WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 5, 931-939. Research Article ISSN 2277– 7105

ABSORBANCE CORRECTION SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF LINAGLIPTINE AND PIOGLITAZONE IN SYNTHETIC MIXTURE

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Article Received on
08 March 2017,

Revised on 26 March 2017,
Accepted on 16 April 2017

DOI: 10.20959/wjpr20175-8375

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ABSTRACT

A new, simple, accurate and sensitive UV - spectrophotometric absorption correction method has been developed for simultaneous determination of linagliptine and pioglitazone in synthetic mixture. Methanol was used solvents. The method is based upon determination of linagliptine at 295nm and pioglitazone 267nm, respectively. Beer's law obeyed the concentration range of 2- 30 µg/ ml for linagliptine and 10-38 for pioglitazone respectively. The percentage recovery was found in the range of 98.8-100.0% for Linagliptine and 98.1-100.1% for pioglitazone. The developed method was validated statistically and by recovery studies. The % RSD value was found to be less than 2. Thus the proposed method was simple, precise, economic, rapid and accurate and can be successfully applied for simultaneous determination of linagliptine and pioglitazone in synthetic mixture.

KEYWORDS: Linagliptine, pioglitazone, Absorbance Correction.

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.

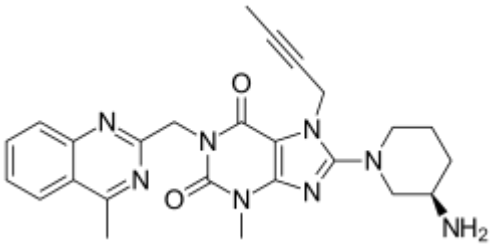

Figure 1: Structure of Linagliptine

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).

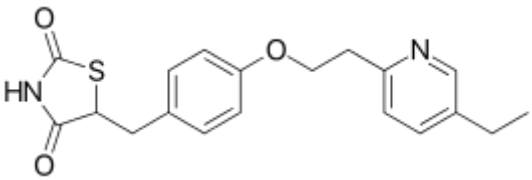

Figure 2: pioglitazone

MATERIAL AND METHODS

Instruments

A Shimadzu model 1700 (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.

Absorbance Correction Method

Standard solution of Linagliptine (10µg/ml) and Pioglitazone (10µg/ml) were scanned in uv range of 200 to 400 nm for determination of wavelength for estimation of Linagliptine and pioglitazone, From the overlain spectra of linagliptine and pioglitazone, the wavelength selected for the estimation of linagliptine was 295 nm, where pioglitazone has no significant absorbance. For estimation of pioglitazone it was 267 nm, where absorbance of pioglitazone is corrected.

$A = a * b * c$

$CX = A1 / a * b$

$CX = A1 / a1 * b$..... 1

$A2 = A (LINA) + A (PIO)$

$A2 = (a2 * CX * b) + (a3 * CY * b)$

$A2 = (a2 * CX) + (a3 * CY)$

$CY = [A2 - (a2 * CX)] / a3$..... 2

Where,

A1 = Absorbance of sample solution at 295 nm

A2 = Absorbance of sample solution at 267 nm

a1 = Absorptivity of Linagliptine at 295 nm

a2 = Absorptivity of Linagliptine at 267 nm

a3 = Absorptivity of Pioglitazone at 267 nm

METHOD VALIDATION

The developed method was validated with respect to linearity, accuracy, intraday and interday precision, limit of detection (LOD) and limit of quantitation (LOQ) and robustness in accordance with the ICH guideline.

Linearity

Linearity was determined over the range of 2 to 30 µg/ml for Linagliptine with correlation coefficient 0.9998 at 295 nm. Linearity of Pioglitazone was determined over the range of 10 to 38 µg/ml with correlation coefficient 0.9995 at 267 nm.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, Intraday precision and Interday precision.

Repeatability

Repeatability of the method was determined by analyzing mixed standard solution of Linagliptine and Pioglitazoneat (10 µg/ml and 18 µg/ml) 6 times without changing the parameters of measurement. The results are reported in terms of relative standard deviation (RSD) in Table-1.

Intermediate Precision

The intraday and inter day precision of the proposed method was performed by analyzing the corresponding responses three times on the same day(intraday) and on three different days (interday) over a period of one week for three different concentrations of standard solutions of Linagliptine and Pioglitazone. Result was showed in Table-1.

Accuracy

Accuracy was checked by recovery study at 3 different concentration levels, i.e., a multilevel recovery study. The tablet samples were spiked with an extra 80,100 and 120% of standard Linagliptine and Pioglitazone and the mixtures were analyzed by proposed method. Results of the recovery study are shown in table 4 suggested that method was accurate for the simultaneous estimation of LINA and PIO in synthetic mixture. Result was showed in Table-2.

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated:

LOD = 3.3 X σ/S

LOQ = 10 X σ/S

Where, σ = the standard deviation of the response,

S = slope of the calibration curve.

Estimation of Linagliptine and Pioglitazone in synthetic mixture

For the estimation of the Linagliptine and Pioglitazone in synthetic mixture Appropriate quantity equivalent to 5 mg LINA and 30 mg PIO was accurately weighed. The powder was transferred to 100 ml volumetric flask and shaken vigorously with methanol for 15 min and the solution was sonicated for 15 minutes and filtered through Whatman filter paper No. 41. Necessary dilutions are made with methanol to give final concentration 5µg/ml of LINA and 30 µg/ml PIO respectively. The absorbance's values were read at 267 and 295 nm and concentration was obtained by solving the absorption correction equations.

RESULTS AND DISCUSSION

An attempt has been made to develop a rapid, sensitive, economic, precise and accurate analytical methods for simultaneous estimation of LINA and PIO in synthetic mixture. The proposed methods are based on spectrophotometric absorption for the simultaneous estimation of LINA and PIO in UV region using methanol as solvent. Beer's law obeyed in the concentration range of 2-30 µg/ ml at 295 nm for LINA, 10-38 µg/ ml for PIO at 267 nm, considering wavelengths selected for method. The correlation coefficient values were found 0.9996, which shows that absorbance of all the drugs was linear with concentration.

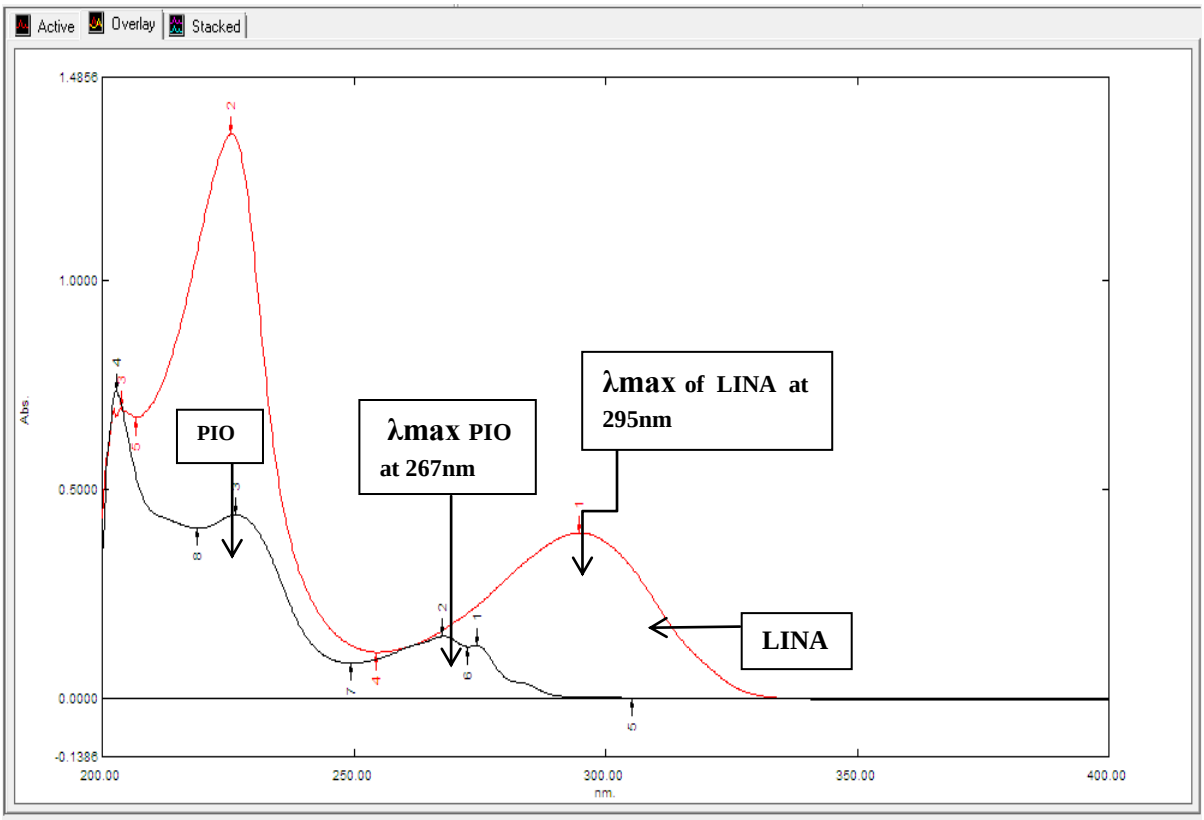

Figure 3: Overlain Spectrum of Linagliptine and Pioglitazone (10µg/ml)

Table 1: Regression data of LINA and PIO by Absorbance Correction Method

Parameters		**LIN (267nm)**	**PIO (295nm)**
Beer's law limit (µg /ml)		2-30	10-38
Regression equation (y = mx +c)		y= 0.0397x + 0.0033	y = 0.0188x – 0.029
Correlation coefficient (r^2)		0.9998	0.9995
LOD (µg/ml)		0.4499	3.2138
LOQ (µg /ml)		1.3634	9.73
Repeatability (% RSD, n = 6)		0.54	0.40
Precision (% RSD, n = 3)	Intraday	0.18-0.58	0.41-0.60
	Interday	0.27-0.76	0.17-0.32

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

Assay level (%)	**Amount taken in µg/ml**		**Amount Add in µg/ml**		**Amount recovered µg/ml**		**%Recovery of standard added**	
	LINA	PIO	LINA	PIO	LINA	PIO	LINA	PIO
80	2	12	1.6	9.6	3.60	21.20	100.19%	98.16%
100	2	12	2	12	3.99	23.80	99.93%	99.22%
120	2	12	2.4	14.4	4.34	26.64	98.80%	100.9%

Table 3: Estimation of Linagliptine and Pioglitazone in synthetic mixture.

synthetic mixture.	Labeled claim (mg/ml)		Amount found (mg/ml)		% Labeled Claim ± S.D (n=6)	
	LINA	PIO	LINA	PIO	LINA	PIO
	5	30	4.96	29.92	99.2 ± 0.013	99.73 ± 0.017

CONCLUSION

The absorbance correction 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.

ACKNOWALEDGEMENT

The authors wish to thank Torrent Research Centre, BHATT, Gandhinagar for providing LINA and PIO pure drug powder as gift sample from Astron Research Ltd, Ahmedabad And we are also hearty thankful to department of Quality assurance of Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University for providing permission and all the facilities to carry out the research work.

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