HPLC METHOD FOR DETERMINATION OF GLIPTIN AS ANTIDIABETIC DRUG IN BULK AND TABLETS

Sanjana Wadhankar*, Nupur Jaiswal, Ishwar Badwaik,

Mahesh Mange, Tanishka Randive

Abstract

Alogliptin benzoate, a member of dipeptidyl peptidase-4 inhibitors, is a recent drug developed by Takeda Pharmaceutical Company for the treatment of Type 2 diabetes; it potentiates the effect of incretin hormones through the inhibition of their degradation. Alogliptin can be used alone or in combination therapy. A new sensitive and rapid HPLC method was developed for the determination of alogliptin benzoate in bulk and pharmaceutical dosage forms; it was validated according to ICH and FDA guidelines. The HPLC analysis was performed on the Agilent 1200 system equipped with a Hypersil Gold Thermo Scientific C18 (250 cm × 4.6 mm) 5 μm column, with a mixture of acetonitrile and ammonium carbonate buffer in the ratio of 55 : 45 v/v as the mobile phase. The proposed method showed excellent linearity, accuracy, precision, specificity, robustness, LOD, LOQ, and system suitability results within theacceptance criteria. In addition, the main features of the developed method are low run time and retention time around 4 min.

1. Introduction

Incretin hormones are secreted in response to eating food from the gastrointestinal tract to the blood stream and can stimulate insulin secretion and help control glucoselevels; that is, they prepare the body against increase in blood glucose. These hormones include glucagon-like peptide-1 and glucose-dependent insulin tropic polypeptide [5, 6]. Dipeptidyl peptidase-4 is an enzyme found in the human body that helps inactivate the incretin hormones, thus terminating their hypoglycemic effect [2]. Alogliptin a member of dipeptidyl peptidase-4 inhibitors is a recent drug developed in 2010 by Takeda Pharmaceutical Company [2, 7], which is used for the treatment of Type 2 diabetes, and it potentiates the effect of incretin hormones through inhibition of their degradation by the dipeptidyl peptidase-4 enzyme . Alogliptin can be used alone or in combination therapy, and it is now approved in theUSA and Europe also .

Alogliptin is 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4- tetrahydropyrimidin-1yl}methyl)benzonitrile ($C_{18}H_{21}N_5O_2$), and its structure is shown in Figure 3 [8]

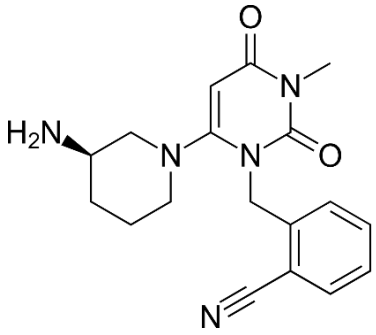


Figure 3 - Chemical structure of Alogliptin.

According to the ICH guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines [9–12].

In this research, a new sensitive and rapid HPLC method was developed for the determination of alogliptin benzoate in pharmaceutical dosage forms, and this method was validated according to ICH and FDA guidelines.

2. Materials and Methods

2.1. Instrumentation - Agilent 1200 HPLC system was used for liquid chromatography method development and validation , equipped with a pump, an auto sampler (ALS), and a Hypersil Gold Thermo Scientific C18 (250 cm × 4.6 mm) 5 μm column, and the detector consisted of UV/VIS operated at 277 nm. Chemstation Software was used for data processing and evaluation.

2.2. Chemicals and Reagents - A pharmaceutical grade sample of alogliptin benzoate (assigned purity 99.4%) , NESINA tablets containing 8.5 mg alogliptin benzoate, Acetonitrile HPLC grade and ammonium carbonate and double distilled water.

2.3. Chromatographic Conditions - The mobile phase was prepared by dissolving 1.0 gm ammonium carbonate in 1000 ml water. From the previous solution, 450 ml was mixed with 550 ml of acetonitrile. Prior to use the mobile phasewas filtered through 0.45 μm membrane filters and degassed by sonication for 10 min. The injection volume was 10 μL, and the flow rate was maintained at 1.0 mL/min.

2.4. Preparation of Standard Solution - A standard solution of alogliptin benzoate was prepared by dissolving an accurately weighed amount of alogliptin benzoate (42.5 mg, which is equivalent to 31.25 mg alogliptin) in 50 ml of the mobilephase, and then 5 mL of the resulting solution was diluted to 25 mL by the same solvent to obtain a standard solution of alogliptin benzoate (170 μg/ml).

2.5. Preparation of Sample Solution - Twenty alogliptin tablets were weighed - triturated in porcelain mortar - mixed - average weight of tablet was calculated. Powder equivalent to 25 mg of alogliptin - a 200 mL volumetric flask - 150 mL of themobile phase was added and sonicated for 30 minutes - solution was filtered through0.45 μm membrane filters.

2.6. Method Validation - The method was validated as per ICH guidelines, and the validation parameters included specificity, linearity, range, accuracy, precision, sensitivity (LOQ and LOD).

2.6.1. Specificity - Specificity of the method was evaluated by injecting 10 μl solutions of standard, sample, blank, and placebo separately.

2.6.2. Linearity - Linear regression analysis was used to evaluate the linearity of thecalibration curve by using the least square linear regression method.

2.6.3. Sensitivity - Limit of detection (LOD)/limit of quantitation (LOQ) of alogliptin benzoate were determined by analysing different solutions of alogliptin benzoate and measuring the signal-to-noise ratio. The limit of detection (LOD) is approximately 3 : 1, while the limit of quantification (LOQ) is 10 : 1 with %RSD () of less than 10%.

2.6.4. Accuracy - The accuracy of the assay method was determined by recovery studies at three concentration levels (50%, 100%, and 150%), i.e., 85, 170, and 255 μg/ml, and three samples from each concentration were injected. The percentage recovery of added alogliptin benzoate and RSD were calculated for each of the replicate samples.

2.6.5. Precision - System precision was established by ten measurements of the standard solution at the 100% concentration levels on the same day. Method precision was established by six assay determinations of the sample solution at the 100% concentration levels on the same day [23]. The RSD of obtained results was calculated to evaluate repeatability results.

2.6.6. Robustness - Robustness of the method was verified by applying minor anddeliberate changes in the experimental parameters. Change was made to evaluate itseffect on the method. Obtained data for each case was evaluated by calculating %RSDand percent of recovery.

2.6.7. Stability of Analytical Solutions - The stability of analytical solutions was determined by analysing the standard and sample preparations at OH at ambient room temperature 30°C. Three injections from each solution were analysed, and the average ofthe peak and the RSD were calculated.

3. Results and Discussion

Table 2 -Results of method optimization

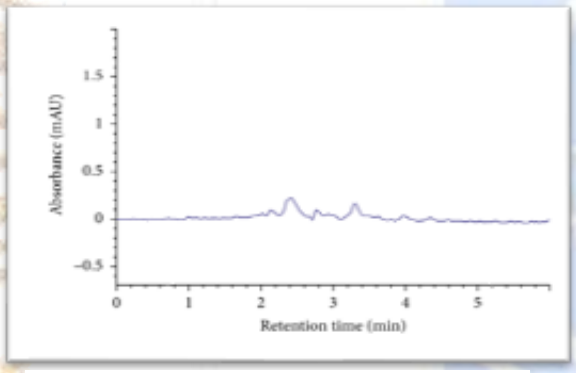
Column used	Mobile phase	Flow rate	Wavelength	Observation	Result
Restek C18, 125 x 4.0 mm i.d., 5 μm	(Buffer : methanol) (45 : 55) v/v	1.0 ml/min	216 nm	Poor resolution 1.4	Method rejected
Thermo Scientific C18, 250 x 4.6 mm i.d., 5 μm	(Buffer : acetonitrile) (25 : 75) v/v	1.0 ml/min	277 nm	Poor resolution 1.6	Method rejected
Thermo Scientific C18, 250 x 4.6 mm i.d., 5 μm	(Buffer : acetonitrile) (45 : 55) v/v	1.0 ml/min	277 nm	Good resolution 2.4	Method accepted

3.1 Method Development and Optimization

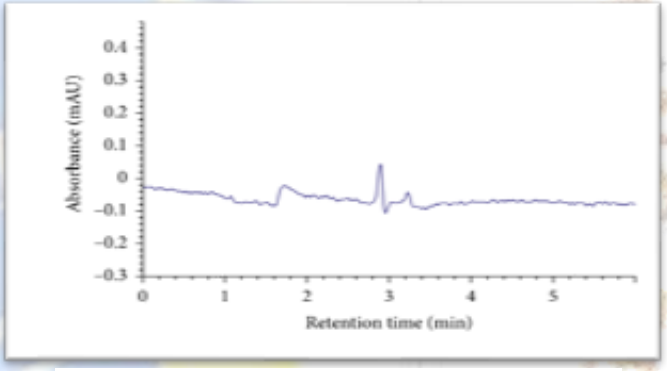
3.2. Method Validation

3.2.1. Specificity

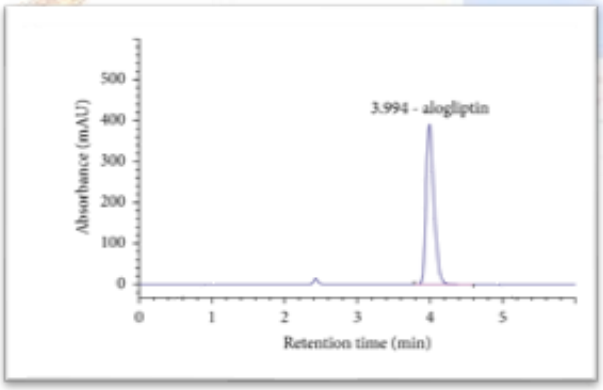
Specificity was evaluated by comparing the chromatograms of mobile phase blank, placebo solution, standard solution, and sample solution (alogliptin 170 μg/ml). For thispurpose, 10 μl from solutions mobile phase blank, standard solution, and sample solution were injected into the HPLC system separately.



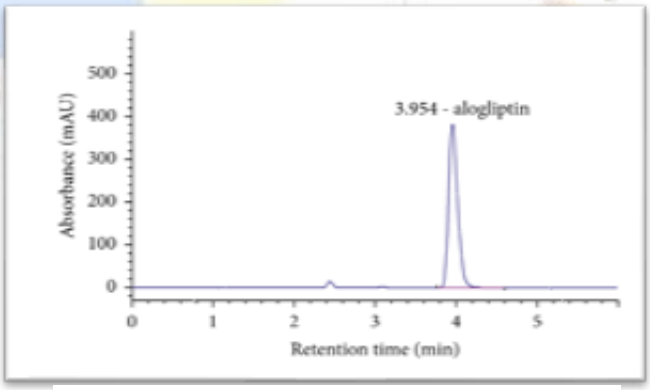
1. Chromatogram of Placebo Solution



2. Chromatogram of Blank Solution

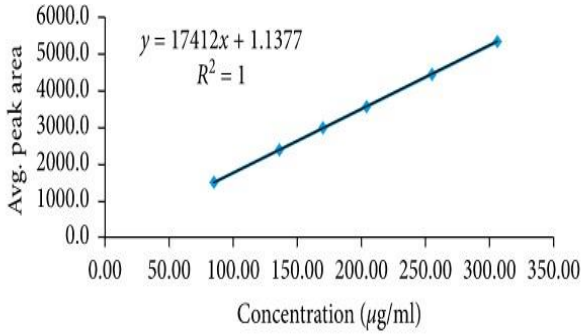


3. Chromatogram of Alogliptin sample solution



4. Chromatogram of Alogliptin standard solution

3.2.2. Linearity and Range



Graph 5 - Standard calibration curve of Alogliptin

Analytical method linearity is defined as the ability of the method to obtain test resultsthat are directly proportional to the analyte concentration, within a specific range.

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

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, while the limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision [24].

3.2.4. Accuracy The accuracy of an analytical procedure expresses the closeness ofresults obtained by that method to the true value

Table 3 – Recovery data of the proposed HPLC method

% spiked level	Replicate number	Peak area	% recovery	Mean %RSD
50	1	1508.4	101.9	101.5
	2	1495.5	101.0	0.43
	3	1503.5	101.6	
100	1	2950.7	99.4	99.4
	2	2950.8	99.4	0.06
	3	2953.8	99.5	
150	1	4443.5	100.2	100.1
	2	4435.4	100.0	0.13
	3	4431.9	99.9	
Mean (% of recovery)	98.0–102.0		100.318	
%RSD	Max 2.00		0.964149	

3.2.5. Precision - The results of both system and method precision showed thatthe method is precise within the acceptable limits. The RSD, tailing factor, and number of theoretical plats were calculated for both solutions; all the results are within limits. Acceptable precision was not more than 2.0% for the RSD and the tailing factor and not less than 1000 for number of plates, as shown in Tables 3 and 4.

Replicate number	RT	Peak area	Number of theoretical plates	Tailing factor
1	3.954	2952	1.32	6274
2	3.956	2951	1.36	6388
3	3.961	2951	1.35	6363
4	3.959	2960	1.33	6364
5	3.961	2953	1.36	6386
6	3.965	2946	1.36	6441
7	3.962	2949	1.38	6479
8	3.965	2950	1.35	6486
9	3.965	2954	1.35	6464
10	3.969	2958	1.33	6471
Average	3.962	2952	1.3	6412
%RSD	—	0.10	—	—

Table 3- System precision data from the standard solution of the proposed HPLC method.

Alogliptin 6.25 mg tablet					
Replicate number	RT	Peak area	Tailing	Plates	% assay
1	4.025	3009	1.54	8086	99.2
2	4.024	3012	1.52	8049	99.2
3	4.027	3009	1.48	8101	99.2
4	4.027	3009	1.49	8105	98.6
5	4.028	3015	1.50	8039	99.3
6	4.027	3012	1.50	8107	99.5
Average	4.026	3011.0	1.5	8081	99.2
%RSD	—	0.1			0.31

Table 4 - Method precision data from the sample solution of the proposed HPLC metho

3.2.6. Robustness

Table 5 - Robustness data of the proposed HPLC method

Parameter		%RSD of standard peak area	%RSD of assay
Column temperature	25°C	0.07	0.15
	30°C (normal)	0.03	0.19
	35°C	0.04	0.2
Wavelength	274 nm	0.06	0.07
	277 nm (normal)	0.03	0.19
	280 nm	0.06	0.17
Mobile phase composition	−5% acetonitrile	0.05	0.20
	Normal	0.03	0.19
	+5% acetonitrile	0.02	0.14
Flow rate	0.8 ml/min	0.04	0.11
	1 ml/min (normal)	0.03	0.19
	1.2 ml/min	0.08	0.23

The results of robustness testing showed that a minor change of method conditions, such as the composition of the mobile phase, temperature, flow rate, and wavelength, is robust within the acceptable limits.Solution Stability

The percent of recovery was within the range of 98.0% to 102.0% and RSD was not more than 2.0%, indicating a good stability of the sample and standard solutions for 24 hr at both conditions. The percent of recovery was within acceptable limits, and the %RSD is within the limit of not more than 2.0%. The tailing factors and number of theoretical plates were found within acceptable limits as well. The results are shown in Table 6.

Table 6- Solutions stability data of the proposed HPLC method.

Parameter		RT	Avg. peak area	RSD peak area (%)	Tailing factor	Recovered (%)	Number of theoretical plates
Standard solution	0 h	4.034	3022.7	0.07	1.5	—	8058
	After 24 h at 30°C	4.035	3021.7	0.2	1.6	100.0	8143
	After 24 h at refrigerator	4.049	2983.7	0.08	1.5	98.7	8137
Sample solution	0 h	4.034	2995.7	0.07	1.5	—	8142
	After 24 h at 30°C	4.035	3001.3	0.3	1.5	100.2	8179
	After 24 h at refrigerator	4.036	3000.0	0.2	1.6	100.1	8188

4. Conclusion

In the present research, a fast, simple, accurate, precise, and linear stability- indicating HPLC method has been developed and validated for alogliptin benzoate, and hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for alogliptin benzoate. In addition, the main features of the developed method are short run time and retention time around 4 min. The method was validated in accordance with ICH guidelines. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions.

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