ISSN-2231-5012

Original Article

UV Spectrophotometric Absorption Correction Method for the Simultaneous Estimation of Pioglitazone HCl, Metformin HCl and Glibenclamide in Multicomponent Formulation

Seema M. Dhole¹, Pramod B. Khedekar², Nikhil D. Amnerkar³

¹ Department of Pharmaceutical Chemistry, J.L. Chaturvedi College of Pharmacy, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-440016, Maharashtra, India.
² University Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-440033, Maharashtra, India.
³ Department of Pharmaceutical Chemistry, Sharad Pawar College of Pharmacy, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-441110, Maharashtra, India

Email: seemadhole@gmail.com

Received 09 January 2013; accepted 22 January 2013

Abstract

The present paper describes simple, accurate, rapid, precise and sensitive UV spectrophotometric absorption correction method for the simultaneous determination of Pioglitazone HCl, Metformin HCl and Glibenclamide in combined tablet dosage form. Ethanol (95%) was used as solvent. The wavelengths selected for the analysis using absorption correction method were 237 nm, 268 nm and 300 nm for estimation of Metformin HCl, Pioglitazone HCl and Glibenclamide, respectively. Beer’s law obeyed in the concentration range of 3-30 µg/mL, 10-100 µg/mL and 1-10 µg/mL for Pioglitazone HCl, Metformin HCl and Glibenclamide, respectively. The mean percentage drug content for Pioglitazone HCl, Metformin HCl and Glibenclamide were found to be 99.48%, 99.77% and 99.35%, respectively and the % RSD value was found to be less than 2 which shows the precision of method. The developed method was validated statistically and by recovery studies. The high recovery and low coefficients of variation conforms the suitability of the method for simultaneous analysis of three drugs in combined tablets. Statistical analysis proves that the method was found to be suitable for the routine quality control analysis of Pioglitazone HCl, Metformin HCl and Glibenclamide in pure and pharmaceutical dosage forms.

Keywords: Pioglitazone HCl, Metformin HCl, Glibenclamide, Absorption correction method, Validation

Introduction

Pioglitazone hydrochloride (PIO), (±)-5-[p-[2-(5-ethyl-2-pyridyl)-ethoxy]benzyl]-2,4-thiazolidinedione hydrochloride is an oral antidiabetic agent used in the treatment of type 2 diabetes mellitus (Figure 1). PIO decreases insulin resistance in the periphery and liver, resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Literature survey reveals that chromatographic and spectroscopic methods are reported for its determination as an individual drug and in combination with other drugs in pharmaceutical formulations and in biological fluids [1-11]. Metformin hydrochloride (MET) chemically, N,N-dimethyl-imidodcarbonimidic diamide hydrochloride is an antidiabetic agent from the biguanide class used in the management of type 2 diabetes (Figure 2). It does not cause insulin release from the pancreas and does not cause hypoglycemia, even in large dose. It decrease hepatic glucose production, decrease intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. It predominant effect is to decrease fasting plasma glucose. Some methods have been reported in the literature for the estimation of MET in the presence of other drugs in formulations [12-19]. Glibenclamide(GLB),5-chloro-N-{2-4}[[[(cyclohexylamino) carbonyl]-amino][sulphonyl]-phenyl][ethyl]-2-methoxy benzamide is a potent, second generation oral sulfonylurea antiabetic agent widely used to lower blood glucose levels in patients with type 2 diabetes mellitus (Figure 3). It acts mainly by inhibiting ATP-sensitive potassium channels in pancreatic beta cells. This inhibition causes cell
membrane depolarization, which cause voltage dependent calcium channels to open, which causes an increase in intracellular calcium in the beta cell, which stimulates insulin release. The literature survey reveals that few methods are reported for estimation of GLB [20-25].

For many patients with type 2 diabetes, monotherapy with an oral antidiabetic agent is not sufficient to reach target glycemic goals and multiple drugs may be necessary to achieve adequate control. The fixed dose combination of PIO, MET and GLB showed significant efficacy in improving the glycemic control in type 2 diabetics. The present work describes a new simple UV spectrophotometric method for the simultaneous determination of PIO, MET and GLB in combined tablet dosage form. The developed method was validated as per ICH guidelines [26].

Materials and Methods

Reagents and chemicals
Pharmaceutically pure sample of PIO, MET and GLB were obtained as generous gifts from USV Lab. Pvt. Ltd., Mumbai, India. A combination of PIO (15 mg), MET (500 mg) and GLB (5 mg) in tablet formulation (Triglycomet, Tristar Formulations Pvt. Ltd., Mettupalayam, Puducherry, India) was obtained from local market commercially. All chemicals were of analytical grade and supplied by Merck Co, Mumbai, India.

Instrumentation
UV spectrophotometric analyses was carried out on Shimadzu 1700 Double beam UV-Vis spectrophotometer, with pair of 1.0cm matched quartz cells.

Experimental condition
According to the solubility characteristics, the common solvent for both the drugs was found to be ethanol (95%). Hence the stock solution was prepared in ethanol (95%) and further dilutions were made up with same solvent.

Preparation of standard stock solution
Accurately weighed quantity 10 mg of each PIO, MET and GLB were transferred in to 10 mL volumetric flask separately. Dissolved in ethanol (95%) and diluted to the mark with same solvent to obtained standard stock solution 1000 μg/mL of each drug.

Study of spectral and linearity characteristics
The aliquot portions of standard stock solutions of PIO, MET and GLB were further diluted with ethanol (95%) to get the concentration of 10 μg/ mL of each drug and the solutions were scanned between the range 400 - 200 nm in 1cm cell against blank and the overlain spectra was recorded. From the overlain spectrum of PIO, MET and GLB in ethanol (95%), it was observed that PIO and MET have zero absorbance at 300 nm, where as GLB has substantial absorbance. Thus GLB was estimated directly at 300 nm without interference of PIO and MET. At 268 nm, MET has zero absorbance. For estimation of PIO, the absorbance of GLB was measured at 268 nm using standard solution of GLB. The contribution of GLB was deducted from the total absorbance of sample mixture at 268 nm. The calculated absorbance was called as corrected absorbance for PIO. At 237 nm, these three drugs were showed the absorbance. To estimate the amount of MET, the absorbance of PIO and MET were corrected for interference at 237 nm by using absorptivity values. A set of three equations (Equation 1, Equation 2 and Equation 3) were framed using absorptivity coefficients at selected wavelengths.

Cz = A3/ax3          …(Eq. 1)
Cy = A2 – ax2 cx/ay2/ az3                   …(Eq. 2)
Cz =A3 – (ax2 cx + ay3cy)/ az3        …(Eq. 3)

Where,
A1, A2 and A3 are absorbance of sample solution at 300 nm, 268 nm and 237 nm, respectively.
ax1, ax2 and ax3, absorptivity coefficients of GLB at 300 nm, 268 nm and 237 nm, respectively.
ay2 and ay3, absorptivity coefficients of PIO at 268 nm and 237 nm, respectively.
az3, absorptivity coefficient of MET at 237 nm. cx, cy and cz are concentrations of GLB, PIO and MET, respectively in mixture.

For spectrophotometric method, the calibration curves for PIO, MET and GLB were prepared in the concentration range of 3-30 μg/mL, 10-100 μg/mL and 1-10 μg/mL, respectively at their respective wavelengths by diluting aliquot portions of standard stock solution of each drug.

Analysis of tablet formulation
Twenty tablets were weighed and their mean weight was determined. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 100 mg of MET was transferred to 50 ml volumetric flask and added a minimum quantity of ethanol (95%) to dissolve the substance and made up to the volume with the same. The solution was sonicated for 15 minutes and filtered through Whatman filter paper No. 42. An aliquot portion of obtained filtrate was diluted to 10 mL with ethanol (95%) to get final concentration within linearity range for analysis of PIO and GLB. From the clear solution, further dilution was made to obtain 50 μg/mL
solution of MET. The absorbance of sample solutions were measured at all selected wavelengths. The content of PIO, MET and GLB in sample solution of tablet was calculated. This procedure was repeated for six times.

**Method Validation**

The optimized UV spectrophotometric method was completely validated according to the procedure described in ICH guidelines and United State Pharmacopoeia for validation of analytical methods. The performance parameters evaluated for the method were linearity, precision, accuracy, limits of detection and quantitation, and ruggedness.

**Linearity:**

Linearity was studied by diluting standard stock solution at six different concentrations (n=3) covering the range of 3-30 µg/mL, 10-100 µg/mL and 1-10 µg/mL, for PIO, MET and GLB, respectively. Calibration curves with concentration verses absorbance were plotted for three drug at their respective wavelengths and the obtained data were subjected to regression analysis using the least squares method.

**Precision:**

The precision of the method was confirmed by repeatability parameter. The repeatability was performed by the analysis of formulation was repeated for six times with the same concentration. It was expressed as percent relative standard deviation (%R.S.D.) of series of measurements.

**Accuracy:**

To check the accuracy of the developed methods and to study interference of formulation additives, analytical recovery experiments were carried out by using standard addition method. Reference standard solution of each drug was added to tablet samples at three different concentrations level. At each level, samples were prepared in triplicate and the mean percentage recoveries and %R.S.D. value were calculated.

**Limit of detection and limit of quantitation:**

The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (4) and (5), respectively.

\[
\text{LOD} = \frac{3.3 \delta}{S}\quad \text{...(Eq. 4)}
\]

\[
\text{LOQ} = \frac{10 \delta}{S}\quad \text{...(Eq. 5)}
\]

where, δ: standard of y-intercept and S: slope of calibration curve.

**Ruggedness:**

The ruggedness test of analytical assay method is defined as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. In present study, determination of PIO, MET and GLB were carried by proposed method between different time intervals, days and analysts. The % R.S.D. was determined.

**Results and Discussion**

An attempt has been made to develop a rapid, sensitive, economic, precise and accurate analytical method for simultaneous estimation of PIO, MET and GLB in combined tablet dosage form. The proposed method is based on UV spectrophotometric absorption correction method for the simultaneous estimation of PIO, MET and GLB in UV region using ethanol 95% as solvent. The overlain spectra of PIO, MET and GLB are shown in Figure 4. Wavelengths 268 nm, 237 nm and 300 nm as absorption maxima were selected for estimation of PIO, MET and GLB, respectively. The absorptivity values at selected wavelengths are listed in Table 1. PIO and MET have zero absorbance at 300 nm, where as GLB has substantial absorbance. At 268 nm, MET has zero absorbance. For estimation of PIO, corrected absorbance was calculated at 268 nm due to the interference of GLB. At 237 nm, these three drugs were showed absorbance. To estimate the amount of MET, the absorbance due to PIO and GLB were corrected for interference at 237 nm by using their absorptivity values.

**Table 1: Absorptivity values**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Wavelength (nm)</th>
<th>Absorptivity values *</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIO</td>
<td>268</td>
<td>97.05</td>
</tr>
<tr>
<td>MET</td>
<td>237</td>
<td>477.55</td>
</tr>
<tr>
<td>GLB</td>
<td>300</td>
<td>46.70</td>
</tr>
</tbody>
</table>

* Average of five determinations.

**Method validation**

**Linearity**

A linear correlation was found between absorbance and concentrations of drugs. The regression analysis data are represented in Table 2. The regression coefficients (r²) obtained was higher than 0.99 for PIO, MET and GLB, which attest the linearity of the method.

**Table 2: Regression analysis data**

<table>
<thead>
<tr>
<th>Regression parameters</th>
<th>PIO</th>
<th>MET</th>
<th>GLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range (µg/ml)</td>
<td>3-30</td>
<td>10-100</td>
<td>1-10</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9992</td>
<td>0.9996</td>
<td>0.9993</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0102</td>
<td>0.0478</td>
<td>0.0068</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0038</td>
<td>0.0066</td>
<td>0.0003</td>
</tr>
</tbody>
</table>
**Precision**
Mean contents of PIO, MET and GLB in precision analysis (n=6) were much closer to labeled claim of respective drugs. The %R.S.D. value was lower than 2%, assure the precision of the method and the results are shown in Table 3.

**Table 3: Results of analysis of tablet formulation**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Labeled claim mg/tablet</th>
<th>Mean (%) (n=6)</th>
<th>Amount Estimated (mg)</th>
<th>S.D.</th>
<th>%R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIO</td>
<td>15</td>
<td>99.48</td>
<td>14.92</td>
<td>0.5350</td>
<td>0.5378</td>
</tr>
<tr>
<td>MET</td>
<td>500</td>
<td>99.77</td>
<td>498.85</td>
<td>0.6784</td>
<td>0.6800</td>
</tr>
<tr>
<td>GLB</td>
<td>5</td>
<td>99.35</td>
<td>4.96</td>
<td>0.7741</td>
<td>0.7792</td>
</tr>
</tbody>
</table>

S.D.: Standard deviation; R.S.D.: Relative standard deviation

Accuracy was investigated by means of recovery studies using the proposed method. The percent recoveries after spiking with additional standard drug afford recovery in the range of 98-102% and the results are listed in Table 4.

**Table 4: Result of recovery studies**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>% Amount added</th>
<th>% Recovery(n=3)</th>
<th>±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIO</td>
<td>80</td>
<td>99.73</td>
<td>0.8221</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.12</td>
<td>0.6850</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>99.31</td>
<td>0.5242</td>
</tr>
<tr>
<td>MET</td>
<td>80</td>
<td>99.66</td>
<td>0.6417</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.05</td>
<td>0.5363</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>99.43</td>
<td>1.0674</td>
</tr>
<tr>
<td>GLB</td>
<td>80</td>
<td>98.90</td>
<td>0.1405</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.82</td>
<td>0.7956</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>99.76</td>
<td>0.7061</td>
</tr>
</tbody>
</table>

S.D.: standard deviation.

**LOD and LOQ**
The LOD and LOQ were found to be 0.08 µg/ml and 0.29 µg/ml for PIO, 0.31 µg/ml and 0.94 µg/ml for MET and 0.21µg/ml and 0.68 µg/ml for GLB, respectively.

**Ruggedness:**
The standard deviation was calculated for each parameter and the % RSD were found to be less than 2%. The low values of the %RSD, as shown in Table 5 indicated the ruggedness of the method.

The data of summary of validation parameters are listed in Table 5.

**Table 5: Validation parameters of evaluated method**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PIO</th>
<th>MET</th>
<th>GLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range(µg/ml)</td>
<td>3-30</td>
<td>10-100</td>
<td>1-10</td>
</tr>
<tr>
<td>Precision, n=6 (%R.S.D.)</td>
<td>0.5378</td>
<td>0.6800</td>
<td>0.7792</td>
</tr>
<tr>
<td>Recovery, n=9 (%±S.D.)</td>
<td>99.39±0.3</td>
<td>99.38±0.3</td>
<td>99.49±0.51</td>
</tr>
<tr>
<td>LOD(µg/ml)</td>
<td>0.08</td>
<td>0.31</td>
<td>0.21</td>
</tr>
<tr>
<td>LOQ(µg/ml)</td>
<td>0.29</td>
<td>0.94</td>
<td>0.68</td>
</tr>
<tr>
<td>Ruggedness (n=3) (%R.S.D.)</td>
<td>0.425</td>
<td>0.663</td>
<td>0.391</td>
</tr>
<tr>
<td>Intraday</td>
<td>0.922</td>
<td>0.742</td>
<td>0.652</td>
</tr>
<tr>
<td>Analysts</td>
<td>0.693</td>
<td>0.5211</td>
<td>0.439</td>
</tr>
</tbody>
</table>


**Analysis of marketed formulation**
The proposed validated method was successfully applied for determination of PIO, MET and GLB in their combined dosage form. The results of analysis of pharmaceutical dosage form by the proposed method (Table 1), expressed as percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets.

**Conclusions**
UV spectrophotometric absorption correction method was developed and validated for the determination of PIO, MET and GLB in combined tablet. The developed method was found to be simple, specific, rapid, precise and accurate from the results of validation parameters. Hence the proposed method could be effectively applied for the routine quality control analysis of PIO, MET and GLB in bulk and pharmaceutical dosage form.

**Acknowledgments**
Authors are thankful to the Manager, USV Lab. Pvt. Ltd., Mumbai, India for providing the gift samples of drugs of PIO, MET and GLB, respectively and also thankful to Dr. K. P. Bhusari, Principal, Sharad Pawar College of Pharmacy, Nagpur for providing experimental facilities for this work.

**References**


Source of support: Nil; Conflict of interest: None declared