Research Article

Dual Wavelength Spectrophotometric Method for the Simultaneous Determination of Linagliptine and Pioglitazone in Synthetic Mixture

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical dual wavelength method for the simultaneous determination of Linagliptine and Pioglitazone sodium in synthetic mixture. The principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". Linagliptine was determined directly at 295.877 nm in methanol. The wavelengths selected for determination of Pioglitazone sodium were 295.877 nm and 237.380 nm in methanol. Regression analysis of Beer's plots showed good correlation in concentration range of 2-30 µg/ml for both the drugs. Lower limit of detection (LOD) for LINA and PIO were found to be 0.54 µg/ml and 0.64 µg/ml respectively. Lower limit of quantification (LOQ) for LINA and PIO were found to be 1.66 µg/ml and 1.95 µg/ml respectively. The % recovery was found to be 98.66% to 100.49% for Linagliptine whereas 98.98% to 100.58% for Pioglitazone. The results of analysis in terms of % label claim was 98.30% ± 0.13 for Linagliptine and 99.04% ±0.06 for Pioglitazone for a synthetic mixture.

Keywords: Linagliptine, Pioglitazone and Dual wavelength.

INTRODUCTION

Linagliptin is described chemically as 1H-Purine-2,6-Dione, 8-[(3R) -3-amino-1-piperidinyl] -7-(2-butyn-1- yl) -3,7-dihydro-3-methyl-1-[(4-methyl-2-quinazolinyl) methyl] -The empirical formula is $C_{25}H_{28}N_8O_2$[1]. Linagliptin is a white to yellowish or only slightly hygroscopic solid substance. It is very slightly soluble in water. Linagliptin is soluble in methanol, sparingly soluble in ethanol, very slightly soluble in isopropanol, and very slightly soluble in acetone.[2] Linagliptin is an oral drug that reduces blood sugar (glucose) levels in patients with type 2 diabetes. Linagliptin is a member of a class of drugs that inhibit the enzyme, DI peptidyl peptidase-4 (DPP-4). Linagliptin reduces blood glucose levels by inhibiting DPP-4 and increasing the levels of GLP-1 and GIP.[3] The chemical structure of Linagliptin is shown in fig. 1.

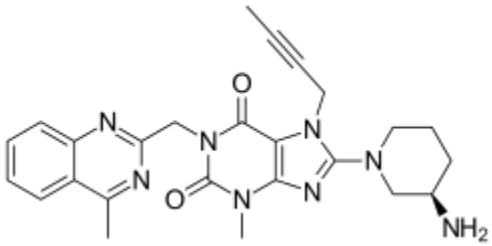

Fig. 1: Structure of Linagliptine

Pioglitazone is described chemically as 5-({4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}methyl)-1,3-thiazolidine-2,4-dione.The empirical formula is $C_{19}H_{20}N_2O_3S$. Pioglitazone is a white to yellowish or only slightly hygroscopic solid substance. Soluble in Methanol; Slightly soluble in ethanol; Very slightly soluble in acetone, acetonitrile; Practically insoluble in water [1,2]. PPARs are found in tissues like adipose tissue, skeletal muscle and liver, which are critical to

insulin action. Activation of PPAR-γ modulates the transcription of a number of insulin-responsive genes involved in the control of glucose and lipid metabolism.[4] The structural formula is shown in fig (2).

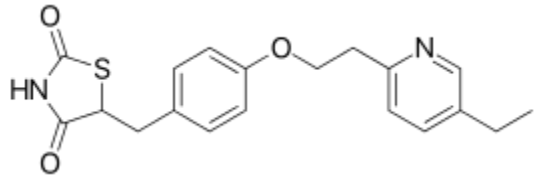

Fig. 2: Structure of Pioglitazone

MATERIAL AND METHODS

Instruments

A Shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Materials and Reagents

Linagliptin was kindly supplied as a gift sample from Torrent Research Centre, BHATT, Gandhinagar, Gujarat, India. and Pioglitazone was kindly supplied as a gift sample from Astron Research Ltd, Ahmedabad, Gujarat, India. Methanol (AR Grade, Finar Chemicals Ltd., Ahmedabad, India)

Preparation of Solutions

Preparation of standard stock solutions

Accurately weighed standard LIN (10 mg) and PIO (10 mg) powder was transferred to separate 100 ml volumetric flask and dissolve in methanol. The flasks were sonicated for 15 min. and diluted up to the mark with methanol to get (100 µg/ml) of standard stock solution of both the drugs (LIN and PIO).

Preparation of Working Standard Solutions

Linagliptine (100 µg/ml):Standard Stock solution (1 ml) was transferred to a 100 ml volumetric flask and diluted up to the mark with methanol.

Pioglitazone (100 µg/ml) :Standard Stock solution (1 ml) was transferred to a 100 ml volumetric flask and diluted up to the mark with methanol.

Dual Wavelength Method

The utility of dual wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the component of interest, independent of the interfering components. From the overlay of two drugs in figure-1, it is evident that direct determination of LINA at 295.877 nm (no absorbance of PIO at 295.877 nm). For estimation of PIO, two wavelengths selected (295.877nm and 237.380 nm) the LINA shows same absorbance whereas PIO shows significant difference in absorbance with concentration. Eight working standard solutions having concentration 2, 6, 10, 14, 18, 22, 26, and 30 µg/ml. the drugs were prepared separately in methanol and the absorbance at 237.380 nm and 295.877 nm were measured and absorptive coefficients were calculated using calibration curve. The concentration of two drugs in the mixture can be calculated using regression equation.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[19].

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2 to 30 µg/ml for both LINA and PIO. Accurately measured standard solutions of LINA AND PIO (0.2, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6, AND 3.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbances of the standard solutions were measured at 237.380 nm and 295.877 nm against methanol as blank.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (*n*= 6) for LINA and PIO (10 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method.

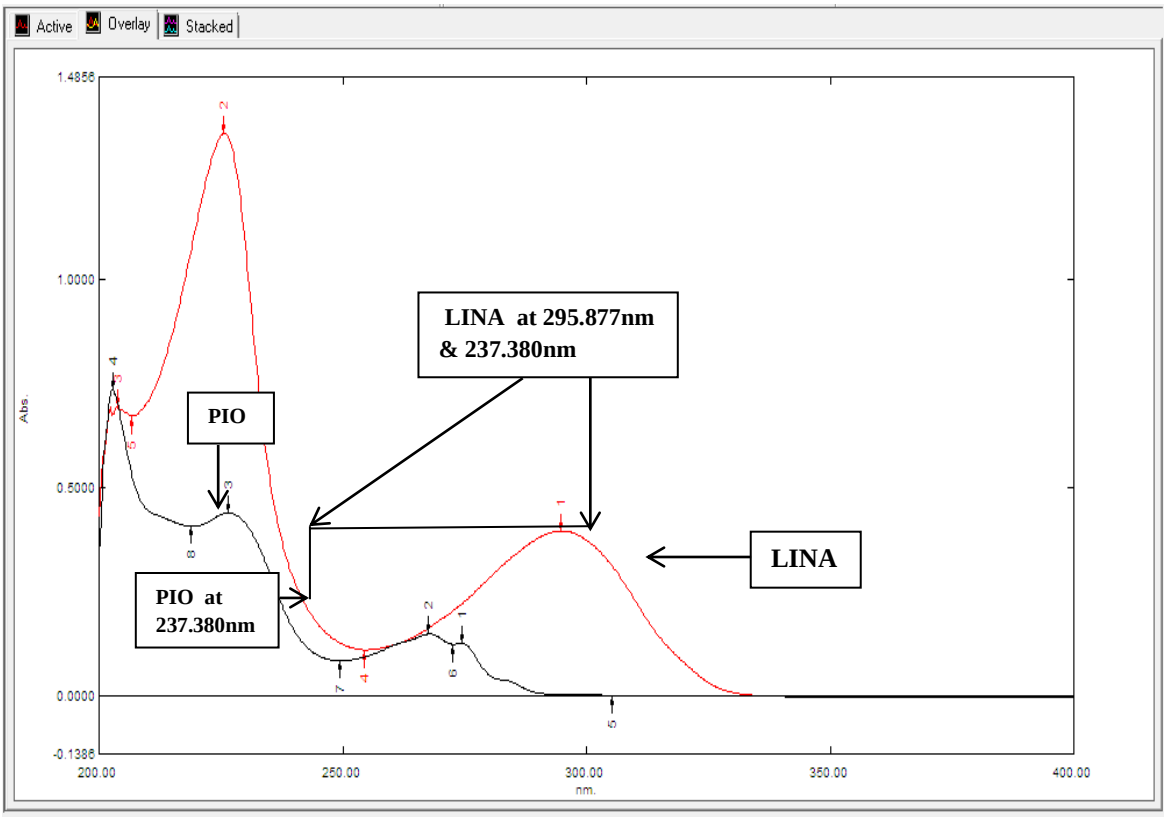

Fig. 3: Overlain absorption spectra of LINA (10µg/ml) and PIO (10µg/ml) in methanol

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of LINA and PIO (10,14,18 µg/ml). The result was reported in terms of relative standard deviation (% RSD) (Table-1)

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of LINA and PIO by the standard addition method. Known amounts of standard solutions of LINA and PIO were added at 80, 100 and 120 % level to prequantified sample solutions of LINA and PIO (2 µg/ml for LINA and 12 µg/ml for PIO). The amounts of LINA and PIO were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times.

Limit of detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[18].

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve. LOD and LOQ for Linagliptine were found to be 0.5482 µg/ml and 1.6612 µg/ml respectively. LOD and LOQ for Pioglitazone were found to be 0.6456 µg/ml and 1.9564 µg/ml.

Analysis of mixture

Amount of sample equivalent to 5 mg Linagliptine and 30 mg Pioglitazone was transferred in 100 ml volumetric flask, 15 ml of methanol was added , sonicated to dissolve and diluted up to mark. The solution was filtered with Whatman filter paper No.41 and filtrate was taken in 100 ml volumetric flask and dilute to mark with methanol then pipette out 1 ml solution in 10 ml volumetric flask and dilute up to

mark with methanol to get a final concentration of LINA (5 µg/ml) and PIO (30 µg/ml). The quantitative determination of Linagliptine is carried out by measurind absorbance at 295.877nm where Pioglitazone show zero absorbance. The quantitative determination of Pioglitazone is carried out by taking absorbance difference at 237.380 nm and 295.877 nm where linagliptine show same absorbance at both wavelength. The amounts of LINA and PIO present in sample solution were calculated by fitting the responses into regression equation for LINA and PIO in proposed method.

RESULTS AND DISCUSSION

Dual wavelength method was developed for the simultaneous spectroscopic estimation of LINA and PIO in synthetic mixture. Methanol was used as the solvent since both the drugs exhibit good solubility in it and no interference due to excipients of formulation were observed. In Dual wavelength method The diluted solutions were scanned over the wavelength range of 200 - 400 nm. From the overlain spectra, Linagliptine (LINA) at 295.877 nm and 237.380 nm give same absorbance but PIO have zero absorbance at 296 nm so, LINA directly measured at 295.877 nm and Pioglitazone(PIO) at the absorbance difference between 237.380 nm and 295.877 nm. For studying Beer's law, two series of different concentrations in range of 2-30 µg/mL for both LINA and PIO were prepared from stock solutions. The calibration curves were constructed at 295.877 nm and 237.380 nm respectively. The absorptivity (A1%, 1 cm) of both the drugs at both the selected wavelengths were determined. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.The recovery experiment was performed by the standard addition method. The mean recoveries were 100.49 ± 0.69and 98.98 ±0.99for LINA and PIO, respectively (Table 2).The results obtained for LINA and PIO were comparable with the corresponding labeled amounts in (Table 3).

CONCLUSION

The dual wavelenth method was developed for simultaneous determination of LINA and PIO in binary mixture. Method was found to be precise and accurate as can be reflected from validation parameters data. Developed method was efficiently applied for determination of LINA and PIO in pharmaceutical formulation and there for method can be extended for the regular QC analysis of both drugs in Synthetic mixture.

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Table 1: Summary of Validation Parameter

Parameters		LIN	PIO
Wavelength (nm)		295.877	237.380
Beer's law limit (µg /ml)		2-30	2-30
Regression equation (y = mx +c)		y= 0.0374x + 0.0167	y = 0.0201x + 0.0386
Correlation coefficient (r^2)		0.9986	0.9992
LOD (µg/ml)		0.5482	0.6456
LOQ (µg /ml)		1.6612	1.9564
Repeatability (% RSD, n = 6)		0.7142	1.1514
Precision (% RSD, n = 3)	Intraday	0.27-0.80	0.58-1.36
	Interday	0.27-0.76	0.84-1.36
Accuracy (% recovery, n = 3)		98.8-100.1	98.1-100.1
% Assay ± S.D. (n=6)		100.3 ± 1.40	99.3 ± 1.11

Table 2: Accuracy (% Recovery Study) data for LINA and PIO

DRUG	Level	Amount taken (µg/ml)	Amount added (%)	% Mean Recovery ± SD (n= 3)
LIN	I	2	80	99.26 ± 1.58
	II	2	100	98.66 ± 1.24
	III	2	120	100.49 ± 0.79
PIO	I	12	80	98.98 ± 0.39
	II	12	100	100.58 ± 0.28
	III	12	120	99.05 ± 0.73

Table 3: Estimation of Linagliptine and Pioglitazone in synthetic mixture

synthetic mixture	Labeled claim (mg/ml)		Amount found		% Labeled Claim ± S.D (n=3)	
	LINA	PIO	LINA	PIO	LINA	PIO
	5	30	5.02	29.14	100.4 ± 0.57	97.13 ± 0.75

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