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Research Article

FIRST ORDER DERIVATIVE AND DUAL WAVELENGTH SPECTROPHOTOMETRY METHODS DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ALOGLIPTIN AND PIOGLITAZONE IN BULK AND DOSAGE FORM

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ABSTRACT

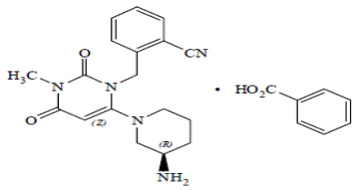
Simple, sensitive, rapid and accurate UV spectroscopic methods have been developed for the Simultaneous estimation of Alogliptin and Pioglitazone bulk and pharmaceutical dosage forms. First order derivative and Dual wavelength methods were developed and validated using solvent methanol. Both methods show linearity at 5-30 µg/ml. The first order derivative spectra of each solution were obtained. ZCP of Alogliptin was found 275.60 nm and ZCP of Pioglitazone was found 268.20 nm. The zero crossing point (ZCP) of Alogliptin at which Pioglitazone is measured and ZCP of Pioglitazone at which Alogliptin is measured. In Dual wavelength method, spectra two wavelengths 270.20 nm and 265 were selected as λ_1 and λ_2 for the estimation of Alogliptin. Pioglitazone shows the same absorbance at these wavelengths. Similarly, wavelengths 280 nm and 271 nm were selected as λ_3 and λ_4 for estimation of Pioglitazone. Alogliptin shows the same absorbance at these wavelengths.The methods were validated based on ICH guidelines. There are simple, sensitive, and reliable and results are reproducible for the routine analysis of Alogliptin and Pioglitazone.

Keywords: Alogliptin, Pioglitazone, Methanol and Validation parameter.

INTRODUCTION

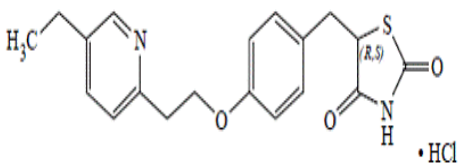
OSENI tablets contain 2 oral anti-hyperglycemic drugsWhich contain Alogliptin and Pioglitazone. Alogliptin is a selective, orally bioavailable inhibitor of the enzymatic activity of dipeptidylpeptidase-4 (DPP-4). Chemically, Alogliptin is prepared as a benzoate salt, which is identified as 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-3, 4-dihydropyrimidin- 1(2H)-yl} methyl) benzonitrilemonobenzoate. Its molecular formula and molecular weight are $C_{18}H_{21}N_5O_2 \bullet C_7H_6O_2$ and 461.51 respectively.

The structural formula is:



Alogliptin benzoate is a white to off-white, crystalline powder, containing one asymmetric carbon in the amino-piperidine moiety. It is soluble in dimethylsulfoxide, sparingly soluble in water and methanol, slightly soluble in ethanol, and very slightly soluble inoctanol and isopropyl acetate. Pioglitazone is an oral antihyperglycemic agent that acts primarily by decreasing insulin resistance. Chemically, pioglitazone is prepared as hydrochloride salt, which is identified as (±)-5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy] phenyl] methyl]-2, 4-thiazolidinedionemonohydrochloride. Molecular formula and molecular weight are $C_{19}H_{20}N_2O_3S$ HCland 392.90 respectively.

The structural formula is:



Pioglitazone hydrochloride is an odorless white crystalline powder that contains one asymmetric carbon in the thiazolidinedione moiety. The synthetic compound is a racemate and the two enantiomers of pioglitazone interconvert in vivo. It is soluble in N, N dimethylformamide, slightly soluble in anhydrous ethanol, very slightly soluble in acetone and acetonitrile, practically insoluble in water, and insoluble in ether.[1] Up to now there are methods developed on Pioglitazone. [2-20]but there are no methods developed on Simultaneous estimation of Alogliptin and Pioglitazone.

MATRIALS AND METHODS

Instrumentation, Reagents and Material

Jasco UV-1800 UV spectrophotometer, Alogliptin, Pioglitazone and Methanol

Marketed formulation

The commercial formulation Oseniin which eachcontains 25mg Alogliptin and 15mg Pioglitazone.

Preparation of standard solution

Preparation of standard stock solution of Alogliptin

Accurately weighed quantity of Alogliptin100 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with Methanol. This will give a stock solution having strength of 1000 µg/ml.

Preparation of working standard solution of Alogliptin

100 µg/ml of Alogliptinsolution was prepared by diluting 10 ml of stock solution to 100 ml with Methanol

Preparation of standard stock solution of Pioglitazone

Accurately weighed quantity of Pioglitazone 100 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with Methanol. This will give a stock solution having strength of 1000 µg/ml.

Preparation of working standard solution ofPioglitazone

100 µg/ml of Pioglitazonesolution was prepared by diluting 10 ml of stock solution to 100 ml with Methanol.

First order derivative method

Derivative Conditions

Mode:Spectrum, Scan speed:Medium, Wavelength range:200-400 nm,Initial base line correction:Methanol, Derivative order:1, the first order derivative spectra of each solution were obtained using smoothing$\Delta\lambda$ = 2.

Determination of wavelength for measurement

2.5 ml of working standard solution of Alogliptin(100 µg/ml) and 2.5 ml of working standard of Pioglitazone (100 µg/ml) was diluted to 10 ml with Methanol to get 25 µg/ml of Alogliptinand 25 µg/ml of Pioglitazone. Each solution was scanned between 200-400 nm. The first order derivative spectra of each solution were obtained.ZCP of Alogliptin was found 275.60 nm and ZCP of Pioglitazone was found 268.20 nm. The zero crossing point (ZCP) of Alogliptin at which Pioglitazone is measured and ZCP of Pioglitazone at which Alogliptin is measured, obtained from the overlain spectra of both. Which shown in figure no. 1.

Preparation of Calibration Curve

Calibration curve for Alogliptin(5-30 µg/ml)

Calibration curve for Alogliptin consisted of different concentrations of standard Alogliptin solution ranging from 5-30µg/ml. The solutions were prepared by pipetting out 0.5, 1, 1.5, 2, 2.5 and 3ml of the working standard solution of Alogliptin (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. The first derivative (D1) curve of each solution against the methanol was recorded. D1 absorbance at ZCP of Pioglitazone was measured and the plot of D1 absorbance vs. concentration was plotted. The straight-line equation was determined. And data was recorded in table no. 1 and figure no. 2- 3.

Calibration curve for Pioglitazone(5-30 µg/ml)

Calibration curve for Pioglitazone consisted of different concentrations of standard Pioglitazone solution ranging from 5-30 µg/ml. The solutions were prepared by pipetting 0.5, 1, 1.5, 2, 2.5 and 3ml of the working standard solution of Pioglitazone (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. The first derivative (D1) curve of each solution against the methanol was recorded. D1 absorbance at ZCP of Alogliptinwas measured and the plot of D1 absorbance vs. concentration was plotted. The straight-line equation was determined. And data was recorded in table no. 1 and figure no. 2- 4.

Validation of proposed method

Linearity

The linearity response was determined by analyzing independent levels of concentrations in the range of 5-30 and 5-30 µg/ml for Alogliptin and Pioglitazone respectively six times. Absorbance of each solution was measured at ZCP of Pioglitazone and Alogliptin respectively using developed method. Calibration curve of D1absorbance vs. concentration was plotted. The correlation coefficient and regression line equations for Alogliptin and Pioglitazonewere determined. Linearity of 6 concentrations were measured six times and recorded in table no. 2.

Precision

Repeatability

6 replicates of 5 µg/ml concentrations of Alogliptinand 5 µg/ml of Pioglitazonewere prepared and absorbance was measured at ZCP of Pioglitazone and Alogliptin respectively. SD and RSD were calculated and recorded in table no. 3.

Intraday Precision

Standard solutions containing 5, 15 and 20 µg/ml Alogliptin and 5, 15 and 20 µg/ml Pioglitazonewere analyzed 3 times on the same day. The absorbance of solutions was measured at ZCP of Pioglitazone and Alogliptin respectively. SD and RSD were calculated and recorded in table no. 4.

Interday precision

Standard solutions containing 5, 15 and 20 µg/ml Alogliptin and 5, 15 and 20µg/ml Pioglitazonewere analyzed 3 times on the three different days. The absorbance of solutions was measured at ZCP of Pioglitazone and Alogliptin respectively. SD and RSD were calculated and recorded in table no. 5.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (80, 100 and 120 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery of Alogliptin and Pioglitazoneby standard addition method.

Preparation of sample solution for % recovery*:*

An accurately weighed powder equivalent to about 100mg Alogliptin and 100mg Pioglitazone was transferred to 100 ml volumetric flask and the volume was made up to the mark using Methanol as solventand aliquate them to make final concentration 10 µg/ml Alogliptin and 10 µg/ml Pioglitazone. The resulting solution was filtered through Whatman filter paper. Absorbance of sample solutions was measured at selected wavelength of Alogliptin and Pioglitazoneand concentration is calculated which is known as pre-analyzed sample.

In pre-analyzed sample 80, 100 and 120 % of Alogiptin and Pioglitazonewas spiked. Absorbance of spiked samples was measured and total amount of drug was calculated and from which % recovery was calculated and recorded in table no. 6 &7.

Limit of Detection (LOD)

The LOD is estimated from the set of 6 calibration curves used to determine method linearity. The LOD may be calculated as;

LOD = 3.3 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Limit of Quantification (LOQ)

The LOQ is estimated from the set of 6 calibration curves used to determine method linearity. The LOQ may be calculated as;

LOQ = 10 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Which are shown in table no 8

Analysis of marketed formulation:

Twenty tablets were weighed and content crushed to obtain a fine powder. An accurately weighed powder equivalent to about 10 mg of Alogliptinwas transferred to 100 ml volumetric flask and the volume was made up to the mark using Methanol as solvent. The solution was sonicated for 20minutes. The solution was filtered through whatman Filter Paper No.42. First 2.5 ml of filtrate were discarded and was diluted to 10 ml with Methanol.Resulting solution contain 25 µg/ml Alogliptin and 15 µg/ml Pioglitazone. The absorbance of the resulting solution was measured at 268.20 nm for Alogliptin and 275.60 nm for Pioglitazone. The concentration of each drug was calculated using equation of straight line.This is shown in figure no. 5 and table no.9.

Development of dual wavelength method

Determination of wavelength for measurement

1 ml of working standard solution of Alogliptin (100 µg/ml) and 1 ml of working standard of Pioglitazone (100 µg/ml) was diluted to

10 ml with Methanol to get 10µg/ml of Alogliptin and 10 µg/ml of Pioglitazone. Each solution was scanned between 200-400 nm. From the overlayspectra two wavelengths 270.20nm and 265 wereselected as λ_1 and $\lambda_2$for the estimation of Alogliptin. Pioglitazone shows thesame absorbance at these wavelengths. Similarly,wavelengths 280 nm and 271 nmwere selected as λ_3 and λ_4 for estimation of Pioglitazone.Alogliptin shows thesame absorbance at these wavelengths which is shown in figure no. 6.

Preparation of Calibration Curve

Calibration curve for Alogliptin(5-30 µg/ml)

Calibration curve for Alogliptin consisted of different concentrations of standard Alogliptin solution ranging from 5-30 µg/ml. The solutions were prepared by pipetting out 0.5, 1, 1.5, 2, 2.5, and 3 ml of the working standard solution of Alogliptin (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. The absorbance of the solutions was measured at 270.20 nm and 265 nm against methanoland the plot of absorbance differences vs. concentration was plotted. The straight-line equation was determined and shown in table no 10and figure no. 7 and 9

Calibration curve for Pioglitazone**(**5-30µg/ml)

Calibration curve for Pioglitazone consisted of different concentrations of standard Pioglitazone solution ranging from 6-22 µg/ml. The solutions were prepared by pipetting out 0.5, 1, 1.5, 2, 2.5, and 3ml of the working standard solution of Pioglitazone (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. The absorbance of the solutions was measured at 280 nm and 271 nmagainst methanoland the plot of absorbance differences vs. concentration was plotted. The straight-line equation was determined and shown in table no. 10and figure no.8and10.

Validation of proposed method

Linearity

The linearity response was determined by analyzing independent levels of concentrations in the range of 5-30 and 5-30 µg/ml for Alogliptin and Pioglitazone respectively 6 times.. Absorbance of each solution was measured at selected wavelength respectively using developed method. Calibration curveof absorbance differences vs. concentration was plotted. The correlation coefficient and regression line equations for Alogliptin and Pioglitazonewere determined. Linearity of 6 concentrations were measured six times and recorded in table no.11

Precision

Repeatability

6 replicates of 5 µg/ml concentrations of Alogliptin and 5 µg/ml of Pioglitazonewere prepared and absorbance was measured at selected wavelength respectively. SD and RSD were calculated and recorded in table no. 2.312.

Intraday Precision

Standard solutions containing 5, 10 and 15 µg/ml Alogliptin and 5, 10 and 15 µg/ml Pioglitazonewere analyzed 3 times on the same day. The absorbance of solutions was measured at selected wavelength respectively. SD and RSD were calculated and recorded in table no.13.

Interday Precision

Standard solutions containing 5, 10 and 15 µg/ml Alogliptin and 5, 10 and 15 µg/ml Pioglitazonewere analyzed on 3 different days. The absorbance of solutions was measured at selected wavelength respectively. SD and RSD were calculated and recorded in table no.14

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drugto the pre analysedsample at 3 different concentration levels (80, 100 and 120 %) taking into consideration percentagepurity of added bulk drug samples. It was determined by calculating the recovery of Alogliptinand Pioglitazone by standard addition method.

Preparation of sample solution for % recovery

An accurately weighed powder equivalent to about 100 mg of Alogliptin and 100 mg of Pioglitazonewas transferred to 100 ml volumetric flask and the volume was made up to the mark using Methanol as solvent and aliquate them to make final concentration 10 µg/ml Alogliptinand 10 µg/ml Pioglitazone. The resulting solution was filtered through Whatman filter paper. Absorbance of sample solutions was measured at selected wavelength of Alogliptin and Pioglitazone and concentration is calculated which is known as pre-analyzed sample.

In pre-analyzed sample 80, 100 and 120 % of Alogliptin and Pioglitazone was spiked. Absorbance of spiked samples was measured and total amount of drug was calculated and from which % recovery was calculated and recorded in table no. 15&16.

Limit of Detection (LOD)

The LOD is estimated from the set of 6 calibration curves used to determine method linearity. The LOD may be calculated as;

LOD = 3.3 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Limit of Quantification (LOQ)

The LOQ is estimated from the set of 6 calibration curves used to determine methodlinearity. The LOQ may be calculated as;

LOQ = 10 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Which are shown in table no17

Analysis of marketed formulation:

Twenty tablets were weighed and content crushed to obtain a fine powder. An accurately weighed powder equivalent to about 10 mg of Alogliptin was transferred to 100 ml volumetric flask and the volume was made up to the mark using Methanol as solvent. The solution was sonicated for 20minutes. The solution was filtered through whatman Filter Paper No.42. First 2.5 ml of filtrate were discarded and was diluted to 10 ml with Methanol. Resulting solution contain 25 µg/mlAlogliptin and 15 µg/ml Pioglitazone. The absorbance of the resulting solution was measured at 270.20 nm & 265 nmfor Alogliptinand 280 nm & 271 nmfor Pioglitazone. The concentration of each drug was calculated using equation of straight line.This is shown in figure no. 11and table no.18.

RESULT AND DISCUSSION

First order derivative method

Selection of wavelength for simultaneous estimation of Alogliptin and Pioglitazone

1.5 ml of working standard solution of Alogliptin (100µg/ml) and 2.5 ml of working standard solution of Pioglitazone (100µg/ml) was pipette out into two separate 10 ml volumetric flask and volume was adjusted to the mark with Methanol to get 25µg/ml of Alogliptin and 25µg/ml of Pioglitazone. Each solution was scanned between 200-400 nm against methanol as a reagent blank for zero order spectra. The first order derivative spectra of each solution were obtained using smoothing ($\Delta\lambda$ = 2, Scaling Factor = 15). The zero crossing points were selected to be 275.60 nm and 268.20 nm for Alogliptin and Pioglitazone respectively. Wavelengths selected for quantitation

were 275.60 nm for Pioglitazone (Zero crossing point for Alogliptin) and 268.20 nm for Alogliptin (zero crossing point for Pioglitazone)

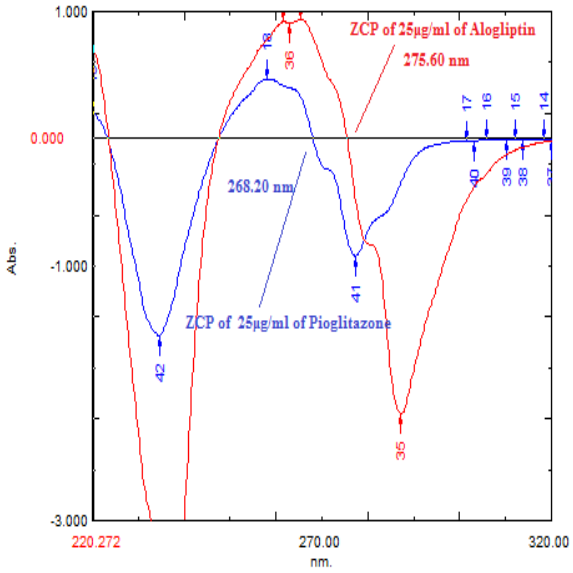


Fig. 1: First order UV spectra of Alogliptin and Pioglitazone showing selection of wavelength for detection

Standard curve

Table 1: Standard curve data for Alogliptin and Pioglitazone

Alogliptin at 268.20 nm		Pioglitazone at 275.60 nm	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
5	0.152	5	0.190
10	0.329	10	0.339
15	0.471	15	0.469
20	0.637	20	0.622
25	0.785	25	0.795
30	0.951	30	0.941

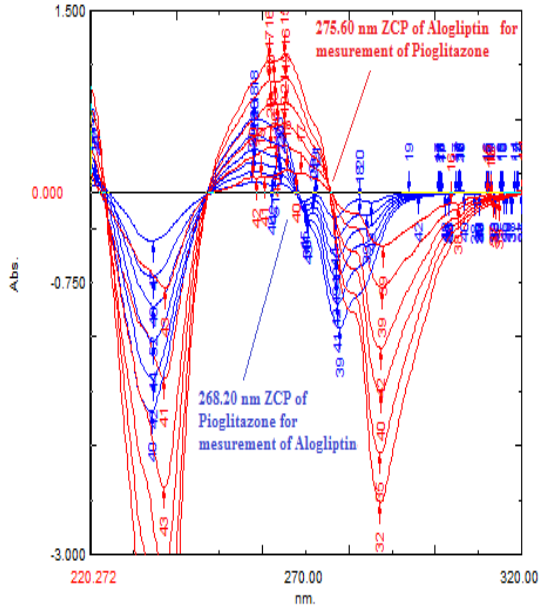


Fig. 2: Standard curve Spectra of Alogliptin and Pioglitazone showing selection of wavelength for detection

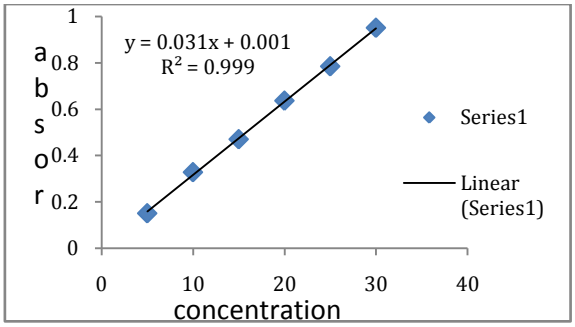


Fig. 3: STD cure for Alogliptin at ZCP of Pioglitazone 268.20 nm

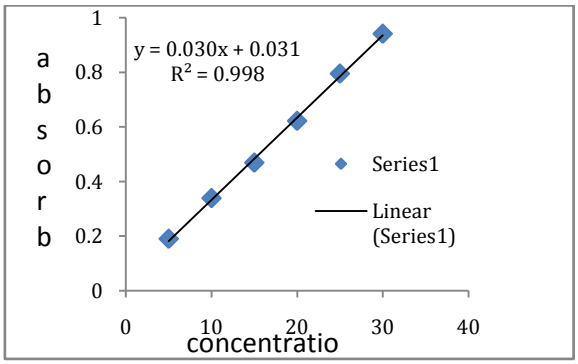


Fig. 4: STD cure for Pioglitazone at ZCP of Alogliptin 275.60 nm

STD curve result

Parameter	Alogliptin at 268.20 nm	Pioglitazone at 275.60 nm
Regression lineEquation	y = 0.0316x + 0.0013	y = 0.0301x + 0.0317
Correlation coefficient	0.9994	0.9985

Method Validation

Linearity

Table 2: Linearity data for Alogliptin and Pioglitazone

Alogliptin at 268.20 nm		Pioglitazone at 275.60 nm	
Concentration (µg/ml)	**D¹Absorbance Mean* ± S.D.**	**Concentration (µg/ml)**	**D¹Absorbance Mean* ± S.D.**
5	0.150±0.00216	5	0.190±0.003162
10	0.329±0.00371	10	0.339±0.003033
15	0.470±0.003312	15	0.469±0.003125
20	0.637±0.002503	20	0.620±0.002927
25	0.784±0.003312	25	0.793±0.002787
30	0.951±0.003327	30	0.941±0.003899

Table 3: Repeatability data for Alogliptin at 268.20 nm and Pioglitazone at 275.60 nm

Alogliptin at 268.20 nm		Pioglitazone at 275.60 nm	
Concentration (µg/ml)	**D¹ Absorbance**	**Concentration (µg/ml)**	**D¹ Absorbance**
5	0.152	5	0.191
5	0.149	5	0.187
5	0.153	5	0.192
5	0.15	5	0.193
5	0.148	5	0.19
5	0.147	5	0.187
Mean	0.149	**Mean**	0.190
SD	0.002317	**SD**	0.00253
%RSD	1.54	**%RSD**	1.33

*n=6

Discussion: The % RSD for Repeatability of both the drugs was found to be less than 2. So, it was concluded that proposed method for estimation of Alogliptin and Pioglitazone is précised in nature.

Precision

Repeatability

Intraday precision

CONCLUSION

The linearity range for Alogliptin was found to be in the range of 5-30 µg/ml and for Pioglitazone5-30 µg/ml. Correlation co-efficient for calibration curve of Alogliptin and Pioglitazone was found to be 0.9994 and 0.9985 respectively.

Table 4: Intraday precision data for estimation of Alogliptin and Pioglitazone

Alogliptin Concentration (µg/ml)	D¹Absorbance* ±S.D.	%RSD	Pioglitazone Concentration (µg/ml)	D¹Absorbance* ±S.D.	%RSD
5	0.149± 0.002517	1.68	5	0.191± 0.002	1.04
10	0.327± 0.002	0.61	10	0.340± 0.001528	0.44
15	0.470± 0.001528	0.32	15	0.470± 0.002	0.42

*n=3

Discussion: The % RSD for Repeatabilityof both the drugs was found to be less than 2.0, so, it was concluded that proposed method for estimation of Alogliptin and Pioglitazone is précised in nature

Interday precision

Table 5: Interday precision data for estimation of Alogliptin and Pioglitazone

Alogliptin Concentration (µg/ml)	Absorbance* ±S.D.	%RSD	Pioglitazone Concentration (µg/ml)	Absorbance* ±S.D.	%RSD
5	0.149±0.002517	1.68	5	0.190± 0.002517	1.32
10	0.326± 0.004	1.22	10	0.334± 0.005508	1.64
15	0.468±0.005033	1.07	15	0.468± 0.005033	1.07

*n=3

Discussion

The % RSD for Repeatability of both the drugs was found to be less than 2 so, it was concluded that proposed method for estimation of Alogliptin and Pioglitazone is précised in nature

Accuracy

Table 6: Accuracy (%Recovery) data for Alogliptin

Level of recovery	Sample amount (µg/ml)	amount added (µg/ml)	amount recovered (µg/ml)	% recovery	AVG	SD	%RSD
80%	10	8	7.90	98.75	99.5	1.520691	1.52
80%	10	8	7.88	98.5			
80%	10	8	8.10	101.25			
100%	10	10	9.96	99.6	98.9	0.754983	0.76
100%	10	10	9.81	98.1			
100%	10	10	9.90	99			
120%	10	12	11.87	98.91	99.60	0.998015	1.0
120%	10	12	11.90	99.16			
120%	10	12	12.09	100.75			

Discussion: Result obtained reveals that % recovery of Alogliptin was within acceptance criteria given in ICH guideline.

Table 7: Accuracy (%Recovery) data for Pioglitazone

Level of recovery	Sample amount (µg/ml)	amount added (µg/ml)	amount recovered (µg/ml)	% recovery	AVG	SD	%RSD
80%	10	8	8.05	100.62	99.29	1.610062	1.62
80%	10	8	7.98	99.75			
80%	10	8	7.80	97.5			
100%	10	10	10.01	101	99.96	0.896289	0.89
100%	10	10	9.95	99.5			
100%	10	10	9.94	99.4			
120%	10	12	11.90	99.16	99.21667	0.916315	0.92
120%	10	12	12.02	100.16			
120%	10	12	11.80	98.33			

Discussion*:* Result obtained reveals that % recovery of Pioglitazone was within acceptance criteria given in ICH guideline.

Limit of DetectionandLimit of Quantitation

Table 8: LOD and LOQ data for Alogliptin and Pioglitazone

Parameters	Alogliptin	Pioglitazone
Mean Slope (n=6)	0.031667	0.0301
SD (n=6)	0.001829	0.002992
LOD (µg/ml)	0.19	0.32
LOQ (µg/ml)	0.57	0.99

DISCUSSION

The proposed method can detect and quantify small amount of drugs with precisely. So, it was concluded that the proposed method is very sensitive in nature.

Analysis of marketed formulation

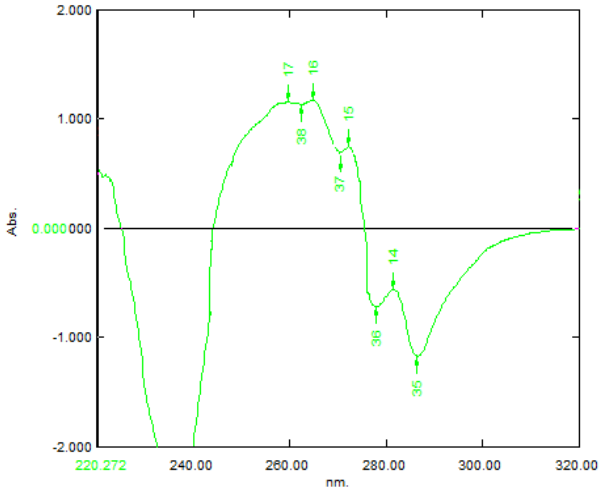


Fig. 5: First order Derivative Spectrum of Marketed formulation

Table 9: Analysis of marketed formulation

Brand name	Drugs	Label Claim (mg)	Amount Found (mg)	% Label Claim*
OSENI	Alogliptin	25	24.95	99.8
	Pioglitazone	15	14.92	99.46

Discussion*:* % Assay of Alogliptin and Pioglitazone was found in an acceptance limit so this method could be used for analysis of this combination.

Dual wavelength method

Selection of wavelength for simultaneous estimation of Alogliptin and Pioglitazone

By appropriate dilutions from the working standardsolutions of 100 µg/ml of Alogliptin and 100 µg/ml of Pioglitazone, thesolutions of Alogliptin (10 µg/ml) and Pioglitazone (10 µg/ml) werepreprepared respectively and scanned over the range of 200- 400 nm and the overlain spectra were observed fordevelopment of suitable method for analysis.

From the overlayspectra two wavelengths 270.20 nm and 265 nm wereselected as λ_1 and $\lambda_2$for the estimation of Alogliptin. Pioglitazone shows thesame absorbance at these wavelengths. Similarly,wavelengths 280 nm and 271 nm were selected as λ_3 and λ_4 for estimation of Pioglitazone.Alogliptin shows thesame absorbance at these wavelengths.

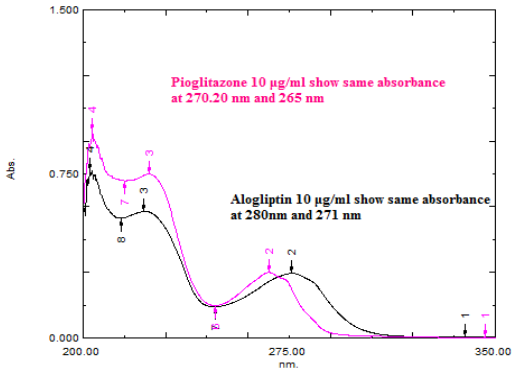


Fig. 6: Zero order UV spectra of Alogliptin and Pioglitazone showing selection of wavelength for detection

Standard curve

Table 10: STD curve for Alogliptin and Pioglitazone

Alogliptinat270.20 nm and 265 nm		Pioglitazone at 280 nm and 271 nm	
Concentration (µg/ml)	absorbance difference *	Concentration (µg/ml)	absorbance difference *
5	0.019	5	0.076
10	0.04	10	0.148
15	0.057	15	0.217
20	0.076	20	0.3
25	0.095	25	0.383
30	0.114	30	0.477

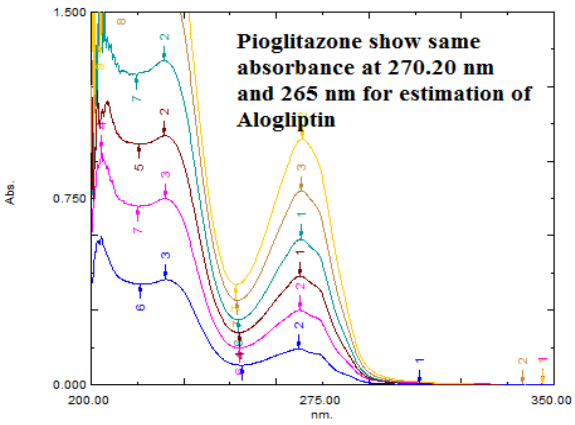


Fig.7: Spectra for Alogliptin and Pioglitazone for different concentration at 270.20 nm and 265 nm where, Pioglitazone has same absorbance and Alogliptin has different absorbance

CONCLUSION

The linearity range for Alogliptin was found to be in the range of 5-30 µg/ml and for Pioglitazone5-30 µg/ml. Correlation co-efficient for calibration curve of Alogliptin and Pioglitazone was found to be 0.999 and 0.998 respectively

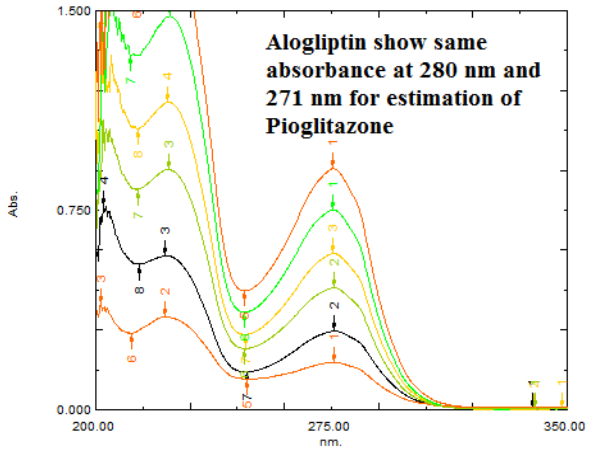


Fig. 8: Spectra for Alogliptin and Pioglitazone for different concentration at 280nm and 271 nm where, Alogliptin has same absorbance and Pioglitazone has different absorbance

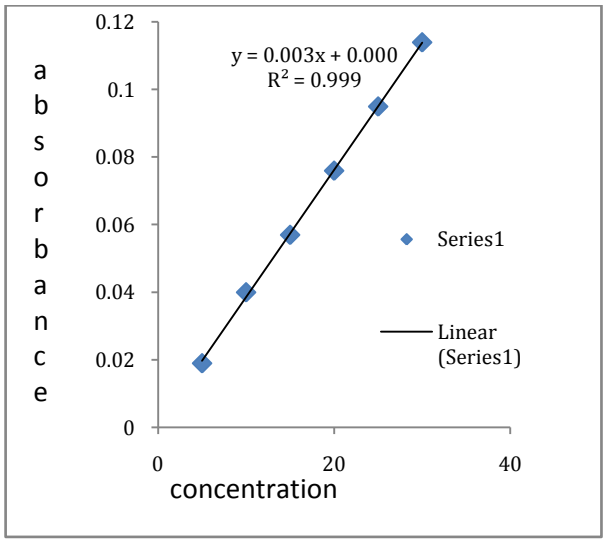


Fig. 9: Calibration curve of Alogliptin at 270.20-265 nm in Methanol

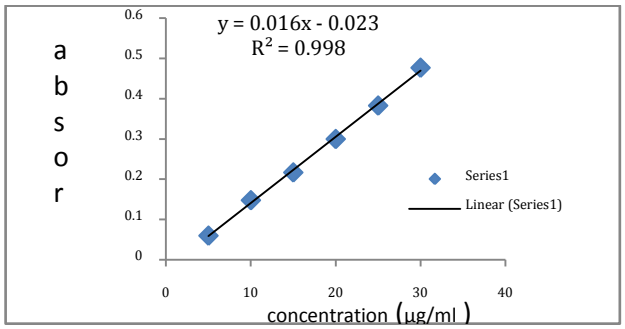


Fig. 10: Calibration curve of Pioglitazone at 280-271nm in Methanol

STD curve resultResult

Parameter	Alogliptin at 270.20-265 nm	Pioglitazone at 280-271nm
Regression lineEquation	y = 0.0038x + 0.0009	y = 0.0164x - 0.0231
Correlation coefficient	0.9995	0.9984

Validation

Linearity

Table 11: Linearity for Alogliptin and Pioglitazone

Alogliptin at 270.20-265 nm		Pioglitazone at 280-271nm	
Concentration (µg/ml)	**Mean absorbance difference *±SD**	**Concentration (µg/ml)**	**Mean absorbance difference *±SD**
5	0.018833 ± 0.000408	5	0.0605 ± 0.000837
10	0.040167 ± 0.000753	10	0.148 ± 0.000632
15	0.0575 ± 0.000548	15	0.217 ± 0.000632
20	0.075667 ± 0.000516	20	0.3 ± 0.000632
25	0.000548 ± 0.0057	25	0.383 ± 0.000632
30	0.000837 ± 0.0073	30	0.477333 ± 0.000516

Precision

I. Repeatability

Table 12: Repeatability data for Alogliptin at 270.20-265 nm and Pioglitazone at 280-271nm

Alogliptin at 270.20-265 nm		Pioglitazone at 280-271nm	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
5	0.04	5	0.147
10	0.04	10	0.147
15	0.039	15	0.148
20	0.04	20	0.149
25	0.04	25	0.148
30	0.041	30	0.147
Mean	0.04	**Mean**	0.147667
SD	0.000632	**SD**	0.000816
%RSD	1.57	**%RSD**	0.55

Discussion

The % RSD for Repeatabilityof both the drugs was found to be less than 2. So, it was concluded that proposed method for estimation of Alogliptin and Pioglitazone is précised in nature.

II. Intraday precision

Table 13: Intraday precision data for Alogliptin at 270.20-265 nm and Pioglitazone at 280-271nm

Alogliptin Concentration (µg/ml)	Absorbance* ± S.D.	%RSD	Pioglitazone Concentration (µg/ml)	Absorbance* ± S.D.	%RSD
10	0.041333±0.000577	1.4	10	0.147667±0.001155	0.77
15	0.056667±0.000577	1	15	0.216333±0.000577	0.26
20	0.077667±0.001155	1.48	20	0.300667±0.001155	0.38

*n=3

Discussion

The % RSD for Repeatabilityof both the drugs was found to be less than 2 so, it was concluded that proposed method for estimation of Alogliptin and Pioglitazone is précised in nature

III. Interday precision

Table 14:Interday precision data for Alogliptin at 270.20-265 nm and Pioglitazone at 280-271nm

AlogliptineConcentration (µg/ml)	Absorbance* ±S.D.	% RSD	Pioglitazone Concentration (µg/ml)	Absorbance* ±S.D.	% RSD
10	0.040333± 0.000577	1.43	10	0.147667± 0.000577	0.39
15	0.057±0.001	1.86	15	0.216±0.001	0.46
20	0.077±0.001	1.29	20	0.301±0.001	0.33

*n=3

Discussion

The % RSD for Repeatabilityof both the drugs was found to be less than 2so, it was concluded that proposed method for estimation of Alogliptin and is précised in nature

Accuracy

Table 15: Accuracy (%Recovery) data for Alogliptin

Level of recovery	Sample amount (µg/ml)	amount added (µg/ml)	amount recovered (µg/ml)	% recovery	AVG	SD	%RSD
80%	10	8	8.15	101.8	100.8	1.322	1.3
			7	7	876	1
80%	10	8	7.95	99.37			
80%	10	8	8.11	101.37			
100%	10	10	10.04	100.4	99.53333	3.372437	1.69
100%	10	10	9.72	97.2			
100%	10	10	9.80	98			
120%	10	12	12.03	100.25	99.41333	0.743124	0.74
120%	10	12	11.86	98.83			
120%	10	12	11.90	99.16			

Discussion

Result obtained reveals that % recovery of Alogliptin was within acceptance criteria given in ICH guideline.

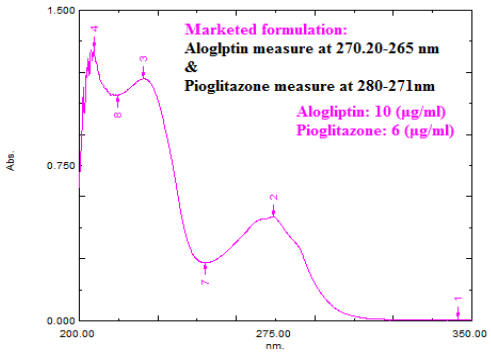


Fig. 11: Spectrum of Marketed formulation

Table 16: Accuracy (%Recovery) data for Pioglitazone

Level of recovery	Sample amount (µg/ml)	amount added (µg/ml)	amount recovered (µg/ml)	% recovery	AVG	SD	%RSD
80%	10	8	8.15	101.87	100.33	1.703614	1.69
80%	10	8	8.05	100.62			
80%	10	8	7.88	98.5			
100%	10	10	10.06	100.6	99.23333	1.350309	1.36
100%	10	10	9.92	99.2			
100%	10	10	9.79	97.9			
120%	10	12	11.83	98.58	99.47	1.136354	1.14
120%	10	12	12.09	100.75			
120%	10	12	11.89	99.08			

Discussion

Result obtained reveals that % recovery of Pioglitazone was within acceptance criteria given in ICH guideline.

Limit of DetectionandLimit of Quantitation

Table 17: LOD and LOQ data for Alogliptin and Pioglitazone

Parameters	Alogliptin	Pioglitazone
Mean Slope (n=6)	0.0038	0.016417
SD (n=6)	0.000408	0.000838
LOD (µg/ml)	0.35	0.16
LOQ (µg/ml)	1.07	0.51

Discussion

The proposed method can detect and quantify small amount of drugs with precisely. So, it was concluded that the proposed method is very sensitive in nature.

Analysis of marketed formulation

Table 18: Analysis of marketed formulation

BRAND NAME	Drugs	Label Claim (mg)	Amount Found (mg)	% Label Claim*
OSENI	Alogliptin	25	25.37	101.48
	Pioglitazone	15	15.55	103.66

Discussion

% Assay of Alogliptin and Pioglitazone was found in an acceptance limit so this method could be used for analysis of this combination.

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