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RESEARCH ARTICLE

CONCURRENT ESTIMATION OF LOTEPREDNOL ETABONATE AND LEVOFLOXACIN BY UV SPECTROPHOTOMETRIC ABSORBANCE RATIO METHOD FROM THEIR COMBINED EYE DROPS DOSAGE FORM

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Abstract

A simple, sensitive, rapid, accurate and precise absorption ratio method (Q value analysis method) has been developed for simultaneous estimation of loteprednol etabonate and levofloxacin in combined dosage form. Ratio of absorbance at two selected wavelengths was calculated. First wavelength is absorption maxima of respective drug and second wavelength is iso-absorptive point at which both drugs give same absorbance. Loteprednol etabonate showed absorbance maxima at 269.29 and levofloxacin showed absorbance maxima at 298.5 nm in methanol. The iso-absorptive point of loteprednol etabonate and levofloxacin was found to be at 269.29 nm. Linearity was constructed in the concentration range of 5-25 µg/mL. Promising values of correlation coefficient for LE (R^2=0.998) and levofloxacin (R^2=0.999) proves that method is linear. Furthermore, the method was successfully validated in terms of various validation parameters as per ICH Q2 (R1) guidelines. The developed method was successfully applied for estimation of both loteprednol etabonate and levofloxacin from its eye drops. Mean % recovery indicated that no interference was observed from excipients present in the formulation. Therefore, developed method can be routinely applied for simultaneous estimation of loteprednol etabonate and levofloxacin from its pharmaceutical dosage form.

Keywords: Loteprednol etabonate, levofloxacin, absorbance ratio method

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1. INTRODUCTION

Loteprednol etabonate (LE), chloromethyl (11β, 17α)-17-[(ethoxycarbonyl) oxy]-11-hydroxy-3-oxoandrosta-1, 4-diene-17-carboxylate [1] (Figure 1) is a topical corticoid anti-inflammatory which is used in ophthalmic solution for the treatment of allergic conjunctivitis, uveitis, acne rosacea, keratitis, iritis, cyclitis, and selected infective conjunctivitis [2–5]. Levofloxacin (LV) is (S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido [1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid[6] (Figure 2). LV, one of the commonly used fluoroquinolone antimicrobials, is the active S-isomer isolated from the racemic ofloxacin. Its antibacterial action is twice as active as the racemate ofloxacin *in vitro*. Because of its excellent antibacterial activity and low frequency of adverse effects on oral administration, LV has been widely used for the treatment of infectious eye diseases [7,8]. The combination comprising LV and LE is available as ophthalmic, otic or nasal pharmaceutical preparations, which is commonly used for treatment of conjunctivitis, keratitis, blepharitis, dacrycystitis, hordeolum, corneal ulcer and ocular infections.

Literature survey reveals that various analytical methods have been reported for the estimation of LE & LV individually. UV Spectrophotometric [9–11] Spectrofluorimetric [12,13], HPTLC [14,15],HPLC [16–20] and UPLC [21] methods have been reported for the estimation of LV, whereas HPLC method [22,23] has been reported for estimation of LE. No analytical method has been reported for estimation of LE and LV simultaneously in pharmaceutical dosage form. Therefore, it was endeavored to develop rapid and simple absorbance ratio methodfor simultaneous estimation of LE and LV from eye drop formulation and to validate the method as per ICH Q2 (R1) guidelines [24] .

2. EXPERIMENTAL WORK

2.1 Chemicals and Reagents

LE (99.9 %) reference standard (RS) and LV (99.9 %) (RS) was provided as a gift sample by Sun Pharmaceutical Industries Ltd. and Ajanta Pharma Limited respectively. Methanol (AR Grade) was purchased from S.D. Fine Chemicals Ltd., Bombay, India. LE& LV Eye Drops were prepared in the laboratory having concentration of 0.5% w/v of LE and 1.5% w/v of LV.

2.2 Instrumentation

A UV-2400, Version 2.21 double beam spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm, and a pair of 10 mm matched quartz cells (Shimadzu, Columbia, MD) was used. An analytical Balance, Citizen CX 220 (Citizen Pvt. Ltd, Germany) of capacity10 to 220 mg was used for weighing. Sonicator (Trans-O-Sonic, Erection & Instrumentation Engineers, Ahmedabad, India) was used for solubilization of drug.

2.3 UV Spectrophotometric conditions

In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drugs and iso-absorptive point, solutions of both drugs having concentration 10µg/mL in methanol were scanned using UV spectrophotometer within the wavelength range of 200 – 400 nm against methanol as blank. Absorption curve showed characteristic absorption maxima at 230.7 nm for LE and 298.5 nm for LV. Both drugs showed same absorbance at 269.29 nm, so it was considered as an iso-absorptive point of both drugs. **(Figure 3)**

2.4 Preparation of standard stock solution

25 mg of LE (RS) and LV(RS) were accurately weighed and transferred to separate 25 mL amber coloured volumetric flasks and diluted up to the mark with methanol to produce a stock solution of 1000 µg/mL concentration of both drugs. 2.5 mL of aliquot of both stock solutions was separately diluted to 25 mL with methanol to give each solution having 100 µg/mL concentration.

2.5 Preparation of sample solution from eye drops

Sample solution was prepared by diluting 0.1 mL of eye drops to 10 mL with methanol in amber coloured volumetric flask. From that, 1 mL of aliquot was diluted to 10 mL with methanol, which correspondingly gives 5 µg/mL and 15 µg/mL concentrations of LE and LV, respectively.

2.6 Method Validation

2.6.1 Linearity: Preparation of calibration curve

For construction of calibration curve, 0.5, 1.0, 1.5, 2.0 and 2.5 mL solutions of both the drugs were taken from standard stock solution in 10 mL separate volumetric flasks to get the concentration of 5, 10, 15, 20 and 25 µg/mL for LE and LV, respectively. Calibration curve was constructed by plotting absorbance versus concentration.

2.6.2 Precision

Intraday and Interday precision was determined by measuring the absorbance of both the drugs three times within a day and on three different days, respectively. For this, absorbance of solution of both drugs having concentration 5, 15 and 25 µg/mL was measured and solutions were analyzed as per UV Spectrophotometric conditions.

2.6.3 Accuracy (% Recovery)

The accuracy of the method was determined by calculating % recoveries for LE and LV by standard addition method at three different levels (80, 100 and 120 %). Known amount of standard solution of LE (0.2, 0.25 and 0.3 mL) and LV (0.6, 0.75 and 0.9 mL) were added to pre-analysed sample solution containing 0.25 mL of LE and 0.75 mL of LV in 10 ml volumetric flask. Solutions were analyzed as per UV Spectrophotometric conditions.

2.6.4 Robustness

Robustness was performed on concentration (15 µg/mL) of LE and LV. Robustness of the method was determined by making changes in λ_{max} of both the drugs by ± 4 nm. The % Assay values were calculated and compared with that of the standard.

2.6.5 Limit of Detection and Limit of Quantification

For this determination, Calibration curve for both the drugs was repeated six times. Further from which, LOD & LOQ were calculated using mathematical equations given below:

LOD = 3.3 x σ/S

LOQ = 10 x σ/S

Where, σ = Standard deviation of the Intercept

S = slope of calibration curve

2.7 Analysis of LE and LV in prepared combinedeye drops dosage form

Assay was determined for 5 µg/mL and 15 µg/mL concentrations of LE and LV, respectively. Solution was analyzed as per UV Spectrophotometric conditions.

3. RESULT AND DISCUSSION

3.1 Optimization of experimental conditions for absorbance ratio method (Q value analysis method)

From the overlain spectrum of LE and LV which is shown in Figure 3, the wavelengths selected for analysis were 269.29 nm (isoabsorptive point) and 298.5 nm (λ_{max} of LV). LE was quantified at 269.29 nm and 298.5 nm (equation 1). LV was quantified at 298.5 nm using equation 2 because LE doesn't show any absorbance at 298.5 nm. The absorbance of the standard and sample solutions was measured. The absorptivity values for both standard drugs at the selected wavelengths were employed for determination of Q values. The concentrations of drugs in sample solution were determined by using the following equations.

$$C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{a y_1} \quad \ldots\ldots 1$$

$$C_y = \frac{A}{ab} \quad \ldots\ldots 2$$

Where, $Q_m = A_2/A_1$, $Q_x = ax_2/ax_1$ & $Q_y = ay_2/ay_1$. A_1 & A_2 are the absorbance of the mixture at 298.5 nm & 269.29 nm respectively; ax_1 and ay_1 are absorptivity of LE and LV respectively at 298.5 nm; ax_2 and ay_2 are absorptivity of LE and LV respectively at 269.29 nm.

3.2 Validation of the Proposed Method

Linearity: A linear relationship was achieved between absorbance and the

concentration of the both the drugs in the range of 5-25 µg/mL. The correlation coefficients of both the drugs for the developed method were found to be not less than 0.998. The results of linearity are shown in table 1.

Precision, LOD and LOQ: Interday and intraday variation in estimation of LE and LV showed that the RSD was always less than 2% during analysis by developed method. These low RSD values show good precision of the method. The results of precision are shown in table 2 and 3.

Accuracy: Recovery studies were carried out by the standard addition method. The results of recovery studies of both the drugs for the developed method are shown in table 4. The % recovery values were in the range of 98-100%, which shows good accuracy of proposed method.

LOD and LOQ: The LOD and LOQ values for both LE and LV were calculated using the equation. The LOD and LOQ values for both the drugs are reported in table 5.

Robustness: The % RSD in robustness studies was found to be less than 2%. The low RSD value indicated robustness of the method. Results of robustness studies for the developed methods are shown in table 6.

3.3 Analysis of LE and LV in prepared combined eye drops dosage form

The proposed method were successfully applied to determine LE and LV in their combined eye drops dosage form. The results obtained for LE and LV were compared with the corresponding labelled amounts. The assay values obtained are shown in table 7.

The proposed method was developed and validated according to ICH guidelines. The results of validation parameters for LE and LV are summarized in table 8.

4. CONCLUSION

The developed method has linear response in the range of 5-25 µg/mL with correlation coefficient of R^2=0.999 (LE) & R^2 = 0.998 (LV). Furthermore, the method was successfully validated in terms of various parameters as per ICH Q2 (R1) guidelines. The developed method was successfully applied for estimation of both LE and LV from its pharmaceutical dosage form, i.e. eye drops. The results of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the formulation. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of LE and LV. Hence, the method can be successfully used for the routine analysis of LE and LV in combined eye drops dosage form.

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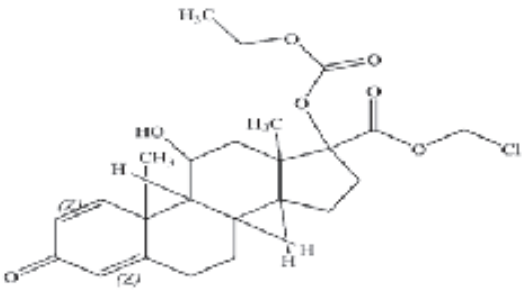

Figure 1: Structure of Loteprednol Etabonate

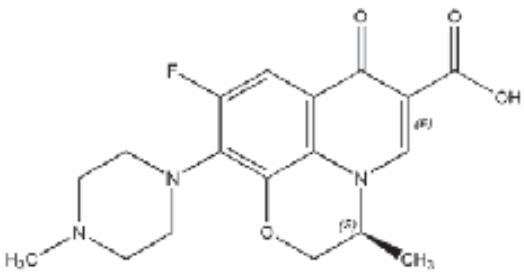

Figure 2: Structure of Levofloxacin

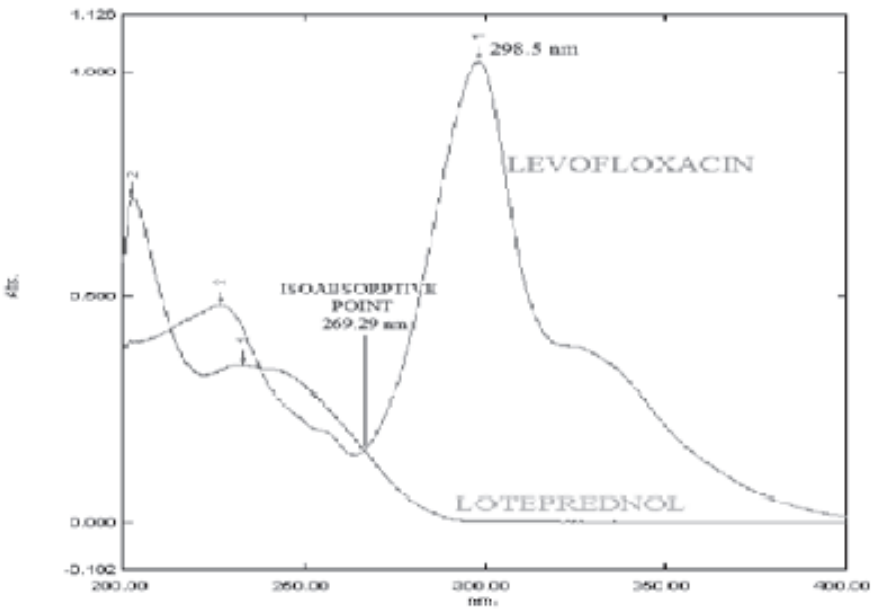

Figure 3: Overlain spectra of LoteprednolEtabonate(10 µg/mL) and Levofloxacin (10 µg/mL) in methanol

Table 1: Linearity of LoteprednolEtabonate and Levofloxacin

Conc. (ppm)	Loteprednol Etabonate		Levofloxacin		Isoabsorptive point	
	269.29 nm		298.5 nm		269.29 nm	
	Mean Absorbance ± S.D. *	% R.S.D.	Mean Absorbance ± S.D. *	% R.S.D.	Mean Absorbance ± S.D. *	% R.S.D.
5	0.1140 ± 0.0019	1.6667	0.531 ± 0.0039	0.8394	0.1136 ± 0.0013	1.1444
10	0.2033 ± 0.0035	1.7216	1.126 ± 0.0116	1.0349	0.2031 ± 0.0034	1.6740
15	0.2935 ± 0.004	1.3628	1.5915 ± 0.0101	0.6392	0.2932 ± 0.0039	1.3301
20	0.3901 ± 0.0051	1.3073	2.193 ± 0.0068	0.3106	0.3896 ± 0.0060	1.5400
25	0.4891 ± 0.0091	1.8605	2.7411 ± 0.0096	0.3517	0.5002 ± 0.0083	1.6593
Linearity Equation	y = 0.018x + 0.017		y = 0.109x - 0.009		y = 0.019x + 0.000	
Correlation Coefficient	0.9989		0.998		0.998	
Slope	0.018		0.109		0.019	
Intercept	0.017		-0.009		0.000	

*** n = 6**

Table 2: Intraday precision of Loteprednol Etabonate and Levofloxacin

Lotepredno lEtabonate

Conc. (ppm)	Mean conc. (ppm) ± S.D.*	% R.S.D.
5	5.15 ± 0.075	1.45
15	14.89 ± 0.205	1.37
25	24.86 ± 0.271	1.09

Levofloxacin

Conc. (ppm)	298.5 nm		269.29 nm	
	Mean conc. (ppm) ± S.D.*	% R.S.D.	Mean conc. (ppm) ± S.D.*	% R.S.D.
5	4.99 ± 0.025	0.50	5.08 ± 0.100	1.96
15	15.00 ± 0.108	0.72	15.18 ± 0.175	1.15
25	25.00 ± 0.055	0.22	25.02 ± 0. 150	0.59

* n=3

Table 3: Interday precision of Loteprednol Etabonate and Levofloxacin

Lotepredno lEtabonate

Conc. (ppm)	Mean conc. (ppm) ± S.D.*	% R.S.D.
5	5.01 ± 0.072	1.43
15	14.99 ± 0.170	1.13
25	24.94 ± 0.346	1.38

Levofloxacin

Conc. (ppm)	298.5 nm		269.29 nm	
	Mean conc. (ppm) ± S.D.*	% R.S.D.	Mean conc. (ppm) ± S.D.*	% R.S.D.
5	4.99 ± 0.028	0.56	5.09 ± 0.076	1.49
15	14.96 ± 0.060	0.40	15.30 ± 0.250	1.63
25	24.99 ± 0.041	0.16	25.02 ± 0.217	0.97

* n=3

Table 4: Recovery (Accuracy study)

Lotepredno lEtabonate

Level of Recovery	Sample Conc. (ppm)	Amount of Std. Added (ppm)	Total amount (ppm)	Amount Recovered (ppm)	% Recovery	Mean % Recovery
80 %	2.5	2.0	4.5	4.47	99.33	100.29
	2.5	2.0	4.5	4.51	100.22	
	2.5	2.0	4.5	4.56	101.33	
100 %	2.5	2.5	5	5.04	100.8	100.03
	2.5	2.5	5	4.96	99.2	
	2.5	2.5	5	5.00	100.1	
120 %	2.5	3.0	5.5	5.52	100.36	100.24
	2.5	3.0	5.5	5.47	99.45	
	2.5	3.0	5.5	5.55	100.91	

Levofloxacin

Level of Recovery	Sample Conc. (ppm)	Amount of Std. Added (ppm)	Total amount (ppm)	Amount Recovered (ppm)	% Recovery	Mean % Recovery
80 %	7.5	6.0	13.5	13.45	99.63	99.98
	7.5	6.0	13.5	13.49	99.93	
	7.5	6.0	13.5	13.55	100.37	
100 %	7.5	7.5	15	15.09	100.6	100.47
	7.5	7.5	15	15.12	100.8	
	7.5	7.5	15	15.00	100.0	
120 %	7.5	9.0	16.5	16.58	100.48	100.32
	7.5	9.0	16.5	16.60	100.61	
	7.5	9.0	16.5	16.48	99.88	

Table 5: Limit of Detection and Limit of Quantification

Drug	LOD (ppm)		LOQ (ppm)	
	298.5 nm	269.29nm	298.5 nm	269.29nm
Levofloxacin	0.06	0.15	0.20	0.50
LoteprednolEtabonate	-	0.05	-	0.19

Table 6: Robustness

Conc.(µg/mL)	LoteprednolEtabonate		Levofloxacin			
	Conc. (µg/mL)		Conc. (µg/mL)		Conc. (µg/mL)	
	267.29 nm	271.29 nm	296.5 nm	300.5 nm	267.29 nm	271.29 nm
15	15.55	11.67	15.09	15.08	13.53	17.47
	15.43	11.53	15.11	15.11	13.59	17.39
	15.50	11.49	14.99	14.98	13.66	17.53
Average	15.49	11.56	15.06	15.05	13.59	17.46
S.D.	0.06	0.09	0.06	0.06	0.06	0.07
R.S.D.	0.38	0.81	0.42	0.45	0.47	0.40
%Assay ± S.D.	103.28 ± 0.388	77.08 ± 0.817	100.42 ± 0.427	100.36 ± 0.450	90.62 ± 0.435	116.42 ± 0.402

Table 7: Analysis of prepared formulation

Drug	Label Claim	%Assay ± S.D. *	% R.S.D.
LoteprednolEtabonate	0.5 % w/v	99.86 ± 0.463	0.46
Levofloxacin	1.5 % w/v	99.63 ± 0.234	0.23

* n=5

Table 8: Summary of Validation Parameters

Parameter	LoteprednolEtabonate	Levofloxacin	
Wavelength	269.29 nm	298.5 nm	269.29 nm
Linearity	5-25 µg/mL	5-25 µg/mL	
Equation	y = 0.018x + 0.018	y = 0.109x - 0.009	y = 0.019x + 0.000
R^2	0.99	0.99	0.99
LOD (µg/mL)	0.16	0.06	0.15
LOQ (µg/mL)	0.49	0.20	0.50
Intraday Precision (%R.S.D., n = 3)	1.30	0.48	1.24
Interday Precision (%R.S.D., n = 3)	1.32	0.37	1.36
% Recovery	100.03-100.29	99.98-100.47	
Robustness (% R.S.D., n=3)	0.60	0.44	
% Assay ± S.D. (n=5)	99.86 ± 0.463	99.63 ± 0.234	