Stability Indicating Rp-Hplc Method for Assay of Dexamethasone in Its Formulation

Asha Deepti[1*], Alapati Sahithi[2]

Abstract

New, simple, reliable, and reproducible stability-indicating RP-HPLC assay method has been developed for quantitative analysis of dexamethasone in formulation using Agilent 1260 DAD Detector. A non-polar analytical chromatographic column Symmetry Waters C18 (150mm×3.9mm, 5µ), was chosen as the stationary phase. The mobile phase used 3.0 OPA Buffer and Acetonitrile. 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 quantifying 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%Accuracy was performed with the concentration ranges 50%, 100%, and 150% and was found to be within the limit. No interference was observed at the main peak Retention time in all stressed conditions. 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 ophthalmic solutions.

KeyWords:OPA Buffer, Dexamethasone, Accuracy, precision

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Introduction

Asthma is a common chronic and complex disease. This involves airway inflammation, intermittent airflow obstruction, and hyper bronchial responsiveness. Inflammation mainly occurs due to an allergic reaction. Three main types of drugs available for anti-inflammatory and anti-allergic effects are corticosteroids, antihistamines, and decongestants [A.M. Watts et al.,2019] [C.H. Gil et al., 2018].

Dexamethasone (Fig. 1) is a corticosteroid that prevents the release of substances in the body that cause inflammation. It is used to treat many conditions, including several skin diseases, severe allergies, and asthma. The effects of dexamethasone are frequently seen within a day and last for about three days [C.A.C. Jessurun et al. 2019].[S. Shaikh et al. 2012]Dexamethasone tablets are available in the market with brands such as Daksone, Decdan, Demisone, Dexona, Dexasone, Decmax, Intradex, etc.

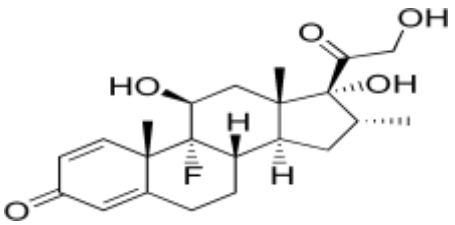


Fig 1: Strcture of Dexamethasone

From the literature survey, it has been observed that few methods were found to analyze the estimation of dexamethasone quantitatively. The official method for assay of dexamethasone is available [Dexamethasone USP monograph, USP29-NF24-Page 643] but with challenging chromatographic conditions, and some authors also reported studies on dexamethasone [T. Thamaraikani et.al 2012]. As per ICH guidelines, a specific and stability-indicating procedure should be included to determine the content of the new drug substance [G.M.S. Gonçalves et al. 2009].

Corresponding author: Asha Deepti
Address: [1]Assistant Professor, Department of Pharmaceutical Analysis GITAM Institute of Pharmacy, GITAM (Deemed to be) University, Visakhapatnam, Andhra Pradesh, India-530045, [2]Research scholar, GITAM Institute of Pharmacy, GITAM (Deemed to be) University, Visakhapatnam, Andhra Pradesh, India-530045 & Assistant professor, Department of Pharmaceutical Analysis, School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad, Telangana, INDIA500088

E-mail: salapati1@gitam.in

The main objective of this study is to develop a simple, suitable, cost-effective and environment-friendly HPLC method required for the analysis and characterization of dexamethasone from dexamethasone finished product

Materials And Methods

Chemicals & Reagents

Dexamethasone working standard and Placebo were a kind gift of Orbicular Pharmaceutical Technologies Pvt. Ltd., Hyderabad Test samples purchased from the market store. HPLC grade Acetonitrile and HPLC Water were purchased from Ranbaxy Fine Chemicals Ltd., India. Analytical grade orthophosphoric acid, NaOH pallets purchased from Merck, India

Instrumentation

A high-performance liquid chromatographic system (Agilent-1260 VWD and DAD detector) with a UV-visible detector was used for the analysis. The data were recorded using Open Lab E.Z. Chrom with Datastore A.01.05 software

Preparation of Standard Solutions

Dexamethasone Standard Stock Preparation: (Concentration 1mg/ml)
Accurately weigh about 20 mg of Dexamethasone Working Standard, and transfer to a 25mL volumetric flask. Add 17mL of Acetonitrile and sonicate to 10mins; makeup to volume with Acetonitrile.

Dexamethasone Acetate Imp Stock Solution

Preparation of Standard Solution: (Concentrations: Dexamethasone 0.05mg/mL)
Transfer 1mL of the Standard stock solution into a 20mL volumetric flask; make up the volume with diluent.
Preparation of Sample Solution: (Concentrations: Dexamethasone 0.05mg/mL)
Accurately weigh about 2g of sample transfer in 20mL volumetric flask, add about 15mL of diluent, and sonicated for 10 mins. Make up the volume with diluent and mix well.

Procedure

Set the Chromatographic conditions described above and equilibrate the column with the mobile phase until a stable baseline is obtained. Inject blank (diluents) solution in duplicate into the chromatograph and record the chromatogram. Inject standard solution six times into the chromatograph, record the chromatograms and measure the peak areas.

System suitability

The system suitability test is a Pharmacopoeia requirement and is used to verify whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from six replicate injections of standard solutions. It was carried out for the parameters like plate number (N), resolution (R), and tailing factor, and the %RSD values were calculated in each case.

Precision

Dexamethasone Standard Stock Preparation: (Concentration 1mg/ml)
Weighed about 20 mg of Dexamethasone Working Standard and transferred to a 25mL volumetric flask. 17mL of Acetonitrile was added and sonicated for 10mins; made up to volume with Acetonitrile.
Preparation of Standard Solution: (Concentrations: Dexamethasone 0.05mg/mL)
1mL of the Standard stock solution was transferred to a 20mL volumetric flask, made up the volume with diluent..6 preparations were prepared and injected into the HPLC system.

Accuracy

The recovery test determined the Accuracy parameter.
Dexamethasone Standard Stock solution: About 20mg of dexamethasone was accurately weighed, transferred to a 20ml volumetric flask, about 15mL of diluents was added, sonicated for 10mins, made up to the volume with diluent, and mixed well. (Concentration 1mg/mL).
Weighed about 2g of Placebo transferred to a 20mL volumetric flask, to this 1mL of Standard stock solution, add about 8mL of diluent, sonicated for 10mins, made up the volume with diluent and mixed well. (Accuracy concentrations (50%, 100% and 150%). Injections of each level were injected, and percent recovery was calculated.

Linearity

Linearity has been performed on different concentrations within 50-150% of the nominal standard attention. This proposed method's linearity was evaluated using a calibration curve to calculate the coefficient of correlation, slope, and intercept values.

Robustness

Robustness is the capacity of the method to remain unaffected by slight, 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.

Forced Degradation Studies

Degradation studies were carried out as per ICH guidelines. The study's objective was to find the degradation products which help establish degradation pathways and the intrinsic stability of drug molecules. To check the selectivity of the proposed method, degradation studies were carried out using acidic, basic, neutral, and oxidative conditions.

Acid degradation

Weighed about 2g of sample transferred to a 10ml volumetric flask, 5mL 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 2 hr at 80°C. After cooling, 5mL of the above solution containing 0.1mg/mL of dexamethasone was transferred to a 10mL volumetric flask, made up the volume with diluent to get 0.05mg/mL concentration of dexamethasone. This solution was injected into the HPLC system, and the chromatogram was recorded.

Alkali degradation

Weighed about 2g of sample and transferred to a 10ml volumetric flask, 5mL of 0.01N NaOH was added, sonicated for 10 mins and made up the volume with 0.01N NaOH.kept it on bench top for 30 mins. Then, 5mL of the above solution containing 0.1mg/mL of dexamethasone was transferred to a 10mL volumetric flask, made up the volume with diluent to get 0.05mg/mL concentration of dexamethasone. Finally, this solution was injected into the HPLC system, and the chromatogram was recorded.

Thermal Degradation

Weighed about 2g of sample and transferred to a 10ml volumetric flask, 5mL of water was added, sonicated for 10 mins, and made up the volume with water. Mixed the contents well and leave undisturbed for 2 hr at 80°C. After cooling, 5mL of the above solution containing 0.1mg/mL of dexamethasone was transferred to a 10mL volumetric flask, made up the volume with diluent to get 0.05mg/mL concentration of dexamethasone. This solution was injected into the HPLC system, and a chromatogram was recorded

Thermal degradation

Weighed about 2g of sample and transferred to a 10ml volumetric flask, 5mL of Diluent was added, sonicated for 10 mins, and made up the volume with diluent. Mixed the contents well and leave undisturbed for 2 hr at 80°C. After cooling, 5mL of the above solution containing 0.1mg/mL of dexamethasone was transferred to a 10mL volumetric flask, made up the volume with diluent to get 0.05mg/mL concentration of dexamethasone. This solution was injected into the HPLC system, and the chromatogram was recorded.

Peroxide degradation

Weighed about 2g of sample and transferred to a 10ml volumetric flask, 5mL 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 2 hr at 80°C. After cooling, 5mL of the above solution containing 0.1mg/mL of dexamethasone was transferred to a 10mL volumetric flask, made up the volume with diluent to get 0.05mg/mL concentration of dexamethasone. This solution was injected into the HPLC system, and a chromatogram was recorded

Photodegradation

10gms of the sample was kept under Sunlight for half day. Weighed about 2g of sample and transferred to a 20 ml volumetric flask, 15mL of diluent was added, sonicated for 10 mins, and made up the volume with diluent. This solution was injected into the HPLC system, and the chromatogram was recorded.

Results & Discussions

Developing the analytical method is a continuous process and essential to confirm and maintain the

quality of the pharmaceutical finished product. Therefore, finalized chromatographic conditions were applied, and performed analytical method validation.

Chromatographic Conditions

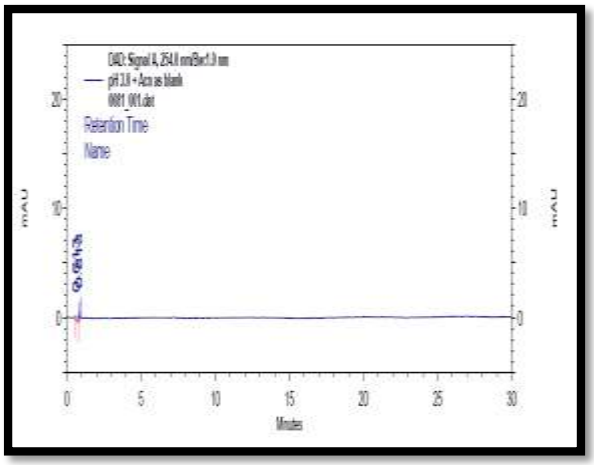
Column Details: Waters, Symmetry, C18, 3.9 mm x 15 cm, 5µ (or) any equivalent Column
Flow Rate: 1.5 ml/min
Run time: 15 minutes for Assay
30 minutes for System Suitability Solution
Column Temperature: 45ºC
Sample cooler: 5°C
Detection W.L.: 254 nm
Injection Volume: 20 µl
Needle wash solution: Acetonitrile

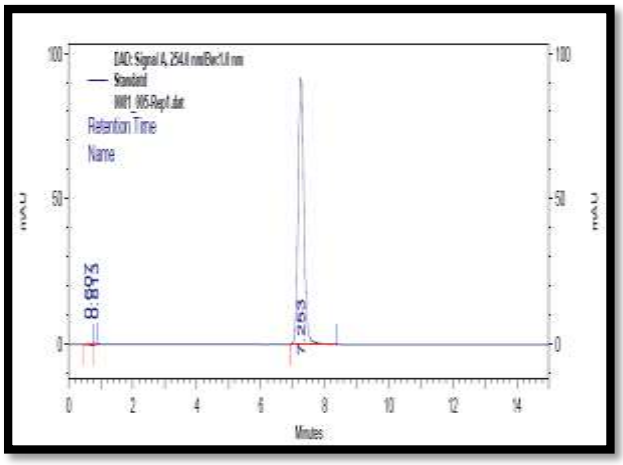
System suitability

System suitability is evaluated by injecting six replicates of freshly prepared standard solutions. Standard peak area, tailing factor, and theoretical plates were measured and tabulated in Tables 1& 2. The results were satisfactory, i.e., tailing factor value is not more than 2.0; area % RSD is not more than 2.0 %, and theoretical plates were above 2000, also evaluated method precision by injecting six different sample preparation from a single batch of the finished product in duplicate, calculate % assay of dexamethasone peak and calculated assay values (NMT 2.0 %) observed satisfactory, i.e., between the limit range of 98.0 to 102.0 % (Table-1&2)

Accuracy

Known amount of dexamethasone was spiked in Placebo at about 50, 100, and 150 % of test concentration. Each accuracy level was prepared in triplicate. First, the amount of dexamethasone recovered was quantified per the developed method. Then, the percentage recovery was calculated from the amount found and added. The results are shown in Table 3. The overall recovery of dexamethasone in the samples was between 98% and 102 % (RSD < 2 %), which is satisfactory for quantifying dexamethasone in the finished product.





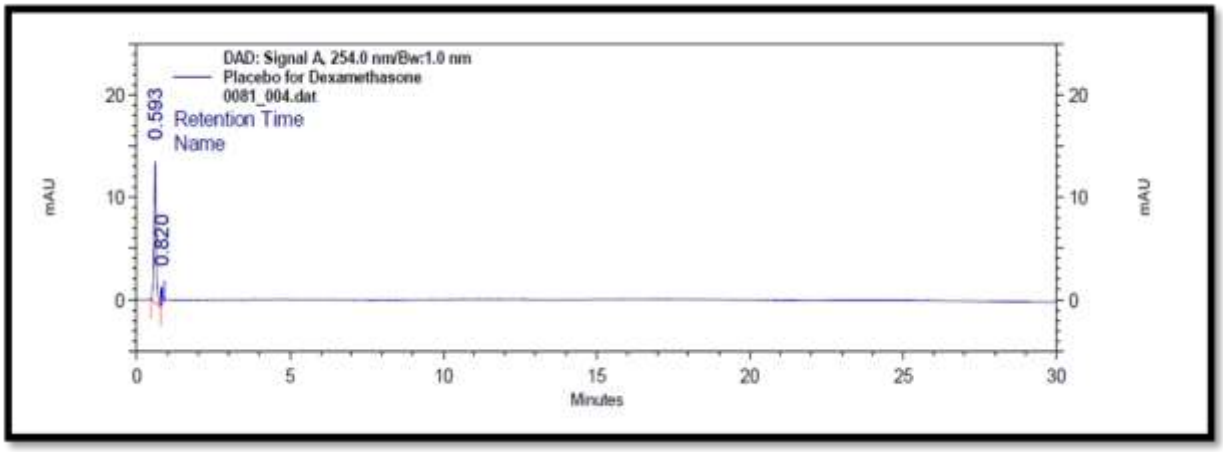


Fig. 2. HPLC chromatogram (a) blank, (b) Sample solution and (c) Placebo

Table 1: Results of precision method and system precision

Dexamethasone	Area
Standard 1	153996639
Standard 2	154073405
Standard 3	153437416
Standard 4	153348371
Standard 5	153569363
Standard 6	154248182
Mean	153778896
SD	374230.239
%RSD	0.24

Table 2: Results of precision method and system precision

Dexamethasone	Area	% Assay
Sample 1	143301022	93.25
Sample 2	143152680	93.00
Sample 3	143301436	93.40
Sample 4	143501221	93.25
Sample 5	143178524	93.48
Sample 6	143178254	93.20
Mean	143251713	
SD	85765	
%RSD	0.05	

Table 3: Accuracy evaluation for quantification of dexamethasone

S.No	Accuracy Level (%)	Concentration (mg/ml)	% Recovery
1.	50	0.025	99.5
2.	100	0.05	98.0
3.	150	0.075	99.2

Linearity: Standard stock solution was used to prepare all linearity levels. All linearity levels were injected in duplicate into the chromatographic system. The correlation coefficient value was calculated and observed within acceptance criteria, i.e., correlation coefficient (R2) 0.996. A graph plotted the concentration (µg/mL) of dexamethasone on the X-axis and the peak areas of dexamethasone on the Y-axis.

Robustness: Robustness was performed by injecting a blank and standard solution in duplicate. The method's robustness was verified by a deliberately slight change in the chromatographic conditions. All the variations observed are found

satisfactory

Forced degradation: Forced degradation is performed on the blank solution (mobile phase), and test sample solution of dexamethasone finished product. Blank and sample solutions of test concentration injected in a single, recorded peak purity factor from each tested stressed condition and calculated percentage assay of dexamethasone peak. The strategy used for forced degradation produces the desired amount of degradation, i.e., 2-20,% and satisfactory degradation is observed. Based on chromatograms generated from all forced degradation experiments, the degradation at each specific condition is shown in Table 4

No interference was observed in Blank and Placebo at main peak Retention time in all stressed conditions. The peak purity of dexamethasone was given in table 4

Table 4 : Peak purity data of Dexamethasone Peak in Forced degradation

Condition	Sample Name	Peak Purity	% Assay	Net Degradation
Acid Degradation	Dexamethasone	1.000000	84.4	15.6
Alkali Degradation	Dexamethasone	1.000000	92.5	7.5
Neutral Degradation	Dexamethasone	1.000000	80.99	19.01
Peroxide Degradation	Dexamethasone	1.000000	86.1	13.9
Thermal Degradation	Dexamethasone	1.000000	85.8	14.2
Photo Degradation	Dexamethasone	1.000000	72.59	27.41

Conclusion

New and simple stability indicating RP-HPLC method for the routine sample analysis of dexamethasone in dexamethasone finished product was developed and validated. The proposed method does not use any buffer in the mobile phase, directly proportional to the stationary phase cost. This developed method can be used for quantitative and qualitative analysis purposes. The proposed method is reproducible, accurate, precise, robust, specific, and linear over the analysis ranges and can resolve the drug from excipients in a concise analytical run time. Solution stability and forced degradation study of dexamethasone prove that the stability-indicating method can be used for routine and stable sample analysis of the finished product. This method can also be used for preparative, characterization, dissolution, content uniformity, and blend uniformity of dexamethasone finished product

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