

NOVEL STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TOBRAMYCIN AND LOTE PredNOL IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, accurate, rapid and precise isocratic stability indicating reversed-phase high-performance liquid chromatographic method has been developed and validated for simultaneous determination of Tobramycin and Loteprednol in combined tablet dosage form. The chromatographic separation was carried out on Zodiac C₁₈ (150 x 4.6mm, 5μ) with a mixture of Phosphate buffer: acetonitrile (50:50%v/v) as a mobile phase at a flow rate of 1.0 mL/min. UV detection was performed at 243nm. The retention times were 2.442 and 3.269 min for Tobramycin and Loteprednol respectively. Calibration plots were linear ($r^2=0.999$) over the concentration range of 3.75-22.5 μg/mL for Tobramycin 6.25-37.5μg/mL for Loteprednol. The method was validated for accuracy, precision, specificity, linearity, robustness, LOD and LOQ. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that developed method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Tobramycin and Loteprednol in bulk and tablet dosage form.

Keywords: Tobramycin; Loteprednol; RP-HPLC; Tablets.

1. INTRODUCTION

Tobramycin (TOBR)¹⁻³ (Fig-1) is an aminoglycoside, broad-spectrum antibiotic produced by *Streptomyces tenebrarius*. Tobramycin can be used in topical or systemic treatment. It is effective against gram-negative bacteria, especially the pseudomonas species. It is a 10% component of the antibiotic complex, produced by the same species. It is chemically (2S,3R,4S,5S,6R)-4-amino-2-[[[(1S, 2S, 3R, 4S,6R)-4,6-diamino-3-[[[(2R,3R,5S,6R)-3-amino-6-(aminomethyl)-5-hydroxyoxan-2-yl]oxy]-2-hydroxycyclohexyl]oxy]-6-(hydroxymethyl)oxane-3,5-diol. Loteprednol (LOTE) (Fig-2) (as Loteprednol etabonate) is a topical corticoid anti-inflammatory. It is used in ophthalmic solution for the treatment of steroid responsive inflammatory conditions of the eye such as allergic conjunctivitis, uveitis, acne rosacea, superficial punctate keratitis, herpes zoster

keratitis, iritis, cyclitis, and selected infective conjunctivitis. As a nasal spray, is used for the treatment and management of seasonal allergic rhinitis. Loteprednol etabonate is synthesized through structural modifications of prednisolone related compounds so that it will undergo a predictable transformation to an inactive metabolite. It is chemically Chloromethyl (1S,2R,10S,11S,14R,15S,17S)-14,17-dihydroxy-2,15-dimethyl-5-oxotetracyclo[8.7.0.0^{2,7}.0^{11,15}] heptadeca-3,6-diene-14-carboxylate. Literature survey⁴⁻¹² reveals that few Spectrophotometric and chromatographic methods were reported for estimation of TOBR and LOTE in single and combination with other drugs. In this study, an attempt has been made to develop an accurate, rapid and reproducible reverse phase HPLC method for simultaneous determination of TOBR and

LOTE in combined tablet dosage form and validate it, in accordance with International Conference on Harmonization (ICH) guidelines.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

The reference samples of TOBR and LOTE (API) were obtained from Pulse Pharmaceuticals, Hyderabad. The branded formulations LACNE gel was procured from the local market. Gel claimed to contain 0.3% TOBR and 0.5% LOTE have been utilized in the present work. All chemicals and reagents used were HPLC grade and purchased from Merck chemicals, India.

2.2. Chromatographic conditions

Separation was performed on an isocratic waters HPLC 2695 system instrument equipped with a with binary pump and variable wavelength PDA detector with auto injector. Data was analysed by using Empower2 software. Degassing of the mobile phase was done by using bath sonicator. A Shimadzu balance was used for weighing the materials. The separation was achieved on a Zodiac C₁₈ (150 x 4.6 mm, 5 μ) analytical column. The mobile phase consisted of phosphate buffer: acetonitrile (50:50%v/v). The flow rate was 1.0 mL/min and UV detection was performed at 243 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45 μ membrane filter (Millipore, Ireland). The injection volume was 10 μ L and all the experiments were performed at ambient temperature.

2.3. Preparation of Standard stock solutions

Accurately weighed 3.75mg of TOBR and 6.25mg of LOTE standard drugs was transferred 25ml clean and dry volumetric flask containing 3/4th volume of diluent and sonicated for 10 minutes. Flask was made up with diluent and labeled as Standard stock solution. (150 μ g/ml of TOBR and 250 μ g/ml of LOTE)

2.4. Preparation of Sample stock solutions

20 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (150 μ g/ml of TOBR and 250 μ g/ml of LOTE).

2.5. Method validation

The developed method was validated according to ICH guidelines. The system suitability was evaluated by six replicate analysis of TOBR and LOTE mixture at concentrations of 1000 μ g/mL and 100 μ g/mL. The acceptance criteria are number of theoretical plates (N) at least 2000 per each peak and tailing factor is not more than 2.0.

2.5.1. Linearity

Standard calibration curves were plotted against the concentration ranging from 3.75-22.5 μ g/mL for TOBR and 6.25-37.5 μ g/mL for LOTE. Different linearity levels were prepared and injected into the HPLC system keeping the injection volume constant.

2.5.2. Precision

Precision of assay was determined by System and Method Precision. Every sample was injected six times. The repeatability of sample application and measurements for peak area were expressed in terms of %RSD.

2.5.3. Specificity

All chromatograms were examined to determine whether compound of interest co-eluted with each other or with any additional excipient peaks. Marketed formulation was analysed to determine the specificity of the optimized method in presence of common excipients.

2.5.4. Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were estimated from signal-to-noise ratio. LOD and LOQ were calculated using 3.3 σ /s and 10 σ /s formulae, respectively. Where, σ is the standard deviation of the peak areas and S is the slope of the corresponding calibration curve.

2.5.5. Robustness

To evaluate robustness of HPLC method a few parameters were deliberately varied. The parameters included are variation of flow rate and Detection Wavelength.

2.5.6. Force Degradation studies

➤ Oxidation

To 1 ml of stock solution, 1 ml of 20% hydrogen peroxide (H₂O₂) was added separately. The solutions were kept for 30 min at 60^oc. For HPLC study, the resultant solution was diluted to obtain 100 μ g/ml and 10 μ g/ml solution and 10 μ L were injected into the system and the chromatograms were recorded to assess the stability of sample.

➤ Acid Degradation Studies

To 1 ml of stock solution of TOBR and LOTE, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain 100µg/ml and 10µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

➤ Alkali Degradation Studies

To 1 ml of stock solution of TOBR and LOTE, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain 100µg/ml and 10µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

➤ Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml and 10µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

➤ Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 300µg/ml, 10µg/ml and 25µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m² in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 100µg/ml and 10µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

➤ Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60°. For HPLC study, the resultant solution was diluted to 100µg/ml and 10µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

3. RESULTS AND DISCUSSION

During the optimization of HPLC method, two columns symmetry C-18 and C-8 analytical column (4.6×250 mm; 5 µm) and (4.6×150 mm; 5 µm), organic solvent (acetonitrile), one buffer (phosphate) were tested. Initially Water: Acetonitrile and Phosphate buffer, were tried in different ratios. Finally mobile phase consisting of mixture of acetonitrile: Phosphate

buffer in ratio 50:50% v/v was selected as mobile phase to achieve clear separation and sensitivity. Flow rates between 0.8 to 1.2 mL/min were studied. A flow rate of 1.0 mL/min gave an optimum signal to noise ratio with reasonable separation time using a C₁₈ Zodiac column (4.6×150 mm; 5 µm), the retention times for TOBR and LOTE were observed to be 2.442 and 3.269 min respectively. Total run time was less than 7 min. The chromatogram at 243 nm showed a complete resolution for all peaks (Fig. 3).

Validity of the analytical procedure as well as the resolution between different peaks of interest is ensured by the system suitability tests. All critical parameters tested meet the acceptance criteria on all days. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks.

Linearity was obtained for TOBR and LOTE in the range of 3.75-22.5 µg/mL and 6.25-37.5 µg/mL. The correlation coefficient (r²) was found to be greater than 0.999 in all instances. The results of calibration studies are summarized in Table 1.

The proposed method afforded high recoveries for TOBR and LOTE in dosage form. Results obtained from recovery studies presented in Table 2 indicate that this assay procedure can be used for routine quality control analysis of binary mixture in sample.

Precision of the analytical method was found to be reliable based on %RSD (<2%) corresponding to peak areas and retention times. As can be seen in Table 3 the %RSD values were less than 2 for System and Method precision. Hence, the method was found to be precise for these two drugs.

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common excipients and impurities. Also the peak areas were compared with standard and percentage purity calculated was found to be within limits. LOD and LOQ were found to be 0.07µg/mL and 0.21µg/mL for TOBR, 0.15µg/mL and 0.45µg/mL for LOTE. In all deliberately varied conditions, the %RSD for replicate injections of TOBR and LOTE were found to be within the acceptable limit. The tailing factors for two peaks were found to be less than 1.5 and the results are shown in Table 4.

The validated method was used in analysis of marketed tablet dosage form. The results for the drugs assay showed good agreement with label claims and the results are shown in Table 5. Degradation studies results were shown in Table 6 and 7.

Table 1: Linearity table for Tobramycin and Loteprednol

Tobramycin		Loteprednol	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
3.75	63178	6.25	128937
7.5	120694	12.5	253509
11.25	181816	18.75	369347
15	243565	25	474971
18.75	302855	31.25	613614
22.5	363017	37.5	732990

Table 2a: Accuracy data for Loteprednol

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	7.5	7.430	99.07	99.45%
	7.5	7.466	99.55	
	7.5	7.496	99.95	
100%	15	14.904	99.36	
	15	14.937	99.58	
	15	14.881	99.20	
150%	22.5	22.490	99.96	
	22.5	22.352	99.34	
	22.5	22.280	99.02	

Table 2b: Accuracy data for Tobramycin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	12.5	12.476	99.81	98.73%
	12.5	12.405	99.24	
	12.5	12.456	99.65	
100%	25	24.886	99.54	
	25	24.895	99.58	
	25	24.795	99.18	
150%	37.5	37.401	99.74	
	37.5	37.022	99.81	
	37.5	37.356	99.24	

Table 3: Precision data of proposed method

S. No.	System Precision		method Precision	
	Tobramycin*	Loteprednol*	Tobramycin*	Loteprednol*
1	242044	473471	235044	445390
2	239658	474567	235658	444567
3	240636	478549	235636	438549
4	239711	478981	234711	442551
5	240134	472187	235330	440819
6	241725	476737	232686	446737
Mean	240651	475749	234844	443102
Std. Dev.	1022.8	2776.2	1117.2	3060.7
%RSD	0.4	0.6	0.5	0.7

Table 4: Robustness for flow rate variation of TOBRA and LOTE

S.NO	Robustness condition	Tobramycin Area %RSD	Loteprednol Area %RSD
1	Flow rate- 0.9	0.9	0.7
2	Flow rate-1.1	0.9	0.4
3	Mobile Phase(55:45)	0.8	0.3
4	Mobile Phase(45:55)	0.7	0.6
5	Temperature-25°C	0.8	0.5
6	Temperature-35°C	0.6	0.7

Table 5: Analysis of marketed formulation by proposed method

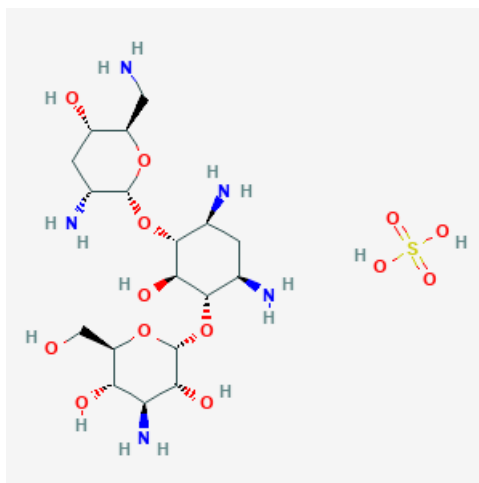
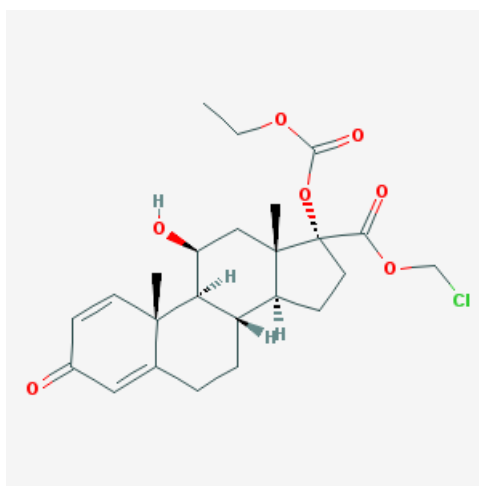
Brand Name	Drug	Labelled claim	Amount found*	% Assay*
LACNE	Tobramycin	0.3%	9.98 mg	99.89
	Loteprednol	0.5%	4.99 mg	99.89

Table 6: Degradation Data of Tobramycin

S. No.	Degradation Condition	% Drug found	% Drug Degraded
1	Acid	94.46	5.54
2	Alkali	95.52	4.48
3	Oxidation	96.34	3.66
4	Thermal	96.85	3.15
5	UV	98.77	1.23
6	Neutral	98.77	1.23

Table 7: Degradation Data of Loteprednol

S. No.	Degradation Condition	% Drug found	% Drug Degraded
1	Acid	94.08	5.92
2	Alkali	96.13	3.87
3	Oxidation	96.92	3.08
4	Thermal	97.82	2.18
5	UV	98.33	1.67
6	Neutral	99.23	0.77

**Fig. 1: Molecular structure of Tobramycin****Fig. 2: Molecular structure of Loteprednol**

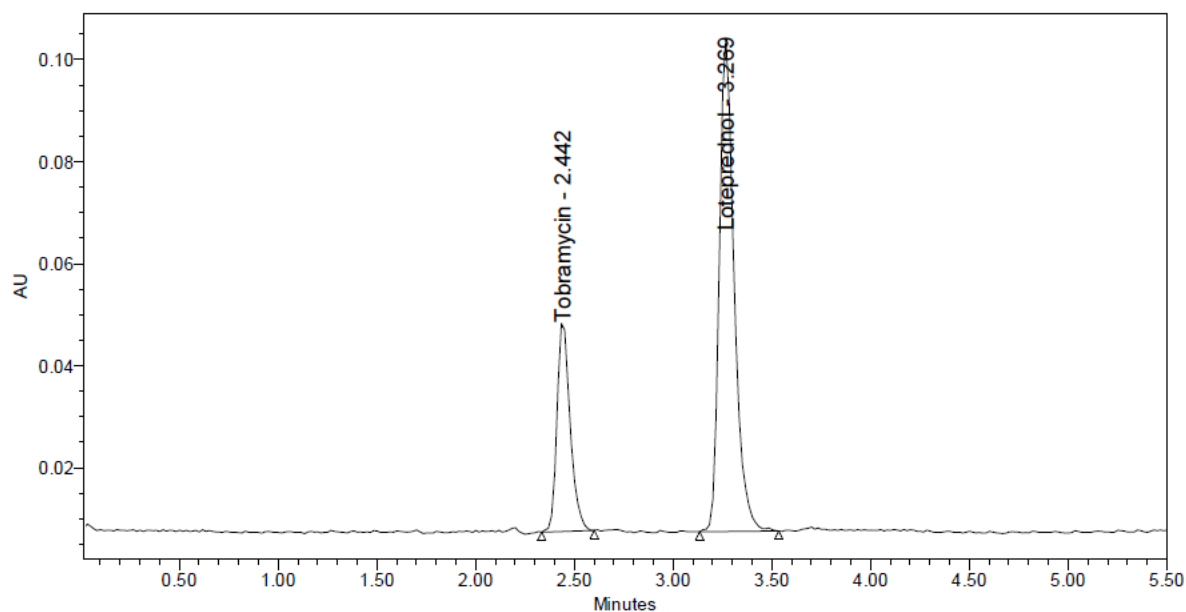


Fig. 3: Typical chromatogram of standard for TOBRA and LOTE

4. CONCLUSION

The developed stability indicating RP-HPLC method is simple, specific, accurate and precise for the simultaneous determination of TOBR and LOTE in combined tablet dosage form. The developed method provides good resolution between TOBRA and LOTE. It was successfully validated in terms of system suitability, linearity, precision, accuracy, specificity, LOD, LOQ and robustness in accordance with ICH guidelines. Thus the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

5. ACKNOWLEDGEMENTS

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6. Conflict of interest

No conflict of interest

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