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RESEARCH PAPER

RP-HPLC Estimation of Alogliptin and Pioglitazone Simultaneously in Combined Tablet Dosage Forms

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ABSTRACT

A high performance liquid chromatographic method was developed to quantify alogliptin and pioglitazone simultaneously in bulk and combined tablet dosage form. The chromatographic analysis was done on a Zorbax C8 column (150 mm x 4.6 mm internal diameter, 5 µm particle size) with a mobile phase of 0.1 M ammonium acetate and methanol (50:50, *v/v*) at 1.0 mL/min. The effluents were monitored at 248 nm and the retention time of alogliptin and pioglitazone were 2.883 min and 4.329 min, respectively. Calibration curves were linear from 6.25-18.75 µg/mL for alogliptin and 11.25-33.75 µg/mL for pioglitazone. The LOD and LOQ values for alogliptin were 0.047 and 0.157 µg/mL, respectively; corresponding values for pioglitazone were 0.085 and 0.284 µg/mL, respectively. The precision for alogliptin and pioglitazone was in the range of 0.094-0.303% and 0.072-0.239%, respectively, with corresponding accuracy of 99.450-99.692% and 100.184-100.422%. The developed and validated method was successfully applied for the simultaneous determination of alogliptin and pioglitazone in tablet formulation.

Keywords: Alogliptin, Pioglitazone, Liquid Chromatography, Tablets, Assay

1. INTRODUCTION

Alogliptin, chemically known as 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl}methyl)benzonitrile (Figure 1), is an oral antihyperglycemic of dipeptidyl peptidase 4 inhibitor class. It is used in the treatment of type II diabetes milletus (1,2). The inhibition of dipeptidyl peptidase 4 by alogliptin increases the quantity of active plasma incretins and glucagon like peptide 1 that helps in glycemic control (3).

Pioglitazone, an anti-diabetic drug, belongs to the thiazolidinedione class of drugs. Chemically it is known as 5-({4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}methyl)-1,3-thiazolidine-2,4-dione (Figure 1). Pioglitazone is prescribed to improve control of blood glucose level in adults with type 2 diabetes mellitus (4). Pioglitazone enhances tissue sensitivity to insulin by acting as a potent and selective agonist at peroxisome proliferator activated gamma receptor in adipose tissue, skeletal muscle and liver (5).

With proper diet and exercise, alogliptin and pioglitazone combination is used in the management of high blood sugar levels caused by type 2 diabetes (6,7). This combination was approved by FDA in 2013 (8). The combination of the

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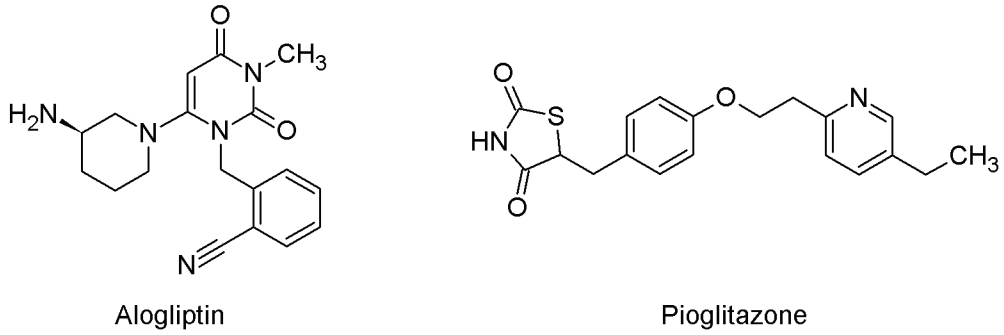

Figure 1. Chemical structures of alogliptin and pioglitazone

selected drugs is not official in any pharmacopeia. Therefore, it is essential to develop effective analytical method for the simultaneous determination of alogliptin and pioglitazone.

UV spectrophotometric methods like first order derivative, dual wavelength, second order derivative and area under curve methods were described by Raval & Srinivasa (9) and Anusha et al. (10) for the simultaneous estimation of alogliptin and pioglitazone in bulk and pharmaceutical dosage forms. High performance thin layer chromatographic method was reported by Komal & Amrita for the simultaneous assay of alogliptin and pioglitazone in combined dosage forms (11). In HPTLC method, separation was achieved on Merck HPTLC aluminum sheets coated with silica gel 60F254, with acetonitrile: 1 % ammonium acetate in methanol (4.5:5.5 *v/v*) as mobile phase and densitometric analysis was performed at 254 nm. Though the UV spectrophotometric methods reported by Raval & Srinivasa (9) and Anusha et al. (10) are simple, they are less selective since they involve measurements in the UV range where there is a possibility of absorbance by the tablet excipients. One of the important validation parameter, method robustness, is not reported in the UV spectrophotometric methods. The HPTLC method described by Komal & Amrita (10) requires costly, sophisticated instrumentation and expertise personnel to operate. Moreover the HPTLC instrument is not commonly available in the developing and under developed countries.

RP-HPLC methods were also applied to the determination of the selected drug combination in bulk and pharmaceutical dosage forms by Raval & Srinivasa (12), Neelima et al. (13), Manzoor et al. (14) and Mokhtar et al. (15). In Raval & Srinivasa method, the separation was carried out on an BDS hypersil C18 (250 mm × 4.6 mm, 5 µm) analytical column using buffer with pH 3.5 and methanol (70:30, *v/v*) as mobile phase at a flow rate of 1.0 mL/min with UV detection at 271 nm (12). Using a Hypersil BDS C18, (250 x 4.6 mm, 5 µm) column as stationary phase and a phosphate buffer of pH 4.8-acetonitrile (45:55, *v/v*) as mobile phase, the selected drugs combination in pharmaceutical formulations was determined by Neelima et al. (13). The detection wavelength was set at 215 nm. In Mokhtar et al. method (14), alogliptin and pioglitazone was chromatographed on Enable C18 (250 mm × 4.6 mm, 5 µm) Column with a mobile phase consisting of phosphate buffer with pH 3.6-acetonitrile (35:65, *v/v*) pumped at a flow rate of 1.0 mL/min with UV-detection at 268 nm. Alogliptin and pioglitazone in tablets was assayed by Mokhtar et al. (15) by carrying out chromatography on an Inertsil ODS-3 (250 mm × 4.6 mm, 5 µm) column using a mixture of methanol and phosphate buffer with pH 3.0 (80:20, *v/v*) as mobile phase at a flow rate of 1 mL/min with UV-detection at 269 nm.

In the present study, a new RP-HPLC method with PDA detector was developed for simultaneous determination of alogliptin and pioglitazone in bulk and combined tablet dosage form. The method validation has been carried out according to the International Conference on Harmonization guidelines (16). The developed and validated RP-HPLC method was successfully applied to combined tablet dosage form.

2. MATERIALS AND METHODS

2.1. Reference standard drugs and tablet dosage forms

Alogliptin and pioglitazone reference standard drugs were provided by Lara Drugs Private Limited (Telangana, India) as gift samples. They are used as received**.** The tablet dosage form, Oseni tablets (strength 25 mg alogliptin and 45 mg pioglitazone), manufactured by Takeda pharmaceuticals America Inc., Deerfield was purchased from the local pharmacy.

2.2. Chemicals and solvents

The HPLC grade methanol was obtained from Merck India Ltd., Mumbai, India. Analytical reagent ammonium acetate was obtained from Sd. Fine Chemicals Ltd., Mumbai, India. Water was obtained using a Milli-Q system.

2.3. Apparatus and HPLC conditions

The Waters Alliance 2695 Module equipped with a 2998 PDA detector with Empower 2 software was used in the current analysis. The Zorbax C8 column (150 mm x 4.6 mm internal diameter, 5 µm particle size) was used. Isocratic mobile phase was composed of 0.1 M ammonium acetate and methanol (50:50, *v/v*) with pH 3.5 (adjusted with orthophosphoric acid: Sd. Fine Chemicals Ltd., Mumbai). The same mobile phase was used as diluent for the preparation of standard solutions of alogliptin and pioglitazone. A flow rate of 1.0 mL/min was maintained. The eluted compounds were monitored at 248 nm. The column temperature was maintained at 30±1 °C. An injection volume of 10 µL was used.

2.4. Standard solutions

A stock standard solution (alogliptin - 250 µg/mL and pioglitazone – 450 µg/mL) was prepared in a 100 mL volumetric flask by dissolving 25 mg of alogliptin and 45 mg of pioglitazone in a final volume of 100 mL mobile phase. Working standard solutions (6.25-18.75 µg/mL for alogliptin and 11.25-33.75 µg/mL for pioglitazone) were prepared from the above stock solution by appropriate dilution with mobile phase.

2.5. Tablet sample solution

Average weight of ten tablets was determined, transferred to a clean dry mortar and grinded into fine powder. Tablet powder equivalent to 25 mg of alogliptin and 45 mg of pioglitazone was then transferred to a 100 mL volumetric flask, 30 mL of mobile phase was added and the flask was sonicated for 10 min to dissolve the drugs completely. The mixture was diluted up to volume with the mobile phase to give a solution containing 250 µg/mL and 450 µg/mL of alogliptin and pioglitazone, respectively. This solution was filtered through 0.45 µm pore size membrane filter. Appropriate dilution (12.50 µg/mL of alogliptin and 22.50 µg/mL of pioglitazone) was prepared in mobile phase for analysis.

2.6. Calibration graph

Working standard solutions equivalent to 6.25-18.75 µg/mL alogliptin and 11.25-33.75 µg/mL pioglitazone were prepared by appropriate dilution of the stock standard solution with the mobile phase. 10 µL aliquot of each solution was injected automatically into the column in triplicate and the chromatograms were recorded. The peak areas of the drugs were determined. Calibration graph was constructed by plotting the mean peak area against drug concentration. The concentration of the unknown was calculated from the calibration graph or from the regression equation derived from the mean peak area-concentration data.

2.7. Estimation of alogliptin and pioglitzone in combined tablet dosage form

10 µL of the tablet sample solution was injected into the HPLC system in triplicate. The chromatograms were recorded. The peak areas were determined. The concentrations of alogliptin and pioglitazone in the combined tablet dosage form were calculated from the corresponding calibration curves or corresponding regression equations.

2.8. Forced degradation

To assess the stability indicating properties of the proposed HPLC method, forced degradation studies were performed. The tablet sample was subjected to acid, alkali, oxidation, thermal and photo degradation

Acid and alkali hydrolysis

Tablet powder equivalent to 25 mg of alogliptin and 45 mg of pioglitazone was transferred to a 100 mL volumetric flask. The powder was mixed with 10 mL of 0.1 N hydrochloric acid (for acid hydrolysis) or 10 mL of 0.1 N sodium hydroxide (for alkali hydrolysis). The solutions were subjected to sonication for 30 min. The samples were neutralized with an amount of acid (for alkali hydrolysis) or base (for acid hydrolysis) equivalent to that of the previously added. The flask was made up to the volume with mobile phase.

Oxidative degradation

Tablet powder equivalent to 25 mg of alogliptin and 45 mg of pioglitazone was transferred to a 100 mL volumetric flask. The contents were mixed with 10 mL of 30% hydrogen peroxide solution. The reaction mixture was allowed to

sonication for 30 min and then the volume of the flask was made up to 100 mL with mobile phase.

Thermal and photo degradation

Tablet sample powder (alogliptin-25 mg and pioglitazone-45 mg) was exposed to 105°C for 30 min in oven (for thermal degradation) or subjected to direct sun light for up to 24 hr (for photo degradation). After the specified time, the tablet powder was cooled and dissolved in 30 mL of mobile phase in a 100 mL volumetric flask. The solution thus prepared was diluted to volume with the mobile phase.

The degraded sample solutions were appropriately diluted with mobile phase to obtain a concentration of 12.50 µg/mL (alogliptin) and 22.50 µg/mL (pioglitazone). The solutions were filtered through 0.45 µm pore size membrane filter. A volume of 10 µL was injected into the HPLC system and the chromatograms were recorded.

3. RESULTS AND DISCUSSION

3.1. Method development

The present study was aimed to establish a sensitive, robust and reliable RP-HPLC method for the simultaneous determination of alogliptin and pioglitazone in combined tablet dosage form. During method development, two different columns like the Hypersil BDS C18 column (250 mm x 4.6 mm internal diameter, 5 µm particle size) and Zorbax C8 column (150 mm x 4.6 mm internal diameter, 5 µm particle size) were tried. Better results (good symmetrical sharp peak, acceptable tailing factor and resolution) were obtained with Zorbax C8 column (150 mm x 4.6 mm internal diameter, 5 µm particle size) maintained at a temperature of 30±1 °C. Hence, the same column and temperature was chosen for analysis.

Various mobile phases (0.1% Orthophosphoric acid: Methanol, 0.1 M NaH_2PO_4: Methanol, 0.1 M KH_2PO_4: Methanol and 0.1 M ammonium acetate: Methanol) with different ratios, flow rate and pH were tried and the responses were recorded. After a series of experiments, highly symmetrical and sharp peaks of alogliptin and pioglitazone with better resolution were obtained at pH 3.5 by using 0.1 M ammonium acetate and methanol (50:50 (*v/v*)) as mobile phase at a flow rate of 1.0 mL/min. The alogliptin and pioglitazone in the selected mobile phase have sufficient absorption at 248 nm, which was therefore chosen for the analysis. Figure 2 shows a typical HPLC chromatogram of alogliptin and pioglitazone using the optimized chromatographic conditions.

3.2. Method validation

The developed RP-HPLC method was validated using ICH guidelines (16).

System suitability test

In order to determine the satisfactory resolution and reproducibility of the method, suitability parameters, including % RSD of retention time, % RSD of peak area, USP plate count and USP tailing factor, were investigated. In order to test the system suitability, standard solution (alogliptin - 12.50 µg/mL and pioglitazone - 22.50 µg/mL) was injected five times into the HPLC system. The results (Table 1) demonstrate the method suitability.

Specificity

Specificity was evaluated by comparing the chromatograms of mobile phase blank, placebo blank, working standard solution (alogliptin 12.50 µg/mL and pioglitazone 22.50 µg/mL) and tablet sample solution (alogliptin 12.50 µg/mL and pioglitazone 22.50 µg/mL). For this purpose, solutions of placebo blank, mobile phase blank, working standard and tablet sample was injected into the HPLC system. The resulting chromatograms are shown in Figure 3. The chromatograms of placebo blank, mobile phase, working standard and tablet sample did not show any peaks other than that of alogliptin and pioglitazone. This confirmed the specificity of the method.

Linearity

Plot of the mean peak area against concentration gave the linear relationship over the concentration range 6.25-18.75 µg/mL for alogliptin and 11.25-33.75 µg/mL for pioglitazone. Using the regression analysis, the linear equation obtained was: $y = 15825\ x - 554$ ($R^2 = 0.9999$) for alogliptin; $y = 61574\ x - 471.2$ ($R^2 = 0.9998$) for pioglitazone where y is the mean peak area, x is concentration in µg/mL and R^2 is the regression correlation.

Limit of detection (LOD) and limit of quantification (LOQ)

The limits of detection and quantification were calculated according to ICH guidelines. The LOD for alogliptin and pioglitazone was 0.047 and 0.085 µg/mL, respectively, while LOQ was 0.157 and 0.284 µg/mL, respectively.

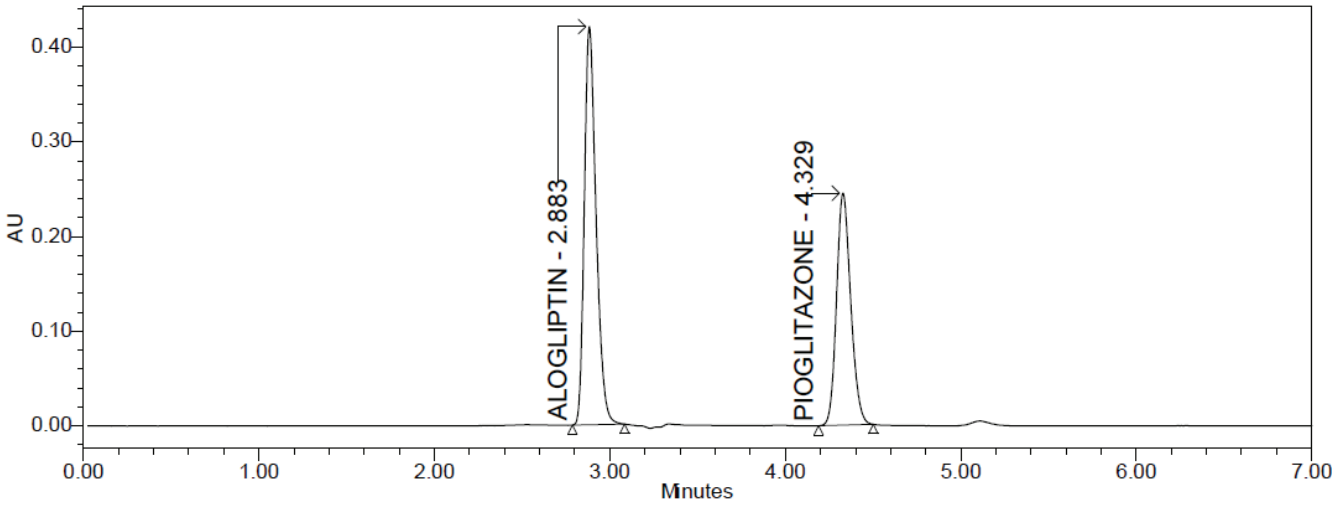

Figure 2. Typical HPLC chromatogram of alogliptin and pioglitazone

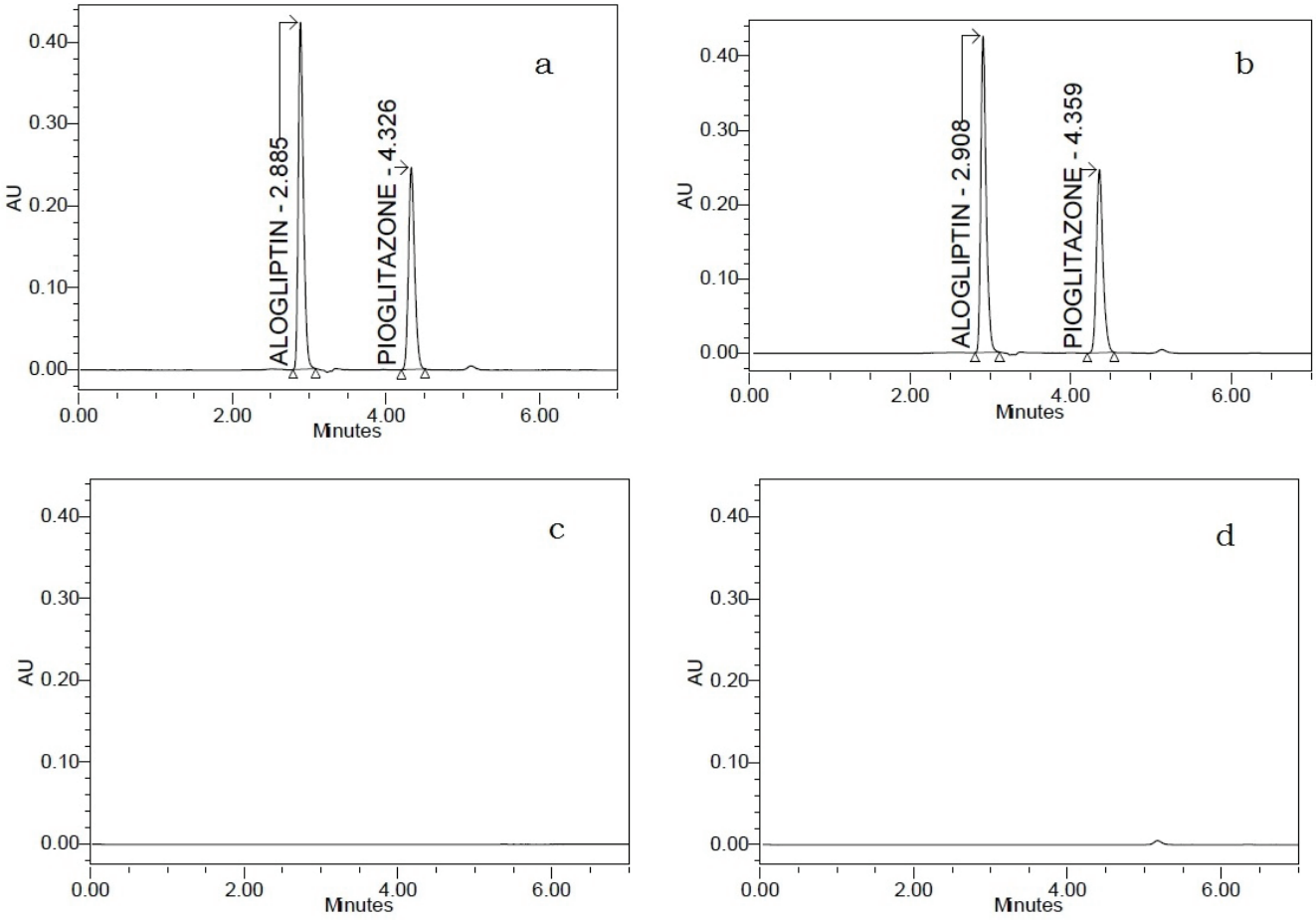

Figure 3. Chromatogram of (A) Alogliptin and pioglitazone working standard solution (B) Tablet sample solution (C) Placebo blank (D) Mobile phase blank

Table 1. System suitability parameters of the proposed HPLC method

Parameters	Alogliptin		Pioglitazone		Recommended limit
	Value*	RSD (%)	Value*	RSD (%)	
Retention time	2.906	0.761	4.361	0.754	RSD ≤2
Peak area	1976065	0.474	1386415	0.864	RSD ≤2
USP resolution	-	-	10.41	0.686	> 1.5
USP plate count	8997	0.365	13896	0.524	> 2000
USP tailing factor	1.322	0.632	1.186	0.461	≤ 2

*Average of five values

Precision and accuracy

The intra-day and inter-day precision and accuracy of the method was tested by applying the proposed HPLC method for the determination of working standard solution of alogliptin and pioglitazone at a concentration of 12.50 µg/mL and 22.50 µg/mL, respectively. The working standard solution was assayed six times on the same day for intra-day analysis and on three consecutive days for inter-day analysis. The precision and accuracy were expressed as % relative standard deviation and % recovery, respectively. The results are shown in Tables 2 and 3. The low values of % RSD and good % recovery values confirm the satisfactory precision and accuracy of the present HPLC method.

Recovery study

The accuracy of the proposed HPLC method was further checked by the standard addition method. For this, the pre-analyzed sample solution was spiked with known concentration of alogliptin and pioglitazone at three different concentration levels (50 %, 100 % and 150 %) the percentage recovery data (Table 4) show that the proposed method was accurate. Common excipients in tablets did not interfere with the assay of alogliptin and pioglitazone indicating the selectivity of the method.

Table 2. Intra-day precision and accuracy data of the proposed HPLC method

Alogliptin		Pioglitazone	
Peak area	**Recovery (%)**	**Peak area**	**Recovery (%)**
1976730	98.53	1381221	98.83
1972402	98.32	1388479	99.35
1972552	98.32	1380884	98.80
1974805	98.44	1382787	98.94
1979467	98.67	1387533	99.28
1971548	98.27	1386181	99.18
Average	**98.43**	**Average**	**99.06**
RSD (%)	**0.152**	**RSD (%)**	**0.239**

Table 3. Inter-day precision and accuracy data of the proposed HPLC method

Alogliptin		Pioglitazone	
Peak area	**Recovery (%)**	**Peak area**	**Recovery (%)**
Day 1			
2026286	101.00	1395606	99.86
2018801	100.63	1393109	99.68
2027031	101.04	1392061	99.60
2020576	100.72	1398160	100.04
2034946	101.44	1393087	99.68
2019810	100.68	1397336	99.98
Average	**100.92**	**Average**	**99.81**
RSD (%)	**0.303**	**RSD (%)**	**0.180**
Day 2			
1988214	99.11	1395715	99.87
1988415	99.12	1392458	99.63
1986324	99.01	1390257	99.47
1988251	99.11	1392548	99.64
1984235	98.91	1395482	99.85
1989325	99.16	1392547	99.64
Average	**99.07**	**Average**	**99.68**
RSD (%)	**0.094**	**RSD (%)**	**0.149**
Day 3			
1984267	98.91	1395157	99.83
1989315	99.16	1396385	99.91
1988329	99.11	1393652	99.72
1983679	98.88	1395480	99.85
1988367	99.11	1396354	99.91
1989634	99.18	1395365	99.84
Average	**99.06**	**Average**	**99.84**
RSD (%)	**0.131**	**RSD (%)**	**0.072**

Robustness

The method robustness was performed by evaluating the influence of small and deliberate changes in HPLC conditions on the system suitability parameters of the proposed HPLC method. The selected conditions are flow rate (±0.1 mL/min) and temperature (±2 °C). Robustness was determined with the working standard solution of

alogliptin and pioglitazone at a concentration of 12.50 µg/mL and 22.50 µg/mL, respectively. The results are summarized in Table 5. In all cases, good separations of both alogliptin and pioglitazone were achieved and the system suitability parameters are well within the acceptable limits, indicating that the proposed HPLC method remained robust under the optimized conditions.

Stability studies

Forced degradation studies were carried out to elucidate the inherent stability characteristics of the aloglitpin and pioglitazone. An ideal stability-indicating HPLC method is one that measures the analytes and also resolves its degradation products. Different stress conditions were applied: acid & base hydrolysis, oxidative, thermal and photo degradation.

Alogliptin and pioglitazone were found to degrade under all the stress conditions employed. Alogliptin was found to be more degraded in thermal degradation and pioglitazone in oxidative degradation condition applied. Less degradation of both the drugs was observed in photolytic degradation. The results of forced degradation studies are shown in Table 6. Chromatograms obtained under different stress conditions are shown in Figure 4. The developed HPLC method could effectively resolve the drugs from their degradation products. This confirms the stability indicating power of the developed HPLC method.

The chromatographic peak purity tool was applied to verify the purity of aloglitpin and pioglitazone peaks in all cases. This was done by calculating purity angle and purity threshold for aloglitpin and pioglitazone peaks. In all cases, aloglitpin and pioglitazone peaks were pure since purity angle was less than purity threshold. This showed that aloglitpin and pioglitazone peaks had no detectable impurity peaks and free of co-eluting degradation products.

3.3. Advantages of the proposed method

The details of the reported RP-HPLC methods for the simultaneous assay of alogliptin and pioglitzone are summarized in Table 7. The proposed RP-HPLC method has the advantages of being more sensitive (12-15), more precise (12-15) and more accurate (12-15) than the reported

Table 4. Recovery data of the proposed HPLC method

Spiked level (%)	Concentration of alogliptin (µg/mL)		Recovery (%)	Mean (%)	Concentration of pioglitazone (µg/mL)		Recovery (%)	Mean (%)
	Added	Found			Added	Found		
50	6.178	6.160	99.709	99.692	11.120	11.163	100.385	100.422
	6.178	6.156	99.652		11.120	11.168	100.433	
	6.178	6.160	99.714		11.120	11.170	100.448	
100	12.375	12.314	99.509	99.450	22.275	22.308	100.150	100.184
	12.375	12.282	99.246		22.275	22.313	100.169	
	12.375	12.325	99.596		22.275	22.327	100.232	
150	18.534	18.483	99.729	99.626	33.360	33.399	100.116	100.221
	18.534	18.450	99.547		33.360	33.445	100.255	
	18.534	18.460	99.601		33.360	33.458	100.291	

Table 5. Robustness data of the proposed HPLC method

Parameter	Alogliptin			Pioglitazone		
	USP Tailing	USP plate count	USP resolution	USP Tailing	USP plate count	USP resolution
Flow rate 1.0 + 0.1 mL/min	1.36	9687	-	1.25	15120	10.71
Flow rate 1.0 – 0.1 mL/min	1.40	8369	-	1.28	13455	10.17
Temperature 30 + 5 °C	1.37	9714	-	1.27	15015	10.70
Temperature 30 - 5 °C	1.42	8508	-	1.29	13532	10.23

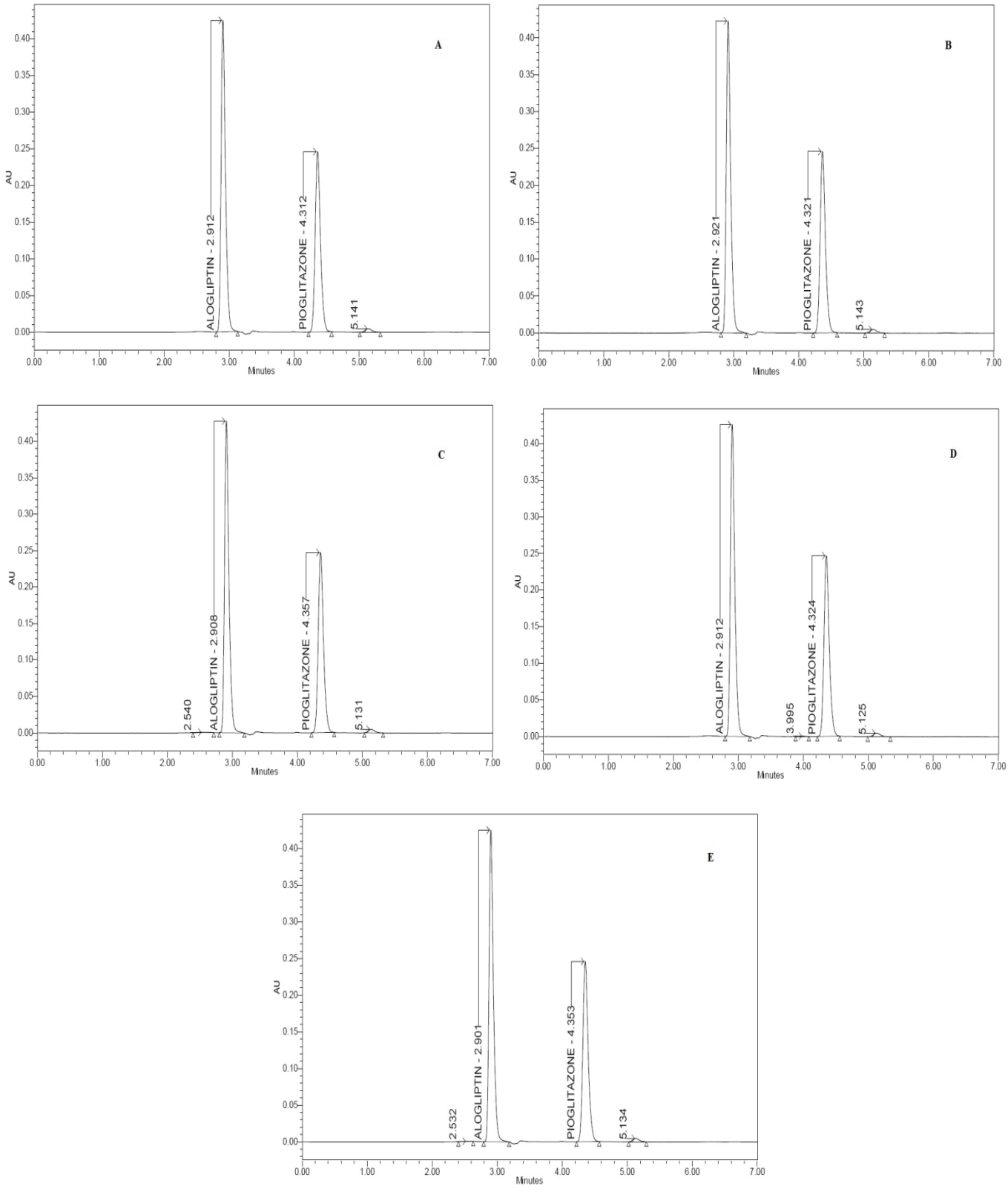

Figure 4. Chromatogram of tablet sample (A) Treated with 0.1 N HCl (B) Treated with 0.1 N NaOH (C) Treated with 30% H_2O_2 (D) Exposed to 105°C (E) Exposed to sunlight

Table 6. Results of degradation studies

Stress condition	Drug	Peak area	Degradation (%)	Assay (%)	Purity angle	Purity threshold	Retention time of degradants
Acid (0.1N HCl)	Alo	1782654	9.82	90.18	0.554	0.659	5.141
	Pio	1888497	4.46	95.54	0.528	0.667	
Base (0.1 N NaOH)	Alo	1790579	9.41	90.59	0.494	0.658	5.143
	Pio	1287248	7.19	92.81	0.299	0.437	
Oxidation (30% H_2O_2)	Alo	1791485	9.37	90.63	0.516	0.659	2.540 & 5.131
	Pio	1283480	7.46	92.54	0.305	0.437	
Dry heat (105°C)	Alo	1705211	13.73	86.27	0.565	0.661	3.995 & 5.125
	Pio	1284260	7.41	92.59	0.306	0.439	
Photolytic (sun light 24 hr)	Alo	1888497	4.46	95.54	0.528	0.667	2.532 & 5.134
	Pio	1290513	6.96	93.04	0.315	0.444	

Alo: Aloglitpin; Pio: Pioglitazone

Table 7. Summary of proposed and reported RP-HPLC methods

Drug	Run time (min)	Linearity (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	RSD (%)	Recovery (%)	Reference
Alo	10	6.25-31.25	0.555	1.680	0.404-1.069	101.01-101.07	Raval & Srinivasa (12)
Pio		3.75-18.75	0.139	0.423	0.553-1.124	99.84-100.77	
Alo	10	31-187	0.339	1.210	0.31	99.87-100.56	Neelima et al. (13)
Pio		75-450	0.516	1.565	0.32	99.62-100.61	
Alo	8	2.5-15	0.034	0.012	0.257	98.44-100.40	Manzoor et al. (14)
Pio		3-18	0.034	0.105	0.230	99.16-100.55	
Alo	6	5-100	0.170	0.500	0.23-1.10	99.12-99.48	Mokhtar et al. (15)
Pio		5-100	0.215	0.650	0.31-1.54	101.38-101.95	
Alo	7	6.25-18.75	0.047	0.157	0.152	99.45-99.69	Proposed
Pio		11.25-33.75	0.085	0.284	0.236	100.18-100.42	

Alo: Aloglitpin; Pio: Pioglitazone

HPLC methods. The total run time of the proposed method was less when compared with the reported HPLC methods (12-14). The less run time may decrease the utilization of solvents, time and cost of analysis. The proposed method has wider range of linearity than the Manzoor et al. (14) method. The validation parameters like system suitability (13) and specificity (12-14) is not reported in some of the reported HPLC methods. The volume of sample for analysis in the proposed method (10 µL) is lesser than the methods (20 µL) of Manzoor et al. (14) and Mokhtar et al. (15).

4. CONCLUSION

In the present study, an attempt was made to develop an accurate, precise, selective and sensitive RP-HPLC method for the simultaneous analysis of alogliptin and pioglitazone in bulk and combined tablet dosage forms. The method was validated in accordance with ICH guidelines. The main features of the developed method are economical, low run time, selective, robust, sensitive and satisfactory precision and accuracy. Therefore, the suggested RP-HPLC method can be used for the simultaneous quantification of alogliptin and pioglitazone in quality control laboratories or industry.

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Algoliptin ve Pioglitazon'un kombine tablet dozaj formunda RP-HPLC yöntemiyle eş zamanlı miktar tayini

ÖZ

Algoliptin ve pioglitazon'un bulk üründe ve kombine tablet dozaj formunda eş zamanlı miktar tayini için bir yüksek basınçlı sıvı kromatografisi (HPLC) yöntemi geliştirildi. Geliştirilen yöntemde; stasyoner faz olarak Zorbax C8 kolon (150 mm x 4.6 mm, partikül büyüklüğü:5 µm), mobil faz olarak 0.1 M amonyum asetat tamponu and metanol (50:50, *h/h*) kullanıldı ve mobil fazın akış hızı 1.0 mL/min olarak belirlendi. Alogliptin ve pioglitazon'un, 248 nm'de, alıkonma zamanları sırasıyla 2.883 ve 4.329 dakika olarak belirlendi. Alogliptin ve pioglitazon için kalibrasyon eğrileri sırasıyla 6.25-18.75 µg/mL ve 11.25-33.75 µg/mL aralığında doğrusal olarak belirlendi. Alogliptin için LOD ve LOQ değerleri 0.047 and 0.157 µg/mL olarak belirlenirken, pioglitazon'un LOD ve LOQ değerleri 0.085 and 0.284 µg/mL olarak tespit edildi. Alogliptin ve pioglitazon için kesinlik değerleri sırasıyla % 0.094-0.303 ve % 0.072-0.239 aralığında ve doğruluk değerleri %99.450-99.692 ve %100.184-100.422'ye karşılık gelecek şekilde belirlendi. Geliştirilen ve valide edilen yöntem, algoliptin ve pioglitazon'un tablette eş zamanlı miktar tayini için başarıyla uygulanabilir bir yöntemdir.

Anahtar kelimeler Alogliptin, Pioglitazon, Sıvı kromatografisi, Tablet, Yöntem geliştirme ve validayon

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