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A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ALOGLIPTINE AND PIOGLITAZONE IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

The proposed study, a new stability- indicating RP-HPLC method has been developed for estimation of Alogliptin and Pioglitazone in bulk and pharmaceutical dosage form. The present method was a sensitive, precise, and accurate RP-HPLC method for the analysis of Alogliptin and Pioglitazone. To optimize the mobile phase, various combinations of buffer and organic solvents were used on Hypersil BDS C18 column. Then the mobile phase containing a mixture of Phosphate Buffer: Acetonitrile in the ratio of 45:55 % v/v was selected at a flow rate of 1.0 ml/min for developing the method and the peaks with good shape and resolution were found resulting in short retention time, baseline stability and minimum noise. The retention times of Alogliptine and Pioglitazone were found to be 3.42 and 5.24 min respectively. Quantitative linearity was obeyed in the concentration range of 31-187 and 75-450 µg/mL of Alogliptin and Pioglitazone respectively. The limit of detection and limit of quantitation were found to be 0.399 µg/mL and 1.21µg/mL (ALO); 0.516 µg/ mL and 1.565 µg/mL (PIO) respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations did not interfere with the estimation of the drugs by the proposed HPLC method.

Key words: Alogliptin, Pioglitazone, Pharmaceutical dosage form

INTRODUCTION

Alogliptine is a potent highly selective DPP-IV inhibitor used in the treatment of type 2 diabetes. Chemically, alogliptine is 2-({6-[(3R)-3-aminopiperidine-1-yl]-3-methyl-2-4-dioxo-3,4-dihydropyrimidin-1(2H)-yl}methyl benzonitrile. Alogliptine stimulate the glucose dependent insulin release by inhibiting the Dipeptideyl Peptidase Inhibitor IV [1, 2, 3, 4], which normally inactivates the glucagon like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP). GLP-1 and GIP are incretin hormones [5] secreted in response to food intake and stimulate insulin secretion. The increased availability of GLP-1 and GIP results in glucose dependent insulin release and better glycemic control. The chemical structure of alogliptine was given in fig 1.

Pioglitazone is oral hypoglycemic thiazolidinediones derivative used in the treatment of type 2 diabetes. Chemically it is (RS)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxy] benzyl) thiazolidine-2, 4-dione [6]. It is selective modulator of peroxisome proliferator activated receptor-gamma (PPAR-γ) [7, 8]. It controls the glucose and lipid metabolism in the muscle, liver and adipose tissue by modulating the transcription of insulin sensitive genes. Pioglitazones also reduces insulin resistance in the liver and increases the expense of insulin dependent glucose. The chemical structure of pioglitazone was given in fig 2.

The review of literature revealed that several analytical methods have been reported for Alogliptin, [9,10,11,12] Pioglitazone [13,14,15 16] in spectrophotometry, HPLC, HPTLC, LC/MS individually and in combination with Metformin. To date, there have been no published reports about the stability indicating studies and simultaneous estimation of Alogliptin and Pioglitazone by HPLC in bulk drug and in pharmaceutical dosage forms. This present study reports for the first time stability indicating simultaneous estimation of Alogliptin and Pioglitazone by RP-HPLC in bulk drug and in pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals and Reagents: Alogliptine and Pioglitazone were obtained as gift sample from Spectrum Pharma Research laboratory in Hyderabad and marketed formulation was purchased from local market. Acetonitrile, Water, were obtained from Merck. Mumbai and Potassium dihydrogen ortho phosphate, Triethylamine, Ortho Phosphoric Acid obtained from RANKEM Mumbai. All solvents used in this work are HPLC grade.

Instrument and chromatographic conditions: RP-HPLC waters 2695 separation module equipped with 2996 Photodiode Array Detector was employed in this method. The Empower 2 software was used for LC peak integration along with data acquisition and data processing. The column used for separation of analytes is Hypersil BDS C18, (250 x 4.6 mm, 5µ). Mobile phase consisting of Phosphate Buffer: Acetonitrile in the ration of 45:55 % v/v at a flow rate of 1.0 ml/min. It was filtered through 0.45µm nylon filter and sonicated for 5 min in ultrasonic bath. Samples were analysed at 215 nm at an injection volume of 10 µL.

Preparation of Phosphate Buffer pH 3.5: Accurately weighed 2.72gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 4.8 with dil. Orthophosphoric acid solution.

Preparation of Solutions:

Alogliptin stock preparation (1250µg/ml): Accurately Weighed and transferred 12.5mg of Alogliptin working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent added, then sonicated for 5 minutes and make up to the final volume with diluent.

Pioglitazone stock preparation (3000µg/ml): Accurately Weighed and transferred 30mg of pioglitazone working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent added, then sonicated for 5 minutes and make up to the final volume with diluent.

Standard Preparations:

Alogliptin Standard Preparation *(125µg/ml):* From the above Alogliptin stock solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Pioglitazone Standard Preparation *(300µg/ml):* From the above pioglitazone stock solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

METHOD VALIDATION

The validation of the method was carried out as per ICH Guidelines [18]. The parameters assessed were specificity, linearity, precision, accuracy, stability, LOD and LOQ.

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.

Accuracy: The accuracy was determined by calculating % recoveries of Alogliptine and pioglitazone. It was carried out by adding known amounts of each analyte corresponding to three concentration levels (50, 100, and 150%) of the labelled claim to the excipients. At each level, six determinations were performed and the accuracy results were expressed as percent analyte recovered by the proposed method.

Precision: Precision of an analytical method is usually expressed as the standard deviation. The repeatability studies were carried out by estimating response of alogliptine and pioglitazone six times. The intra-day and inter-day precision studies (intermediate precision) were carried out by estimating the corresponding responses three times on the same day and on three different days for three different concentrations and the results are reported in terms of relative standard deviation.

Linearity: The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits a linear response and is directly proportional over the relevant concentration range for the target concentration of the analyte. The linear regression

data for the calibration plot is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance.

Robustness: Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase, flow rate and temperature. This deliberate change in the method has no affect on the peak tailing, peak area and theoretical plates and finally the method was found to be robust.

Limit of Detection & Limit of Quantitation: The LOD can be defined as the smallest level of analyte that gives a measurable response and LOQ was determined as the lowest amount of analyte that was reproducibly quantified. These two parameters were calculated using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations, LOD=3.3 × s/s and LOQ=10 × s/s, where s = standard deviation, S= slope of the calibration curve.

Assay of Alogliptine and Pioglitazone in Tablet: Assay of marketed product was carried out by using the developed method. Sample solutions were prepared and injected into RP-HPLC system. The sample solution was scanned at 215 nm. The % drug estimated was found to be 99.86. The chromatogram showed two single peaks of Alogliptine and Pioglitazone was observed with retention times of 3.42 and 5.26 min (Figure 3)

Forced Degradation studies: Stress studies are performed according to ICH guidelines under conditions of hydrolysis (acidic and alkaline), photolysis, oxidation, and thermal studies.

Oxidation: To 1 ml of stock solution of Alogliptin and Pioglitazone, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 125µg/ml & 300µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of s tock s solution Alogliptin and Pioglitazone, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 125µg/ml & 300µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 1 ml of stock solution Alogliptin and Pioglitazone, 1ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 125µg/ml & 300µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105^0c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 125µg/ml & 300µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 125µg/ml & 300µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 125µg/ml & 300µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS & DISCUSSIONS

Optimized Chromatographic conditions: To establish and validate an efficient method for analysis of these drugs in pharmaceutical formulations, preliminary tests were performed. Different chromatographic conditions were employed for the analysis of the alogliptine and pioglitazone in both bulk and pharmaceutical dosage form. Finally the analysis was performed by using Phosphate Buffer: Acetonitrile in the ration of 45:55 % v/v at a flow rate 1.0 ml/min. Samples were analysed at 215nm at an injection volume of 10 µL and separation was carried by using Hypersil BDS C18, (250 x 4.6 mm, 5µ) column. The proposed method was optimized to give a sharp peak with minimum tailing for alogliptine and pioglitazone. (Fig 4). The optimized conditions were given in table 1.

Forced degradation studies were performed to establish the stability indicating property and specificity of the proposed method. Degradation studies were carried out under conditions of hydrolysis, dry heat, oxidation, UV light and photolysis and the drug substances were degraded in all conditions. Acid and base hydrolysis was performed by exposing the drug substances with 2N HCl and 2N NaOH at 60 ^{o}C for 30min and it was showed degradation of Alogliptin and Pioglitazone with degraded products peak at retention time 2.88 min. Degradation studies under oxidative conditions

were performed by heating the drug sample with 20% H_2O_2 at 60 ^oC and degraded product peaks were observed. Both Alogliptin and Pioglitazone are sensitive to acid and alkali and there was no degradation occurs under UV light and thermal conditions. The results of forced degradation studies were given in table 2. Precision was evaluated by a known concentration of Alogliptin and Pioglitazone was injected six times and corresponding peaks were recorded and % RSD was calculated and found within the limits. The low % RSD value was indicated that the method was precise and reproducible and the results were shown in the table (Table 3). Accuracy of the method was proved by performing recovery studies on the commercial formulation at 50, 100 and 150% level. % Recoveries of Alogliptin and Pioglitazone ranges from 99.61 to 100.62% in simultaneous equation method and the results were shown in the (Table 4). Linearity was established by analyzing different concentrations of alogliptine and pioglitazone respectively. The calibration curve was plotted with the area obtained versus concentration of both Alogliptin and Pioglitazone (Fig 5&6). In the present study six concentrations were chosen ranging between 31-181 µg/mL of alogliptine and 75-450 µg/mL of pioglitazone. The regression equation and correlation coefficient for Alogliptin and Pioglitazone was found to be y=12696x-4635. And R^2=0.9990 and y=15904x-1066. and R^2=0.9990 respectively and results were given in table 5. Robustness of the method is the ability of the method to remain unaffected by small deliberate changes in parameters like flow rate, mobile phase composition and column temperature. To study the effect of flow rate of the mobile phase it was changed to 0.1 units from 1.0 mL to 0.9 mL and 1.1 mL. The effect of column temperature also checked by changing temperature to ± 5 ^oC. This deliberate change in the above parameters has no significant effect on chromatographic behaviour of the samples and results were given in table 6. LOD and LOQ of alogliptine and pioglitazone were evaluated based on relative standard deviation of the response and slope of the calibration curve. The detection limits were found to be 0.399 µg/mL and 0.516 µg/mL for alogliptine and pioglitazone respectively. The quantitation limits were found to be 1.211 µg/mL and 1.565 µg/mL for alogliptine and pioglitazone respectively. The results were given in the table 7.

CONCLUSION

A new stability- indicating RP-HPLC method has been developed for estimation of Alogliptine and Pioglitazone in bulk and pharmaceutical dosage form. The developed method was validated and it was found to be simple, sensitive, precise, robust and it can be used for the routine analysis of Alogliptine and Pioglitazone in both bulk and pharmaceutical dosage forms. The forced degradation studies were carried out in accordance with ICH guidelines and the results revealed suitability of the method to study stability of Alogliptine and Pioglitazone under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations. Finally it was concluded that the method is simple, sensitive and has the ability to separate the drug from degradation products and excipients found in the dosage form.

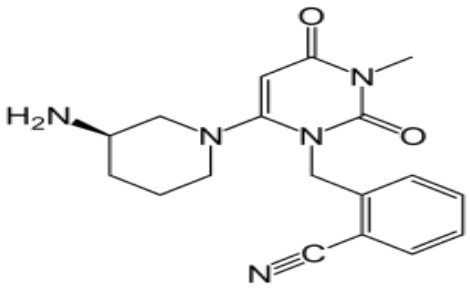

Fig 1: Chemical Structure of Alogliptine

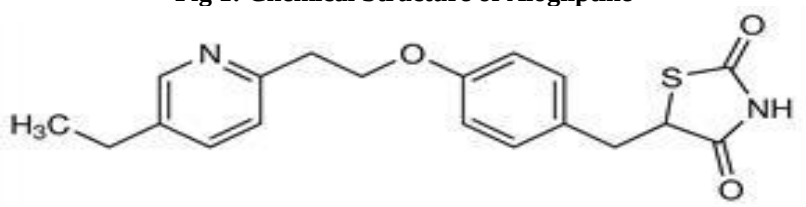

Fig 2: Chemical Structure of Pioglitazone

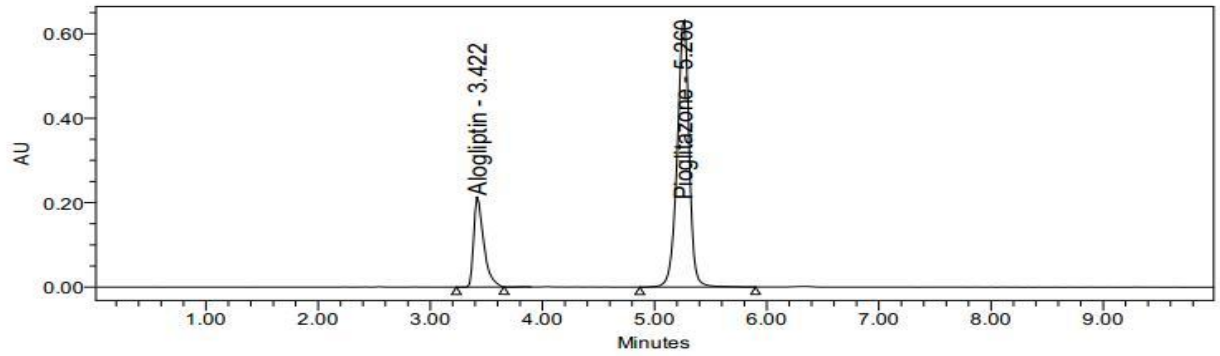

Fig 3: A typical chromatogram of Alogliptine and Pioglitazone in tablet dosage form

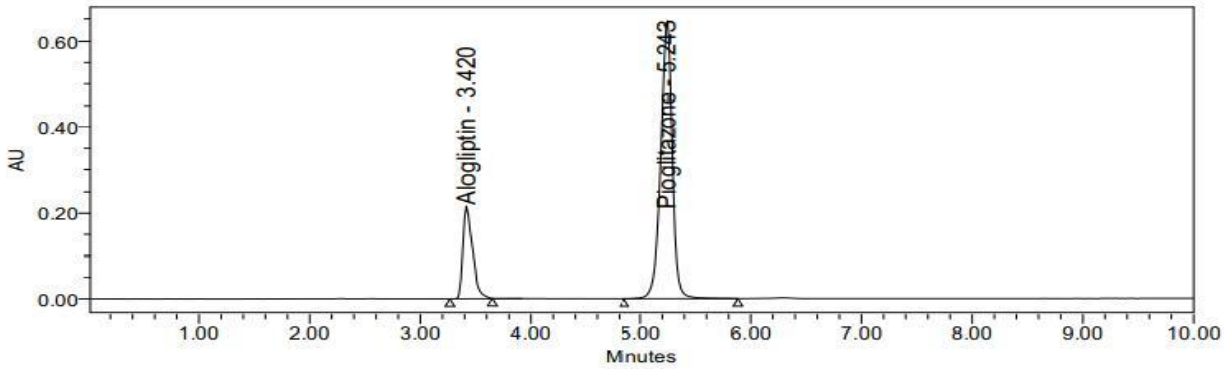

Fig 4: Standard Chromatogram of Alogliptine and Pioglitazone

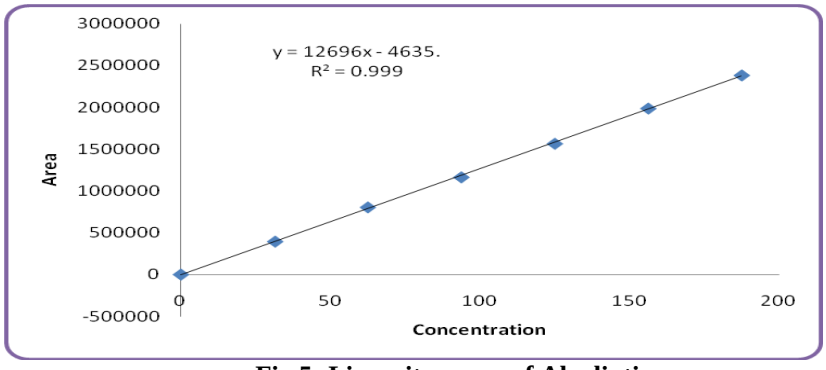

Fig 5: Linearity curve of Alogliptine

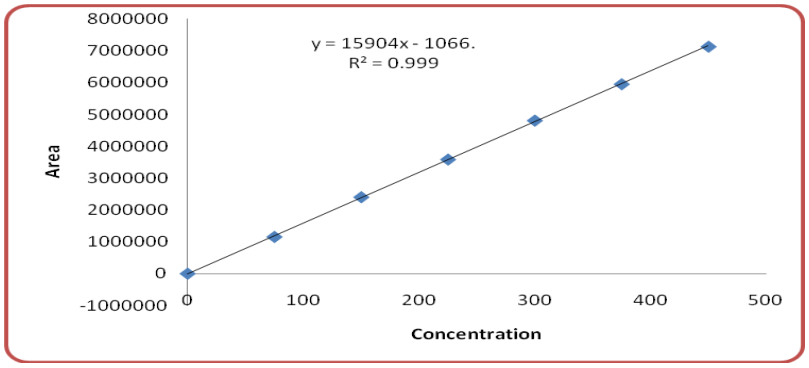

Fig 6: Linearity curve of Pioglitazone

Table-1: Optimized Chromatographic conditions

Parameter	Condition
Mobile Phase	Phosphate Buffer: Acetonitrile (45:55 % V/V) pH adjusted to 4.8
Column	Hypersil BDS C18, 250 x 4.6 mm, 5µ.
Wave length	215nm
Flow rate	1.0 mL/min
Injection volume	10µL
Run time	10 min
Diluent	Water: Acetonitrile (50:50)

Table 2: Results of Forced Degradation Studies

S.No	Injection	Alogliptine		Pioglitazone	
		% Assay	% Degradation	% Assay	% Degradation
1	Acid Degradation	87.79	12.21	90.44	9.56
2.	Base Degradation	90.99	9.01	91.14	8.86
3.	Peroxide	93.62	6.38	94.14	5.86
4.	Thermal Degradation	94.43	5.57	94.08	5.92
5.	UV Degradation	98.68	1.32	98.07	1.93
6.	Neutral degradation	98.91	1.09	98.56	1.44

Table 3: Precision method of proposed RP-HPLC method

Drug	Mean Area	% RSD
Alogliptine	1503754	0.31
Pioglitazone	4680752	0.32

Table 4: % Recovery Data for Alogliptine and Pioglitazone

Drug	Spiked Level %	% Recovery	% RSD
Aloglitpine	50	99.87	0.630
	100	99.99	0.758
	150	100.56	0.656
Pioglitazone	50	99.62	0.819
	100	99.79	0.608
	150	100.61	0.155

Table 5: Results of Linearity

S.No	Alogliptine		Pioglitazone	
	Conc. (µg/ml)	Peak Area	Conc. (µg/ml)	Peak Area
1	31.25	394560	75	1155742
2	62.50	804549	150	2403371
3	93.75	1164367	225	3586362
4	125	1566629	300	4810406
5	156.25	1987897	375	5952903
6	187.50	2384765	450	7132185

Table 6: Robustness Data

Parameters	Changed Condition	Mean Peak Area		USP plate count	
		ALG	PIO	ALG	PIO
Flow rate (mL/min)	0.8ml	1728685	5343001	6871	13211
	1.0ml	1502579	4669632	6576	12398
	1.2ml	1389559	4295709	6544	12153
Temperature (±5)	25^0C	1501832	4660587	6708	12598
	30 ^{0}C	1502579	4669632	6576	12398
	35^0C	1486135	4609506	6694	12677
Mobile phase (±5%)	50:50 %v/v	1485501	4754021	7016	12714
	45:55 %v/v	1502579	4669632	6576	12398
	40:60 %v/v	1535490	4661642	6635	12362

ALG-Aloglitpine **PIO-**Pioglitazone

Table 7: Results of LOD and LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Aloglitpine	0.399	1.211
Pioglitazone	0.516	1.565

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