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## Simultaneous estimation of moxifloxacin hydrochloride and dexamethasone sodium phosphate in bulk and in eye drops by UV-Spectrophotometry

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### ABSTRACT

Moxifloxacin Hydrochloride (MOX) is a 4<sup>th</sup> generation fluoroquinolone broad spectrum antibiotic and Dexamethasone Sodium Phosphate (DSP) is glucocorticoid class of steroids. These two drugs are used in combination for eye infection. Two simple, rapid and economical simultaneous equation (Method-I) and Q-Analysis UV-Spectrophotometric (Method-II) methods have been developed for simultaneous estimation of MOX and DSP in eye drop. The methods involved solving simultaneous equations and Q-value Analysis based on measurement of absorbance at wavelengths, 288.2 nm ( $\lambda_{max}$  of MOX), 241.6 nm ( $\lambda_{max}$  of DSP) and 249 nm (Iso-absorptive point). MOX & DSP followed linearity in the concentration range of 2 – 12  $\mu\text{g/mL}$  and 4 – 32  $\mu\text{g/mL}$ , respectively with coefficient correlation greater than 0.999. The quantity of drugs estimated by proposed methods are in excellent accord with label claimed. The methods were validated statistically and for accuracy, precision, ruggedness and by recovery studies

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### KEYWORDS

Moxifloxacin hydrochloride;  
Dexamethasone sodium phosphate;  
Simultaneous equation method;  
Q- Absorbance ratio method.

### INTRODUCTION

Moxifloxacin Hydrochloride (MOX) chemically is 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<sup>[1]</sup>. Dexamethasone Sodium Phosphate (DSP) chemically is 9- fluoro-11b, 17, 21-trihydroxy-16a-methylpregna-1, 4-diene-3, 20-dione 21-(dihydrogen phosphate) disodium salt<sup>[1-3]</sup>. A fixed dose combination of MOX and DSP is available for treatment of eye infection. The review of the literature revealed that several methods have been developed for quantitative determination of MOX in pharmaceutical

formulation. This includes RP-HPLC, and hyphenated techniques such as, LC-MS have been reported for the determination of MOX single in biological fluids<sup>[4-7]</sup>. One RP-HPLC<sup>[8]</sup>, some UV-Spectrophotometric<sup>[9-12]</sup>, and HPTLC<sup>[13]</sup> 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 high proportion of phosphate buffer and methanol<sup>[14]</sup>.

It has been reported that DSP has been estimated by LC-MS in human plasma in combination with dexamethasone<sup>[15]</sup>, and in ear drops by RP-HPLC in

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combination with neomycin sulphate, polymyxin B sulphate<sup>[16]</sup>.

To our knowledge no method has been yet reported for simultaneous estimation of both the drugs in combined dosage form. In present work, a successful endeavor has been made to estimate both these drugs simultaneously by two simple UV-spectrophotometric methods (simultaneous equation method, Q-Absorbance ratio method)<sup>[17]</sup>. These methods are validated according to the ICH guidelines<sup>[18]</sup>.

## MATERIAL AND METHODS

### Chemicals

MOX and DSP were obtained from Cadila Pharmaceutical Ltd., Dholka, Ahmadabad, India as a gift sample. Eye drop (Milflox DM) was purchased from Indian market, containing 5 mg/ml of MOX and 1 mg/ml of DSP.

### Instrumentation

A UV-Visible spectrophotometer (Shimadzu-2450, UV Probe 2.21 software) with spectral bandwidth 1 nm was employed for all spectroscopic measurements, using a pair of 1.0 cm matched quartz cells.

### Selection of common solvent

Double distilled water was selected as common solvent for studying spectral characteristics of drug. The selection was made after assessing the solubility of both the drugs in different solvents.

### Preparation of stock standard solutions

Stock standard solutions of MOX and DSP were separately prepared by dissolving 10 mg in 100 mL water to obtain concentrations 100 µg/mL each.

### Simultaneous equation method (Method-I)

From the stock solution of 100 µg/mL, working standard solution of drugs were prepared by appropriate dilution and scanned in the UV-region i.e. 400 – 200 nm. From the overlain spectra (Figure 1)

Two wavelengths, 288.2 nm ( $\lambda_{\max}$  of MOX) and 241.6 nm ( $\lambda_{\max}$  of DSP) were selected for the formation of simultaneous equation. Standard solutions were prepared in concentration 2-12 µg/mL for MOX

and 4 - 32 µg/mL for DSP. The absorbances of these standard solutions were measured at 288.2 nm and 241.6 nm and calibration curves were plotted at these wavelengths. Two simultaneous equations (in two variables  $C_1$  and  $C_2$ ) were formed using these E (1%, 1cm) values (TABLE 1).

$$A_1 = 932.667 C_{\text{MOX}} + 37.83 C_{\text{DSP}} \quad (1)$$

$$A_2 = 278.66 C_{\text{MOX}} + 270.0 C_{\text{DSP}} \quad (2)$$

Where,  $C_{\text{MOX}}$  and  $C_{\text{DSP}}$  are the concentration in g/100 mL in sample solution.  $A_1$  and  $A_2$  are absorbance of mixture at selected wavelength 288.2 nm and 241.6 nm, respectively.

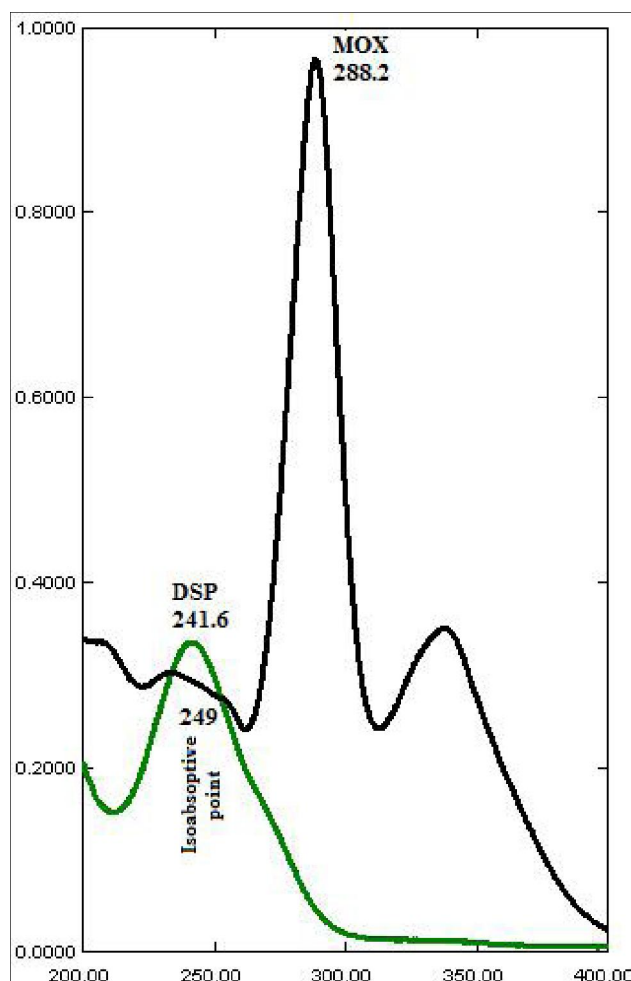


Figure 1 : Overlain spectra of MOX and DSP

By applying the Cramer's rule to equation 1 and 2, the concentration  $C_{\text{MOX}}$  and  $C_{\text{DSP}}$ , can be obtained as follows;

$$C_{\text{MOX}} = (A_2 \times 37.83) - (A_1 \times 270.0) / -241276.5 \quad (3)$$

$$C_{\text{DSP}} = (A_1 \times 278.66) - (A_2 \times 932.667) / -241276.5 \quad (4)$$

### Q-Absorbance ratio method (Method-II)

From the overlain spectrum of MOX and DSP, two wavelengths were selected one at 288.2 nm,  $\lambda_{\text{max}}$  of MOX and other at 249 nm which was isoabsorptive point for both the drugs. The E (1%, 1 cm) values for both the drugs at selected wavelengths (TABLE 1).

**TABLE 1 : E (1%, 1cm) value of MOX and DSP at 241.6 nm, 288.2 nm and 249 nm**

Sr. No	(E 1%, 1cm) at 241.6 nm		(E 1%, 1cm) at 288.2 nm		(E 1%, 1cm) at 249 nm	
	MOX	DSP	MOX	DSP	MOX	DSP
Mean	278.667	270	932.667	37.83	272.66	272.333
SD*	1.03279	0.894427	1.03279	1.0327	1.966	1.3662
%RSD**	0.307062	0.33126	0.1107	0.3706	0.72116	0.5546
SEE***	0.377	0.971	1.09	0.419	2.067	1.34

SD\*- Standard deviation

%RSD\*\*- % Relative standard deviation

SEE\*\*\*- Standard error of estimate

The method employed Q-values, concentrations of drugs in sample solutions were determined using following formula;

$$C_{\text{DSP}} = \frac{Q_m - Q_y}{Q_x - Q_y} X \frac{A}{ax_1} \quad (5)$$

$$C_{\text{MOX}} = \frac{Q_m - Q_x}{Q_y - Q_x} X \frac{A}{ay_1} \quad (6)$$

Where, A1 and A2 are the absorbances of mixture at 249 nm and 288.2 nm,  $ax_1$  (272.33),  $ax_2$  (37.83),  $ay_1$  (272.66) and  $ay_2$  (932.667) are absorptivities E (1%, 1 cm) of MOX and DSP at 249 nm and 288.2 nm and  $Q_m = A2/A1$ ,  $Q_y = ay_2/ay_1$  and  $Q_x = ax_2/ax_1$ .

### Assay of eye drop formulation (ophthalmic solution) by Method-I and Method-II

From the contents of six eye drops an accurately measured volume of ophthalmic solution equivalent to 10 mg of MOX and 2 mg DSP was transfer into 100 mL volumetric flask containing 25 mL of water, to it 8 mg of DSP working standard was added and volume was made up to the mark with water, filtered through 0.45  $\mu\text{m}$  Whatmann filter paper. An appropriate volume of solution was further diluted with water to obtain concentration 10  $\mu\text{g/mL}$  of MOX and 10  $\mu\text{g/mL}$  of DSP. Absorbance of sample solution were recorded at 241.6 nm, 249 nm and 288.2 nm and the concentration of two drugs in the sample were

determined using equations 3, 4 (Method-I) and 5, 6 (Method-II). The analysis procedure was repeated for six times with ophthalmic solution. The results of analysis are reported in (TABLE 2).

**TABLE 2 : Results of simultaneous estimation of marketed formulation Milfloxx DM**

Method	Eye drop content	Label claim mg/mL	% Label claim	% RSD
I	MOX	5	99.12	1.29
	DSP	1	101.83	1.03
II	MOX	5	99.35	1.51
	DSP	1	100.4	1.19

### % Recovery studies

To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method. A known amount of drug was added to pre-analyse ophthalmic solution at 80, 100 and 120% level and percentage recoveries were calculated. The results of recovery studies were satisfactory and are presented in (TABLE 3).

**TABLE 3 : Results for recovery studies**

Level of recovery	Amount of drug added $\mu\text{g/mL}$	Drug	Method-I			Method-II		
			% Recovery	% RSD	SEE	% Recovery	% RSD	SEE
80%	3.2	MOX	100.66	1.14	0.71	99.99	0.005	0.76
	3.2	DSP	102	1.38	0.56	98.97	0.08	0.56
100%	4	MOX	100.55	0.22	0.25	100.61	0.12	0.21
	4	DSP	101.45	0.17	0.36	99.01	0.54	0.48
120%	4.8	MOX	102.6	0.54	0.98	102.9	0.65	0.88
	4.8	DSP	102.9	0.33	0.95	102.9	0.25	0.89

## RESULTS AND DISCUSSION

MOX and DSP followed linearity in the concentration range of 2 - 12  $\mu\text{g/mL}$  and 4 - 32  $\mu\text{g/mL}$ , respectively. Marketed brand of eye drop was analyzed and amount of MOX and DSP determined by proposed method I & II was found to be 99.2, 99.35% for MOX and 101.83, 100.4% for DSP. The proposed methods were validated as per ICH guideline. The accuracy of method was determined by calculating mean percentage recovery. The % recoveries range from 98.97 to 102.9 for MOX and DSP in both these methods. Precision was calculated as repeatability (% RSD is less than 2) and inter and intraday variations

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(%RSD is less than 2) for both drugs and validation parameters are presented in (TABLE 4).

**TABLE 4 : Validation parameter**

Parameters	MOX	DSP		
<b>Linear regression data</b>				
Linearity range [ $\mu\text{g/mL}$ ]	2 – 12	4- 32		
Correlation coefficient [ $r^2$ ]	0.9996	0.9994		
Chi square	0.00031	0.0008		
Standard error of estimate	0.00751	0.00646		
Residual standard deviation	0.00672	0.00609		
<b>Sensitivity</b>				
LOD [ $\mu\text{g/mL}$ ]	0.056	0.098		
LOQ [ $\mu\text{g/mL}$ ]	0.25	0.78		
Sandell's sensitivity $\mu\text{g/cm}^2/0.001/\text{abs unit}$	0.01072	0.037037		
<b>Precision [% RSD]</b>	<b>Method - I</b>		<b>Method - II</b>	
	<b>MOX</b>	<b>DSP</b>	<b>MOX</b>	<b>DSP</b>
Intra – day [n=3]	1.19	0.59	0.98	0.63
Inter – day [n=3]	1.5	0.63	0.89	0.59
Repeatability [ n=6]	0.8	0.38	0.97	0.93

The proposed methods were found to be simple, accurate and rapid for the routine determination of MOX and DSP in eye drop formulation. To study the validity and reproducibility of proposed methods, recovery experiments were carried out. The methods were validated in terms of linearity, accuracy, precision, specificity and reproducibility.

## CONCLUSION

These two methods are simple can be successfully used for simultaneous estimation of moxifloxacin hydrochloride and dexamethasone sodium phosphate in combined dosage form.

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