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R E S E A R C H A R T I C L E

Analytical Method Development and Validation for the Simultaneous Estimation of Alogliptin and Pioglitazone by Using RP-HPLC

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A B S T R A C T

A new method was established for simultaneous estimation of Alogliptin and Pioglitazone by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Alogliptin and Pioglitazone by using Thermosil C18 column (4.0×125mm) 5µ, flow rate was 0.7 ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate bufferpH 3(pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. The instrument used is (Shimadzu HPLC Auto Sampler, Separation model; number SPD20A, LC Solutois) (photodiode array detector 996) the retention times were found to be 2.566 mins and 3.417mins. The % purity of Alogliptin and Pioglitazone was found to be 97.89% and 100.03% respectively. The system suitability parameters for Alogliptin and Pioglitazone such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Alogliptin and Pioglitazone 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 99.55% and 99.47%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively.

Keywords: Thermosil C18 column, Alogliptin and Pioglitazone, RP-HPLC, Methanol.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Alogliptin tradename Nesina and Vipidiais an oral anti-diabetic drug in the DPP-4 inhibitor (gliptin) class. Alogliptin does not decrease the risk of heart attack and stroke. Like other members of the gliptin class, it causes little or no weight gain, exhibits relatively little risk of hypoglycemia, and has relatively modest glucose-lowering activity. Alogliptin and other gliptins are commonly used in combination with metformin in people whose diabetes cannot adequately be controlled with metformin alone.

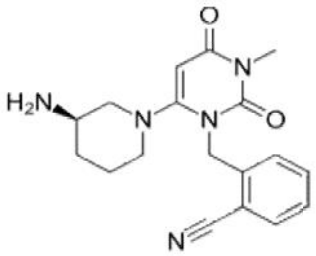

Fig 1: Structure of Alogliptin

Pioglitazone, sold under the brand name Actos among others, is a medication used to treat diabetes mellitus type 2. It may be used with metformin, a sulfonylurea, or insulin. Use is recommended together with exercise and diet. It is not recommended in diabetes mellitus type 1. It is taken by mouth

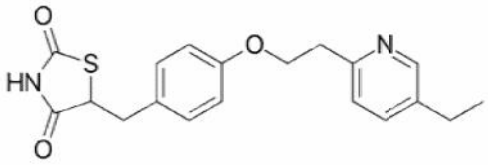

Fig 2: Structure of Pioglitazone

2. Materials and Methods

Instrumentation:

HPLC Auto Sampler: Shimadzu Model number SPD20ASoftware LC Solutions, Detector: Photo diode array detector, Thermosil C18 Column (4.0×1.25mm, 5µ), Sonicator: Model number SE60US Enertech , U.V double beam spectrophotometer: PG Instrument Model number T60 Software UV Win5, pH meter: ADWA Model number AD102U, Digital Weighing machine: a Model number ER200A .

Chemicals:

Alogliptin and Pioglitazone, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4,

Optimized chromatographic conditions

Column : Thermosil C18 (4.0×125 mm) 5.0µm
Mobile phase ratio: Methanol: Sodium acetate buffer (70: 30 % v/v)
Detection wavelength : 252 nm
Flow rate : 0.7 ml/min
Injection volume : 10µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 8min
Retention time : 2.566 & 3.417 mins

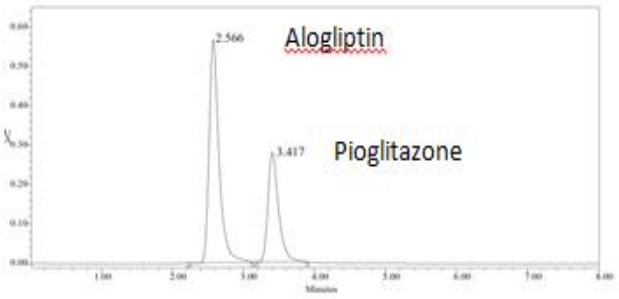

Fig 3: Chromatogram from optimized conditions

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Sample solution preparation:

25.02mg of Alogliptin and 30.02 mg Pioglitazone tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation:

25mg Alogliptin and 30 mg Pioglitazone in working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Method Validation

- ✓ System Suitability
- ✓ Linearity
- ✓ Specificity
- ✓ Precision (Repeatability & Intermediate precision)
- ✓ Accuracy
- ✓ Limit of Detection and Limit of Quantification
- ✓ Robustness

3. Results and Discussion

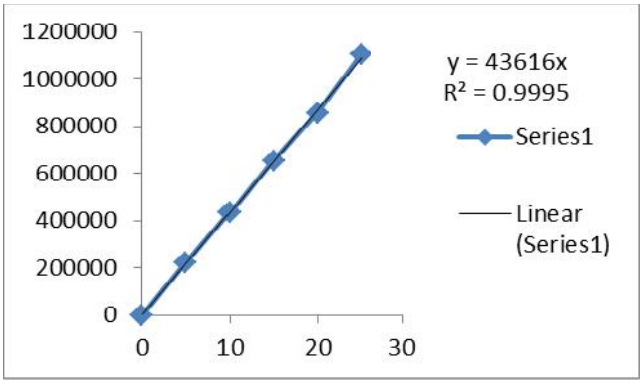

Fig 4: Showing calibration graph for Alogliptin

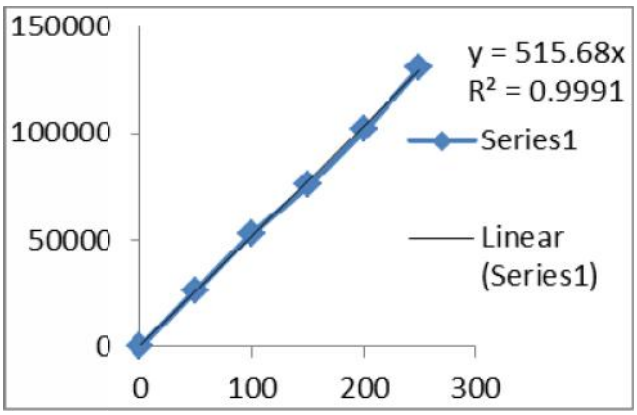

Fig 5: Showing calibration graph for Pioglitazone

Table 1: Results for system suitability

S.No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Alogliptin	2.566	22712	157429	5105	1.3	1.4
2	Pioglitazone	3.417	22254	13239	3788	1.4	1.3

Table 2: Showing assay results

S.No	Name of compound	Amount taken	%purity
1	Alogliptin	754.7	97.89%
2	Pioglitazone	735.6	100.03%

Table 3: Linearity Results for Alogliptin

S.No	Linearity Level	Concentration	Area
1	I	5 µg/ml	221543
2	II	10 µg/ml	436277
3	III	15 µg/ml	654999
4	IV	20 µg/ml	856124
5	V	25 µg/ml	1102139
Correlation Coefficient			0.999

Table 4: Showing accuracy results for Pioglitazone

S.No	Linearity Level	Concentration	Area
1	I	50 µg/ml	26472
2	II	100 µg/ml	52841
3	III	150 µg/ml	75655
4	IV	200 µg/ml	101541
5	V	250 µg/ml	130567
Correlation Coefficient			0.999

Table 5: Showing accuracy results for Alogliptin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	2630409	5	4.96	99.91%	99.55%
100%	5277055	10	9.98	99.18%	
150%	7514836	15	15.02	99.60%	

Table 6: Showing accuracy results for Pioglitazone

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1366666	0.5	0.99	99.53%	99.47%
100%	2777487	1.0	1.05	99.38%	
150%	4151234	1.5	1.495	99.52%	

Table 7: Showing% RSD results for Alogliptin

	Peak Name	Rt	Area	Height(µV)
1	Alogliptin	2.755	5223559	541538.3
2	Alogliptin	2.687	5208511	485548.5
3	Alogliptin	2.632	5323569	574440.4
4	Alogliptin	2.612	5259147	557413.5
5	Alogliptin	2.616	5273463	565020.1
Mean			5257650	
Std.Dev.			45206.4	
%RSD			0.86	

Table 8: Showing %RSD results for Pioglitazone

	Peak Name	Rt	Area	Height(µV)
1	Pioglitazone	3.616	2742453	238643.4
2	Pioglitazone	3.634	2762750	271543.5
3	Pioglitazone	3.460	2797670	281711.6
4	Pioglitazone	3.446	2793578	274499.8
5	Pioglitazone	3.437	2778483	276713.0
Mean			2774987	
Std.Dev.			22806.9	
%RSD			0.82	

Table 9: Showing results for intermediate precision of Alogliptin

	Peak Name	Rt	Area	Height(µV)
1	Alogliptin	2.756	5698542	539568.1
2	Alogliptin	2.688	5682534	536985.4
3	Alogliptin	2.633	5695846	539584.1
4	Alogliptin	2.613	5689452	534569.8
5	Alogliptin	2.617	5636591	534985.5
Mean			5600593	
Std.Dev.			203577.3	
%RSD			0.44	

Table 10: Showing results for intermediate precision of Pioglitazone

	Peak Name	Rt	Area	Height(µV)
1	Pioglitazone	3.617	2624315	231325.6
2	Pioglitazone	3.635	2623598	231315.4
3	Pioglitazone	3.461	2623541	231250.1
4	Pioglitazone	3.447	2624987	231342.6
5	Pioglitazone	3.438	2635698	231765.2
Mean			2626428	
Std.Dev.			5215.78	
%RSD			0.19	

Table 11: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Alogliptin	373625.50	581075863	3.17
Pioglitazone	5772.40	476579210	0.0172

Table 12: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Alogliptin	372727.80	574265980	5.80
Pioglitazone	5761.30	478828490	0.212

Table 13: Showing system suitability results for Alogliptin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing

1	0.8	5339	1.4
2	1	4668	1.3
3	1.2	5216	1.4

Table 14: Showing system suitability results for Pioglitazone

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	**1**	**6089**	**1.2**
3	1.2	6998	1. 3

Table 15: Showing system suitability results for Alogliptin

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6232	1.4
2	***Actual**	**4668**	**1.3**
3	5 % more	6387	1.4

Table 16: Showing system suitability results for Pioglitazone

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3
2	***Actual**	**6089**	**1.2**
3	5 % more	4817	1.2

4. Conclusion

The RP-HPLC method developed and validated allows a simple and fast quantitative determination of Alogliptin and Pioglitazone by RP-HPLC method. All the validation parameters were found to be within the limits according to ICH guidelines. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be simple, accurate, precise, rugged and robust.

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