.8) and pumped at a flow rate of 1.0 ml/ min, in an isocratic mode, gave good result at 222 nm. The retention time was 2.642 min for ALG as in Figure 2.

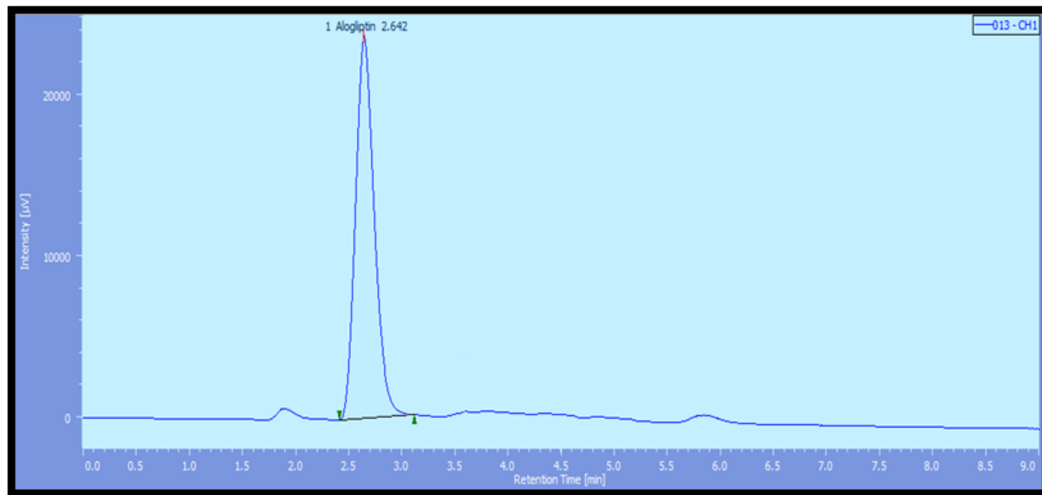

Figure 2. A typical chromatogram of alogliptin in sample solution (10µg/ml)

4.1.1 System suitability tests

According to USP 2007 (7), system suitability tests are an integral part of liquid chromatographic methods in the course of optimizing the conditions of the proposed method. System suitability tests are used to verify that the resolution and reproducibility were adequate for the analysis performed. The parameters of these tests are column efficiency (number of theoretical plates), tailing of chromatographic peak and repeatability as %R.S.D of peak area for six injections and reproducibility of retention as %R.S.D of retention time. The results of these tests are listed in Table 1.

Table 1. System suitability tests for RP-HPLC method for the determination of alogliptin in bulk

Parameters	Obtained Values
	Alogliptin
Theoretical plates (N)	6735
Asymmetry(Tailling factor)	1.365
LOD (µg/ml)	0.00137046
LOQ (µg/ml)	0.00415292

4.2. Method validation

4.2.1 Linearity

Linearity was studied for ALG. A linear relationship between area under the peak (AUP) and concentration (C) was obtained. The linearity of the calibration curve was validated by the high value of correlation coefficient was shown in fig 3. The analytical data of the calibration curve including standard deviation for the slope and intercept (m, c) are summarized in Table 2.

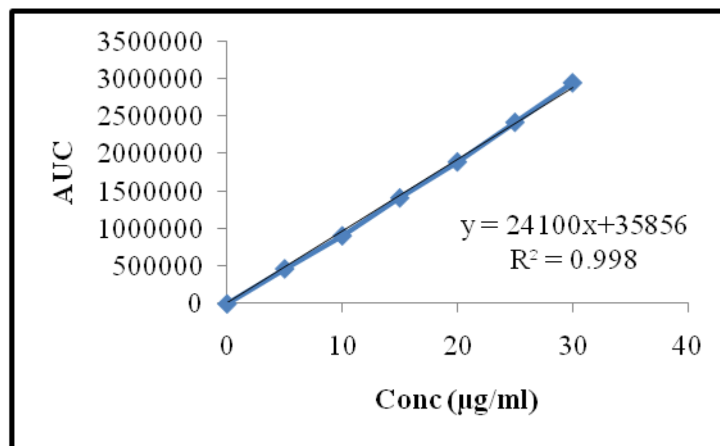

Fig 3. Calibration curve of pure Alogliptin benzoate

4.2.2 Accuracy. Accuracy of the results was calculated by % recovery of by standard addition technique applied for tablets, all carried out in triplicates. The results obtained including the mean of the recovery and standard deviation are displayed in Table 2.

4.2.3 Precision. The repeatability of the method was assessed by six determinations for each of the three concentrations of ALG (5-10-15 µg/ml) representing 50-100-150%, respectively. The repeatability of sample application and measurement of peak area of active compound were expressed in terms of percentage relative standard deviation (%R.S.D.) and found to be less than 1% in the three concentrations. Results for the determination of precision are displayed in Table 2.

4.2.4 Specificity. Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation products and related substances. The chromatograms of the samples were checked for the appearance of any extra peaks. No chromatographic interference from any of the excipients was found at the retention times of the

examined compounds (Figure 2). In addition, the chromatogram of each compound in the sample solution was found identical to the chromatogram received by the standard solution at the wavelength applied. These results demonstrate the absence of interference from other materials in the pharmaceutical formulations and therefore confirm the specificity of the proposed methods.

Table 2. Results obtained for RP-HPLC method for the determination of alogliptin in tablet

Parameters	Results
Retention time	2.642
Wavelength of detection	222
Range of linearity	5-30 µg/ml
Regression equation	Y=24100x +35856
Regession coefficient(r^2)	0.998
Slope(m)	24100
Intercept(c)	35856
Precision Intraday % RSD Interday % RSD	 0.7345 0.3847
Accuracy	0.1537
LOD (µg/ml)	0.00137046
LOQ (µg/ml)	0.00415292

4.2.5 Limit of detection and limit of quantification

Limit of detection (LOD) which represents the concentration of analyte at S/N ratio of 3 and limit of quantification (LOQ) at which S/N is 10 were determined experimentally for the proposed methods and results are given in Table 2.

5. CONCLUSION

The proposed RP-HPLC method proved to be simple, accurate and reproducible for the determination of ALG in a reasonable run time. The method was validated showing satisfactory data for all the

method validation parameters tested. The developed method can be conveniently used by quality control laboratories.

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