DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF MOXIFLOXACIN AND PREDNISOLONE ACETATE IN PHARMACEUTICAL PREPARATION

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ABSTRACT

A simple and sensitive first order derivative spectrophotometric method was developed for the simultaneous estimation of Moxifloxacin and Prednisolone Acetate in pharmaceutical dosage form. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra was obtained in methanol and the determinations were made at 258 nm (ZCP of Moxifloxacin) for Prednisolone Acetate and 303 nm (ZCP of Prednisolone Acetate) for Moxifloxacin. The two drugs comply with beer’s-lambert’s law over the linearity range of 2-40 µg/ml for Moxifloxacin and 5-80 µg/ml for Prednisolone Acetate. The method was validated as per 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.

KEYWORDS: Moxifloxacin, Prednisolone Acetate, First order derivative spectrophotometric method, Zero crossing point, Pharmaceutical dosage form, Validation.
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. 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." It is official in Indian Pharmacopoeia (IP), United State Pharmacopoeia (USP), British Pharmacopoeia (BP) and European Pharmacopoeia (EP). IP, USP, BP and EP describe LC method for its determination. Literature review reveals RP-HPLC, HPTLC and spectrophotometry methods for determination of MOX in alone. Prednisolone Acetate (PRD) (Figure 2) is chemically (11β). It is a topical anti-inflammatory agent for ophthalmic use. 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 conditions such as asthma, uveitis, pyoderma gangrenosum, rheumatoid arthritis. This drug is official in Indian Pharmacopoeia (IP), United State Pharmacopoeia (USP), British Pharmacopoeia (BP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP). IP, USP, EP, BP and JP describe LC method for its estimation. Literature survey reveals HPLC and spectrophotometry 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 pharmacopoeia; hence no official method is available for simultaneous estimation of these two drugs. Literature survey reveals RP-HPLC, Stability indicating HPLC, HPTLC and spectrophotometry (simultaneous equations method) methods for estimation of MOX and PRD in combined dosage form. Literature survey reveals only single spectrophotometric method based on simultaneous equation for estimation of this two drugs in mixture; hence, it is thought of interest to developed and validate alternative spectrophotometric method for simultaneous estimation of MOX and PRD in combined dosage form. The present manuscript describe 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 Pharmaceutical 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 100 μg/ml of each drugs. Aliquots of MOX and PRD and were suitably diluted with methanol to obtain the final concentration in the range of 2 to 40 μg/ml and 5 to 80 μg/ml for MOX and PRD, respectively.

Methodology

The standard solution of MOX (20 μg/ml) and PRD (20 μg/ml) were scanned separately in the UV range of 200-400 nm. The zero-order spectra thus obtained was then processed to obtain first-derivative spectra. The two spectra were overlain and it appeared that MOX showed zero crossing at 258 nm, while PRD showed zero crossing at 303 nm. At the zero crossing point (ZCP) of MOX (258 nm), PRD showed a first-derivative absorbance, whereas at the ZCP of PRD (303 nm), MOX showed a first-derivative absorbance. Hence 303 and 258 nm was selected as analytical wavelengths for determination of MOX and PRD, respectively. These two wavelengths can be employed for the determination of MOX and PRD without any interference from the other additives in their combined dosage formulation.

VALIDATION OF THE DEVELOPED METHOD

The method was validated as per the International Conference on Harmonization (ICH) guidelines. [23]
Linearity (Calibration curve)
The calibration curves were plotted a concentration range of 2 - 40 μg/ml for MOX and 5 – 80 μg/ml for PRD. Accurately measured standard solutions of MOX (0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml) and PRD (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. First-derivative absorbance was measured at 303 nm for MOX and 258 nm for PRD. The calibration curves were constructed by plotting derivative absorbances versus concentrations and the regression equations were calculated.

Method precision
Repeatability
The standard solutions of MOX and PRD (10 μg/ml and 20 μg/ml) was prepared. The absorbance was measured at selected wavelength six times on same day without changing the parameters of the developed method and % RSD was calculated.

Intraday and Interday precision
The intraday variation (% RSD) was determined by analysis of three standard solutions of MOX and PRD (10, 20 and 30 μg/ml and 10, 30 and 50 μg/ml) three times on the same day. Interday variation (% RSD) was determine by analysis of three standard solutions of MOX and PRD (10, 20 and 30 μg/ml and 10, 30 and 50 μg/ml) three times on the three different days for period of one week and % RSD was calculated.

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 were 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 (5 μg/ml) and PRD (10 μg/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 limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated:
LOD = 3.3 X σ/S
LOQ = 10 X σ/S
Where, σ = 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 paper no. 41. 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.

**RESULT AND DISCUSSION**

In first derivative spectrophotometric method, the foremost and prime need is that the drugs should comply with the beer’s law at selected wavelengths. Linear correlation was obtained between absorbance and concentration of MOX and PRD in the concentration ranges of 2-40 μg/ml and 5-80 μg/ml, respectively. The standard solutions of MOX and PRD were scanned separately in the UV range and zero-order spectra (Figure 3) thus obtained was then processed to obtain first-derivative spectra. The two derivative spectra showed maximum absorbance at 258 nm (ZCP of MOX) for PRD and 303 nm (ZCP of PRD) for MOX. First-derivative absorbances (D1) were recorded 303 nm for MOX and 258 nm for PRD (Figure 4). First derivative spectra give good quantitative determination of both the drugs at their respective wavelengths without any interference from the excipients in their combined dosage formulation.

**Table 1: Recovery Data of MOX and PRD by first order derivative spectrophotometric Method**

<table>
<thead>
<tr>
<th>% Level (n=3)</th>
<th>Amount of drug taken (μg/ml)</th>
<th>Amount standard added (μg/ml)</th>
<th>% Recovery ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOX</td>
<td>PRD</td>
<td>MOX</td>
</tr>
<tr>
<td>50%</td>
<td>5</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>100%</td>
<td>5</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>150%</td>
<td>5</td>
<td>10</td>
<td>7.5</td>
</tr>
</tbody>
</table>

S.D. is standard deviation and n is number of replicate.
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=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EYE DROPS</td>
<td>MOX 5</td>
<td>PRD 10</td>
<td>MOX 4.96</td>
</tr>
</tbody>
</table>

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

Table 3: Regression analysis data and summary of validation parameters by proposed first order derivative spectrophotometric method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First derivative spectrophotometric Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOX</td>
</tr>
<tr>
<td>Wavelength</td>
<td>303 nm</td>
</tr>
<tr>
<td>Beer’s Law Linearity Range (μg/ml)</td>
<td>2-40</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0059</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0024</td>
</tr>
<tr>
<td>Correlation Coefficient (r²)</td>
<td>0.9993</td>
</tr>
<tr>
<td>Accuracy ± S.D. (% Recovery, n= 3)</td>
<td>100.7 ± 1.19</td>
</tr>
<tr>
<td>Repeatability (% RSD , n= 6)</td>
<td>0.87</td>
</tr>
<tr>
<td>Intraday Precision %RSD (n = 3)</td>
<td>0.53 - 0.91</td>
</tr>
<tr>
<td>Interday Precision %RSD (n = 3)</td>
<td>0.79 - 0.94</td>
</tr>
<tr>
<td>LOD (μg/ml)</td>
<td>0.19</td>
</tr>
<tr>
<td>LOQ (μg/ml)</td>
<td>0.59</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
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 % recoveries were found to be in the range of 99.33 – 101.60% for MOX and 99.53 – 100.80% 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 303 nm were found to be 0.19 μg/ml and 0.59 μg/ml, respectively. The LOD and LOQ for PRD at 258 nm were found to be 1.29 μg/ml and 3.90 μg/ml, respectively. All the regression and validation parameters are summarized in 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 first order derivative spectrophotometric 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.

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