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Analytical Method Development and Validation for the Determination of Loteprednol Etabonate and Tobramycin in Combined Dosage Form

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ABSTRACT:

A newer, simple, rapid, accurate, precise and sensitive method was developed and validated for determination of Loteprednol Etabonate (LOTE) and Tobramycin (TOBRA) in combined dosage form. The method employed was First order derivative. Concentration range of 15-35 µg/ml for Loteprednol Etabonate and 9-21 µg/ml for Tobramycin for the proposed method. First order Derivative method, wherein wavelengths selected were 223.62nm(ZCP of Tobramycin) for Loteprednol Etabonate and 300 nm(ZCP of Loteprednol) for Tobramycin. The results of the analysis were analyzed and validated statistically and recovery study was carried out as per ICH guidelines.

Key words: Loteprednol Etabonate (LOTE) and Tobramycin (TOBRA), First Order derivative method, ZCP (Zero Cross point)

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INTRODUCTION:

Loteprednol Etabonate (LOTE) is Chloromethyl 17-ethoxycarbonyloxy- 11-hydroxy-10,13-dimethyl-3-oxo- 7,8,9,11,12,14,15, 16-octahydro- 6*H*-cyclopenta[a] phenanthrene-17-carboxylateis corticosteroid used as the treatment of inflammation of the eye due to allergies.[1]

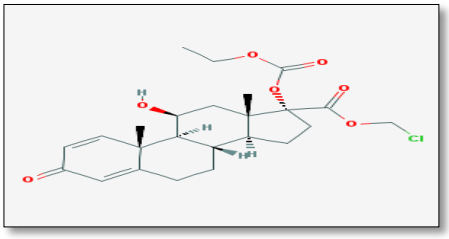

Figure : 1 Structure of Loteprednol Etabonate

Tobamycin is(2*S*,3*R*,4*S*,5*S*,6*R*)-4-amino-2-{[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-3-{[(2*R*,3*R*,5*S*,6*R*)-3-amino-6-(aminomethyl)-5-hydroxyoxan-2-yl]oxy}-2-hydroxycyclohexyl]oxy}-6-(hydroxymethyl)oxane-3,5-diol. It is ananti bacterial agent used for the treatment of pseudomonas aeruginosa lung infections.[2]

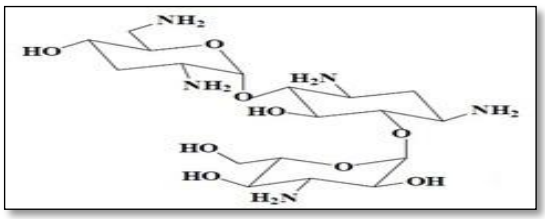

Figure : 2 Structure of Tobramycin

Liquid chromatography is the only available official method for the estimation of Tobramyin[3,4] and no official method available for Loeprednol Etabonate. LOTE and TOBRA combination is not official in any pharmacopoeia, hence no official method is available for estimation of these two drugs in combined dosage forms.

Literature survey revealed that one RP-HPLC method reported for determination of the drug [5]. The aim of the present work was to develop simple, sensitive, accurate, and precise methods for routine analysis. The proposed method was validated according to ICH guidelines [6].

Tobramycin itself does not provide UV spectra. So it becomes necessary to derivatized tobramycin.In which amino group of tobramycin is derivatized into nitro group.

2,4 – Dinitrophenyl Derivative [7]

The halogen atom in 1-chloro-2,4-dinitrobenzene is reactive and coloured crystalline compounds (usually yellow/red) are formed with primary and with secondary amines-

$2,4\text{-}(NO_2)_2C_6H_3Cl + R.NH_2 \rightarrow 2,4\text{-}(NO_2)_2C_6H_3.NH.R + HCl$

Dissolve 1.0 gm(or 10ml)of amine and 1.0 gm of 1-chloro-2,4-dinitro-benzene in 5-10 ml of ethanol, add a slightly excess of anhydrous potassium carbonate or of powdered fused sodium acetate, reflux the mixture on a water bath for 20-30 minutes and then pour into water. Wash the precipitated solid with dilute sodium carbonate solution, followed by dilute hydrochloric acid. Recrystallise from ethanol, dilute ethanol or glacial acetic acid.

So equivalent to 50mg of amine was calculated from the formulation and synthesized LOTEPRED-T was than analyzed.

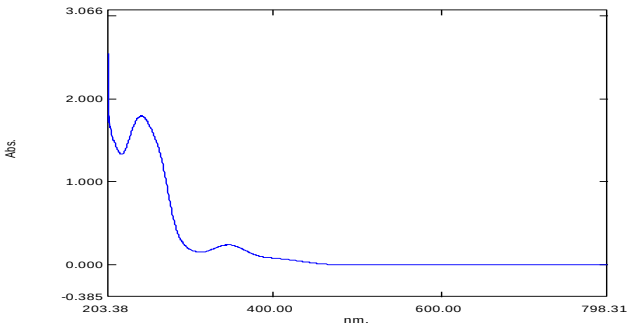

Figure: 3 UV spectra of formulation (25ppm Lote & 15ppm Tobra)

In formulation benzalkonium chloride is present as a preservative & along with all other excipients it has no interference with UV spectra.

MATERIALS AND METHODS

Apparatus

Instrument used was an UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800, software – UV probe, version 2.42) with a pair of 1 cm matched quartz cells. All weighing was done on Mettler Toledo electronic analytical balance.

Reagents and chemicals

Loteprednol Etabonate (LOTE) and Tobramycin (TOBRA) was kindly supplied as a gift samples from Aarti Industry,Vapi, Gujarat (India).

Methanol used as solvent and all calibrated glass wares were used throughout the work.

Marketed formulation

Eye drop formulation LOTEPRED-T was purchased from the local market.

Preparation of standard stock solution

The standard stock solution of LOTE and TOBRA was prepared by dissolving 10 mg of each API in 10 ml of different volumetric flaskin methanoland volume make up with Methanol to produce 1000 µg/ml of each solution.

The standard stock solution of formulation was prepared by dissolving equivalent to 10 mg of each API in 10 ml of different volumetric flask in methanol and volume make up with Methanol to produce 1000 µg/ml of each solution.

Preparation of second stock solution

The second stock solution of LOTE and TOBRA was prepared by dissolving 10 mg of each API in 10 ml of different volumetric flask in methanol and volume make up with Methanol to produce 100µg/ml of each solution.

The second stock solution of formulation was prepared by dissolving equivalent to 10 mg of each API in 10 ml of different volumetric flask in methanol and volume make up with Methanol to produce 100 µg/ml of each solution.

Preparation of working standard solution

Accurately measured second stock solutions of LOTE and for TOBRA were transferred to a series of volumetric flask separately and prepare 15,20,25,30,35ppm of LOTE and 9,12,15,18,21ppm of TOBRA.

Accurately measured second stock solution of formulation was transferred to a series of volumetric flask separately and prepare 25ppm of LOTE and 15ppm of TOBRA.

Selection of analytical wavelength

Standard solutions of LOTE (15µg/ml) and TOBRA (9µg/ml) were scanned in the range of 200 to 800 nm than convert into derivative spectra. From the overlain spectra, Zero Crossing Point was found at 223.62nm for LOTE and 300nm for TOBRA. For the First Order Derivative method 223.62nm and 300nm wereselected as analytical wavelengths.

Method : First Order Derivative Method[8,9]

The overlain spectra were converted to first order derivative spectra and from these overlain Derivative Spectra (Fig 3) Zero Crossing Point was found at 223.62nm for LOTE and 300nm for TOBRA selected for the First Order Derivative method of two drugs. The absorbance at 223.62nm for LOTE and 300nm for TOBRA was measured

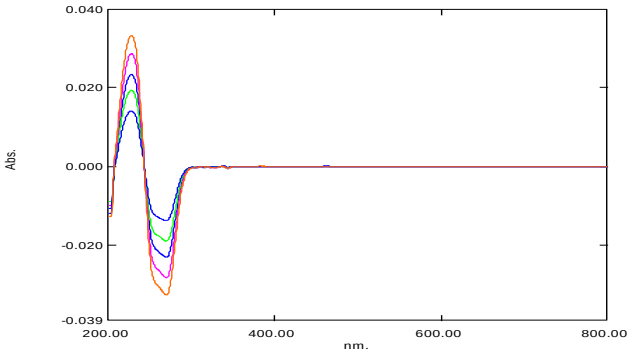

Figure 4: Derivative spectra of Loteprednol Etabonate

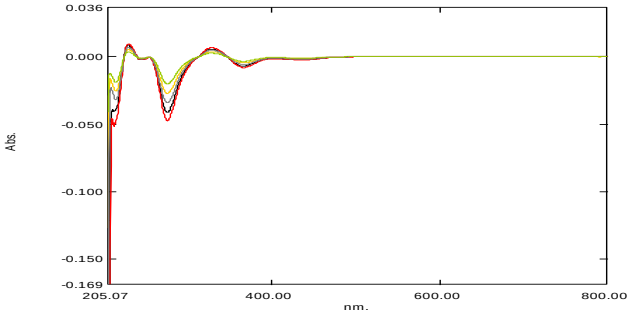

Figure 5: Derivative spectra of Tobramycin

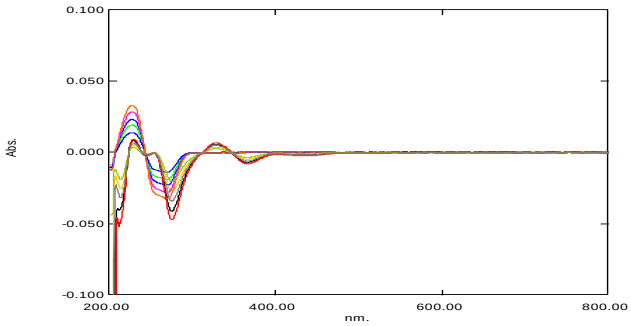

Figure 6: Overlain Derivative spectra of Loteprednol Etabonate and Tobramycin

Validation of the proposed method

Linearity (calibration curve)

The calibration curves were plotted over a concentration range of 15-35µg/ml for LOTE and 9-21 µg/ml TOBRA. Accurately measured standard stock solutions of LOTEand for TOBRA were transferred to a series of volumetric flask separately and prepare 15,20,25,30,35ppm of LOTE and 9,12,15,18,21ppm of TOBRA. The absorbance of solution was measured. These spectra were converted to first order derivative spectra and Absorbance at 223.62nm for LOTE and 300nm for TOBRA was measured for First Order Derivative method.The calibration curves were constructed by plotting dA/dλ versus concentration for First Order Derivative method.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of LOTE (25 µg/ml) and TOBRA (15 µg/ml) without changing the parameters of derivative method.

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposedmethod was determined by estimating the correspondingresponses of the drug 3 different concentrations three times on the same day and on 3 differentdays over a period of one week for LOTE (20, 25 and 30µg/ml) and for TOBRA (12, 15 and 18µg/ml). The results wereereported in terms of relative standard deviation (**Table5**).

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of LOTE and TOBRA by the

standard addition method. Known amount of standard solutions of LOTE and TOBRAwere added to formulation having LOTE (15µg/ml) and TOBRA (9 µg/ml). The amounts of LOTE and TOBRA were obtained by applying regression line equations.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification(LOQ) of the drug were derived by calculating the signal to- noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on harmonization (ICH) guidelines.

LOD = 3.3 × σ/S
LOQ = 10 × σ/S
Where, σ= the standard deviation of Y-intercept of 6calibration curves and

S = the mean slope of the 6 calibration curves.

RESULT AND DISCCUSION

The proposed method was validated as per ICH guideline. Method discussed in the present work provide a convenient and accurate way for analysis of LOTE and TOBRA. In First Order Derivative method, wavelengths selected were 232.62 nm for LOTE(ZCP of TORA) and 300 nm for TOBRA (ZCP of LOTE).. The plot of absorbance versus respective concentrations of LOTE and TOBRA were found to be linear in the concentration range of 15-35 µg/ml for LOTE and 9-21 µg/ml for TOBRA with correlation coefficient 0.9986 at 223.62 nm for LOTE and 1.0000 at300nm for TOBRA as shown in table 3 and figures 4-5.Precision was calculated in terms of repeatability, intraday and interday precision was found to be in acceptance range (Table 3). The accuracy of method was determined by standard addition method. The % recovery ranges from 98.52-100.73 % for LOTE and 98.55-101.48 % for TOBRA (Table 4).

Table.2 Linearity data of LOTE at 223.62nm

Sr. No.	Conc. (µg/ml)	Absorbance at 223.62nm Mean ± S.D (n=3)
1.	15	0.0133±0.000216
2.	20	0.0178±0.000236
3.	25	0.0218±0.000236
4.	30	0.0264±0.0003
5.	35	0.0322±0.000205

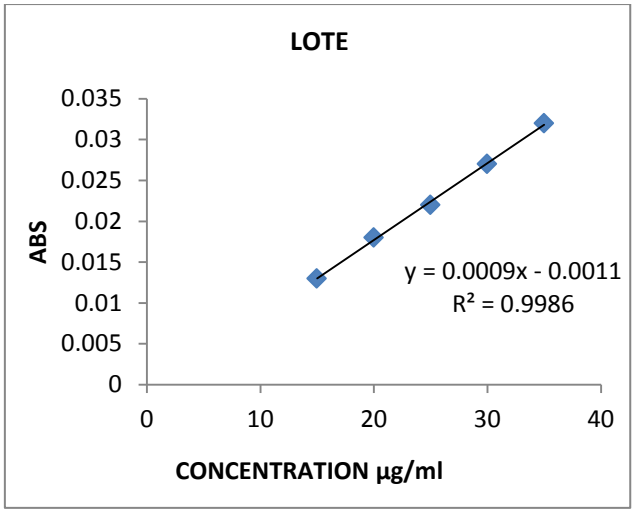

Figure 7: Calibration curve of standard LOTE at 223.62 nm

Table.2 Linearity data of TOBRA at 300nm

Sr. No.	Conc. (µg/ml)	Absorbance at 300 nm Mean ± S.D (n=3)
1.	9	-0.00403±0.0000471
2.	12	-0.0051±0.0000816
3.	15	-0.00597±0.0000471
4.	18	-0.00703±0.0000471
5.	21	-0.00803±0.0000471

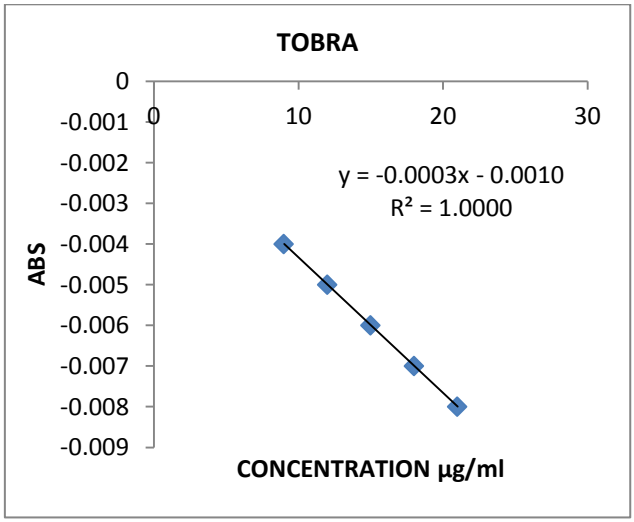

Figure: 8 Calibration curve of standard TOBRA at 300 nm

Table3: Recovery study

Concentration (µg/ml) (formulation)		Spiked level (µg/ml)			% recovery ±SD (n=3)	
LOTE	TOBRA	LOTE		OBRA	LOTE	TOBRA
15	9	0%	0	0	-	-
15	9	80%	12	7.2	99.83 % ± 0.9805	98.55 % ± 0.2922
15	9	100%	15	9	100.73% ± 0.3061	101.48% ± 0.5232
15	9	120%	18	10.8	98.52% ± 0.3111	99.48 % ± 0.7165

Table 4: Results of estimation of LOTE and TOBRA in marketed formulation

Marketed Formulation(Tab)	Labeled claim		Amount Obtained		% Assay(n=3)	
	LOTE		LOTE		LOTE	
LOTEPRED-T	35µg/ml	21µg/ml	34.55µg/ml	20.66µg/ml	99.12% ±0.3879	98.96% ±0.5720

This method can be successfully used for simultaneous estimation of LOTE and TOBRAin their combined dosage form. Marketed Formulation wasanalyzed and results obtained were within the range of 98-102% (**Table 4**).

Table 5: Summary of validation parameters

Parameters (First Order Derivative)	LOTE	TOBRA
ZCP	**223.62nm**	**300nm**
Conc. Range (µg/ml)	15-35	9-21
Regression Equation	Y=0.0009x-0.00011	Y=-0.0003x-0.00010
Slope (m)	0.0009	-0.0003
Intercept (C)	-0.00011	-0.00010
Regression coefficient (r^2)	0.9986	1.000
Repeatability (n = 6) % R.S.D.	0.75664	0.62286
Intraday Precision (n = 3) % R.S.D.	0.84274	1.01118
Interday Precision (n = 3) % R.S.D.	1.28451	1.03127
LOD (µg/ml)(n=6)	2.55617	0.0568
LOQ (µg/ml)(n=6)	7.74597	0.17213

CONCLUSION

The lower value of relative standard deviation for repeated measurement indicates that the method is precise. The value of % recovery is approximately 100%, which indicates that these methods can be used for estimation of these two drugs in combined dosage form without any interference due to the other components present in the formulations. Hence this study presents simple, accurate, precise and rapid spectroscopic analytical method for the estimation of these two drugs in combined dosage form.

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