Simultaneous Determination of Moxifloxacin Hydrochloride and Dexamethasone Sodium Phosphate in Eye Drops by HPLC and Absorbance Correction Method

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ABSTRACT

High performance liquid chromatography and Absorbance correction methods were applied for the simultaneous determination of Moxifloxacin hydrochloride and Dexamethasone sodium phosphate in eye drops. Chromatographic separation was achieved on reversed-phase C18 column (25 cm × 4.6 mm id, 5 µm) in the isocratic mode using methanol-water-triethylamine (60:40:0.75, v/v/v; pH adjusted to 3.25 ± 0.05 with orthophosphoric acid) as the mobile phase at a flow rate 0.8 mL/min. Quantitation was achieved with UV detection at 254 nm. In the proposed HPLC method, the concentration range of 5-30 and 1-6 µg/mL, with mean recoveries of 101.46 ± 1.57 and 101.50 ± 1.64 % for moxifloxacin hydrochloride and dexamethasone sodium phosphate respectively was obtained. In the Absorbance correction method, quantification was achieved over the concentration range of 10-50 and 2-10 µg/mL, with mean recoveries of 100.49± 0.62 and 100.36 ± 1.09 % for moxifloxacin hydrochloride and dexamethasone sodium phosphate respectively. Determination was performed at 244.2 nm and 357 nm for moxifloxacin hydrochloride and dexamethasone sodium phosphate. The proposed methods were successfully applied for the analysis of synthetic mixtures and pharmaceutical formulations of moxifloxacin hydrochloride and dexamethasone sodium phosphate without any interference from common excipients. The results obtained by applying the proposed methods were statistically compared by the Student’s t-test.

Keywords: Moxifloxacin hydrochloride, Dexamethasone sodium phosphate, High performance liquid chromatography, Absorbance Correction method, Simultaneous determination

INTRODUCTION

Moxifloxacin hydrochloride (MOXI) (1-cyclopropyl-7-((S,S)-2,8-diazabicyclo(4.3.0)non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid) is a broad spectrum antibacterial agent, belonging to the group of fluoroquinolones [1-2]. Moxifloxacin hydrochloride is active against a wide variety of gram-positive and gram-negative organism, use in the treatment of conjunctivitis, chronic bronchitis and community acquired pneumonia [3]. Dexamethasone sodium phosphate (DEXP) (disodium 9α-fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dioxo-1,4-pregnadien-21-yl phosphate) is a synthetic glucocorticoid with varied pharmacological activities including anti-inflammatory[1-3].

Literature survey reveals that HPLC [3-6], HPTLC [7-10], LC-MS [11-12], Capillary electrophoresis [13] and UV spectroscopy [14] method has been reported for determination of moxifloxacin hydrochloride and dexamethasone sodium phosphate alone or in combination with other drugs in pharmaceutical formulations and in human plasma. Moxifloxacin hydrochloride and dexamethasone sodium phosphate eye drops are not official in any pharmacopoeia. No isocratic HPLC method and spectrophotometric methods are available for simultaneous determination of moxifloxacin hydrochloride and dexamethasone sodium phosphate, so there was a need to develop and validate...
method for their determination in combination. Simultaneous estimation by UV-Visible spectroscopy can be done by various methods [15-17]. However, absorbance correction method is relatively rapid and simple. No isocratic HPLC method and spectrophotometric methods are available for simultaneous determination of MOXI and DEXP, so there was a need to develop and validate method for their determination in combination. Thus, the aim of this work is to determine both drugs simultaneously by simple, rapid, and selective HPLC and absorbance correction method which could be used for quality control and routine analysis.

EXPERIMENTAL SECTION

2.1 Instrumentation
All absorption spectra were recorded with a Shimadzu UV-1700 Pharmaspec, UV-Visible double-beam spectrophotometer with 1 cm quartz cuvettes (Shimadzu Corporation, Kyoto, Japan). The spectral bandwidth was 0.2 nm.

HPLC instrument (Shimadzu, Kyoto, Japan) was equipped with a model series LC-10 AS pump, Rheodyne 7725i injector with a 100 µL loop and SPD-10A UV-Visible detector. A Grace smart reversed-phase C18 column (Grace Discovery and Division; 5 µm, 25 cm × 4.6 mm id) was used as the stationary phase. Class CR10 software was used for data acquisition.

2.2 Reference substances, reagents and chemicals
MOXI standard was procured from Astron Research Ltd. (Ahmedabad, India). DEXP standard was procured from Indiana Opthalmics (Surendranagar, India).

For HPLC work, methanol (RFCL Ltd., India), phosphoric acid (RFCL Ltd., India) and triethylamine (RFCL Ltd., India) were of HPLC grade. Triple distilled water was used throughout the experiment.

2.3 Pharmaceutical Dosage form
Milfloxy-DM eye drops (Batch No. CBJ0029) manufactured by Sun Pharmaceuticals and Occumox-DM eye drops (Batch No. 28) manufactured by Sunways Pvt. Ltd. were used for analysis. Each eye drops claimed to contain 0.5 % w/v MOXI (as free base) and 0.1 % w/v DEXP.

2.3 Preparation of Standard Solutions
2.3.1 For HPLC method
Stock standard solutions of MOXI (as free base, 2 mg/mL) and DEXP (2 mg/mL) were prepared separately in methanol. The stock solutions were diluted with mobile phase to prepare final concentrations of 200 and 40 µg/mL for MOXI and DEXP respectively.

2.3.2 For Absorbance correction method
Stock standard solutions of MOXI (as free base, 250 µg/mL) and DEXP (as free base, 250µg/mL) were prepared separately in 0.1 N HCl.

2.4 HPLC Method
The mobile phase was filtered through Millipore filter paper type HV (0.45 µm) and degassed by sonication. A flow rate of 0.8 mL/min was maintained. The injection volume was 20 µL. The detector was set at 254 nm. Aliquots equivalent to 0.25-1.5 mL of MOXI working standard solution and 0.25-1.5 mL of DEXP working standard solution were transferred separately into 10-mL volumetric flasks from their respective working standard solution and completed to volume with mobile phase. Calibration graphs for both MOXI and DEXP were obtained by plotting the peak area against concentration, and the corresponding regression equations were calculated.

2.5 Absorbance Correction Method
Appropriate aliquots from the stock solutions of MOXI and DEXP were used to prepare three different sets of dilutions. Series A, B and C as follows. Series A consisted of different concentrations of MOXI (10-50 µg/mL). Aliquots of MOXI stock standard solution (250 µg/mL) was transferred separately into a series of 25 mL volumetric flask and diluted with 0.1 N HCl to obtain final concentration of 10-50µg/mL. Series B consisted of different concentrations of DEXP (2-10 µg/mL). Aliquots of DEXP stock standard solution (250 µg/mL) was transferred separately into a series of 25 mL volumetric flask and diluted with 0.1 N HCl to obtain final concentration of 2-10 µg/mL. Series C comprised of mixture of MOXI and DEXP having varying concentrations of MOXI (10-50 µg/mL) and DEXP (2-12 µg/mL).
Aliquot equivalent to 1-5 mL of MOXI stock standard solution and 0.2-1.0 mL of DEXP stock standard solution were transferred into 25 mL volumetric flasks and completed to volume with 0.1 N HCl. The absorbance of the solutions of series A and C were measured at 244.2 nm ($\lambda_1$) and 357 nm ($\lambda_2$) while absorbance of the solutions of series B was measured at 244.2 nm ($\lambda_1$). The difference in absorbance between 244.2 nm and 357 nm is due to DEXP and this difference was plotted against DEXP concentration. The absorbance at 357 nm is due to MOXI only and was plotted against MOXI concentration.

2.6 Preparation of Pharmaceutical Samples
2.6.1 For HPLC method
Milflox-DM/Occumox-DM eye drops (1 mL, 0.5 % w/v MOXI and 0.1 % w/v DEXP) was transferred in 10 mL volumetric flask and diluted up to mark with mobile phase. Take 0.2 mL from above solution in 10 mL volumetric flask and diluted up to mark with mobile phase to obtain final concentration of MOXI (10 µg/mL) and DEXP (2 µg/mL). The amount of MOXI and DEX was computed using respective equation of straight line.

2.6.2 For Absorbance correction method
Milflox-DM/Occumox-DM eye drops (0.5 mL, 0.5 % w/v MOXI and 0.1 % w/v DEXP) was transferred in 10 mL volumetric flask and diluted up to mark with 0.1 N HCl. Take 1 mL from above solution in 10 mL volumetric flask and diluted up to mark with 0.1 N HCl to obtain final concentration of MOXI (25 µg/mL) and DEXP (5 µg/mL). The absorbance of final sample solution was measured at 244.2 nm and 357 nm. The amount of MOXI and DEX was computed using respective equation of straight line.

RESULTS AND DISCUSSION
3.1 HPLC method
The developed HPLC method has been applied for simultaneous determination of MOXI and DEXP. To optimize the chromatographic conditions for the separation of MOXI and DEXP, mobile phase composition, the effect of pH and wavelength of detection were investigated. During the method development work, Grace smart RP - C18 column (250 mm × 4.6 mm i.d, 5 µm particle size) gave the most suitable resolution. A satisfactory separation was obtained with mobile phase consisting of methanol-water-triethylamine (60:40:0.75, v/v/v; pH adjusted to 3.25 ± 0.05 with orthophosphoric acid), with flow rate of 0.8 mL/min. Quantitation based on peak area was achieved with UV detection at 254 nm. The specificity of HPLC method is illustrated in Figure 1, which show complete separation of compounds in mixtures. The average retention time ± standard deviation (SD) for MOXI and DEXP were found to be 4.214 ± 0.02 and 6.998 ± 0.05 respectively, for seven replicates.

External standard calibration method was applied for analysis of MOXI and DEXP. A linear relation was obtained between peak area and the concentration of the drug in the range of 5-30 µg/mL and 1-6 µg/mL for MOXI and DEXP respectively.

![Figure 1 Chromatogram of 25 µg/mL MOXI (RT = 4.230 min) and 5 µg/mL DEX (RT = 6.992 min)](image)

3.2 Absorbance Correction method
Absorbance correction method uses the measurement of absorbance at two selected wavelengths, one usually at $\lambda_{max}$ of one drug where other drug also shows considerable absorbance and other being the wavelength at which one of the two drugs is having zero absorbance. From the overlaid spectrum of MOXI and DEXP in 0.1 N HCl
Regression analysis for series A and C shows no difference in the equations of straight line and thus indicates that there is no interference of DEXP in the determination of MOXI. Regression analysis for series B and C shows no difference in the equations of straight line and thus indicated that there is no interference of MOXI in the determination of DEXP (Table 1).

The proposed methods have been validated as per ICH guideline [18] for method validation (Table 2). The proposed methods have been applied for determination of two drugs in Occumox-DM and Mifloxy-DM EyeDrops (Table 3) and validity of the Absorbance correction method was further assessed by applying the standard addition method, which proved that excipients did not interfere during the determination of the two drugs.

![Absorption spectra of Moxifloxacin hydrochloride (10 µg/mL) and Dexamethasone sodium phosphate (10 µg/mL) in 0.1 N HCl.](image)

Figure 2: Absorption spectra of Moxifloxacin hydrochloride (10 µg/mL) and Dexamethasone sodium phosphate (10 µg/mL) in 0.1 N HCl.

Table 1: Regression equation of Series A, B and C in Absorbance correction method

<table>
<thead>
<tr>
<th>Series</th>
<th>Concentration (µg/mL)</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10-50</td>
<td>Y = 0.023X + 0.007</td>
<td>0.9999</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>Y = 0.025X + 0.004</td>
<td>0.9998</td>
</tr>
<tr>
<td>C</td>
<td>10-50</td>
<td>Y = 0.025X + 0.001</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

Y is absorbance; X is concentration; *Regression equation for MOXI and †Regression equation for DEXP.

Table 2: Summary of validation parameter for the proposed methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HPLC method</th>
<th>Absorbance correction method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range (µg/mL)</td>
<td>MOXI</td>
<td>DEXP</td>
</tr>
<tr>
<td></td>
<td>5-30</td>
<td>1-6</td>
</tr>
<tr>
<td>Slope</td>
<td>296705.40</td>
<td>40883.20</td>
</tr>
<tr>
<td>Intercept</td>
<td>49885.02</td>
<td>16191.21</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9998</td>
<td>0.9998</td>
</tr>
<tr>
<td>Repeatability RSD (%)</td>
<td>0.43</td>
<td>0.38</td>
</tr>
<tr>
<td>Intraday RSD (%)</td>
<td>1.14-1.54</td>
<td>0.51-1.37</td>
</tr>
<tr>
<td>Interday RSD (%)</td>
<td>1.35-1.81</td>
<td>0.90-2.39</td>
</tr>
<tr>
<td>Accuracy (% Recovery±S.D.)</td>
<td>101.46 ± 1.57</td>
<td>101.50 ± 1.64</td>
</tr>
<tr>
<td>LOD (µg/mL)</td>
<td>0.20</td>
<td>0.13</td>
</tr>
<tr>
<td>LOQ (µg/mL)</td>
<td>0.60</td>
<td>0.40</td>
</tr>
</tbody>
</table>

*Average of six experiments
Table 3 Determination of MOXI and DEXP in pharmaceutical formulation by HPLC and Absorbance correction method

<table>
<thead>
<tr>
<th>Sample</th>
<th>MOXI % Recovery ± S.D.</th>
<th>DEXP % Recovery ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occumox-DM</td>
<td>100.34 ± 1.29</td>
<td>99.25 ± 1.08</td>
</tr>
<tr>
<td>Milflox-DM</td>
<td>100.93 ± 0.62</td>
<td>100.25 ± 0.41</td>
</tr>
</tbody>
</table>

*Label claim: MOXI (0.5 % w/v) and DEXP (0.1 % w/v)

Average of three determinations

3.4 Validation of the Methods

3.4.1 Linearity and range

The linearity of the HPLC and absorbance correction methods for the determination of MOXI and DEXP was evaluated by analyzing a series of different concentrations of each drug. In this study six concentrations were chosen for each drug in HPLC method and five concentrations were chosen for each drug in Absorbance correction method. Each concentration was repeated six times. The linearity of the calibration graphs was validated by the high value of the correlation coefficient. The calibration range was established through consideration of the practical range necessary, according to each drug concentration present in the pharmaceutical products to give accurate, precise and linear results. The calibration range of the proposed method is given in Table 2.

3.4.2 Precision

Evaluation of the precision estimates repeatability and intermediate precision were performed at three concentration levels for each drug on three different days. The low values of relative standard deviation (% RSD) of the intraday and interday determinations (Table 2) show that there was no statistically significant difference between the mean results obtained from one day to another.

3.4.3 Detection and quantitation limits

According to ICH recommendation, the approach based on the standard deviation of the response and slope was used for determining the limit of detection (LOD) and limit of quantitation (LOQ). The calculated values for HPLC and absorbance correction method are given in Table 2.

3.4.4 Accuracy

The interference of excipients in the pharmaceutical formulations was studied in detail by proposed methods. To perform this study, standard addition method was applied to the pharmaceutical formulation containing these compounds. This study was performed by addition of known amounts of studied drugs to a known concentration of the commercial pharmaceutical product. The excellent recoveries of standard addition method (Table 2) prove the good precision and accuracy of the proposed methods. Consequently, the excipients in the studied pharmaceutical formulations do not interfere in the analysis of these compounds.

3.4 Analysis of Pharmaceutical formulations

The proposed HPLC and absorbance correction methods were applied to the simultaneous determination of MOXI and DEXP in Occumox-DM and Milflox-DM EyeDrops. Three replicated determinations were made. Satisfactory results were obtained for each compound in good agreement with label claims (Table 3).

3.5 Statistical Analysis

Results of the proposed methods were statistically compared using Paired t test at 95% confidence interval. The values of the calculated t were less than the tabulated values, which revealed that there was no significant difference in the developed methods (Table 4).

Table 4 Statistical Comparison of developed methods using Student’s t-test

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MOXI</th>
<th>DEXP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance</td>
<td>100.79</td>
<td>100.63</td>
</tr>
<tr>
<td>T calc</td>
<td>0.89</td>
<td>0.93</td>
</tr>
<tr>
<td>t critical</td>
<td>2.015</td>
<td>2.015</td>
</tr>
<tr>
<td>P (T&lt;4) one tail</td>
<td>0.214</td>
<td>0.353</td>
</tr>
<tr>
<td>T calc &lt; t critical</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

CONCLUSION

The developed HPLC and Absorbance correction methods are precise, specific, and accurate. Statistical analysis proves that the methods are suitable for the analysis of MOXI and DEXP as a bulk drug and in pharmaceutical formulation without any interference from the excipients. HPLC method gives a good resolution between MOXI and
DEXP within a short time. Absorbance correction method is less expensive, simple, rapid, and more flexible than HPLC.

REFERENCES