Stability Indicating RP-HPLC Method Development and Validation for Dexamethasone

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Abstract: A new, simple, reliable and reproducible stability indicating RP-HPLC assay method has been developed for quantitative analysis of dexamethasone from dexamethasone tablets. This developed method has been validated according to ICH guideline with respect to system suitability, specificity, precision, linearity, accuracy and robustness. An isocratic condition of mobile phase water (0.1% orthophosphoric acid):acetonitrile in a ratio of 60:40, v/v at a flow rate of 1.0 mL/minute over RP 2.5 Fortis C18, 100 × 4.6 mm, 2.5 µm, column was at 27 ºC maintained. This method is specific and showed excellent linear response with correlation coefficient (R2) values of 0.999. In forced degradation, the proposed method has been investigated with different stress conditions as hydrolytic, oxidative, thermal and humid as recommended by ICH guidelines. An accurate and reliable reversed-phase HPLC method for the analysis of dexamethasone in dexamethasone tablets was developed and validated successfully.

Keywords: Dexamethasone, RP-HPLC, Forced degradation.

1. INTRODUCTION

Dexamethasone Tablet is a glucocorticoid medicine. It is used in the management of arthritis (inflammation and tenderness of one or more joints), skin, blood, intestinal, and adrenal gland disorders, allergic reactions, asthma, cerebral oedema (an accretion of excess fluid around the brain), etc.1 The muscles adjoining the airways also tend to tighten, which makes the already clogged airways even narrower.2 Ordinarily three types of the drug which describe the doctor for anti-inflammatory and anti-allergic effect is corticosteroids, antihistamines and decongestants.3-5

From literature study, observed that very few methods were found to quantitative analysis for estimation of dexamethasone. Official method for assay of dexamethasone available 6 but with challenging chromatographic conditions and some authors also reported study on dexamethasone.7-12 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 tablet dosage form.

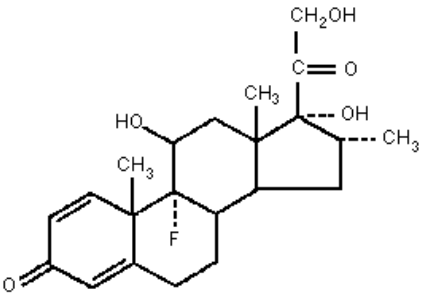

Figure. 1: Structure of dexamethasone.

2. MATERIALS AND METHODS

Dexamethasone working standard and Placebo were a kind gift of Nayantara Chemicals, Aurangabad, India. Test samples purchased from market store. HPLC grade Acetonitrile and HPLC Water were purchased from Ranbaxy Fine ChemicalsLtd., 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. The data were recorded using Chemstation 10.02 software. Preparation of mobile phase: Transferred 1 mL of orthophosphoric acid in 1000 mL HPLC grade water and mixed. Prepared a mixture of water (0.1% orthophosphoric acid):acetonitrile (60:40), v/v, sonicated to degas.

Preparation of standard solution (100 ppm): Accurately weighed and transferred 50 mg of dexamethasone working standard in to 50 mL volumetric flask, added about 20 mL of acetonitrile and sonicated to dissolve, cool and diluted upto the mark with diluent. Transferred 5 mL of this solution into 50 mL volumetric and diluted up to mark with mobile phase.

Preparation of sample solution (100 ppm): Finely crushed the average weight of 20 tablets of Decdan (0.5 mg) (Wockhardt) with mortar and pestle. Accurately weighed and transferred sample powder equivalent to 2.5 mg of dexamethasone into 25 mL volumetric flask, added about 15 mL of mobile phase

and sonicated to dissolve. Cool the solution and diluted up to the mark with mobile phase.

Preparation of placebo solution: Accurately weighed and transferred 400 mg of placebo powder into a 25 mL volumetric flask, added about 15 mL of mobile phase and sonicated to dissolve and diluted up to mark with mobile phase.

Detection method: The analysis was carried out at 27 ºC under isocratic condition. The mobile phase was run at a flow rate of 1.0 mL/min for 10 min. The injection volume was 20 µL for blank, placebo, standard and sample solution. Before analysis, every standard and sample were filtered through 0.45 µm nylon syringe filter. The analysis was monitored with UV detection at 240 nm.

Method Development: Various trials performed with respect to mobile phase and stationary phase to optimize the suitable chromatographic conditions. Determined peak purity and the UV spectrum from injection of dexamethasone standard solution is represented in Fig. 2.

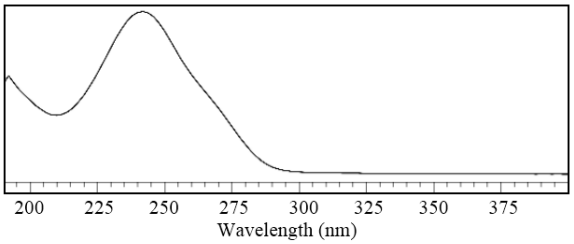

Fig. 2. UV spectrum of dexamethasone

Maximum absorbance of dexamethasone peak observed at 240 nm and it has also been confirmed the purity factor is within the threshold limit [8]. Based on the obtained data, it is decided to performe analytical method validation on 240 nm with proposed detection method.

Method validation

System suitability: To determine system suitability dexamethasone standard solution was prepared and injected for six times into HPLC system. The mean, SD and % RSD for peak areas of dexamethasone was calculated.

Specificity: The placebo solution containing excipients without dexamethasone were injected. To evaluate the specificity of the method blank, placebo and sample solution were injected.

Precision: The precision of assay method was assessed with respect to repeatability and reproducibility. Sample of a single batch were prepared six times and analyzed as per test method, % assay of dexamethasone for six samples calculated for method precision.

Accuracy: In this study, successive analysis (n = 3) for three different concentrations of standard mixtures (50, 100 and 150 %) was carried out to determine the accuracy of proposed analytical method.

Linearity: Linearity has been performed on different concentrations within 50-150% of nominal standard concentration. The linearity of this proposed method was evaluated by using calibration curve to calculate the coefficient of correlation, slope and intercept values.

Robustness: Robustness is a capacity of the method to remain unaffected by small deliberate variations in method parameters. The effect of the following deliberate changes in chromatographic conditions was monitored, e.g. detector wavelength: ± 2 nm, flow rate: ± 10 % and temperature: ± 2 ºC.

Solution stability: Solution stability has been performed for standard solution and sample test solution.

Forced degradation: Forced degradation study carried out to determine stability of a drug substance in finished product, establishing degradation pathway [9,10] and to resolve stability related problems [11]. The stability study performed by applying the physical stress (acid, alkali, hydrogen peroxide, heat and humidity) to the product [12,13]. Degradation products observed in forced degradation study are potential degradation products, these products may not be generate in relevant stability or storage conditions but they assist in developing degradation pathway [14].

Acid degradation: Accurately weighed and transferred sample powder equivalent to 2.5 mg of dexamethasone into a 25 mL volumetric flask, added about 15 mL of mobile phase and sonicated to dissolve. Added 2 mL of 0.1N HCl solution, shake well and kept on bench top for 30 min and then added 2 mL of 0.1N NaOH solution and finally mixed to neutralized, diluted up to the mark with mobile phase.

Alkali degradation: Accurately weighed and transferred sample powder equivalent to 2.5 mg of dexamethasone into a 25 mL volumetric flask, added

about 15 mL of mobile phase and sonicated to dissolve. Added 1 mL of 0.01 N NaOH solution, shake well and kept on bench top for 30 min and then added 1 mL of 0.01N HCl solution and finally mixed to neutralized, diluted up to the mark with mobile phase.

Oxidation degradation: Accurately weighed and transferred sample powder equivalent to 2.5 mg of dexamethasone into a 25 mL volumetric flask, added about 15 mL of mobile phase and sonicated to dissolve. Added 2 mL of 5 % H2O2 solution, shake well and kept on bench top for 30 min, diluted up to the mark with mobile phase.

Thermal degradation: About 20 tablets exposed to 60 ºC for 15 h and then crushed finely with mortar and pestle.

Accurately weighed and transferred sample powder equivalent to 2.5 mg of dexamethasone into a 25 mL volumetric flask, added about 15 mL of mobile phase and sonicated to dissolve and diluted upto the mark with mobile phase.

Humidity degradation: About 20 tablets exposed for humidity at 75 % RH for 15 h and then crushed finely with motor pestle. Accurately weighed and transferred sample powder equivalent to 2.5 mg of dexamethasone into a 25 mL volumetric flask, added about 15 mL of mobile phase and sonicated to dissolve and diluted up to mark with mobile phase.

3. RESULTS AND DISCUSSION:

In this study, a HPLC method for quantitative analysis of dexamethasone in tablet dosage form was developed and validated.

1. System suitability parameters:

System suitability is performed by injecting the standard solution having six replicate injection as per specified in the US Pharmacopeia. The results of system suitability parameters are shown in table.

Table 1: Results of system suitability parameters

Sample No.	Response		
	Weight of standard(mg) Dexamethasone	Area	Retention Time
1	25.00	1553764	5.189
2		1555281	5.182
3		1552724	5.196
4		1554926	5.112
5		1555680	5.213
6		1557235	5.265
Mean			1554935
%RSD			0.10

2. Specificity: Individual solutions were prepared, injected into the system by using optimize chromatographic conditions. A chromatogram of blank (diluents) shown in figure. 3

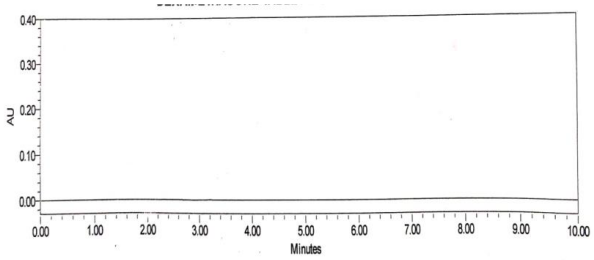

Figure. 3: Chromatogram of blank solution at 254 nm

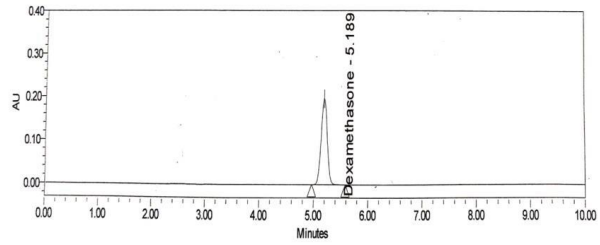

Figure. 4: Chromatogram of standard solution of dexamethasone (0.1 µg / mL) at 254 nm

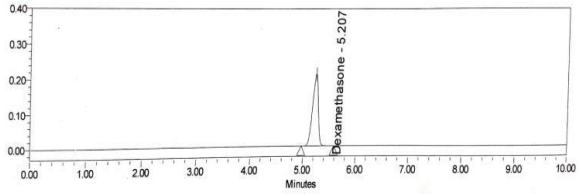

Figure. 5: Chromatogram of Dexamethasone Tablet (0.1 µg / mL) at 254 nm

3. Linearity:

Table 2: Linearity of Dexamethasone

% conc. Of sample	Mean response (area)	Statistical analysis	
50	788155.00	Correlation	0.999
80	1314321.5		
100	1605121.5	Intercept	10635.634
120	1900940.00		
150	2400069.5	Slope	15784.582

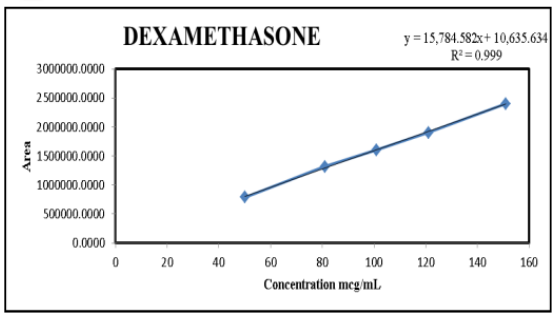

Figure. 6: Linearity of dexamethasone

4. Accuracy: Prepared dexamethasone solutions at three levels (50%, 100%, and 150%) each in triplicate and analysed as per the method. The result is summarized in the following table

Table 3: Data sheet for Accuracy

% conc. Sample	Theoretical concentration (µg/ml)	Concentration recovered (µg/ml)	% recovery	Mean % recovery
50%-1	49.894	49.912	100.0	100.5
50%-2	51.110	52.965	101.6	
50%-3	50.770	50.823	100.1	
100%-1	101.896	100.543	98.6	99.9
100%-2	100.412	100.187	99.7	
100%-3	102.525	103.987	101.4	
150%-1	151.092	150.514	99.6	100.4
150%-2	150.225	151.309	100.7	
150%-3	149.997	151.752	101.1	

Mean recovery	**100.26**
SD	**0.321**
%RSD	**0.320**

Percentage recovery value obtained for the sample solution of dexamethasone is about 98.6-101.6, which is within the specified limit. The relative standard deviation value was found to be less than 2%.

5.Precision:

a. System precision: Perform the analysis of standard solution of dexamethasone injected six times.
b. Method precision: Six spiked sample solutions preparations were prepared and injected on the HPLC.

Table 4: Observations for system precision

Injection	Area
1	1576184
2	1579225
3	1574762
4	1573897
5	1573516
6	1579248
Mean	**1576138.666**
SD	**2568.81**
%RSD	**0.16**

Table 5: Observations for Method Precision

Injection no.	Area	%Area
1	1526568	100.7
2	1536549	101.4
3	1491626	98.4
4	1490628	98.4
5	1528237	100.9
6	1499877	99.0
Mean	1512248	99.8
SD	20479.17	1.352
%RSD	1.35	1.35

c. Ruggedness (Intermediate precision): same procedure of method precision is followed by another Analyst by using same lot on different instrument, different column and on different day. The data obtained from Analyst-II are summarized in table no.6

Table 6: Observation for ruggedness (intermediate precision)

Sample	Analyst-1 % assay	Analyst-2 %assay
1	99.2	98.6
2	99.3	98.7
3	100.3	99.5
4	99.5	100.3
5	99.7	100.7
6	99.3	100.4
Mean	**99.5**	**99.7**
SD	**0.4086**	**0.9055**
%RSD	**0.4104**	**0.9082**

6. LOQ and LOQ:

LOD and LOQ were found to be 0.5370µg/ml and 1.6273µg/ml respectively.

Force degradation study

1. Acid degradation:

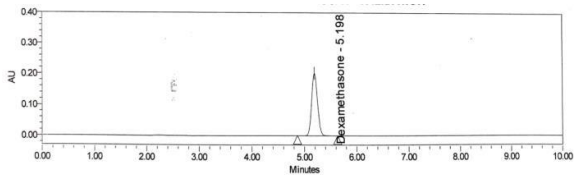

Fig. 7: Chromatogram of Dexamethasone on treatment with Acid

2. Base Degradation:

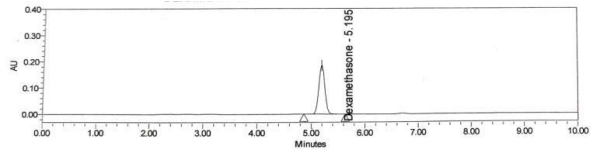

Figure 8: Chromatogram of Dexamethasone on treatment with Base

3. Peroxide Degradation (H2O2):

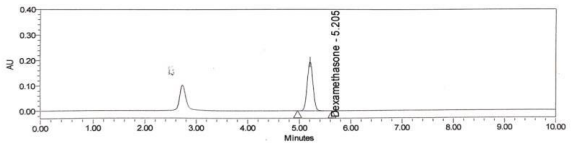

Figure 9: Chromatogram of Dexamethasone on treatment with 10% hydrogen peroxide

4. Photolytic Degradation:

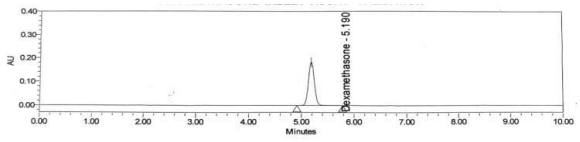

Figure 10: Chromatogram of Dexamethasone on Photolytic degradation

5. Heat degradation:

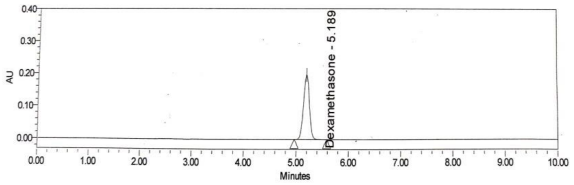

Figure 11: Chromatogram of Dexamethasone on Heat degradation

Table 7: Observations of Forced degradation

Condition	% Assay	%Degradation	Purity angle	Purity threshold
Control	100.6	-	-	-
Acid Degradation	101.5	-2.3	0.024	0.210
Base Degradation	93.9	6.3	0.028	0.224
Peroxide Degradation	99.9	0.7	0.041	0.226
Photolytic Degradation	92.5	8.8	0.035	0.212
Heat Degradation	99.3	3.1	0.038	0.215

Assay of tablet formulation:

Table 8: % Assay of tablet formulation

	Standard 1 (mean of six replicating injections)	Standard 2 (mean of two replicating injections)	Dexamethasone (mean of two replicating injections)	% correlation	% Assay
Area	1553764	1623783	1538725	100.0%	99.4%

4. CONCLUSION

A new and simple RP-HPLC technique for the routine sample analysis of dexamethasone in dexamethasone tablet was developed and validated. In system suitability parameter the % RSD of six replicate injections was found to be NMT 1.5%. In Specificity Retention time of sample solution found to be comparable with retention time of Dexamethasone standard solution. The data demonstrates that there is no interference in blank and placebo at the retention time of Dexamethasone peak. In linearity Correlation coefficient was 0.999 & in accuracy the RSD of %recovery was found to be 0.32. LOD and LOQ were found within the limit of LOD &LOQ. Present developed method does not use any buffer in mobile phase, so that it helps to increase the life of the column. This developed method can be used for quantitative as well as qualitative analysis purpose. The proposed method is reproducible, accurate, precise, robust, specific and linear over the analysis ranges and also able to resolve the drug in a very short analytical run time.

5. CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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