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Analytical Method Validation of Benzalkonium Chloride Assay in Loteprednol Etabonate and Tobramycin Ophthalmic

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Keywords: *Benzalkonium Chloride, Loteprednol Etobanate & Tobramycin Ophthalmic*

ABSTRACT

As a part of analytical method validation, Assay and Related substances test parameters of the ophthalmic suspension covering both the actives were validated. The analytical method validation was carried out satisfactorily covering the parameters like Precision, Accuracy, Robustness and Linearity. Apart from assay and related substances test parameters, method development and validation of particle size distribution test parameter was also carried. Standard solution and sample solutions were stable up to 63 hours and 62 hours at 25°C respectively. The test method is checked for Specificity, Solution Stability, Accuracy& Method Precision found meeting the predetermined acceptance criteria. The test method is Specific, Accurate, Precise and Robust for the content of Benzalkonium Chloride in Loteprednol Etabonate and Tobramycin Ophthalmic Suspension 0.5% and 0.3%. Hence, this method can be introduced into the routine use for measuring the for content of Benzalkonium Chloride in Loteprednol Etabonate and Tobramycin Ophthalmic Suspension 0.5% and 0.3%.

INTRODUCTION:

Analytical Method Development & Validation[1-5]:

An in-house analytical method development for Assay and Related substances was developed and it is learned that methods were found stable indicating nature.

As a part of analytical method validation, Assay and Related substances test parameters of the ophthalmic suspension covering both the actives were validated. As a part of method validation activity, forced degradation study was also carried. The analytical method validation was carried out satisfactorily covering the parameters like Precision, Accuracy, Robustness and Linearity. Apart from assay and related substances test parameters, method development and validation of particle size distribution test parameter was also carried. The validated method was applied while analyzing the stability exposed and photostability exposed samples.

Analytical method validation for the content of Benzalkonium Hydrochloride in Loteprednol Etabonate & Tobramycin Suspension 0.5% & 0.3% has been carried using the below mentioned following parameters.

- System suitability[6-12]
- Precision
- System precision
- Method precision
- Intermediate precision
- Stability in analytical solution
- Mobile phase stability
- Linearity
- Accuracy
- Range
- Robustness

- Specificity
- Forced Degradation

System Suitability and System precision:

Objective: To perform System suitability for assay of Benzalkonium chloride in Loteprednol Etabonate and Tobramycin Ophthalmic Suspension 0.5% & 0.3%.

Preparation of Buffer (pH-6.0): Accurately weighed and transferred about 5.45 g of Potassium dihydrogen phosphate transfer in a 2000 mL beaker containing water and Sonicated to dissolve, adjusted the pH to 6.0 with sodium hydroxide solution and sonicated to degas.

Mobile phase: Mixed 1850mL of Buffer (pH- 6.0) and 3150mL of Acetonitrile. Sonicated to degas.

Diluent: Mixed water and Acetonitrile in the ratio of 60:40 (v/v) respectively.

Table No.:1. Details of Chromatographic Conditions:

HPLC Column: Phenomenex, Luna, CN 250mm×4.6mm, 100A°,5µ,(Part Number-00G-4255-E0)	
Wave length: 210 nm	**Flow Rate :** 1.2 mL/min
Column Oven Temperature: 30°C	**Injection Volume :** 60 µL
Sample Compartment Temperature: 25°C	**Run Time :** 45 mins
Elution mode: Isocratic	

Standard Stock Preparation (For 96.1%Benzalkonium chloride):

Accurately weighed and transferred 53.50 mg of Benzalkonium chloride standard (96.1%) into a clean and dry 50 mL volumetric flask. Added 30 mL of diluent and sonicated to dissolve, made up to the volume with diluent and mixed well.

Standard Preparation: (20 ppm)

Further transferred 1 mL of the above solution into a 50 mL volumetric flask, made up to the volume with diluent, and mixed well.

Observations:

Table No.:2. Details of System Precision.

S. No	Injection	Area		
		C-12 Homologue	**C-14 Homologue**	**Average of C-12&C-14 Homologue**
1	Standard-01	16556536	5082497	21639033
2	Standard-02	16572757	5097486	21670243
3	Standard-03	16557297	5072819	21630116
4	Standard-04	16631484	5110685	21742169
5	Standard-05	16599274	5117660	21716934
6	Standard-06	16679875	5068360	21748235
Average				21691122
SD				51778.14
RSD				0.24

Acceptance criteria: % RSD for average area for C12 and C14 Homolog peaks from Standard Preparation of six injections should not be more than 2.0.

Conclusion: % RSD was found in the acceptance criteria.

Table No.:3. Details of System Suitability.

System Suitability Parameter	**Results**	
Tailing factor for C12 and C14 Homolog peaks from Standard preparation	**C12 Homologue**	**C14 Homologue**
	1.22	1.10
Theoretical plates for C12 and C14 Homolog peaks from Standard preparation	12014	14133
Resolution between C-12 and C-14 Homologue from Standard preparation	4.99	

Acceptance criteria:

- Tailing factor for C12 and C14 Homolog peaks from Standard Preparation Should be not more than 2.0.

- Theoretical plates for C12 and C14 Homolog peaks from Standard Preparation Should be not more than 2000.

- The resolution between C12 and C14 Homologue from the Standard solution should be not less than 2.0

Conclusion: All the system suitability parameters were found in the acceptance criteria.

Specificity:

Objective: To perform specificity for assay of Benzalkonium chloride in Loteprednol etabonate and Tobramycin ophthalmic suspension 0.5%&0.3%.

Weighed 2.01400 g of Placebo for BKC of Loteprednol Etabonate and Tobramycin ophthalmic suspension 0.5% and 0.3% sample into 10 mL volumetric flask , volume made up to the mark with diluent mixed well and vortex for 5min. Centrifuged the placebo at 6000 RPM for 10 minutes and injected a supernatant layer.

Preparation of Sample:

Weighed 2.01625 g of Loteprednol Etabonate and Tobramycin ophthalmic suspension 0.5% and 0.3% sample into 10 mL volumetric flask, volume made up to the mark with diluent mixed well and vortex for 5min. Centrifuged the sample solution at 6000 RPM for 10 minutes and injected supernatant layer.

Preparation of impurity stock solutions: (100 ppm)

Prednisolone:

Weighed 1.059 mg of Prednisolone impurity into 10mL volumetric flask, dissolved in methanol and made up to volume with methanol.

Prednisolone-17-β-Hydroxy acid:

Weighed 0.999 mg of Prednosolone-17-β-Hydroxy acid impurity into 10mL volumetric flask, dissolved in methanol and made up to volume with methanol.

Prednisolone-17-acid, 17-ethyl carbonate:

Weighed 1.087 mg of Prednisolone-17-acid, 17-ethyl carbonate impurity into 10mL volumetric flask, dissolved in methanol and made up to volume with methanol.

11-keto Loteprednol Etabonate:

Weighed 1.009 mg of 11-keto Loteprednol Etabonate impurity into 10mL volumetric flask, dissolved in methanol and made up to volume with methanol.

Loteprednol Etabonate methyl ester:

Weighed 1.006 mg of Loteprednol Etabonate methyl ester impurity into 10mL volumetric flask, dissolved in methanol and made up to volume with methanol.

1, 2 Dihydro Loteprednol Etabonate:

Weighed 1.046 mg of 1, 2 Dihydro Loteprednol Etabonate impurity into 10mL volumetric flask, dissolved in methanol and made up to volume with methanol.

1, 2 Dihydro diethyl carbonate:

Weighed 1.072 mg of 1, 2 Dihydro diethyl carbonate impurity into 10mL volumetric flask, dissolved in methanol and made up to volume with methanol.

Note: Diluted 1 mL of Each individual impurity (100 ppm) solution to10 mL (10 ppm) separately with diluent and mixed well.

Preparation of Spiked Sample:

Weighed 2.04868g of Loteprednol Etabonate and Tobramycin ophthalmic suspension 0.5% and 0.3% sample into 10 mL volumetric flask, added 0.5mL of each impurity solution(100ppm) volume made up to the mark with diluent mixed well and vortex for 5min. Centrifuged the sample solution at 6000 RPM for 10 minutes and injected the supernatant layer.

Procedure:

Injected Blank, placebo for BKC, individual impurities (10ppm), standard BKC, Sample and Spiked sample into the above chromatographic conditions and observed chromatograms.

Observation:

No blank, Placebo and known impurity interference at the retention time of C12 and C14 homologue peaks of BKC.

Note: a. Tobramycin & its degradants are UV inactive, so no need to inject for specificity

Table No.:4. Details of Impurity

Standard/Impurity details	**Retention time**	
	C-12 Homologue	**C-14 Homologue**
Standard	23.403	27.873
Sample	23.157	27.700
Prednisolone impurity	2.81	
Prednisolone 17 Beta Hydroxy acid impurity	2.57	
Prednisolone 17-Acid 17-Ethyl Carbonate impurity	2.58	
Loteprednol Etabonate Methyl Ester	3.56	
Loteprednol Etabonate 11-Keto impurity	4.03	
1,2-Dihydro Loteprednol Etabonate impurity	3.92	
1,2-Dihydro Diethyl carbonate impurity	3.96	

Conclusion: Method is found to be specific.

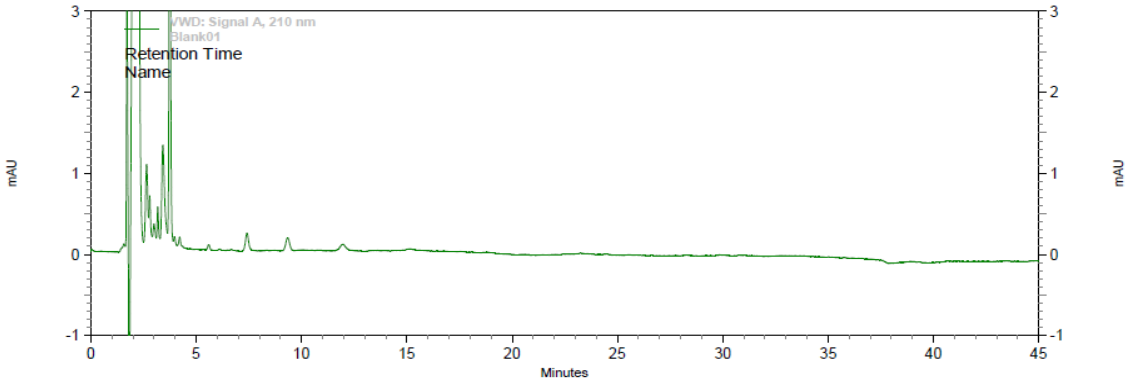

Figure No.:1. Chromatogram of Blank.

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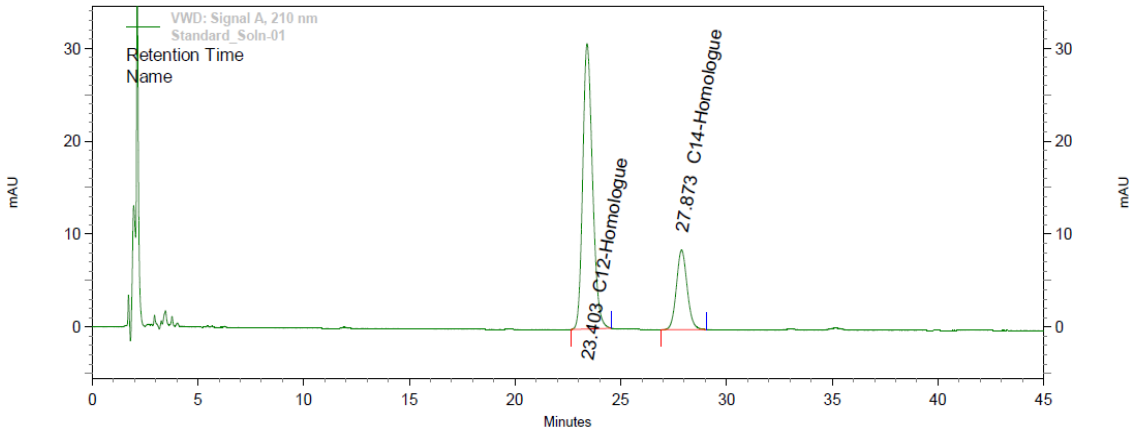

Figure No.:2. Chromatogram of Placebo.

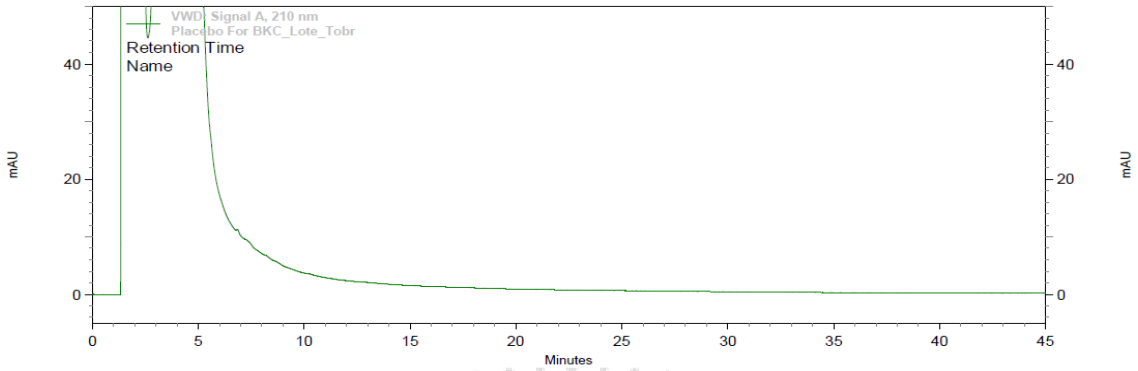

Figure No.:3. Chromatogram of Standard

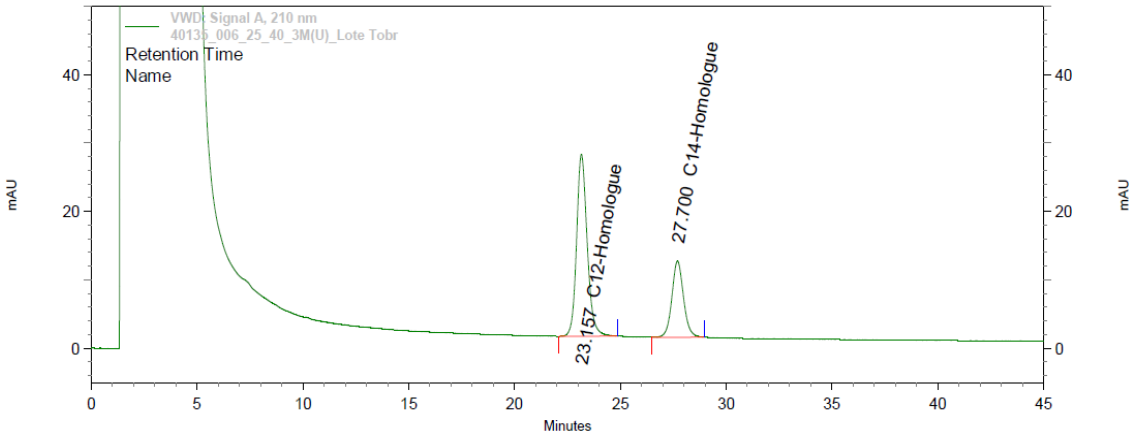

Figure No.:4. Chromatogram of Sample

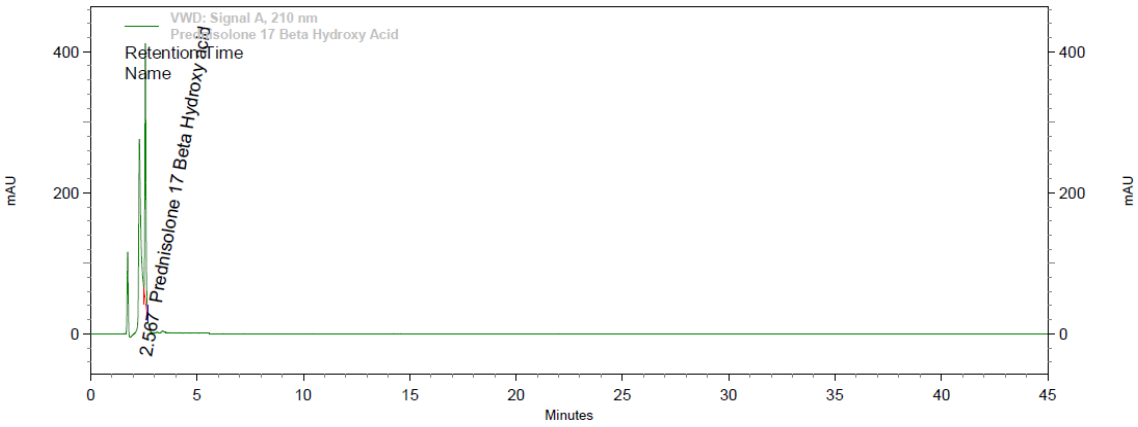

Figure No.:5. Chromatogram of Prednisolone Impurity.

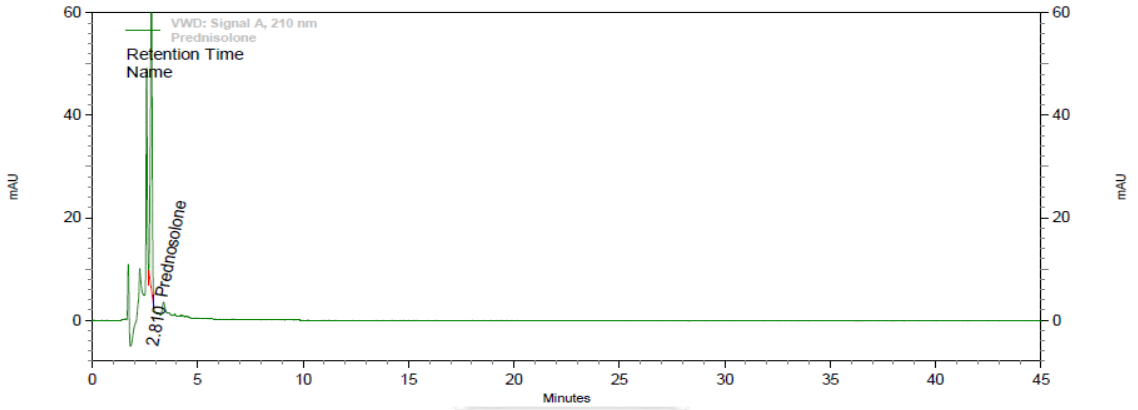

Figure No.:6. Chromatogram of Prednisolone 17-Beta Hydroxy acid impurity.

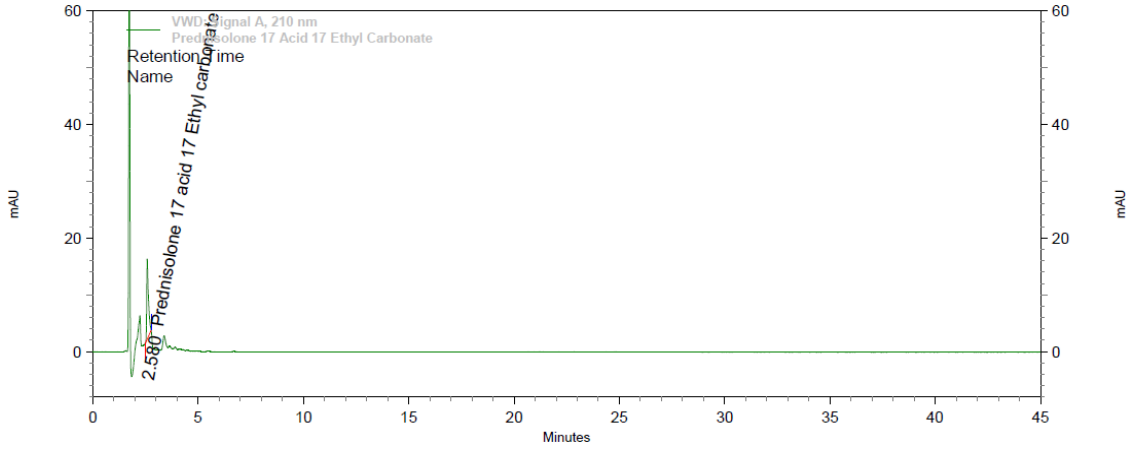

Figure No.:7. Chromatogram of Prednisolone 17-Acid 17-Ethyl Carbonate impurity.

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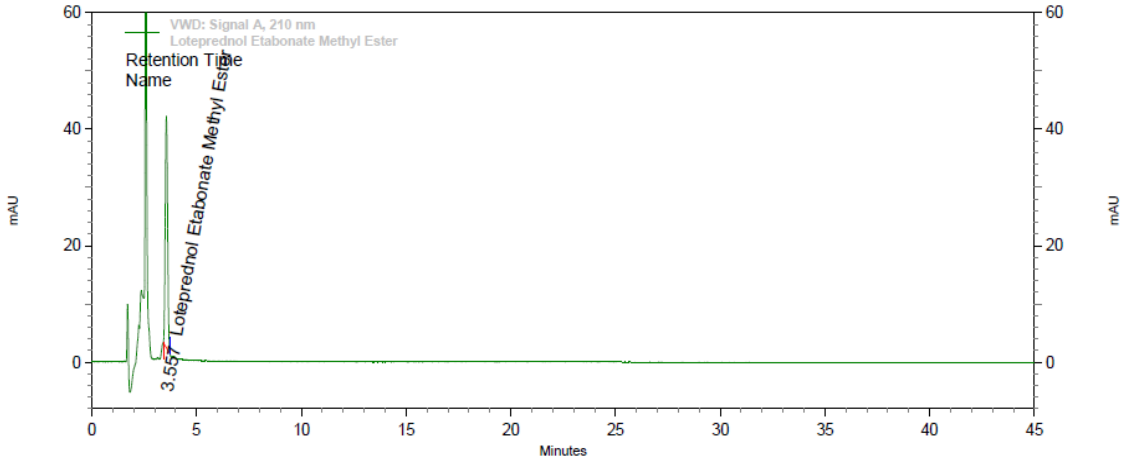


Figure No.:8. Chromatogram of Loteprednol Etabonate Methyl Ester impurity

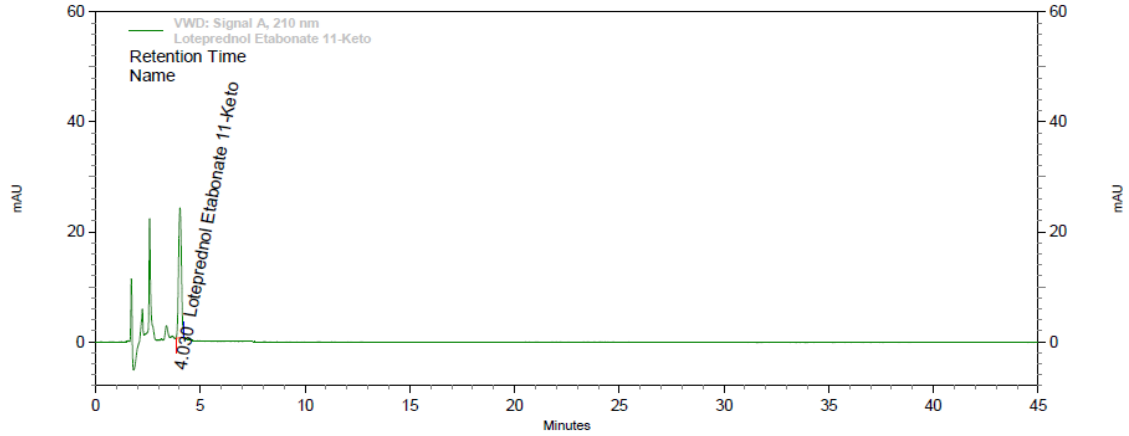


Figure No.:9. Chromatogram of Loteprednol Etabonate 11-Keto impurity.

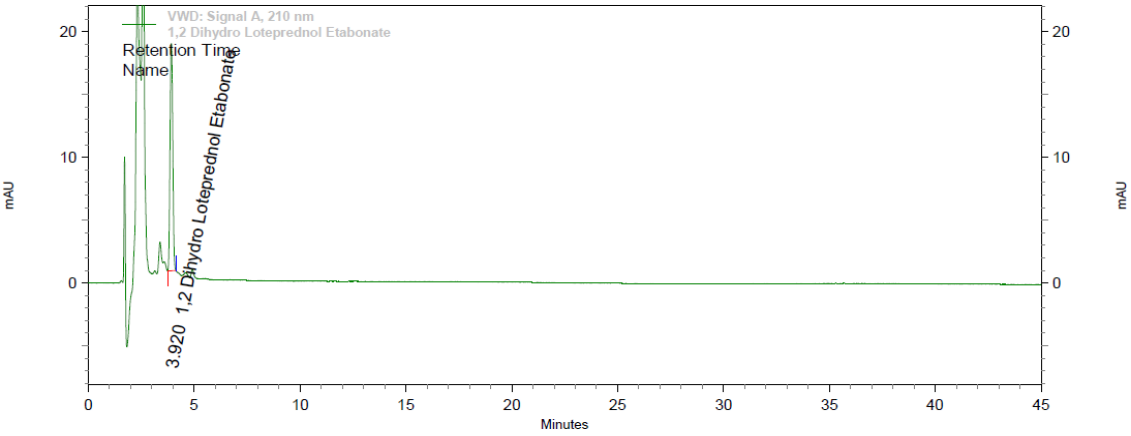


Figure No.:10. Chromatogram of Loteprednol 1,2-Dihydro Loteprednol Etabonate impurity

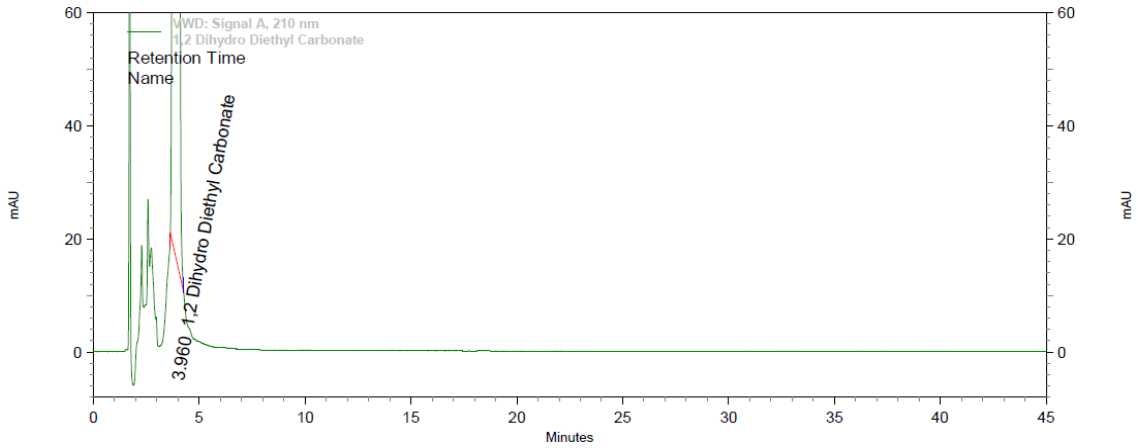


Figure No.:11. Chromatogram of 1,2-Dihydro Diethyl carbonate impurity.

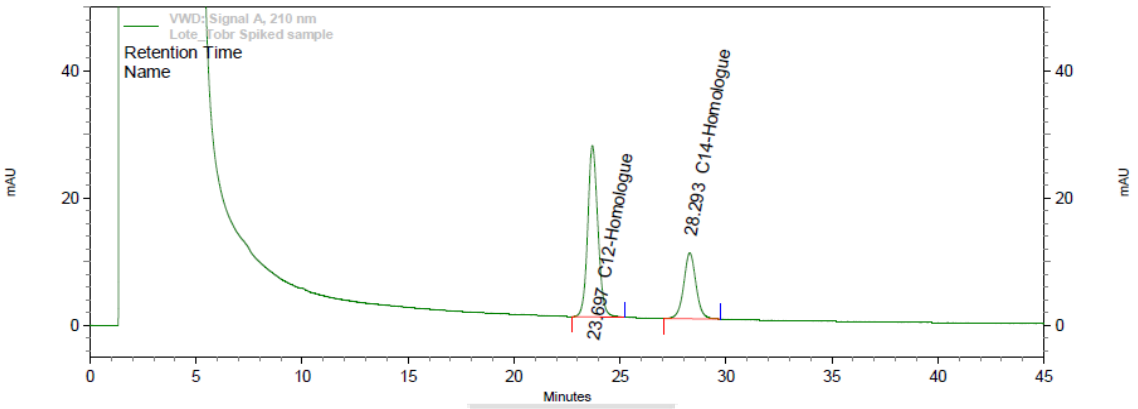


Figure No.:12. Chromatogram of Spiked Sample.

Method Precision:

Objective: To perform method precision for assay of Benzalkonium chloride in Loteprednol Etabonate and Tobramycin ophthalmic suspension 0.5%& 0.3%.

Preparation of Method Precision Sample-1:

Weighed 2.06537g of Loteprednol Etabonate and Tobramycin ophthalmic suspension 0.5% and 0.3% sample into 10 mL volumetric flask, volume made up to the mark with diluent mixed well and vortex for 5min. Centrifuged the sample solution at 6000 RPM for 10 minutes and injected the supernatant layer.

The remaining 5 preparations were prepared as per above procedure.

Table No.:5. Observations of Method Precision.

S. No	Injection	Weight of Sample in g	% Assay
1	Preparation-01	2.06537	101.3
2	Preparation-02	2.06548	101.3
3	Preparation-03	2.04782	102.5
4	Preparation-04	2.04902	101.8
5	Preparation-05	2.04905	102.5
6	Preparation-06	2.04905	102.7
Average			102.0
SD			0.63
% RSD			0.6

Acceptance criteria: %RSD of Assay of BKC from six preparations should not be more than 2.0%.

Conclusion: This method was precise.

Accuracy:

Objective: To perform accuracy for assay of Benzalkonium chloride in Loteprednol etabonate and Tobramycin ophthalmic suspension 0.5%&0.3%.

Preparation of Accuracy stock solution:

Accurately weighed and transferred 51.85mg of Benzalkonium chloride standard (96.1%) in to a clean and dry 50 mL volumetric flask. Added 30 mL of diluent and sonicated to dissolve, made up to the volume with diluent and mixed well. Further diluted 1mL of this solution to 10mL with diluent and mixed well.

Preparation of Accuracy solution (50%):

Weighed 2.04832 g of placebo for BKC into 10 mL volumetric flask, added 1mL of standard stock solution, then volume made up to the mark with diluent mixed well and vortex for 5min. Centrifuged the sample solution at 6000 RPM for 10 minutes and injected supernatant layer.

Preparation of Accuracy solution (100%):

Weighed 2.04910 g of placebo for BKC into 10 mL volumetric flask, added 2.0 mL of standard stock solution, then volume made up to the mark with diluent mixed well and vortex for 5min. Centrifuged the sample solution at 6000 RPM for 10 minutes and injected supernatant layer.

Preparation of Accuracy solution (150%):

Weighed 2.06572 g of placebo for BKC into 10 mL volumetric flask, added 3mL of standard stock solution, then volume made up to the mark with diluent mixed well and vortex for 5min. Centrifuged the sample solution at 6000 RPM for 10 minutes and injected supernatant layer.

Table No. 6. Details of Accuracy Parameter Observation.

Accuracy Level	**µg added(ppm)**	**µg found (ppm)**	**% Accuracy**
50%	9.9656	10.0841	101.2
100%	19.9311	19.9195	99.9
150%	29.8967	27.7046	99.4

Conclusion: The Accuracy values were found to be in the range of 97.0%-103.0%.

Linearity:

Objective: To perform Linearity for assay of Benzalkonium chloride in Loteprednol etabonate and Tobramycin ophthalmic suspension 0.5%&0.3%.

Preparation of Linearity stock solution:

Diluted 2mL of standard stock solution to 20mL with diluent and mixed well.

Table No.:7. Details of Preparation of Linearity Solutions

S. No.	Name of solution	Linearity stock taken in mL	Diluted to mL	Conc.(ppm)
1	Linearity_25%	0.5	10	5.14
2	Linearity_50%	1.0	10	10.28
3	Linearity_100%	2.0	10	20.57
4	Linearity_125%	2.5	10	25.71
5	Linearity_150%	3.0	10	30.85
6	Linearity_200%	4.0	10	41.13

Table No.:8. Details of Linearity Observation**.**

Concentration (%)	Concentration (ppm)	Area
25	5.14	5267054
50	10.28	9977435
100	20.57	22236582
125	25.71	26066690
150	30.85	31026424
200	41.13	42015787
Slope		1020466
Intercept		29004.5461
Correlation		0.999
% Bias		0.13

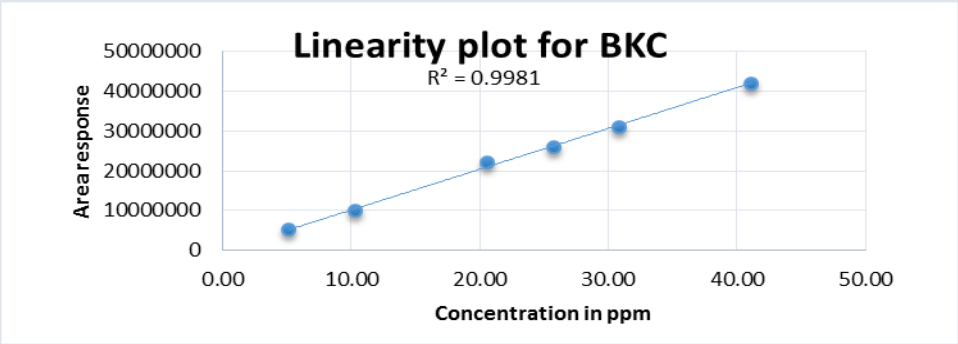


Figure No.:13. Linearity Graph**.**

Acceptance Criteria: The correlation coefficient should not less than 0.999.

Conclusion: Method is linear.

Robustness study: Objective: To perform a robustness study for assay of Benzalkonium chloride in Loteprednol etabonate and Tobramycin ophthalmic suspension 0.5%&0.3%.

Table No.:9. Details of Robustness Study Parameter**.**

Name of the Parameter	**Retention time(min)**		**Tailing factor**		**Plate count**		**Resolution**
	C12	**C14**	**C12**	**C14**	**C12**	**C14**	
As Such	23.40	27.87	1.22	1.10	12014	14133	4.99
Low Wavelength (207)	24.29	28.90	1.20	1.08	11792	13929	4.92
High Wavelength (213)	24.37	29.00	1.21	1.08	11737	13989	4.92
Low Flow (1.1mL)	26.25	31.31	1.21	1.04	10837	13813	4.88
High Flow (1.3mL)	22.16	26.37	1.22	1.06	10779	13333	4.76
Low Temperature	25.28	30.10	1.18	1.05	10994	13631	4.82
High Temperature	23.25	27.47	1.24	1.05	11574	12338	4.55
Low pH (5.8)	21.28	24.81	1.34	1.08	14650	15770	4.73
High pH (6.2)	26.72	31.40	1.29	1.14	14879	16216	5.02
Low Organic (39:61)	26.65	31.94	1.40	1.20	12701	15722	5.38
High Organic (35:65)	21.71	24.853	1.40	1.20	14595	15886	4.17
Acceptance Criteria			**NMT 2.0**		**NLT 2000**		**NLT 2.0**

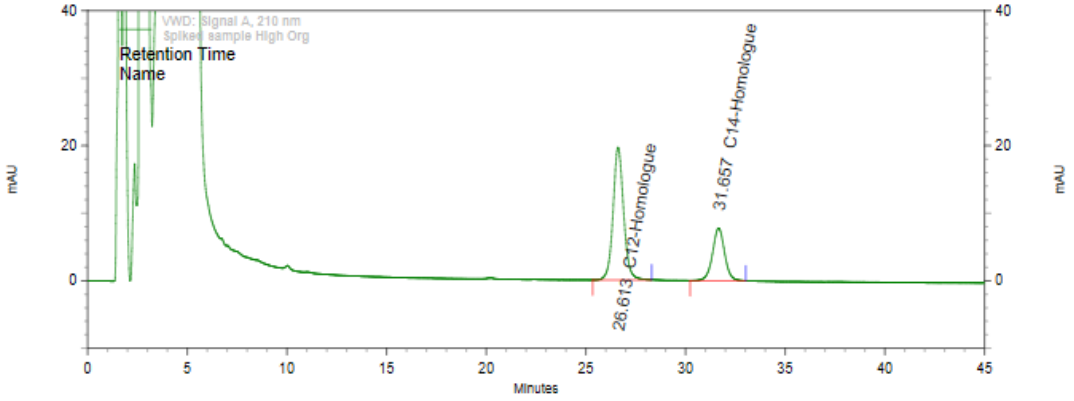
Preparation of Buffer Solution: Weighed and dissolved 5.45g of potassium dihydrogen phosphate in 2000mL of water and mixed well.

Note: From the above buffer solution pH-5.8, pH-6.0 and pH-6.2 solutions were prepared for robustness parameters.

Procedure:

Injected Blank, Standard, Sample, Placebo and Spiked sample in to chromatography.

Observation:



Conclusion: The method was found robust.

Solution Stability: Objective: To perform solution stability for assay of Benzalkonium chloride in Loteprednol Etabonate and Tobramycin ophthalmic solution 0.5&0.3%

Table No.:10. Details of Observations of Solution Stability for Standard Solution**:**

Name	**Initial at 25°C**	**22 Hours at 25°C**	**63 Hours at 25°C**
BKC Area in standard	21639033	21934523	21788235
% Difference With Initial	NA	-1.37	-0.69

Table No.: 11. Solution stability for Sample solution details.

Name	**Initial at 25°C**	**18 Hours at 25°C**	**62 Hours at 25°C**
BKC Area in sample	22718360	22527048	22349625
%Difference With Initial	NA	0.84	1.62

CONCLUSION:

Standard solution and sample solutions was stable up to 63 hours and 62 hours at 25°C respectively. The test method is checked for Specificity, Solution Stability, Accuracy& Method Precision found meeting the predetermined acceptance criteria. The test method is Specific, Accurate, Precise and Robust for the content of Benzalkonium Chloride in

Loteprednol Etabonate and Tobramycin Ophthalmic Suspension 0.5% and 0.3%. Hence, this method can be introduced into the routine use for measuring the for the content of Benzalkonium Chloride in Loteprednol Etabonate and Tobramycin Ophthalmic Suspension 0.5% and 0.3%.

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