A simple, accurate and precise High Performance Liquid Chromatographic (HPLC) method has been developed for simultaneous determination of Pioglitazone and Alogliptin in bulk and dosage form. The method has been validated as per the guidelines of ICH. The separation is achieved on BDS hypersil C18, 250mm × 4.6mm, 5µ (particle size) column with flow rate 1.0 mL per minute in isocratic mode using Buffer pH 3.5. Methanol (70:30) as mobile phase. Column oven temperature is maintained at 25°C and observations are recorded at 271 nm. The linearity range was found to be in the range of 3.75-18.75μg/ml for Pioglitazone and 6.25-31.25 μg/ml for Alogliptin. Correlation coefficient for calibration curve of Pioglitazone and Alogliptin was found to be 0.9997 and 0.9993 respectively. The method is simple, accurate, reproducible and short and can be used for simultaneous analysis of Pioglitazone and Pioglitazone.

Pioglitazone benzoate is a white to off-white, crystalline powder, containing one asymmetric carbon in the aminopiperidine moiety. It is soluble in dimethylsulfoxide, sparingly soluble in water and methanol, slightly soluble in ethanol, and very slightly soluble in octanol 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-[(5-ethyl-2-pyridinyl)ethoxy]phenyl]-methyl}-2,4-thiazolidinedionemonohydrochloride. Its Molecular formula & Molecular weight are C19H20N2O5HCl & 392.90 respectively.

The structural formula is:

\[ \text{Pioglitazone benzoate} \]

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. (OSENI: http://www.rxlist.com/oseni-drug.htm) But up to now there is no HPLC methods develop for simultaneous
estimation Pioglitazone and Pioglitazone Analysis of the drug is important for development of drugs in their formulation and their use in therapies, for which we require standard analytical procedures. (Jajow Swapna et al., 2012; Effat et al., 2008; Vinod et al., 2011; Dhirender et al., 2011; Ramzia et al., 2012; Gadapa and Upendra, 2013; Madhukar et al., 2011; Lalit and Navpreet, 2011; Bhat et al., 2008; Sane et al., 2004; Karthik et al., 2008; Narasimha et al., 2011; Surendra and Sravani, Ravi, 2012; Sonali et al., 2012; Ramzia et al., 2012; Srinivasulu et al., 2010; Adukondalu et al., 2011; Pranshu et al., 2012). The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, Limit of quantitation, Linearity and range, Robustness (Ludwig, 2007; International Conference on Harmonisation, Guidance for industry in; Q2B Validation on Analytical Procedures: Methodology, 1996). As quality control process is not static some form of validation/verification should continue till the validated procedure is in use. It should not be a concept that once the method is initially developed and validated it is forgotten.

MATERIALS AND METHODS

Chromatographic methods offer an advantage in terms of sensitivity and selectivity. These methods can be used for routine analysis of dosage forms where two or more drugs are present together. HPLC method was developed for simultaneous estimation of Pioglitazone and Alogliptin.

Selection of Mobile Phase

After assessing the solubility of drugs in different solvents as well on the basis of literature survey, the standard solution of Pioglitazone and Alogliptin were injected into the HPLC system by using different solvent systems. Different mobile phases were tried in order to find the best conditions for the separation of both the drugs. It was found that Buffer pH 3.5: Methanol (70:30) give satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase was determined to be Buffer pH 3.5: Methanol (70:30) which show in Figure 1.

Selection of Detection Wavelength

The Detection of wavelength was done in UV SIMADZU 1800 instrument. The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study standard drug solutions of 10µg/ml Pioglitazone and 10µg/ml Alogliptin were, therefore, prepared in solvent mixtures of mixture of Buffer pH 3.5: Methanol (70:30) This drug solution was than scanned in the UV region of 200-400 nm and the spectrum was recorded 271nm which is shown in Figure 2.

Optimized Chromatographic Conditions

The HPLC used for the method was SPD-20AT UV DETECTOR to optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase pH, flow rate, and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor and column efficiency were calculated. The conditions that gave the best resolution, symmetry and capacity factor were selected for estimation. Buffer preparation (pH3.5), Mobile phase: Buffer pH 3.5: Methanol (70:30) Flow rate: 1.0 mL/min, Wavelength: 271nm, Column: BDS hypersil C18, 250mm × 4.6mm, 5µ(particle size) and Injection volume: 20 micro liter

Preparation of standard solutions

Preparation of mobile phase

700 ml of Buffer (pH 3.5) & 300ml Methanol were mixed and used as mobile phase. Use mobile phase as a diluents.

Preparation of STD Stock solution of Pioglitazone

Stock solution of Pioglitazone: 10mg of Pioglitazone was taken as working standard into a 100ml volumetric flask. Added 60ml mobile phase and dissolve, make up volume with mobile phase (100 µg/ml)

Figure 1. Selection of Mobile Phase
Preparation of STD Stock solution of Alogliptin

Stock solution of Alogliptin: 10mg of Alogliptin was taken as working standard into a 100ml volumetric flask. Add 60ml mobile phase and dissolve, make up volume with mobile phase (100 μg/ml)

Calibration curve for the 3.75-18.75μg/ml Pioglitazone and 6.25-31.25μg/ml Alogliptin

Appropriate volume of aliquots from standard Pioglitazone and Alogliptin stock solutions were transferred to same volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with mobile phase give a solution containing 3.75, 7.5, 11.25, 15 & 18.75 μg/ml Pioglitazone and 6.25, 12.5, 18.75,25 & 31.25 μg/ml Alogliptin. Each of these mixed standard solutions was chromatographed for 10 minutes run time using mobile phase at 271nm at flow rate of 1 ml/min. The graphs were plotted for peak area vs. concentration for both the drugs. Data is recorded in Table 1 and Figure 3, 4 and 5.

Validation of proposed HPLC method

Analysis of marketed formulation

Solution-1: Sample was taken equivalent to 10mg of Pioglitazone (10ml sample) into a 100ml volumetric flask. Added 60ml of mobile phase and shaken for 15 minutes to dissolve. Made up with mobile phase. Filtered this solution with 0.45micron membrane filter Solution-2: solution-1 was diluted into a 10ml volumetric flask to get 11.25 μg/ml Pioglitazone and 18.75 μg/ml Alogliptin.

The prepared sample solution was chromatographed for 10 minutes run time using mobile phase at 271nm at flow rate of 1 ml/min. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated by fitting peak area responses into the equation of the straight line representing the calibration curves for Pioglitazone and Alogliptin. And result shown in Table 2 and Figure 6.

Table 1. STD curve data for Alogliptin and Pioglitazone

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Pioglitazone</th>
<th>Alogliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.75</td>
<td>693.638</td>
<td>12.5</td>
</tr>
<tr>
<td>7.5</td>
<td>1387.276</td>
<td>12.5</td>
</tr>
<tr>
<td>11.25</td>
<td>1414.558</td>
<td>18.75</td>
</tr>
<tr>
<td>15</td>
<td>1464.079</td>
<td>25</td>
</tr>
<tr>
<td>18.75</td>
<td>1962.437</td>
<td>31.25</td>
</tr>
</tbody>
</table>

$y = 129.88x + 21.95$  
y = 98.374x + 31.162

Figure 2. Detection of wavelength 271nm

Table 2. Analysis of marketed formulation

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Label Claim (μg/ml)</th>
<th>Amount Found (μg/ml)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIOGLITAZONE</td>
<td>25</td>
<td>15.20</td>
<td>103.5</td>
</tr>
<tr>
<td>ALOGLIPTIN</td>
<td>25</td>
<td>25.44</td>
<td>101.7</td>
</tr>
</tbody>
</table>

Table 3. Summary of HPLC method for Pioglitazone & Alogliptin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pioglitazone</th>
<th>Alogliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength detection(nm)</td>
<td>3.75-18.75μg/ml</td>
<td>6.25-31.25μg/ml</td>
</tr>
<tr>
<td>Beer’s law limit (μg/ml)</td>
<td>STD CURVE</td>
<td></td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 129.88x + 21.95</td>
<td>y = 98.374x + 31.162</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.9997</td>
<td>0.9993</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>3.6661</td>
<td>9.4908</td>
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<tr>
<td>System suitability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retention time:4.3</td>
<td>Teoretical Plate: 453</td>
<td>Theoretical Plate: 7270</td>
</tr>
<tr>
<td>Tailing factor:1.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resolution:6.072</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solvent suitability</td>
<td>15μg/ml</td>
<td>25μg/ml</td>
</tr>
<tr>
<td>PRECISION</td>
<td>INTRA DAY</td>
<td>INTRA DAY</td>
</tr>
<tr>
<td>INTERDAY</td>
<td>658.06573</td>
<td>751.38665</td>
</tr>
<tr>
<td>Repeatability</td>
<td>101.64±0.4098</td>
<td>101.46±0.4098</td>
</tr>
<tr>
<td>ACCURACY</td>
<td>100.77±0.5371</td>
<td>100.09±0.4028</td>
</tr>
<tr>
<td>Robustness</td>
<td>11.25μg/ml</td>
<td>18.75 μg/ml</td>
</tr>
<tr>
<td>Flow rate +2</td>
<td>1348.2264</td>
<td>1544.6444</td>
</tr>
<tr>
<td>Flow rate -2</td>
<td>1297.7138</td>
<td>1481.0312</td>
</tr>
<tr>
<td>M.P. +2</td>
<td>M.P. +2</td>
<td>M.P. +2</td>
</tr>
<tr>
<td>pH +2</td>
<td>pH +2</td>
<td>pH +2</td>
</tr>
<tr>
<td>LOD</td>
<td>0.1398</td>
<td>0.5554</td>
</tr>
</tbody>
</table>

Figure 3, 4 and 5.
Figure 3. STD curve linearity

Figure 4. STD curve for Pioglitazone

Figure 5. STD curve for Alogliptin

Figure 6. Analysis of marketed formulation
Acceptance Criteria
The % RSD for area response obtained from six replicate injections of Standard solution should be ≤ 2.0 %, Tailing factor should be ≤ 2.0, Theoretical should be ≥ 2000 and Resolution should be ≥ 2.0 in Standard solution.

Solvent suitability
Recorded in Table 3.

Linearity
The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. The linearity response was determined by analyzing independent levels of concentrations in the range of 3.75-18.75μg/ml and 6.25-31.25μg/ml for Pioglitazone and Alogliptin respectively six times. Peak area of each solution was measured.

Precision
Repeatability
6 replicates of standard mixture solution having and Pioglitazone (15μg/ml) and Alogliptin (25μg/ml) were prepared and chromatograms were recorded and RSD was calculated and shown in Table 3.

Intraday precision
Standard solutions containing 3.75, 11.25 & 18.75μg/ml Pioglitazone and 6.25, 18.75 & 31.25 μg/ml Alogliptin were analyzed 3 times on the same day. Chromatogram of each sample was recorded. SD and RSD were calculated and shown in Table 3.

Interday precision
Standard solutions containing 3.75, 11.25 & 18.75μg/ml Pioglitazone and 6.25, 18.75 & 31.25 μg/ml Alogliptin were analyzed 3 times on three different days. Chromatogram of each sample was recorded. SD and RSD were calculated and shown in Table 3.

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 Pioglitazone and Alogliptin by standard addition method.

Preparation of sample solution for % recovery
An accurately weighed powder was transferred to 100 ml volumetric flask; dissolved and the volume was made up to the mark using mobile phase to prepare 3.75 μg/ml & 6.25 μg/ml Pioglitazone and Alogliptin respectively. The prepared sample solution was chromatographed for 10 minutes using mobile phase at flow rate of 1 ml/min. concentration of Pioglitazone and Pioglitazone was calculated which is known as pre-analyzed sample. In pre-analyzed sample 80, 100 and 120% of Pioglitazone and Pioglitazone was spiked. Chromatogram of each spiked solutions was taken and total amount of drug was calculated and from which % recovery was calculated. This is shown in Table 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
The LOD and LOQ are estimated from the set of 6 calibration curves used to determine method linearity.

LOD = 3.3 x (SD / Slope)
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. This is shown in Table 3.

Robustness

The robustness of an analytical method was carried out to confirm that the method remained unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The standard solution was injected five times for each varied conditions of flow, column temperature, pH, and mobile phase ratio and chromatograms were recorded in table no. 3 Change in Conditions for Robustness like Change in flow rate, M.P. and pH.

RESULTS AND DISCUSSION

High Performance Liquid Chromatographic (HPLC) method has been developed for simultaneous determination of Pioglitazone and Alogliptin in bulk and dosage form. The linearity range was found to be in the range of 3.75-18.75μg/ml for Pioglitazone and 6.25-31.25μg/ml for Alogliptin with using mobile phase Buffer pH 3.5: Methanol (70:30). Correlation coefficient for calibration curve Pioglitazone and Piogliptazin were found to be 0.9997 and 0.9993 respectively. The method is Accurate and precise which be used for simultaneous analysis of Pioglitazone and Alogliptin.

Acknowledgement

The author wishes to thanks mates who helped me lot for my work. And how can I forget U. Srinivas, my guide who suggests me in all way.

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