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METHOD DEVELOPMENT AND VALIDATION OF ALOGLIPTIN AND PIOGLITAZONE BY RP-HPLC METHOD IN BULK AND ITS MARKETED DOSAGE FORM

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ABSTRACT

A simple, rapid, specific and accurate Reverse Phase High Performance Liquid Chromatographic Method have been developed for the validation of Alogliptin & Pioglitazone in bulk as well as in the marketed pharmaceutical dosage form. This separation was performed on a Develosil ODS C18 (4.6mm×250mm, 5µm) column with Acetonitrile: Methanol: 1% Orthophosphoric acid (50:30:20% v/v) as mobile phase at a flow rate of 1.0 ml/ min with UV detection at 242 nm; the constant column temperature was Ambient. The run time under these chromatographic conditions was within 10 min. The retention time of Alogliptin and Pioglitazone was found to be 2.24 and 5.44. The calibration plot was linear over the concentration range of 30-70µg/ ml for Alogliptin and 60-140µg/ ml for Pioglitazone with limit of detection 0.63 & 1.20 for Alogliptin & Pioglitazone and quantification values 1.70 & 3.40 µg/ ml respectively. The mean % assay of marketed formulation was found to be 100.25% and 100.20%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2.The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Alogliptin and Pioglitazone in bulk and marketed pharmaceutical dosage form dosage form.

KEYWORDS: Alogliptine, Pioglitazone, RP-HPLC, Orthophosphoric acid, Methanol, ICH validation guidelines, Osinitablets, Sonicator, Waters HPLC, PDA detector.

INTRODUCTION

Pharmaceutical analysis comprises of the procedures necessary to determine the identity,

strength, quality and purity of substances of therapeutic importance. It also deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher pressure. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

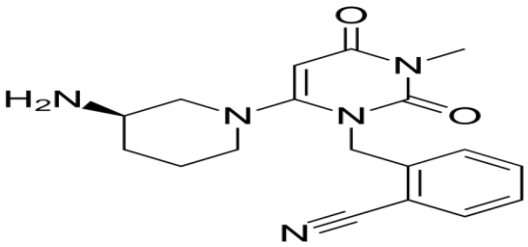
Advantages

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance.

HPLC Instrumentation

- Mobile phase and reservoir
- Solvent degassing system
- Pump
- Injector
- Colum
- Detector
- Data system

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



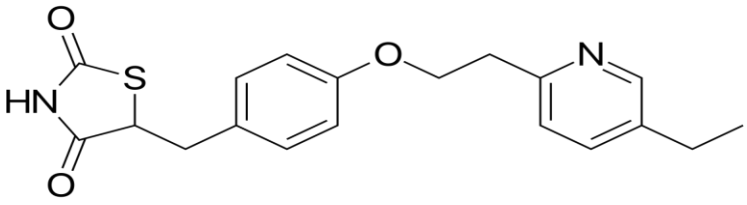
Alogliptin is a selective, orally-bioavailable inhibitor of the enzymatic activity of dipeptidyl peptidase-4 (DPP-4). Chemically, Alogliptin is prepared as a benzoate salt and exists predominantly as the R- enantiomers (>99%). It undergoes little or no chiral conversion in vivo to the (S)-enantiomers. Alogliptin was found to be soluble in dimethyl sulphoxide, sparingly soluble in water and methanol, slightly soluble in ethanol, and very slightly soluble in Octanol and isopropyl acetate.

Mechanism of Action

Alogliptin inhibits dipeptidyl peptidase 4 (DPP-4), which normally degrades the incretins like 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 in glycemic control. GIP and GLP-1 stimulate glucose dependent secretion of insulin in pancreatic beta cells.

Side Effects: Severe pain in upper stomach spreading to your back, Nausea and vomiting, Loss ofappetite, Fast heart rate, Itching, Dark urine, Clay-colored stools, Jaundice (yellowing of the skin or eyes).

Pioglitazone: 5-({4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}methyl)-1,3-thiazolidine-2,4-Dione.



Pioglitazone is an orally-active thiazolidinedione with anti-diabetic properties and potential anti-neoplastic activity. Pioglitazone activates Peroxisome Proliferators-Activated Receptor Gamma (PPAR-gamma), a ligand-activated transcription factor, thereby inducing cell differentiation and inhibiting cell growth and angiogenesis. Pioglitazone was found to be

freely soluble in DMSO, dimethyl formamide, practically insoluble in Water, Ether, slightly soluble in Ethanol, Acetone and Acetonitrile.

Mechanism of Action

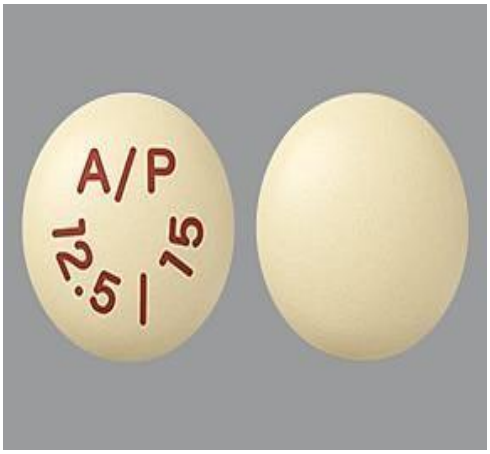
Pioglitazone acts as a selective agonist at Peroxisome Proliferators Activated Receptor Gamma (PPARγ) in target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPAR-gamma receptors increases the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization. In this way, Pioglitazone enhances both the tissue sensitivity to insulin and reduces the production of glucose via the liver (hepatic gluconeogenesis). Thus, insulin resistance associated with type 2 diabetes mellitus is improved without an increase in insulin secretion by pancreatic β cells.

Side Effects

Swelling (Edema), when used in combination with Sulfonylurea or Insulin, Low blood sugar (Hypoglycemia), Upper respiratory infection, Headache, Heart failure, Sinus infection, Fracture of bone & Sore throat (Pharyngitis)

Uses: Treat Type – 2 Diabetes

Marketed Formulation



Literature review showed that the methods were not reported by using Methanol, Acetonitrile & 1% Orthophosphoric acid in suitable ratio for Alogliptin and Pioglitazone. Hence, the current method was focused on the development of accurate, simple, precise analytical method and was validated according to ICH guidelines.

Experiment

Instruments used: RP-HPLC, UV-Visible Spectrophotometer, Ultra Sonicator, pH meter and weighingbalanc**e.**

Chemicals used: Alogliptin and Pioglitazone pure samples (Sura Labs, Hyderabad.), Methanol,Orthophosphoric acid, Acetonitrile, Oseni tablets available from local market.

Method

Preparation of stock solution

10 mg of Alogliptin and Pioglitazone working standard drugs were accurately weighed and transferred into a 10ml clean dry volumetric flasks and 7ml of methanol was added and sonicated to dissolve it completely and made volume up to the mark with the methanol. Further, 0.5mlof Alogliptin and 1ml of Pioglitazone was pipetted out from the above stock solution into a 10ml volumetric flask and diluted up to the mark with Methanol.

Detection of wavelength

The standard solution is scanned for wavelength in UV-Visible Spectrophotometer from 400-200nm and the wavelength is found to be 242nm.

Preparation of 1% Ortho-phosphoric acid

280 ml of 90% **Orthophosphoric acid** (concentrated H_3PO_4) was measured and **diluted** to 1 Lwith distilled/deionized water.

Preparation of Mobile Phase

500ml of Acetonitrile, 300ml of Methanol & 200ml of 1% Orthophosphoric acid were mixed into a 1000ml volumetric flask and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45µ filter under vacuum filtration.

Method Development and Optimization:

The method was developed by varying different conditions and parameters like columns like phenomenox column, Symmetry ODS column, Devilosil ODS column etc., and mobile phases like acetonitrile, methanol, water and phosphate buffer & 1% Orthophosphoric acid etc.,

The optimized method is observed in the following conditions:

Instrument: Waters HPLC 2965 module, auto sampler, with PDA detector,

Mobile phase ratio: Acetonitrile: Methanol: 1% Orthophosphoric acid (50:30:20) V/V

Column: Develosil ODS C18 (4.6×250mm, 5µm)

Column temperature: Ambient

Wavelength: 242nm

Flow rate: 1ml/min

Injection volume: 10µl

Run time: 10min

This method is considered as optimized because the tailing factor is less than 2 and plate count is more than 2000.

Validation

The present method is validated for the following parameters like Accuracy, Precision, Linearity, Range, Robustness, LOD, LOQ and System suitability etc., as per the ICH guidelines.

Validation Parameters

Assay

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Alogliptin and Pioglitazone working standards into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume upto the mark with the same solvent. (Stock solution). Further pipette 0.5ml of Alogliptin and 1ml of Pioglitazone was pipetted out from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample Solution

Weight 10 mg equivalent weight of Alogliptin and Pioglitazone marketed tablet (Oseni tablet powder) sample into a 10mL clean dry volumetric flask and add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity

The solutions were prepared in the concentration range of 30-70ppm of Alogliptin and 60-140ppm of Pioglitazone. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Slope = y = mx + c.

Precision

The Precision was calculated for Intermediate precision and Repeatability. The solutions were prepared and injected 3 times and the % the RSD and standard deviation were calculated.

Accuracy

The different concentrations like 50%, 100% and 150% spiked concentrations were prepared and injected. The chromatograms are recorded and the peak responses are measured. Calculate the Amount found and Amount added for Alogliptin and Pioglitazone and calculate the individual % recovery and mean % recovery values.

Robustness

The analysis was performed in different conditions to find the variability of test results like change in flow rate and mobile phase and observe the change in the retention time, tailing factor and theoretical plate count.

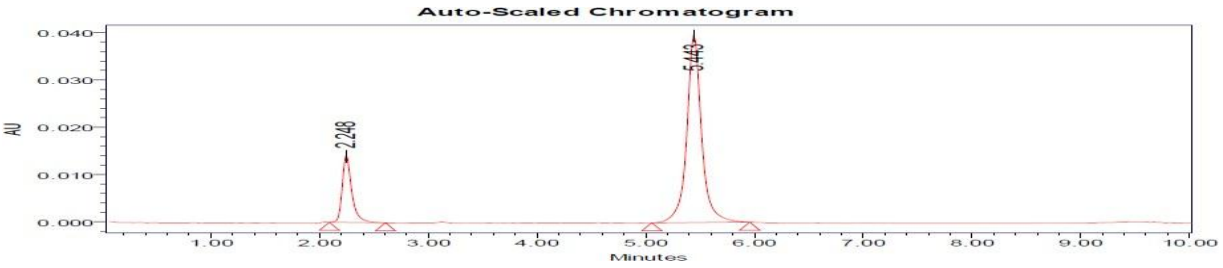
System suitability

5 mg of Alogliptin and 10mg of Pioglitazone working standards were accurately weighed and transferred into a 10ml clean dry volumetric flasks and 7ml of diluent was added and sonicated to dissolve it completely and made the volume up to the mark with the same solvent. (stock solution) Further, 0.5 ml of Alogliptin and 1 ml of Pioglitazone solutions were pipetted out from the above stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent (primary stock solution). The standard solution (primary stock solution)

was injected for five times and the peak areas for all five injections are recorded in HPLC. The %RSD for all the replicates of injections was calculated.

RESULTS

Optimized chromatogram



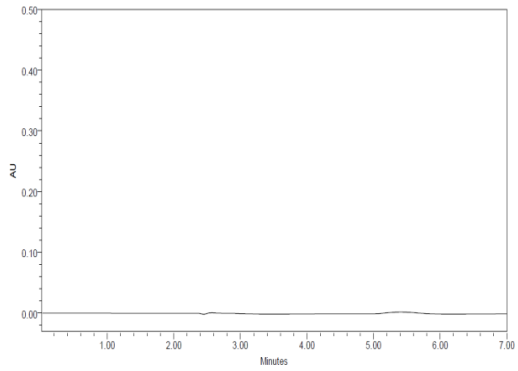
S. No.	Peak name	Rt	Area	Height	USP Resolution	USP Tailing	USP platecount
1	Alogliptin	2.248	98645	15246	---	1.15	7856
2	Pioglitazone	5.443	465824	56248	4.36	1.06	5695

Observation: From the above chromatogram, it was observed that the Alogliptin and Pioglitazone peaks are well separated and they show proper retention time, resolution and plate count. Hence, it can be concluded that, there is no further requirement to either increase or decrease the retention time and theoretical plates or resolution factor. So, this trial is considered as a final method.

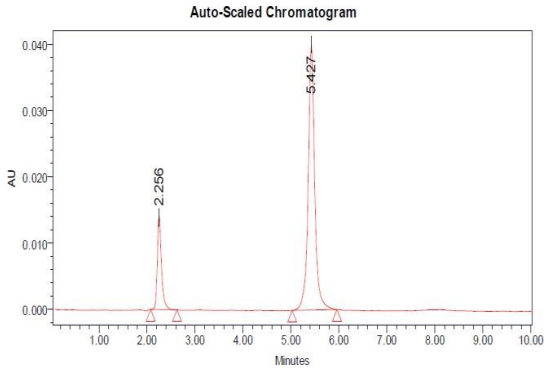
Validation

Assay

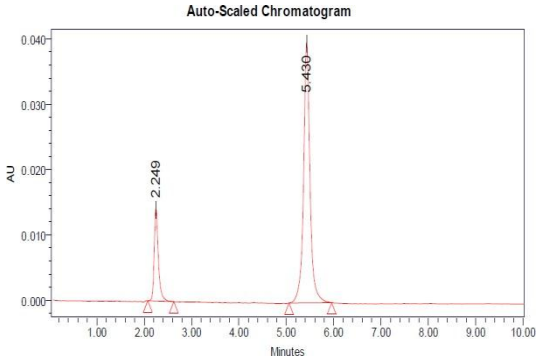
Blank injection



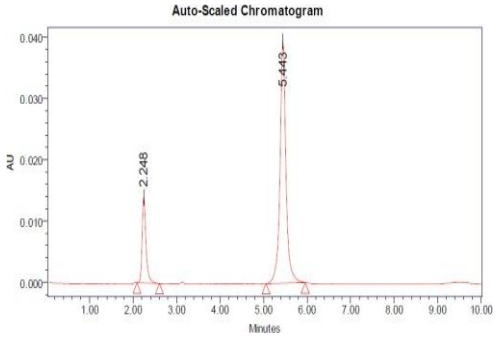
Standard Injection 1



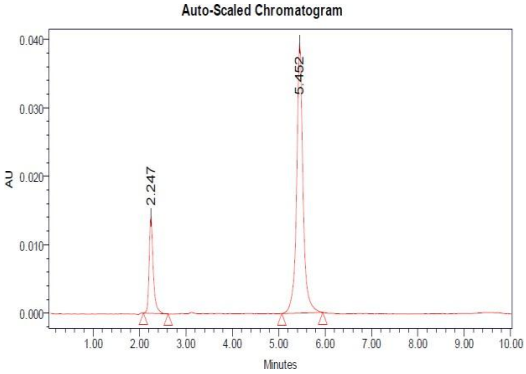
Standard Injection 2



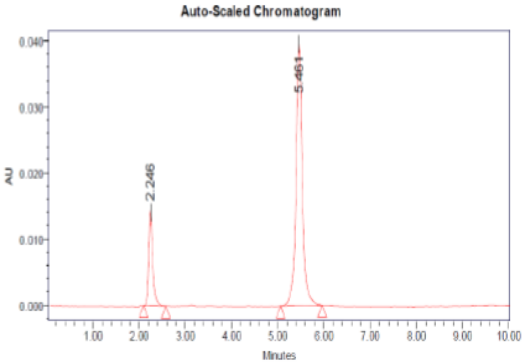
Standard Injection 3



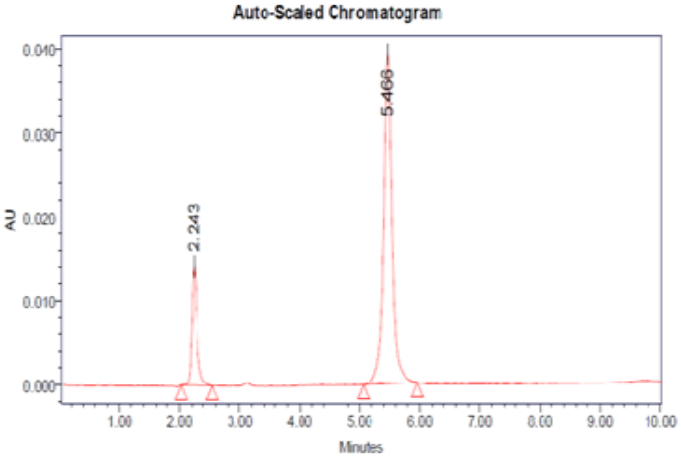
Sample Injection 1.



Sample Injection 2.



Sample Injection 3



Assay result for standard Alogliptin & Pioglitazone

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP platecount	Injection
1	Alogliptin	2.256	98685	15365	----	1.16	7885	1
2	Pioglitazone	5.427	465875	56898	4.27	1.05	5698	1
3	Alogliptin	2.249	98568	15687	---	1.15	7854	2
4	Pioglitazone	5.430	468547	56857	4.13	1.06	5628	2
5	Alogliptin	2.248	98574	15698	----	1.16	7863	3
6	Pioglitazone	5.443	468958	56258	4.19	1.05	5682	3

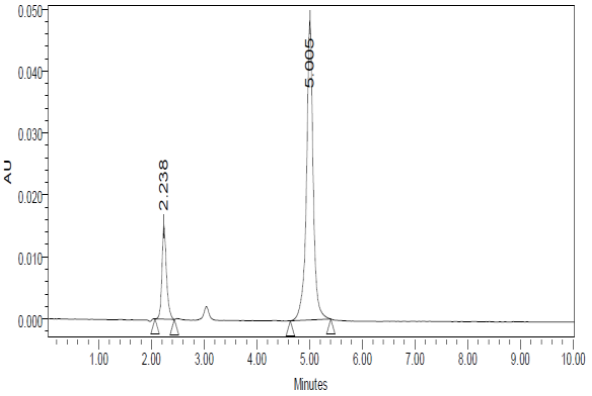
Assay result for sample Alogliptin & Pioglitazone

S.No.	Name	Rt	Area	Height	USP Tailing	USP platecount	Injection
1	Alogliptin	2.247	99689	16528	1.16	7985	1
2	Pioglitazone	5.452	478598	57847	1.06	5789	1
3	Alogliptin	2.246	99854	16352	1.15	7928	2
4	Pioglitazone	5.461	476895	57898	1.05	5784	2
5	Alogliptin	2.243	99865	16587	1.16	7982	3
6	Pioglitazone	5.466	478512	57854	1.06	5763	3

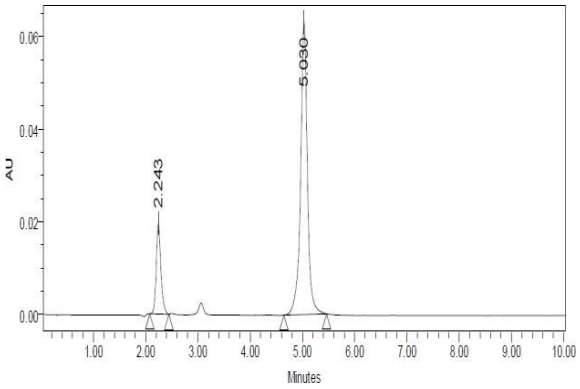
The % purity of Alogliptin and Pioglitazone in pharmaceutical dosage form was found to be 99.76% &100.45%.

Linearity

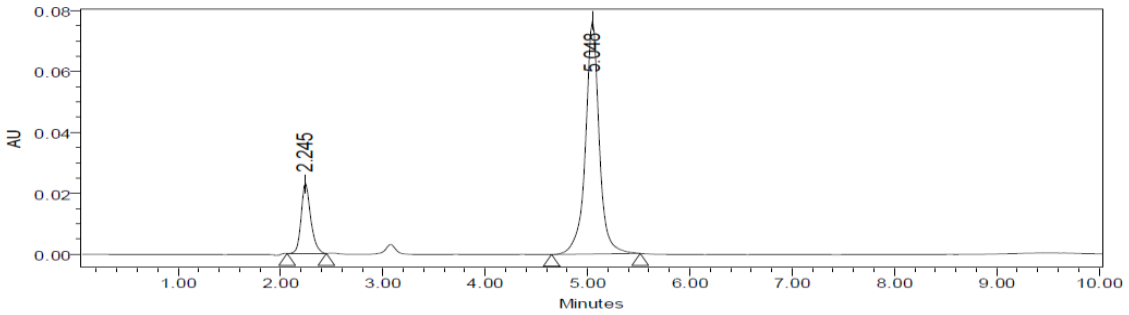
30ppmofAlogliptin & 60ppm of Pioglitazone



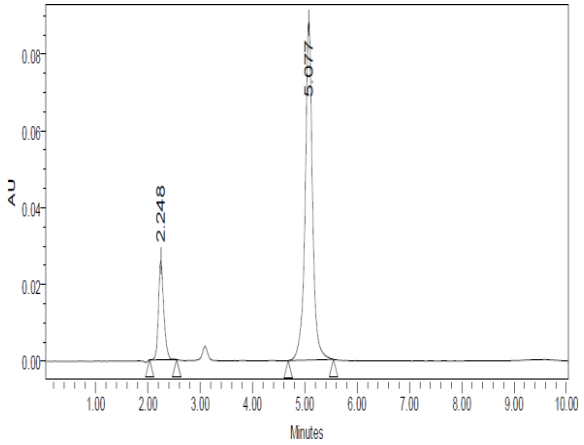
40ppmofAlogliptin & 80ppm of Pioglitazone



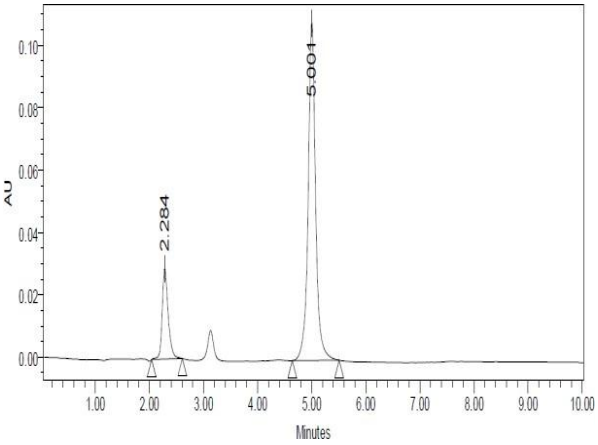
50ppmofAlogliptin & 100ppm of Pioglitazone



60ppmofAlogliptin & 120ppm of Pioglitazone

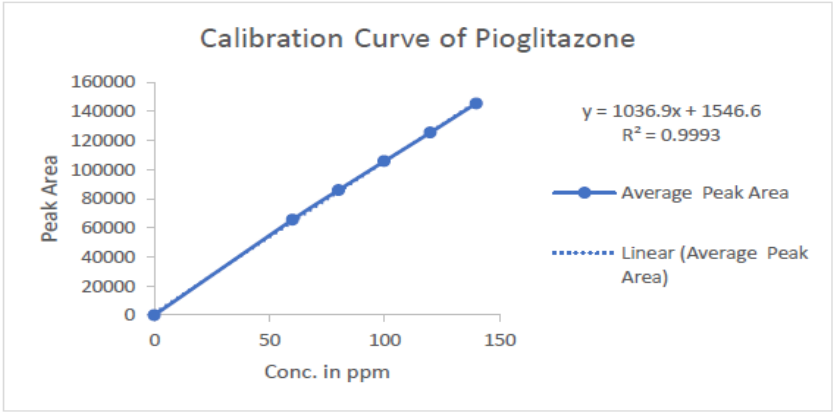


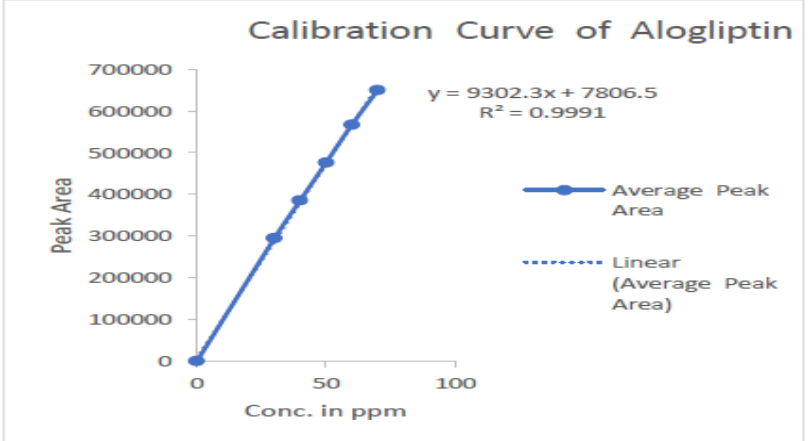
70ppmofAlogliptin & 140ppm of Pioglitazone



Alogliptin

Concentration g/ml	Average Peak Area
30	65453
40	85784
50	105645
60	125365
70	145487





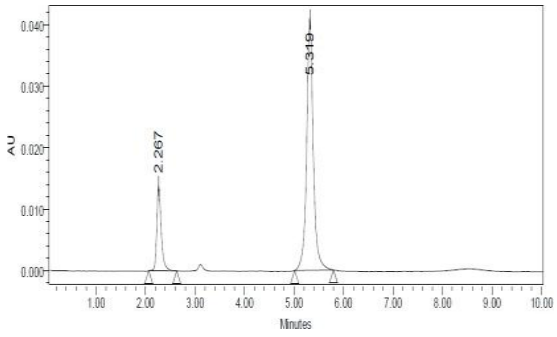
Pioglitazone

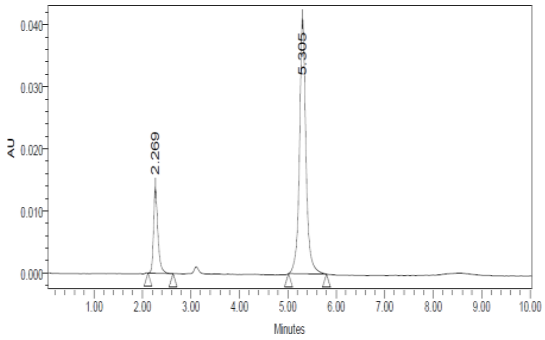
Concentration □g/ml	Average Peak Area
60	294679
80	385468
100	475824
120	566845
140	649587

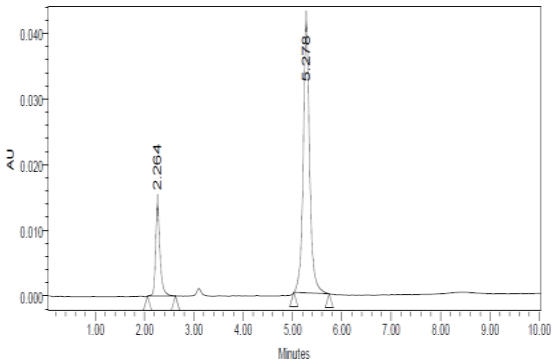
- The co-relation co-efficient of Alogliptin and Pioglitazone drug was found to be 0.999.
- The Linearity test for Pioglitazone was validated and was found to be within the limit.

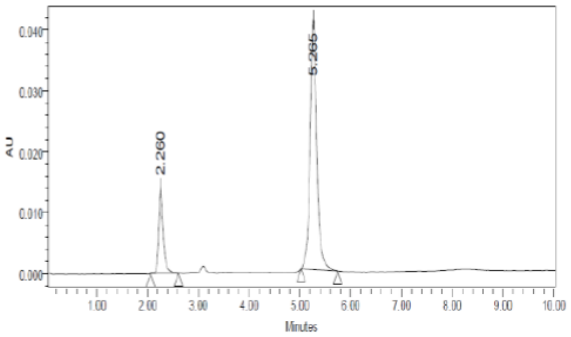
Precision

Repeatability









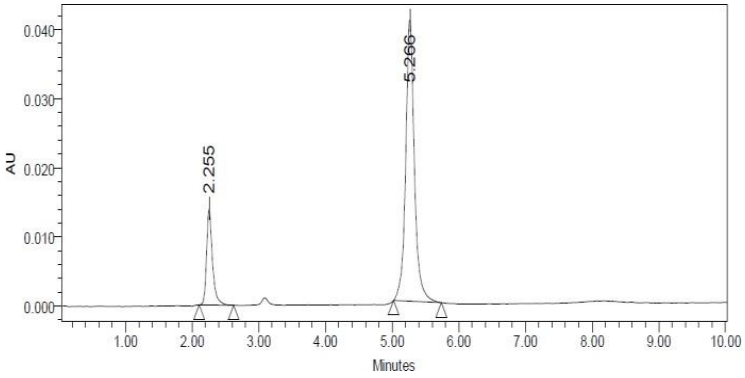


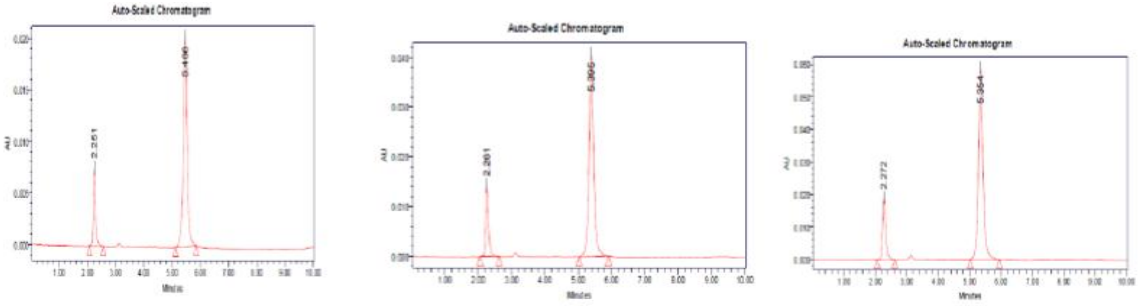
Table showing repeatability results for Alogliptin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Alogliptin	2.269	98645	15468	7898	1.15
2	Alogliptin	2.255	98568	15685	7856	1.16
3	Alogliptin	2.252	98569	15874	7874	1.16
4	Alogliptin	2.267	98574	15784	7859	1.15
5	Alogliptin	2.260	98598	15685	7864	1.16
Mean			98590.8			
Std. Dev			32.66037			
% RSD			0.33127			

Table showing repeatability results for Pioglitazone

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Pioglitazone	5.274	465855	56895	7859	1.16
2	Pioglitazone	5.266	465689	56845	7846	1.15
3	Pioglitazone	5.265	465352	56421	7826	1.15
4	Pioglitazone	5.278	469857	56879	7842	1.16
5	Pioglitazone	5.305	465872	56254	7843	1.16
Avg			466525			
Std.Dev			1874.324			
% RSD			0.401763			

Accuracy



50% concentration **100% concentration** **150% concentration**

Table showing Accuracy results

%Concentration (atspecificationLevel)	Area	Amount Added (ppm)	AmountFound (ppm)	% Recovery	Mean Recovery
50%	**241382.3**	**25**	**25.110**	**100.44%**	**100.20%**
100%	**474115**	**50**	**50.129**	**100.258%**	
150%	**704753**	**75**	**74.924**	**99.898%**	

Acceptance Criteria

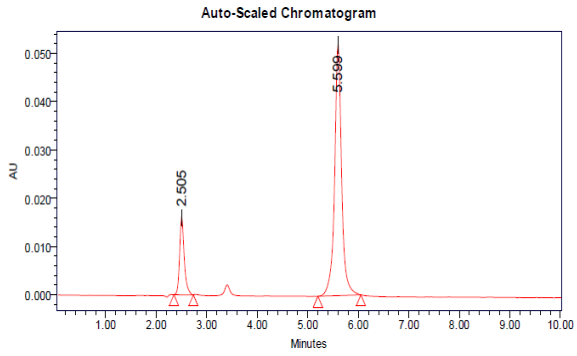
The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

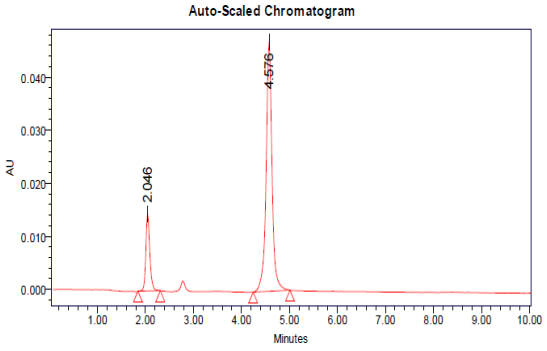
Limit of detection: It was calculated from calibration curve slope and it was found to be 0.63µg/ml for Alogliptin & 1.2µg/ml for Pioglitazone.

Limit of quantification: It was calculated from calibration curve value and it was found to be 1.7µg/ml forAlogliptin & 3.4 µg/ml for Pioglitazone.

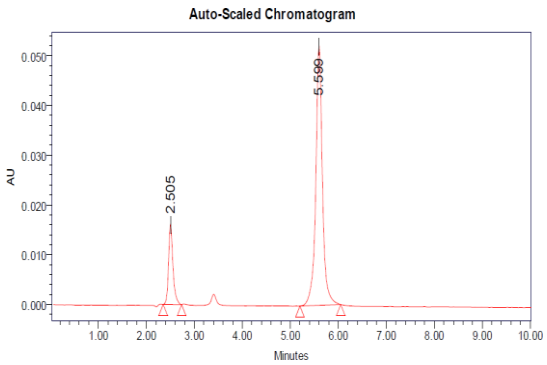
Robustness



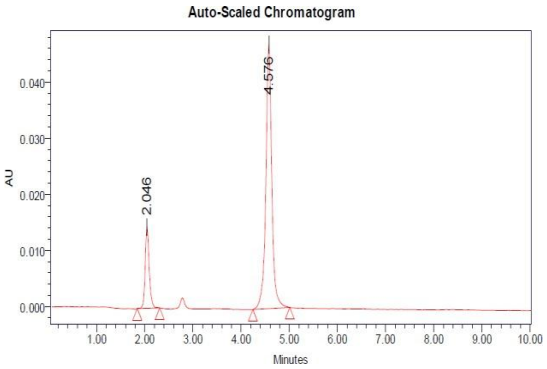
Flow rate 0.9 ml/min



Flow rate 1.1 ml/min



Less organmic phase composition



More organic phase composition

Robustness Values For Alogliptin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 ml/min	98645	2.248	7856	1.15
Less Flow rate of 0.9 ml/min	99867	2.505	7562	1.16
More Flow rate of 1.1 ml/min	92564	2.046	7451	1.13
Less organic phase	91254	2.505	7246	1.12
More organic phase	90255	2.068	7189	1.14

Robustness values for Pioglitazone

Parameter used for sampleanalysis	Peak Area	Retention Time	Theoreticalplates	Tailing factor
Actual Flow rate of 1.0 ml/min	465824	5.443	5695	1.06
Less Flow rate of 0.9 ml/min	489865	5.599	5265	1.02
More Flow rate of 1.1 ml/min	436522	4.576	5365	1.01
Less organic phase	425874	5.610	5187	1.03
More organic phase	416985	4.769	5359	1.04

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000

CONCLUSION

In the present study/work, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Alogliptin and Pioglitazone in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation orpurification steps.

Alogliptin and Pioglitazone were freely soluble in DMSO, sparingly soluble in methanol, ethanol, and Acetonitrile and practically insoluble in water. Acetonitrile: Methanol: 1% Orthophosphoric acid combination was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The tailing factor and theoretical plate countvalues are within the acceptance criteria.

The results expressed in Tables for RP-HPLC method were promising. The developed method is optimized and validated as per the ICH guidelines. All the results of the validation parameters are within the acceptance criteria & all the parameters were validated as per the ICH guidelines. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Alogliptin and Pioglitazone in bulk drug and in Pharmaceutical dosage forms.

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