Simultaneous Estimation of Flurbiprofen and Gatifloxacin by Dual Wavelength UV Spectroscopy Method in an Eye Drops

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ABSTRACT

The aim of the present work is to develop and validate a simple, fast and reliable UV method for the determination of Flurbiprofen and Gatifloxacin in pharmaceutical combined dosage form. The principle of dual wavelength method is used for determination of single wavelength of Flurbiprofen (246nm and 268nm). For Gatifloxacin single wavelength 325nm is appropriate. The method was validated under the range from 0.3-1.5µg/ml and 3-15 µg/ml for Flurbiprofen and Gatifloxacin respectively. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonisation (ICH), to determination of both drugs in pharmaceutical dosage form. The suitability of this method checked by quantitative determination of Flurbiprofen and Gatifloxacin. Validation parameters like Linearity, Accuracy, Precision and Recovery study were undertaken. Considering the possible worldwide development of counterfeit pharmaceutical dosage forms, the proposed method could be useful for the quality control laboratories in developing countries.

Keywords: Flurbiprofen, Gatifloxacin, UV- Visible Spectroscopy, Validation.

INTRODUCTION

Flurbiprofen\(^1\), 3-[(1R)-1-hydroxy-2-(methyl amino) ethyl] phenol hydrochloride Flurbiprofen, a nonsteroidal anti-inflammatory agent (NSAIA) of the propionic acid class, is structurally and pharmacologically related to fenoprofen, ibuprofen, and ketoprofen and has similar pharmacological actions to other prototypica NSAIs. Flurbiprofen exhibits anti inflammatory, analgesic, and antipyretic activities while gatifloxacin\(^2\) 1-cyclopropyl-6-fluro-8methoxy-7-[3-methylpiperaizne-1yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. It should be used only to treat or prevent infections. No simultaneous Spectrophotometric method has been reported for both the components in a combined dosage forms. The aim of this paper was to develop Dual wavelength spectroscopy method for estimating Flurbiprofen and Gatifloxacin in their mixture form.

Figure 1: Chemical structures of (A) Gatifloxacin (B) Flurbiprofen

MATERIALS AND METHODS

Shimadzu UV-1700 a double beam spectrophotometer, connected to a computer loaded with Shimadzu UV Probe 2.34 software was used for all the Spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1 cm quartz cells over the range of 200-400 nm. Pure drug sample of Flurbiprofen and Gatifloxacin was kindly gifted by Provizer Pharma, surat, Gujarat.

Methods

Preparation of standard solutions\(^3,4\)

Gatifloxacin\(^3\) stock solution: Accurately weighted 10 mg Gatifloxacin was taken in 10 ml volumetric flasks and then diluted with mixture of distilled water and methanol (40:60) up to the mark (1000 µg/ml). 1ml of this solution was transferred in 10 ml volumetric flask and diluted up to mark with solvent mixture (100 µg/ml).

Gatifloxacin working solution: 0.3, 0.6, 0.9, 1.2 and 1.5 ml of resultant solution was transferred in 10 ml volumetric flask and diluted up to mark with solvent mixture to get concentrations of 3, 6, 9, 12 and 15 µg/ml respectively.

Flurbiprofen\(^4\) stock solution: Accurately weighted 100 mg Flurbiprofen was taken in 100 ml volumetric flask and then diluted with solvent mixture water: methanol (40:60) up to the mark (1000 µg/ml). 10 ml of this solution was transferred in 100 ml volumetric flask and diluted up to mark with solvent mixture (100 µg/ml). Now again 1ml of this solution was transferred in 10ml volumetric flask and diluted up to mark with solvent mixture (10 µg/ml).

Flurbiprofen working solution: 0.3, 0.6, 0.9, 1.2 and 1.5 ml of resultant solution was transferred in 10 ml volumetric flask and diluted up to mark with solvent mixture to get concentrations of 0.3, 0.6, 0.9, 1.2 and 1.5 µg/ml respectively.
Selection of $\lambda_{\text{max}}$

The absorption spectra of the solutions of Flurbiprofen and Gatifloxacin were recorded in the range of 200-400 nm zero order spectra (figure 6.1, 6.2) and converted to Dual wavelength method.

Detection of Flurbiprofen at $\lambda=246$ and 268nm where at these two wavelength shows absorbance difference of Gatifloxacin is almost zero.

Similarly detection of Gatifloxacin at $\lambda=325$ where Flurbiprofen shows zero absorbance, (Figure 6.3, 6.4) to construct two separate calibration curves for both the drugs. Method showed good linearity in concentration range 0.3-1.5 ppm and 3 – 15 ppm respectively for both drugs.

Dual wavelength method

This method is applicable to calculate the concentration of component of interest found in a mixture containing it along with some unwanted interfering component. The absorbance difference between two points of the mixture spectra is directly proportional to the concentration of the analyte irrespective of the interferent. In this method two wavelengths were selected ($\lambda_1$, $\lambda_2$) where the drug A showing equal absorbance (or difference between absorbance is zero) and drug B showing some response. Then different concentrations of drug A and drug B are prepared to confirm that at all different concentrations of drug A the difference between absorbance at two selected wavelengths ($\lambda_1$, $\lambda_2$) remain zero, and at all different concentration of drug B difference between absorbance at two selected wavelength ($\lambda_1$, $\lambda_2$) showing linear response. So the calibration curve is prepared for absorbance difference verses conc. of drug B (Absorbance difference is zero for drug A). Similarly same procedure for estimation of drug A two wavelengths were selected where drug B showing same absorbance (difference between absorbance is zero) and drug showing linear response. From the overlay spectra two wavelengths 246 nm and 268 nm were selected as $\lambda_1$ and $\lambda_2$ for the estimation of B. A shows the same absorbance at these wavelengths. Similarly, wavelengths at 325nm were selected as $\lambda_1$ and $\lambda_2$ for estimation of A.

RESULTS AND DISCUSSION

Determination of Flurbiprofen at two wavelengths shows 268nm and 246nm while for Gatifloxacin at 325nm.

Method Validation

Linearity and Range

The linearity response was determined by analyzing solutions having concentrations in the range of 0.3-1.5µg/ml and 3-15 µg/ml for Flurbiprofen and Gatifloxacin respectively from same solution. Absorbance of each solution was measured using developed method. Calibration curve of Absorbance vs. Concentration was plotted. The correlation coefficient and regression line equations for Flurbiprofen and Gatifloxacin were determined.

![Calibration curve of GAT](image)

**Figure 3: ‘Calibration curve of GAT’**

![Calibration curve of FLU](image)

**Figure 4: ‘Calibration curve of FLU’**

Table 1: ‘Regression analysis data for proposed method’

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Gatifloxacin</th>
<th>Flurbiprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda$</td>
<td>325nm</td>
<td>$A\lambda_{268}-A\lambda_{246}$</td>
</tr>
<tr>
<td>Beer’s law Limit (µg/ml)</td>
<td>3-15</td>
<td>0.3-1.5</td>
</tr>
<tr>
<td>Regression equation ($y = mx + c$)</td>
<td>$y = 0.030x + 0.345$</td>
<td>$y = 0.025x + 0.011$</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.998</td>
<td>0.996</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.030</td>
<td>0.025</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.345</td>
<td>0.011</td>
</tr>
</tbody>
</table>
Six replicates of standard mixture solution having Flurbiprofen (0.9 µg/ml) and Gatifloxacin (9µg/ml) were prepared and absorbance were recorded and RSD was calculated. Intraday and Interday precision for Dual wavelength spectroscopy method were measured in term of %RSD. The experiment was repeated three times in a day for intraday and on three different days for interday precision. The limit for %RSD is NMT 2%.

**LOD and LOQ**

Calibration curves were repeated 6 times and standard deviation of intercept were calculated. Then LOD and LOQ were measured as follows.

\[
\text{LOD} = 3.3 \times \text{SD} / \text{Slope of calibration curve}
\]

\[
\text{LOQ} = 10 \times \text{SD} / \text{Slope of calibration curve}
\]

SD = Standard deviation of intercepts

**Accuracy**

Accuracy of the method was confirmed by recovery study from prepared laboratory sample at three level of standard addition (80%, 100% and 120%) of label claim.

**Table 2: 'Summary of Validation Parameter”**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GAT</th>
<th>FLU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy(n=3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100.2 ±0.003</td>
<td>99.79±0.008</td>
</tr>
<tr>
<td>2</td>
<td>100.5 ±0.005</td>
<td>99.80±0.02</td>
</tr>
<tr>
<td>3</td>
<td>99.31 ±0.003</td>
<td>99.98±0.010</td>
</tr>
<tr>
<td>Method precision (Repeatability) (% RSD, n = 6),</td>
<td>0.48</td>
<td>0.66</td>
</tr>
<tr>
<td>Interday (n = 3) (% RSD)</td>
<td>0.33-1.02</td>
<td>0.84-1.36</td>
</tr>
<tr>
<td>Intraday (n = 3) (% RSD)</td>
<td>0.60-0.94</td>
<td>0.76-1.26</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.30</td>
<td>0.07</td>
</tr>
<tr>
<td>LOQ(µg/ml)</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Assay ± S. D (n = 3)</td>
<td>99.71 ± 0.75</td>
<td>99.61 ± 0.44</td>
</tr>
</tbody>
</table>

**Preparation of sample solution for % recovery**

1 mg Flurbiprofen and 10 mg Gatifloxacin was accurately weighed and transferred to volumetric flask of 100ml capacity and aliquot them to make final concentration 1µg/ml Flurbiprofen and 10µg/ml Gatifloxacin. Then absorbance of each sample solutions was taken at selected wavelength for Flurbiprofen and Gatifloxacin and concentration is calculated which is known as pre-analyzed sample.

In pre-analyzed sample 80, 100 and 120 % of Flurbiprofen and Gatifloxacin was spiked absorbance of each spiked solutions was taken and total amount of drug was calculated and from which % recovery was calculated.

**Procedures for the analysis of Eye Drops**

Sample: Flurbiprofen and Gatifloxacin

Brand name: Flubigat (Flurbiprofen 0.03% w/v, Gatifloxacin 0.3% w/v)

Manufacturer: Entod Pharmaceuticals, Bandra, Mumbai, India.

**Preparation of sample solution**

Applicability of proposed method was tested by analyzing marketed formulation. Take 2ml of sample solution (Equivalent to 1mg of FLU and 10mg of GAT and dilute up to 100ml of mixture of methanol: water(60:40) and then sonicate it to make FLU (1µg/ml) + GAT (10µg/ml) shows in Table 3.

**Table 3: ‘Analysis of market formulation”**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. In dosage form (%w/v)</th>
<th>Conc. Found (µg/ml) ± S.D n=6</th>
<th>Assay± S.D</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAT</td>
<td>10µg/ml</td>
<td>9.96±0.081µg/ml</td>
<td>99.71 ± 0.75</td>
<td>1.22</td>
</tr>
<tr>
<td>FLU</td>
<td>1µg/ml</td>
<td>0.97±0.041µg/ml</td>
<td>99.61 ± 0.44</td>
<td>0.61</td>
</tr>
</tbody>
</table>

**Discussion**

Linearity was obeyed in concentration range of 0.3-1.5µg/ml and 3-1.5µg/ml for FLU and GAT, respectively. As the values of % RSD of all precision study were within the acceptable limits (less than 2 %), the method provides good precision and reproducibility. The % RSD (less than 2 %) of accuracy study indicated that the method was accurate. Results of the recovery study were found to be within the acceptance criteria.

**CONCLUSION**

The proposed method is based on dual wavelength data processing and only requires measurement of absorbance at selected wavelengths. Interference studies revealed that the common excipients and other additives usually present in the pharmaceutical formulation did not interfere in the proposed method for estimation of both drugs. The proposed method was found to be simple, rapid, economical, accurate and precise. It can be useful for routine in process quality control and simultaneous estimation of Flurbiprofen and Gatifloxacin from their combined dosage form.

**REFERENCES**

1. http://www.drugbank.ca/DB0144
2. http://www.drugbank.ca/DB00712


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