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RP- HPLC Method for Simultaneous Determination of Moxifloxacin HCL And Dexamethasone Sodium Phosphate in Bulk and In Ophthalmic Solution

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Abstract

A RP-HPLC method for the simultaneous estimation of MOX and DSP in bulk drug and in combined ophthalmic solution has been developed. Separation of the drugs were carried out on the Qualisil C_8 (5 µ, 250 mm × 4.60 mm) column at ambient temperature using a mobile phase consisting of methanol and water (75: 25, *v/v*), pH of mobile phase adjusted to 3. Flow rate was 1.0 mL/min & detection was carried out at 240 nm. The average retention time for MOX and DSP was found to be 2.22, and 7.26 min, respectively. The calibration range was 10 – 60 µg/mL (r^2 = 0.999) for MOX and 2 - 12 µg/mL (r^2 = 0.999) for DSP, respectively. The method has successively been applied for determination of MOX and DSP in combined ophthalmic solution. There was no interference from the excipients commonly present in the ophthalmic solution. The drug contents for MOX and DSP was found to be 99.97 % and 99.68 %, respectively. The % recovery was found to be within the limits of the acceptance criteria with average % recovery 99.35 - 100.79 % (MOX) and 99.43 - 99.96 % (DSP). Precision of the method was studied as intra-day, inter-day and repeatability, the % RSD value for each parameter was found to be less than 2, indicate high precision of the method. For MOX, LOD and LOQ was found to be 0.30 µg & 0.91 µg, respectively. For DSP, the LOD and LOQ was found to be 0.10 µg & 0.30 µg. According to USP, system suitability tests are an integral part of chromatographic methods. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions.

Keywords: Moxifloxacin Hydrochloride; Dexamethasone Sodium Phosphate; RP-HPLC.

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1. Introduction

Moxifloxacin Hydrochloride (MOX) is chemically 1-cyclopropyl-7-[(1S, 6S)-2, 8-diazabicyclo [4.3.0] non-8-yl]-6-fluoro-8-methoxy-4-oxo-quinoline-3-carboxylic acid hydrochloride is a 4th generation fluoroquinolone broad spectrum antibiotic implicated in the treatment of conjunctivitis [1]. The drug is official in British Pharmacopeia [1,2].

In literature, several analytical methods such as RP-HPLC, UPLC and some hyphenated techniques such as LC/MS/MS, LC-MS have been reported for the determination of MOX single and in combination in biological fluids [3]. Few RP-HPLC, UV-Spectrophotometric, and HPTLC methods have been studied for determination of MOX in bulk and in pharmaceutical formulations. One RP-HPLC method has been studied for determination of moxifloxacin in combination with dexamethasone acetate in ear drop using SHIM-PACK VP-ODS (150 mmx 4.6 mm, 5 µm) column, with column temperature of 30°C [4]. The mobile phase

consisted of phosphate buffer solution (which contained 0.02 mol L^{-1} sodium dihydrogen phosphate and 0.3% triethylamine)-methanol (40:60 v/v) pH was adjusted to 3.0 using phosphoric acid [5].

Dexamethasone Sodium Phosphate (DSP) is chemically 9- fluoro-11b, 17, 21-trihydroxy-16a-methylpregna-1, 4-diene-3, 20-dione 21-(dihydrogen phosphate) disodium salt, is glucocorticoid class of steroids mainly used as anti-inflammatory and immunosuppressant [6,7]. The drug is official in Indian Pharmacopoeia, British Pharmacopoeia, United State Pharmacopeia and European Pharmacopoeia [8]. In literature, HPLC-MS/ESI and One RP-HPLC methods was found for determination of DSP in biological fluids, one RP-HPLC, and UV-spectrophotometric method have been studied for estimation of DSP in combination with other drugs in bulk and in pharmaceutical formulations [9].

The chemical structures of both drugs are as shown in (Figure 1).

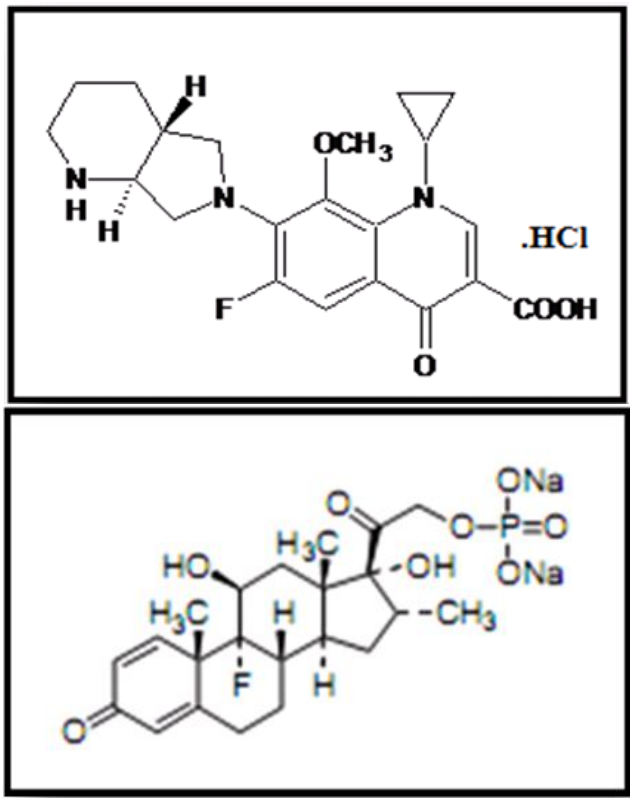

Figure 1. Chemical structure of MOX (a) and DSP (b)

Combination of both these drugs in eye drop formulation is used for treatment of superficial eye infections and available in Indian market [10].

To our knowledge no RP-HPLC method has been studied so far for the simultaneous determination of MOX and DSP in combined dosage form [11].

Therefore, an objective of this work is to develop and validated RP-HPLC method for the simultaneous determination of both these drugs in their combined dosage form. The second objective is to validate the method as per the ICH guidelines [12].

2. Materials And Methods

Moxifloxacin Hydrochloride, Dexamethasone Sodium Phosphate was obtained from Cipla, Mumbai, and Cadilla pharmaceutical ltd, Ahmadabad, India as a gift sample respectively. Potassium dihydrogen ortho-phosphate (AR Grade), ortho-phosphoric acid (AR Grade), methanol (HPLC Grade), was purchased from Merck (India) Ltd., Worli, and Mumbai, India. Ophthalmic solution (Milflox DM) was purchased from Indian market, containing Moxifloxacin Hydrochloride (5 mg/ml), Dexamethasone Sodium Phosphate (1 mg/ml).

Double distilled water of HPLC grade was prepared by distillation system.

A RP-HPLC method for the simultaneous estimation of MOX and DSP in bulk drug and in combined ophthalmic solution has been developed. Separation of the drugs were carried out on the Qualisil C_8 (5 µ, 250 mm × 4.60 mm) column at ambient temperature using a mobile phase consisting of methanol and water (75: 25, *v/v*), pH of mobile phase adjusted to 3. Flow rate was 1.0 mL/min. Before analysis both the mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed for 15 min in an ultrasonicator. The detection of these drugs was carried out at 240 nm. The average retention time for MOX and DSP was found to be 2.22, and 7.26 min, respectively. The UV- spectra of these two drugs in double distilled water is shown in (Figure 2).

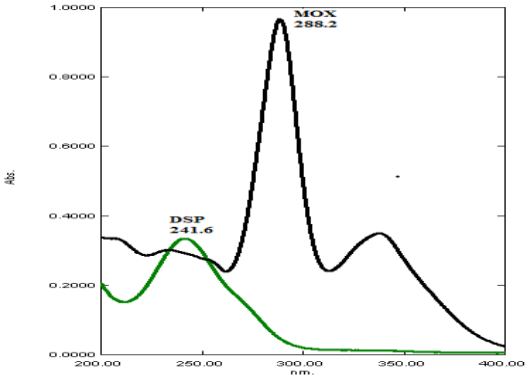

Figure 2. A typical overlay spectrum of standard MOX and DSP

2.1 Preparation of Stock Standard Solutions

Stock standard solutions were prepared separately by dissolving 10 mg of MOX and 10 mg of DSP in 100 mL

methanol to obtain concentration 100 µg/mL of MOX and DSP, each.

2.2 Method Validation

The HPLC method was validated in accordance with ICH guidelines.

2.3 Precision

The precision of the method was studied as intra-day and inter-day. Intra-day precision was determined by analyzing, the three different concentrations 20 µg mL^{-1}, 30 µg mL^{-1} and 40 µg mL^{-1} of MOX and 4 µg mL^{-1}, 6 µg mL^{-1} and 8 µg mL^{-1} of DSP, respectively, for three times in the same day. Day to Day variability was assessed using above mentioned three concentrations analyzed on three different days for inter-day precision.

2.4 Specificity and Selectivity

Specificity of the method was ascertained by analysing drug standard and sample. The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

2.5 Accuracy

To the pre-analyzed sample solution of 20 µg mL^{-1} of MOX and 4 µg mL^{-1} of DSP, a known amount of drug standards of MOX and DSP were added at 80, 100 and 120 % level and re-analysed using proposed method. The analysis was repeated for three times at each level.

2.6 Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, where 'ASD' is the average standard deviation and 'S' is the slope of the calibration curve.

2.7 Robustness

Robustness of the method was studied by making deliberate variations in the chromatographic conditions such as variation of pH (± 0.1) of the mobile phase; flow rate (± 0.1 mL min^{-1}) and change in mobile phase composition (methanol (± 2 mL): water (± 2 mL)). The robustness of the method was studied by using sample solution containing 20 µg mL^{-1} of MOX and 4 µg mL^{-1} solution of DSP, respectively.

2.8 Ruggedness

Ruggedness of the method was studied by two different analysts using same experimental and environmental conditions. It was performed by injecting 20 µg mL^{-1} of MOX and 4 µg mL^{-1} solution of DSP. Peak area was measured for same concentration solutions for six times.

3. Results and Discussion

3.1 Selection of Chromatographic Conditions and Optimization of Mobile Phase

Mobile phase was optimized with a view to separate MOX and DSP using Qualisil RP C-8 column (250 mm x 4.6 mm i.d., 5 µm. Initially, methanol and water in the various proportions were tried as mobile phase but the splitting of the peaks for both these drugs was observed. Therefore, after adjustment of pH of aqueous phase, methanol and water in 75:25 % v/v was tried for resolution of both these drugs. Good resolution and symmetric peaks were obtained for both drugs when the pH of the mobile phase (aqueous phase) was adjusted to 3.0. The flow rate of the mobile phase was 1.0 mL min^{-1}. Under optimum chromatographic conditions, the retention time for MOX and DSP was found to be 2.22 and 7.26 min, respectively when the detection was carried out at 240 nm. A typical chromatogram of two drugs is shown in (Figure 3).

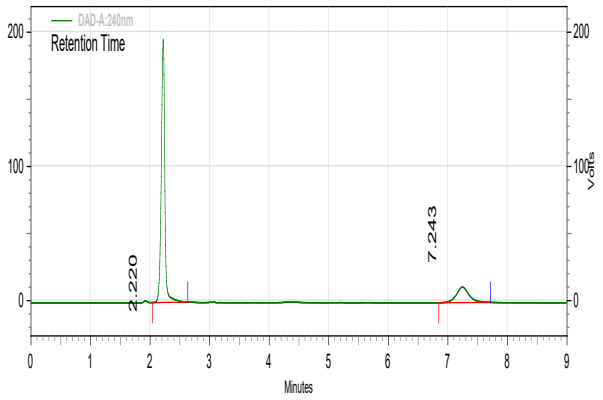

Figure 3: Chromatogram obtained for MOX and DSP standard in methanol: water (75:25 v/v), pH 3.0 (aqueous phase) as a mobile phase.

3.2 Linearity

The linearity was determined separately for MOX and DSP. Solutions of these drugs at six different concentrations were analyzed and calibration curves were constructed by plotting peak area against the respective concentrations. The method was evaluated by determination of the correlation coefficient and intercept values. The MOX and DSP followed linearity in the concentration range of 10 - 60 µg mL^{-1} and 2 - 12 µg mL^{-1}; respectively. The results are shown in Table 1.

Table 1: Linearity Studies.

Parameter	MOX	DSP
Linearity [µg mL^{-1}]	10 – 60	2 – 12
Linearity Equation	Y = 26468 X + 49911	Y = 26041 X + 5698
Slope±SD	26468± 436.03	26041±155.00
Intercept±SD	49911± 8267.66	5698± 774.64
Correlation Coefficient±SD	0.999± 0.0001	0.999± 0.0001

The precision study was evaluated on the basis of % RSD value. The intra-day precision for moxifloxacin hydrochloride and dexamethasone sodium phosphate was found to be in the range 0.21 - 0.62 and 0.16 - 0.61 %, respectively. And, the inter-day precision for MOX and DSP was found to be in the range 0.40 - 0.77 and 0.14 - 0.49 %, respectively. The low values of % RSD indicate high precision of the method. Results of precision study are shown in Table 2.

Table 2: Results from Precision studies

Conc. [µg/mL]	MOX		Conc. [µg/mL]	DSP	
	Amount Found in µg mL^{-1} [n= 3]±SD	% RSD		Amount Found in µg mL^{-1} [n= 3] ±SD	% RSD
Intra – day Precision					
20	19.98±0.04	0.21	4	4.02 ±0.02	0.61
30	29.59± 0.18	0.62	6	5.99± 0.01	0.29
40	40.30± 0.19	0.47	8	7.99± 0.01	0.16
Inter – day Precision					
20	19.94± 0.08	0.40	4	4.01±0.01	0.48
30	29.72± 0.23	0.77	6	6.01±0.008	0.14
40	39.93± 0.21	0.54	8	7.97± 0.03	0.49

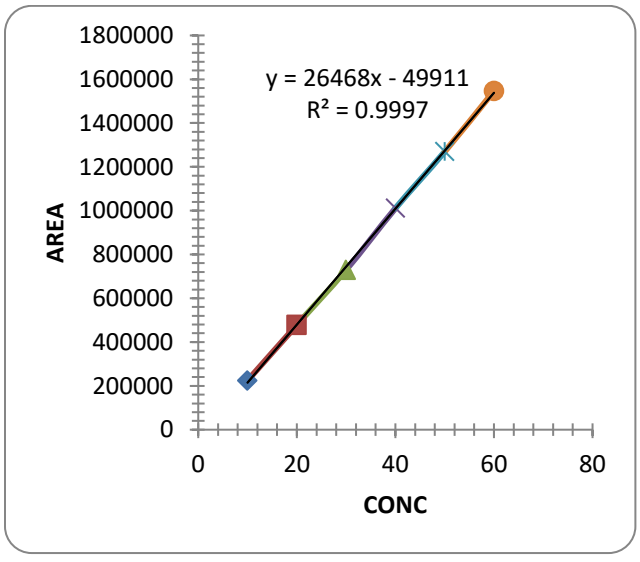

Fig. 4. Linearity of MOX

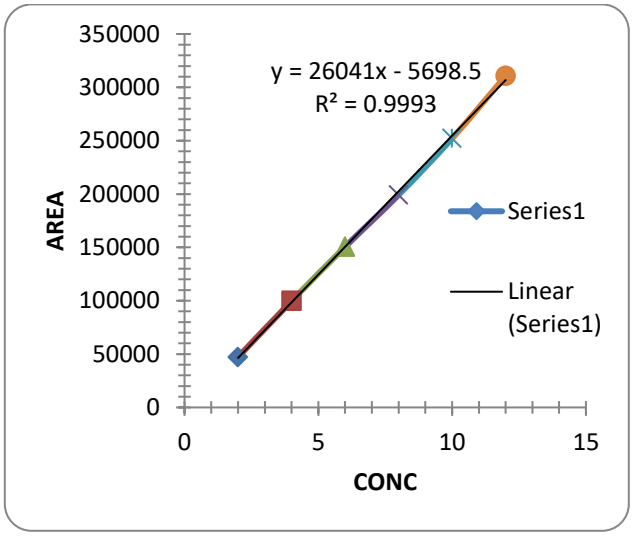

Fig. 5. Linearity of DSP

3.3 Specificity and Selectivity

Specificity of the method was ascertained by comparing the chromatogram obtained from ophthalmic solution and standard drug. The retention times of the standard drugs and the drugs from ophthalmic solutions were same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the ophthalmic solution. The method is quite selective. There was no other interfering peak around the retention time of MOX and DSP; also, the base line did not show any significant noise.

3.4 Accuracy

The accuracy of the method studied at three different concentration levels i.e. 80 %, 100 % and 120 % showed affordable % recoveries in the range of 99.35 - 100.79 % for MOX and 99.43 - 99.96 % for DSP. The results are shown in Table 3. The low value of % RSD indicates accuracy of the method.

Table 3: Recovery Study

Drugs	Label claimed (mg mL^{-1})	Amount of standard drug added (%)	% Drug Recovered [n = 3]	% R.S.D.
MOX	5	80	100	0.57
		100	100.79	0.90
		120	99.35	0.16
DSP	1	80	99.49	1.19
		100	99.43	0.92
		120	99.96	1.25

3.5 Sensitivity

The LOD for MOX and DSP was found to be 0.30 and 0.10 µg mL^{-1}, respectively. The LOQ for MOX and DSP was found to be 0.91 and 0.30 µg mL^{-1}, respectively. The low values of LOD and LOQ indicates adequate sensitivity of the method.

3.6 Robustness and Ruggedness study

Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. The content of the drugs were not adversely affected by these changes as evident from the low values of % RSD (less than 2 %) indicating robustness of the method.

When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged and % RSD (less than 2 %) indicating ruggedness of the method.

3.7 Analysis of marketed tablet formulation

Six replicates of the sample's solutions (20 ìL) were injected for quantitative analysis. The amounts of moxifloxacin hydrochloride and dexamethasone sodium phosphate estimated were found to 99.97 % and 99.68 %, respectively.

3.8 System Suitability Test

According to USP, system suitability test is integral part of liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

The results obtained from validation of the methods and system suitability studies are summarized in Table 4.

Table 4: Summary of system suitability study

Parameter	MOX	DPS
Retention time [t_R]	2.22	7.26
Theoretical plates [N]	7169	5891
Capacity factor [k']	21	67
Resolution [Rs]	-	21.13
Asymmetry [T]	0.90	1.08

4. Conclusion

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately rugged and robust and can be used for simultaneous determination of MOX and DSP in ophthalmic solution.

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