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DEVELOPED AND VALIDATED Q-ABSORPTION RATIO METHOD FOR THE SIMULTANEOUS ESTIMATION OF LOTEPREDNOL ETABONATE AND MOXIFLOXACIN HCL IN THE PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Loteprednol Etabonate and Moxifloxacin HCl are available in the market as combined ophthalmic formulation in ratio of 1:1 for the treatment of Post-operative inflammatory condition. The aim of the present work was to develop a simple, precise, sensitive and economical UV-Spectrophotometric method for the simultaneous estimation of Loteprednol Etabonate and Moxifloxacin HCl in the combined formulation. The wavelengths selected were 242.60 nm (λ_{max} of Loteprednol Etabolate) and 265 nm (Iso-absorptive point). Beer's law was followed in the range of 5-30 µg/ml for both the drugs Loteprednol Etabonate and Moxifloxacin HCl with good recovery results for the accuracy. The reproducibility of the method was determined (Interday and Intraday), the relative standard deviation was found to be below 2%. The method was validated as per ICH guideline and successfully applied for the formulation analysis. A simple, accurate, precise, sensitive and economical UV Spectrophotometric method for the simultaneous estimation of Loteprednol Etabonate and Moxifloxacin HCl in the combined formulation has been developed.

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INTRODUCTION

Moxifloxacin HCl (MOXI) (Fig. 1) is a fourth generation fluroquinolone, the antimicrobial activity of which depends upon inhibition of DNA gyrase (bacterial topoisomerase II), an enzyme necessary for DNA replication, transcription, repair and recombination. Moxifloxacin has in-vitro and in-vivo activities against wide range of gram+ve and gram-ve bacteria.[1-2] It is chemically 31-cyclopropyl-6-fluoro-8-methoxy-7-[(4a,7as)-octhydro-6H-pyrrolo[3,4-b]pyridine-6-yl]-4-oxo-1,4-dihydroquinolone-3-carboxylic acid hydrochloride. Loteprednol Etabonate (LOTE) (Fig. 2) is a corticosteroid derivative, anti-inflammatory activity which depends upon induction of Phospholipase A2 inhibitory proteins, an enzyme necessary for

release of inflammatory mediator from phospholipids membrane. It is chemically Loteprednol Etabonate Chloromethyl 17-ethoxycarbonyloxy- 11-hydroxy- 10,13-dimethyl-3-oxo- 7,8,9,11,12,14,15, 16-octahydro- 6*H*-cyclopenta phenanthrene-17-carboxylate. Clinically a combination of Moxifloxacin HCL and Loteprednol Etabonate is being used in the treatment of post operative steroidal inflammatory condition where risk of bacterial population high.

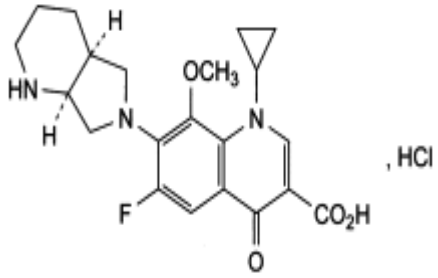

Figure. 1: Structure of Moxifloxacin

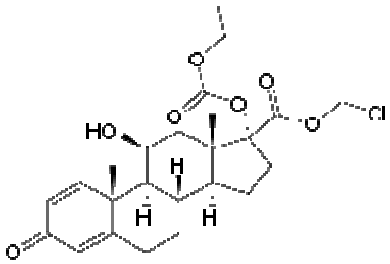

Figure 2: Structure of Loteprednol

The combination of Moxifloxacin HCl and Loteprednol Etabonate is not official in any official pharmacopoeia. A literature survey revealed that only a few methods based on HPLC[3-8],HPTLC[9-11], Spectrometry[12-15] were reported for the determination of Moxifloxacin Hydrochloride and HPLC[16] was reported for the determination of Loteprednol Etabonate but no single method is reported for the simultaneous estimation of Moxifloxacin HCl and Loteprednol Etabonate in pharmaceutical dosage form.

The objective of the present study was to develop a simple precise, accurate and economic analytical method with better detection range, for the estimation of Moxifloxacin HCl & Loteprednol Etabonate in bulk and pharmaceutical formulation. In the analytical method developed, Methanol was used as analytical media, as both drugs were found to be stable in Methanol so Methanol used as a solvent for this method. The developed method was validated as per ICH guidelines. [17]

MATERIAL AND METHODS:

Instrumentation:

Shimadzu model 1800 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 (UV Probe version 2.33).

Materials and Reagents:

MOXI and LOTE bulk powder was gifted by Cipla Healthcare Pvt. Ltd., Goa, India and Molecular Laboratory, Ahmadabad, India respectively. The commercial fixed dose combination product was procured from the local market. Methanol AR Grade was procured from S.D. Fine Chemicals Ltd., Mumbai, India.

Standard and Test Solutions:

Preparation of Standard solution: An accurately weighed quantity of MOXI (10 mg) and LOTE (10 mg) were transferred to a separate 100 mL volumetric flask and dissolved and diluted to the mark with Methanol to obtain standard solution having concentration of MOXI (100 µg/mL) and LOTE (100 µg/mL).

Preparation of Test solution: From the Ophthalmic formulation, Mahaflox-LP (0.5 % w/v MOXI & 0.5 % w/v LOTE), 1.0 ml taken in 100 ml volumetric flask and the volume was adjusted to mark with Methanol. These working sample solutions diluted with Methanol which having strength 50 µg/mL of MOXI & 50 µg/mL of LOTE.

Stock solution: The Aliquot portions of stock standard solutions of MOXI and LOTE were diluted appropriately with Methanol to get a series of concentration between 5 - 30 (µg/ml) of MOXI and LOTE. The absorbance of each solution was measured at 265 nm and 242.60 nm in 1 cm cell against solvent blank. The graphs plotted as

concentration Vs absorbance at selected wavelengths are shown in Fig. 3 to 5.

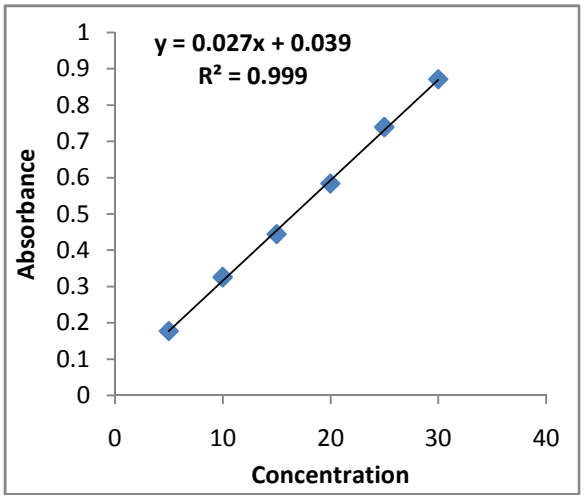

Figure. 3: Calibration Curve of LOTE at

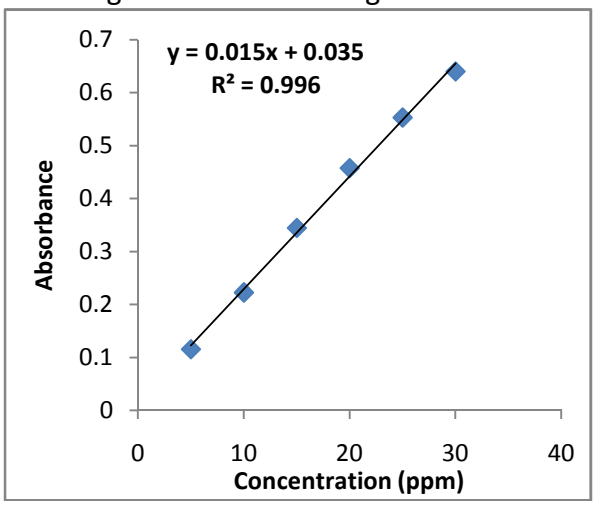

Figure 4: Calibration Curve of MOXI at

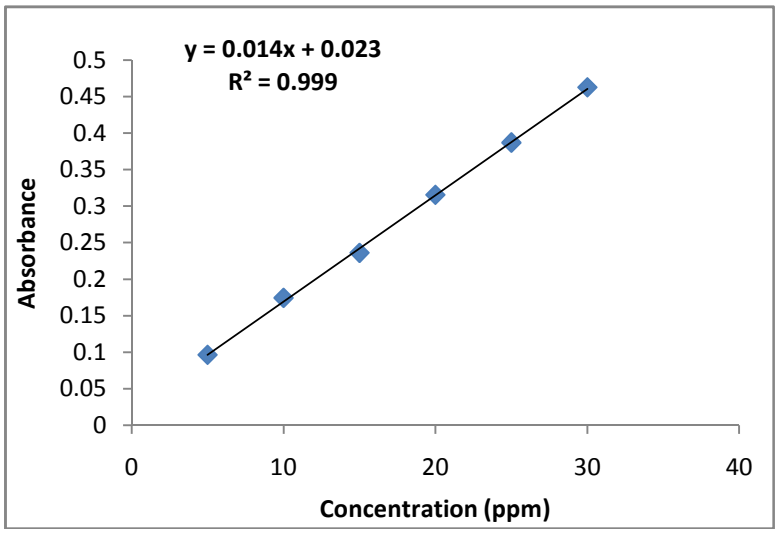

Figure 5: Calibration Curve at Isoabsorptive Point

METHODS:

This method is applicable to the drugs that obey Beer's law at all wavelengths and the ratio of absorbances at any two wavelengths is a constant value, independent of concentration or path length**.** Two wavelengths, 265 nm (Isoabsorptive point) and 242.60 nm (λmax of LOTE) were selected for the formation of Q-absorbance equation. The absorptivity co-efficient of each drug at both the wavelengths were determined. The concentration of individual components, MOX and LOTE may be calculated using the following equations

$C_{LOTE}= (Q_m–Q_{MOXI}/Q_{LOTE}–Q_{MOXI})*A_1/ax_1$ ..... (1)

$C_{MOXI}= (Q_m–Q_{LOTE}/Q_{LOTE}–Q_{MOXI})*A_1/ay_1$ ..... (2)

Where, $Q_m= A_2 / A1$, $Q_{LOTE} = ax_2 / ax_1$ & $Q_{MOXI} = ay_2/ay_1$;

A_1 and A_2 are absorbance of sample solution at Isoabsorptive point (265 nm) and λ_{max} of LOTE (24.60 nm) respectively; ax_1 and ax_2 are the absorptivities of LOTE at 265 and 242.60 nm respectively and ay_1 and ay2 are the absorptivities of MOXI at the two wavelengths respectively. [18-20]

Q-Absorption Ratio Method: As shown in Fig. 3, the overlain spectra of both drugs show a reproducible Isoabsorptive point at 265 nm. Thus estimation of drugs by Q-absorbance ratio equation method was carried out at 265 nm

(Isoabsorptive point) and 242.60 nm (λ_{max} of LOTE). The standard solutions of MOXI and LOTE were prepared to determine the absorptivity values of the subject analyte at the two selected wavelengths. The method showed good linearity in the range of 5-30 µg/mL for MOXI and 5-30 µg/mL for LOTE.

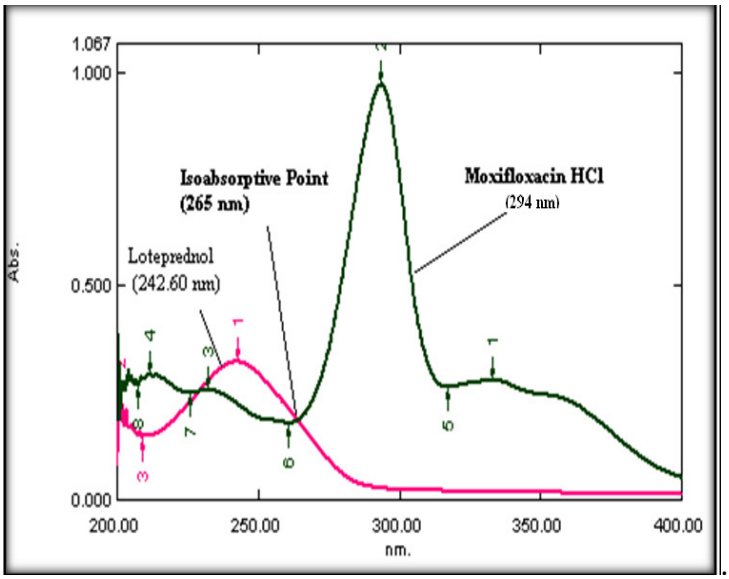

Figure 6: Overlain Absorption Spectra of Moxifloxacin HCl and Loteprednol Etabonate

METHOD VALIDATION:

The developed methods were validated for parameters like linearity, precision, accuracy, LOD, LOQ. The data for which are presented in the following **Tables 1-2.** The low value of % R.S.D. Indicates that all the methods are precise and accurate.

TABLE 01: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETER FOR THE PROPOSED METHOD (n=5)

SR NO.	PARAMETER	LOTE	MOXI	MOXI & LOTE at ISOABSORPTIVE POINT
1	Linearity range µg/mL	5-30	5-30	5-30
2	Slope	0.027	0.015	0.014
3	Intercept	0.039	0.035	0.039
4	Regression Co-efficient	0.999	0.997	0.999
5	Limit of Detection (LOD) µg/mL	0.549	0.630	0.418
6	Limit of Quantification (LOQ) µg/mL	1.665	1.912	1.269
7	Repeatability % RSD (n = 6)	0.446	0.574	0.607
8	Interday Precision % RSD (n = 3)	0.36 – 0.90	0.20 – 0.36	0.46 – 1.05
9	Interday Precision % RSD (n = 3)	0.36 – 0.87	0.35 – 0.96	0.56 – 0.77

(n = number of repetition) R.S.D = Relative standard deviation

TABLE 2: RECOVERY DATA FOR THE PROPOSED METHOD (n=5)

DRUG	AMOUNT TAKEN (µg/mL)	AMOUNT ADDED (µg/mL)	AMOUNT ADDED %	% MEAN RECOVERY ± S.D.
LOTE	10	5	50 %	98.40 ± 1.10
	10	10	100 %	98.47 ± 0.80
	10	15	150 %	100.34 ± 1.27
MOXI	10	5	50 %	100.80 ± 1.14
	10	10	100 %	101.05 ± 0.85
	10	15	150 %	101.34 ± 0.45

(n = number of repetition) S.D. = Standard deviation

ANALYSIS OF MARKETED FORMULATION:

The MOXI & LOTE solution were prepare of 50 (µg/mL) from marketed formulation The solution was filtered through Whattman filter paper no. 41.The absorbance of sample solution was measured at 265 nm and 242.60 nm in 1 cm cell against blank data shown in Table 3.

TABLE 3: RESULT OF ANALYSIS OF FORMULATION (n=5)

DRUG	LABEL CLAIM (mg/mL)	AMOUNT FOUND (mg/mL)	% MEAN RECOVERY ± S.D.
MOXI	5	5.04	100.86 ± 0.335
LOTE	5	4.91	98.33 ± 0.240

S.D. = Standard deviation

RESULT AND DISCUSION:

The optical characteristics such as Beer's law limits correction coefficients, slope and intercept of regression equation are summarized in Table 1. The value obtained for determination of Moxifloxacin HCl and Loteprednol Etabonate in formulation by developed method is summarized in Table 3. To evaluate the validity and repeatability of the method, known amounts of pure drug was added to preanalyzed formulation and mixture were analyzed formulation and mixture were analyzed by developed method and percent recoveries are given in Table 2. The law value of standard deviation and % R.S.D (less than 2 % at each step of validation) as given in Table 1-3 confirms the precision of the method.

CONCLUSION:

In conclusion, the developed spectrophotometric method is simple, sensitive, accurate and reproducible and can be used for routine simultaneous determination of Moxifloxacin HCl and Loteprednol Etabonate in bulk as in formulation mixture.

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