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DEVELOPMENT AND VALIDATION OF A NOVEL RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ALOGLIPTIN BENZOATE AND PIOGLITAZONE HCL IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

RP-HPLC method have been developed for the simultaneous estimation of Alogliptin Benzoate and Pioglitazone HCl in pharmaceutical dosage form. RP-HPLC method was carried on Phenomenex C-18 column (150 mm × 4.6 mm, 5 µm) with Phenomenex Security Guard Cartridges C18(4x3mm) using a mobile phase acetonitrile: methanol: water: (30:22:48) as a mobile phase at 1.0ml/min flow rate at 268 nm. The linearity is found in the range of 10-50 µg/ml and 12-60 µg/ml with regression coefficient (r^2= 0.998, and r^2=0.997) for Alogliptin Benzoate and Pioglitazone HCl respectively. Alogliptin Benzoate and Pioglitazone hydrochloride have eluted at 4.6 and 6.5 mints respectively. This method is accurate and precise and can be employed for routine analysis of Alogliptin benzoate and Pioglitazone hydrochloride in different pharmaceutical dosage forms.

Keywords *– Alogliptin Benzoate, Pioglitazone HCl, RP-HPLC, ICH guidelines.*

1. INTRODUCTION

Alogliptin Benzoate is Antidiabetic drug. 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl}methyl)benzonitrile.[1]It is dipeptidyl peptidase-4 (DDP-4) inhibitor, and act by increasing glucose dependent insulin release.DPP-4 inhibitors are used for type-2 diabetes mellitus alone or in combination with other antidiabetic drugs.[2]

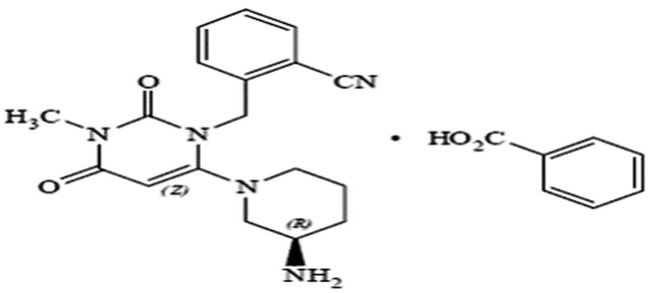

Fig.1: Chemical Structure of Alogliptin Benzoate

Pioglitazone Hydrochloride is a thiazolidinedione with hypoglycemic action to treat diabetes mellitus.[3] 5-({4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}methyl)-1,3-thiazolidine-2,4-dione.[4] pioglitazone hydrochloride is an antidiabetic agent used in the treatment of type-

2 diabetes mellitus (also called as non-insulin-dependent diabetes mellitus NIDDM).It decreases the insulin resistance in the periphery and liver, results in increases the insulin-dependent glucose disposal and decreases hepatic glucose output.[5]

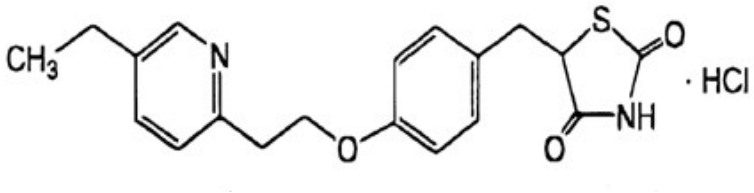

Fig.2: Chemical Structure of Pioglitazone Hydrochloride

Analysis of fixed dose combination (FDC) is quite exigent job for pharmaceutical Analysist in adherence with strict rules and regulations about the safety and quality of manufactured products in the pharmaceutical industry. Analytical research and development for estimation of Alogliptin benzoate and Pioglitazone HCl was found to be interesting and challenging job. Few methods reported for the determination of these drugs. Hence, the present work is to develop reverse phase high performance liquid chromatographic (RP-HPLC) method for determination of Alogliptin benzoate and Pioglitazone HCl in dosage form.

2. MATERIALS AND METHODS

2.1 Materials

Alogliptin Benzoate and Pioglitazone Hydrochloride were obtained as a gift sample from Cipla Pharm Ltd. Mumbai and USV Pharm Ltd. Mumbai, India. The tablet samples of PIOZ – 10® 30 mg were obtained from local market. HPLC grade acetonitrile LiChrosolv®, methanol LiChrosolv®, water LiChrosolv® were purchased from Merk Specialities Pvt. Ltd. Mumbai.

2.2 Instrumentation

The analysis was performed using Younglins Acme 9000 series quaternary gradient pump SP930D.HPLC system accomplished with UV 730D UV-Visible detector with 20µl Rheodyne injector. The data was processed on Autochrom-3000 software. Column C18 (150×45,5µ) Phenomenex with UV method analysis was performed on UV-Visible Double Beam Spectrophotometer Shimadzu 1800. All chemicals were weighed using Electronic Balance AY220 (Shimadzu, Japan). Mobile phase filtered through a Nylon 6,6 membrane 0.45 µm 47 mm filters (Pall India Pvt. Ltd., Mumbai) using vacuum pump. Ultra Sonicator (Microlean-103) was used for degassing the mobile phase. The solution were filtered through 0.45µ syringe filter (Phenomenex).

2.3 Chromatographic Conditions

The chromatographic separation was performed Analytical Column: Phenomenex C18 column (150 mm × 4.6 mm, 5 µm) using mobile phase Acetonitrile: Methanol: Water (30:22:48) at a flow rate of 1.0 ml/min with iscocratic elution. The injection volume was 20 µl and the run time was 10 min. Detection was carried out at 268 nm.

2.4 Preparation of Standard Stock Solution

a) Standard Stock Solution of ALG

10mg of standard ALG was weighed and transfered to a 10ml volumetric flask then dissolved in the methanol LiChrosolv® and the volume was made up to the mark with methanol to obtain conc. of 1000µg/ml of ALG and labeled as 'Std Stock ALG'.

b) Standard Stock Solution of PIO

12mg of standard PIO was weighed and transferred to a 10ml volumetric flask then dissolved in the methanol LiChrosolv® and the volume was made up to the mark with solvent to obtain conc. of 1200µg/ml of PIO and labeled as 'Std Stock PIO'.

c) Combined Standard Stock Solution of ALG and PIO

1ml of 'Std Stock ALG' and 1ml of 'Std Stock PIO' was transferred to 10 ml volumetric flask and diluted to 10 ml with methanol to get 'Std Stock MIX AP' (100µg/ml ALG and 120µg/ml PIO).

2.5 Method Validation

The developed analytical method as per the ICH Q2 (R1) guideline it is suitable for the intended purpose with respect to various parameters such as specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness, system suitability.[10,11]

2.6 Specificity

The chromatogram of standard solution of mixture of Alogliptin Benzoate and Pioglitazone Hydrochloride was compared with formulation to observe the interference of excipient.

2.7 Linearity

1, 2, 3, 4 and 5ml of 'Std Stock MIX AP' were transferred in a series of 10ml volumetric flasks. The volume was made up to the mark with methanol to obtain the conc. of 10, 20, 30, 40 and 50µg/ml of ALG and 12, 24, 36, 48 and 60µg/ml of PIO. The solutions were filtered through 0.45µ syringe filter and 20µl injected into the HPLC system and their chromatogram were recorded for 10mins. Under the chromatographic conditions as described above after getting a stable baseline. Peak areas were recorded for all the peaks. Calibration curves of ALG and PIO were constructed by plotting the peak area of ALG v/s conc. of ALG and peak area of PIO v/s conc. of PIO, respectively. The correlation coefficient (r2) of least square linear regression for ALG and PIO was calculated.

2.8 Range

The range of analytical method was decided from the interval between upper and lower level of calibration curves.

2.9 Precision

The precision of an analytical method was studied by performing Repeatability and intermediate precision.

a) Repeatability

30µg/ml of ALG and 36µg/ml of PIO solution was filtered through 0.45µ syringe filter and 20µl injected into the HPLC system and its chromatogram was recorded under the same chromatographic conditions after getting a stable baseline. Peak area was recorded. The procedure was repeated for six times and calculate the RSD.

b) Intermediate Precision

Intra-day Precision: Intra-day precision was determined by analyzing the standard solution of ALG (30µg/ml) and PIO (36µg/ml) at 8.00am and 4.00pm on same day following the procedure of repeatability and calculate the RSD.

2.10 Accuracy

20 Tablets (PIOZ 30) were weighed and finely powdered; an accurately weighed tablet powder (66.33mg) equivalent to 10 mg of PIO was dissolved and diluted to 20ml with methanol. 0.4 ml of above solution was transferred in four different 10ml volumetric flask labeled as 0%, 80%, 100% and 120%. Then 0, 1.6, 2, 2.4ml of 'Std Stock MIX AP' (100µg/ml ALG and 120µg/ml PIO) were added and volume was made up to the mark with mobile phase. All the solutions were filtered through syringe filter and injected into the HPLC system and their chromatograms were recorded under the same chromatographic conditions after getting a stable baseline. Peak areas were recorded and percent recoveries were calculated.

2.11 Limit of Detection

LOD calculated by the following formulae.

LOD = 3.3(SD/S)

Where, SD- Standard deviation; S- Slope of Curve

2.12 Limit of Quantitation

LOQ calculated by the following formulae.

LOQ = 10(SD/S)

Where, SD- Standard deviation; S- Slope of Curve

2.13 Robustness

Combined standard solution of ALG (20µg/ml), PIO (24µg/ml) was prepared and analyzed at different flow rates (0.9, 1.0, 1.1 ml/min) separately.

2.14 System Suitability

Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they comply with the recommended limit or not.

4. RESULTS AND DISCUSSION

In order to develop RP-HPLC method for combination of Alogliptin Benzoate and Pioglitazone Hydrochloride in bulk and pharmaceutical formulation. The chromatographic conditions were optimized in order to find the best conditions for the separation of Alogliptin Benzoate and Pioglitazone Hydrochloride. Different mobile phase like acetonitrile, methanol, water in varying proportions of mobile phases were tried for better resolution.

After several combinations of mobile solvents with stationary phase C18, the above method has been optimized i.e. acetonitrile: methanol: water in the ratio of 30:22:48 respectively using C18 column which has given good resolution (r^2 for ALG 0.998 and PIO 0.997) and capacity factor, acceptable system suitability. Chromatographic peak of both drugs are identified by overlaying individual drug with chromatograph of mixture is shown in Fig. 3 and 4. Both drugs eluted within 10 mins which will reduce the analysis time and cost. The optimized chromatographic conditions are given in Table-1. The representative chromatogram of standard Alogliptin Benzoate and Pioglitazone Hydrochloride is shown in Fig.5

Table-1: Optimized Chromatographic Conditions

Parameters	Optimized Conditions
HPLC system	Younglins Acme 9000 series quaternary gradient pump system with Autochrom-3000 software
Column	C-18 column (150 mm × 4.6 mm, 5 µm) with Phenomenex Security Guard Cartridges C18(4x3mm)
Mobile phase	acetonitrile: methanol: water: (30:22:48 v/v/v)
Flow rate	1mL/min
Detection wavelength	268nm
Injection volume	20 µL
Concentration of standard Alogliptin Benzoate and Pioglitazone Hydrochloride	10µg/ml and 12µg/ml

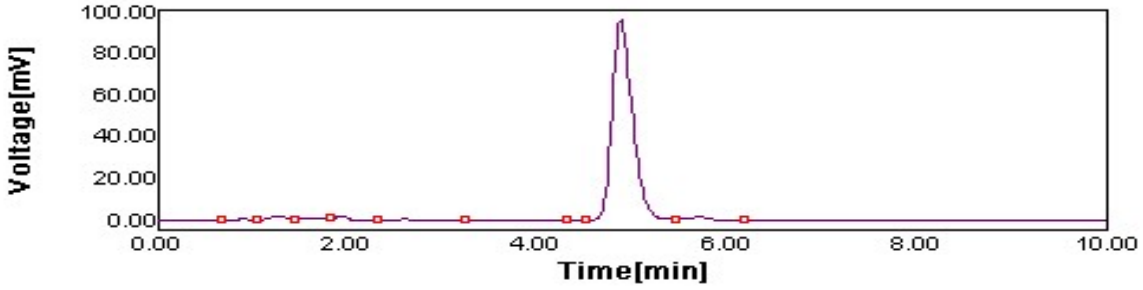

Fig.3: Chromatogram of ALG (10 µg/ml) in optimized chromatographic conditions

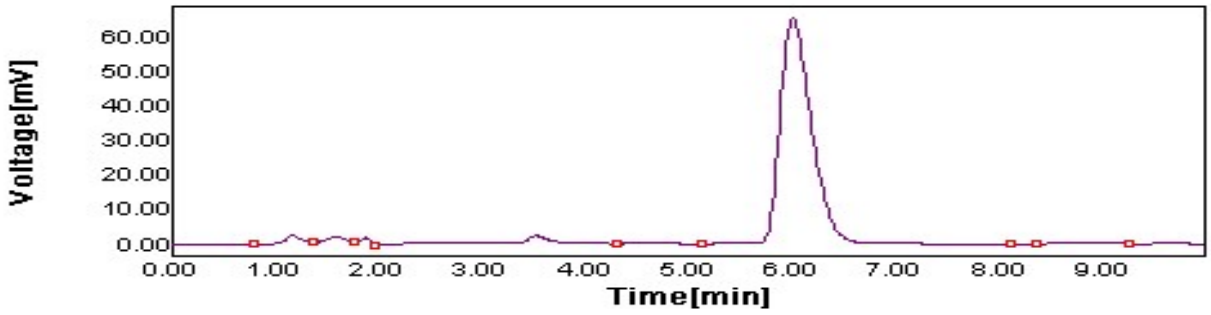

Fig.4: Chromatogram of PIO (12 µg/ml) in optimized chromatographic conditions

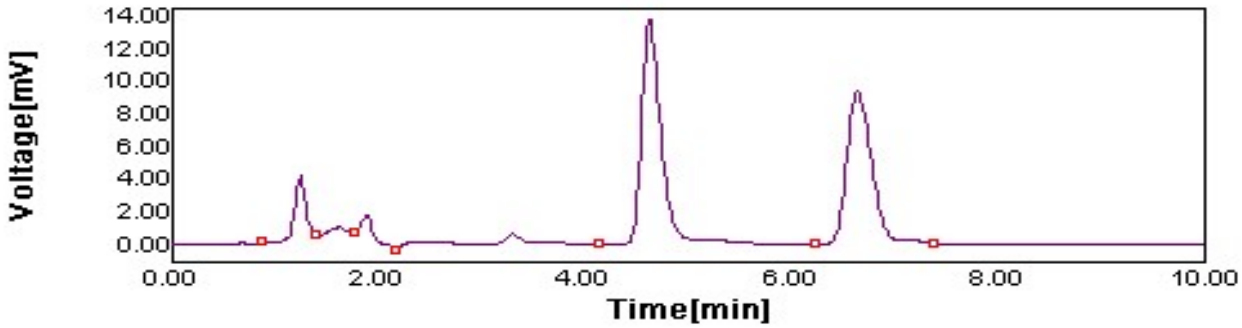

Fig.5: Representative Chromatogram of standard ALG (10µg/ml) & PIO (12µg/ml) in optimized chromatographic conditions

The chromatogram for the Specificity of Pioglitazone hydrochloride marketed tablet dosage form showed peak at a retention time of 6.0 min and retention time of both drugs in standard mixture shows peak at 6.3 and 4.6 min in Fig 6.

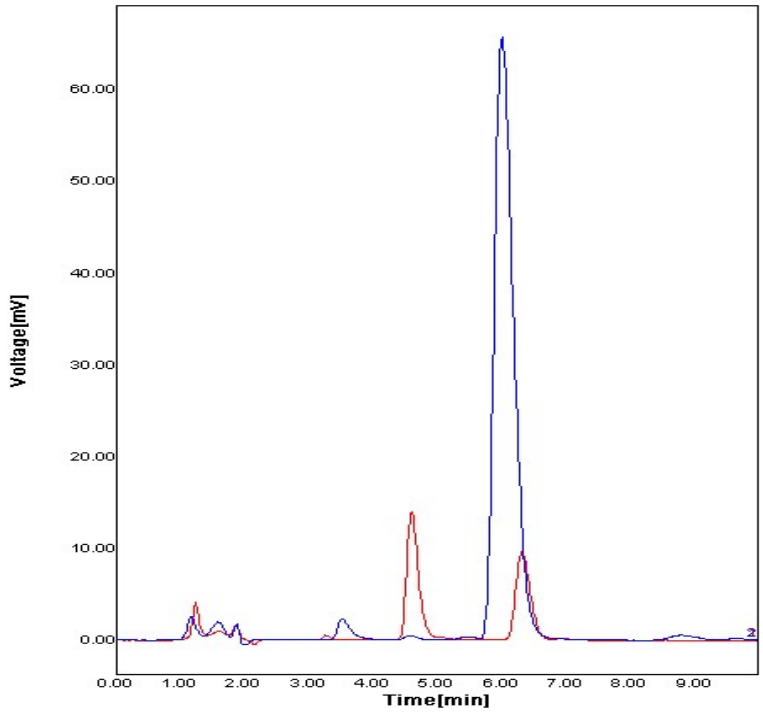

Fig.6: Overlain Chromatograms of sample and standard solution of similar concentrations of drugs

Table-2: Report for accuracy of Pioglitazone hydrochloride

Sr. No.	Level of % Recovery	Amount of 'Sample Stock-A' (ml)	Amount of Standard Drug Added (µg/ml)		Total Amount Found (µg/ml)		Amount Recovered (µg/ml)		% Recovery	
			ALG	PIO	ALG	PIO	ALG	PIO	ALG	PIO
1	0	0.4	0	0	0	17.9	0	0	0	0
2	80	0.4	16	19.2	15.6	36.9	15.6	19	97.5	98.9
3	100	0.4	20	24	19.5	41.2	19.5	23.3	97.5	97
4	120	0.4	24	28.8	23.4	46.1	23.4	28.2	97.5	97.9

The peak response is proportional to concentration and linear in the range of 10-50µg/ml and 12-60µg/ml, respectively for ALG and PIO showed in Fig7.The correlation coefficient is 0.998 and 0.997.

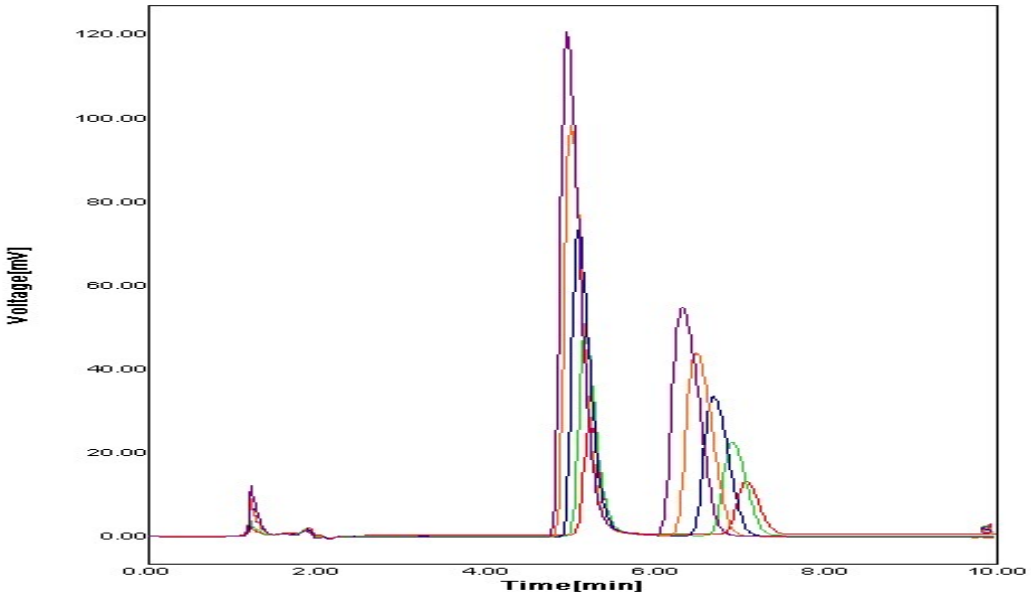

Fig.7 : Overlain Chromatograms of serial dilutions of ALG and PIO in optimized chromatographic conditions

Table-3: Response of ALG at various linearity levels

Conc. of ALG (µg/ml)	Peak Area (mV)
0	0
10	357
20	689
30	1115
40	1535
50	1962

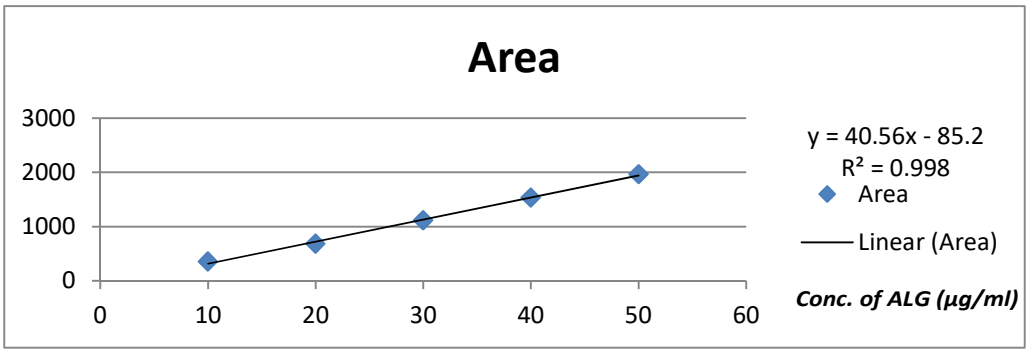

Fig.8 : Calibration curve of ALG of RP-HPLC method

Table-4: Response of PIO at various linearity levels

Sr. No.	Conc. of PIO (µg/ml)	Peak Area (mV)
1.	12	230
2.	24	427
3.	36	681
4.	48	935
5.	60	1197

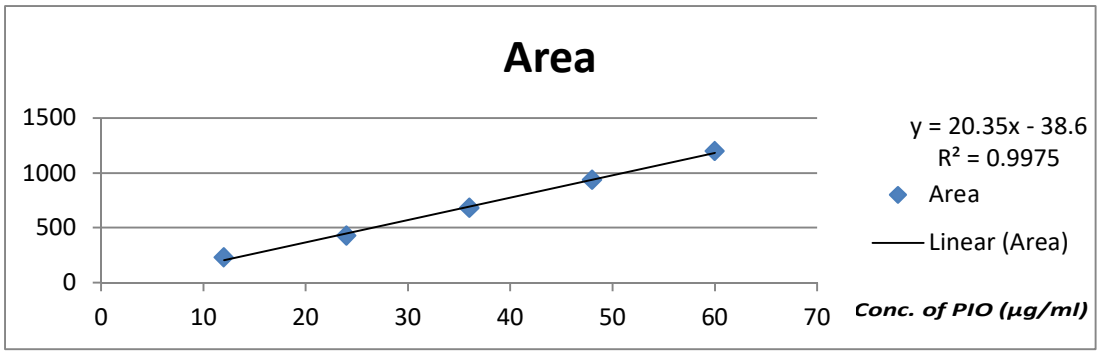

Fig.9 : Calibration curve of PIO of RP-HPLC method

Table-5: Shows the range is from10-50µg/ml for ALG and 12-60µg/ml for PIO

Parameters	ALG	PIO
Linearity Range (µg/ml)	10-50	12-60

The data for precision is represented in Table – 6 and 7. The %RSD was found to be 0.7 for ALG and 1.8 for PIO HCl at intraday and 1.9 for ALG and 1.5 for PIO HCl at 8 am and 1.0 for ALG and 0.2 for PIO HCl at 4 pm for inter day precision studies. Thus the developed method was précised at repeatability and intermediate precision level.

Table-6: Shows the results of intra –day precision of ALG and PIO

Inj.	Peak Area(mV)of ALG	Peak Area(mV)of PIO
1	1519	830
2	1503	857
3	1506	862
4	1498	865
5	1484	865
6	1502	865
SD	11.43	13.75
RSD	0.7	1.8

Table-7: Shows the results of inter–day precision of ALG and PIO

Inj.	Peak Area(mV) at 8am		Peak Area(mV) at 4pm	
	ALG	PIO	ALG	PIO
1	1300	800	1386	828
2	1274	783	1350	829
3	1326	795	1360	831
4	1338	804	1352	828
5	1276	779	1344	831
6	1310	810	1358	832
SD	25.98	12.08	14.71	1.72
RSD	1.9	1.5	1.08	0.20

Detection limit is calculated based on standard deviation of response and slope in Table 8 shows that limit of detection data of ALG and PIO.

Table-8: Limit of Detection data of ALG and PIO

Parameter	ALG	PIO
LOD (µg/ml)	2.71	3.03

Quantification limit is calculated based on standard deviation of response and slope in Table 9 shows that limit of quantification data of ALG and PIO.

Table-9: Limit of Quantitation data of ALG and PIO

Parameter	ALG	PIO
LOQ (µg/ml)	8.24	11.01

Due to change in the flow rate and wavelength no significant changes were found in the chromatogram, the method developed is robust shows in Table 10 and 11.

Table-10: Result of Robustness Study: Variation in Flow Rate (ml/min)

Flow Rate (ml/min)	Analyte	Retention Time (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
0.9	ALG	3.7	1.4	3296	9.57
	PIO	7.4	1.3	3616	
1.0	ALG	4.6	1.3	3712	5.06
	PIO	6.3	1.2	4506	
1.1	ALG	3.0	1.4	3105	8.96
	PIO	5.9	1.3	3198	

Table-11: Result of Robustness Study: Variation in Wavelength (nm)

Wavelength (nm)	Analyte	Retention Time (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
270	ALG	3.3	1.5358	4205	9.454
	PIO	6.5	1.2806	3227	
268	ALG	4.6	1.3696	3712	5.0610
	PIO	6.3	1.2064	4506	
266	ALG	3.3	1.4442	3676	9.1844
	PIO	6.4	1.3628	3261	

System suitability testing the study of resolution, retention, tailing factor and capacity factor shows system is suitable for this method in Table 12.

Table-12: Results of System Suitability Parameters

Analyte	Retention Time (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
ALG	4.6	1.3696	3712	3.8799
PIO	6.3	1.2064	4506	5.0610
Required limits	--	T < 2	N > 2000	R >2

4. CONCLUSION

In conclusion, the HPLC method is simple, accurate, reproducible method for estimation of ALO and PIO in bulk and pharmaceutical formulation. The short chromatographic time makes this method suitable for processing of multiple samples in short time. The method

shows no interference by the excipients. The statistical parameters and recovery data reveals the good accuracy and precision. This method could be useful and suitable for the estimation of the ALG & PIO in bulk and pharmaceutical formulations.

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6. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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