Research Article ISSN: 0976-5700

Estimation of Pioglitazone Hydrochloride and Alogliptin Benzoate in Combination by Second Order Derivative Spectrophotometery Method

Dimal A Shah[1*], Unnati Gajjar[1], Khushboo Agarwal[1], Falgun A Mehta[1], Usmangani K Chhalotiya[2], Vandana B Patel[1]

[*1]Babaria Institute of Pharmacy, Varnama, Vadodara, Gujarat, India.

[2]Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Gujarat, India.

Abstract

Diabetes mellitus, a disease characterized by hyperglycaemia is spreading like epidemic in most of the developing and developed countries. A simple and accurate method for analysis of Pioglitazone Hydrochloride (PIO) and Alogliption benzoate (ALO) in their combination was developed using second order derivative spectrophotometry. PIO and ALO were quantified using second derivative responses at 267 nm and 278 nm prepared in methanol. The calibration curves were found to be linear in the concentration range of 10-30 µg/mL for both PIO and ALO. The method was validated as per ICH guideline and found to be accurate and precise. Developed method was successfully applied for the estimation of PIO and ALO in their synthetic mixture.

1. Introduction

Pioglitazone HCl (PIO) is chemically, (RS)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl) thiazolidine-2,4-dione. The chemical formula of PIO is C19H20N2O3S · HCl and a molecular weight of 356.4387 g/mole **(1-2)**. Pioglitazone is chemically thiazolidinedione's anti-diabetic drug. It acts as agonist for the peroxisome proliferator activated receptor (PPAR). It activates insulin responsive genes that regulate carbohydrate and lipid metabolism. It lowers the hepatic glucose production **(3-5)**. Alogliptin Benzoate (ALO) is chemically2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl}methyl)benzonitrile benzoate. It has chemical formula $C_{18}H_{21}N_5O_2$, $C_7H_6O_2$ and a molecular weight of 339.39164 g/mole **(6-7).** Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (incretin hormones) hormones may cause insulin release from the pancreatic β cells but they are inactivated by the DPP-4 enzyme. Alogliptin inhibit the DPP-4 enzyme and slows the inactivation of the incretin hormones. GLP-1 also lower glucagon secretion from pancreatic alpha cell, reducing hepatic glucose production **(6-7).** The clinical study proved that the combination of PIO and ALO is effective in reducing the Blood glucose level.

PIO is official in Indian Pharmacopoeia **(8).** A literature survey performed for quantitative analysis of these drugs showed that analytical methods have been reported for the estimation of PIO alone and in combination with other drugs by Spectrophotometric methods **(9-10)**, LC-MS **(11)** and HPLC **(12-30)**. Literature survey showed that Spectrophotometric method **(31-32)** and HPLC **(33-38)** have been reported for the analysis of ALO.

There is HPLC methods **(39-40)**, reported for the estimation of PIO and ALO in combination.

The present study involves development of second order derivative spectrophotometric method which is simple, less time consuming and provides accurate and precise result compared to chromatographic methods.

2. Experimental

2.1. Apparatus

2.1.1. Spectrophotometer

All the spectra and derivative responses were recorded on double beam UV-visible spectrophotometer (Model 1800, Shimadzu Ltd., Japan) containing two matched quartz cells with 1 cm path length.

2.1.2. Electronic balance

All the drugs and chemicals were weighed on Electronic balance (Model AUX220, Shimadzu Ltd., Japan).

2.2. Reagents and Materials

2.2.1. Pure samples

Pioglitazone HCl was obtained as a sample from Blue Cross Lab. Ltd, Thane, India and Alogliptin Benzoate was procured from Swapnaroop drugs and pharmaceuticals Ltd, Ahmedabad, India.

Chemicals and Reagents Methanol of analytical reagent grade (SD fine Chem. Ltd, Baroda, India) was used as a solvent in the developed method.

2.3. Second Order Derivative Spectrophotometric Method

2.3.1. Preparation of standard stock solutions

Accurately weighed 10 mg PIO and 10 mg ALO were transferred in to two separate 10 mL volumetric flask and dissolve in 5 mL of methanol and volume was made up the mark with methanol to yield 1000 µg/mL of both the solution of PIO and ALO. The solutions were further diluted with methanol to obtain final stock solution of 100 µg/mL of both PIO and ALO, respectively.

2.3.2. Selection of wavelengths

The stock solutions of PIO and ALO were diluted appropriately with methanol to obtain solution containing 10 µg/mL of both PIO and ALO. The spectra were scanned between 400-200 nm. These

zero order spectra of PIO and ALO were treated to obtain second order derivative response with delta lambda of 8 nm and scaling factor of 100.

The second order derivative the spectra of both the drugs were overlapped. The Zero crossing point (ZCPs) value of ALO at which the PIO showed derivative response were recorded and PIO was estimated. For the analysis of PIO, wavelength 267 nm was selected, where derivative response for ALO was zero. Similarly, 278 nm wavelength was used for estimation of ALO, where response for PIO was zero. Characteristic wavelengths (ZCPs) for PIO and ALO were confirmed by changing different concentration of both drugs.

2.3.3. Calibration curves for PIO and ALO

The calibration of PIO and ALO were prepared by using different dilutions. Aliquots were prepared from the working standard in different 10 mL of volumetric flasks and diluted with the methanol to obtain the calibration range of 10-30 µg/ mL of PIO and 10-30 µg/ mL of ALO. The spectra of calibration were analyzed between 400-200 nm and converted to obtain corresponding derivative second order spectra. The response was measured at 267 nm for analysis of PIO and 278 nm for analysis of ALO. Calibration curve were plotted and regression equations determined for both the drugs **(21)**.

2.4. Method Validation

Validation of developed method was carried out as per ICH guidelines41 and validation parameters like linearity and range, accuracy, precision, LOD and LOQ, specificity and robustness were studied by following procedure.

2.4.1. Linearity and range

The Linearity study was conducted by plotting calibration curve in the range of 10-30 µg/mL for PIO at 267 nm and 10-30 µg/mL for ALO at 278 nm. The calibration curve was plotted between concentration vs absorbance and regression equation was determined (n=5).

2.4.2. Precision

The inter day and intraday precision was carried out by estimation of the derivative responses at 3 times on the same day and on 3 different days. The 3 different concentrations (lowest, medium and highest) were studied and relative standard deviation (RSD) was determined.

The repeatability studies were performed by analysing PIO (20 µg/mL) and ALO (20 µg/mL) six times and RSD was determined.

2.4.3. Accuracy

The accuracy study was performed by recovery study using standard addition technique. The standard drug was added in to the sample solution in the three-different concentration level (80%, 100% and 120%). The sample was prepared in triplicate and analyzed. The mean % recovery and RSD was determined.

2.4.4. Ruggedness

The Ruggedness of the analytical method was checked by changing the experimental conditions like different analyst and reagent (methanol) supplier.

2.4.5. Specificity

Specificity of PIO and ALO were analyzed by using excipients like microcrystalline cellulose (9.5%), talc (0.27%) and magnesium stearate (0.63%) used for preparation of synthetic mixture. Amount of drug recovered was calculated and interference of excipients were noted.

2.4.6. Laboratory prepared mixtures

From the working standard different aliquots of PIO were taken in different 10 mL volumetric flasks and to the same flask aliquots of ALO stock solutions were added and volume was diluted up to the mark with the methanol to achieve the concentration of 0, 10, 20,30 and 30 µg/ mL of PIO and 0,10,20,30 and 30 µg/ mL of ALO. Scanned the spectra of solutions and second order derivative responses were recorded at 267 nm and 278 nm. The percentage of drug was estimated by regression equation of calibration curve.

2.5. Analysis of Synthetic mixture

Excipients [mannitol (44.69%), micro crystalline cellulose (9.5%), Croscarmellose (3.8%), magnesium stearate (0.63%), Hydroxy propyl methyl cellulose (0.24%), Hydroxy propyl cellulose (1.62%), Talc (0.27%) and standard drugs (PIO (20%), ALO (16.66%)) were weighed and mixed to obtain synthetic mixture. Powder equivalent to 10 mg of PIO (8.33 mg ALO) was weighed and taken in to 10 mL of volumetric flask. Methanol was added in to volumetric flask and flask was sonicated for 10 minutes. The solution was filtered using filter paper (what man) and volume was diluted up to the mark with methanol to yield 1000 µg/mL PIO (and 833 µg/mL ALO). One mL aliquot was taken from the above solution to make the solution 100 µg/mL PIO and 83.3 µg/mL ALO. Two mL aliquot from the above solution was taken in 10 mL volumetric flask and diluted up to the mark with methanol to obtain concentration of 20 µg/mL PIO and 16.6 µg/mL ALO. Solution was scanned between 400-200 nm and derivatized to obtained 2nd order derivative response.

3. Results and Discussion

3.1. Derivative spectrophotometric method:

The zero order overlaid spectra of PIO and ALO in methanol are shown in **Figure 1**. ALO significantly contributes to the absorbance of PIO at the maximum absorbance value so, derivative method was preferred for the estimation of PIO and ALO in presence of each other. The second order spectra (Derivative 2 - D2) of PIO and ALO showed a ZCPs of ALO at 267 nm, where PIO gives significant derivative response, while the D2 spectrum of PIO has zero absorbance at 278 nm where ALO gives significant derivative response **(Figure 2)**. The ZCPs of drugs remained constant and no shift was observed. Therefore, 267 nm was used for the analysis of PIO and 278 nm was used for the analysis of ALO.

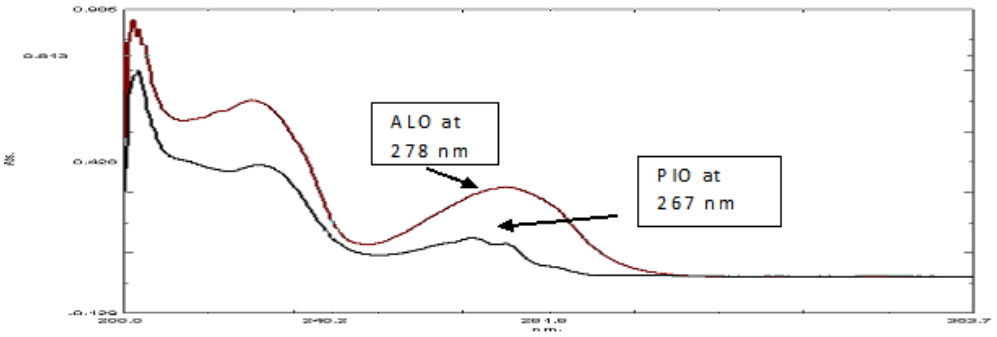

Figure 1. Overlaid zero order spectra of solutions of 10 µg/ml PIO and ALO.

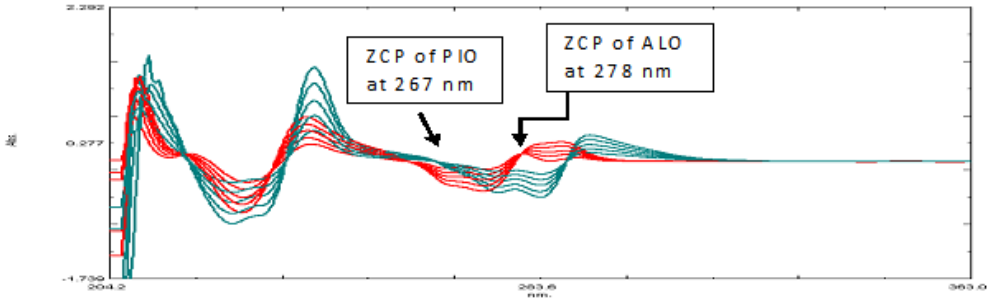

Figure 2: Overlaid second order derivative spectra PIO and ALO.

With increase in the concentration of PIO, the D2 response at 267 nm increased. The responses for PIO were found to be linear in the range of 10-30 µg/mL, with a correlation coefficient value of 0.997. Similarly, the derivative responses for ALO at 278 nm were linear in the range of 10-30 µg/ mL with correlation coefficient value of 0.998. The regression analysis of the calibration curves is shown in **Table 1.**

Table 1: Regression analysis of Calibration curve

Parameter	PIO	ALO
Linearity (µg/mL)	10-30	10-30
Correlation coefficient (r)	0.997	0.998
Slope of Regression	0.011	0.013
Standard deviation of slope	0.033	0.058
Intercept of Regression	-0.062	0.007

The recoveries of PIO and ALO were observed to be in the range of 97.65-101.01% and 97.69-101.28% respectively, which are satisfactory. Precision studies were carried out to study the intra-day and inter-day variability of the responses. The low RSD value showed the precision of

the method **(Table 2)**. Specificity study was performed by preparing synthetic mixture using excipient. Excipients did not interfere with the response of any of the drug. In ruggedness study, no significant change in the response was observed and the % recovery for ALO and PIO were observed to be more than 98 % for both ALO and PIO. The validation parameters are summarized in **Table 2**.

Table 2: Summary of validation parameters

Parameters	Pioglitazone HCl	Alogliptin Benzoate
Detection limit (µg/ml)	2	3
Quantitation limit (µg/ml)	10	10
Accuracy (%)	97.65-101.01%	97.69-101.28%
Precision (RSD[a], %)		
Intra-day precision (n=3)	0.690 - 1.273%	0.22 - 0.39%
Inter-day precision (n=3)	0.20 - 1.36%	0.39 - 0.80%
Repeatability study (n = 6)	0.31%	0.39%

aRSD is relative standard deviation and 'n' is number of determinations.

The proposed method was used for estimation of PIO and ALO in different laboratory prepared mixtures. The mean recoveries are as shown in Table 3, which indicate the suitability of method for the analysis of both the drugs. The mean recoveries indicate that the method is accurate and precise. The proposed method was used for the determination of PIO and ALO in their synthetic mixture. The results obtained were comparable with the corresponding labelled amounts **(Table 4)**.

Table 3: Determination of PIO and ALO in laboratory prepared mixtures by proposed first order derivative spectrophotometer and LC method.

Sr. No.	PIO		ALO	
	Amt. taken b	% Found± SDc	Amt. taken b	% Found ± SDc
1	30	99.00 ± 1.23	0	-
2	30	99.69 ± 0.64	10	99.20 ± 0.47
3	20	98.63 ± 1.22	20	98.46 ± 1.25
4	10	96.36± 0.74	30	99.23 ± 1.29
5	0	-	30	98.20 ± 0.93

b amount of drug taken in µg/ mL; c average of three determinations; SD is standard deviation.

Table 4: Analysis of synthetic formulation.

Synthetic mixture	Labelled Amount (mg)		% Recovery[d]	
	PIO	ALO	PIO	ALO
A	20	16.6	99.21 ± 0.50	98.26 ± 0.50

d mean value µ standard deviation of three determinations; Synthetic mixture contains 10 mg of PIO and 8.33 mg of ALO.

4. Conclusion

A derivative spectrophotometric (second order) method has been developed for the estimation of PIO and ALO in their synthetic mixture. The method was validated as per ICH guideline and found to be simple, accurate and precise. This method (second order spectrophotometric method) is having advantage that it is simple, requires less analysis time and the cost of analysis is less compare to reported chromatographic methods. The method was applied in the estimation of PIO and ALO in their combined synthetic mixture.

5. Acknowledgement

The authors are thankful to Blue Cross Laboratory, Mumbai, India and Swapnaroop drugs and pharmaceuticals Ltd, Ahmedabad, India for providing gift sample of PIO and ALO respectively.

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Citation: Shah DA, Gajjar U, Agarwal K, et al. Estimation of Pioglitazone Hydrochloride and Alogliptin benzoate in combination by Second Order Derivative Spectrophotometery method. International Research Journal of Pharmaceutical Sciences. 2017; 8: 001-010.