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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF ALOGLIPTIN AND PIOGLITAZONE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:

This is a simple, economic, sensitive stability indicating RP-HPLC method for the simultaneous estimation of Alogliptin and Pioglitazone in bulk and pharmaceutical Formulation. The method was carried out on octa-decyl C18 column (5 µm, 25 cm x 4.6 mm, i.d) using methanol: water in the ratio of 70:30 and pH of the mobile phase up to 3 was adjusted with OPA at a flow rate of 1.0 ml/min. The wavelength for Alogliptin and Pioglitazone at 235 nm was found to be appropriate. The retention time of Alogliptin and Pioglitazone was found to be 2.45 and 6.68 min, respectively. The regression equation for Alogliptin and Pioglitazone were found to be as y = 8.288 x + 0.026 and y= 27.26 x + 38.28 with correlation coefficient (R2) 0.999 and 0.999, respectively. The developed method is found to be robust, accurate and sensitive which can be used for estimation of combination of Alogliptin and Pioglitazone in pharmaceutical dosage forms.

Keywords: Alogliptin, Pioglitazone, RP-HPLC, Simultaneous estimation, Stability Study

INTRODUCTION:

Alogliptin hydrochloride (ALG), (Figure 1) chemically is 2-({6-[(3R)-3-Aminopiperidin-1-yl] -3-methyl- 2, 4-dioxo- 3,4- dihydropyrimidin-1(2H)-yl} methyl) benzonitrile[1] with a molecular formula of $C_{18}H_{21}N_5O_2$ and a molecular weight of 339.39 g·mol^{-1}. It is White to

off-white crystalline powder in colour which is freely soluble in organic solvents, and is practically insoluble in water. Alogliptin is a selective DPP-4 inhibitor. DPP-4 inhibitors lower blood glucose by preventing the breakdown of glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide, thus prolonging the activity of these peptides. Alogliptin is a selective DPP-4 inhibitor. DPP-4 inhibitors lower blood glucose by preventing the breakdown of glucagonlike peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide, thus prolonging the activity of these peptides. Alogliptin has demonstrated high selectivity to DPP-4 relative to other related serine proteases. It has exhibited more than 14,000-fold selectivity over the related serine proteases DPP-8 and DPP-9.

Pioglitazone (PGZ) chemically described as (*RS*)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxy] benzyl) thiazolidine-2,4-dione[2] having empirical formula $C_{19}H_{20}N_2O_3S$ and molecular mass 356.44 g·mol^{-1}. It is white to pale white in colour which is freely soluble in water, sparingly soluble in alcohol and methanol. Pioglitazone improves glycaemic control in people with Type 2 diabetes by improving insulin sensitivity through its action at PPAR gamma 1 and PPAR gamma 2, and affects lipid metabolism through action at PPAR alpha. The results of these interactions include increases in glucose transporters 1 and 4, lowered free fatty acids, enhanced insulin signalling, reduced tumour necrosis factor alpha (TNF alpha) and remodelling of adipose tissue. Together, these can increase glucose uptake and utilisation in the peripheral organs and decrease gluconeogenesis in the liver, thereby reducing insulin resistance.

LITERATURE SURVEY:

Literature survey reveals various analytical method validation for Alogliptin including HPLC [3-6] HPLC pioglitazone [7-8] with metformin [9]. While Pioglitazone described UV[10] HPLC [11-19] and stability study with LC-MS-MS [20].

CHEMICALS AND REAGENTS:

Reference standards of Alogliptin and Pioglitazone were obtained as gift sample from Hetero Laboratories, Hyderabad, India. Pharmaceutical formulation was purchased from local market (Brand: Oceni tablet labelled claim ALG 25 mg and PGZ 15 mg per tablet make Takeda). The HPLC grade solvents used were of E-Merck (India) Ltd., Mumbai. HPLC grade methanol and ortho phosphoric acid (Merck, Mumbai, India) were used in the analysis. HPLC grade water was prepared using Millipore purification system.

INSTRUMENTS:

Younglin (S.K) gradient system UV Detector with Autochro-3000 database software, RP C_{18} column (250×4.6 mm), particle size 5µ) was used. Sonicator: PCi mumbai, Model No.3.5L 100H.

Chromatographic conditions:

Various combinations of mobile phases were screened with respect to resolution, theoretical plate capacity factors and other system suitability parameters. Finally the separation was performed with freshly prepared mobile phase consist of methanol: water in the ration of 70:30 and pH up to 3 was adjusted with OPA with isocratic programming at a flow rate of 1.0 ml/min. 248 nm wavelength, injection volume of 20 µL and ambient temperature was maintained during the entire process to obtain symmetric peaks of ALG and PGZ.

Preparation of standard solution:

All solutions were prepared on weight basis and solution concentrations were also measured on weight basis to avoid the use of an internal standard Pharmaceutical formulation. Standard stock solution was prepared by dissolving separately 25 mg of ALG and 15 mg of PGZ in 100 ml clean dry volumetric flask. Dissolved and diluted with methanol up to the mark and filtered through 0.45 µm membrane filter. This gives the concentration of stock solution 250 µg/ml for ALG and 150 µg/ml for PGZ.

Linearity study:

From the prepared standard stock solutions of both, 0.5 ml, 1.0, 1.5, 2.0, 2.5 and 3.0 ml were transferred to 10 ml volumetric flask and volume made up to the mark with the optimized mobile phase to obtain concentration of 25-150 µg/ml for ALG, while 15 to 90 µg/ml for PGZ respectively. Volume of 20 µL of each sample was injected with the help of Hamilton Syringe. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak area *vs* the drug concentration.

VALIDATION OF PROPOSED METHOD:

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Accuracy:

It was done by recovery study using standard addition method at 80%, 100%and 120%level; known amount of standard ALG and PGZ were added to pre-analyzed sample (75 µg/ml of ALG; 45 µg/ml of PGZ) and subjected them to the proposed HPLC method.

Precision:

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Intraday and Interday Precision:

Intraday precision were determined by analyzing, the three different concentrations 50 µg/ml, 75 µg/ml and 100 µg/ml of ALG, while 30 µg/ml, 45 µg/ml and 60 µg/ml of PGZ for three times in the same day. Day to day variability were assessed using abovementioned three concentrations analyzed on three different days, over a period of one week.

Repeatability:

It is measured by multiple injections of a homogenous sample of 75 µg/ml of ALG and 45 µg/ml of PGZ that indicates the performance of the HPLC instrument under chromatographic conditions.

Robustness:

To evaluate robustness few parameters were deliberately varied. The parameters include variation of flow rate, percentage of methanol using 75 µg/ml solution of ALG and 45 µg/ml of PGZ.

Sensitivity:

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD= 3.3 SD/S and LOQ= 10 SD/S, where SD is the residual standard deviation and S is the slope of the line.

Specificity and selectivity:

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

Ruggedness:

From stock solutions, sample solutions of ALG (75 µg/ml) and PGZ (45 µg/ml) were prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times.

System suitability test:

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

Analysis of Pharmaceutical formulation:

To determine the contents of drugs in conventional tablets (Brand: Oceni tablet labelled claim ALG 25 mg and PGZ 15 mg per tablet make Takeda). Twenty tablets were weighed, their mean weight determined and they were finely powered. Powder equivalent to 25 mg ALG was transferred into a 100 ml volumetric flask containing 50 ml methanol. The resulting solution was sonicated for 30 min and diluted to 100 ml with methanol. The solution was filtered, using 0.45µm filter (Millifilter, Milford, MA). Excipients were separated by filtration. The solution was further diluted with optimised mobile phase to get concentration 75 µg/ml of ALG and 45 µg/ml of PGZ which were subjected to proposed method and amount of ALG and PGZ were determined.

RESULTS AND DISCUSSION:

Optimization of chromatographic conditions:

The primary target in developing this stability indicating HPLC method is to achieve the resolution between Alogliptin, Pioglitazone and its degradation products. To achieve the separation of degradation products, octadecyl silane C_{18} stationary phase and freshly prepared mobile phase consist of methanol: water in the ration of 70:30 and pH up to 3 was adjusted with OPA with isocratic programming at a flow rate of 1.0 ml/min. 248 nm wavelength, injection volume of 20 µL and ambient temperature was maintained during the entire process to obtain symmetric peaks of ALG and PGZ. The tailing factor obtained was less than two and retention time was about 2.45 and 6.68 min for ALG and PGZ (Figure 2). This developed method was found to be specific and method was validated as per international guideline.

Linearity study:

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Alogliptin and Pioglitazone were found to be as 25-150 µg/ml and 15-90 µg/ml respectively (TABLE 1). The regression equation for ALG and PGZ were found to be as $y = 8.2885x + 0.0267$ and $y = 27.264x + 38.28$ with correlation coefficient (R^2) 0.9993 and 0.9991, respectively (Figure 3, 4).

Method Validation:

Accuracy:

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 80%, 100%and 120% concentration levels. Known amounts of standard ALG and PGZ were added to the pre-analyzed samples and were subjected to the proposed HPLC method. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 99.58 to 100.55 % for ALG and 99.11-100.99 % for PGZ. Results of recovery studies is shown in Table 2.

Precision:

Precision was evaluated by carrying out six independent sample preparations of a single sample by intra-day and inter-day precision. The sample preparation was carried out in same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is precise shown in Table 3.

Repeatability:

It is measured by multiple injections of a homogenous sample of 150 µg/ml of ALG and 45 µg/ml of PGZ and the % R. S. D. was found to be less than 2 (Table 4).

Robustness of the method:

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in optimized method parameters were done. The effects of change in flow rate, pH retention time, and in mobile phase ratio were studied. The method was found to be unaffected by small changes like +/- 10% in flow rate, +/- 0.2 change in pH, shown in Table 5.

Sensitivity:

LOQ and LOD can be determined based on visual evaluation, signal-to-noise approach and standard deviation of the response and slope. Limit of detection of ALG and PGZ was determined 1.656 and 0.617, respectively. Limit of quantitation of ALG and PGZ was determined 5.019 and 1.869, respectively.

Specificity and selectivity:

The method is quite selective. There were no other interfering peak around the retention time of ALG and PGZ; also the base line did not show any significant noise.

Ruggedness:

Different analyst carried out precision studies in a similar manner carried out by first analyst. The % Assay was found to be 99.40-99.58%, and 99.60-99.80% of ALG and PGZ, respectively. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is rugged, shown in Table 6.

System suitability test:

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. The tailing factor, capacity factor, and theoretical plates for ALG and PGZ were in the acceptance criteria as per the ICH guidelines (Table 7).

Analysis of Pharmaceutical formulation:

The assay procedure was repeated for six times; the percentage content of ALG and PGZ in the tablet formulation was determined as 98.76-101.77 % and 98.36-101.79 % respectively (Table 8).

Procedure for Forced Degradation Study:

Forced degradation of each drug substances and the drug product was carried out under acidic, basic, oxidative stress, thermolytic and photolytic, conditions. Thermal degradation of drug was carried out in solid state. While remaining all studies were carried out in solution form. Solutions were prepared by dissolving drug with distilled water, aqueous hydrochloric acid, aqueous sodium hydroxide, or aqueous hydrogen peroxide solution, which is further diluted with mobile phase to achieve a concentration of 150 µg/ml each of ALG and 90 µg/ml for PGZ. These solutions were kept for 1 Hr. For thermal stress, samples of drug was placed in a controlled-temperature oven at 50^{o}C for 1 hr. Solutions of drug substances and drug product were also kept at 80 ◦C for 48 h. For photolytic stress, samples of drug in solution state, was irradiated with UV radiation having peak intensity at 254 and 366 nm. The degradation studies (Figure 4-7) were tabulated in Table 09.

CONCLUSION:

The present study was conducted to develop and validate a simple, sensitive and reproducible RP-HPLC method for quantitative determination of Alogliptin and Pioglitazone with stressed stability studies under different conditions. The developed chromatographic assay fulfilled all the requirements to be identified as simple, specific, selective and reliable method, including accuracy, linearity, recovery and precision data.

Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of Alogliptin and Pioglitazone in pharmaceutical formulations without any interference from the Excipients and degraded peaks.

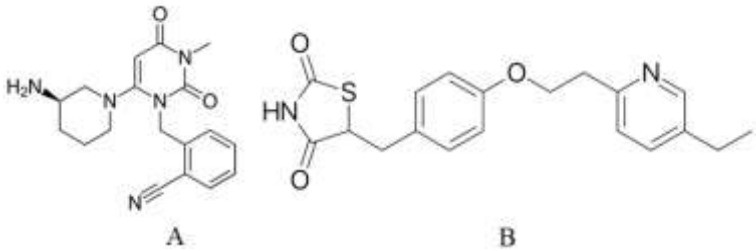

FIGURE 1: CHEMICAL STRUCTURES OF ALG [A] AND PGZ [B]

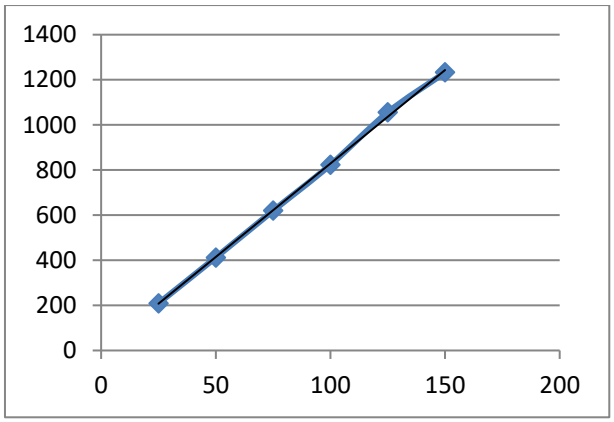

Figure 2: Calibration Curve of Alogliptin

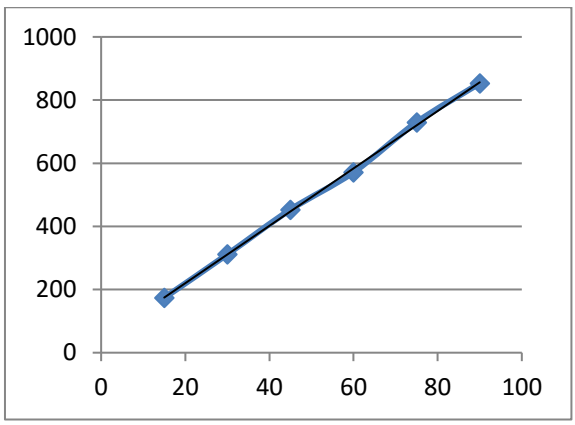

Figure 3: Calibration Curve of Pioglitazone

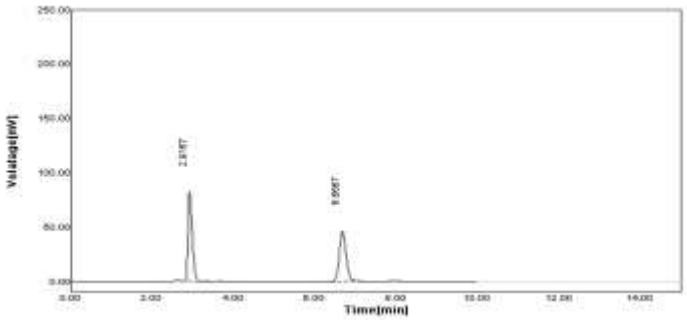

Figure 4: Chromatogram of Standard Alogliptin Hydrochloride and Pioglitazone at 248 nm

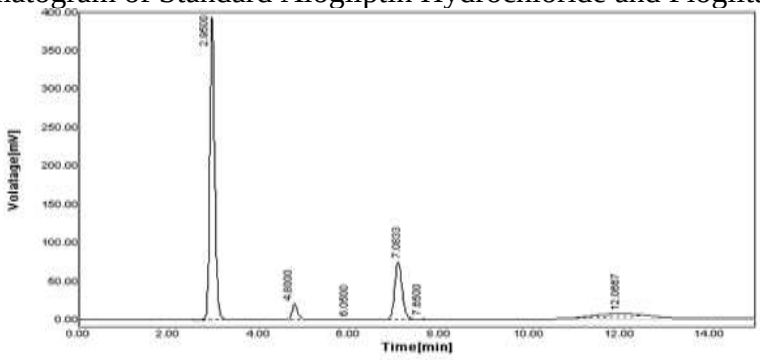

Figure 5: Acidic Degradation (1N, HCl) After 1 Hr

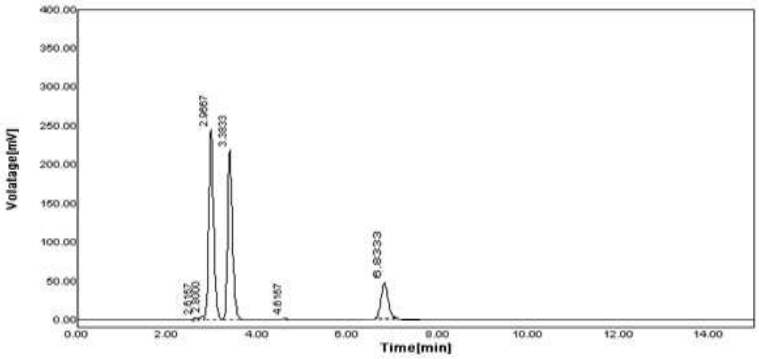

Figure 6: Alkaline Degradation (1 N NaOH) After 1 Hr

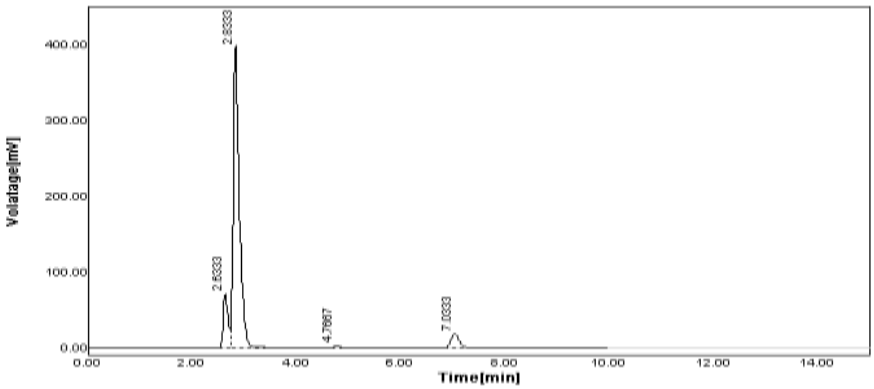

Figure 7: Per Oxide Degradation (30% H_2O_2) After 1 Hr

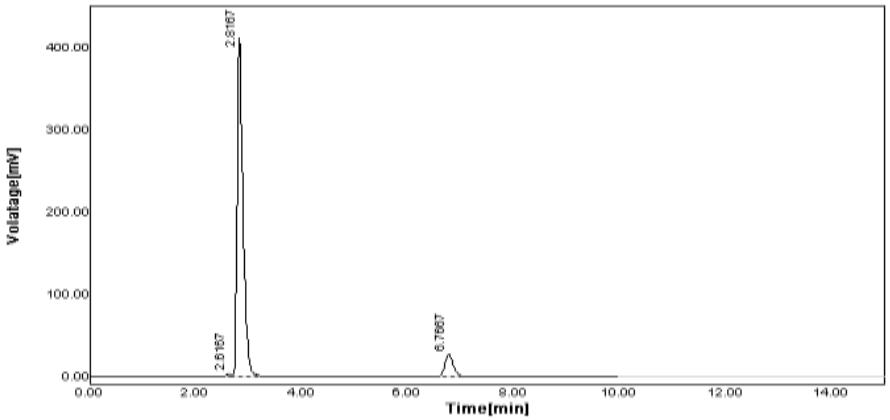

Figure 8: Heat Degradation at 50^{o}c For 1 Hr

Table 1: Linearity Study of ALG And PGZ

Sr. No.	**ALG**				**PGZ**			
	Conc [µg/ml]	Mean peak area [n=5]	± SD	%RSD	Conc [µg/ml]	Mean peak area [n=5]	± SD	%RSD
01	25	208.8	± 3.70	1.96	15	173.6	3.05	
02	50	412.2	± 6.87	1.78	30	311.4	5.98	
03	75	619.4	± 5.81	0.98	45	453	7.28	
04	100	823.2	± 7.12	0.87	60	571.6	4.83	
05	125	1055.4	± 9.34	1.09	75	729.6	5.90	
06	150	1232.6	± 13.24	1.28	90	853.2	8.87	

Table 2: Results of Recovery Studies of ALG And PGZ

Drug	Initial Amt [µg/ml]	Amt added [µg/ml]	Amt recovered ± S.D. [µg/ml, n = 3]	% Recovery	% RSD
ALG	50	0	50.29 ± 0.67	100.39	0.89
	50	40	49.89 ± 0.89	99.81	1.49
	50	50	49.69 ± 1.09	99.58	1.45
	50	60	50.49 ± 1.28	100.55	1.42
PGZ	30	0	30.15 ± 0.27	100.99	1.83
	30	24	30.07 ± 0.20	100.57	1.67
	30	30	30.08 ± 0.24	100.51	1.58
	30	36	29.84 ± 0.18	99.11	1.02

Table 3: Results Of Precision Studies of ALG And PGZ (Intra-Day And Inter-Day)

Drug	Conc. [µg/ml]	Intra day Amt Found [µg/ml]		Inter day Amt Found [µg/ml]	
		Mean ± SD	% RSD [n= 3]	Mean ± SD	% RSD [n= 3]
ALG	50	49.87 ± 4.16	0.34	49.51 ± 8.50	0.69
	75	74.37 ± 10.21	0.55	74.77 ± 7.64	0.41
	100	99.50 ± 6.66	0.27	99.50 ± 9.45	0.38
PGZ	15	15.25 ± 2.00	0.27	15.09 ± 2.00	0.28
	30	30.25 ± 3.06	0.27	30.09 ± 5.57	0.50
	45	45.39 ±5.51	0.35	45.65 ± 5.03	0.34

Table 4: Results of Repeatability Study of ALG And PGZ

ALG	PGZ

Sr. No.	Concentration [µg/ml]	Peak area	Concentration [µg/ml]	Peak area
1	50	628	30	374
2	50	611	30	380
3	50	623	30	386
4	50	618	30	378
5	50	621	30	366
6	50	636	30	382
Mean ± SD		622.833 ± 8.56	Mean ± SD	377.66 ± 6.97
% RSD		1.37	% RSD	1.84

Table 5: Robustness Evaluation of the HPLC Method

Chromatographic conditions (ALG)	T Tailing	K' Capacity Factor	N Theoretical Plate
A: Mobile phase pH			
4.6	1.26	1.23	2683.9
4.8	1.22	1.27	2683.5
5.0	1.21	1.33	2625.5
Mean ± SD	1.23 ± 0.02	1.27 ± 0.05	2678.63 ± 36.80
B: Flow rate (ml/min.)			
0.90	1.23	0.98	2723.8
1.0	1.16	1.08	2818.9
1.1	1.15	1.09	2768.7
Mean ± SD	1.18 ± 0.04	1.05 ± 0.06	2770.47 ± 47.57
C: Percentage MeOH in mobile phase (v/v)			
60	1.09	1.22	2646.2
70	1.06	1.13	2687.4
80	1.19	1.18	2638.3
Mean ± SD	1.11 ± 0.06	1.17 ± 0.04	2657.3 ± 26.36
Chromatographic conditions (PGZ)	T Tailing	K' Capacity Factor	N Theoretical Plate
A: Mobile phase pH			
4.6	1.28	0.99	7591.4
4.8	1.23	1.09	7632.5
5.0	1.25	1.15	7414.7
Mean ± SD	1.25 ± 0.02	1.07 ± 0.02	7546.2 ± 111.72
B: Flow rate (ml/min.)			
0.90	1.26	0.76	7587.3
1.0	1.29	1.10	7668.8
1.1	1.22	0.88	7423.5
Mean ± SD	1.25 ± 0.03	0.91 ± 0.17	7593.2 ± 72.82
C: Percentage MeOH in mobile phase (v/v)			
90	1.18	0.87	7623.8
70	0.94	0.95	7667.3
50	1.23	0.87	7433.2
Mean ± SD	7.296 ± 2.95	1.213 ± 0.73	7574.77 ± 124.51

Table 7: System Suitability Test For ALG And PGZ

System suitability parameters	Proposed method for ALG	Proposed method for PGZ
Retention time (Rt)	2.9333	6.9167
Capacity factor (K')	1.18	0.99
Theoretical plate (N)	2838.7	74.65.8
Tailing factor (T)	1.16	0.95

Table 8: Analysis of Tablet Formulation

Drug	Label claim [mg]	Amount found [mg]	Amount found [%]±SD	%RSD
ALG	25	24.67	98.68 ± 0.43	0.46

PGZ	15	15.06	100.4± 0.68	1.13

Table: 09: Forced Degradation of ALG and PGZ

Sample Exposure condition	Total Number of products with their Rt	ALG		PGZ	
		Degradation remained (150 µg/ml)	Recovery (%)	Degradation remained (30 µg/ml)	Recovery (%)
Acidic, 1N, 1 h	5 (2.95, 4.80, 6.05, 7.08, 7.65)	136.224	90.81	28.25	94.18
Basic, 1N, 1 h	6 (2.61, 2.80, 2.95, 3.38, 4.51, 7.20)	122.22	81.48	13.28	44.29
Per oxide, 30 %, 1 h	4 (2.63, 2.83, 4.76, 7.03)	128.50	85.67	20.92	69.73
Heat, 50 °C, 1 h	3 (2.61,2.81,6.766)	136.58	91.05	22.20	74.01

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