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Analytical Method Development of Related Substance Test Parameter-Ii of Tobramycin in Loteprednol and Torbamycin Ophthalmic Suspension

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Keywords: *Neamine, Nebramine, Kanamycin B, Tobramycin in Loteprednol and Torbamycin Ophthalmic Suspension*

ABSTRACT

To develop an Analytical method for the estimation of Related Substances of Loteprednol in Loteprednol Etabonate and Tobramycin Ophthalmic Suspension 0.5% and 0.3%Since Loteprednol Etabonate drug substance as well as drug product (Suspension) Methods not official in any pharmacopeia. So for quantification of Loteprednol Etabonate and its impurities, an in-house method was developed for the estimation of RS of Loteprednol Etabonate. Neamine, Nebramine and Kanamycin B these impurities are monitored in Method-I. These are listed here only for information. For unknown impurities the RRF is calculated as 1.0. Total Impurities= Sum of Impurities obtained in the Related Substances Method-I + sum of Impurities obtained in the Related Substances Method-II.

INTRODUCTION

Delivery of medication to the human eye is an integral part of medical treatment. Ophthalmic suspension is one of the most interesting and challenging endeavors facing pharmaceutical scientist. Ophthalmic suspension are specialized dosage forms designed to be instilled onto the external surface of the eye (topical), administered inside (intraocular), adjacent to the eye (periocular) or used in conjunction with any special device[1].

Ophthalmic suspension are similar to parenteral dosage form in their requirements for sterility as well as consideration for osmotic pressure (tonicity), preservation, and tissue compatibility, avoidance of pyrogens and particulate matter and suitable packaging. (USP, BP, Ph Eu, and JP) requirements.

Drugs are administered to the eye for local effects such as bacterial infection, miosis, mydriasis, or to reduce intraocular pressure.

OPHTHALMIC SUSPENSION[2-5]

Ophthalmic suspensions contain solid particles dispersed in a liquid vehicle; they must be homogeneous when shaken gently and remain sufficiently dispersed to enable the correct dose to be removed from the container. Sediment may occur, but this should disperse readily when the container is shaken, and the size of the dispersed particles should be controlled.

The active ingredient and any other suspended material must be reduced to a particle size small enough to prevent irritation and damage to the cornea. A good ophthalmic suspension is one in which the particle size distribution lies between 1-10 um.

CHARACTERISTICS OF AN IDEAL SUSPENSION[6-10]

In almost all suspensions, insoluble solids separate on standing. The settling of particles influences product performance to a large extent. Therefore, it is important to enumerate the ideal characteristics of suspensions here.

- The solid particles should be of such a size that they do not settle rapidly.
- Even if sediment is formed, it should not form a hard cake at the bottom of the container.

- Even if sedimentation occurs, it should be possible to easily redisperse it on moderate shaking.

- The viscosity of the suspension should be such that the product can be easily poured from the bottle.

- The nature (particle size and viscosity) of suspension should be such that it can be easily injected through the needle of a syringe.

- Suspensions for topical use should spread when applied and leave a film of medicament at the site of application.

- Suspension should resist microbial attack. Normally preservatives are included in the formulation.

Methodology for Related Substances − I:

Note: For Chromatographic conditions, Blank Preparation, System suitability preparation and Sample Preparation, Refer Assay Method.

Standard Solution:

Preparation-1: (From Assay Standard preparation):

Pipette and transfer 1mL of Assay standard solution into 100mL volumetric flask, dilute and make up to the volume using HPLC water.

Preparation-2:

Weigh and transfer accurately about 12 mg of Tobramycin WS/RS into a 50mL volumetric flask add 20mL of HPLC water and 1mL of 1N sulfuric acid solution and mix well to dissolve completely and make up to the volume using HPLC water.

Dilute 1mL of the above solution to 100 mL using HPLC water.

Placebo Solution: Weigh accurately about 4 grams of the Placebo solution to 50mL volumetric flask add 20mL of diluent and sonicate about 2 minutes and make up to the volume using HPLC water and mix well.

Derivatized blank. Standard, sample and placebo Preparation: Heat all the solutions at the same temperature (60±2°C) and for same time (50±5 minutes).

To separate 50mL volumetric flasks transfer 4mL of Standard solution (Preparation-1 or Preparation-2), 4mL of Placebo solution, 4 mL of blank and 4 mL of sample solution. To each flask add 10mL of solution A and Solution C shake, and insert the stopper, place the flasks in a constant temperature bath at 60±2°Cand heat for 50±5 minutes. Remove the flasks from the water bath and allow to stand at room temperature for 10 minutes, add about 20mL of Acetonitrile and allow the solutions to attain room temperature and make up to the volume using Acetonitrile.

Procedure:

1. Equilibrate the column using mobile phase to get a stable baseline.

2. Inject Derivatized blank preparation (one), System suitability preparation (one) and Derivatized standard preparation (six replicates) into the chromatographic system.

System Suitability:

a. The resolution between the p-naphtholbenzein (RRT 0.6) and Tobramycin peaks should be not less than 4.0.

b. USP tailing factor/Asymmetry of Tobramycin peak from standard as recorded by software should not be more than 2.0.

c. USP Plate count/Theoretical plates of Tobramycin peak from the standard should not be less than 2000 as recorded by software.

d. % RSD of six replicate injections of standard area for Tobramycin should not be more than 5.0.

3. If system suitability parameter passes then inject Derivatized sample preparation (one),

Derivatized Placebo preparation (one (If required)) into the chromatographic system and record Chromatogram.

4. Determine the peaks of the solvents and the derivatization agent on the basis of the chromatogram of the derivatization blank solution.

Calculation:

Calculate the % of each impurity of Tobramycin in the portion of Loteprednol Etabonate and Tobramycin Ophthalmic Suspension 0.5%/0.3%.

$$\text{Content of impurity in \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{100}{LC} \times \frac{1}{RRF} \times Wt/mL$$

AT : Area of peak response of Known and Unknown impurities in the test preparation

AS : Average Area of peak response of Tobramycin in the Standard preparation.

WS : Weight of Tobramycin standard taken in mg.

DS : Dilution for standard preparation

DT : Dilution for Test preparation

WT : Weight of Test sample taken in g

P : Potency of Tobramycin on as is basis

LC : Labeled claim of Tobramycin in mg/mL

RRF : Relative response factor for known impurities

Wt/mL : Weight per mL (in g/mL).

Table No.:1. Details of RRT'S and RRF values for known impurities. [Tobramycin]

S. No.	Impurity Name	RRT of the Impurity	RRF
01	Nebramine	0.84	1.13
02	Neamine	0.60	1.23
03	Kanamycin B	0.72	0.86

Related Substances II of Tobramycin

Based on the evaluation of the different trial, a separate method was developed for the estimation of some of the Impurities like RRT 0.36, Apramycin, Deoxystreptamine kanosaminide, RRT 0.73 and Unknown Impurities.

Method Background:

In this Method for Related Substances (Method-II) of Tobramycin in Loteprednol etabonate and Tobramycin Ophthalmic Suspension 0.5% and 0.3% Apramycin, Deoxystreptamine Kanosaminide and 0.73 RRT Impurities are monitored.

Since there was a blank interference for Apramycin in the related substances Method-I, Apramycin impurity was monitored in the Method-II. Apramycin is a Process related impurity, this impurity was identified by injecting the Impurity standard in the Method-II. No further study was performed for this impurity and RRF value to be taken as 1.0.

RRT 1.04 Impurity is process-related impurity, since this peak is not identified in the In-house method; this impurity is not being monitored in the In-house method for related substances of Tobramycin.

Methodology

Chemicals and Reagents:

Water	: HPLC grade
Acetonitrile	: HPLC grade
Conc. Sulphuric acid	: AR grade or equivalent
Phosphoric acid	: AR grade or equivalent
2, 4-dinitro fluorobenzene	: AR grade or equivalent
Ethanol	: AR grade or equivalent
Tris (hydroxymethyl) amino methane	: AR grade or equivalent

Dimethyl sulfoxide : AR grade or equivalent

1N Sulfuric acid Preparation:

Transfer 2.85 mL of Conc. Sulfuric acid into 100mL volumetric flask containing 20mL of water and mix well and makeup to volume with water.

Mobile Phase A: Mix 50mL of Acetonitrile, 950mL of HPLC water and 0.8mL of phosphoric acid.

Mobile Phase B: Mix 750mL of Acetonitrile, 250mL of HPLC water and 0.8mL of phosphoric acid.

Table No.:2. Chromatographic Conditions of Tobramycin

HPLC Column	**:**	Grace - Alltech Altima, Phenyl, 250 x 4.6 mm,5µm or Equivalent
UV Detection	**:**	365 nm
Flow Rate	**:**	1.2 mL/min
Injection Volume	**:**	45µL
Run Time	**:**	53 minute
Column Oven Temperature	**:**	25°C

Table No.:3. Gradient Programe of Tobramycin

Time (minutes)	0	14	25	35	40	50	50.01	53
Mobile Phase A	79	66	30	30	20	5	79	79
Mobile Phase B	21	34	70	70	80	95	21	21

Solution -A: (2, 4-dinitrofluorobenzene reagent)

Weigh and transfer 1.0g of 2, 4-dinitro fluorobenzene into a 100mL volumetric flask dissolve in ethanol and make up to the volume with ethanol and homogenize the solution.

Note: Store in a refrigerator, this solution may be used for 1 day.

Solution- B: Tris (hydroxymethyl) amino methane (TRIS) reagent preparation

Weigh and transfer 1.5g of Tris (hydroxymethyl) amino methane into a 100mL volumetric flask and make up to the volume using HPLC water and homogenize the solution.

Note: Store in a refrigerator, this solution may be used for 1 day.

Solution -C:

Pipette and transfer 10mL of Solution-B into a 50mL volumetric flask and make up to the volume using DMSO and homogenize the solution.

Note: Store in a refrigerator, this solution may be used within 3-4 hrs.

Blank Stock Solution: Pipette 1.0 mL of 1N sulfuric acid into 50mL volumetric flask and then fill the flask to the mark with distilled water and homogenize.

Blank Solution:

Pipette 10mL of the blank solution into a 50mL volumetric flask and then filled the flask up to the mark with distilled water and homogenize.

System Suitability Stock Solution from API:

Weigh and transfer accurately about 110 mg of Tobramycin WS/API into 100 mL volumetric flask add 40mL of water and adjust the pH of the solution to 6.0 with 1N Sulphuric acid solution and mix well then make up to the volume with water.

(OR)

System Suitability Stock Solution from USP:

Weigh and transfer accurately about 22 mg of Tobramycin USP into 20 mL volumetric flask add 4mL of water and adjust the pH of the solution to 6.0 with 1N Sulphuric acid solution and mix well then make up to the volume with water.

System Suitability Solution-1:

Dilute the 10 mL of the system suitability stock solution from API/WS to 50 mL volumetric flask and makeup to the mark with water.

(OR)

Dilute the 5 mL of the system suitability stock solution from USP to 25mL volumetric flask and makeup to the mark with water.

System Suitability Solution-2:

Dilute and transfer 10 mL of the system suitability stock solution to 50 mL volumetric flask and heat at 100°C for 8 hours. Allow the solution to attain room temperature and dilute using HPLC water up to the volume.

Standard Stock Solution:

Weigh and transfer accurately about 12 mg of Tobramycin WS/RS into a 50mL volumetric flask added 20mL of HPLC water and 1mL of 1N sulfuric acid solution and mixed well and make up to the volume using HPLC water.

Standard Solution: Pipette and transfer 1mL of the above solution to 200 mL volumetric flask dilute and make up to the volume using HPLC water.

Sample Solution:

Weigh accurately about 4grams of the sample solution to 50mL volumetric flask add 20mL of water and sonicate about 2 minutes and make up to the volume using HPLC water and mix well.

Placebo Solution:

Weigh accurately about 4grams of the placebo solution to 50mL volumetric flask add 20mL of water and sonicate about 2 minutes and make up to the volume using HPLC water and mix well.

Derivatized Blank, Placebo, Standard (0.36 ppm) and Sample solution (72 ppm): Heated all the solutions at the same temperature (60°C) and for same time (50±5 minutes).

To separate 50mL volumetric flasks transfer 15 mL of System suitability solution-1 from WS/API or USP, 15mL of System suitability solution-2, 15mL of Standard solution, 15mL of Sample solution, 15mL of Placebo solution and 15mL of Blank solution. To each flask add 10mL of solution A and 10mL of Solution C shake well, and insert the stopper, place the flasks in a constant temperature bath at 60±2°Cand heat for 50±5 minutes. Remove the flasks from the water bath and allow to stand at room temperature for 10 minutes, add about 10mL of Acetonitrile and make up to the mark with Acetonitrile.

Derivatized solutions kept at room temperature should be analyzed at least after 16 hrs.

Note: Standard preparation stable about 20 hrs at room temperature.

Sample preparation stable about 41 hrs at room temperature.

Note: If the sample solution is turbid after Derivatization, then add Acetonitrile in the solution in warm condition only, till the solution becomes clear.

Procedure:

1. Equilibrate the column using mobile phase to get a stable baseline for about 2 hrs.

2. Inject Derivatized Blank (one), Derivatized System suitability solution-1 (one), Derivatized System suitability solution-2 (one), and Derivatized standard preparation (six) into the chromatographic system and check the system suitability parameters.

System Suitability:

a. USP tailing factor/Asymmetry of Tobramycin peak from standard as recorded by software should not be more than 2.0.

b. USP Plate count/Theoretical plates of Tobramycin peak from standard should not be less than 2000 as recorded by the software.

c. % RSD of six replicate injections of standard area for Tobramycin should not be more than 5.0.

3. If system suitability parameter passes then inject Derivatized Placebo preparation (one, if required) and Derivatized sample preparation (one) into the chromatographic system and record chromatograms.

4. After every six injections of Derivatized sample preparation inject Derivatized blank preparation for reducing the carryover of main peak.

5. From the chromatogram of sample preparation, measure the peak responses disregarding any peak corresponding to those obtained in the Derivatized Blank solution and subtracting the quantities of any such peaks found at the relative retention time of 0.36 from those found in the derivatized test solution.

6. Impurities are identified based on the relative retention times. Deoxystreptamine, kanosaminide and Nebramine are identified by comparing the chromatograms of System suitability solution-2 and system suitability solution-1 since their peaks are larger in the system suitability solution-2 solution.

7. For unknown peak determination disregard any peaks found in the chromatogram of Derivatized System suitability solution-1.

CALCULATION:

Calculate the % of each impurity of Tobramycin in the portion of Loteprednol Etabonate and Tobramycin Ophthalmic Suspension 0.5%/0.3%

$$\text{Content of impurity in \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{100}{LC} \times \frac{1}{RRF} \times Wt/mL$$

AT : Area of peak response of Known/Unknown impurity in the test preparation

AS : Average Area of peak response of Tobramycin in the Standard preparation.

WS : Weight of Tobramycin standard taken in mg.

DS : Dilution for standard preparation

DT : Dilution for Test preparation

WT : Weight of Test sample taken in g

LC : Labeled claim of Tobramycin in mg/mL

P : Potency of Tobramycin on as-is basis

RRF : Relative response factor

Wt/mL : Weight per mL (in g)

Table No.:4. Details of RRT'S of Tobramycin Known Impurities:

S. No.	Impurity Name	Impurity RRT	RRF
01	Apramycin	0.64	0.55
02	Deoxystreptamine kanosaminide	0.66	1.0
03	Specified Impurity	0.73	1.0
04	Neamine	0.89	1.0
05	Nebramine	0.94	1.0
06	Kanamycin B	0.96	1.0

Neamine, Nebramine and Kanamycin B these impurities are monitored in Method-I. These are listed here only for information. For unknown impurities the RRF is calculated as 1.0

Total Impurities= Sum of Impurities obtained in the Related Substances Method-I + sum of Impurities obtained in the Related Substances Method-II

Method Development for Related Substances of Loteprednol Etabonate

To develop an Analytical method for the estimation of Related Substances of Loteprednol in Loteprednol Etabonate and Tobramycin Ophthalmic Suspension 0.5% and 0.3%.

Since Loteprednol Etabonate drug substance as well as drug product (Suspension) Methods not official in any pharmacopeia. So, for quantification of Loteprednol Etabonate and its impurities, an inhouse method was developed for the estimation of RS of Loteprednol Etabonate.

Methodology:

Mobile Phase Preparation:

Mobile phase A:0.1% Formic acid in Water.

Mobile phase B:0.1% Formic acid in Methanol.

Diluent: Mix Acetonitrile and HPLC water in the ratio of 90:10 respectively and mix well.

Table No.: 5. Chromatographic Conditions.

HPLC Column	**:**	Zorbax 300SB C8 (4.6x150) mm, 3.5um
UV Detection	**:**	245 nm
Flow Rate	**:**	1.0 mL/minute
Injection Volume	**:**	10µL
Run Time	**:**	70 Minutes
Sample Temperature	**:**	5°C
Elution Mode	**:**	Low Pressure Gradient
Column Oven Temperature	**:**	35°C

Table No.:6. Gradient Programme Details.

Time (min)	0	55	60	61	70
Mobile Phase A %	60	30	30	60	60
Mobile Phase B %	40	70	70	40	40

Blank Preparation: Diluent use as a blank.

Standard Stock Preparation:

Weigh accurately about 20.0mg of Loteprednol Etabonate Standard in a 50mL volumetric flask, add 35mL of diluent and sonicate to dissolve. Dilute up to the volume with diluent and mix well.

Standard solution preparation (1ppm):

Dilute further 5mL of above standard stock to 100mL with diluent, and mix well.

Dilute 5mL this solution to 100mL with diluent and mix well.

Note: Standard solution is stable up to 18 hours at 5°C.

1, 2 Dihydro Diethyl Carbonate impurity preparation (100ppm):

Accurately weigh and transfer about 1mg of 1, 2 Dihydro Diethyl Carbonate impurity into a 10 mL clean and dry volumetric flask. Add 5 mL of diluent and sonicate to dissolve. Dilute up to the volume with diluent and mix well.

1,2 Dihydro Loteprednol Etabonate impurity stock preparation: (100 ppm)

Accurately weigh and transfer about 1mg of 1,2 Dihydro loteprednol etabonate impurity into a 10 mL clean and dry volumetric flask. Add about 5 mL of diluent and sonicate up to dissolve, dilute up to the volume with diluent and mix well.

System suitability solution preparation:

Transfer 10 mL of Standard stock solution into a 20 mL clean and dry volumetric flask and add 1mL of 1,2 Dihydro diethyl carbonate impurity stock solution and 1,2 Dihydro loteprednol etabonate impurity stock solution, dilute up to the volume with diluent and mix well.

Note: System suitability solution can be used till the impurity peak will be appears.

Sample solution preparation (500ppm):

Weigh accurately about 2.0g of sample in 20 mL clean and dry volumetric flask. Add 10mL of diluent and shake vigorously for 2 minutes and dilute up to the volume with diluent and mix well. Centrifuge at 3500rpm for 10 minutes, filter through 0.45um nylon syringe filter.

Note: Sample solution is stable up to 30 hours at 5°C.

Placebo solution preparation:

Weigh accurately about 2.0g of placebo in 20mL clean and dry volumetric flask. Add 10mL of diluent and shake vigorously for 2 minutes and dilute up to the volume with diluent and mix well. Centrifuge at 3500 rpm for 10 minutes, filter through 0.45um nylon syringe filter.

Procedure:

1) Inject blank preparation (one), System suitability solution (one) standard preparation (six replicates) and sample preparation, placebo preparation into the chromatographic system.

2) From the chromatogram of sample preparation, measure the response of Loteprednol Etabonate known impurities peaks and unknown impurities peaks.

3) Disregard the peaks due to blank and placebo preparation.

System Suitability Criteria:

i. Resolution between Loteprednol Etabonate and 1, 2 Dihydro Diethyl Carbonate impurity should be not less than 1.5.

ii. Resolution between Loteprednol Etabonate and 1,2 Dihydro Loteprednol Etabonate impurity from system suitability solution as recorded by software should be not less than 1.2.

iii. USP Plate count/Theoretical plates of Loteprednol Etabonate peak from first injection of standard preparations recorded by software should be not less than 2000.

iv. USP tailing factor/Asymmetry of Loteprednol Etabonate peak from first injection of standard preparation as recorded by software should be not more than 2.0.

4) % RSD of six replicate injections of standard should be not more than 5.0 for Loteprednol Etabonate peak.

Calculation: For Known Impurities of Loteprednol Etabonate.

Calculate % Impurity of Loteprednol Etabonate by using the following formula.

$$\% \text{ Impurity} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{100}{LC} \times \frac{1}{RRF} \times \text{Wt/mL}$$

Where,

AT : Area of known impurity peak from sample solution.

AS : Average Area of Loteprednol Etabonate peak from the standard solution.

WS : Weight of Loteprednol Etabonate standard taken in mg.

DS : Dilution of standard preparation.

WT : Weight of sample taken in gm.

DT : Dilution of sample preparation.

P : Potency of standard in % on as is basis.

LC : Label claim of Loteprednol Etabonate in mg/mL.

RRF : Relative response factor.

Wt/mL : Weight per mL of sample (gm/mL)

For Unknown Impurities:

Calculate % Impurity of Loteprednol Etabonate by using the following formula.

$$\% \text{ Impurity} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{100}{LC} \times Wt/mL$$

Where,

AT : Area of Unknown impurity peak from sample solution.

AS : Average Area of Loteprednol Etabonate peak from the standard solution.

WS : Weight of Loteprednol Etabonate standard taken in mg.

DS : Dilution of standard preparation.

WT : Weight of sample taken in gm.

DT : Dilution of sample preparation.

P : Potency of standard in % on as-is basis.

LC : Label claim of Loteprednol Etabonate in mg/mL.

Wt/mL : Weight per mL of sample (gm/mL)

Total impurities = Sum of Known and Unknown impurities.

Table No.:7. Impurity Details of Loteprednol Etabonate

S. No	Name	~ RRT	RRF
1.	Prednisolone	0.27	1.20
2.	Prednisolone 17-Beta Hydroxy Acid	0.30	1.33
3.	Prednisolone 17-Acid 17-Ethyl Carbonate	0.80	1.10
4.	Loteprednol Etabonate 11-Keto	0.88	1.00
5.	Loteprednol Etabonate Methyl Ester	0.91	1.06
6.	1,2-Dihydro Loteprednol Etabonate	0.97	1.04
7.	Loteprednol etabonate	1.00	1.00
8.	1,2-Dihydro Diethyl Carbonate	1.03	0.57

7.3 Specificity: Standard Preparation: (1ppm)

Weighed accurately 20.41mg of Loteprednol Etabonate Standard in 50mL volumetric flask, mixed with 4mL of diluent, sonicated, mixed well, made up to that mark with diluent.

Further from above stock took 5mL dilution in 100mL volumetric flask, mixed with 4mL of diluent, sonicated, mixed well, and made up to that mark with diluent.

Further from above stock took 5mL dilution in 100mL volumetric flask, mixed with 4mL of diluent, sonicated, mixed well, and made up to that mark with diluent.

Tobramycin Impurity Preparation: Apramycin Impurity:

Took approximately small pinch of Apramycin Impurity in HPLC vial diluted with methanol and water, mixed well, and sonicated.

Spiked impurity of Neamine, Nebramine and Kanamycin:

Impurities Stock preparation:

Weighed and transferred 2.807mg of Nebramine, 1.814 mg of Kanamycin B, 2.213 mg of Neamine and 3.268mg of Apramycin individually in 25mL volumetric flasks and added about 0.5mL of 1N sulfuric acid solution each diluted and made up to the volume using HPLC water and mixed well.

Sample Preparation:

Weighed accurately 2.05g of Loteprednol and Tobramycin Ophthalmic Suspension sample in 20mL volumetric flask, mixed well with diluent, sonicated, made up to that mark with diluent, filtered through 0.45um nylon syringe filter.

Placebo Preparation of Loteprednol and Tobramycin Ophthalmic Suspension: Weighed accurately 2.10g of Loteprednol and Tobramycin Ophthalmic Suspension sample in 20mL volumetric flask, mixed well with diluent, sonicated, made up to that mark with diluent, filtered through 0.45um nylon syringe filter.

Procedure:

Injected Blank (Diluent), Standard of Loteprednol Etabonate 1ppm, Apramycin Impurity, Spiked Impurity of Neamine, Nebramine, Kanamycin, Sample of Loteprednol Etabonate and Tobramycin Suspension, Placebo of Loteprednol Etabonate and Tobramycin Suspension.

Table No.:8. Observation of RT & RRT of Loteprednol Etabonate and All Impurities

NAME	RT (Min)	RRT (Min)
Loteprednol Etabonate (1 ppm)	35.847	1.00
Prednisolone Impurity (100ppm)	9.833	0.27
Prednisolone 17B Hydroxy Acid Impurity (100ppm)	10.869	0.30
Prednisolone 17Acid 17 Ethyl Carbonate Impurity (100ppm)	28.593	0.80
Loteprednol Etabonate 11- Keto (100 ppm)	31.363	0.88
Loteprednol Etabonate Methyl Ester (100 ppm)	32.640	0.91
1,2 Dihydro Loteprednol Etabonate (100 ppm)	34.620	0.97
1,2 Dihydro Diethyl Carbonate (100ppm)	36.695	1.03
1,2 Dihydro Diethyl Carbonate (5ppm)	36.695	1.03
Apramycin Impurity	3.34	0.09
Spiked impurity of Nebramine	2.4	0.07
Spiked impurity of Neamine	3.3	0.092
Spiked impurity of kanamycin	6.3	0.18

Various Chromatograms references of above method are given below.

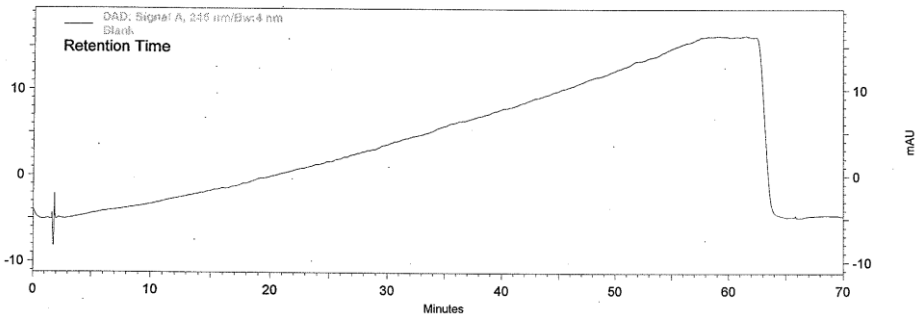

Figure No.1: Chromatogram of Blank [Loteprednol Etabonate]

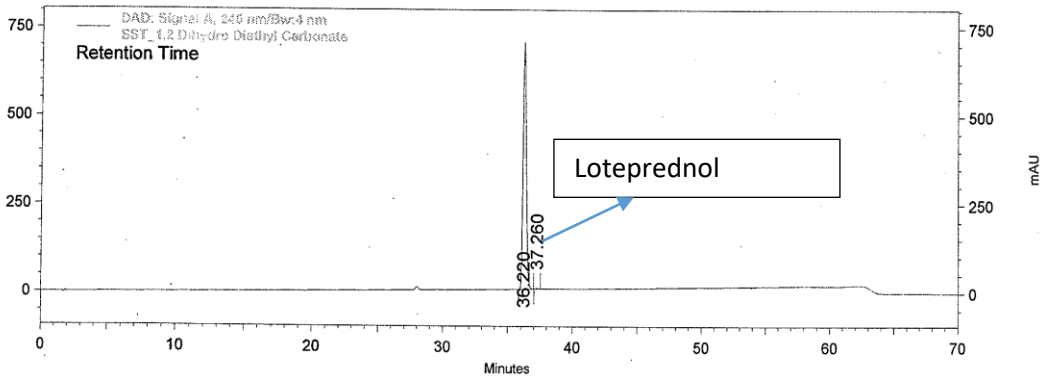

Figure No.:2. Chromatogram of System Suitability [Loteprednol Etabonate]

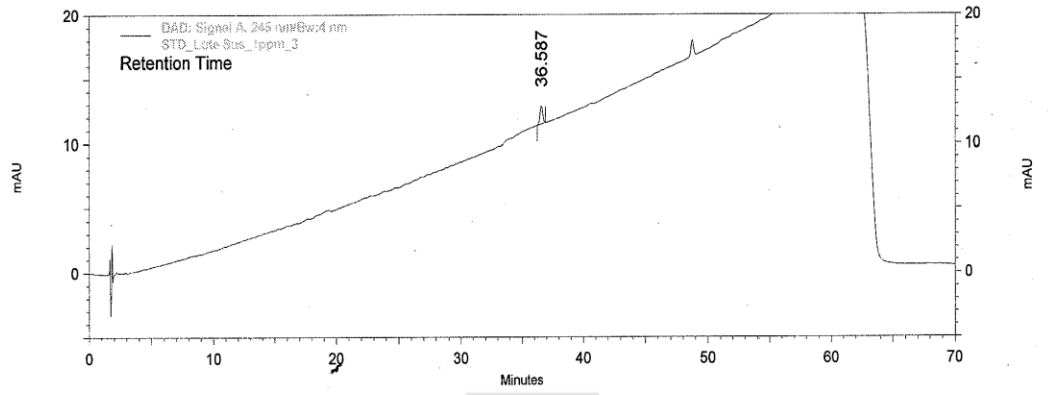

Figure No.:3. Chromatogram of Standard of Loteprednol Etabonate

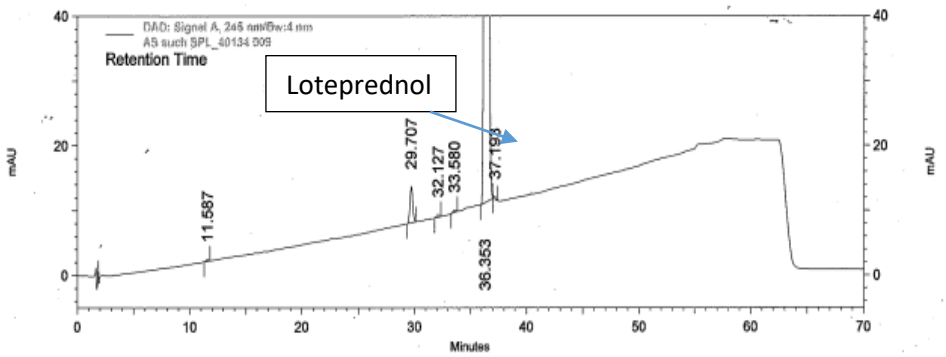

Figure No.:4. Chromatogram of Sample [Loteprednol Etabonate]

CONCLUSION:

No interference was observed of Tobramycin Impurities with respect to Loteprednol Etabonate impurities and main peak. Hence Method is specific.

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