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METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUSESTIMATION OF ALOGLIPTIN AND PIOGLITAZONE IN COMBINED TABLET DOSAGE FORMS BY RP-HPLC

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Abstract
A high performance liquid chromatographic method was developed to quantify alogliptin and pioglitazone simultaneously in bulk and combined tablet dosage form. The chromatographic analysis was done on a Zorbax C8 column (150 mm x 4.6 mm internal diameter, 5 µm particle size) with a mobile phase of 0.1 M ammonium acetate and methanol (50:50, *v/v*) at 1.0 mL/min. The effluents were monitored at 248 nm and the retention time of alogliptin and pioglitazone were 2.883 min and 4.329 min, respectively. Calibration curves were linear from 6.25-18.75 µg/mL for alogliptin and 11.25-33.75 µg/mL for pioglitazone. The LOD and LOQ values for alogliptin were 0.047 and 0.157 µg/mL, respectively corresponding values for pioglitazone were 0.085 and 0.284 µg/ mL, respectively. The precision for alogliptin and pioglitazone was in the range of 0.094-0.3 03 % and 0.072-0.239%, respectively, with corresponding accuracy of 99.450-99.692% and 100.184-100.422%. The developed and validated method was successfully applied for the simultaneous determination of alogliptin and pioglitazone in tablet formulation.

Keywords: Alogliptin, Pioglitazone, Liquid Chromatography, Tablets, Assay

Introduction
Alogliptin, chemically known as 2-({6-[(3R)-3- aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl}methyl)benzonitrile (Figure 1), is an oral antihyperglycemic of dipeptidyl peptidase 4 inhibitor class. It is used in the treatment of type II diabetes milletus (1,2). The inhibition of dipeptidyl peptidase 4 by alogliptin increases the quantity of active plasma incretins and glucagon like peptide 1 that helps in glycemic control (3). Pioglitazone, an anti-diabetic drug, belongs to the thiazolidinedione class of drugs. Chemically it is known as 5-({4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}methyl)-1,3- thiazolidine-2,4-dione (Figure 1). Pioglitazone is prescribed to improve control of blood glucose level in adults with type 2 diabetes mellitus (4). Pioglitazone enhances tissue sensitivity to insulin by acting as a potent and selective agonist at peroxisome proliferator activated gamma receptor in adipose tissue, skeletal muscle and liver (5). With proper diet and exercise, alogliptin and pioglitazone combination is used in the management of high blood

sugar levels caused by type 2 diabetes (6,7). This combination was approved by FDA in 2013 (8). The combination of theselected drugs is not official in any pharmacopeia. Therefore,it is essential to develop effective analytical method for the simultaneous determination of alogliptin and pioglitazone.

UV spectrophotometric methods like first order derivative, dual wavelength, second order derivative and area under curve methods were described by Raval & Srinivasa (9) and Anusha et al. (10) for the simultaneous estimation of alogliptin and pioglitazone in bulk and pharmaceutical dosage forms. High performance thin layer chromatographic method was reported by Komal & Amrita for the simultaneous assay of alogliptin and pioglitazone in combined dosage forms (11). In HPTLC method, separation was achieved on Merck HPTLC aluminum sheets coated with silica gel 60F254, with acetonitrile: 1 % ammonium acetate in methanol (4.5:5.5 *v/v*) as mobile phase and densitometric analysis was performed at 254 nm. Though the UV spectrophotometric methods reported by Raval & Srinivasa (9) and Anusha et al. (10) are simple, they are less selective since they involve measurements in the UV range where there is a possibility of absorbance by the tablet excipients. One of the important validation parameter, method robustness, is not reported in the UV spectrophotometric methods. The HPTLC method described by Komal & Amrita (10) requires costly, sophisticated instrumentation and expertise personnel to operate. Moreover the HPTLC instrument is not commonly available in the developing and under developed countries. RP-HPLC methods were also applied to the determination of the selected drug combination in bulk and pharmaceutical dosage forms by Raval & Srinivasa (12), Neelima et al. (13), Manzoor et al. (14) and Mokhtar et al. (15). In Raval & Srinivasa method, the separation was carried out on an BDS hypersil C18 (250 mm × 4.6 mm, 5 μm) analytical column using buffer with pH 3.5 and methanol (70:30, *v/v*) as mobile phase at a flow rate of 1.0 mL/min with UV detection at 271 nm (12). Using a Hypersil BDS C18, (250 x 4.6 mm, 5 μm) column as stationary phase and a phosphate buffer of pH 4.8-acetonitrile (45:55, *v/v*) as mobile phase, the selected drugs combination in pharmaceutical formulations was determined by Neelima et al. (13). The detection wavelength was set at 215 nm. In Mokhtar et al. method (14), alogliptin and pioglitazone was chromatographed on Enable C18 (250 mm × 4.6 mm, 5 μm) Column with a mobile phase consisting of phosphate buffer with pH 3.6-acetonitrile (35:65, *v/v*) pumped at a flow rate of 1.0 mL/min with UV-detection at 268 nm. Alogliptin and pioglitazone in tablets was assayed by Mokhtar et al. (15) by carrying out chromatography on an Inertsil ODS-3 (250 mm × 4.6 mm, 5 μm) column using a mixture of methanol and phosphate buffer with pH 3.0 (80:20, *v/v*) as mobile phase at a flow rate of 1 mL/min with UV-detection at 269 nm. In the present study, a new RP-HPLC method with PDA detector was developed for simultaneous determination of alogliptin and pioglitazone in bulk and combined tablet dosage form. The method validation has been carried out according to the International Conference on Harmonization guidelines (16). The developed and validated RP-HPLC method was successfully applied to combined tablet dosage form.

2. MATERIALS AND METHODS

2.1. Reference standard drugs and tablet dosage forms

Alogliptin and pioglitazone reference standard drugs were provided by Lara Drugs Private Limited (Telangana, India) as gift samples. They are used as received**.** The tablet dosage form, Oseni tablets (strength 25 mg alogliptin and 45 mg pioglitazone), manufactured by Takeda pharmaceuticals America Inc., Deerfield was purchased from the local pharmacy.

2.2. Chemicals and solvents

The HPLC grade methanol was obtained from Merck India Ltd., Mumbai, India. Analytical reagent ammonium acetate was obtained from Sd. Fine Chemicals Ltd., Mumbai, India.
Water was obtained using a Milli-Q system.

2.3. Apparatus and HPLC conditions

The Waters Alliance 2695 Module equipped with a 2998 PDA detector with Empower 2 software was used in the current analysis. The Zorbax C8 column (150 mm x 4.6 mm internal diameter, 5 µm particle size) was used. Isocratic mobile phase was composed of 0.1 M ammonium acetate and methanol (50:50, *v/v*) with pH 3.5 (adjusted with orthophosphoric acid: Sd. Fine Chemicals Ltd., Mumbai). The same mobile phase was used as diluent for the preparation of standard solutions of alogliptin and pioglitazone. A flow rate of 1.0 mL/min was maintained. The eluted compounds were monitored at 248 nm. The column temperature was maintained at 30• }1 oC. An injection volume of 10 µL was used.

2.4. Standard solutions

A stock standard solution (alogliptin - 250 µg/mL and pioglitazone – 450 µg/mL) was prepared in a 100 mL volumetric flask by dissolving 25 mg of alogliptin and 45 mg of pioglitazone in a final volume of 100 mL mobile phase. Working standard solutions (6.25-18.75 µg/mL for alogliptin and 11.25-33.75 µg/mL for pioglitazone) were prepared from the above stock solution by appropriate dilution with mobile phase.

2.5. Tablet sample solution

Average weight of ten tablets was determined, transferred to a clean dry mortar and grinded into fine powder. Tablet powder equivalent to 25 mg of alogliptin and 45 mg of pioglitazone was then transferred to a 100 mL volumetric flask, 30 mL of mobile phase was added and the flask was sonicated for 10 min to dissolve the drugs completely. The mixture was diluted up to volume with the mobile phase to give a solution containing 250 µg/mL and 450 µg/mL of alogliptin and pioglitazone, respectively. This solution was filtered through 0.45 µm pore size membrane filter. Appropriate dilution (12.50 µg/mL of alogliptin and 22.50 µg/mL of pioglitazone) was prepared in mobile phase for analysis.

2.6. Calibration graph

Working standard solutions equivalent to 6.25-18.75 µg/ mL alogliptin and 11.25-33.75 µg/mL pioglitazone were prepared by appropriate dilution of the stock standard solution with the mobile phase. 10 µL aliquot of each solution was injected automatically into the column in triplicate and the chromatograms were recorded. The peak areas of the drugs were determined. Calibration graph was constructed by plotting the mean peak area against drug concentration. The concentration of the unknown was calculated from thecalibration graph or from the regression equation derived from the mean peak area-concentration data.

2.7. Estimation of alogliptin and pioglitzone in combined tablet dosage form

10 µL of the tablet sample solution was injected into the HPLC system in triplicate. The chromatograms were recorded. The peak areas were determined. The concentrations of alogliptin and pioglitazone in the combined tablet dosage form were calculated from the corresponding calibration curves or corresponding regression equations.

2.8. Forced degradation

To assess the stability indicating properties of the proposed HPLC method, forced degradation studies were performed. The tablet sample was subjected to acid, alkali, oxidation, thermal and photo degradation

Acid and alkali hydrolysis

Tablet powder equivalent to 25 mg of alogliptin and 45 mg of pioglitazone was transferred to a 100 mL volumetric flask. The powder was mixed with 10 mL of 0.1 N hydrochloric acid (for acid hydrolysis) or 10 mL of 0.1 N sodium hydroxide (for alkali hydrolysis). The solutions were

subjected to sonication for 30 min. The samples were neutralized with an amount of acid (for alkali hydrolysis) or base (for acid hydrolysis) equivalent to that of the previously added. The flask was made up to the volume with mobile phase.

Oxidative degradation

Tablet powder equivalent to 25 mg of alogliptin and 45 mg of pioglitazone was transferred to a 100 mL volumetric flask. The contents were mixed with 10 mL of 30% hydrogen peroxide solution. The reaction mixture was allowed to

Thermal and photo degradation

Tablet sample powder (alogliptin-25 mg and pioglitazone-45 mg) was exposed to 105oC for 30 min in oven (for thermal degradation) or subjected to direct sun light for up to 24 hr (for photo degradation). After the specified time, the tablet powder was cooled and dissolved in 30 mL of mobile phase in a 100 mL volumetric flask. The solution thus prepared was diluted to volume with the mobile phase. The degraded sample solutions were appropriately diluted with mobile phase to obtain a concentration of 12.50 µg/mL (alogliptin) and 22.50 µg/mL (pioglitazone). The solutions were filtered through 0.45 µm pore size membrane filter. A volume of 10 µL was injected into the HPLC system and the chromatograms were recorded.

Results and Discussions

The present investigation reported in the thesis was aimed to develop a new method development and validation for the simultaneous estimation of Pioglitazone and Alogliptinby RP-UPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Pioglitazone and Alogliptinby RP-UPLC method.Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Pioglitazone and Alogliptinin pharmaceutical dosage form.

4.1.1. Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Pioglitazone and Alogliptinwas obtained and the isobestic point of Pioglitazoneand Alogliptinshowed absorbance's maxima at 280nm. The spectrums are shown in Fig. 17

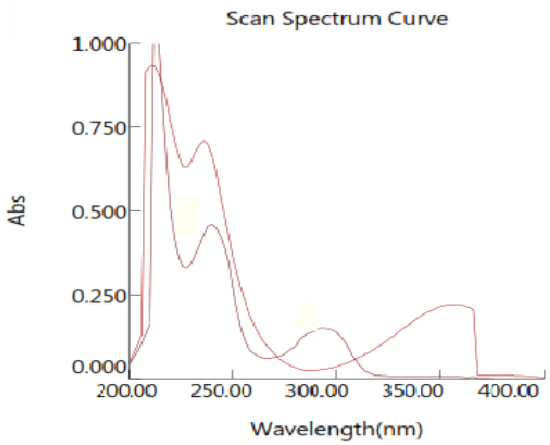


Fig.No.17. Spectrum showing overlapping spectrum of MET and ALO

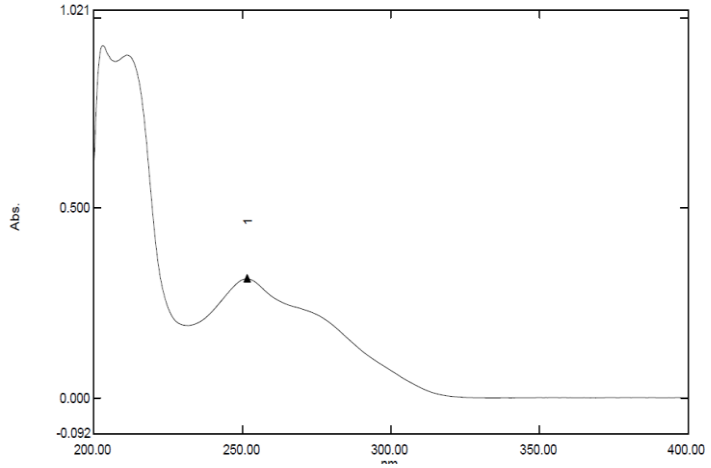


Fig.No.18. Spectrum showing wavelength of Pioglitazone

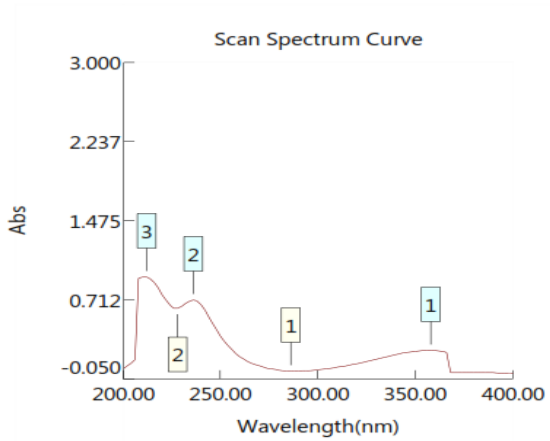


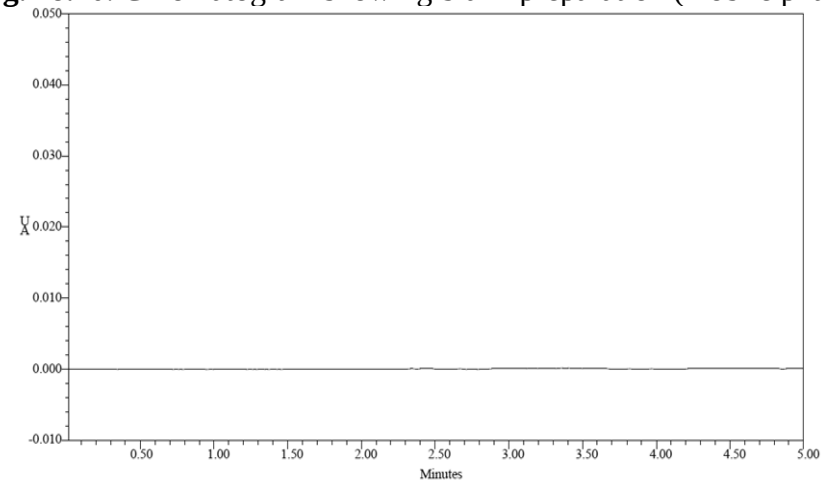
Fig.No.19. Spectrum showing wavelength of Alogliptin

The chromatographic method development for the simultaneous estimation of Pioglitazone and Alogliptinwere optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of Pioglitazoneand Alogliptin in API and pharmaceutical dosage form by RP-UPLC method.

Optimized chromatographic conditions for simultaneous estimations of Pioglitazone and Alogliptinby RP-UPLC method

Column: Water BEH X Bridge RP C_{18}2.1×50 mm 1.7 µm
Column temperature: Ambient
Wavelength: 280nm
Mobile phase ratio: 60:20:20 methanol: ACN: phosphate buffer pH 7
Flow rate: 0.3 ml/min
Auto sampler temperature: Ambient
Injection volume: 4 µl
Run time: 3.0 minutes

Fig.No.20. Chromatogram showing blank preparation (mobile phase)



Assay calculation for Pioglitazone and Alogliptin

The assay study was performed for the Pioglitazone and Alogliptin. Each three injections of sample and standard were injected into chromatographic system. The chromatograms are shown in Fig. No.21-23 and results are tabulated in Table.NO.5.

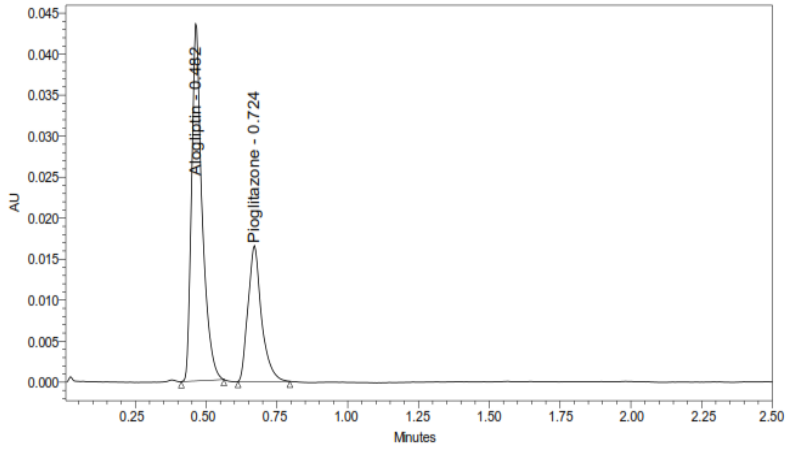
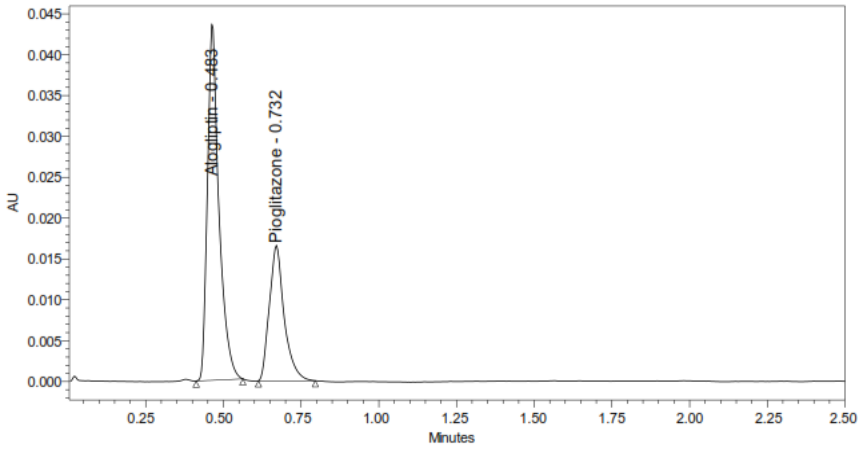


Fig.No.21. Chromatogram showing assay of sample injection-1, 2, 3



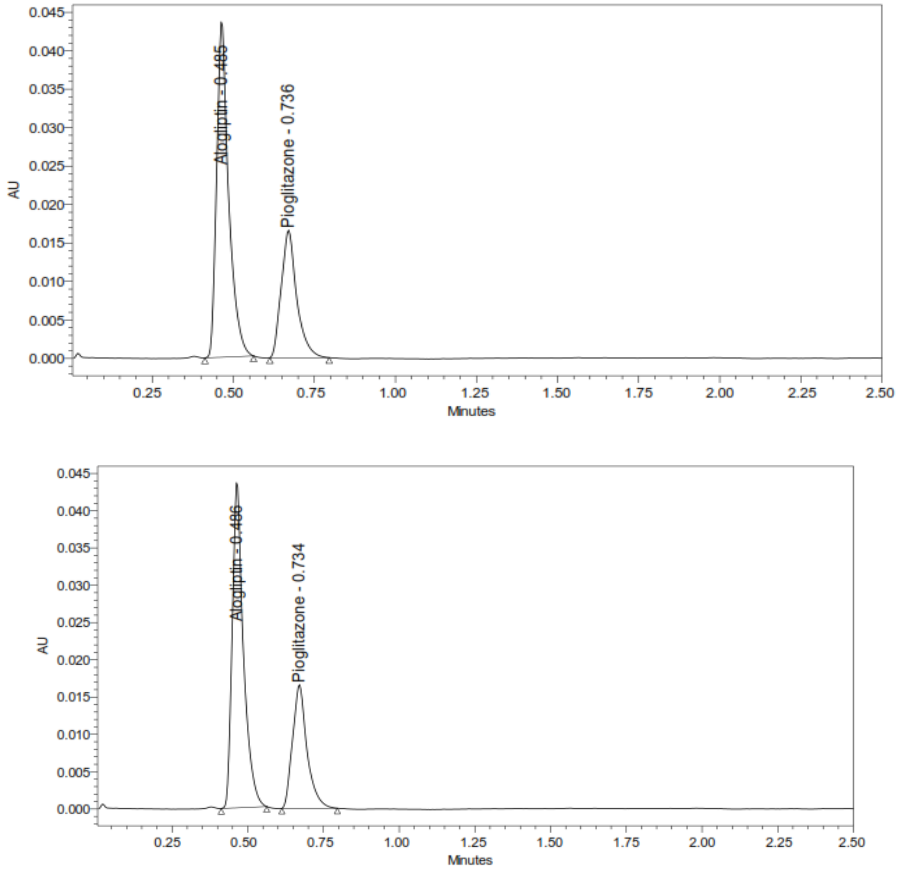


Fig.No.22. Chromatogram showing standard of sample injection -1,2,3.

Assay Results: (Alogliptin)

$$\frac{449143.7}{447408.3} * \frac{40}{100} * \frac{1.5}{10} * \frac{100}{95.2} * \frac{10}{1.5} * \frac{476}{200} * \frac{99.8}{100} * 100 = 100.19\%$$

Assay Results: (For Pioglitazone)

$$\frac{219119}{217707} * \frac{25}{100} * \frac{1.5}{10} * \frac{100}{95.2} * \frac{10}{1.5} * \frac{476}{125} * \frac{99.8}{100} * 100 = 100.45\%$$

Sample and Standard Details

S. No.	Samples	% Assay
1	Alogliptin & Pioglitazone Tablets 12.5 mg & 30 mg	100.19 % for Alogliptin
2	Alogliptin & Pioglitazone	100.45% for Pioglitazone

VALIDATION REPORT

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The chromatograms are shown in Fig.No.23-25.

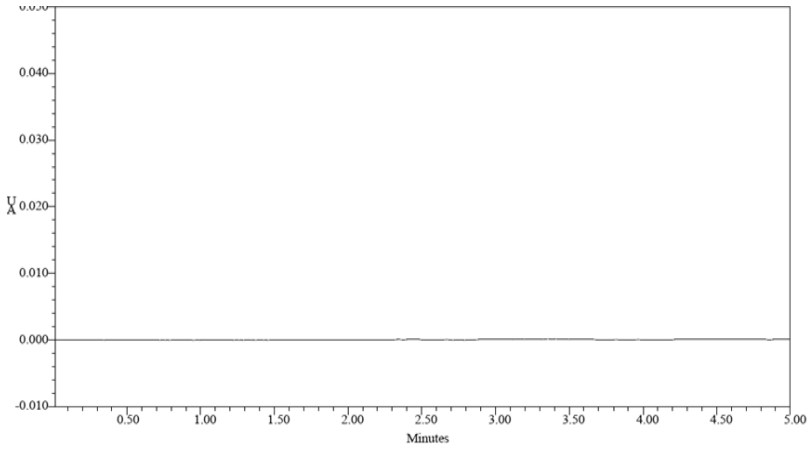


Fig.No.23. Chromatogram showing blank (mobile phase preparation)

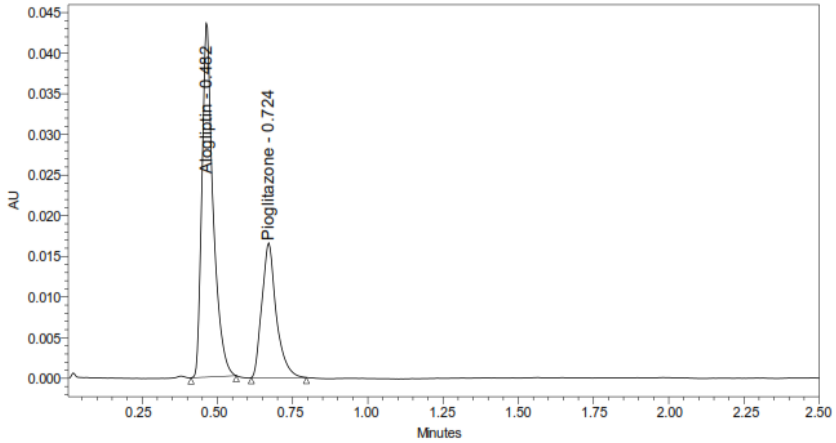


Fig.No.24. Chromatogram showing standard injection

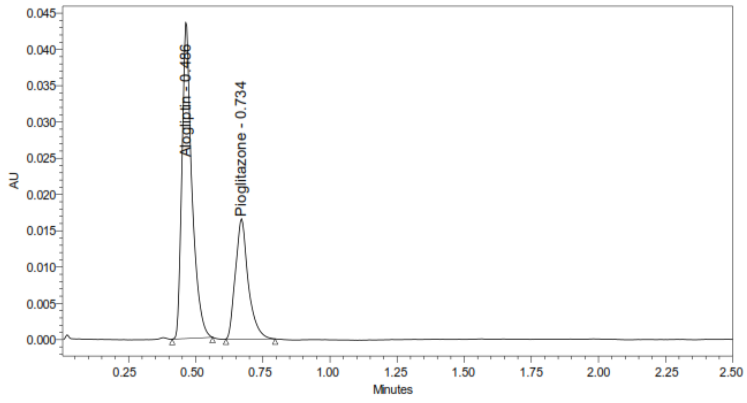


Fig.No.25. Chromatogram showing sample injection

The specificity test was performed for Pioglitazone and Alogliptin. It was found that there was no interference of impurities in retention time of analytical peak.

Linearity

The linearity study was performed for the concentration of 30ppmto150 ppm of Pioglitazone12.5 ppm to 62.5ppm ofAlogliptin level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The results are tabulated in Table. No6-7.Calibration graph for PIO andALOare shown in Fig.No.26.

Tabel No.7. Linearity Results for Alogliptin:

S. No	Linearity Level	Concentration	Area
1	I	12.5	148475
2	II	25	286753
3	III	37.5	445725
4	IV	50	596836
5	V	62.5	745622
Correlation Coefficient			0.999

Tabel No.8. Linearity Results for Pioglitazone:

S. No	Linearity Level	Concentration	Area
1	I	30	71914
2	II	60	140828
3	III	90	215732
4	IV	120	286753
5	V	150	357562
Correlation Coefficient			0.999

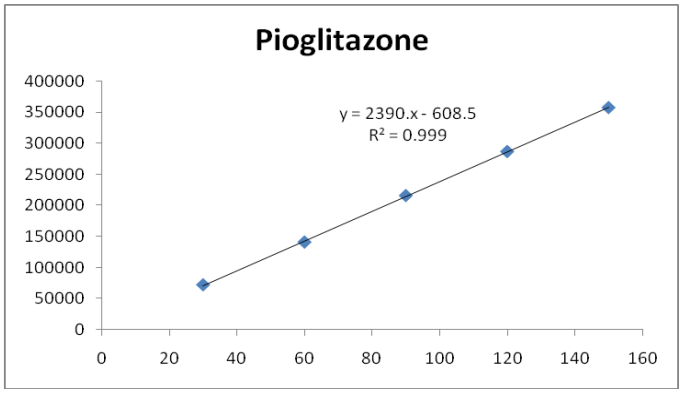


Fig.No.28. Showing calibration graph for Pioglitazone

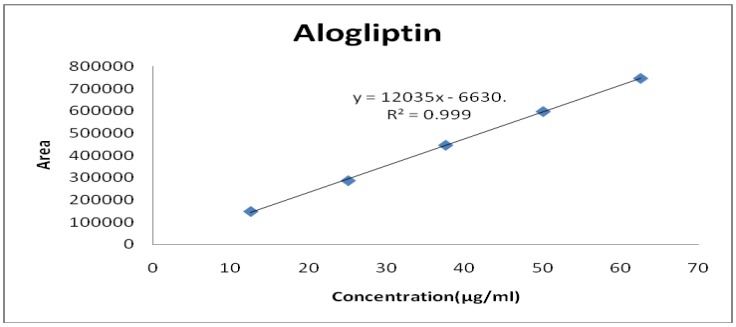
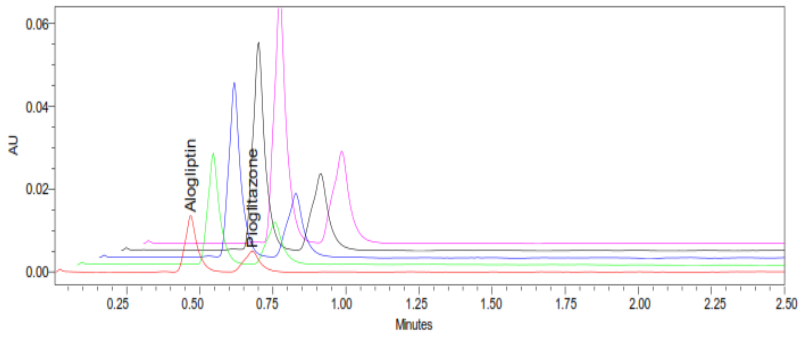


Fig.No.28. Showing calibration graph for Alogliptin

OVERLAY DIAGRAM:



The linearity study was performed for concentration range of 30µg/ml-150µg/ml of Pioglitazone and 12.5µg/ml-62.5 µg/ml of Alogliptinand the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999)respectively.

Accuracy

The accuracy study was performed for 50%, 100% and 150 % for Pioglitazone and Alogliptin. Each level was injected in triplicate into chromatographic system. The area of each l evel was used for calculation of % recovery. Chromatograms are shown in Fig.No.29-31 results are tabulated in Table.No.8-9

Table.No.8. The accuracy results for Alogliptin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	225703.3	12.5	12.59	100.69	100.39
100%	448469.7	25	25.01	100.04	
150%	675482.7	37.5	37.67	100.45	

Table.No.9. The accuracy results for Pioglitazone

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	109553.3	30	30.13	100.44	100.39
100%	219228.7	60	60.30	100.50	
150%	327988.3	90	90.21	100.24	

Precision

- ❖ Repeatability
- ❖ Intermediate Precision

Repeatability

The standard solution was injected for six times and measured the area for all five injections in UPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision/Ruggedness

The standard solution was injected for six times and measured the area for all fiveinjections in UPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Repeatability

The precision study was performed for five injections of Pioglitazone and Alogliptin. Each standard injection was injected into chromatographic system.

The area of each Standard injection was used for calculation of % RSD.

The chromatograms are shown in Fig.No.34-38and results are tabulated in Table.No.10-11.

Table.No.10. Showing% RSD results for Pioglitazone& Alogliptin

Injection	Area for Alogliptin	Area for Pioglitazone
Injection-1	448662	218753
Injection-2	446873	214829
Injection-3	446352	216426
Injection-4	447562	218452
Injection-5	447529	216468
Injection-6	446244	217567

Average	447203.7	217082.5
Standard Deviation	907.4	1468.9
%RSD	**0.2**	**0.7**

The precision study was performed for the %RSD of Pioglitazone and Alogliptin was found to be 0.4 and 0.2(NMT 2).

Intermediate precision/Ruggedness

The intermediate precision study was performed for six injections of Pioglitazone and Alogliptin. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD. The chromatograms are shown in Fig.No.39-43 and results are tabulated in Table.12-13.

The results are summarized for Alogliptin and Pioglitazone

Injection	Area for Alogliptin	Area for Pioglitazone
Injection-1	448776	218573
Injection-2	445735	218562
Injection-3	447673	214652
Injection-4	448673	215354
Injection-5	445876	216454
Injection-6	448676	216457
Average	447568.2	216675.3
Standard Deviation	1424.2	1618.5
%RSD	0.3	0.7

The intermediate precision was performed for %RSD of Pioglitazone and Alogliptinwas found to be 0.7 and 0.3 respectively (NMT 2).

DETECTION LIMIT

LIMIT OF DETECTION: (forAlogliptin)

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 56 µV
Signal Obtained from LOD solution: 172 µV
S/N = 172/56 = 3.07

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

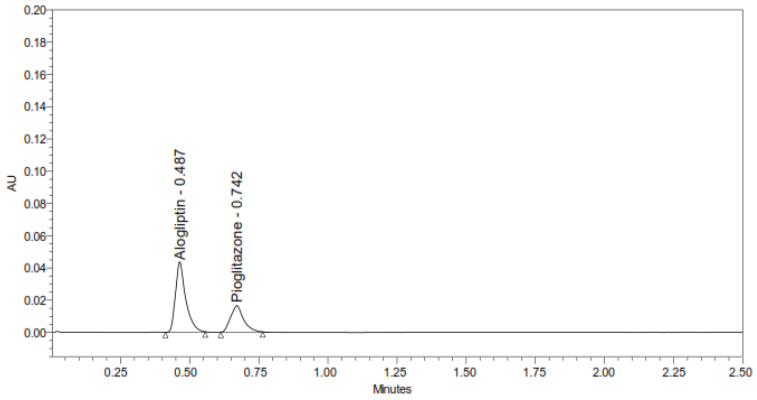
LIMIT OF DETECTION: (forPioglitazone)

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 56 µV
Signal Obtained from LOD solution: 165µV
S/N = 165/56 = 2.95

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.



LOD Chromatogram

LIMIT OF QUANTIFICATION:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 56 µV
Signal Obtained from LOQ solution: 5651µV
S/N = 565/56 = 10.09

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.

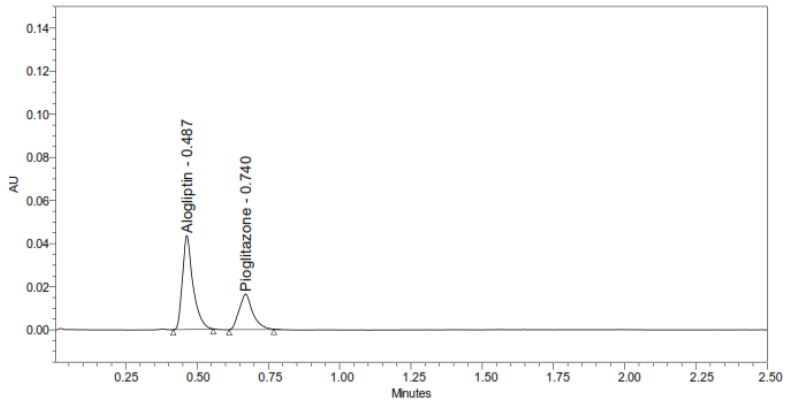
LIMIT OF DETECTION: (for Pioglitazone)

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 56 µV
Signal Obtained from LOQ solution: 556µV
S/N = 556/56 = 9.93

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.



LOQ Chromatograms

Procedure for LOD and LOQ:

The LOD and LOQ solutions was prepared injected, for three times and measured the area for all three injections in UPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Robustness

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Pioglitazone and Alogliptin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.The chromatograms are shown in Fig.No.44-47and results are tabulated in Table.No.16-19.

The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.03ml/min. The method is robust only in less flow condition.

Table.No.16 System suitability results for Alogliptin:

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Tailing	USP Plate Count
1	0.27	1.46	4626.92
2	0.3	1.46	4725.92
3	0.33	1.46	4865.39

System suitability results for Pioglitazone:

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	0.27	3.31	1.29	6132.29
2	0.3	3.18	1.29	6256.39
3	0.33	3.02	1.29	6352.29

***** Results for actual flow (0.3 ml/m

On evaluation of the above results, it can be concluded that the variation in±5%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%.

Table.No.18. System suitability results for Alogliptin:

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	1.46	4762.23
2	*Actual	1.46	4725.92
3	10% more	1.46	4767.76

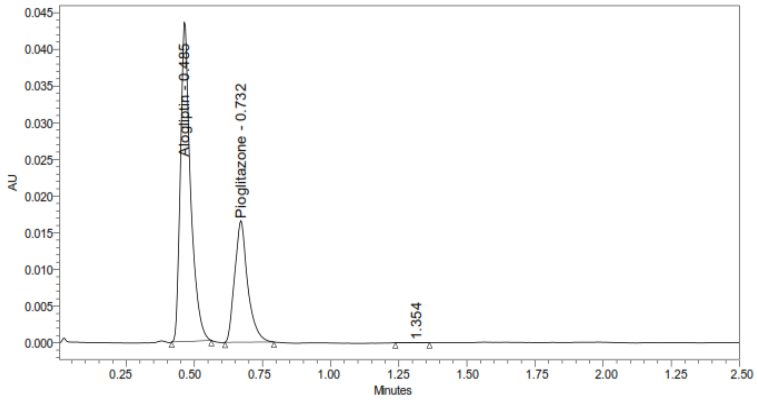
System suitability results for Pioglitazone:

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	10% less	3.37	1.29	6214.27
2	*Actual	3.18	1.29	6256.39
3	10% more	2.96	1.29	6232.23

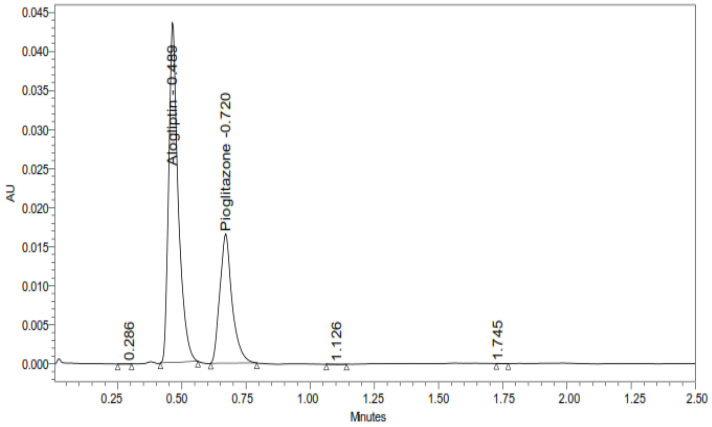
Results for actual Mobile phase composition (60:20:20 methanol: ACN: phosphate buffer pH 7)

STABILITY STUDIES:

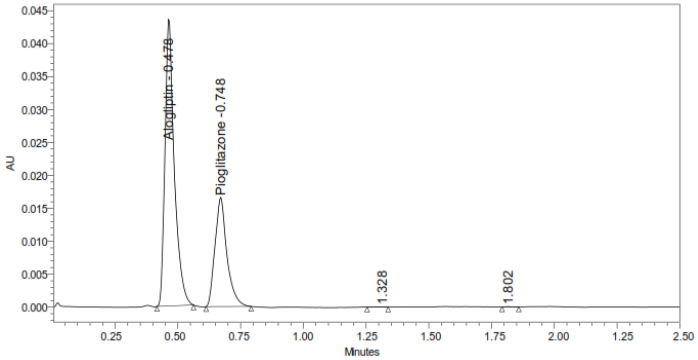
Acid degradation:



Base Degradation:



Thermal Degradation:



Peroxide Degradation:-

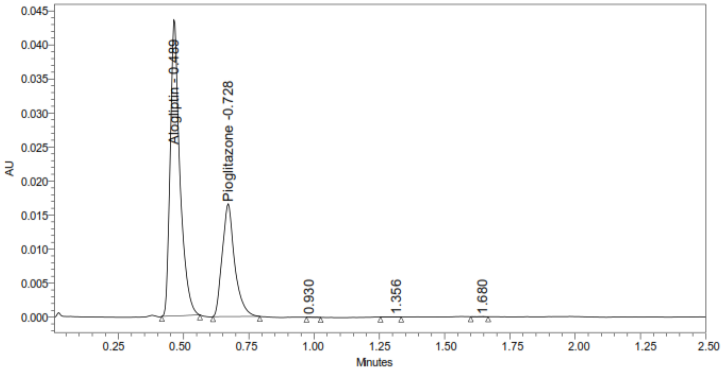


Photo degradation:

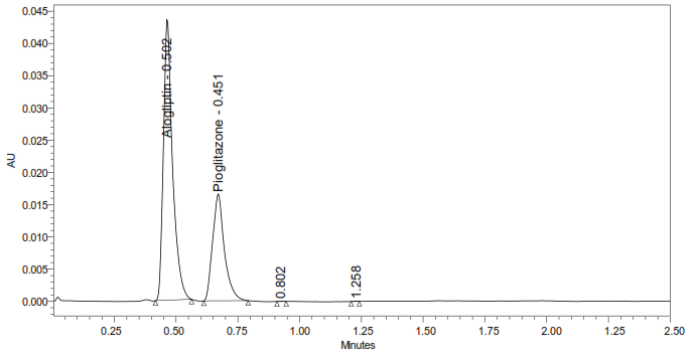


Table 16: Results of forced degradation

	Alogliptin		Pioglitazone	
Sample Name	**Area**	**% Degraded**	**Area**	**% Degraded**
Standard	447408.3		217707	
Acid	436522	2.43	207853	4.53
Base	428673	4.19	196762	9.62
Peroxide	439657	1.73	206752	5.03
Thermal	430876	3.70	199672	8.28
Photo	421862	5.71	195534	10.18

DISCUSSION:

The developed method for the separation of Pioglitazone and Alogliptin was found to be less time ,less economic than the exstisting methods .the previous methods are developed in tri ethyl amine and present method was developed in phosphate buffer ,separation between two peaks are very good than the exstiting method. It is easy to prepare the buffers than the exstiting method. The validation also showing all parameters are within the limits.

Summary and Conclusion

A new method was established for simultaneous estimation of Pioglitazone and Aloglipt in by RP-UPLC method. The chromatographic conditions were successfully developed for the separation of Pioglitazone and Alogliptin by using Waters BEH C18 column (4.6×50mm)3.7µm, flow rate was 0.3 ml/min, mobile phase ratio was 60:20:20 methanol: ACN: phosphate buffer pH 7, detection wave length was280nm. The instrument used was WATERS UPLC Auto Sampler, Acquity module, photo diode array detector 2996, Empower-software version-2. The retention times were found to be 0.482 mins and 0.735mins. The % purity of Pioglitazone and Aloglipt in was found to be 104.4% and 103.39% respectively. The system suitability parameters for Pioglitazone and Alogliptin such as theoretical plates and tailing factor were found to be 993, 1.23and 5775, 1.12, the resolution was found to be 10.18. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Pioglitazone and Alogliptin was found in concentration range of 50µg-250µg and 5µg-25µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.1and 1.4, % RSD for intermediate precision analyst 1 was 0.5 and 0.6 and intermediate precision analyst 2 was 0.8 and 0.3respectively. The precision study was precision, robustness and repeatability. LOD value was 0.39 and 0.7 and LOQ value was1.18and 2.12 respectively.

Hence the suggested RP-UPLC method can be used for routine analysis of Pioglitazone and Alogliptinin API and Pharmaceutical dosage form.

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