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Development and Validation of Stability Indicating RP-HPLC Method for Simulteneous Estimation of Loteprednol Etabonate and Levofloxacin in Bulk Drug

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ABSTRACT

To develop RP-HPLC method for simultaneous estimation of Loteprednoletabonate and levofloxacin in synthetic mixture. In HPLC method the retention time of loteprednol and levofloxacin was found to be 5.68 and 12.08 min, respectively. Linearity was observed in the concentration rang 50-150µg/ml and 5- 15µg/ml with correlation coefficient of loteprednol and Levofloxacin (R2) 0.998 and (R2) 0.997, respectively. The Mean recovery were found to be in the range 99.72 - 100.47% and 99.68 - 100.87% respectively. A simple, precise, rapid, economic, specific and accurate stability indicating RP-HPLC method have been developed and validate for the simultaneous estimation of Loteprednol and Levofloxacin in its bulk drug. All method validation parameters lie within its acceptance criteria as per ICH Q2(R1) guideline. The developed method can be used for routine analysis of Loteprednol and Levofloxacin in bulk drug and in pharmaceutical dosage form.

Key Words: Loteprednol, levofloxacin, RP-HPLC

INTRODUCTION

EYEINFECTION

Disease (eyeinfection)[1-2]

The human eye, which is constantly exposed to the external environment, is a unique organ serving as the window four body. Ocular disease with its complications, due to microorganisms, is a significant health problem worldwide particularly in the leastincome countries [1]. Ocular infections can damage the structure of the eye which canlead to reduced vision or even blindness if it is inappropriately diagnosed and treated. The most frequently affected parts of the eye due to microorganisms are the conjunctiva, eyelid, and cornea. Conjunctivitis, blepharitis, and macrocystis are considered the most common manifestations of external eye infections [2]. These pathogenic micro organisms include bacteria, fungi, viruses, and parasites.

Levofloxacin[3]

Levofloxacin is used to treat a variety of bacterial infections. This medication belongs to a class of drug knows a squinoloneanti-biotics. It works by stopping the growth of bacteria. This anti-biotic treats only bacterial infection.
Mechanism faction: Inhibition of topoisomerase (DNA gyrase) enzymes, which inhibits relaxation of super coiled DNA and promotes breakage of double stranded DNA.

Use: Levofloxacin is used to treat bacterial infection.

Loteprednol Etabonate[4]

Loteprednol etabonate was introduced for the treatment of steroid responsive inflammatory conditions of the palpebral and bulbar conjunctiva, cornea, and anteriorsegmentof theocular globe.

Mechanism faction: Loteprednol etabonate specifically induces phospholipase A2inhibitoryproteins(collectively called lipocortin's), which inhibit the release of arachidonic acid, there by inhibiting the biosynthesis of potentmediators of inflammation, such as prostaglandins and leukotrienes.

Use: This medication is used to treat certain eye conditions due to inflammation orinjury. It is also used after eye surgery. Loteprednol works by relieving symptoms such as swelling, redness, and itching.It belongsto aclassof drugsknown ascorticosteroids.

High Performance Liquid Chromatography12-14

The term chromatography meaning "color writing," was first discovered by Mikhail Tweet, a Russian botanist who separated plant pigments on chalk (CACO3) packed in glass columns in 1903. High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of online detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds. By the 1980's HPLC was commonly used for the separation of chemical compounds. Computers and automation added to the convenience of HPLC.

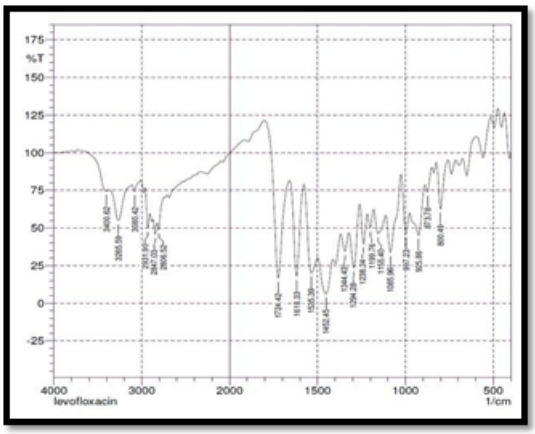
Liquid chromatography (LC) is a physical separation technique conducted in the liquid phase. Analyte is forced to flow through a column under high pressure. Then it is separated into its constituent components by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). Four major separation modes of HPLC are normal phase, reversed phase, ion exchange chromatography, and size exclusion chromatography (gel permeation and gel filtration chromatography.

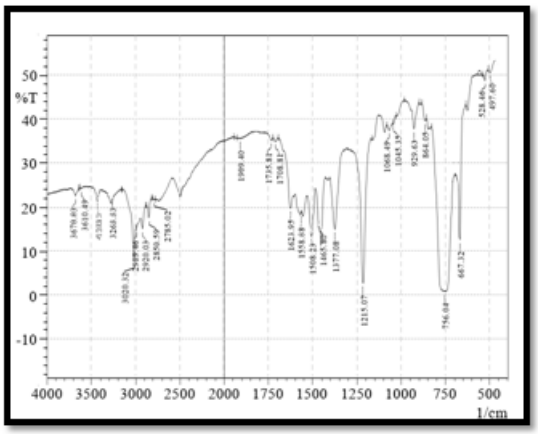
MATIRIALS AND METHOD

Melting Point Determination of Loteprednol Etabonate and Levofloxacin:
Melting point of Drugs

Sr. No.	APIs	Melting point (˚C)	
		Reported	Measured
1	Loteprednol Etabonate	220.5 to 223.5 °C	220-222˚C
2	Levofloxacin	225 to 227 °C	226-227˚C

Identificationby FTIR Spectroscopy:





IR Spectra of sample Levofloxacin IR Spectra of sample Loteprednol Etabonate
IR Spectral interpretationofLevofloxacin

Sr No.	Functional Group	Frequency (cm^{-1})
1	-COOH	3265
2	-CH3	2931
3	C=O	1724
4	C-N	1294
5	F (halogen group)	1085

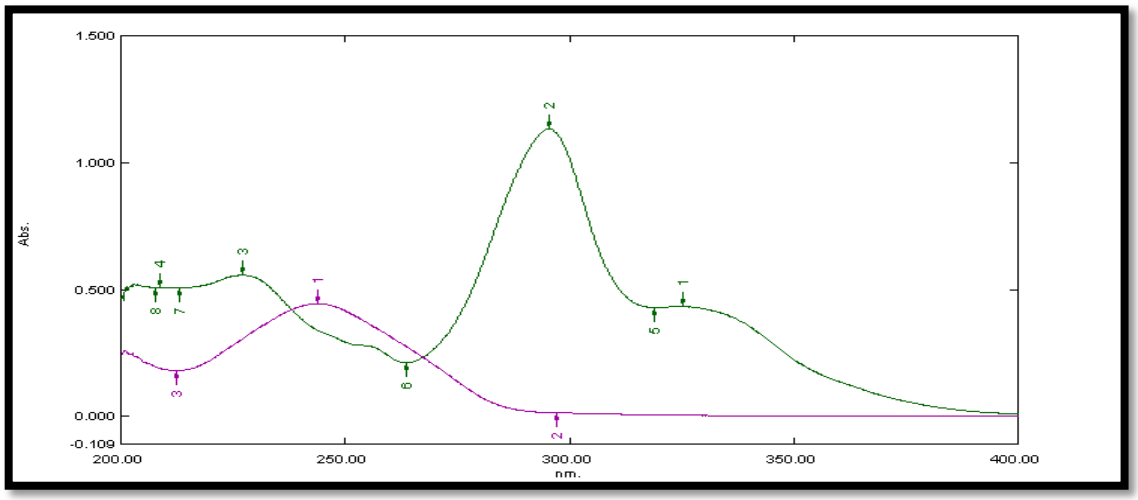
IR Spectral interpretation of Loteprednol Etabonate

Sr. No.	Functional Group	TheoreticalPeaks (cm^{-1})	PracticalPeaks (cm^{-1})
1	C-H (stretch)	2800-2900	2853
2	C-N (stretch)	1000-1350	1137
3	N-H (stretch)	3100-3500	3363
4	C-O (stretch)	1300-1200	1215
5	C=O (stretch)	1600-1770	1623
6	C=C (stretch)	1475-1610	1503
7	C=Cl (stretch)	540-785	756

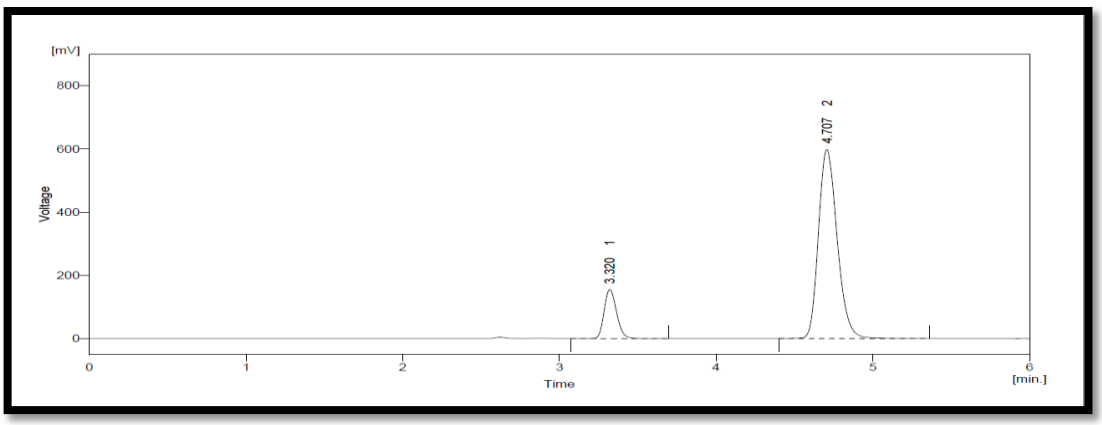
Solubility data of Loteprednol Etabonate and Levofloxacin
Solubility Data

Solvent	Loteprednol Etabonate	Levofloxacin
Water	Freelysoluble	Freely soluble
0.1 N HCl	Slightlysoluble	Slightly soluble
0.1 N NaOH	Highlysoluble	Soluble
Methanol	Freelysoluble	Freelysoluble

Selection ofWavelength:



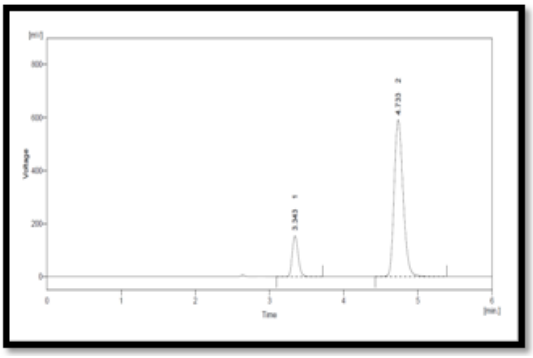
UV spectrum of Loteprednol Etabonate and Levofloxacin for detection of wavelength.
Chromatography:

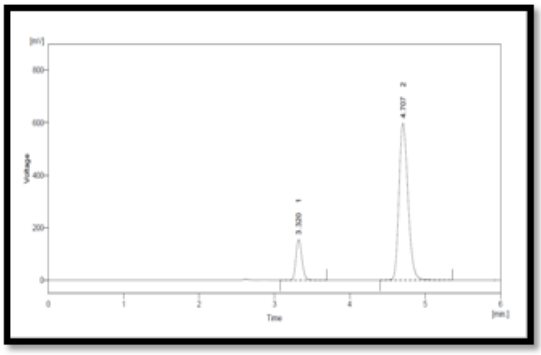


HPLC Chromatogram of Levofloxacin2 ppm and Loteprednol Etabonate50 ppm in Buffer (pH 5.0): Methanol (50:50) (Final)

Mobile Phase was selected based on the review of literature. Various mobile phases were tried. Trial contains various mobile phases which consisted of Methanol, Water, Phosphate Buffer in different proportions with various pH and different volumes at flow rate 1 ml/min were tried. On the basis of various trials the mixture of Buffer (pH 5.0) : Methanol (50:50)d

7.3 Stability Indicating Method for Simultaneous Estimation ofLevofloxacinand LoteprednolEtabonate done by RP-HPLC





Loteprednol Etabonate and Levofloxacin Loteprednol Etabonate and Levofloxacin sample Standard for stability

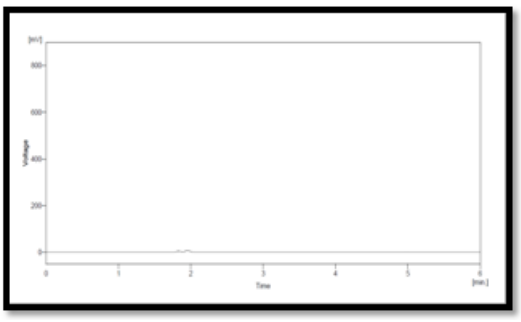
Fored Degradation:

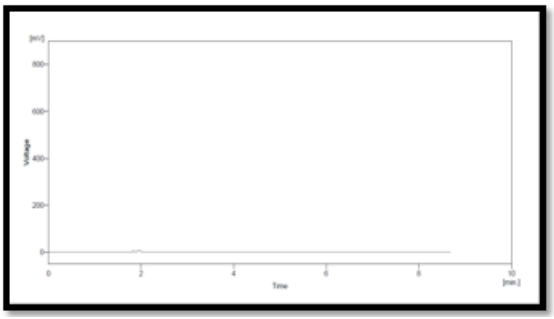
Acid degradation

Acid decomposition studies were performed by Refluxing 1 ml of stock solution was transferred in to 10 ml of volumetric flask. 2 ml of 0.1 N Hydrochloride solutions was added and mixed well and put for 5 hrs at 70 ºC 250 ml roundBottom flask. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 50 µg/ml for Loteprednol Etabonate and 2 µg/ml for Levofloxacin.

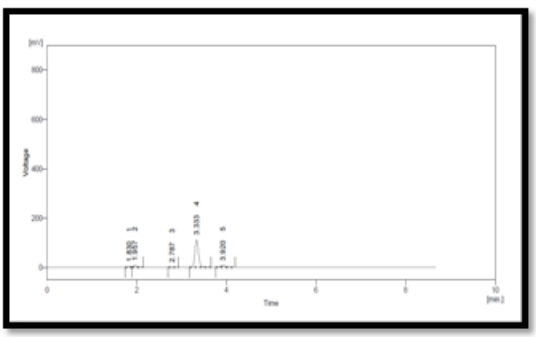
Base degradation

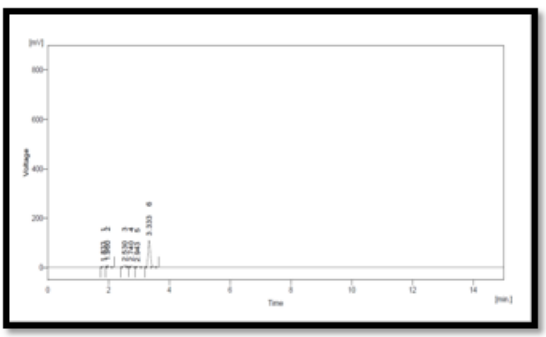
Basic decomposition studies were performed by refluxing 1 ml of stock solution was transferred in to 10 ml of volumetric flask. 2 ml of 0.1 N NaOH solutions was added and mixed well and put for 6 hrs at 70 ºC 250 ml round bottom flask. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 50 µg/ml for Loteprednol Etabonate and 2µg/ml for Levofloxacin.



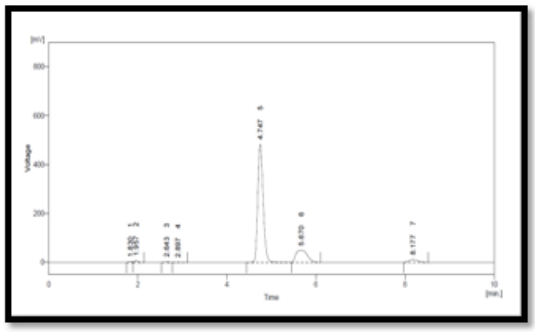


Acid Degradation Blank **Base Degradation Blank**

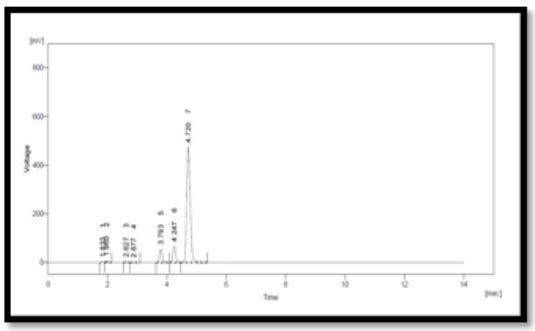




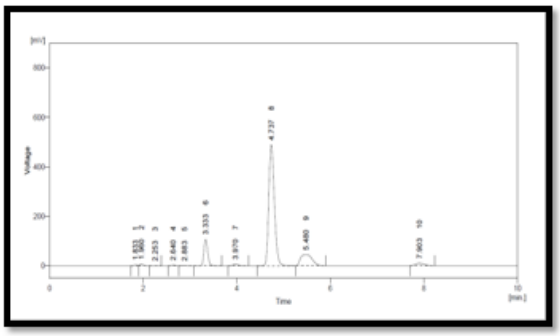
Levofloxacin Acid Degradation Standard Levofloxacin Base Degradation Standard



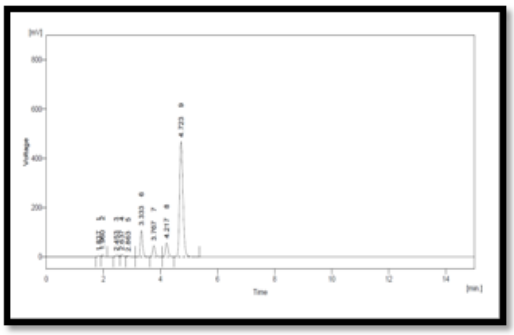
Loteprednol Etabonate Acid Degradation Standard



Loteprednol Etabonate Base Degradation Standard



Loteprednol Etabonate and Levofloxacin Acid Degradation Sample



Loteprednol Etabonate and Levofloxacin Base Degradation Sample Calculation for Stability

Drugs	Area
LoteprednolEtabonate	4936.824
Levofloxacin	844.565

Loteprednol Etabonate and Levofloxacin for stability

Parameter	Sample	
	Area	%Degradation
Acid	603.711	28.52
Base	587.895	30.39
Oxidation	630.229	25.38
Photo	686.227	18.75
Thermal	593.39	29.74

Levofloxacin % Degradation

Parameter	Sample	
	Area	%Degradation
Acid	4091.43	17.12425
Base	3895.506	21.09287
Oxidation	3410.381	30.91953
Photo	3854.245	21.92865
Thermal	4128.013	16.38323

Loteprednol Etabonate% Degradation

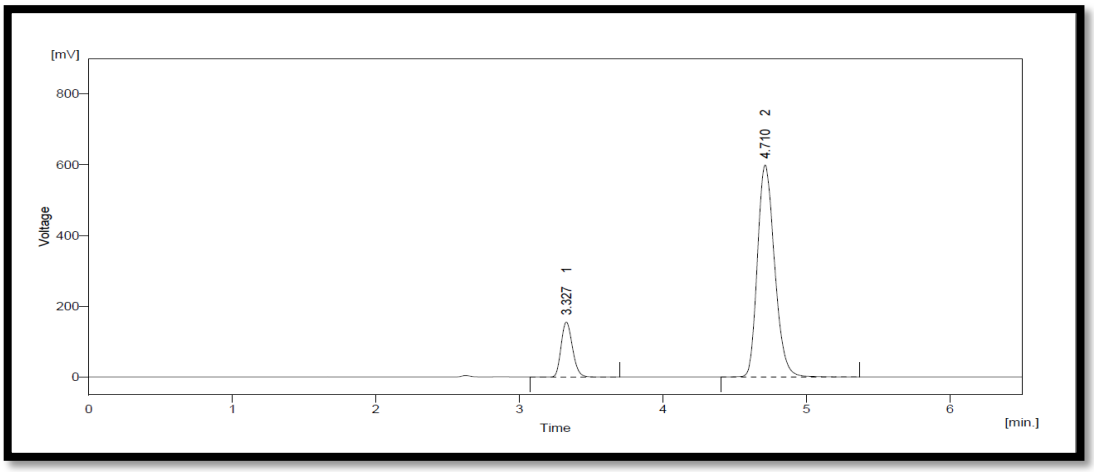
Validation of RP-HPLC Method:

Levofloxacinstandard stock solution: (20 µg/mL)

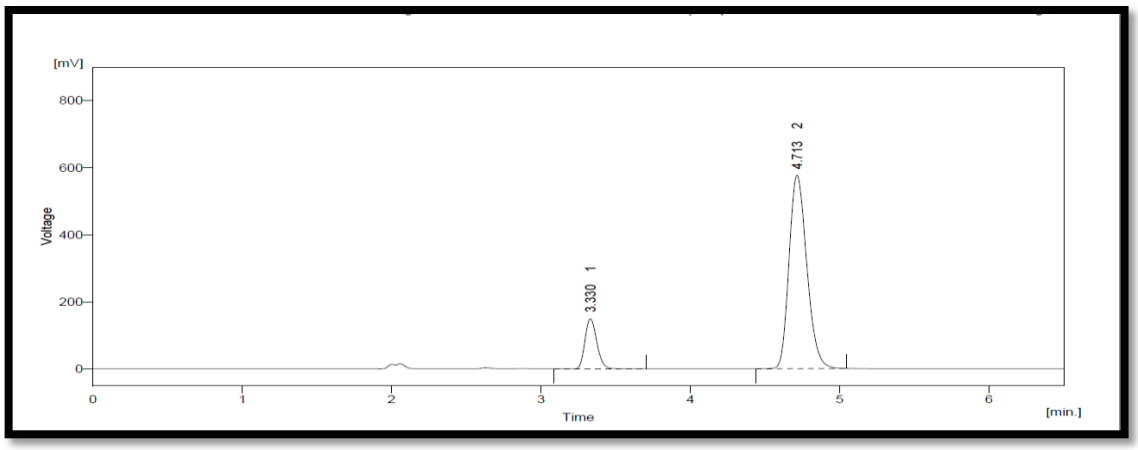
A 2 mg of HBB was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with mobile phase.

LoteprednolEtabonate standard stock solution: (500 µg/mL)
A 50 mg of Loteprednol Etabonate was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with mobile phase. Take 10ml from this solution and Transfer to 100ml volumetric flask and made up the Volume with the Mobile phase

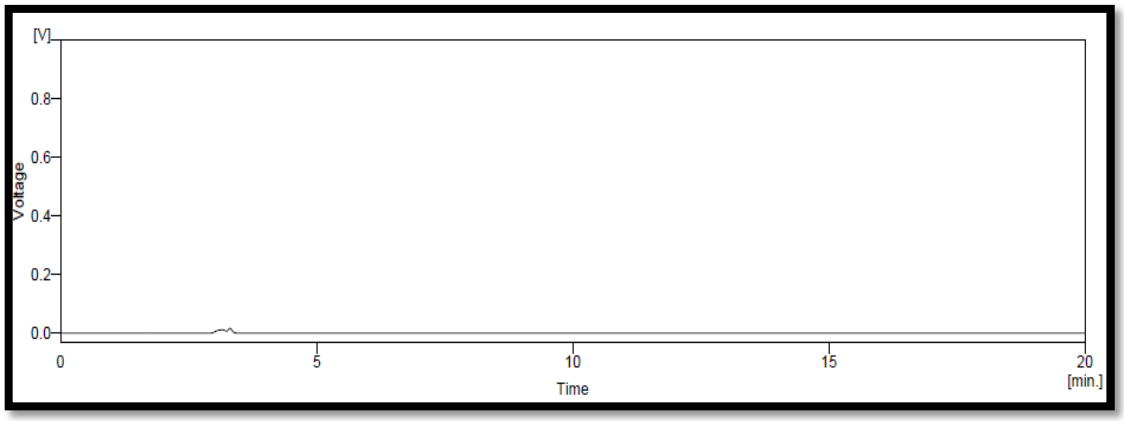
Specificity:



Chromatogram of Levofloxacin and LoteprednolEtabonate Standard for Specificity



Chromatogram of Levofloxacin and Loteprednol Etabonate sample for Specificity



Chromatogram of Levofloxacinand LoteprednolEtabonateBlank for specificity

Linearity and range:

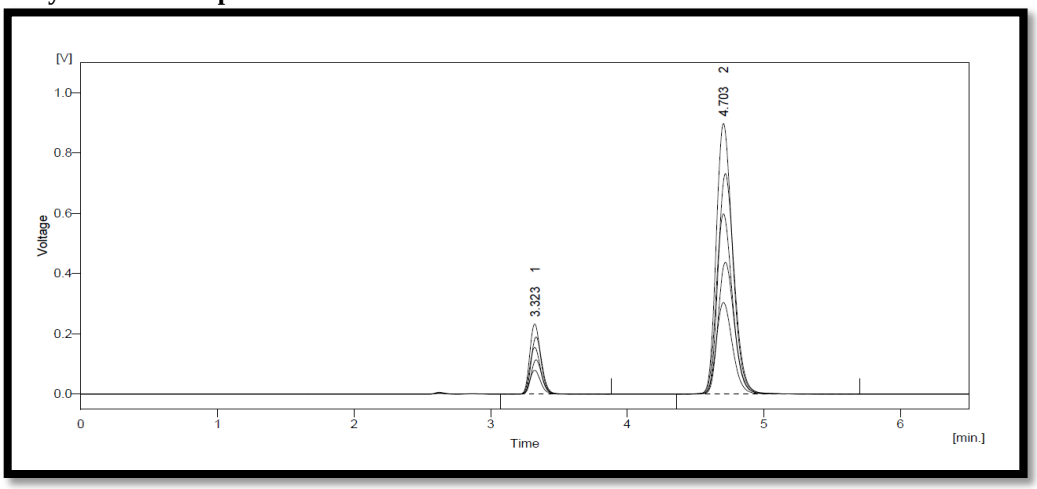
Sr.No	Concentration (µg/ml)	Peak Area

1	1	430.105
2	1.5	621.961
3	2	847.982
4	2.5	1040.391
5	3	1273.655

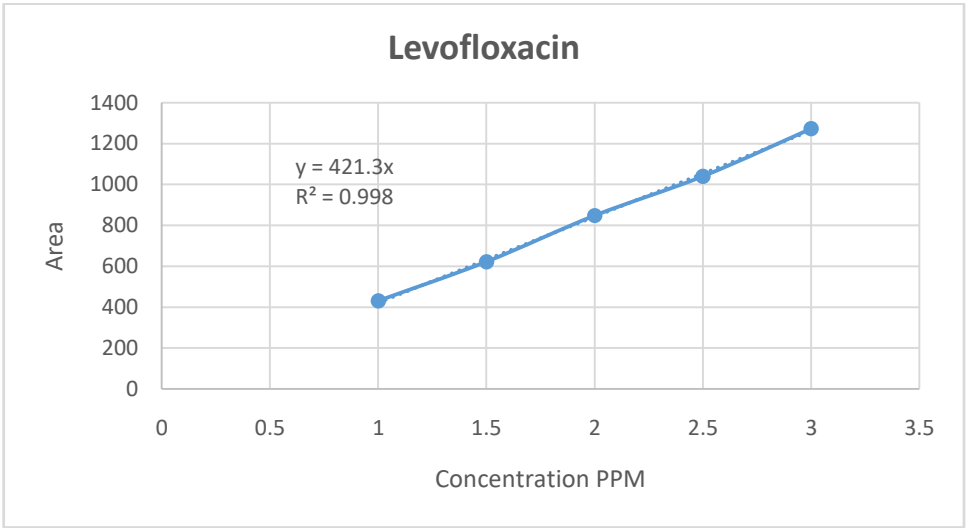
Linearity data for Levofloxacin

Sr.No	Concentration (µg/ml)	Peak Area
1	25	2517.111
2	37.5	3636.106
3	50	4923.711
4	62.5	6082.409
5	75	7446.404

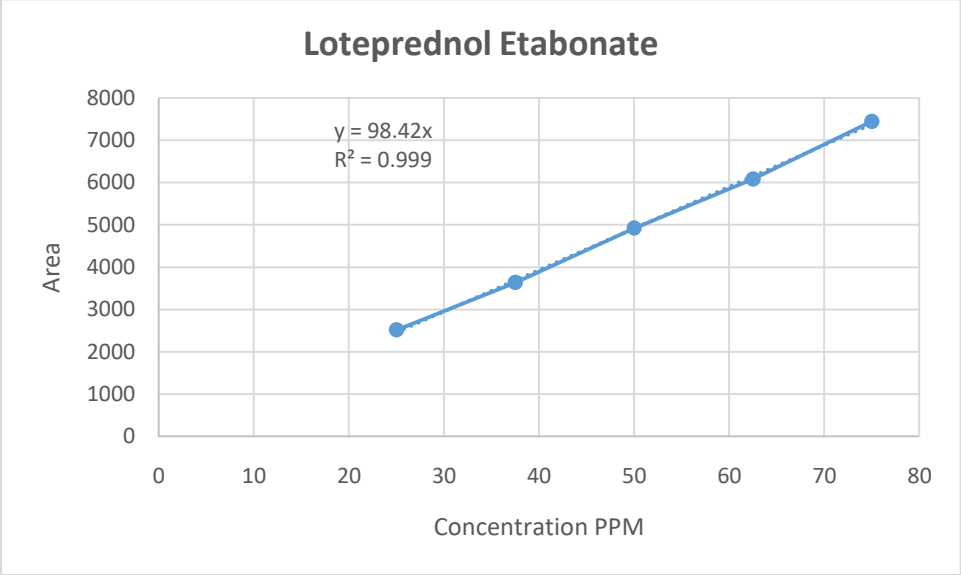
Linearity data for LoteprednolEtabonate



Overlay chromatogram of different concentrations of mixtures of LoteprednolEtabonate and Levofloxacinfor linearity



Calibration Curve of Levofloxacin(1-3 µg/ml)



Calibration Curve of LoteprednolEtabonate (25-75 µg/ml).

Precision

I. Repeatability

The data for repeatability of peak area measurement for LoteprednolEtabonate(50 µg/ml) and Levofloxacin (2 µg/ml) based on six measurements of same solution of LoteprednolEtabonate (50 µg/ml) and Levofloxacin (2 µg/ml). The % RSD for LoteprednolEtabonateand Levofloxacin was found to be 0.5280 and 0.3739 respectively.

Levofloxacin				
Sr No.	**Conc. (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	2	847.956 847.155 853.119 848.838 854.802 847.97	849.9733±3.1788	0.3739

Repeatability data for Levofloxacin

LoteprednolEtabonate				
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	50	4956.633 4951.608 4986.296 4961.373 4996.102 4922.712	4962.454 ±26.202	0.5280

Repeatability data for LoteprednolEtabonate

II. Intraday precision

Standard solution containing (1, 2, 3 µg/ml) of Levofloxacinand (25,50,75 µg/ml) of Levofloxacin were analysed three times on the same day and % R.S.D was calculated.

	Levofloxacin		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	1	424.703 ± 1.0822	0.254
2	2	856.778± 3.470	0.405
3	3	1272.3913± 5.2716	0.414

Intraday precision data for estimation of Levofloxacin

	LoteprednolEtabonate		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	25	2482.292 ± 3.732	0.150
2	50	5000.660 ± 28.846	0.576
3	75	7438.857 ± 30.996	0.416

Intraday precision data for estimation of LoteprednolEtabonate

III. Interday precision

Standard solution containing (1,2,3 µg/ml) of Levofloxacinand (25,50,75 µg/ml) of LoteprednolEtabonatewere analysed three times on the different day and % R.S.D was calculated

	Levofloxacin		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	1	421.478 ± 3.597	0.8535
2	2	849.944± 7.245	0.8524
3	3	1278.5± 5.597	0.4377

Interday precision data for estimation of Levofloxacin.

	LoteprednolEtabonate		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	25	2460.137 ± 31.437	1.2778
2	50	4955.970 ± 61.494	1.2408
3	75	7470.511 ± 37.322	0.4995

Interday precision data for estimation of LoteprednolEtabonate

Accuracy:

For LoteprednolEtabonate

50 µg/ml drug solutions were taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 220 nm. The amount ofLoteprednolEtabonatewas calculated at each level and % recoveries were computed.

For Levofloxacin

2 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 243 nm. The amount of Levofloxacin was calculated at each level and % recoveries were computed.

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	1	0.8	0.8075	100.944	100.571 ± 0.6478
2		1	0.8	0.7985	99.8235	
3		1	0.8	0.8075	100.946	
4	100 %	1	1	1.0011	100.111	100.325 ± 0.2179
5		1	1	1.0031	100.318	
6		1	1	1.0054	100.547	
7	120 %	1	1.2	1.1981	99.847	99.985 ± 0.5446
8		1	1.2	1.1942	99.523	
9		1	1.2	1.2070	100.586	

Recovery data for Levofloxacin

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	25	20	20.1361	100.680	99.6788 ± 1.7258
2		25	20	19.5371	97.6859	
3		25	20	20.1339	100.6696	
4	100 %	25	25	24.9699	99.8797	100.5386 ± 0.977
5		25	25	25.0185	100.0743	
6		25	25	25.4154	101.6616	
7	120 %	25	30	29.8913	99.6378	99.5888 ± 0.799
8		25	30	29.6298	99.7663	
9		25	30	30.1087	100.3623	

Recovery data for Loteprednol Etabonate

LOD and LOQ:
Limit of Detection:

Levofloxacin	LoteprednolEtabonate
LOD = 3.3 x (SD / Slope) = 3.3 x (12.4693 /421.38) = 0.0976µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (71.0611/98.42) = 2.382 µg/ml

Limit of Detection data for Levofloxacin and Loteprednol Etabonate

Limit of Quantitation:

Levofloxacin	Loteprednol Etabonate
LOQ = 10 x (SD / Slope) = 10 x (12.4693/421.38) = 0.295µg/ml	LOQ = 10 x (SD / Slope) = 10 x ((71.0611/98.42) = 7.220µg/ml

Limit of Quantitation data for Levofloxacin and Loteprednol Etabonate

Robustness:

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (±0.2) 5.2 and 4.8.
3. Ratio of Mobile phase was changed (±2) Buffer: Methanol (52:48) and Buffer: Methanol (48:52)

SR NO.	Area at Flow rate (+ 0.2 ml/min)	Area at Flow rate (- 0.2 ml/min)	Area at pH (+0.2)	Area at pH (-0.2)	Area at Mobile phase(+2)	Area at Mobile phase(-2)
1	2282.685	878.606	855.633	868.529	840.382	860.79
2	2354.415	875.087	859.95	868.557	843.762	848.87
3	2368.847	872.713	853.941	869.428	849.686	859.08
% R.S.D	1.9760	0.3386	0.3617	0.05883	0.5576	0.7525

Robustness data for Levofloxacin.

SR NO.	Area at Flow rate (+ 0.2 ml/min)	Area at Flow rate (- 0.2 ml/min)	Area at pH (+ 0.2)	Area at pH (- 0.2)	Area at Mobile phase (+2)	Area at Mobile phase (-2)
1	3333.63	5136.09	5001.41	5076.64	4912.14	5031.40
2	3282.56	5115.49	5001.07	5042.38	4909.36	5021.29
3	3374.72	5115.45	4991.10	5081.61	4966.24	5021.23
% R.S.D	1.3863	0.2323	0.1171	0.4215	0.6505	0.1165

Robustness data for LoteprednolEtabonate

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