Development and Validation of New RP-HPLC Method for the Determination of Alogliptin Benzoate in Bulk Form and Dosage Form

Research Article

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Abstract: Chromatography was performed by isocratic reverse phase separation using an Agilent Eclipse plus C18 column of particle size 5µ, (150×4.6mm). The separations were achieved at the UV detection of 276nm using the mobile phase of Phosphate buffer: Methanol in the ratio of 60:40. Flow rate was 1ml/min and the injection volume was set at 20 µl with 10mins of runtime. The retention time was observed at 4.4 mins for Alogliptin benzoate. The method was validated by using various validation parameters like accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ). The standard curve was linear over a working range of 5-30 µg/ml and gave an average correlation factor 0.999. The limit of detection and the limit of quantification were found to be 0.55µg/ml and 1.86µg/ml respectively. The method showed good recoveries and relative standard deviations of intra and inter day assay less than 2. This method can be easily and conveniently used for routine analysis of Alogliptin in bulk and tablet dosage forms.

Keywords: Alogliptin benzoate, RP-HPLC, Accuracy, Precision, Linearity, LOD, LOQ.

I. INTRODUCTION-

The analytical technique of High Performance Liquid Chromatography (HPLC) is used astronomically throughout the pharmaceutical industry. HPLC play an important and critical role in the field of pharmaceutical industries and analysis, since it is used to provide information on the composition of drug related samples and to detect the raw ingredient used to make them i.e., qualitative and quantitative.

Reverse-phase high-performance liquid chromatography (RP-HPLC) involves the separation of molecules on the basis of hydrophobicity. [1]

Alogliptin benzoate is a selective, orally bioavailable, pyrimidinedione based inhibitor of dipeptidyl peptidase-4 (DPP-4), with hypoglycemic activity. Used to treat type-2 diabetes mellitus In addition to its effects on glucoselevels, alogliptin may inhibit inflammatory responses by preventing the toll like receptor-4 (TLR-4) mediated formation of proinflammatory cytokines.

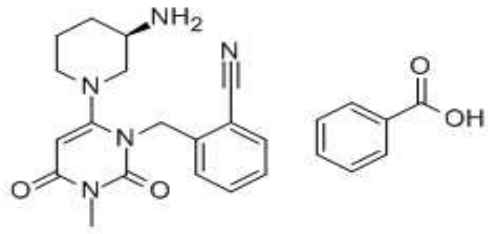

Figure 1: Structure of Alogliptin benzoic acid

Alogliptin Benzoate 2-[[6-[(3R)-3-Amino-1-piperidinyl]-3,4-dihydro-3-methyl-2,4-dioxo-1(2H)- pyrimidinyl]methyl]-benzonitrile monobenzoate. Molecular formula: $C_{18}H_{21}N_5O_2 \cdot C_7H_6O_2$ Molecular weight: 461.522 g/ mol. Solubility: Soluble in dimethyl sulfoxide, sparingly soluble in water and methanol, slightly soluble in ethanol and Pka: 8.5 [2]

Mechanism of Action: Upon eating, concentrations of the incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released into the bloodstream from the small intestine to cause glucose-dependent insulin release. These hormones are inactivated by the DPP-4 enzyme within minutes. Thus, inhibitors of DPP-4 will result increase concentrations of these incretin hormones. [3]

On literature survey it was found that very few analytical methods are available for the determination of Alogliptin benzoate. In view of the need for a suitable method for routine analysis of Alogliptin benzote, attempts are made to develop simple, precise and accurate analytical methods for its estimation. Analytical validation is the corner stone of the process validation. Without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purpose to do. Hence, there is a need to validate the new methods developed.

II. MATERIALS AND METHODS-

Chemicals and reagents-

Authentic sample of alogliptin benzoate purchased from Swapnroop Drugs Pvt. Ltd., Aurangabad. HPLC grade methanol (Merck), Analytical grade potassium dihydrogen phosphate and o-phosphoric acid buffer were used as the solvents throughout the experiment. HPLC grade water obtained by using Direct-Q water purification system (Millipore, Milford, USA) was used in HPLC study.

INSTRUMENTATION-

Analysis was performed on the Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC- 10AT VP pump) (40MPa or 400barr), rheodyne injector, UV variable wavelength detector, standard cell and Agilent syringe was used. The separations were achieved on an Agilent Extended C18 column (5µm, 4.6x150mm). Analytical weighing balance (Shimadzu AUX 220) was used for weighing, sonicator (EQUITRON230VAC, 50Hz), vacuum pump (SUPER FIT), filtration kit (TARSONS) and Nylon membrane filter (Merck Millipore) for solvents and sample filtration was used throughout the experiment. Double beam UV-Visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software-single channel was used for acquisition, evaluation and storage of chromatographic data.

Buffer Preparation

Phosphate Buffer solution of 10mM was prepared by dissolving 0.68g of Potassium dihydrogen phosphate in 500ml HPLC grade water. The pH of the resulting solution was adjusted 3.1 by using o-phosphoric acid. Filtered and sonicated.

Chromatographic condition

After several trials with the different combination and ratio of solvents, the mobile phase phosphate buffer: methanol (60:40v/v) at pH 3.1, a good peak obtained, at Retention time (R_t) 4.40 min for Alogliptin benzoate. Wavelength was selected by scanning the standard drug over a wide range of wavelength 200 nm to 400 nm. The component show reasonably good response and maximum peak at 276nm.

Preparation of Standard

50mg of Alogliptin benzoate is transferred to a 50ml volumetric flask, dissolved and made up to mark with methanol to give stock solution of concentration 1000µg/ml. From stock solution 1-6 dilutions were prepared between 5-30µg/ml by using phosphate buffer: methanol (50:50v/v). Stock solution and working solution were filtered through the nylon membrane filter (0.45µ) and sonicated for 10 min prior to use.

Selection of detection wavelength

For the selection of detection wavelength 10 µg/ml Alogliptin benzoate solution was prepared by appropriate dilution from the standard solution. This drug solution was than scanned in the range of 200-400nm. From the spectrum λmax of Alogliptin benzoate is found to be 276nm was selected for the analysis.

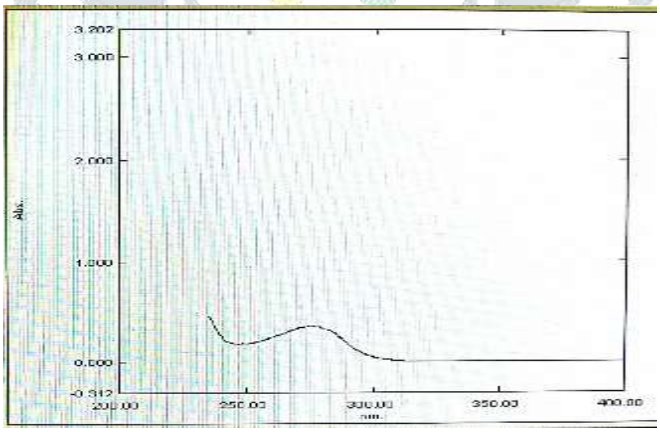

Figure 2: Absorbance maxima of Alogliptin benzoate

Calibration Curve for Alogliptin benzoate

Replicates of each Standard solutions (5, 10, 15, 20, 25, 30 µg.mL^{-1}) were injected using a 20 µL fixed loop system and the solution were chromatographed for 10minutes using mobile phase at a flow rate of 1.0 ml/min. The graph was plotted for peak area vs. concentration for Alogliptin benzoate.

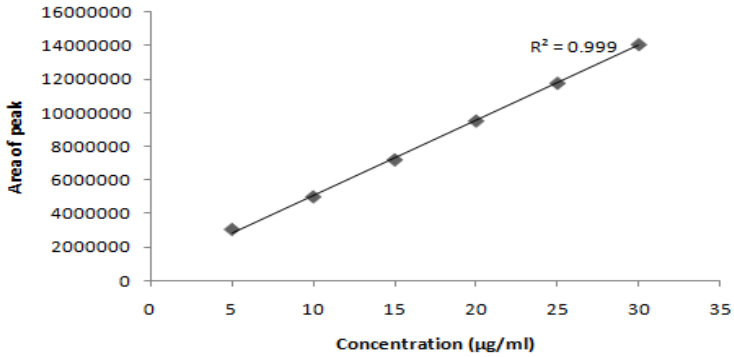

Figure 3: Calibration curve for Alogliptin benzoate

III. VALIDATION OF THE DEVELOPED HPLC METHOD

The developed method was validated according to ICH guidelines with respect to accuracy, precision, linearity, specificity, robustness, limit of detection (LOD), limit of quantification (LOQ), ruggedness and system suitability.

Specificity and selectivity

Specificity is a procedure to detect quantitatively the analyte in presence of the components that may be expected to be present in the sample matrix The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.. While selectivity is a procedure to detect the analyte qualitatively in presence of components that may be expected to be presented in the sample matrix. The excipients in tablet formulation were spiked in pre weighted quantity of drugs and then absorbance was measured and calculations were done to determine the quantity of the drugs.

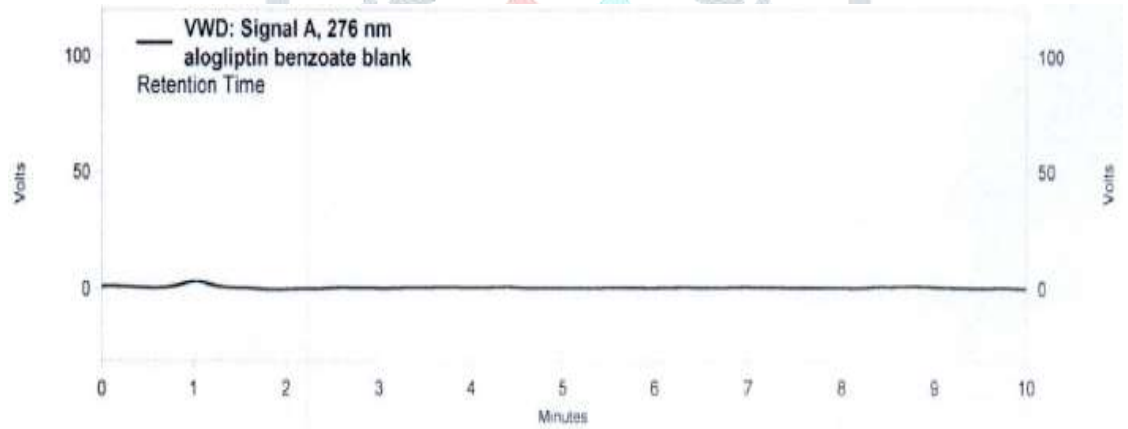

Figure 4: Blank chromatogram

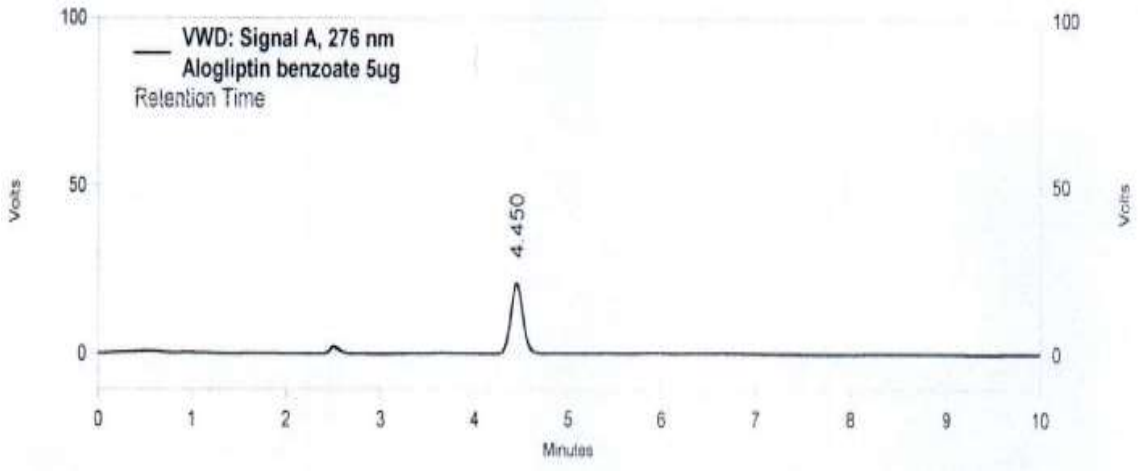

Figure 5: Standard chromatogram of Alogliptin benzoate

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. Linearity was tested for the range of concentrations 5-30µg/ml. Six dilutions of each concentration were prepared separately. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, regression data and calibration data values.

Table 1: Results of linearity of Alogliptin benzoate

Sl. No.	Concentration (µg/ml)	Area
1	5	3056505
2	10	5003577
3	15	7208993
4	20	9534718
5	25	11786384
6	30	14095763

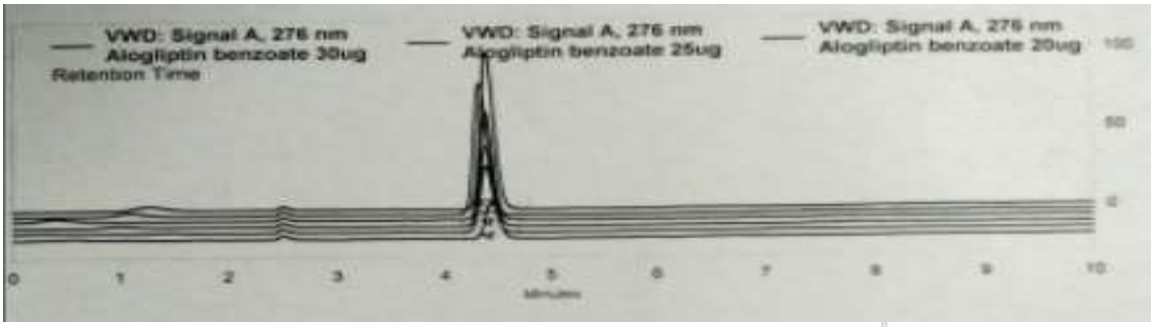

Figure 6: Linearity chromatogram of Alogliptin benzoate

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. It provides an indication of random error results and was expressed as coefficient of variation (RSD). Variation of results within the same day (intra day), variation of results between days (inter day) were analyzed.

Intraday precision was determined by analyzing Alogliptin benzoate for two times in the same day i.e. Morning and afternoon. Inter day precision was determined by analyzing the drug daily for two days i.e. Day 1 and Day 2.

Table 2: Intraday and interday Precision study of Alogliptin benzoate

Sl. No.	Intraday			Interday		
	Replicates	Area		Replicates	Area	
		Morning	Afternoon		Day-1	Day-2
1	Replicate 1	11692256	11660862	Replicate 1	11682157	11702514
2	Replicate 2	11786384	11692256	Replicate 2	11759541	11695012
3	Replicate 3	11730040	11721536	Replicate 3	11702154	11729868
4	Replicate 4	11707812	11788153	Replicate 4	11695391	11745147
5	Replicate 5	11728563	11685498	Replicate 5	11707730	11681350
6	Replicate 6	11699250	11692314	Replicate 6	11698475	11711431
Average		11724051	11706770	**Average**	11707575	11710887
SD		34150.68	44319.07	**SD**	26859	23361.57
% RSD		0.291	0.378	**% RSD**	0.229	0.199

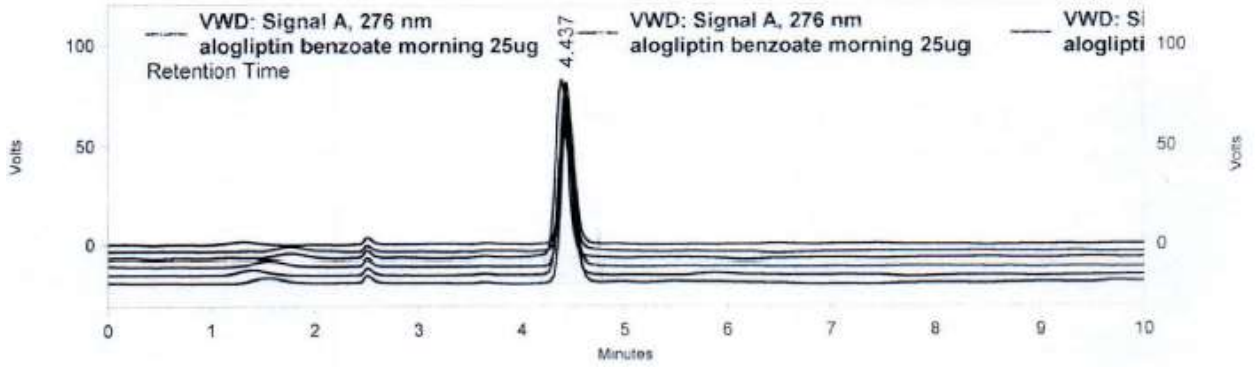

Figure 7: Intraday precision Chromatogram (morning)

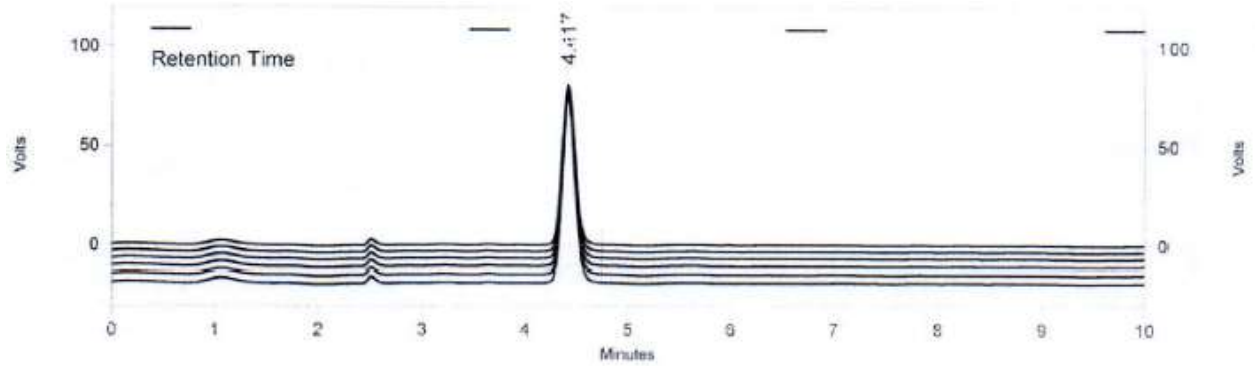

Figure 8: Intraday precision Chromatogram (Afternoon)

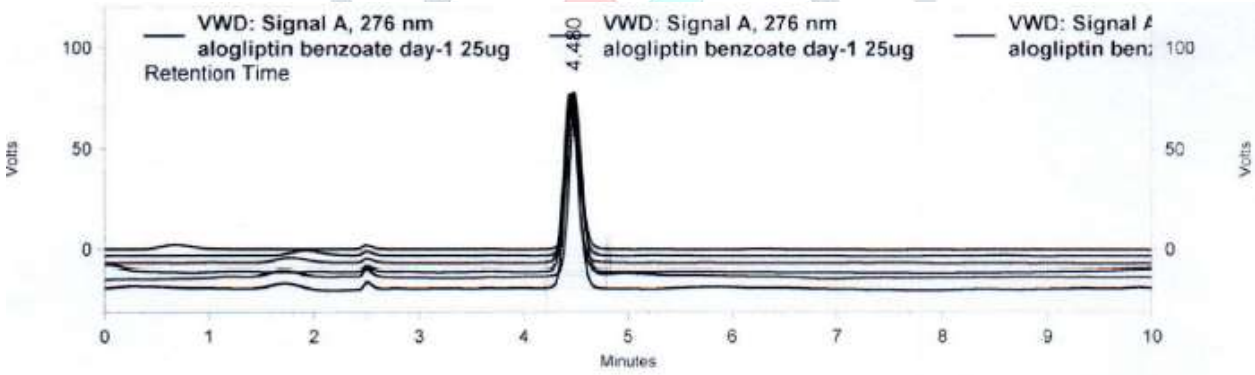

Figure 9: Interday precision Chromatogram (Day-1)

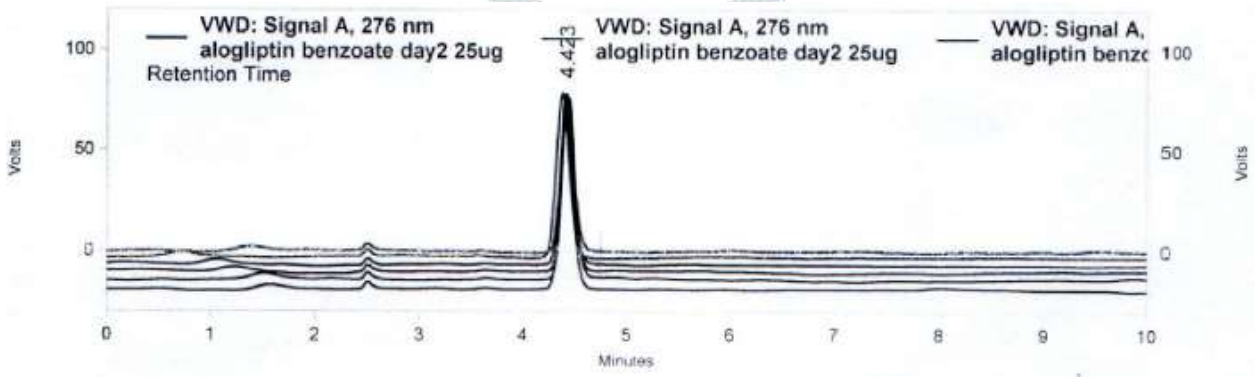

Figure 10: Interday precision Chromatogram (Day-2)

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. The procedure for the preparation of the solutions for Accuracy determination at 80%, 100% and 120% level were prepared.

For 80% Accuracy for Alogliptin benzoate

12 µg of the pure drug was added to 40 µg of formulation

For 100% Accuracy for Alogliptin benzoate

20 µg of the pure drug is added to 50 µg of formulation
For 120% Accuracy for Alogliptin benzoate
30 µg of the pure drug is added to 60 µg of formulation

Table 3: Accuracy study of Alogliptin benzoate

Spiking %	Amount of Sample (µg/ml)	Amount of Drug Added (µg/ml)	Amount of Drug recovered (µg/ml)	% recovery	% RSD
80	15	12	27.2	100.9	0.74
100	20	20	40.1	100.25	0.25
120	25	30	54.6	99.27	0.72

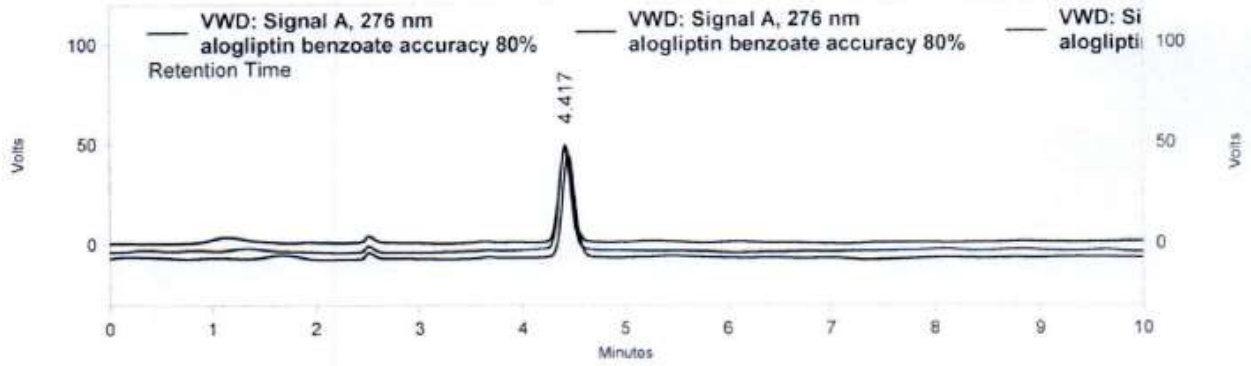

Figure 11: Chromatogram for accuracy (80%)

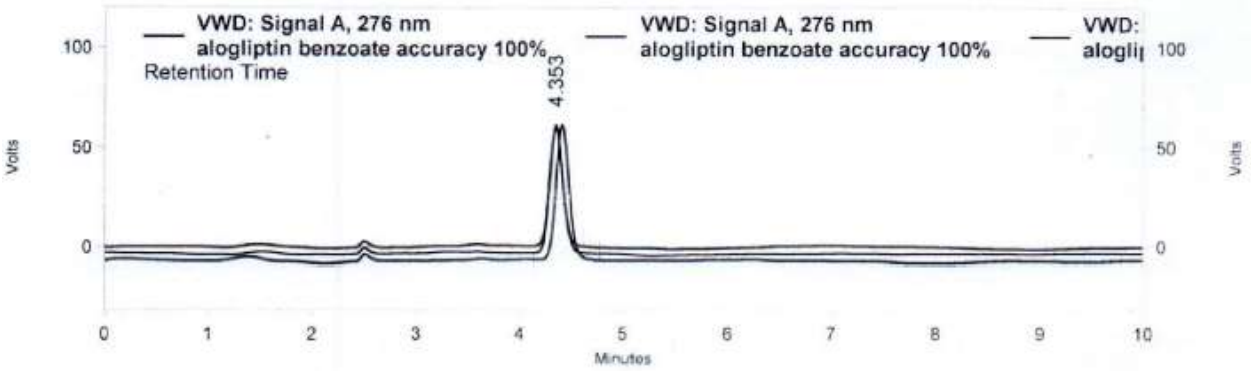

Figure 12: Chromatogram for accuracy (100%)

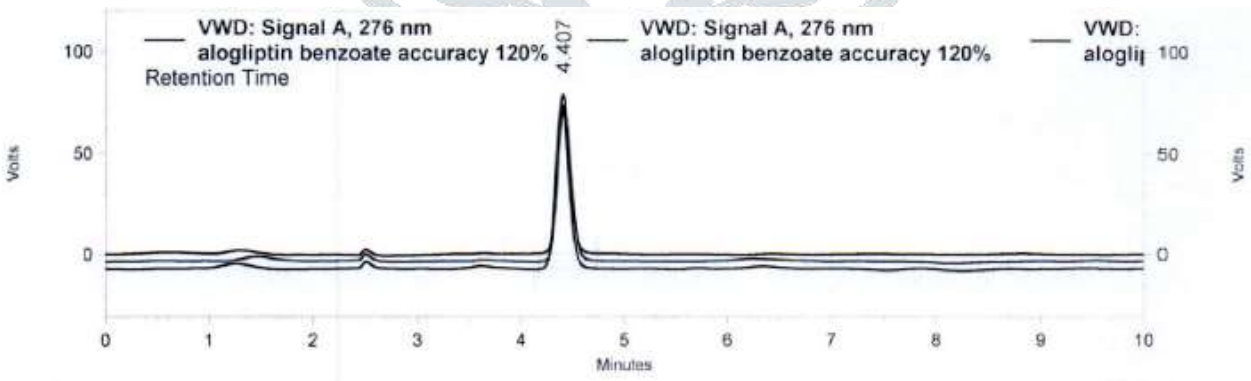

Figure 13: Chromatogram for accuracy (120%)

Robustness

As defined by the ICH, the robustness of an analytical procedures describes to its capability to remain unaffected by small and deliberate variation in the chromatographic conditions such as making small changes in flow rate (± 0.1 ml/min), detection wavelength (±5nm).

Table 4: Robustness study of Alogliptin benzoate

Sl. No.	Parameter	Optimized	Used	Retention time
1	Flow rate	1ml/min	0.9ml/min	4.853min

			1.1ml/min	4.017min
2	Wavelength	276nm	271nm	4.401min
			281nm	4.433min

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of Detection is the lowest concentration in a sample that can be detected. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined. LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. A signal to noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

Table 5: LOD and LOQ for the estimation of Alogliptin benzoate

Name of drug	LOD (µg/ml)	LOQ (µg/ml)
Alogliptin benzoate	0.55	1.86

IV. RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug ALOGLIPTIN being non-polar is preferably analyzed by reverse phase columns and accordingly Agilent Extended C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. First of all, maximum absorbance was found to be at 276nm. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Phosphate buffer: Methanol (pH adjusted to 3.1 using O-Phosphoric acid) in the ratio of 60:40 v/v. Injection volume was selected to be 20µl which gave a good peak area. Run time was selected to be 10 min because analyte elutes at around 4.4±0.03 min. The analytical method was found linear over the range of 5-30µg/ml. The percent recovery was found to be 99.27-100.9. Both Intraday and Interday precision was found to be well within range. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise.

V. CONCLUSION

A New validated RP-HPLC method has been developed for the quantitative determination of Alogliptin benzoate in bulk and pharmaceutical tablet dosage forms. From the above results, method was found to be accurate, precise, linear, specific, system suitable, robust proved to be sensitive, convenient, cost effective, reproducible and have short run time which makes the method rapid for the estimation of Alogliptin benzoate in oral solid dosage form. Hence it can be concluded that this method may be employed for the routine quality control analysis of Alogliptin benzoate in pharmaceutical preparations and active pharmaceutical ingredient (API).

VI. ACKNOWLEDGEMENTS

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VII. REFERENCES

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