Spectrophotometric Method Based on Q-Absorbance Ratio for Simultaneous Determination of Moxifloxacin and Prednisolone Acetate in Pharmaceutical Preparation

**Keywords:** Moxifloxacin, Prednisolone Acetate, Q-Absorbance ratio, Isoabsorptive point, Pharmaceutical dosage form, Validation

**ABSTRACT**

A simple and sensitive spectrophotometric method based on Q-Absorbance ratio was developed for the simultaneous estimation of Moxifloxacin and Prednisolone Acetate in pharmaceutical dosage form. Q-Absorbance ratio method based on formation of Q-absorbance equations at two wavelengths, one is an isoabsorptive point and another is the \( \lambda_{\text{max}} \) of one of the two drugs. Absorbances are measured at two selected wavelengths; one is 226.2 nm (isoabsorptive point) and another being 243 nm \( [\lambda_{\text{max}} \text{ of Prednisolone Acetate}] \). The two drugs comply with Beer’s Lambert’s law over the linearity range of 5-35 \( \mu \)g/ml. The method was validated as per International Conference on Harmonization (ICH) guidelines in terms of linearity, accuracy (recovery study), precision (repeatability, intraday, interday precision), limit of detection and limit of quantification. All the validation parameters were found to be within acceptable limits. The method was found to be simple, sensitive, rapid, cost effective, accurate, and precise for the routine analysis of both the drugs in pharmaceutical dosage form.
INTRODUCTION

Moxifloxacin (MOX) (Figure 1) is chemically 1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3. It is a broad spectrum antibacterial drug that is used for the treatment of bacterial infections\(^1\). Its antibacterial spectrum includes enteric gram (−) rods, atypical bacteria, and *streptococcus pneumoniae* and anaerobic bacteria. It differs from earlier antibacterials of the fluoroquinolone class such as levofloxacin and ciprofloxacin in having greater activity against gram-positive bacteria and anaerobes. Because of its potent activity against the common respiratory pathogen *streptococcus pneumoniae*, it is considered a "respiratory quinolone."\(^2\) It is official in Indian Pharmacopoeia (IP)\(^3\), United State Pharmacopoeia (USP)\(^4\), British Pharmacopoeia (BP)\(^5\) and European Pharmacopoeia (EP)\(^6\). IP, USP, BP and EP describe LC method for its determination. Literature review reveals RP-HPLC\(^7\), HPTLC\(^8\) and spectrophotometry\(^9\) methods for determination of MOX in alone. Prednisolone Acetate (PRD) (Figure 2) is chemically Pregna-1, 4-diene-3, 20-dione,21-(acetyloxy)-11,17-dihydroxy-(11β). It is a topical anti-inflammatory agent for ophthalmic use.\(^1\) Prednisolone is a corticosteroid drug with predominant glucocorticoid and low mineralocorticoid activity, making it useful for the treatment of a wide range of inflammatory and autoimmune condition such as asthma, uveitis, pyoderma gangrenosum, rheumatoid arthritis\(^2\). This drug is official in Indian Pharmacopoeia (IP)\(^10\), United State Pharmacopoeia (USP)\(^11\), British Pharmacopoeia (BP)\(^12\), European Pharmacopoeia (EP)\(^13\) and Japanese Pharmacopoeia (JP)\(^14\). IP, USP, EP, BP and JP describe LC method for its estimation. Literature survey reveals HPLC\(^15\) and spectrophotometry \(^16\)-\(^18\) methods for determination of PRD alone. The present invention relates to a fixed dose combination comprising one or more antibiotics and one or more steroidal anti-inflammatory agents for the treatment of ocular infections. The combination is not official in any Pharmacopeia hence no official method is available for simultaneous estimation of these two drugs. Literature survey reveals RP-HPLC\(^19\), Stability indicating HPLC\(^20\), HPTLC\(^21\) and spectrophotometry (simultaneous equations method)\(^22\) methods for estimation of MOX and PRD in combined dosage form. The present invention describes new simple, accurate, precise and sensitive...
UV spectrophotometric method based on absorbance correction for simultaneous estimation of MOX and PRD in combined ophthalmic formulation.

MATERIALS AND METHODS

Materials

Pure sample of MOX was obtained from Taj Pharmaceuticals Ltd, Ahmedabad, Gujarat. PRD was provided as a gift sample from Maharshi Pharma Chem Private Ltd, Ahmedabad, Gujarat. Methanol (S. D. Fine Chemicals Ltd, Mumbai) was used in the study as solvent. All the chemicals used were of analytical grade. 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) were used in the study.

Methods

Preparation of Standard Stock and Working Standard Solutions

Standard stock solution of MOX and PRD was prepared by accurately weighing 10 mg of pure drug powder to separate 100 ml calibrated volumetric flask, dissolved and diluted up to mark with methanol to obtain 100µg/ml each drug. Aliquots of MOX and PRD and were suitably diluted with methanol to obtain the final concentration in the range of 5 to 35 µg/ml respectively.

Methodology

Q-Absorbance ratio method utilizes the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two drugs. From the overlay spectra of two drugs, it is observed that MOX and PRD show an isoabsorptive point at 226.2 nm. The second wavelength chosen is 243 nm, which is the λ-max of PRD. The working standard solution ranging from 5-35 µg/ml for both drugs were prepared and the absorbances at 226.2 nm (Isoabsorptive point) and 243 nm (λ-max of PRD) were measured and absorptivity coefficients were calculated using calibration curve. The absorptivity values determined for MOX at 226.2 nm is (aX1), and at 243 nm is (aX2) and the absorptivity values for PRD at 226.2 nm is (aY1), at 243 nm is (aY2).
The concentration of MOX and PRD in the mixture can be calculated by following equations

\[ C_X = \left[ \frac{(Q_M - Q_Y)}{(Q_X - Q_Y)} \right] \times \frac{A_1}{aX_1} \quad \ldots \ldots \ldots \ldots \ldots \ldots (1) \]

\[ C_Y = \left[ \frac{(Q_M - Q_X)}{(Q_Y - Q_X)} \right] \times \frac{A_1}{aY_1} \quad \ldots \ldots \ldots \ldots \ldots \ldots (2) \]

Where, \( A_1 \) and \( A_2 \) are absorbances of mixture at 226.2 nm and 243 nm, \( Q_M = A_2 / A_1 \), \( Q_X = aX_2 / aX_1 \) and \( Q_Y = aY_2 / aY_1 \).

**Determination of Analytical Wavelengths**

Aliquots portion of 2.0 ml of standard stock solution of MOX and PRD were transferred to separate 10 ml volumetric flask, diluted to mark with methanol to obtain concentration of 20 \( \mu \)g/ml for each MOX and PRD. The resultant solutions were scanned in UV range of 200 nm to 400 nm against methanol as a blank to obtain overlain spectra. From the overlay spectra of two drugs, it is observed that MOX and PRD show an isoabsorptive point at 226.2 nm. The second wavelength chosen is 243 nm, which is the \( \lambda \)-max of PRD. Absorbances are measured at these two selected wavelengths.

**VALIDATION OF THE DEVELOPED METHOD**

The method was validated as per the rules of International Conference on Harmonization (ICH) guidelines \(^{[23]}\).

**Linearity (Calibration curve)**

The calibration curves were plotted over a concentration range of 5-35 \( \mu \)g/ml for each MOX and PRD. An appropriate volume of standard stock solution of each MOX and PRD in the range of \((0.5, 1, 1.5, 2, 2.5, 3, 3.5 \text{ ml})\) were transferred into series of separate 10 ml volumetric flasks separately and volume was made up to mark with methanol to get concentrations in the range of 5–35 \( \mu \)g/ml for both drugs respectively. The absorbances of solution were then measured at 226.2 nm and 243 nm. The calibration curves were constructed and linear regression equations were calculated for the drugs.

**Method precision**

**Repeatability**

The standard solutions of MOX and PRD \((10 \mu \text{g/ml and } 20 \mu \text{g/ml})\) were prepared. The absorbance was measured by UV spectrophotometer six times on same day without changing
the parameters of the developed method and % RSD was calculated.

Intraday and Interday precision

The intraday variation (% Relative standard deviation [RSD]) was determined by analysis of three standard solutions of MOX and PRD (10, 20 and 30μg/ml for both drugs) three times on the same day. Interday variation (% RSD) was determined by analysis of three standard solutions of MOX and PRD (10, 20 and 30μg/ml for both drugs) three times on the three different days for period of one week.

Accuracy (recovery study)

The accuracy of an analytical procedure is the closeness of agreement between the value which is accepted as true value and the value found. The recovery experiment was carried out by adding known amount of standard solution of MOX and PRD at 50%, 100%, and 150% level to prequantified sample solution of MOX (5μg/ml) and PRD (10μg/ml). The amount of MOX and PRD were analyzed by proposed method.

Limit of detection and limit of quantification

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio by the use of standard deviation of the response and the slope of the calibration curve. The LOD and LOQ were calculated using signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated:

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

Where, \(\sigma\) = the standard deviation of the response,

\(S\) = slope of the calibration curve.

Determination of MOX and PRD in their combined ophthalmic formulation

The eye-drop (1.0 ml) containing 5 mg of MOX and 10 mg of PRD was transferred to 25 ml volumetric flask. Methanol (10 ml) was added and sonicated for 20 min. The volume is adjusted up to the mark with methanol. The solution was then filtered through Whatman filter.
The solution was suitably diluted with methanol to get a final concentration of 5 μg/ml of MOX and 10 μg/ml of PRD. The resulting solution was analyzed by proposed methods.

RESULTS AND DISCUSSION

In Q-absorbance ratio spectrophotometry method, the foremost and prime need is that both the drugs should comply with the beer’s law at all the wavelength. MOX and PRD obeyed linearity in the concentration range of 5-35 μg/ml in methanol at their respective λ-max and Isoabsorptive point with correlation coefficient (r² > 0.99). The overlain absorption spectra of MOX and PRD showing Isoabsorptive point in methanol is shown in (Figure 3).

Table 1: Recovery Data of MOX and PRD by Q-absorbance ratio Method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level</th>
<th>Amount taken (μg/ml)</th>
<th>Amount added (%)</th>
<th>% Mean recovery ± S.D. (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOX</td>
<td>I</td>
<td>5</td>
<td>50</td>
<td>98.66 ± 0.80</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>5</td>
<td>100</td>
<td>99.71 ± 1.50</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>5</td>
<td>150</td>
<td>99.52 ± 0.75</td>
</tr>
<tr>
<td>PRD</td>
<td>I</td>
<td>10</td>
<td>50</td>
<td>99.73 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>10</td>
<td>100</td>
<td>100.12 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>10</td>
<td>150</td>
<td>99.76 ± 0.47</td>
</tr>
</tbody>
</table>

S.D. is standard deviation and n is number of replicates.

Table 2: Analysis of MOX and PRD in ophthalmic formulation by developed method

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Label claim (mg)</th>
<th>Amount found(mg)</th>
<th>% Label claim ± S.D (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EYE DROPS</td>
<td>MOX</td>
<td>PRD</td>
<td>MOX</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>4.96</td>
<td>10.1</td>
</tr>
</tbody>
</table>

S.D is standard deviation and n is number of replicates.
Table 3: Regression analysis data and summary of validation parameters by proposed Q- absorbance method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MOX</th>
<th>PRD</th>
<th>MOX &amp; PRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>243</td>
<td>243</td>
<td>226.2</td>
</tr>
<tr>
<td>Beer’s law limit (µg/ml)</td>
<td>5 to 35</td>
<td>5 to 35</td>
<td>5 to 35</td>
</tr>
<tr>
<td>Regression equation (y = a + bc)</td>
<td>Y = 0.0177X + 0.0043</td>
<td>Y = 0.0353X - 0.0016</td>
<td>Y = 0.0238X + 0.0016</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0177</td>
<td>0.0353</td>
<td>0.0238</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0043</td>
<td>0.0016</td>
<td>0.0016</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9995</td>
<td>0.9986</td>
<td>0.9991</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.41</td>
<td>0.16</td>
<td>0.22</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>1.26</td>
<td>0.48</td>
<td>0.68</td>
</tr>
<tr>
<td>Repeatability (% RSD, n = 6)</td>
<td>0.8017</td>
<td>0.4479</td>
<td>0.8996</td>
</tr>
<tr>
<td>Precision (% RSD, n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday</td>
<td>0.529-0.432</td>
<td>0.161-0.887</td>
<td>0.240-0.271</td>
</tr>
<tr>
<td>Intraday</td>
<td>0.388-1.073</td>
<td>0.162-1.033</td>
<td>0.268-0.645</td>
</tr>
<tr>
<td>Accuracy ± S.D. (% Recovery, n= 3)</td>
<td>99.29 ± 1.01</td>
<td>99.87 ± 0.65</td>
<td>-</td>
</tr>
<tr>
<td>% Assay ± S.D.(n=3)</td>
<td>99.20 ± 0.79</td>
<td>101.0 ± 0.92</td>
<td>-</td>
</tr>
</tbody>
</table>

LOD = Limit of detection, LOQ = Limit of quantification, RSD = Relative standard deviation, S. D. = Standard deviation, n = number of replicates
**FIGURE 1: STRUCTURE OF MOXIFLOXACIN**

**FIGURE 2: STRUCTURE OF PREDNISOLONE ACETATE**

**Figure 3: Overlain Spectrum of MOX (20 μg/ml) and PRD (20 μg/ml)**

Citation: Pinal B. Patel et al. Ijprr.Human, 2017; Vol. 8 (4): 45-54.
The validation parameters were studied at all the selected wavelengths for the developed method. All the validation parameters were found to be within acceptable limits. The percent coveries were found to be in the range of 98.66 - 99.52 % for MOX and 99.73 – 100.12 % for PRD (Table 1). The precision of method was determination by repeatability, intraday, interday precision and was expressed as the % RSD which indicates good method precision (Table 3). The LOD and LOQ for MOX at 243 nm were found to be 0.41 μg/ml and 1.26μg/ml, respectively. The LOD and LOQ for PRD at 243 nm were found to be 0.16 μg/ml and 0.48μg/ml, respectively. LOD and LOQ for both drugs at isoabsorptive point (226.2nm) were found to be 0.22μg/ml and 0.68μg/ml, respectively (Table 3). The proposed spectrophotometric method was successfully applied to determine MOX and PRD in pharmaceutical dosage form. MOX and PRD content in marketed eye drops were found to be 99.20 % and 101.0 %, respectively indicates non-interference from excipients (Table 2).

CONCLUSION

The Q – absorbance ratio method was developed for simultaneous determination of MOX and PRD 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 MOX and PRD in pharmaceutical formulation and there for method can be extended for the routine QC analysis of both drugs in formulation.

ACKNOWLEDGEMENT

The authors are thankful to Taj Pharmaceuticals Ltd, Ahmedabad, Gujarat and Maharshi pharma Chem Private Ltd, Ahmedabad, Gujarat for providing gift sample of MOX and PRD for research. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva – 382711, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

REFERENCES