Simultaneous Spectrophotometric Determination of Pioglitazone Hydrochloride and Glimepiride in Tablets

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Two simple, accurate and precise methods for simultaneous estimation of pioglitazone hydrochloride and glimepiride in combined dosage form have been described. First method employs formation and solving of simultaneous equation using 268.6 nm and 227.6 nm as two analytical wavelengths. Second method is absorbance ratio method, 268.6 nm for estimation of pioglitazone hydrochloride and 251.5 nm, isobestic point, where two drugs shows equal absorptivity, for the estimation of glimepiride. Both the methods allow the simultaneous determination of pioglitazone hydrochloride and glimepiride in the concentration ranges employed for this purpose. The result of analysis has been validated statistically and by recovery studies and assay of tablets with standard deviation < 1.0 % was found.

Pioglitazone hydrochloride (PIO-H), a thiazolidinedione antidiabetic drug and chemically it is (±)-5-(4-(2-(5-ethyl-2-pyridinyl)ethoxy phenyl) methyl)-2,4-thiazolidinedione HCl. Glimepiride (GLIM) is a sulphonylurea antidiabetic drug and chemically it is trans-3-ethyl-2,5-dihydro-4-methyl-N-(2-((4 methyl cyclohexylamino)carbonyl)amino)sulfonyl)phenyl)ethyl-1,2-oxo-IH pyrrole)-carboxamide. The combination of pioglitazone hydrochloride (PIO-H) and glimepiride (GLIM) is newly introduced in market and used in the treatment of Type II DM (NIDDM). Both the drugs are non-official, reports are available for estimation of PIO-H by HPLC4 and GLIM by HPLC5 and derivative UV spectrophotometry8. But there is no evidence in literature for simultaneous estimation of these drugs in combination products. Hence, in the present investigation, a simple rapid and reproducible method was developed for simultaneous estimation of PIO-H and GLIM from their combined dosage forms.

UV/Vis double beam spectrophotometer, model SHIMADZU UV-2401 with 1 cm UV matched quartz cells were used. Gift samples of PIO-H were obtained from Wockhardt Research Centre, Aurangabad and GLIM from Glenmark Pharmaceuticals, Nashik. Tablet of brand
Piozone-G containing PIO (15 mg) and GLIM (2 mg) were procured from a local pharmacy store.

Standard stock solution was prepared by dissolving 25 mg of each in 25 ml of methanol (E. Merck) to get a concentration of 1 mg/ml. Standard solutions of 100 μg/ml each was prepared in methanol. From the overlain spectra two wavelengths 268.6 and 227.6 nm, were selected for the formation of simultaneous equation. For absorbance ratio method, 251.5 nm is the isobestic point and was taken as the wavelength for estimation of PIO-H and the wavelength used for estimation of PIO-H is 268.6 nm. The absorptivities E (1%, 1 cm) of both the drugs at both the wavelengths for the respective methods were determined. These calculated values were the mean of six independent determinations. Calibration curve was plotted between absorbance for both the drugs at both the wavelengths against the concentration. Six laboratory mixtures were prepared in the ratio 1:1 and dilution of 30 μg/ml was prepared. The absorbance of the solution was measured at 268.6, 227.6 and 251.5 nm. Quantitative estimation of these drugs were carried out by solving the simultaneous equations, 

\[ C_x = A_y \frac{A_y}{A_x} \text{ax}_2 \text{ay}_2 \ldots \ldots \ldots \ldots \ldots (1) \]

\[ C_y = A_x \frac{A_x}{A_y} \text{ax}_2 \text{ay}_2 \ldots \ldots \ldots \ldots \ldots (2) \]

where, \(A_1\) and \(A_2\) are absorbances of the mixture at 268.6 and 227.6 nm, respectively, \(a_x\) and \(a_y\) are absorbivities of x at 268.6 and 227.6 nm, respectively, Cx is the concentration of the PIO-H and Cy is the concentration of the GLIM.

Quantitative estimation of these drugs was carried out by absorbance ratio method by solving equations,

\[ C_x = \frac{(Q_x - Q_y)(Q_x - Q)}{(Q_x - Q)} \text{ax}_2 \text{ay}_2 \ldots \ldots \ldots \ldots \ldots (3) \]

\[ C_y = \frac{(Q_x - Q_y)(Q_x - Q)}{(Q_x - Q)} \text{ax}_2 \text{ay}_2 \ldots \ldots \ldots \ldots \ldots (4) \]

where, \(C_x\) and \(C_y\) are concentration of PIO-H and GLIM, respectively, \(Q_x\) is the ratio of absorbivity of PIO-H at 268.6 nm and 251.5 nm, \(Q_y\) is the ratio of absorbitivity of GLIM at 268.6 nm and 251.5 nm, \(a_x\) is the absorbitivity of pure PIO-H at isobestic wavelength, \(a_y\) is the absorbitivity of pure PIO-H at isobestic wavelength, \(Q_w\) is the absorbance ratio at 268.6 nm and 251.5 nm of mixture, and A is the absorbance of mixture at isobestic wavelength.

Twenty tablets of brand Piozone-G (Nicholas Piramal India Limited, Mumbai, label claim 15 mg of PIO and 2 mg of GLIM) were weighed, average weight determined and finely powdered. Appropriate quantity of powder from each tablet equivalent to 15 mg of PIO was accurately weighed and following Standard addition method, 13 mg of pure GLIM was added to achieve 1:1 ratio shaken vigorously for 15 min and filtered. Necessary dilutions of filtrate were made with methanol to give final concentration 30 μg/ml. The absorbances were read at 227.6, 268.6 and 251.5 nm and concentration was obtained by solving the simultaneous equations and absorbance ratio equations as shown in Table 1. Validation of proposed method was carried out by performing recovery studies by standard addition method in which preanalysed samples were taken and standard drug was added at three different levels. Results are shown in Table 1.

The overlain spectra of both drugs showed the \(\lambda_{max}\) of PIO-H at 268.6 nm and \(\lambda_{max}\) of GLIM at 227.5 nm. Both satisfies the criteria for simultaneous equation method of each drug should absorb at the \(\lambda_{max}\) of the other drug and both differ by 20 nm. Hence 268.6 nm was selected for estimation of PIO-H and 227.6 nm for estimation of GLIM. Absorbances were determined at both the wavelengths. Calibration curves were plotted and regression analysis was carried out. Both these drugs obeyed linearity individually and in mixture with the concentration range of 10-60 μg/ml with correlation coefficient \((r<1)\). The absorbitivity was then calculated and along with absorbance, values were submitted in the Eqns. 1 and 2 to obtain concentrations of drugs.

### Table 1: Assay Results of Pioglitazone and Glimepiride in Commercial Formulations by Method 1 and 2

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Statistical data</th>
<th>% Label claim</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method 1</td>
<td>Method 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PIO</td>
<td>GLIM</td>
</tr>
<tr>
<td>Marketed</td>
<td>Mean</td>
<td>99.41</td>
<td>100.18</td>
</tr>
<tr>
<td>formulation</td>
<td>SD</td>
<td>0.238</td>
<td>0.795</td>
</tr>
<tr>
<td>(Piozone-G)</td>
<td>CV</td>
<td>0.239</td>
<td>0.793</td>
</tr>
</tbody>
</table>

Method 1 is the simultaneous equation method while method 2 is absorbance ratio method. Values for recovery are mean for three determinations, SD is standard deviation, CV is coefficient of variation.

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Absorbance ratio method\textsuperscript{12} depends on the property that, for a substance which obeys Beer's Law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length. Quantitatively, absorbances are measured at two wavelengths one being the $\lambda_{\text{max}}$ of PIO-H at 268.6 nm for estimation of PIO-H, and the other being a wavelength of equal absorbity of two components i.e. isosbestic point 251.5 nm for estimation of GLIM. Both the methods were successfully used to estimate the amount of PIO-H and GLIM present in marketed tablet formulations containing PIO-H and GLIM. The assay values for tablets, by both the methods are in the range 99.4-99.6 % and 100.2-100.2 % for PIO and GLIM, respectively. The results obtained were comparable with the corresponding labeled amounts (Table 1).

By observing the validation parameters, accuracy, precision expressed as coefficient of variation (CV) ruggedness (interday, intraday, different analysts), specificity, linearity (correlation coefficient, $r^2<1$) and range, both the methods were found to be specific, accurate, precise, reproducible and rugged. Hence both the methods can be employed for routine analysis of tablets for assay.

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**Wound Healing Activity of Leaves of *Artocarpus heterophyllus***

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The ethanol extract of dried leaves of *Artocarpus heterophyllus* Lam. and different crude fractions such as petroleum ether (40-60°), butanol, butanone and methanol were tested for various phytoconstituents and screened for wound healing properties using incision, excision and dead space (granulation) wound models in albino rats. The methanol fraction had exhibited the most significant wound healing property followed by butanol, butanone fractions and ethanol extract, whereas petroleum ether fraction was least effective.

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