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Development of stability indicating method for the simultaneous estimation of alogliptin and pioglitazone in bulk and tablet dosage form by reversed-phase ultra-performance liquid chromatography method

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ABSTRACT

The objective of the work is to develop and validate a new reverse phased ultra-performance chromatography method and its stability studies for the simultaneous estimation of alogliptin and pioglitazone in bulk and tablet dosage form. The column of the method was BEH C18 (2.1× 50 mm, 1.7 µ) used as a stationary phase and the mobile phase was 45:55 v/v of phosphate buffer (pH 3) and methanol, respectively. The injection volume was 2 µl and flow rate was maintained at 0.3 ml/minute. The wavelength was 280 nm and the runtime was 3 minutes. The retention time of alogliptin was 0.4 minutes and pioglitazone was 0.529 minutes. The Linearity of the alogliptin was 6.25–37.5 µg/ml and pioglitazone was 15–90 µg/ml. The newly developed method could be used for the routine analysis of pure drug and its formulations in accordance with the ICH Q2 (R1) guidelines.

INTRODUCTION

Alogliptin (Fig. 1) decreases the incretin glucose dependent insulinotropic polypeptide (GIP) and glucagon like peptide (GLP-2) (Cabrera *et al*., 2013) increases the plasma incretin concentration, which can controls the glucose levels in the blood (Marino and Cole, 2015). GIP and GLP-2 are stimulating the glucose dependent pancreatic beta cells. It inhibits the activity of GIP and GLP-2 (Cyrus and Vijay, 2010).

Pioglitazone (Fig. 2) is a thiazolidine class of anti-diabetic drug (Brunetti *et al*., 2010). It is an agonist of peroxisome proliferator activated receptor gamma and it activates the insulin responsive genes then increase the production insulin (Al-Majed *et al*., 2016; Lincoff *et al*., 2007).

On extensive survey of literature, very few reverse phased high-performance chromatography (RP-HPLC) methods have been reported for the estimation of alogliptin and pioglitazone combined dosage form (Vasanthi *et al*., 2017). The present developed reversed-phase ultra-performance liquid chromatography (RP-UPLC) method was accurate, precise, and robust for the simultaneous estimation of alogliptin and pioglitazone in Active Pharmaceutical Ingredient and tablet dosage form. The developed RP-UPLC method showed better resolution and low retention time, very good separation efficiency, and faster elution and tiny amount of sample consumed when compared to the (Vasanthi *et al*., 2017) reported RP-HPLC methods.

MATERIALS AND METHODS

Instruments used

The liquid chromatographic system was made up of Waters-Acquity, Japan, UPLC equipped with auto sampler and

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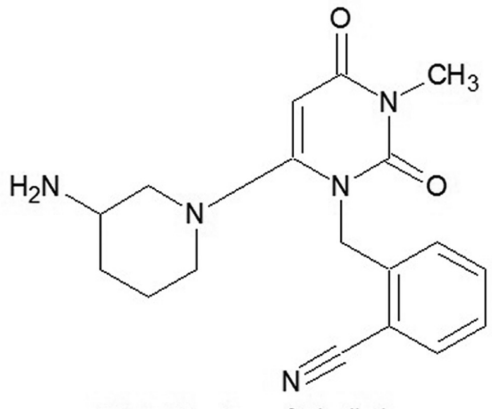

Figure 1. Structure of alogliptin.

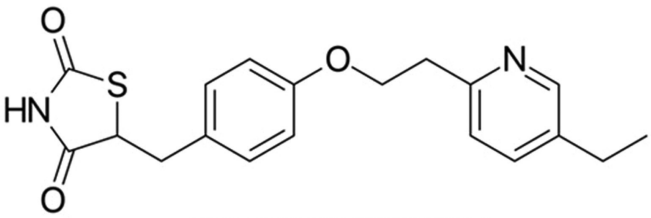

Figure 2. Structure of pioglitazone.

2996 PDA detector with Empower 2 software. Chromatographic separation was performed on waters BEH C_{18} (2.1 × 50 mm, 1.7 µ) column.

Chemicals used

Gift samples of alogliptin and pioglitazone are procured from Pharma Train, Hyderabad, India. HPLC grade water and methanol are purchased from Merck Laboratories, Mumbai, India. Potassium dihydrogen phosphate ($K_2H_2PO_4$) was obtained from Finar Chemicals Pvt. Limited, Ahmedabad, Gujarat, India.

Chromatographic conditions

Column	: Waters BEH C_{18} (2.1 mm*50 mm, 1.7 µ)
Mobile phase ratio	: Phosphate buffer (pH 3):Methanol (45:55)
Wavelength	: 280 nm
Flow rate	: 0.3 ml/minute
Injection volume	: 2 µl
Run time	: 3 minute

Preparation of mobile phase

450 ml (45%) of phosphate Buffer and 550 ml (55%) of Methanol was taken and ultra sonicated for 10–15 minutes for degassing, further filtered. pH was adjusted to 3 with orthophosphoric acid (Raval and Srinivasa, 2014).

Preparation of standard

12.5 mg of alogliptin and 30 mg of pioglitazone was taken in 100-ml volumetric flask then 70 ml of diluent was added and dissolved completely, the volume was made up to the mark (Stock solution). 1.5 ml of stock solution was pipette out into 10ml volumetric flask and the volume was made up to the mark with diluent (Neelima *et al*., 2014).

Validation of analytical method

The developed RP-UPLC method was validated for System suitability, Specificity, Linearity, Accuracy, Precision, Robustness, and stability studies. The validation in accordance with ICH guidelines $Q_2(R_1)$.

System suitability

It was performed before each validation to check the retention time, theoretical plates, tailing factor, and resolution determined to five suitability injections (Manzoor *et al*., 2015).

Specificity

It was carried out to determine the analyte in the presence of other compounds, such as impurities, degradants, and matrix. In specificity study standard injection was compared to the running blank injection.

Linearity

From the standard stock solution, appropriate aliquots of alogliptin and pioglitazone were taken in different volumetric flask and make up the volume up to the mark with diluent to obtain different concentrations are 6.25, 12.5, 18.75, 25, 31.25, and 37.5 µg/ml for alogliptin and 15, 30, 45, 60, 75, and 90 µg/ml for pioglitazone, respectively (Haribabu *et al*., 2017). The solutions are injected into 2 µl fixed loop system and then chromatograms were recorded. The calibration curve was plotted by concentration Vs Peak area.

Precision

Both intraday and inter day was carried out for six injections in the day and between the days.

Accuracy

Recovery was obtained by adding known quantities of pure standard in three different concentrations 50%, 100%, and 150% to the pre-analyzed sample formulation. The amount of drug found, amount of drug recovered, and percentage recovery were calculated for the confirmation of the method accuracy (Komal and Amrita, 2015).

Robustness

It is an analytical procedure to measure the capacity to remain unaffected by small variations in the method parameter. Some typical variations impacts on method were flow rate, temperature, pH of mobile phase, and mobile phase composition.

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation were made to evaluate the impact on the method. From the Standard solution, 18.75 ppm of alogliptin and 45 ppm of pioglitazone was prepared and analyzed using the varied flow rate and wave length change along with actual conditions.

Forced degradation studies

1.5 ml from the stock solution was taken in 5 different 10ml volumetric flasks. The required aliquots are prepared and the solutions are exposed to the stress conditions, such as acidic,

alkaline, peroxide, thermal, and photolytic conditions (Mokhtar *et al*., 2016). Then, finally the amount of drug degraded in stress conditions was calculated. The data were shown in Table 10.

RESULTS AND DISCUSSION

System suitability

The retention time of alogliptin was 0.403 minutes and pioglitazone was 0.529 minutes. Three system suitability injections were injected into the chromatographic column. Then, the tailing factor, theoretical plates and resolution was calculated. The results are shown in Table 1 and the values were found to be within the limit.

Specificity

Comparing the results of standard solution along with running blank solution, there was no interference in blank.

Linearity

For the determining the linearity, plot the graph peak versus concentration and calculate the correlation coefficient. The correlation coefficient and regression equation of alogliptin (Fig. 4) was found to be $Y = 797.9x - 228.7$ ($r^2 = 0.9997$) and pioglitazone (Fig. 5) was found to be $Y = 2157.4x - 40.1$ ($r^2 = 0.9998$). The results are shown in Table 2 and Figure 3.

Accuracy

The mean percentage recovery of alogliptin was 100.34% and pioglitazone was 100.30%. It was present within the limit, hence the method was accurate and it is shown in Tables 3 and 4.

Precision

It was carried out in intraday and intermediate day for the % Relative Standard Deviation (RSD) calculation. The results were found to be under accepted criteria, i.e., 2%. It is shown in Tables 5 and 6.

LOD and LOQ

The Limit of Detection (LOD) of alogaliptin and pioglitazone found to be 2.91 and 2.96 µg/ml. The Limit of Quantification (LOQ) of alogliptin was 10.4 and 10.09 µg/ml for pioglitazone, respectively. The results are shown in Table 7.

Robustness

The variation of flow rate and wavelength was found to be (+10%), which was affected by the method. The standard solutions were injected under the selected robust conditions. The system suitability parameters, such as theoretical plates, tailing factor and resolution was observed and measured. Results shows >2000 theoretical plate count, < 2 of tailing factor and resolution was found to be > 2 (See Tables 8 and 9). Hence the method was robust and the results were shown in Figures 6 to 9.

Forced degradation studies

The working standards of alogliptin and pioglitazone were placed in different stress conditions, such as acid, base, thermal, peroxide, and photolytic conditions, and then observe the amount of drug degraded in above stress conditions. The results of degradation study of standard solutions were stable in all the selected stress conditions and there is no observe the much deviation. The results obtained were compared with existing methods and found to be stable in all stress conditions, but existing

Table 1. System suitability parameters for alogliptin and pioglitazone.

S. no	Peak name	RT	Area	Height	USP tailing	USP plate count
1	Alogliptin	0.403	14,601	2,825	1.16	3,083
2	Alogliptin	0.412	14,715	2,847	1.13	3,479.74
3	Alogliptin	0.415	14,585	2,822	1.13	3,260.44
4	Pioglitazone	0.529	96,100	15,802	1.31	3,760.10
5	Pioglitazone	0.535	97,019	15,953	1.37	3,205.44
6	Pioglitazone	0.541	95,651	15,728	1.33	3,815.62

Table 2. Linearity results.

S. no	Linearity level	Alogliptin		Pioglitazone	
		Concentration	Area	Concentration	Area
1	I	6.25	14,8485	15	32,573
2	II	12.5	159,870	30	64,978
3	III	18.75	172,969	45	96,453
4	IV	25	185,137	60	128,673
5	V	31.25	196,786	75	162,527
6	VI	37.5	208,365	90	195,462

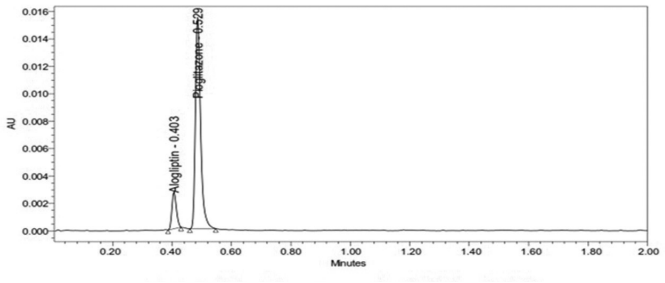

Figure 3. Optimized chromatogram for alogliptin and pioglitazone.

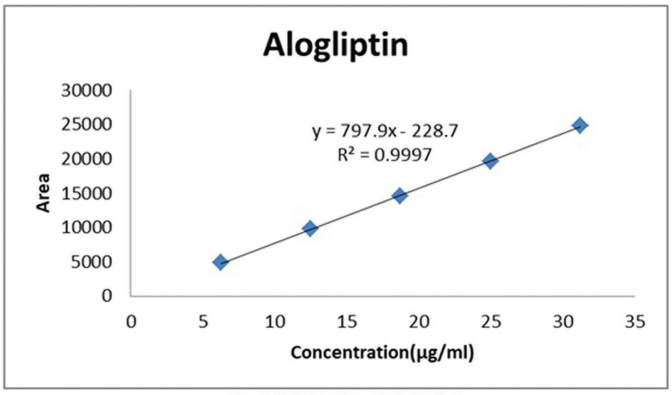

Figure 4. Linearity graph of alogliptin.

Table 3. The accuracy results for alogliptin.

%Concentration (at specification Level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	7,352	6.25	6.27	100.28	
100%	14,665	12.5	12.50	100.04	100.34%
150%	22,155	18.75	18.89	100.73	

Table 4. The accuracy results for pioglitazone.

%Concentration (at specification Level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	48,228	15	15.00	100.01	
100%	96,846	30	30.12	100.41	100.30%
150%	327,988.3	45	45.22	100.47	

Table 5. Results for intraday precision.

Injection	Area for alogliptin	Area for pioglitazone
Injection-1	14,572	96,756
Injection-2	14,497	96,245
Injection-3	14,756	96,786
Injection-4	14,678	96,458
Injection-5	14,565	96,452
Injection-6	14,767	96,753
Average	14,639.2	96,575.0
Standard Deviation	111.1	222.1
%RSD	0.8	0.2

Table 6. Results for intermediate precision.

Injection	Area for alogliptin	Area for pioglitazone
Injection-1	14,872	96,836
Injection-2	14,756	96,486
Injection-3	14,582	96,435
Injection-4	14,643	96,856
Injection-5	14,869	96,456
Injection-6	14,668	96,786
Average	14,731.7	96,642.5
Standard Deviation	121.2	203.0
%RSD	0.8	0.2

Table 7. Results for LOD and LOQ.

Parameter	Alogliptin	Pioglitazone
LOD	2.91 µg/ml	2.96 µg/ml
LOQ	10.4 µg/ml	10.09 µg/ml

methods were stable in thermal and photolytic conditions only. The data are shown in Table 10.

DISCUSSION

Present developed RP-UPLC method showed better results when compared to the reported RP-HPLC method by (Vasanthi *et al*., 2017). In the reported method, where C_{18} column (250 × 4.6 mm, 5 µm) was used, then slow elution of solutes at

Table 8. Robustness results for change in flow rate.

S. no	Flow Rate (ml/minute)	Alogaliptin			Pioglitazone	
		USP tailing	USP plate count	USP resolution	USP tailing	USP plate count
1	0.27	1.46	4,626.92	3.31	1.29	6,132.29
2	0.3	1.46	4,725.92	3.18	1.29	6,256.39
3	0.33	1.46	4,865.39	3.02	1.29	6,352.29

*Results for actual flow (0.3 ml/minute) have been considered from Assay standard.

Table 9. Robustness results for change in wavelength.

S. no	Change wavelength	Alogaliptin			Pioglitazone	
		USP tailing	USP plate count	USP resolution	USP tailing	USP plate count
1	270 nm	1.46	4,762.23	3.37	1.29	6,214.27
2	*280 nm	1.46	4,725.92	3.18	1.29	6,256.39
3	290 nm	1.46	4,767.76	2.96	1.29	6,232.23

* Results for actual wavelength 280 has been considered from accuracy standard.

Table 10. Degradation results for alogliptin and pioglitazone.

Sample name	Alogliptin		Pioglitazone	
	Area	% Degraded	Area	% Degraded
Standard	14,633.7	-	96,256.7	-
Acid	14,356	1.90	95,568	0.72
Base	13,677	6.54	94,682	1.64
Peroxide	13,978	4.48	93,562	2.80
Thermal	14,078	3.80	93,968	2.38
Photo	14,262	2.54	93,027	3.36

Table 11. Stastical comparison of previous method and present method.

Chromatographic parameter	Vasanthi *et al.*, 2017	Ramesh *et al.*, 2019
Method	RP-HPLC	RP-UPLC
Column	Symmetry C_{18} (250 × 4.6 mm, 5 µm)	BEH C_{18} (2.1 × 50 mm, 1.7 µ)
Flow rate	1.0 ml/minute	0.3 ml/minutes
Injection volume	20.0 µl	2µl
Retention time	Alogliptin: 2.234 minutes	Alogliptin: 0.403 minutes
	Pioglitazone: 3.294 minutes	Pioglitazone: 0.529 minutes
Resolution	3.56	6.95

2.234 minutes (alogliptin) and 3.294 minutes (pioglitazone) was observed, in newly developed RP-UPLC method the BEH C_{18} column (2.1 × 50 mm, 1.7 µ) was employed, the solutes are eluted rapidly and showed retention time at 0.403 minutes (alogliptin) and 0.529 minutes (pioglitazone), retention time difference between the two methods was noted of 2 minutes. In RP-UPLC method, mobile was prepared by ecofriendly chemicals such as 45 % of phosphate buffer (3.0 pH) and 55% of methanol, but in the reported RP-HPLC method, the mobile phase was buffer (pH 4.0) and acetonitrile with the ratio of 20:80%, it contains more organic phase then compared to the present method. When compared the stability indicating studies between both methods, in RP-HPLC method, when solution was placed in acid, base,

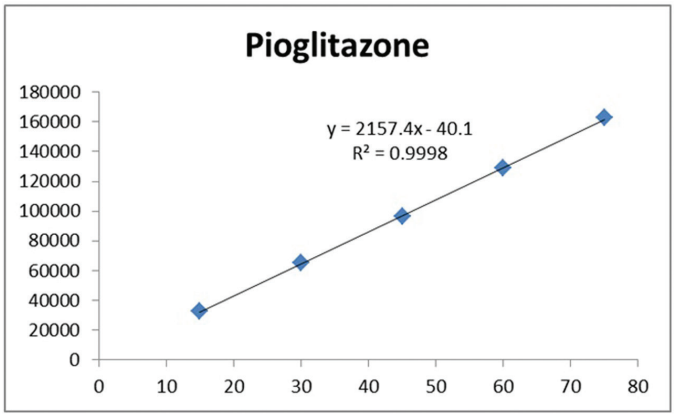

Figure 5. Linearity graph of pioglitazone.

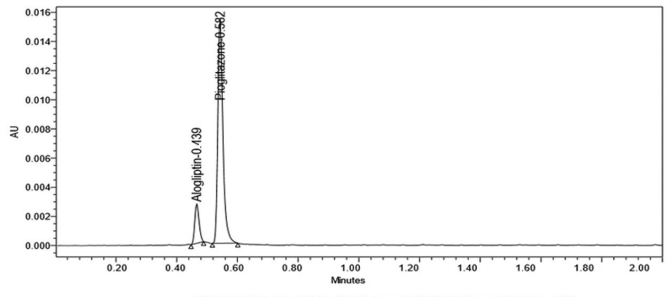

Figure 6. Less flow chromatogram of alogliptin and pioglitazone.

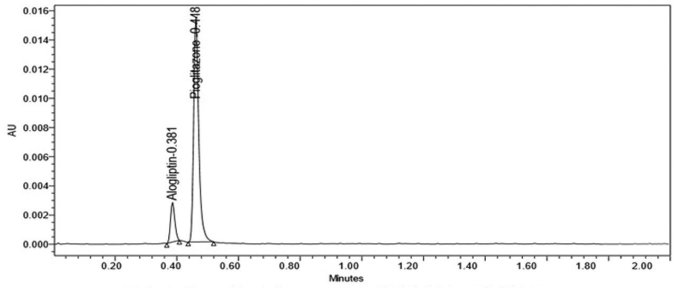

Figure 7. More flow chromatogram of alogliptin and pioglitazone.

thermal, oxidative, and photolytic stress conditions, it was stable in thermal and photolytic conditions. In acidic and basic conditions more amount of drug degraded. In the newly developed RP-UPLC method, there was not much amount of drug was degraded under selected stress conditions, such as acid, base, thermal, and photolytic. It was most stable in acidic conditions. In RP-HPLC method, high amount of sample was consumed because 20 µl of injection volume and flow rate was maintained at 1.0 ml/minute. But in the current RP-UPLC method, little amount of sample was consumed because 2 µl of injection volume and flow rate was maintained at 0.3 ml/minute. The advantages of RP-UPLC method was rapid analysis, faster elution when compared to the reported RP-HPLC methods. The data are shown in Table 11.

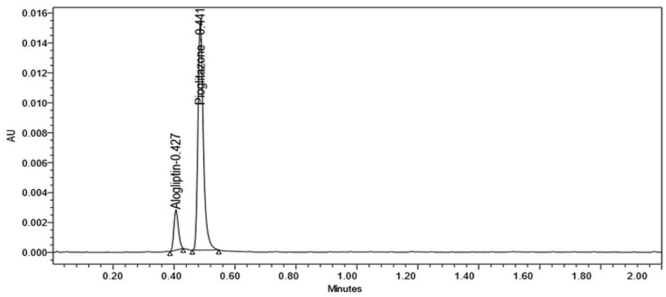

Figure 8. Less wave chromatogram of alogliptin and pioglitazone.

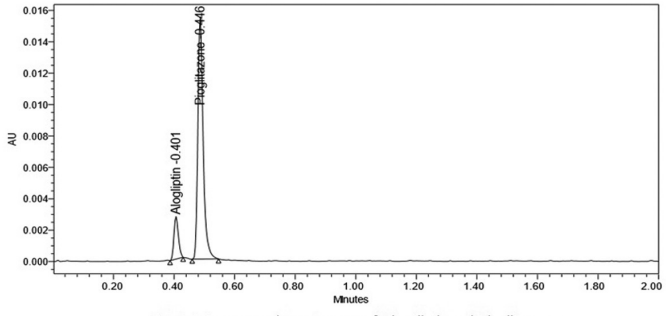

Figure 9. More wave chromatogram of alogliptin and pioglitazone.

CONCLUSION

In the present study, a new RP-UPLS method was developed for the simultaneous estimation of alogliptin and pioglitazone. The new method was validated according to the ICH guidelines. The method was consumed less solvents with high resolution and short run time was observed. When compared to reported RP-HPLC methods, the current RP-UPLC method was found to precise, accurate, and robust, and it can be used for the routine analysis of pharmaceutical formulations.

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CONFLICT OF INTEREST

All the authors declared that they have no conflict of interest.

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