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DEVELOPMENT AND VALIDATION OF NOVEL RP-HPLC METHOD FORSIMULTANEOUS ESTIMATION OF LOTEPREDNOL AND TOBRAMYCIN IN PHARMACEUTICAL DOSAGE FORMS

Suggu Sarika*, V. Swathi

Department of Pharmacy, Sri Sivani College of Pharmacy, Affiliated to JNTUK, Srikakulam- 532402, Andhra Pradesh, INDIA

***Corresponding Author E-mail: sarikareddy994@gmail.com**

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ABSTRACT

A simple, Accurate, precise technique was developed for the simultaneous estimation of Loteprednol and Tobramycin in pharmaceutical dosage form. Chromatogram was run through Discovery- C_{18}column (250 x 4.6mm, 5µ) column. Mobile phase containing Water: Methanol Buffer taken in the proportions 50:50v/v was pumped through column at flow rate of 1.0ml/min. Temperature was maintained at 30°C. Optimised wavelength selected was 243nm. Retention time of Tobramycin and Loteprednolwere observed to be 2.442min and 3.269min. %RSD of the Tobramycin and Loteprednolwere and observed to be 0.4 and 0.6 respectively. %Recovery was obtained as99.45% for Tobramycin and98.73% for Loteprednol respectively. LOD, LOQ values obtained from regression equations of Tobramycin and Loteprednol were 0.07, 0.21 and 0.15, 0.45 respectively. Regression equation of Tobramycinis y = 16107x + 952.5, and y = 19370x + 4432 of Loteprednol . Retention times were decreased and that run time was decreased, so the technique developed was simple and conservative that can be embraced in regular quality control test in industries.

INTRODUCTION

Loteprednol is a corticosteroid used to treat inflammations of the eye. It is also used after eye surgeries. Loteprednol works by relieving symptoms such as swelling, redness and itching. Tobramycin is an aminoglycoside antibiotic that kills susceptible bacteria by blocking bacterial protein synthesis. Tobramycin treats only bacterial eye infections and does not work for other type of eye infections . Loteprednol and Tobramycin solution is a combination of a steroid and an antibiotic. Loteprednol reduces swelling and inflammation. Tobramycin works by killing the bacteria or by preventing it from growing.

Loteprednol and Tobramycin solution is used to treat inflammation or swelling in the eye caused by a bacterial infection. [1-2]

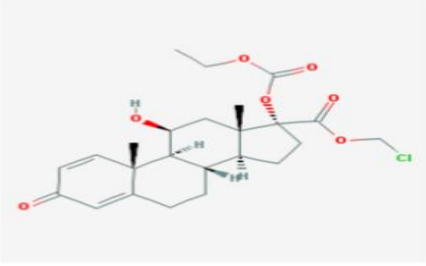

Fig-1: structure of Loteprednol

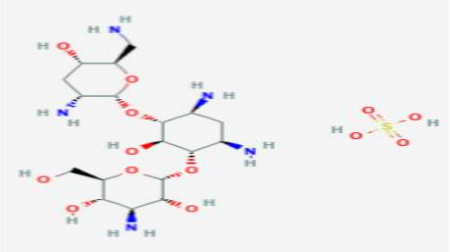

Fig-2: structure of Tobramycin

MATERIALS and METHODS

Preparation of buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Buffer: 0.01N Potassium di hydrogen orthophosphate: Accurately weighed 1.36gm of Potassium di hydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Tri ethylamine then PH adjusted to 3.0 with dil. Ortho phosphoric acid solution.

Diluent: Based upon the solubility of the drugs, diluents were selected. Acetonitrile and water are taken in the ratio of 50:50 v/v.

Stock solution:

Preparation of Standard stock solutions: Accurately weighed 3.75mg of Tobramycin, 6.25mg of Loteprednol and transferred to individual 25ml volumetric flasks separately. 3/4thof diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labelled as Standard stock solution 1and 2. (150µg/ml of Tobramycin and 250µg/ml of Loteprednol).

Preparation of Sample stock solutions: 5 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 (150µg/ml of Tobramycin and 250µg/ml of Loteprednol).

Working solution:

Preparation of Standard working solutions: 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (15µg/ml Tobramycin of and 25µg/ml of Loteprednol).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (15µg/ml of Tobramycin and 25µg/ml of Loteprednol).

Procedure: Sample solutions (10µL) in duplicates were injected and the peak responses were measured. % assay were calculated for Loteprednol and Tobramycin.[3]

RESULTS AND DISCUSSION:

Method validation: Specificity, linearity, range, Accuracy, precision, Repeatability,Intermediate precision,limit of detection,limit of Quantification,Robustness.

SPECIFICITY: The system suitability for specificity was carried out to determine whether there is an interference of any impurities in retention time of analytical peak. The specificity study was performed by injecting blank. It was found that there was no interference of impurities in retention time of analytical peak.

LINEARITY: To establish the linearity of the method, serial dilutions were prepared to obtain the mixture of Loteprednol and Tobramycin ranging from 6.25ml to 37.5ml and 3.75ml to 22.5ml, all the solutions were filtered through a 0.45µm Millipore filters. The final solution was injected in duplicate manner keeping the injection volume 10µl. Calibration curve was plotted between mean peak area and concentration. The correlation coefficient and slope were determined from the calibration curve. The linearity chats of Loteprednol and Tobramycin was shown in figure no.5 and 6 . The correlation coefficient was found to be 0.999 for both drugs and hence the method was set to be linear. They were tabulated in table 1.[4]

ACCURACY: Accuracy was evaluated by standard addition method of three known concentration of the drug and the spiked solution were analysed.

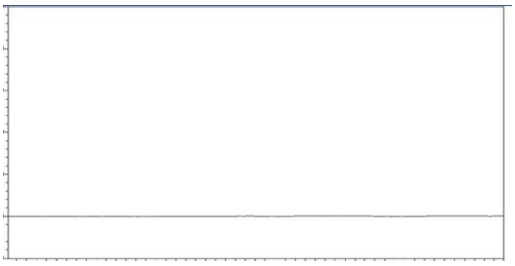

Fig-3: Chromatogram showing blank

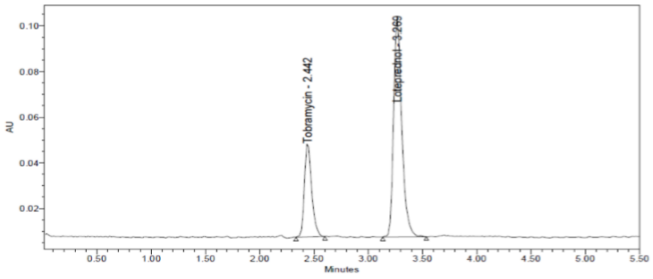

Fig-4: Chromatogram showing optimised condition

Table 1: Linearity results 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

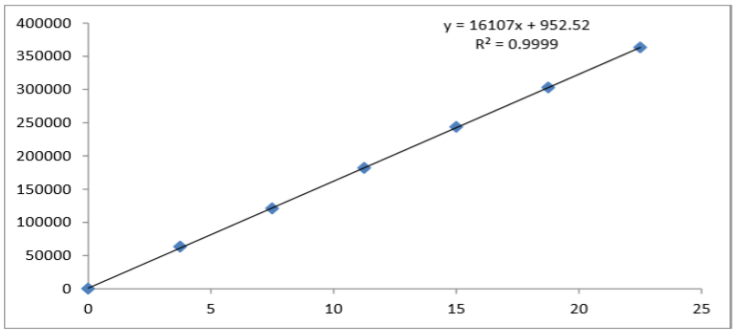

Fig-5: showing calibration curve of Tobramycin

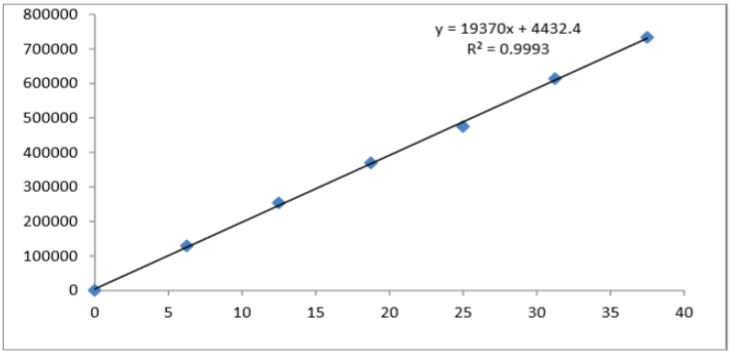

Fig-6: showingcalibration curve of Loteprednol

Table 2 : Accuracy data for Tobramycin

% 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	99.55	
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	22.380	99.02	

Table 3 : Accuracy data for Loteprednol

% 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	24.895	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-4: Intermediate precision data for Tobramycin and Loteprednol

S.NO	Area of Tobramycin	Area of Loteprednol
1	235044	445390
2	235658	444567
3	235636	438549
4	234711	4442551
5	235330	440819
6	232686	446737
Mean	234844	443102
S.D	1117.2	3060.7
% RSD	0.5	0.7

Table-5: Repeatability results for Tobramycin and Loteprednol

S.NO	Area of Tobramycin	Area of Loteprednol
1	240714	474364
2	239900	473487
3	238965	475844
4	239316	474948
5	238657	474612
6	238681	473299
Mean	239372	474426
S.D	804.6	945.9
% RSD	0.3	0.2

Table-6: LOD and LOQ data for Tobramycin and Loteprednol

Molecule	LOD	LOQ
Tobramycin	0.07	0.21
Loteprednol	0.15	0.45

Table-7: Robustness data for Tobramycin and Loteprednol

S.no	Condition	% RSD of Tobramycin	%RSD of Loteprednol
1	Flow rate (-) 0.9ml/min	0.9	0.7
2	Flow rate (+) 1.1ml/min	0.9	0.4
3	Mobile phase (+) 65B:35A	0.8	0.3
4	Mobile phase (-) 55B:45A	0.7	0.6
5	Temperature (-) 25°C	0.8	0.5
6	Temperature (+) 35°C	0.6	0.7

The recovery of the added drug was determined by calculating the pre-analysed drug concentration with concentration of spiked drug. The % recovery was calculated and the result was reported in table no. 2 & 3. [(5)]

PRECISION: The precision of the analytical method was studied by injecting six replicates of standard and sample concentration on the same day and another day. The concentration of Loteprednol and Tobramycinwere injected atintermediate precision and repeatability. The %RSD was calculated and results were reported at table no. 4 and 5.[(6)]

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ): The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting six replicates of mobile phase followed by three concentration of the drug. The LOD was defined as the concentration which yields a signal-to-noise ratio 3:1 while the LOQ was calculated to be the lowest concentration that could be measured with signal-to-noise ratio 10:1. The LOD & LOQ were calculated by measuring the standard deviation of the response and slope. The result of LOD &LOQ was tabulated in table no. 6. ([(7)]

ROBUSTNESS: The small deliberate changes in method like flow rate was made but there were no recognized change in the result and are within the range as per ICH guide lines. Robustness condition like flow minus (0.9ml/min), flow plus (1.1ml/min), temperature ambient was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed %RSD was found to be within the limits and results were tabulated in table no.7.[(8)]

CONCLUSION:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Tobramycin and Loteprednol in Tablet dosage form. Retention time of Tobramycin and Loteprednol were found to be 2.442min and 3.269 min.. %RSD of the Tobramycin and Loteprednol were and found to be 0.4 and 0.6 respectively. %Recovery was obtained as 99.45% and 98.73% for Tobramycin and Loteprednol respectively. LOD, LOQ values obtained from regression equations of Tobramycin and Loteprednol were 0.07, 0.21 and 0.15, 0.45respectively. Regression equation of Tobramycin is y = 16107x + 952.5, and y = 19370x + 4432 of Loteprednol. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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