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A stability indicating RP-HPLC method for related impurities of dexamethasone in tobramycin and dexamethasone otic suspension

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***Abstract*---**A Stability Indicating RP-HPLC Method for Related Impurities of Dexamethasone in Trobramycin and Dexamethasone Otic Suspension USP 0.3% and 0.1%. This RP-HPLC method is also validated for various parameters as per ICH guidelines. The system suitability parameters proved that the method is suitable for the quantification of Dexamethasone. System suitability parameters were within the limits as indicated by good resolution. The precision was within the acceptance criteria of %RSD i.e., not more than 2%. Linearity was observed in concentration range of LOQ to 200% and the Correlation coefficient was found to be within the limit. Accuracy was performed with the concentration ranges 50%, 100%, and 200% and was found to be within the limit i.e., 85 to 115%. Stability was evaluated by subjecting the ophthalmic suspension to thermal, acidic, basic, oxidative, and UV stress condition. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and can be used as a quality – control tool for routine analysis of Dexamethasone in Trobramycin and Dexamethasone Otic Suspension USP 0.3% and 0.1%.

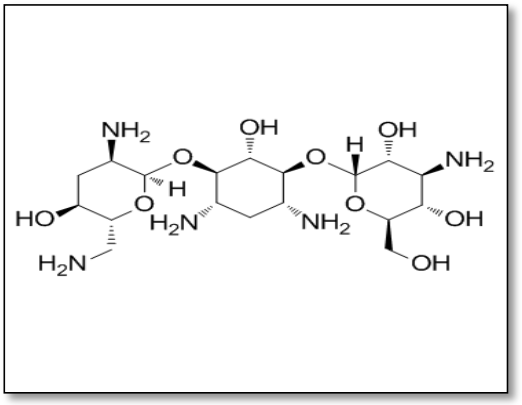
***Keywords*---**Otic, Stability, quantification, thermal, acidic, stress condition, Suspension, USP

1. Introduction

Tobramycin (Russ H et al.,) (Fig-1) is an amino-glycoside, 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

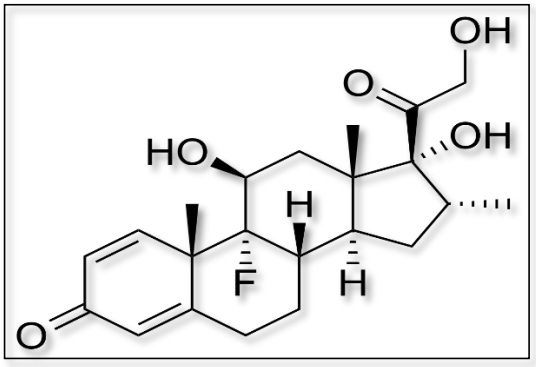
complex, produced by the same species. It is chemically (2S,3R,4S,5S,6R)-4-amino-2-{[(1S,2S,3R,4S,6R)-4,6-diamino3{[(2R,3R,5S,6R)-3-amino-6-(aminomethyl)-5-hydroxyoxan-2-yl]oxy}-2-hydroxycyclohexyl] oxy}-6-(hydroxymethyl) oxane-3,5-diol.

Fig No 1 Structure of Tobramycin



Dexamethasone (Fig. 2) is a kind of corticosteroids and prevents the release of substances in the body that cause inflammation. It is used in the treatment of many conditions including a number of skin diseases, severe allergies and asthma. Dexamethasone chemically is 9-fluoro-11β, 17, 21-trihydroxy-16α-methylpregna-1, 4-diene, 3, 20-dione. It is a synthetic adrenocortical steroid used to treat many different conditions such as allergic disorders, skin conditions, ulcerative colitis, arthritis, lupus, psoriasis, or breathing disorders The effects of dexamethasone are frequently seen within a day and last for about three days (Sreelaksmi et al).

Fig No 1 Structure of Dexamethasone



From literature survey, it has been observed that few methods found to quantitative analysis for estimation of dexamethasone. Official method for assay of dexamethasone available but with challenging chromatographic conditions and some authors also reported study on dexamethasone (S. Shaikh et al.,). As per ICH guideline a specific and stability-indicating procedure should be included to determine the content of the new drug substance (Thamaraikani et al.,) The main objective of this study is to develop a simple, suitable, cost effective and

environment friendly HPLC method required for analysis of dexamethasone from dexamethasone finished product.
Dexamethasone working standard were a kind gift of Remidium Laboratories, Hyderabad, India. Test samples purchased from market store. HPLC grade Acetonitrile and HPLC Water were purchased from Ultra Fine Chemicals Ltd., India. Analytical grade orthophosphoric acid, HCl, NaOH pallets and H2O2 purchased from Merck, India. High performance liquid chromatographic system (Agilent (1100) Gradient System) equipped with UV-visible detector was used for the analysis.

2. Method Development

Various trials performed with respect to mobile phase and stationary phase to optimize the suitable chromatographic conditions. Method was done using Agilent 1260 UV/PDA Detector. A non-polar analytical chromatographic column Waters, Symmetry, C18, 3.9 mm x 150 mm, 5µ (or) any equivalent Column was chosen as the stationary phase. With Flow Rate: 1.5 mL/min Run time: For standard: 15mins; For Sample: 60 mins, Column Temperature: 35°C, Detection WL: 254nm and Injection Volume: 50 µL

2.1 Preparation of Standard Solutions:

Dexamethasone Standard Stock Preparation: Accurately weigh about 25mg of Dexamethasone Working Standard transfer to a 25mL volumetric flask. Add 15mL of Acetonitrile and sonicate for 3 mins; make up to volume Acetonitrile. (Concentration:1 mg/ml).
Preparation of Standard Solution A: Transfer 1mL of the Standard stock solution (1mg/mL) into 25mL volumetric flask, make up the volume with diluent (Concentration: 0.04mg/mL).
Preparation of Standard Solution B: Transfer 1mL of the Standard solution A (0.04mg/mL) into 10mL volumetric flask, make up the volume with diluent (Concentration: 0.004mg/mL).

2.1.1 Dexamethasone Acetate Imp Stock Solution:

2.0 mg of Dexamethasone Acetate Imp in 1ml of Acetonitrile. (Concentration: 2mg/mL)
2.1.2 System Suitability Solution preparation: Transfer 2mL of the Standard stock solution(1mg/mL), 1mL of the Dexamethasone Acetate impurity Stock(2mg/mL) into 10mL volumetric flask, make up the volume with diluent (Concentrations: Dexamethasone 0.2mg/mL and Dexamethasone Acetate impurity 0.2mg/mL).
2.1.3 Preparation of Sample Solution: Accurately weigh about 2.0gm of sample in 10mL volumetric flask, add about 4mL of diluent, and sonicate for 5mins, make up volume with diluent and mix well. (Concentrations: 0.2mg/mL).
2.1.3 Preparation of Placebo: Accurately weigh about 2.0gm of Placebo in 10mL volumetric flask, add about 4mL of diluent, and sonicate for 5mins, make up volume with diluent and mix well.

2.2 Procedure:

Set the chromatographic conditions as described above and equilibrate the column with mobile phase till a stable baseline is obtained. Inject diluent (as

blank) solution in duplicate into the chromatograph and record the chromatogram. Inject standard solution for six times into the chromatograph record the chromatograms and measure the peak areas**.**

Fig-3 Chromatogram for Standard:

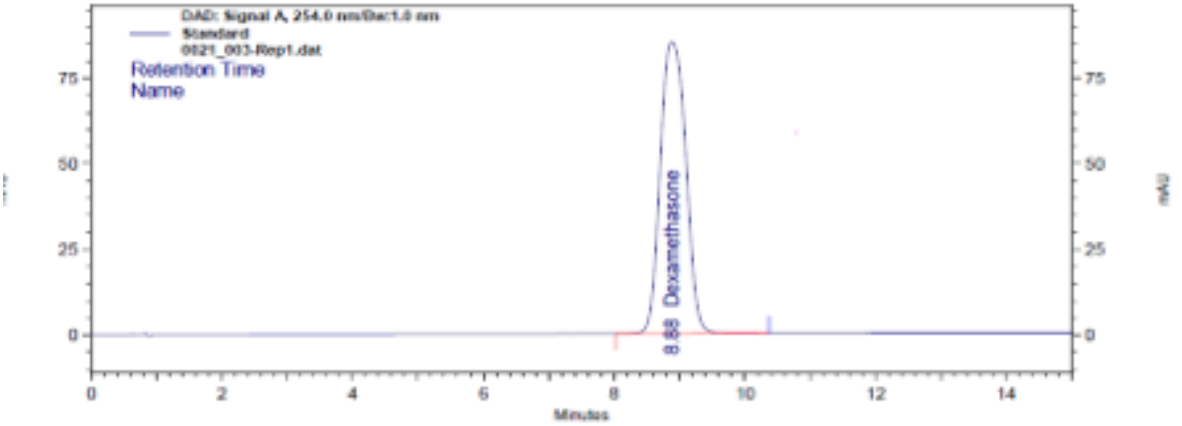


Fig-4 Chromatogram for System suitability:

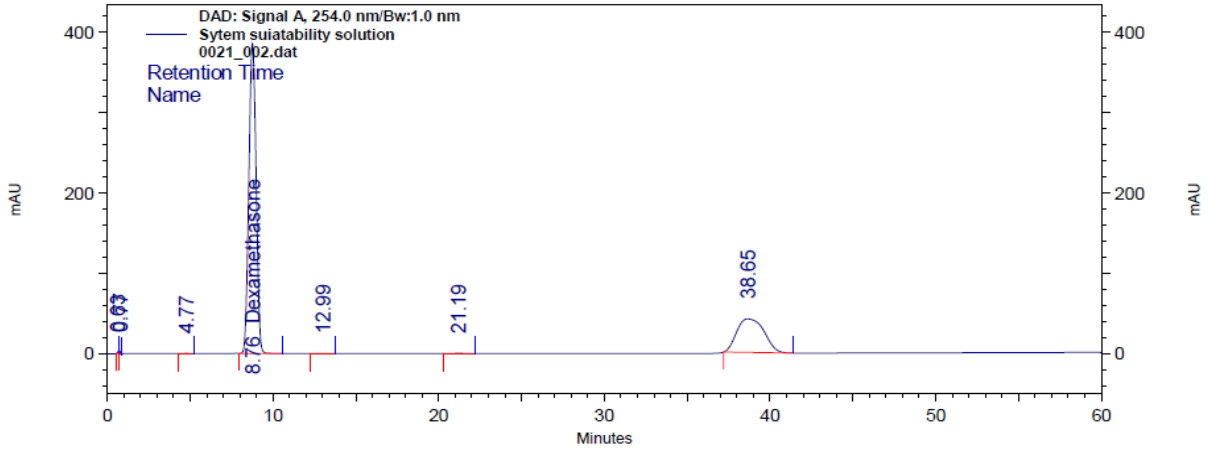


TABLE 1 System Suitability

S. No	Name of the compound	Retention time(mins)	Area	USP Plate Count	USP Resolution	USP Tailing
1	Dexamethasone	8.76	1574556877	1723	0.00	1.03
2	Dexamethasone Acetate	38.65	641723744	2805	15.89	1.24

3. Validations of RP-HPLC method

3.1 System Precision:
Preparation of Standard Solutions:
Dexamethasone Standard Stock Preparation:
Accurately weighed about 25.25 mg of Dexamethasone Working Standard and transferred to a 25mL volumetric flask. 15mL of Acetonitrile was added and sonicated for 3mins; made up the volume with Acetonitrile. (Concentration 1.0mg/ml).
Preparation of Standard Solution – A: Transferred 1mL of the Standard stock solution, into 25mL volumetric flask, made up the volume with Acetonitrile (Concentrations: 0.04mg/mL).
Preparation of Standard Solution – B: Transferred 1mL of the Standard solution A into 10mL volumetric flask, made up the volume with diluent (Concentrations: 0.004mg/mL).

Table 2- System suitability Results

Dexamethasone	Area
Standard 1	4078673
Standard 2	4095825
Standard 3	4097963
Standard 4	4119881
Standard 5	4123177
Standard 6	4131259
Mean	4107796
SD	20106.1
%RSD	0.5

3.2 Method Precision:

Table 3- Method Precision Results

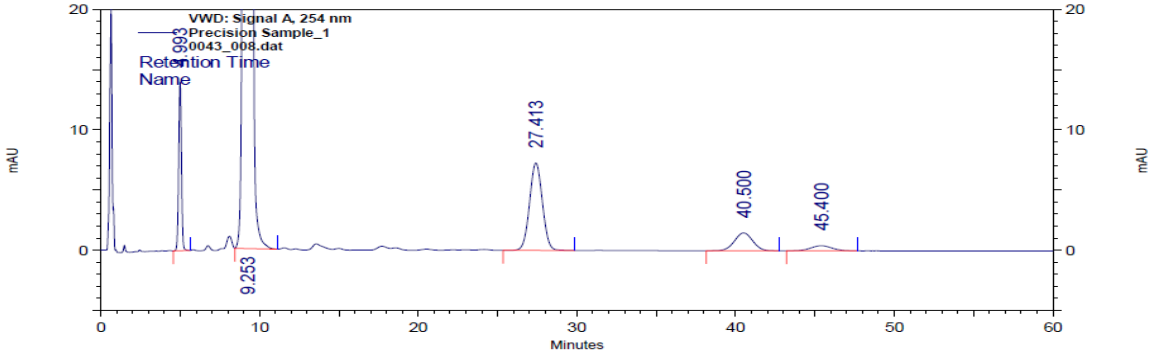
S. No	Weight of Sample (gm)	Diluent added (mL)	Sonication time	9,11 Epoxide Impurity Stock Solution (Conc: 0.1mg/mL)	17-Carboxy-17-deoxy Impurity Stock Solution Conc: 0.052mg/mL)	Dexamethasone-21-Acetate Impurity Stock Solution Conc: 0.1mg/mL)	21-dehydro, 17 deoxy Impurity Stock Solution Conc: 0.1mg/mL)	Made up to volume with Mobile phase
1.	2.02424	2	5min	0.2mL	1mL	0.2mL	0.2mL	10mL
2.	2.00950	2		0.2mL	1mL	0.2mL	0.2mL	
3.	2.02259	2		0.2mL	1mL	0.2mL	0.2mL	
4.	2.02107	2		0.2mL	1mL	0.2mL	0.2mL	

5.	2.01937	2		0.2mL	1mL	0.2mL	0.2mL	
6.	2.02001	2		0.2mL	1mL	0.2mL	0.2mL	

Table 4 Impurities – Retention Times, Relative Retention Times and Relative Response Factors:

S.No	Name of compound	Retention time (approx.)	RRT	RRF
1	9,11 Alpha Epoxide	5.93	0.647	1.23
2	17-Carboxy-17 Deoxy	27.41	2.96	1.05
3	Dexamethasone-21-Acetate	40.50	4.37	1.37
4	21-Dehydro-17-deoxy	45.40	4.90	4.67
5	Dexamethasone	9.25	1	1

Fig 5 Chromatogram for Method Precision



3.3 LOQ Precission

Table 5 LOQ Dexa-21 Acetate

Dexa-21 Acetate	
Sample_1	128976
Sample_2	143891
Sample_3	143735
Sample_4	136300
Sample_5	146936
Sample_6	137166
AVG	139501
STD	6617.44
%RSD	4.7

Table 6 LOQ 9,11Alpha Epoxide

9,11Alpha Epoxide	
Sample_1	255736
Sample_2	266415
Sample_3	264462
Sample_4	260527
Sample_5	265011
Sample_6	257043
AVG	261532
STD	4456.16
%RSD	1.7

Table 7 LOQ Dexa-21 Acetate Acetate

21-Dehydro 17-Deoxy	
Sample_1	96640
Sample_2	81609
Sample_3	86820
Sample_4	90467
Sample_5	86174
Sample_6	82228
AVG	87323
STD	5601.31
%RSD	6.4

Table 8 LOQ Dexa-21

17-Carboxy 17-Deoxy	
Sample_1	269867
Sample_2	264402
Sample_3	270675
Sample_4	259174
Sample_5	263390
Sample_6	252663
AVG	263362
STD	6765.88
%RSD	2.6

3.4 Linearity:

The linearity calibration curve (Peak area vs. Concentration) of Dexamethasone impurity was checked over the concentration ranges LOQ to 200% the linearity was evaluated by linear regression analysis.

Table 9 Linearity Dexamethasone

S. No	Linearity Level (%)	Linearity Preparations: Dexamethasone Standard Solution A (Stock conc. 0.05mg/mL)	Final concentration (ppm)	Area
1	**LOQ**	0.05mg/mL ×0.02ml/20ml	0.05	78006
2	10	0.05mg/mL ×0.02ml/10ml	0.10	136680
3	25	0.05mg/mL ×0.05ml/10ml	0.26	344989
4	50	0.05mg/mL ×0.1ml/10ml	0.51	656934
5	100	0.05mg/mL ×0.2ml/10ml	1.02	1399427
6	200	0.05mg/mL ×0.4ml/10ml	2.04	2481866
Correlation Coefficient I				0.998
Slope				1223009.48
Intercept				38477

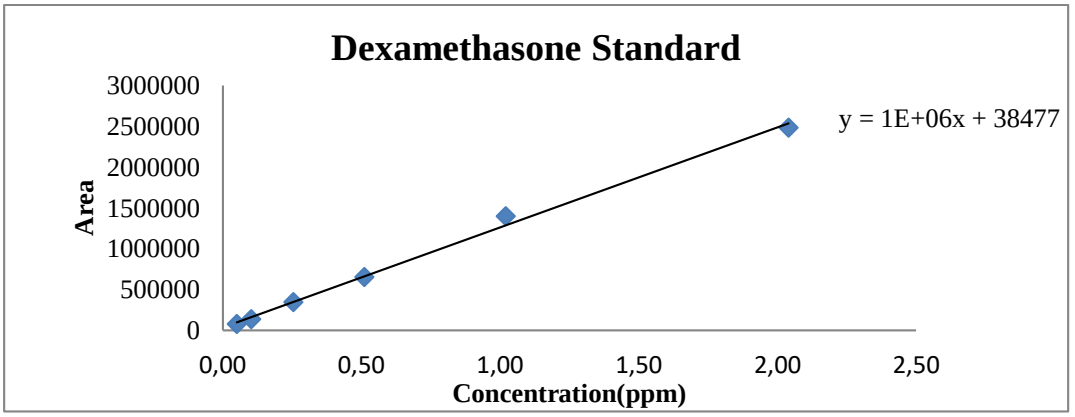


Table 10 Linearity 9,11 Alpha Epoxide

S. No	Linearity Level (%)	Linearity Preparations	Final concentration (ppm)	Area
		9,11Alpha Epoxide Impurity		
1	LOQ	0.005mg/mL ×0.4ml/20ml	0.10	109348
2	10	0.102mg/mL ×0.02ml/10ml	0.20	196940
3	25	0.102mg/mL ×0.05ml/10ml	0.51	473516
4	50	0.102mg/mL ×0.1ml/10ml	1.02	937445
5	100	0.102mg/mL ×0.2ml/10ml	2.05	1983247
6	200	0.102mg/mL ×0.4ml/10ml	4.10	4054964
Correlation Coefficient I				1.000
Slope				991525.16
Intercept				27084

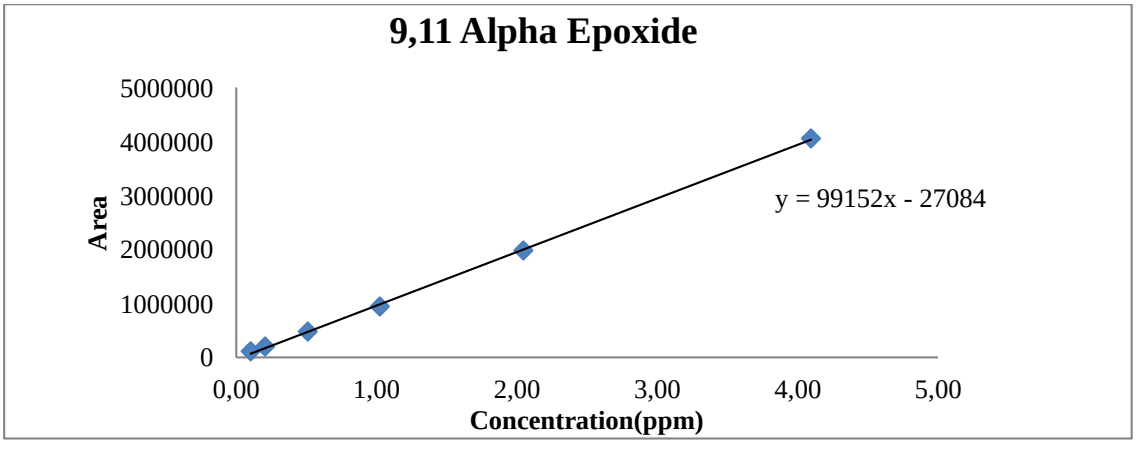


Table 11 Linearity 17-Carboxy-17-deoxy Impurity

S.No	Linearity Level (%)	Linearity Preparations	Final concentration (ppm)	Area
		17-Carboxy-17-deoxy Impurity		
1	LOQ	0.0052mg/mL ×0.4ml/20ml	0.11	242874
2	10	0.052mg/mL ×0.1ml/10ml	0.53	552304
3	25	0.052mg/mL ×0.25ml/10ml	1.32	1525580
4	50	0.052mg/mL ×0.5ml/10ml	2.64	2783789
5	100	0.052mg/mL ×1.0ml/10ml	5.29	5724467
6	200	0.052mg/mL ×2.0ml/10ml	10.57	12456437
Correlation Coefficient I				0.999
Slope				1167719.68
Intercept				-10115

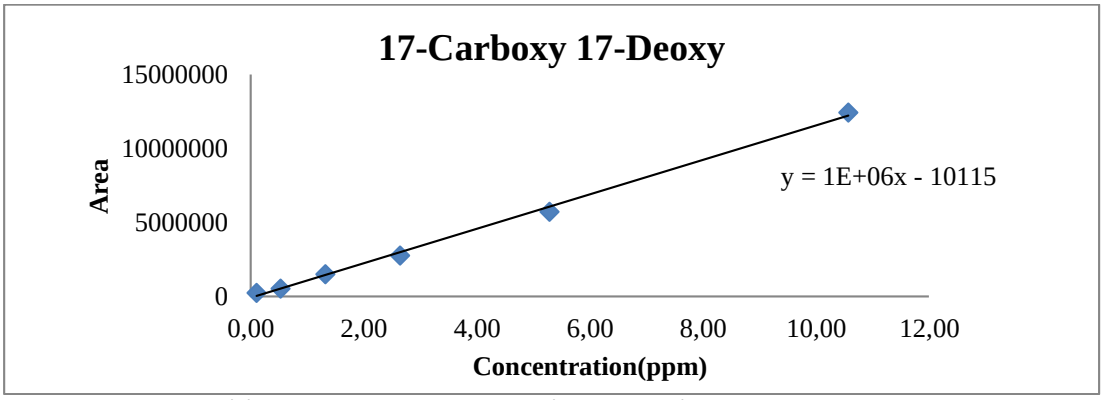


Table 12 Linearity 17-Carboxy-17-deoxy Impurity

S. No	Linearity Level (%)	Linearity Preparations	Final concentration (ppm)	Area
		Dexamethasone-21-Acetate Impurity		
1	LOQ	0.005mg/mL ×0.4ml/20ml	0.10	91684
2	10	0.100mg/mL ×0.02ml/10ml	0.20	166163
3	25	0.100mg/mL ×0.05ml/10ml	0.50	423392
4	50	0.100mg/mL ×0.1ml/10ml	1.01	1184468
5	100	0.100mg/mL ×0.2ml/10ml	2.01	1822787
6	200	0.100mg/mL ×0.4ml/10ml	4.02	3609379
Correlation Coefficient I				0.996

Slope	893778.91
Intercept	47428

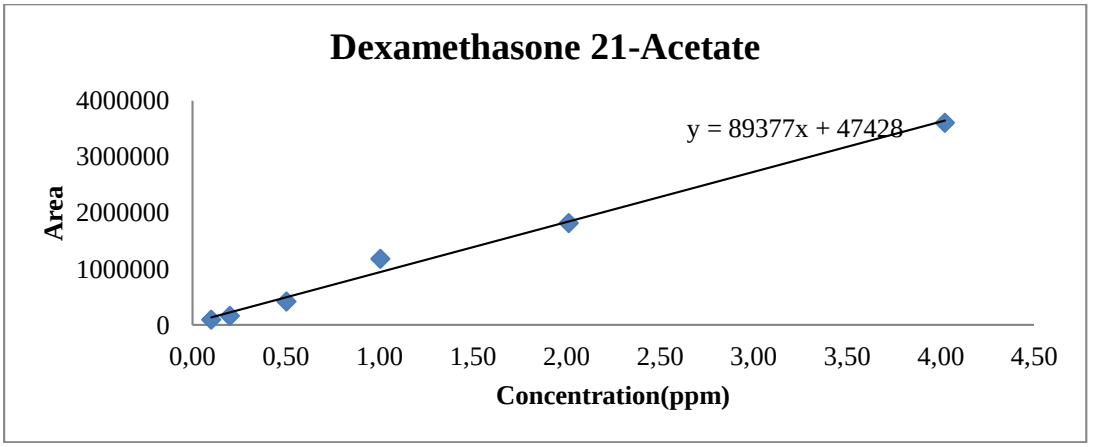
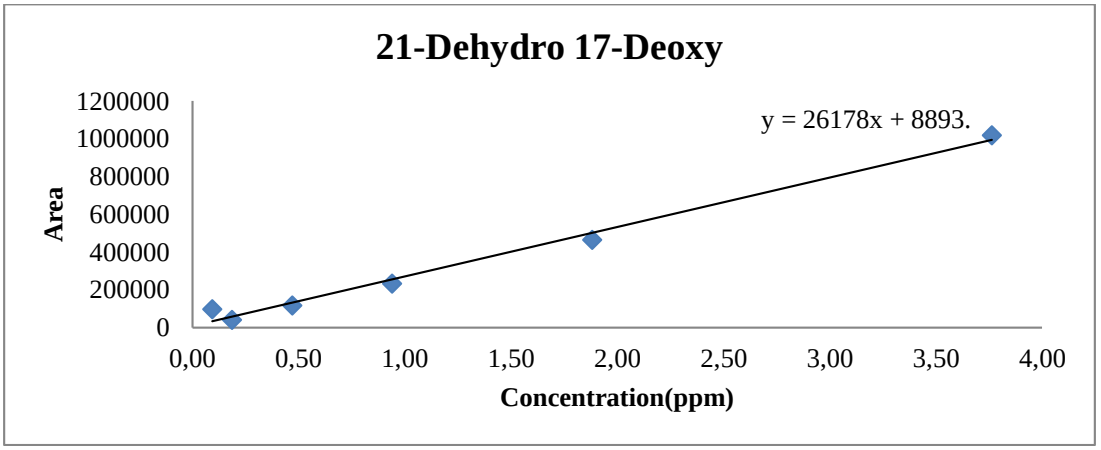


Table 13 Linearity Dexamethasone-21-Acetate Impurity

S.No	Linearity Level (%)	Linearity Preparations	Final concentration (ppm)	Area
		21-dehydro-17-Deoxy Impurity		
1	LOQ	0.005mg/mL ×0.4ml/20ml	0.09	98508
2	10	0.094mg/mL ×0.02ml/10ml	0.19	41088
3	25	0.094mg/mL ×0.05ml/10ml	0.47	117034
4	50	0.094mg/mL ×0.1ml/10ml	0.94	234046
5	100	0.094mg/mL ×0.2ml/10ml	1.88	464864
6	200	0.094mg/mL ×0.4ml/10ml	3.76	1018454
Correlation Coefficient I				0.995
Slope				261785.09
Intercept				8893



From the above observation, the correlation coefficient for the Dexamethasone and their
Impurities was found to be within the limit.

3.5 Accuracy

Accuracy was performed with the concentration range 50%, 100% and 200% was found to be within the limit i.e., 85 to 115%. The % Recovery of 50%, 100% and 200% was found to be within the limits.

Table 14 Accuracy Results

S. No	Sample Name	Concentration (mg/ml)			% Recovery		
		50%	100%	200%	50%	100%	200%
1.	9,11 Epoxide Impurity	0.001	0.002	0.004	88.0	85.3	101.2
2.	17-Carboxy-17-deoxy Impurity	0.026	0.052	0.104	102.9	103.6	101.9
3.	Dexamethasone-21-Acetate Impurity	0.001	0.002	0.004	107.4	97.7	99.1

4.0 Forced Degradation Studies:

Degradation studies were carried out as per ICH guidelines. The objective of the study was to find out the degradation products, which in turn help in the establishment of degradation pathways and the intrinsic stability of drug molecule. In order to check the selectivity of the proposed method, degradation studies were carried out by using acidic, basic, neutral, oxidative conditions**.**

4.1 Acidic degradation:

Test sample Preparation: Weighed about 4.03049g of sample and transferred to a 10ml volumetric flask, 6mL of 0.1NHCl was added, sonicated for 10 mins and made up the volume with 0.1N HCl. Mixed the contents well and leave undisturbed for 30mins at 80°C. After cooling 5mL of the above solution was transferred to a 10mL volumetric flask, made up the volume with diluent. This solution was injected into the HPLC system and chromatogram was recorded.

4.2 Alkali degradation:

Test sample Preparation:

Weighed about 4.03138g of sample and transferred to a 10ml volumetric flask, 6mL of 0.01N NaOH was added, sonicated for 10 mins and made up the volume with 0.01N NaOH. Mixed the contents well and leave undisturbed for 30mins at 80°C. After cooling 5mL of the above solution was transferred to a 10mL volumetric flask, made up the volume with diluent. This solution was injected into the HPLC system and chromatogram was recorded.

4.3 Neutral degradation:

Test sample Preparation:

Weighed about 4.03794g of sample and transferred to a 10ml volumetric flask, 6mL of water was added, sonicated for 10 mins and made up the volume with water. Mixed the contents well and leave undisturbed for 30mins at 80°C. After cooling 5mL of the above solution was transferred to a 10mL volumetric flask, made up the volume with diluent. This solution was injected into the HPLC system and chromatogram was recorded.

4.4 Thermal degradation:

Test sample Preparation:

Weighed about 4.03817g of sample (B.No. 40192-003A) and transferred to a 10ml volumetric flask, and leave undisturbed for 30mins at 80°C. After cooling 5mL of the above solution was transferred to a 10mL volumetric flask, made up the volume with diluent. This solution was injected into the HPLC system and chromatogram was recorded.

4.5 Peroxide degradation:

Test sample Preparation:

Weighed about 4.04564g of sample (B.No.: 40192-003A) and transferred to a 10ml volumetric flask, 6mL of 0.1%H2O2 was added, sonicated for 10 mins and made up the volume with 0.1%H2O2. Mixed the contents well and leave undisturbed for 30mins at 80°C. After cooling 5mL of the above solution was transferred to a 10mL volumetric flask, made up the volume with diluent. This solution was injected into the HPLC system and chromatogram was recorded.

4.6 Photo degradation:

Test sample Preparation: About 4gms of sample was kept under UV Cabinet for 2hrs.

Weighed about 2.02276gms of above sample and transferred to a 10 ml volumetric flask, 7mL of diluent was added, sonicated for 5 mins and made up the volume with diluent. This solution was injected into the HPLC system and chromatogram was recorded.

Table 15 Peak purity data of Dexamethasone Peak in Forced degradation:

Condition	Sample Name	Peak Purity	% Assay	Total % of Impurities	Mass Balance
Acid Degradation	Dexamethasone	1.000	88.72	3.01	91.73
Alkali Degradation	Dexamethasone	1.000	87.57	2.03	89.60
Neutral Degradation	Dexamethasone	1.000	86.40	3.14	89.31
Peroxide Degradation	Dexamethasone	1.000	86.17	2.26	96.21
Thermal Degradation	Dexamethasone	1.000	93.95	2.03	88.43
Photo Degradation	Dexamethasone	1.000	92.09	2.05	94.14

5. Conclusion

Method was done using Agilent 1260 UV/PDA Detector. A non-polar analytical chromatographic column Waters, Symmetry, C18, 3.9 mm x 150 mm, 5µ (or) any equivalent Column was chosen as the stationary phase. With Flow Rate: 1.5 mL/min Run time: For standard: 15mins; For Sample: 60 mins, Column Temperature: 35°C, Detection WL: 254nm and Injection Volume: 50 µL

This RP-HPLC method is also validated for various parameters as per ICH guidelines. The system suitability parameters proved that the method is suitable for the quantification of Dexamethasone. System suitability parameters were within the limits as indicated by good resolution. The precision was within the acceptance criteria of %RSD i.e., not more than 2%. Linearity was observed in concentration range of LOQ to 200% and the Correlation coefficient was found to be within the limit. Accuracy was performed with the concentration ranges 50%, 100%, and 200% and was found to be within the limit i.e., 85 to 115%. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and can be used as a quality – control tool for routine analysis of Dexamethasone in Trobramycin and Dexamethasone Otic Suspension USP 0.3% and 0.1%.

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