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

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ABSTRACT
A new, simple, accurate and sensitive UV - spectrophotometric absorpt ion correction method has been developed for simultaneous determinat ion 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_{8}O_{2}. 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. 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, DPP-4 (Dipeptidyl peptidase-4). Linagliptin reduces blood glucose levels by inhibiting DPP-4 and increasing the levels of GLP-1 and GIP.\textsuperscript{3} The chemical structure of Linagliptin is shown in fig. 1.

![Figure 1: Structure of Linagliptine](image)

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.\textsuperscript{1,2} PPARs are found in tissues like adipose tissue, skeletal muscle and liver, which are critical to insulin action. Activation of PPAR-\(\gamma\) modulates the transcription of a number of insulin-responsive genes involved in the control of glucose and lipid metabolism.\textsuperscript{4} The structural formula is shown in fig (2).

![Figure 2: pioglitazone](image)

**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 interday 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:

\[ \text{LOD} = 3.3 \times \sigma/S \]
\[ \text{LOQ} = 10 \times \sigma/S \]

Where, \( \sigma \) = 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.
Table 1: Regression data of LINA and PIO by Absorbance Correction Method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LIN (267nm)</th>
<th>PIO (295nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s law limit (µg /ml)</td>
<td>2-30</td>
<td>10-38</td>
</tr>
<tr>
<td>Regression equation (y = mx +c)</td>
<td>y = 0.0397x + 0.0033</td>
<td>y = 0.0188x – 0.029</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9998</td>
<td>0.9995</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.4499</td>
<td>3.2138</td>
</tr>
<tr>
<td>LOQ (µg /ml)</td>
<td>1.3634</td>
<td>9.73</td>
</tr>
<tr>
<td>Repeatability (% RSD, n = 6)</td>
<td>0.54</td>
<td>0.40</td>
</tr>
<tr>
<td>Precision (%) Intraday</td>
<td>0.18-0.58</td>
<td>0.41-0.60</td>
</tr>
<tr>
<td>RSD, n = 3</td>
<td>0.27-0.76</td>
<td>0.17-0.32</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Assay level (%)</th>
<th>Amount taken in µg/ml</th>
<th>Amount Add in µg/ml</th>
<th>Amount recovered µg/ml</th>
<th>%Recovery of standard added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LINA</td>
<td>PIO</td>
<td>LINA</td>
<td>PIO</td>
</tr>
<tr>
<td>80</td>
<td>2</td>
<td>12</td>
<td>1.6</td>
<td>9.6</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>12</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>120</td>
<td>2</td>
<td>12</td>
<td>2.4</td>
<td>14.4</td>
</tr>
</tbody>
</table>
Table 3: Estimation of Linagliptine and Pioglitazone in synthetic mixture.

<table>
<thead>
<tr>
<th>synthetic mixture.</th>
<th>Labeled claim (mg/ml)</th>
<th>Amount found (mg/ml)</th>
<th>% Labeled Claim ± S.D (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LINA</td>
<td>PIO</td>
<td>LINA</td>
<td>PIO</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>4.96</td>
<td>29.92</td>
</tr>
</tbody>
</table>

**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.

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