



**DEVELOPMENT AND VALIDATION OF STABILITY INDICATING  
RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF  
ALOGLIPTIN AND PIOGLITAZONE AND THEIR DOSAGE FORMS  
IN BIORELEVANT DISSOLUTION MEDIA**

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**ABSTRACT**

This study was aimed to develop Alogliptin and Pioglitazone marketed formulation and to develop stability indicating HPLC method for their simultaneous estimation of Alogliptin and Pioglitazone in pure forms and in its final dosage forms according to the ICH guidelines. Isocratic mode HPLC method was performed; the flow rate was 1.0 ml/min, injected volume 10 $\mu$ L, the mobile phases consist of 0.1% OPA: Methanol in the ratio of 30:70 and UV detection was carried out at 280nm. Alogliptin and Pioglitazone and their combined dosage form were exposed to thermal, oxidative, acid base hydrolytic stress conditions, the stressed samples were analyzed. The method was

validated with respect to linearity, precision, accuracy, system suitability, and robustness. The used method is specific for the estimation of Alogliptin and Pioglitazone in presence of their degradation products and impurities. The method was linear over the range of 12.5–62.5 $\mu$ g/mL and 30–150 $\mu$ g/mL for Alogliptin and Pioglitazone respectively. The mean recoveries for the accuracy studies were found to be within the limits for Alogliptin and Pioglitazone respectively. The percentage of relative standard deviation (%RSD) was found to be less than critical value. Our developed analytical method is a stability indicating, economical and easy method which is useful in the quality control of Alogliptin and Pioglitazone in pharmaceutical tablet dosage forms.

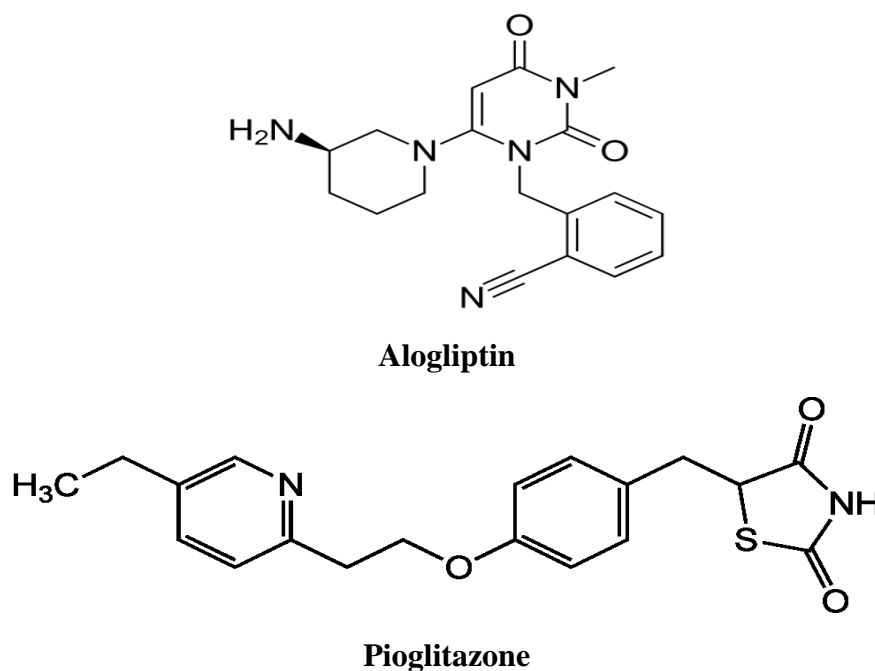
**KEY WORDS:** Alogliptin and Pioglitazone, ICH Guidelines, Method Development, Validation.

## INTRODUCTION

Alogliptin 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<sup>[1, 2]</sup> 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<sup>[3]</sup> secretion, inducing satiety, reducing food intake, and reducing gastric emptying. Alogliptin does not undergo extensive metabolism. Two minor metabolites that were detected are N-demethylated Alogliptin (<1% of parent compound) and N-acetylated Alogliptin (<6% of parent compound). The N-demethylated metabolite<sup>[4]</sup> is active and an inhibitor of DPP-4. The N-acetylated metabolite is inactive. Cytochrome enzymes that are involved with the metabolism of Alogliptin are CYP2D6 and CYP3A4 but the extent to which this occurs is minimal. Approximately 10-20% of the dose is hepatically metabolized by cytochrome enzymes.

Pioglitazone 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<sup>[5, 6]</sup> 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 (PPAR $\gamma$ ).<sup>[4]</sup> 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.<sup>[5]</sup> PPAR $\gamma$  in particular is abundantly<sup>[7]</sup> expressed in lipid cells (adipocytes), where it plays a central role in lipid production and regulation of lipid metabolism. Pioglitazone acts as a selective agonist at Peroxisome Proliferator Activated Receptor Gamma (PPAR $\gamma$ ) in target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPAR-gamma receptors increases the transcription<sup>[8, 9]</sup> of insulin-responsive genes involved in the control of glucose production, transport, and

utilization. In this way, Pioglitazone both enhances tissue sensitivity to insulin and reduces the production of glucose<sup>[10]</sup> via the liver (hepatic gluconeogenesis). Thus, insulin resistance associated with type 2 diabetes mellitus is improved without an increase in insulin secretion by pancreatic  $\beta$  cells.



**Fig-1: Chemical Structures of Alogliptin and Pioglitazone.**

Alogliptin and Pioglitazone in combination used for the treatment of type 2 diabetes. Literature survey have few analytical methods are available for the simultaneous estimation of Alogliptin and Pioglitazone (Fig.1) in pharmaceutical formulations by using UV and HPLC. Hence, we made an attempt to develop a simple method for the simultaneous estimation of Alogliptin and Pioglitazone by RP-HPLC in pharmaceutical dosage forms. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines & pharmacopeias.<sup>[11-13]</sup>

## MATERIALS AND METHODS

Alogliptin and Pioglitazone standard is obtained as a generous gift sample from Syncorp Clinecare Technologies Pvt. Ltd., Hyderabad, India. Alogliptin and Pioglitazone tablets labeled to contain Alogliptin and Pioglitazone (12.5mg & 30mg) manufactured by Takeda Pharmaceuticals North America, Inc, were purchased from local market. All the chemicals used were of HPLC grade, obtained from S D Fine-Chem Limited, Mumbai, India. All HPLC solvents and solutions were filtered through Nylon membrane filter of 0.45 $\mu$  pore size.

### **HPLC Instrumentation & Conditions**

The HPLC system was used are WATERS, Software: Empower2, 2695 separation module, UV detector. UV/VIS spectrophotometer LABINDIA UV 3000+, pH meter (Adwa – AD 1020), Weighing machine (Afcoset ER-200A), Pipettes and Burettes, Beakers (Borosil).

### **PREPARATION OF THE ALOGLIPTIN & PIOGLITAZONE STANDARD & SAMPLE SOLUTIONS**

#### **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 sonicated 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 and transfer equivalent to 25 mg of Alogliptin and 60 mg of Pioglitazone sample into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicated 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.

#### **Mobile Phase Preparation**

Accurately measured 300 ml (30%) of 0.1% OPA Buffer and 700 ml (60%) of Methanol were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45  $\mu$  filter under vacuum filtration.

#### **Diluent Preparation**

##### **Preparation of blank Fasted State Simulated Intestinal Fluid (FaSSIF)**

Accurately weighed 1.74g of Sodium hydroxide pellets, 19.77g of Sodium dihydrogen orthophosphate, and 30.93g of Sodium chloride dissolve in 5 L of purified water and adjust the pH 6.5 exactly by using 1N Hydrochloric acid.

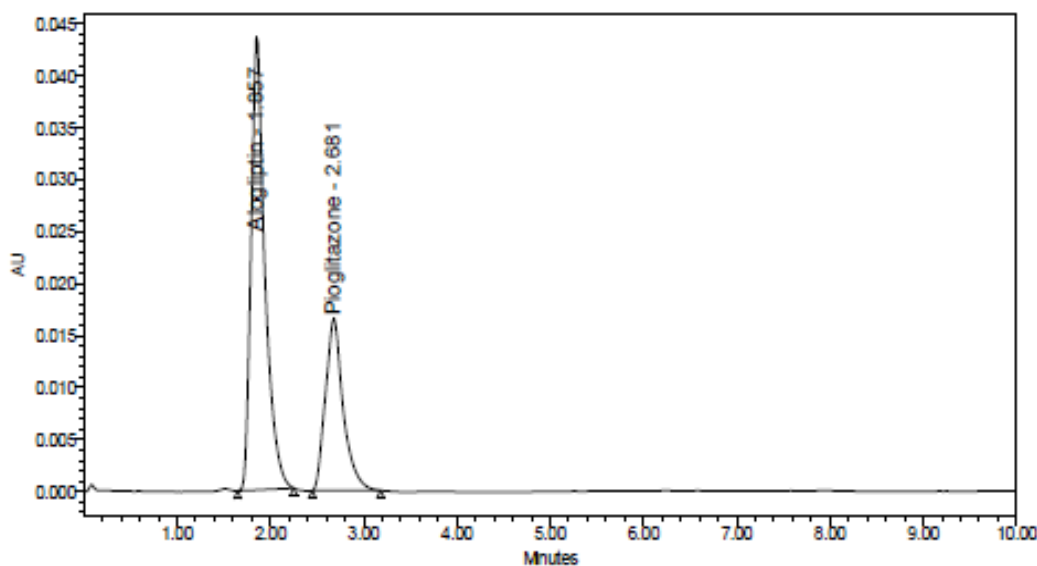
### Preparation of FaSSIF

Accurately weighed 3.3g of sodium taurocholate dissolve in 500 mL blank FaSSIF solution, add 11.8 mL of a solution to 100mg/mL lecithin in methylene chloride, and forming an emulsion. The methylene chloride was eliminated under vacuum at 40°C. Then draw a vacuum for 15 minutes at 250mbar and also followed by 15 minutes at 100mbar. These results gave in a clear, micellar solution, having no perceptible odor for methylene chloride. After that, it was cool to room temperature and adjusts the volume upto 2L with blank FaSSIF.

### METHOD DEVELOPMENT

#### *Optimized Chromatographic Conditions*

Instrument used	:	Waters HPLC with auto sampler and UV detector.
Temperature	:	Ambient (25° C)
Mode of separation	:	Isocratic mode
Column	:	Agilent Eclipse Column (4.6 x 150mm, 5µm)
Mobile phase	:	0.1% OPA: Methanol (30: 70)
Flow rate	:	1 ml per min
Wavelength	:	280 nm
Injection volume	:	10 µl
Run time	:	10 min.



**Fig-2: Optimized Chromatographic Condition.**

## METHOD VALIDATION

### System Suitability Studies

System-suitability<sup>[14]</sup> tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Tailing factor for the peaks due to Alogliptin and Pioglitazone in Standard solution should not be more than 2. Theoretical plates for the Alogliptin and Pioglitazone peaks in Standard solution should not be less than 2000. Resolution for the Alogliptin and Pioglitazone peaks in standard solution should not be less than 2.

### Precision

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

### Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision<sup>[15]</sup> was performed on different day. The standard solutions prepared in the precision were injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

### Specificity

For Specificity Blank and Standard are injected into system. There is no any interference of any peak in blank with the retention time of the analytical peaks.

### Accuracy

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Alogliptin & Pioglitazone and calculate the individual recovery<sup>[15]</sup> and mean recovery values.

### Linearity

Inject each level into the chromatographic system and measure the peak area. Plot a graph<sup>[16]</sup> of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

**Robustness**

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

**Degradation Studies**

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies<sup>[17, 18]</sup> on the Alogliptin and Pioglitazone using the proposed method.

**Hydrolytic degradation under acidic condition**

Pipette 1.5 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60°C for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

**Hydrolytic degradation under alkaline condition**

Pipette 1.5 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60°C for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

**Thermal induced degradation**

Alogliptin and Pioglitazone sample was taken in Petri dish and kept in Hot air oven at 110<sup>0</sup> C for 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

**Oxidative degradation**

Pipette 1.5 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

**Photo degradation**

Pipette 1.5 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

**RESULTS AND DISCUSSION****System Suitability Studies (For Alogliptin)**

1. Tailing factor Obtained from the standard injection is 1.46
2. Theoretical Plates Obtained from the standard injection is 4725.92

**System Suitability Studies (For Pioglitazone)**

1. Tailing factor Obtained from the standard injection is 1.29
2. Theoretical Plates Obtained from the standard injection is 6256.39
3. Resolution Obtained from the standard injection is 3.18

**Precision****Table-1: The results are summarized for Alogliptin and Pioglitazone.**

<b>Injection</b>	<b>Area for Alogliptin</b>	<b>Area for Pioglitazone</b>
Injection-1	448662	218836
Injection-2	446873	218753
Injection-3	446352	214829
Injection-4	447562	216426
Injection-5	447529	218452
Injection-6	446244	216468
<b>Average</b>	447203.7	217567
<b>Standard Deviation</b>	907.4	217082.5
<b>%RSD</b>	0.2	1468.9

**Acceptance Criteria:** The % RSD for the area of six standard injections results should not be more than 2%.

**Intermediate Precision/Ruggedness****Table-2: The results are summarized for Alogliptin and Pioglitazone.**

<b>Injection</b>	<b>Area for Alogliptin</b>	<b>Area for Pioglitazone</b>
Injection-1	448776	218573
Injection-2	445735	218562
Injection-3	447673	214652
Injection-4	448673	215354
Injection-5	445876	216454
Injection-6	448676	216457



<b>Average</b>	447568.2	216675.3
<b>Standard Deviation</b>	1424.2	1618.5
<b>%RSD</b>	0.3	0.7

**Acceptance Criteria:** The % RSD for the area of six standard injections results should not be more than 2%.

### Specificity

For Specificity Blank and Standard are injected into system. There is no any interference of any peak in blank with the retention time of the analytical peaks.

### Accuracy

**Table-3: 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	

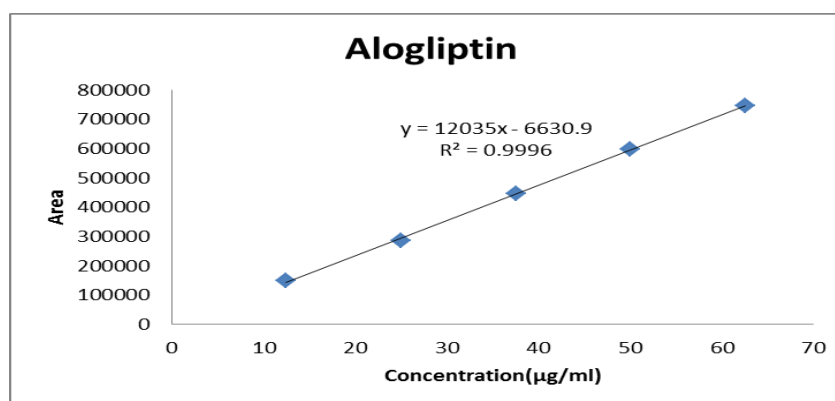
**Table-4: The accuracy results for Pioglitazone.**

%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	

### Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102.0%.

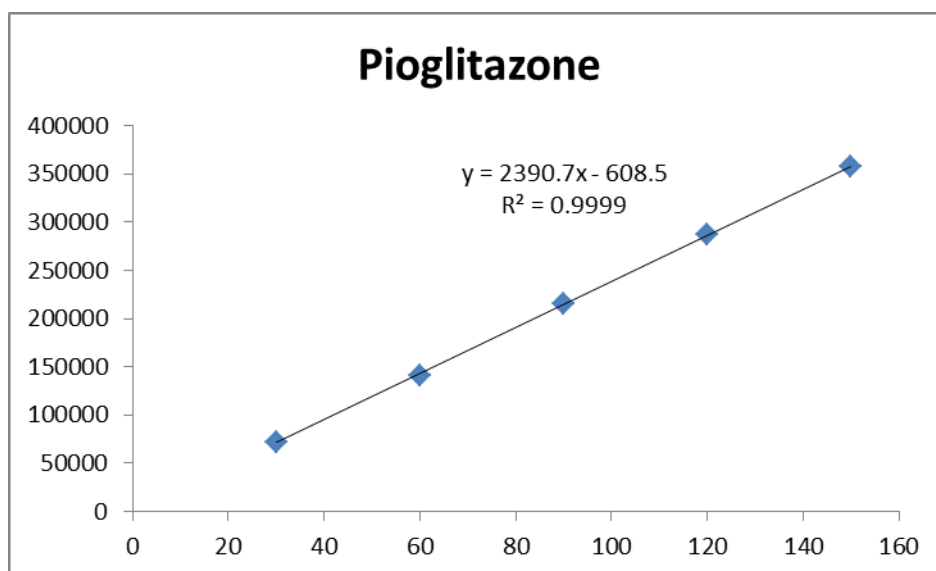
### Linearity



**Fig-3: Linearity graph for Alogliptin.**

**Table-5: 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

**Fig-4: Linearity graph for Pioglitazone.****Table-6: 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

**Acceptance Criteria:** Correlation coefficient should be not less than 0.999.

#### Detection Limit: (For Alogliptin)

##### Calculation of S/N Ratio

Average Baseline Noise obtained from Blank : 56  $\mu$ V

Signal Obtained from LOD solution : 172  $\mu$ V

$$S/N = 172/56 = 3.07$$

**Acceptance Criteria**

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

**Quantification Limit: (For Alogliptin)****Calculation of S/N Ratio**

Average Baseline Noise obtained from Blank: 56  $\mu$ V

Signal Obtained from LOQ solution: 5651 $\mu$ V

$$S/N = 565/56 = 10.09$$

**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  $\mu$ V

Signal Obtained from LOD solution: 165 $\mu$ V

$$S/N = 165/56 = 2.95$$

**Acceptance Criteria**

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

**Limit of Quantification: (For Pioglitazone)****Calculation of S/N Ratio**

Average Baseline Noise obtained from Blank: 56  $\mu$ V

Signal Obtained from LOQ solution: 556 $\mu$ V

$$S/N = 556/56 = 9.93$$

**Acceptance Criteria**

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

**Robustness****A. The flow rate was varied at 0.9 ml/min to 1.1ml/min.**

Standard solution 37.5 ppm of Alogliptin & 90 ppm of Pioglitazone was prepared and analysed using the varied flow rates along with method flow rate.

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  $\pm 10\%$ .

**Table-7: System suitability results for Alogliptin.**

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Tailing	USP Plate Count
1	0.9	1.46	4626.92
2	1.0	1.46	4725.92
3	1.1	1.46	4865.39

**Table-8: System suitability results for Pioglitazone.**

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	0.9	3.31	1.29	6132.29
2	1.0	3.18	1.29	6256.39
3	1.1	3.02	1.29	6352.29

\* Results for actual flow (1ml/min) have been considered from Assay standard.

#### **B. The Organic composition in the Mobile phase was varied from $\pm 10\%$ .**

Standard solution 37.5 ppm of Alogliptin & 90 ppm of Pioglitazone was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

On evaluation of the above results, it can be concluded that the variation in 10%.

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  $\pm 10\%$ .

**Table-9: 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

**Table-10: 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 (30:70) Buffer: Methanol has been considered from Accuracy stand.

### Degradation Studies

**Table-11: Degradation results for Alogliptin and Pioglitazone.**

Sample Name	Alogliptin		Pioglitazone	
	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

### CONCLUSION

The results of the analysis of pharmaceutical dosage form indicated that the developed RP-HPLC method is highly accurate, precise and robust and are in good agreement with the labeled claim of the drug.

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Alogliptin and Pioglitazone API.

Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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