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DETERMINATION OF LOTEPREDNOL ETABONATE AND TOBRAMYCIN IN COMBINED DOSAGE FORM USING RP-HPLC METHOD

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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 Reverse-Phase High Performance Liquid Chromatography (RP-HPLC). Linearity was obtained in the range of 15-35 µg/ml forLoteprednol Etabonate and 9-21 µg/ml for Tobramycin. Chromatographic separation was achieved isocratically at 25°C ± 0.5°C on Enable C18 column (250 x 4.6 mm, 5µm) with a mobile phase composed of acetonitrile : Water (55:45v/v) pH adjusted to 4 with OPA at flow rate of 1.0 ml/min and selected wavelength is 243nm. The retention time of Tobramycin and Loteprednol Etabonate was found to be 5.857 min and 7.479 min respectively.The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus the proposed method was successfully applied for simultaneous determination of Loteprednol Etabonate and Tobramycin in routine analysis.

Keywords: Loteprednol Etabonate (LOTE) and Tobramycin (TOBRA), RP-HPLC, Validation.

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-carboxylate is corticosteroid used as the treatment of inflammation of the eye due to allergies [1].

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 an anti bacterial agent used for the treatment of pseudomonas aeruginosa lung infections [2].

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].

MATERIALS AND METHODS

Apparatus

Instrument HPLC (Shimadzu LC-20 AT) system used consisted of Isocratic pump and injection volume 10µL. Detector consists of Ultraviolet detector, the column used was Enable C18 column (250 x 4.6 mm, 5µm), Mettler Toledo electronic analytical balance.

Reagents and chemicals

Loteprednol Etabonate (LOTE) and Tobramycin

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(TOBRA) was kindly supplied as a gift samples from Aarti Industry, Vapi, Gujarat (India) and Shreeji Pharma International, Gujarat (India) respectively. Acetonitrile and Water (HPLC grade) were used.

Marketed formulation

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

Chromatographic Condition

Column : Enable C_{18} G (250*4.6 mm, 5µm)
Run time :10 min
Temperature : 30^{o}C
Injection Volume : 10 µl
Flow rate : 1 ml/min
Mobile phase : Acetonitrile: water (55:45) pH adjusted to 4 with OPA
Detection wavelength : 243 nm

Selection of Wavelength

Standard solution of LOTE (25 µg/ml) and TOBRA (15 µg/ml) were prepared in methanol and scanned between 200-800 nm using UV-visible spectrophotometer. Wavelength was selected from the overlay spectra of both the drugs

Selection of Mobile phase

Based on solubility, partition co-efficient and polarity of drugs, different mobile phases in varying concentrations were tried and the suitable was selected for the combination of LOTE and TOBRA. The finally selected mobile phase is acetonitrile : water (55:45v/v) (pH- 4 with OPA).

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 flask in methanol and volume made up with Mobile phase 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 made up with Mobile phase 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 made up with Mobile phase 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 made up with Mobile phase l 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 prepares 25ppm of LOTE and 15ppm of TOBRA.

Calibration curve of LOTE and TOBRA

Calibration curve were prepared by taking appropriate aliquots 1.5,2..0 ,2.5 ,3.0 and 3.5 ml from the stock solution of LOTE(10 µg/ml) and 0.9, 1.2, 1.5, 1.8, 2.1 ml from the stock solution of TOBRA (10 µg/ml) in 10 ml vol. flask and make up the vol. with mobile phase to give 15-35µg/ml of LOTEand 9-21 µg/ml of TOBRA. The standard solution was run for 10 min. using mobile phase at a flow rate of 1.0 ml/min. The graph of peak area vs conc. was plotted, regression equation and correlation co-efficient for both drugs were obtained.

Final Mobile Phase Optimization

ACN: Water pH adjusted with OPA pH:4. (55:45), Flow rate: 1 ml/min.

Validation of the proposed method

Specificity is a procedure to detect quantitatively the analyte in the presence of components that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in the presence of components that may be expected to be present in the sample matrix. Specificity of the developed method was established by spiking of LOTEand TOBRA in hypothetical placebo and expressing that analytes peak was not interfered from excipients.

Mobile phase was used as blank. Standard and sample were prepared. Blank, standard preparation and sample preparation were prepared and analyzed.

Linearity (calibration curve) and Range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 15-35 µg/ml and 9-21 µg/ml for LOTE and TOBRA respectively (n = 3). The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient and regression line equations for LOTE and TOBRA were calculated. The standard solution was run for 10 min. using mobile phase at a flow rate of 1.0 ml/min. The graph of peak area vs conc. was plotted, regression equation and correlation co-efficient for both drugs were obtained.

Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) standard solutions of LOTE

(25 µg/ml) and TOBRA (15 µg/ml) under the same chromatographic condition and measurements of peak area, retention time and tailing factor. Percentage relative standard deviation (RSD) or coefficient of variation (CV) should not be more than 2 %.

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses of the drug 3 different concentrations three times on the same day and on 3 different days over a period of one week for LOTE (20, 25 and 30 µg/ml) and for TOBRA (12, 15 and 18 µg/ml). The results were reported in terms of relative standard deviation.

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 TOBRA were 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.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ= the standard deviation of Y-intercept of 6 calibration 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 RP-HPLC method, wavelengths selected were 243nm for LOTE and TOBRA both. The plot of area 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 0.995 for LOTE and 0.996 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 99.59-100.03 % for LOTE and 99.17-99.56 % for TOBRA.

This method can be successfully used for simultaneous estimation of LOTE and TOBRA in their combined dosage form. Marketed Formulation was analyzed and results obtained were within the range of 98-102% .

Fig 1. Structure of Loteprednol Etabonate

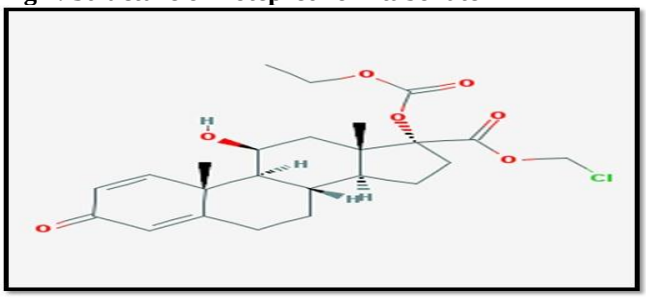

Fig 2. Structure of Tobramycin

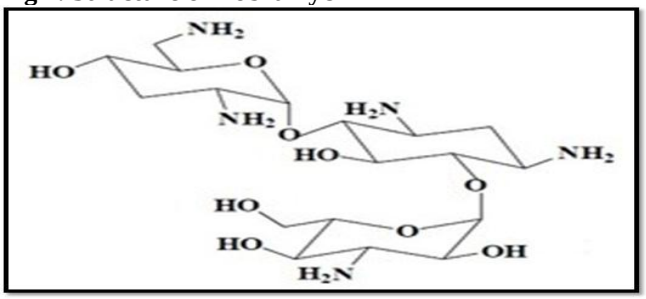

Fig 3. Chromatogram of Acetonitrile: water (55:45v/v), pH 4 with OPA , Flow rate: 1 ml/min.

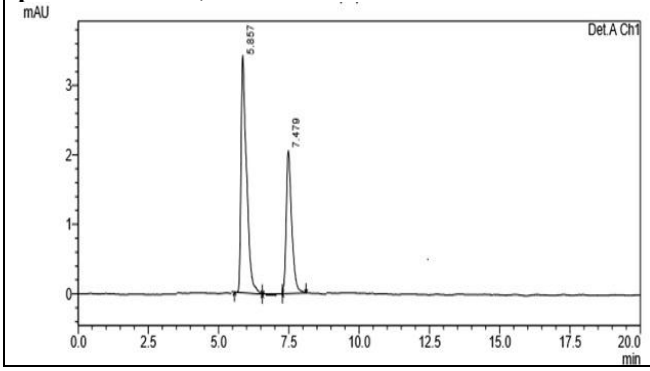

Fig 4. Linearity of Loteprednol Etaboante and Tobramycin

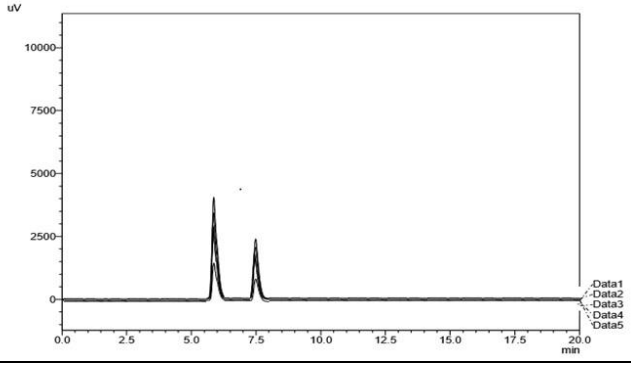

Fig 5. Calibration curve of standard LOTE

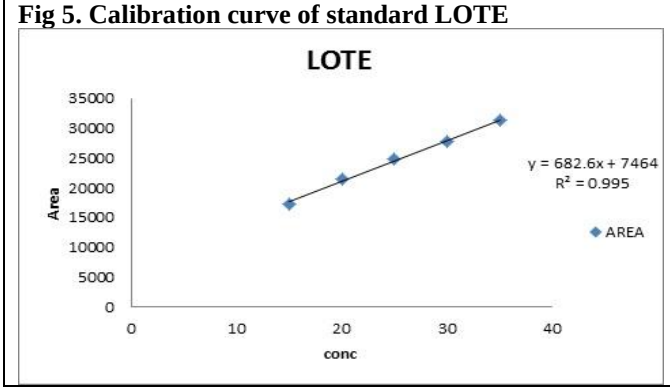

Fig 6. Calibration curve of standard TOBRA

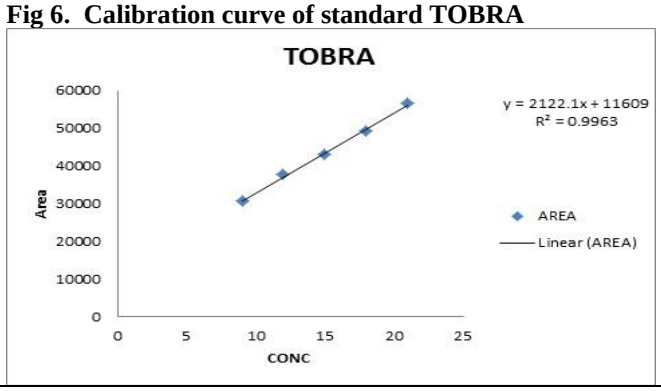

Table 1. Result for system suitability test

Parameters	Data obtained	
	LOTE	TOBRA
Retention time	7.479 min.	5.857 min.
Theoretical plates / column	32240	48872
Tailing factor	1.472	1.933
Resolution	1.724	

Table 2. Linearity data of LOTE

Sr. No.	Conc.(µg/ml)	Absorbance at 223.62nmMean ± S.D (n=3)
1.	15	17269±0.471405
2.	20	21526±1.41421 4
3.	25	24912±0.471405
4.	30	0.0264±0299832
5.	35	0.0322±0.942809

Table 3. Linearity data of TOBRA

Sr. No.	Conc.(µg/ml)	Absorbance at 300 nmMean ± S.D (n=3)
1.	9	30564±0.94281
2.	12	37803±0.4714
3.	15	42964±1.41421
4.	18	49147±0.4714
5.	21	56723±0.94281

Table 4.Recovery study

Concentration (µg/ml) (formulation)		Spiked level (µg/ml)			% recovery ± SD (n=3)	
LOTE	TOBRA	LOTE		TOBRA	LOTE	TOBRA
15	9	0%	0	0	-	-
15	9	80%	12	7.2	100.03 % ± 0.4252	99.56 % ± 0.5235
15	9	100%	15	9	99.59% ± 1.0185	99.17% ± 0.6800
15	9	120%	18	10.8	98.86% ± 0.7537	99.25 % ± 0.7213

Table 5.Results of estimation of LOTE and TOBRA in marketed formulation

Marketed Formulation(Tab)	Labeled claim		Amount Obtained		% Assay(n=3)	
	LOTE	TOBRA	LOTE	TOBRA	LOTE	TOBRA
LOTEPRED-T	35µg/ml	21 µg/ml	34.93µg/ml	21.10 µg/ml	99.79% ±0.8005	100.5% ±0.4920

Table 6.Summary of validation parameters

Parameters	LOTE at 243nm	TOBRA at 243nm
Conc. Range (µg/ml)	15-35	9-21
Regression Equation	Y=682.5x + 7464	Y= 2122X +11609
Slope (m)(n=3)	682.6	2122
Intercept (C)(n=3)	7464	11609
Regression coefficient (r^2)	0.995	0.996
Repeatability (n = 6) % R.S.D.	0.6987	0.5660
IntradayPrecision (n = 3) % R.S.D.	0.8677	0.8093
InterdayPrecision (n = 3) % R.S.D.	1.3604	1.1400
LOD (µg/ml)(n=6)	1.2462	3.7764
LOQ (µg/ml)(n=6)	0.3466	1.0503

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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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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