World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

A NEW SEPARATION TECHNIQUE FOR METHOD DEVELOPMENT AND VALIDATION OF ALOGLIPTIN AND PIOGLITAZONE IN ITS PURE AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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Article Received on 20/09/2019 **Article Revised on 10/10/2019** **Article Accepted on 30/10/2019**

ABSTRACT

A short selective, precise, accurate and sensitive for the estimation of Alogliptin and Pioglitazone was done by RP-HPLC. The estimation of Alogliptin and Pioglitazone was done by RP-HPLC. The assay of Alogliptin and Pioglitazone was performed with tablets and the % assay was found to be 99.93 and 99.95 which shows that the method is useful for routine analysis. The linearity of Alogliptin and Pioglitazone was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.8 for Alogliptin and Pioglitazone which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.6 for Alogliptin and Pioglitazone which shows that the method is repeatable when performed in different days also.

KEYWORDS: Alogliptin and Pioglitazone, Validation, stability indicating method, degradationproducts.

INTRODUCTION

Alogliptin,2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl}methyl) benzonitrile is a selective, orally-bioavailable inhibitor of enzymatic activity of dipeptidyl peptidase-4 (DPP-4). Chemically, alogliptin is prepared as a benzoate salt and exists predominantly as the R-enantiomer (>99%). It undergoes little or no chiral conversion in vivo to the (S)-enantiomer. FDA approved January 25, 2013. Alogliptin inhibits dipeptidyl peptidase 4 (DPP-4), which normally degrades the incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon like peptide 1 (GLP-1). The inhibition of DPP-4 increases the amount of active plasma incretins which helps with glycemic control. GIP and GLP-1 stimulate glucose dependent secretion of insulin in pancreatic beta cells. GLP-1 has the additional effects of suppressing glucose dependent glucagon secretion, inducing satiety, reducing food intake, and reducing gastric emptying.

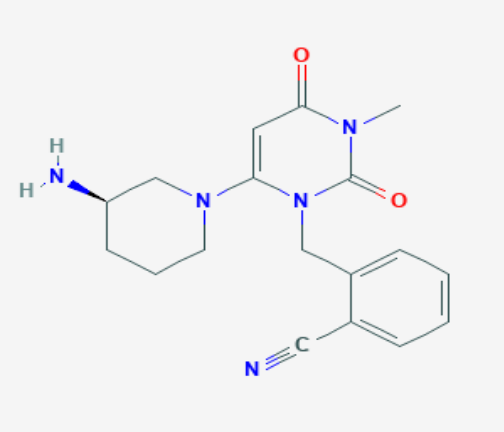

Fig. 1: Chemical Structure of Alogliptin, 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl}methyl)benzonitrile

Pioglitazone, 5-({4-[2-(5-ethylpyridin-2-yl)ethoxy] phenyl} methyl)-1,3-thiazolidine-2,4-dione is a medication belonging to the thiazolidinedione class of drugs that are used as adjuncts to diet, exercise, and other diabetes medications to manage type 2 diabetes mellitus. The thiazolidinedione class of medications exerts its pharmacological effect primarily by promoting insulin sensitivity and the improved uptake of blood glucose. Following entry into fat cell nuclei, pioglitazone selectively binds to the Peroxisome Proliferator-

Activated Receptor Gamma (PPARγ). PPARs are ligand-activated transcription factors that are involved in the expression of more than 100 genes, and affect numerous metabolic processes, notably lipid and glucose homeostasis. PPARγ in particular is abundantly expressed in lipid cells (adipocytes), where it plays a central role in lipid production and regulation of lipid metabolism.

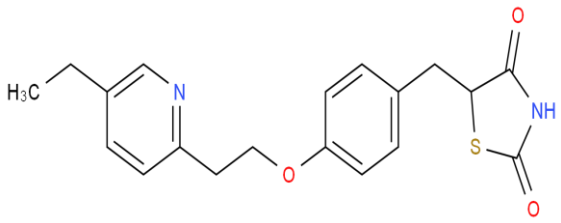

Fig.2 Chemical Structure of Pioglitazone, 5-({4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl} methyl)-1,3-thiazolidine -2, 4-dione

Experimental

Optimized chromatographic conditions

Instrument used	:	Waters HPLC with auto sampler and UV detector.
Temperature	:	Ambient
Column	:	Inertsil ODS (150 x 4.6, 5µm)
Buffer	:	phosphate buffer
pH	:	3.0
Mobile phase	:	70% buffer 30% Acetonitrile
Flow rate	:	1 ml per min
Wavelength	:	248 nm
Injection volume	:	20 µl
Run time	:	15 min.

Preparation of Buffer and Mobile Phase

Preparation of pH3 phosphate buffer

Accurately weigh and transfer 3.5gms of potassium di hydrogen orthopjosphate dissolved in 1000 ml of HPLC water Ph was adjusted up to 3.0. Final solution was filtered through 0.44 µm Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase

Accurately measured 700 ml (70%) of above buffer and 300 ml (30%) of Acetonitrile HPLC were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Preparation Of The Alogliptin& Pioglitazone Standard & Sample Solution

Standard Solution Preparation

Accurately weigh and transfer 25 mg of Alogliptin and 60 mg of Pioglitazone working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 25 mg of Alogliptin and 60 mg Pioglitazone sample into a 100 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate it up to 15 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is Filtered through 0.45 micron Injection filter. (Stock solution).

Further pipette 1.5ml of Alogliptin and Pioglitazone from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject 20 µl of the standard, sample into the chromatographic system and measure the areas for Alogliptin and Pioglitazone peaks and calculate the %Assay by using the formulae.

RESULTS AND DISCUSSIONS

Validation

The analytical method was validated with respect to parameters such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

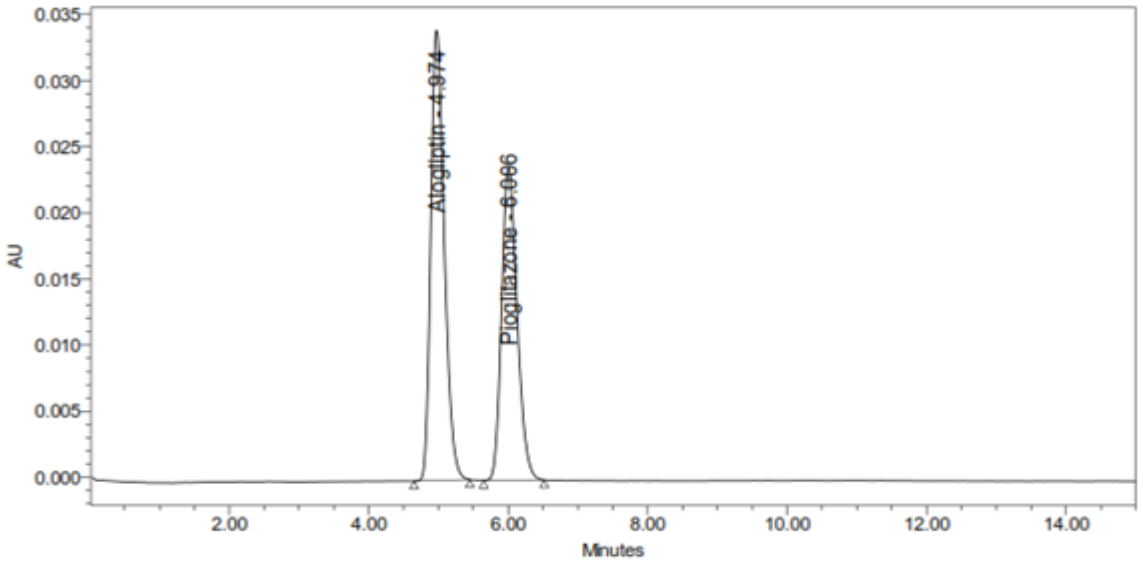

Fig. 3: Standard Chromatogram of Alogliptin and Pioglitazone.

Table 1: Results of system suitability parameters.

S.No.	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Alogliptin	4.974	463731	34151		1.29	3122.36
2	Pioglitazone	6.006	373273	23654	2.69	1.22	3422.48

Table 2: Results of Linearity of Alogliptin and Pioglitazone.

S. No.	Alogliptin		Pioglitazone	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	12.5	163126	30	123687
2	25	324879	60	258151
3	37.5	484999	90	374272
4	50	622089	120	500737
5	62.5	774838	150	622363

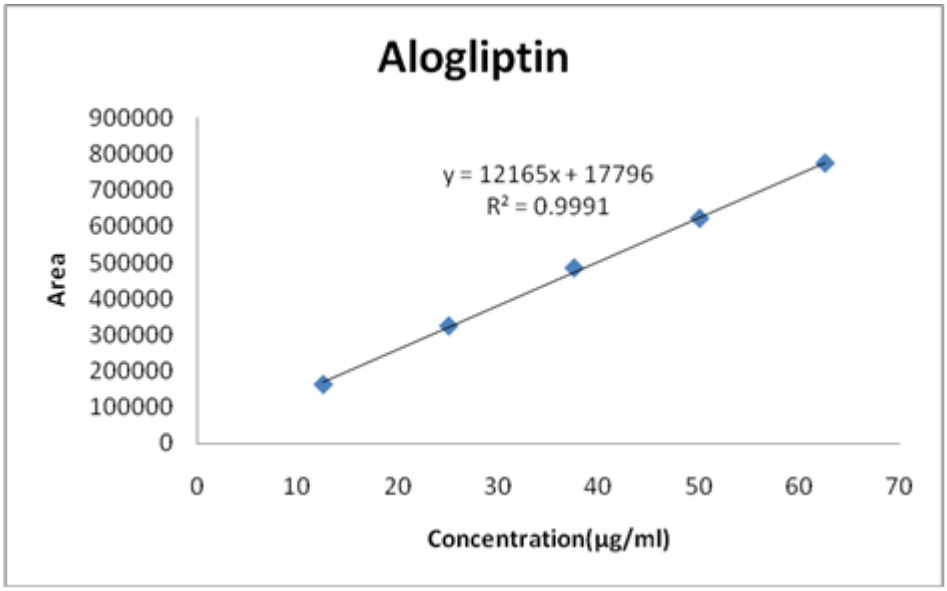

Figure 4: Calibration graph for Alogliptin.

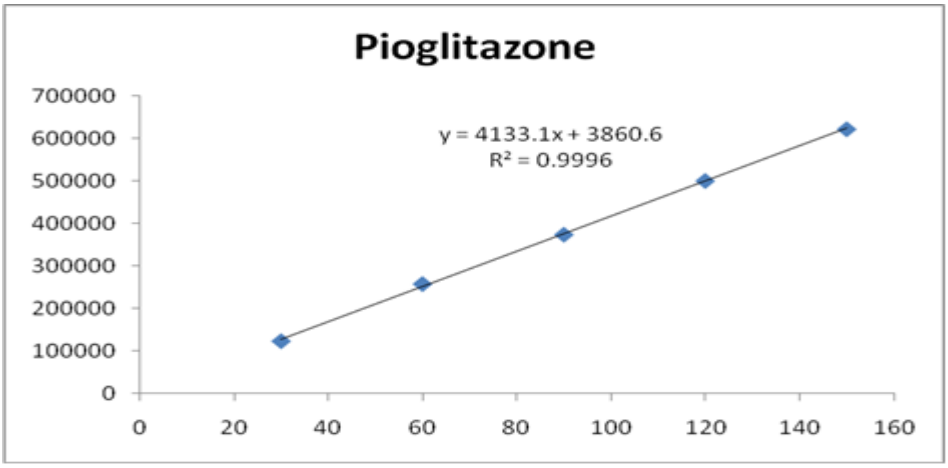

Figure 5: Calibration graph for Pioglitazone.

Table 3. Results of Precision for Alogliptin.

Injection	Area
Injection-1	469199
Injection-2	466480
Injection-3	463505
Injection-4	465113
Injection-5	463129
Injection-6	460972
Average	464733.0
Standard Deviation	2876.4
%RSD	0.6

Table 4: Results of Precision for Pioglitazone.

Injection	Area
Injection-1	378542
Injection-2	370422
Injection-3	377395
Injection-4	375692
Injection-5	375700
Injection-6	372893
Average	375107.3
Standard Deviation	2985.9
%RSD	0.8

Table 5: Accuracy (recovery) data for Alogliptin.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	233775.3	12.5	12.53	100.28	99.60
100%	462242.7	25	24.78	99.14	
150%	695121.3	37.5	37.27	99.39	

Table 6: Accuracy (recovery) data for Pioglitazone.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	188250.7	30	30.06	100.19	100.15
100%	374491	60	59.79	99.66	
150%	567073.3	90	90.54	100.60	

Table 7: Results of LOD.

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Alogliptin	43	132	3.07
Pioglitazone	43	127	2.95

Table 8: Results of LOQ.

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Alogliptin	43	434	10.09
Pioglitazone	43	427	9.93

Degradationstudies

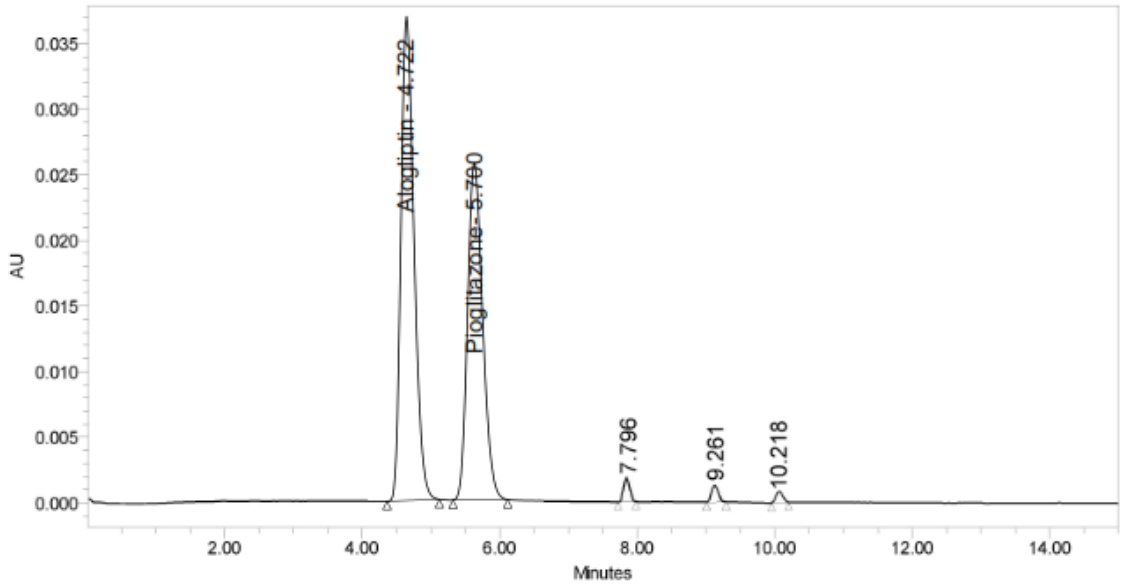

Figure 6: Chromatogram showing Acid degradation.

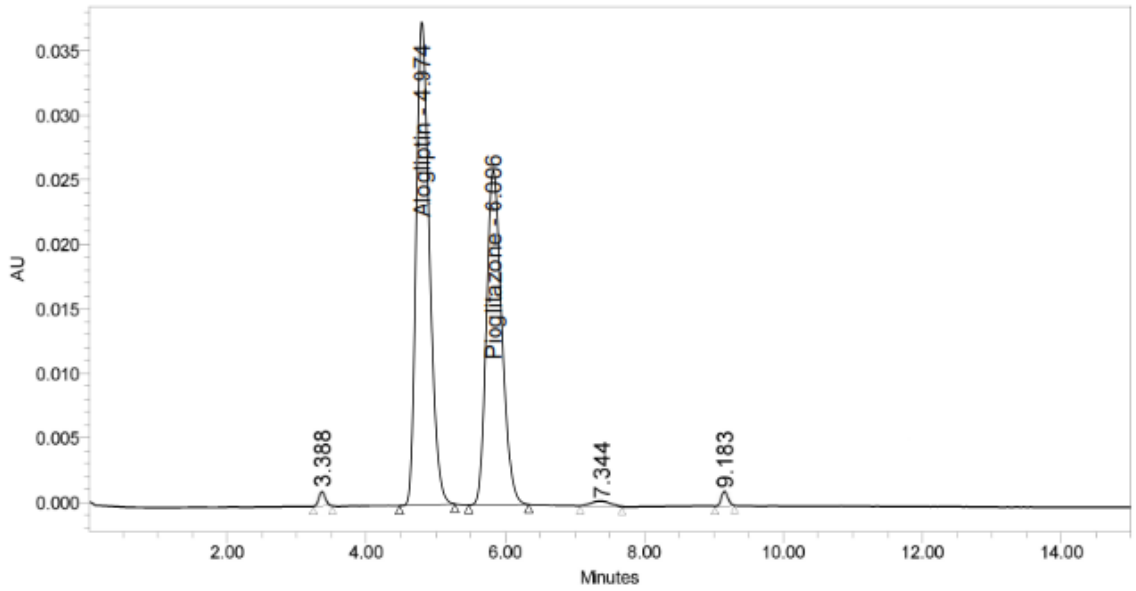

Figure 7: Chromatogram showing Base degradation.

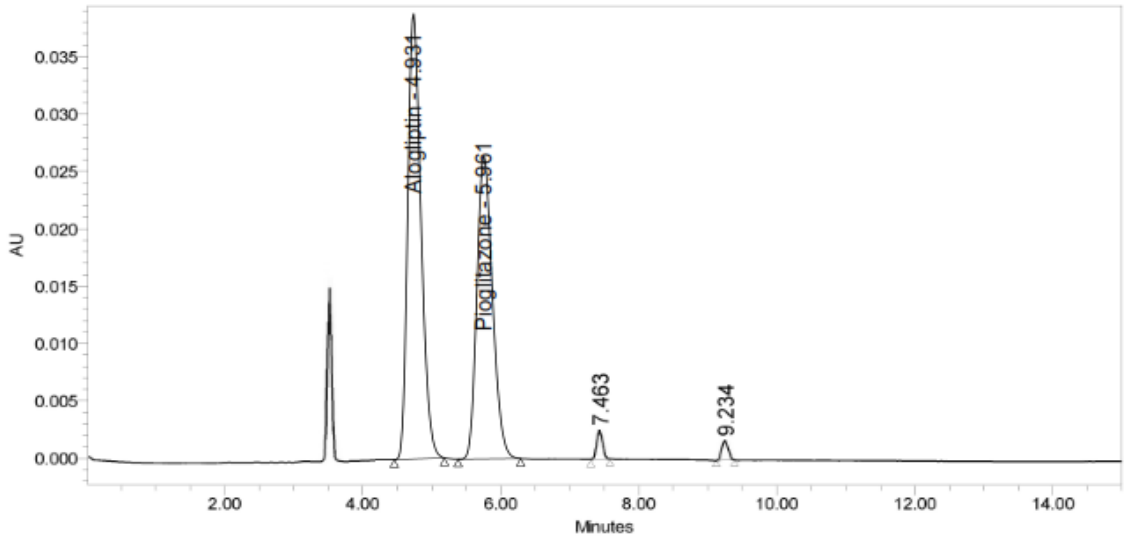

Figure 8: Chromatogram showing Peroxide degradation.

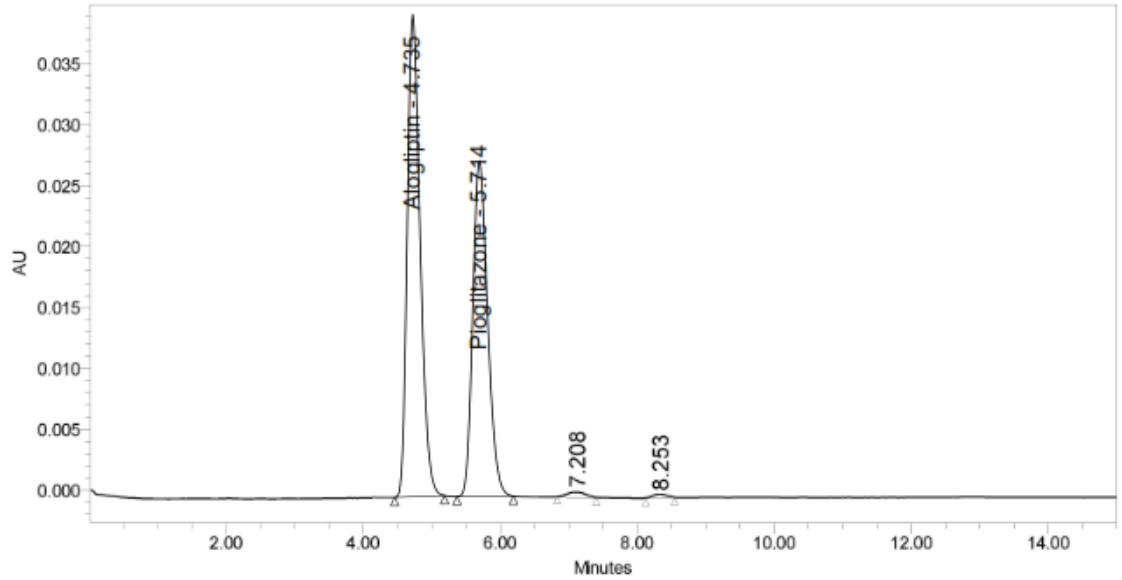

Figure 9: Chromatogram showing Thermal degradation.

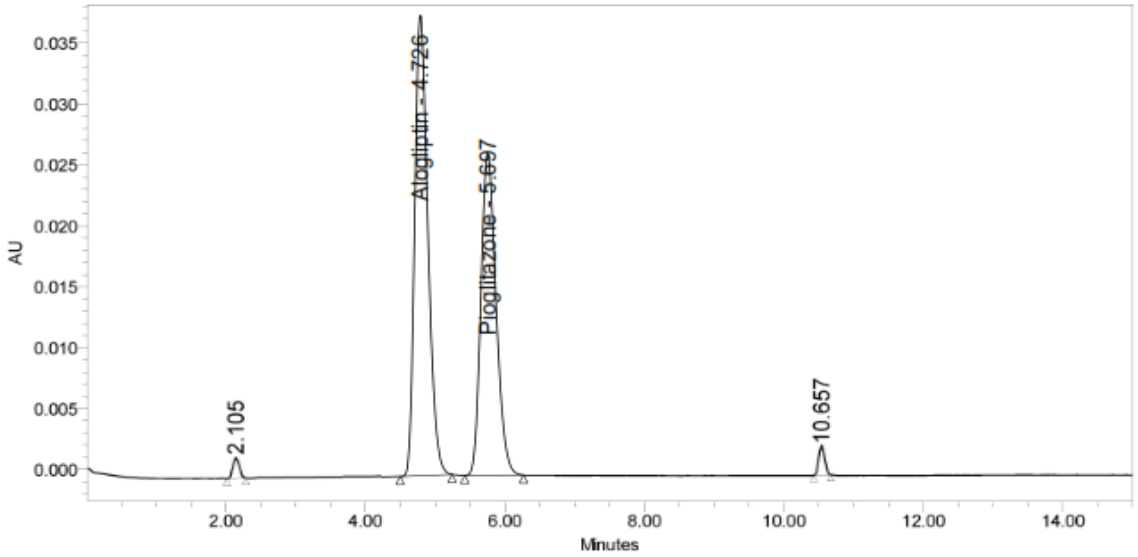

Figure 10: Chromatogram showing Photo degradation.

Table 9: Results for Stability of Alogliptin andPioglitazone.

Sample Name	Alogliptin		Pioglitazone	
	Area	% Degraded	Area	% Degraded
Standard	465326.7		375025.0	
Acid	446578	4.03	359788	4.06
Base	453567	2.53	362545	3.33
Peroxide	439786	5.49	343876	8.31
Thermal	448788	3.55	349675	6.76
Photo	437675	5.94	351989	6.14

Table 10: Results of Assay for Alogliptin and Pioglitazone.

	Label Claim (mg)	% Assay
Alogliptin	12.5	99.93
Pioglitazone	30	99.95

SUMMARY AND CONCLUSION

The estimation of Alogliptin and Pioglitazone was done by RP-HPLC.

The assay of Alogliptin and Pioglitazone was performed with tablets and the % assay was found to be 99.93 and 99.95 which shows that the method is useful for routine analysis.

The linearity of Alogliptin and Pioglitazone was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity.

The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.8 for Alogliptin and Pioglitazone which shows that the method is precise.

The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.6 for Alogliptin and Pioglitazone which shows that the method is repeatable when performed in different days also.

The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 99.60% and 100.15% for Alogliptin and Pioglitazone. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

The acceptance criteria for LOD and LOQ are 3 and 10. The LOD and LOQ for Alogliptin was found to be 3.07 and 10.09 and LOD and LOQ for Pioglitazone was found to be 2.95 and 9.93.

The robustness limit for mobile phase variation and flow rate variation are well within the limit, the % degradation results are in limits. Which shows that the method is having good system suitability and precision under given set of conditions.

BIBLIOGRAPHY

1. Gurudeep Chatwal and Sham Anand, Instrumental methods of chemical analysis. Himalaya publishers, 7th edition, 1992; 2: 624-2.639.
2. Skoog et al., Principles of Instrumental Analysis. Barkhanath publishers, 8th edition, 973-995.
3. Hobart.H.Willard et al., Instrumental methods of analysis, CBS Publications and Distributors, New Delhi, 1st edition, 1986; 529-563.
4. Sethi P.D., Quantitative analysis of Drugs & Pharmaceuticals. CBS publishers and distributors, New Delhi, 3rd edition, 2001; 1-120.
5. Janeyulu.Y & Marayyah, Quality Assurance & Quality Management in Pharmaceutical Industry. Pharma book publishers, Hyd, edition, 2005; 78-108.
6. Vogel's Text book of quantitative chemical analysis. Published by Dorling Kindersley pvt.ltd. 6th edition, 289-304.
7. Lloyd R. Snyder et al., practical HPLC method development. John Wiley & sons publishers, 2nd edition, 350-400.
8. Knevel A.M. &.Digengl F.E, Jenkins Quantitative Pharmaceutical Chemistry, Mc Graw Hill Book Co.
9. Daniel W.Armstrong, Bonded Phase material for Chromatographic separations, 1985, U.S.Patent, 4539399.
10. Sastry, C.S.P., Singh, N.R., Reddy, Methods of Analysis, edition, 1986; 316.