

Application of Ratio Spectra Derivative Method for Simultaneous Estimation of Moxifloxacin Hydrochloride and Loteprednol Etabonate from Ophthalmic Formulation

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Gamit *et al.*: Estimation of Moxifloxacin Hydrochloride and Loteprednol Etabonate from Ophthalmic Formulation

A simple, precise and accurate ultraviolet-visible spectrophotometer method has been developed by applying ratio spectra derivative method and validated for the simultaneous estimation of moxifloxacin hydrochloride and loteprednol etabonate from ophthalmic formulation. Absorbance of moxifloxacin hydrochloride and loteprednol etabonate was recorded at their respective wavelength and then zero order spectra was converted to first order derivative spectra after dividing respective zero order spectra with divisor concentration. Developed method was linear at concentration range 4-20 µg/ml, ($r^2=0.99$) and ($r^2=0.99$), for loteprednol etabonate and moxifloxacin hydrochloride respectively. The linear regression equations were $y=0.005x+0.001$ for loteprednol etabonate and $y=0.118x-0.062$ for moxifloxacin hydrochloride. The results of analysis were validated which showed that proposed method was simple, precise and accurate.

Key words: Moxifloxacin hydrochloride, loteprednol etabonate, ratio spectra derivative method

Quantitative analysis of any drug is an important tool in the pharmaceutical industry. It is important to determine that the raw material, intermediate products as well as final products meet its specifications and are of required quality. Multicomponent dosage forms are known to be effective due to their combined mode of action in the body. The complexities of these dosage forms including the presence of multiple drug entities possess considerable challenge to the analytical chemist during development of assay procedure. Combination of Moxifloxacin Hydrochloride (MOX) and Loteprednol Etabonate (LOT) is used to treat conjunctivitis^[1-4].

MOX is a 4th generation synthetic fluoroquinolone antibacterial agent^[5]. It 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 (fig. 1). Various High Performance Liquid Chromatography (HPLC), High-Performance Thin-layer Chromatography (HPTLC) and spectroscopic methods are available for estimation of MOX in Active Pharmaceutical Ingredient (API)

and pharmaceutical dosage form^[6-18].

Chemically, LOT is 7-(ethyl carbonate) chloromethyl 11 β ,17-dihydroxy-3-oxoandrost-1,4-diene-17 β -carboxylate (fig. 2). It was estimated in bile and blood by HPLC^[19] and in combination with other drug by RP-HPLC and spectroscopic method^[20-23]. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of LOT and MOX in their combined synthetic mixture or dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of LOT-E and MOX-I in synthetic mixture or combined dosage forms. Combination of MOX and LOT in ophthalmic formulation was estimated by spectrophotometric Q-absorbance ratio method. Absorbance ratio

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method uses the ratio of absorbances at two selected wavelengths, one which is an iso-absorptive point and other being the λ_{\max} of one of the two components^[24].

Spectrophotometry methods are considered to be suitable for the simultaneous estimation of the drugs present in multicomponent dosage forms. Ratio Spectra Derivative method used for the simultaneous estimation of MOX and LOT because it has advantage to suppress matrix effect, resolve the overlay spectra, high number of analytical signal and maxima and minima, easy measurement and rapid operation. So, it was thought of our interest to develop an analytical method for simultaneous estimation of MOX and LOT by ratio spectra derivative method. Numbers of multicomponent dosage forms are available with ingredients, which provide a challenge for analyst for estimation of active drugs by different analytical methods in fixed dosage combination

(Table 1).

MATERIALS AND METHODS

Procurement of active pharmaceutical ingredient and formulation:

MOX was procured from Cadila Pharmaceuticals Ltd., Dholka and LOT was procured from Arti healthcare Ltd., Mumbai as a gift sample. Formulation named Moxinix-LP eye drops was procured from the market which contained MOX and LOT 0.5 % w/v individually.

Reagents and instrument:

Methanol (AR Grade) was purchased from Lobachem, India. Ultraviolet (UV)-Visible Spectrophotometer (UV-1800, Shimadzu Corporation, Japan) was used to carry out experiments.

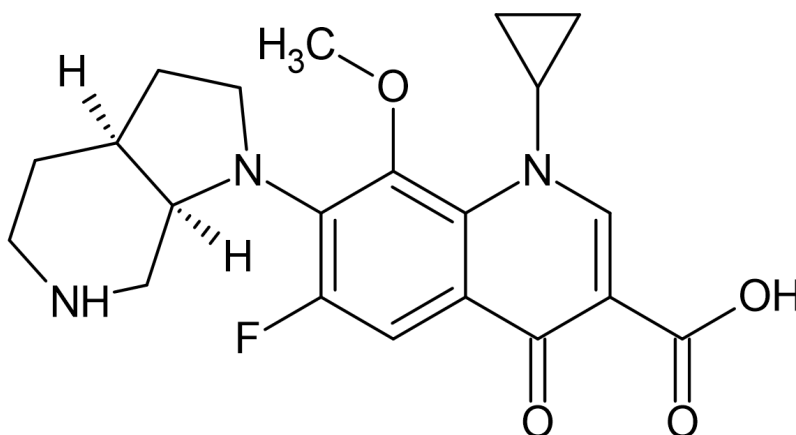


Fig. 1: Chemical structure of MOX

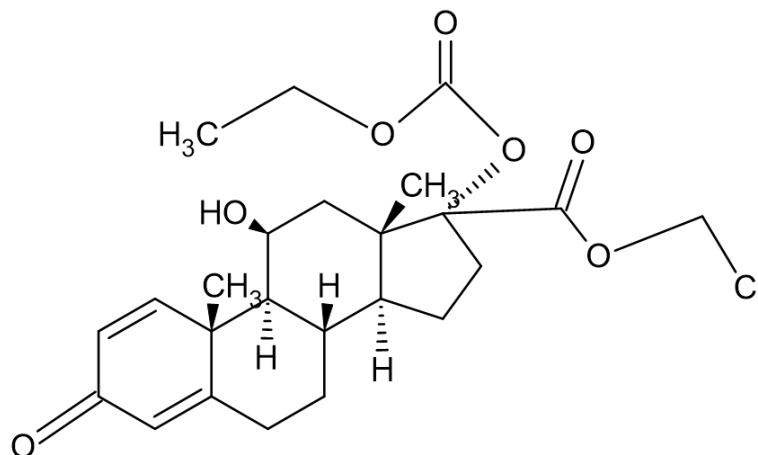


Fig. 2: Chemical structure of LOT

TABLE 1: OPTIMIZED PARAMETER FOR RATIO DERIVATIVE METHOD

Parameter	Optimized value
Wavelength range	200-400 nm
Concentration range	4-20 µg/ml
Divisor concentration for MOX	16 µg/ml LOT
Divisor concentration for LOT	12 µg/ml MOX
Smoothing factor	8 nm
Scaling factor	1
Analytical wavelength for MOX	285 nm
Analytical wavelength for LOT	238 nm
Solvent	Methanol

Preparation of standard stock solution:

MOX standard stock solution (100 µg/ml): 10 mg of standard MOX was weighed and transferred to a 100 ml volumetric flask and dissolved in 30 ml methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 100 µg/ml of MOX.

LOT standard stock solution (100 µg/ml): 10 mg of standard LOT was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in 30 ml methanol. The flask was shaken, and volume was made up to the mark with methanol to give a solution containing 100 µg/ml of LOT.

Selection of analytical wavelength:

10 µg/ml solutions of MOX and LOT were prepared by transferring 1 ml from the standard stock solution of MOX and LOT to the 10 ml volumetric flask and diluted up to the mark with methanol and spectrums were recorded between 200-400 nm. The spectrum of MOX and LOT was recorded and peak maxima of both the drugs were found. The peak maximum of MOX was 294 nm and a peak maximum of LOT was 243 nm were obtained.

Calibration curve for the MOX (4-20 µg/ml):

Appropriate volume of aliquots 0.4, 0.8, 1.2, 1.6, 2 ml from standard MOX stock solutions were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 4, 8, 12, 16 and 20 µg/ml. Absorbance of each solution against the methanol at wavelengths 294 nm were measured and after dividing all zero order spectra with divisor concentration (LOT 16 µg/ml) and

converted this ratio spectra into first derivative, the absorbance measured at 285 nm. Results are shown in Table 2.

Calibration curve for the LOT (4-20 µg/ml):

Appropriate volume of aliquots 0.4, 0.8, 1.2, 1.6, 2 ml from standard LOT stock solutions were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 4, 8, 12, 16 and 20 µg/ml. Absorbance of each solution against the methanol at wavelength of 243 nm were measured and after dividing all zero order spectra with divisor concentration (MOX 12 µg/ml) and converted this ratio spectra into first derivative, the absorbance measured at 238 nm. Results are shown in Table 2.

Sample preparation of MOX and LOT from ophthalmic formulation:

The quantity of eye drops equivalent to 1 ml volume was taken in 100 ml volumetric flask, 30 ml methanol added to the flask, the flask was shaken and volume was made up to the mark with the methanol to obtain final concentration of 50 µg/ml LOT and MOX. From the above solution 2.5 ml of aliquot was taken and transferred to the 10 ml volumetric flask, the volume was made up to the mark with methanol to get a concentration 12.5 µg/ml of MOX and LOT. Solution filtered with the help of whatman filter paper (Grade 42). This solution further used for estimation of MOX and LOT.

Validation of UV Method:

Linearity: Linearity was established by taking absorbance at 285 nm and 238 nm respectively of MOX and LOT at concentration range 4-20 µg/ml.

Precision: Interday and intraday precision is expressed in percentage Relative Standard Deviation (% RSD). For interday evaluation, data was taken on 3 consecutive d and for intraday data was taken 3 times in same day for precision study, concentration selected for both MOX and LOT is 8, 12 and 16 µg/ml. The result obtained for each concentration was subjected to statistical treatment to determine mean and % RSD. The results of the studies are shown in Table 3 and Table 4 for intraday and interday precision respectively^[25-31].

Accuracy: Accuracy for method was confirmed by recovery study from marketed formulation at three levels of standard addition 50 %, 100 % and 150 %. Concentration selected for MOX and LOT to perform accuracy study was 8 µg/ml. Results of accuracy studies are shown in Table 5.

Repeatability: Repeatability was performed by recording absorbance for MOX and LOT at selected concentration, 8 µg/ml. The results are shown in Table 6. LOD and LOQ values were determined to establish sensitivity of the developed analytical method for MOX and LOT.

Assay: From the calibration curve, the line equation was constructed using the least square regression analysis. Amount of MOX and LOT present in tablet was calculated from line equation using area of peak corresponded to MOX and LOT. Results are shown in Table 7.

RESULT AND DISCUSSION

For selection of divisor concentration, all the concentration of range was tested individually. 16 µg/ml LOT and 12 µg/ml MOX selected as a divisor concentration because it gives high correlation coefficient (fig. 3 and fig. 4). Derivative spectra calculating with smoothing factor 8 nm and scaling factor 1, it gives best derivative spectra. So this is considered for determination. Parameters selected for optimization of method are mentioned in Table 1.

Linearity range for LOT and MOX were 4-20 µg/ml, ($r^2=0.99$) and ($r^2=0.99$), for LOT and MOX respectively. The linear regression equations are $y=0.118x-0.062$ for MOX and $y=0.005x+0.001$ for LOT shown in Table 2, fig. 5 and fig. 6.

TABLE 2: CALIBRATION DATA OF LOT AND MOX

Concentration	LOT (238 nm)		MOX (285 nm)	
	Absorbance±standard deviation	% RSD	Absorbance±standard deviation	% RSD
4	0.022±0.00037	1.68	0.422±0.00047	0.11
8	0.042±0.00068	1.61	0.868±0.00057	0.06
12	0.066±0.00115	1.66	1.368±0.00074	0.05
16	0.089±0.00089	1.02	1.822±0.00106	0.05
20	0.109±0.00115	1.05	2.323±0.00372	0.16

TABLE 3: INTRADAY PRECISION OF MOX AND LOT

Concentration (µg/ml)	MOX		LOT	
	Absorbance±standard deviation	% RSD	Absorbance±standard deviation	% RSD
8	0.868±0.000577	0.06	0.042±0.00057	1.35
12	1.366±0.0036	0.26	0.064±0.00057	0.78
16	1.824± 0.0045	0.24	0.087±0.0015	1.14

TABLE 4: INTERDAY PRECISION OF MOX AND LOT

Concentration (µg/ml)	MOX		LOT	
	Absorbance±standard deviation	% RSD	Absorbance±standard deviation	% RSD
8	0.868±0.001	0.11	0.041±0.00057	1.38
12	1.366±0.0023	0.16	0.065±0.0015	1.53
16	1.8255±0.0055	0.3	0.086±0.0017	1.93

TABLE 5: ACCURACY OF MOX AND LOT (n=3)

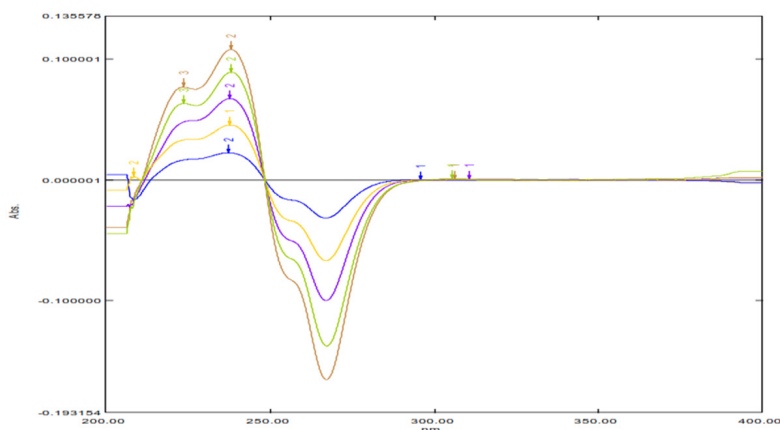
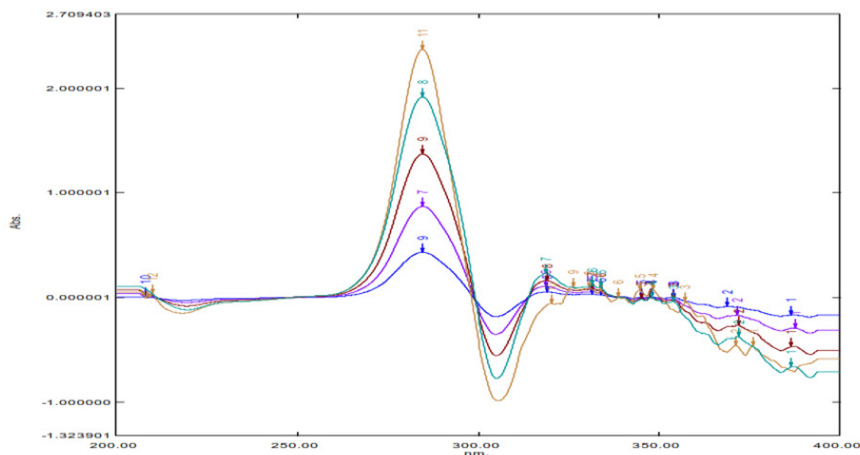
Concentration ($\mu\text{g}/\text{ml}$)	Level (%)	MOX		LOT	
		Mean % recovery \pm standard deviation	% RSD	Mean % recovery \pm standard deviation	% RSD
8	50 %	98.56 \pm 0.53	0.54	96.94 \pm 4.74	4.89
	100 %	100 \pm 1.08	1.08	99.00 \pm 1.02	1.03
	150 %	98.98 \pm 0.26	0.26	98.7 \pm 0.46	0.46

TABLE 6: REPEATABILITY OF MOX AND LOT (n=6)

Concentration ($\mu\text{g}/\text{ml}$)	MOX		LOT	
	Mean \pm standard deviation	% RSD	Mean \pm standard deviation	% RSD
8	0.868 \pm 0.00057	0.06	0.041 \pm 0.00057	1.39

TABLE 7: ASSAY MOX AND LOT (n=3)

API	Label claim (%)	Amount obtain (%)	(%) Mean \pm standard deviation	% RSD
MOX	0.5	0.51	100.13 \pm 0.152	0.152
LOT	0.5	0.49	98.6 \pm 1.154	1.17

**Fig. 3: Ratio derivative spectra of LOT by using MOX-12 $\mu\text{g}/\text{ml}$ as a divisor concentration at 238 nm (smoothing factor=8 nm), range (4-20 $\mu\text{g}/\text{ml}$)****Fig. 4: Ratio derivative spectra of MOX by using LOT-16 $\mu\text{g}/\text{ml}$ as a divisor concentration at 285 nm (smoothing factor=8 nm), range (4-20 $\mu\text{g}/\text{ml}$)**

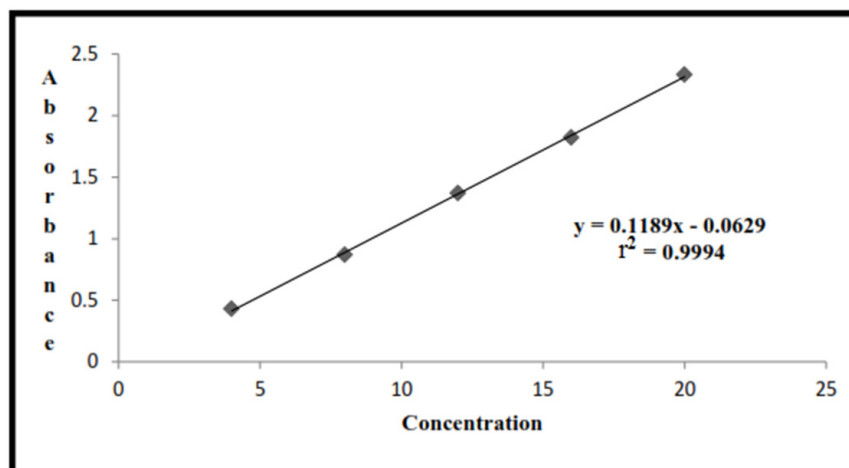


Fig. 5: Calibration curve of MOX by using LOT 16 µg/ml as divisor concentration at 285 nm

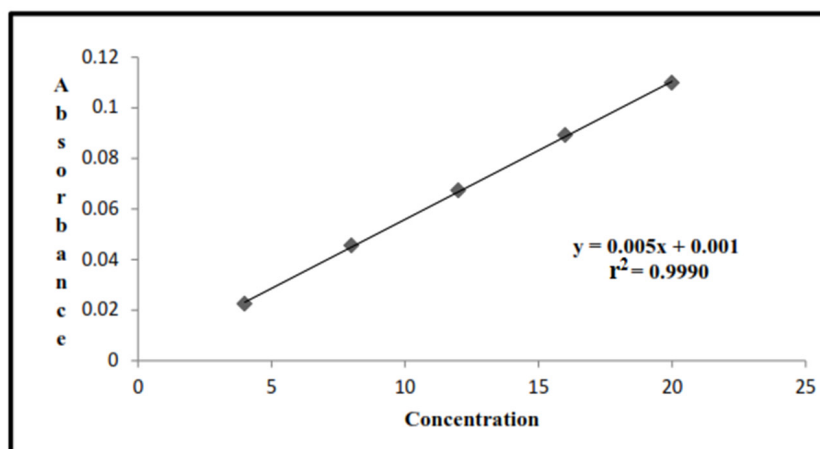


Fig. 6: Calibration curve of LOT by using MOX 12 µg/ml as a divisor concentration at 238 nm

The correlation coefficient value for plot of concentration of MOX and LOT was found approaching 1.0, which ensured the linearity of area for the concentration range selected for the studies. The correlation coefficient value for plot of concentration of MOX and LOT was found approaching 1.0, which ensured the linearity of area for the concentration range selected for the studies. The results of precision studies are shown in Table 3 and Table 4, which showed that showed that % RSD value for all three selected concentrations was within the recommended limit. Intraday precision was 0.06 %-0.26; % RSD was 0.78 %-1.35 %; interday precision was found to 0.11-0.30; % RSD was 1.38 %-1.93 % for MOX and LOT respectively. As the value for % RSD was very low, it may confirmed that method was precise.

Accuracy studies were carried out at three different level, i.e., 50, 100 and 150 %. Concentration of the sample solution selected was 8 µg/ml for both

MOX and LOT. Results of accuracy studies are shown in Table 5. The results of repeatability for MOX and LOT are shown in Table 6 for selected concentration of 8 µg/ml. LOD was found to 0.01 µg/ml and 0.56 µg/ml for MOX and LOT respectively. LOQ was found to be 0.042 µg/ml and 1.70 µg/ml for MOX and LOT respectively. Assay of MOX and LOT was performed from eye drops. Results are shown in Table 7.

Accurate and precise method was developed for simultaneous estimation of MOX and LOT. Method was validated according to International Council for Harmonisation guideline. The % RSD value is less than 2 obtained. This method can be applied for the simultaneous estimation of MOX and LOT from ophthalmic formulation. The excipients usually present in the pharmaceutical formulation did not interfere with estimation of MOX and LOT.

Conflict of interest:

The authors declared no conflict of interests.

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