Stability Indicating RP-HPLC Method Development and Validation for Dexamethasone

Pankaj N. Kulkarni[iD], C.K. Jadhav, A.S. Nipate, Alaknanda M. Dodake-Supekar and C.H. Gill[*]

Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, India

*Corresponding author: Tel : +91 70 28247050, E-mail: chgill16@gmail.com; pankajnkulkarni@gmail.com

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 ℃ maintained. This method is specific and showed excellent linear response with correlation coefficient (R^2) 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.

INTRODUCTION

Asthma is a common chronic and complex disease. This involves airway inflammation, intermittent airflow obstruction, and bronchial hyper responsiveness. Inflammation mostly occurs due to an allergic reaction. There are three main types of drugs available for anti-inflammatory and antiallergic effect are corticosteroids, antihistamines and decongestants [1,2].

Dexamethasone (Fig. 1) 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. The effects of dexamethasone are frequently seen within a day and last for about three days [3,4]. Dexamethasone tablets are available in market with different brand names such as Daksone, Decdan, Demisone, Dexona, Dexasone, Decmax, Intradex, *etc*.

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 [5] but with challenging chromatographic conditions and some authors also reported study on dexamethasone [6]. As per ICH guideline a specific and stability-indicating procedure
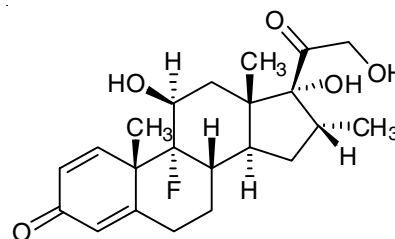

Fig. 1.   Chemical structure of dexamethasone (m.f.: $C_{22}H_{29}FO_5$; m.w.: 392.5 g/mol

should be included to determine the content of the new drug substance [7]. The main objective of this study is to develop a simple, suitable, cost effective and environment friendly HPLC method required for analysis and characterization of dexamethasone from dexamethasone finished product.

EXPERIMENTAL

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 Chemicals

Ltd., India. Analytical grade orthophosphoric acid, HCl, NaOH pallets and H_2O_2 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 ℃ 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.
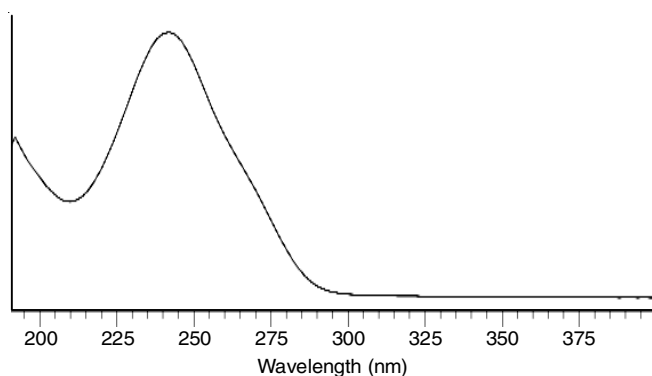

Fig. 2. UV spectrum of dexamethasone

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 ℃.

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 % H_2O_2

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.

RESULTS AND DISCUSSION

Development of analytical method is continuous process and very essential to confirm and maintain the quality of pharmaceutical finished product. Finalized chromatographic conditions were applied and performed analytical method validation.

System suitability: System suitability evaluated by injecting six replicates of freshly prepared standard solution. Standard peak area, tailing factor, theoretical plates were measured and tabulated in Table-1. 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 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).

Specificity: To perform the specificity of this method blank, placebo and sample solution were injected and evaluate the interference from main peak, no interference observed from

TABLE-1
RESULTS OF PRECISION METHOD AND SYSTEM PRECISION

Standard No.	Dexamethasone		Tailing factor	Theoretical plate counts
	Assay (%)	Peak area		
1	99.0	4594	0.93	9502
2	98.7	4580	0.95	9529
3	99.5	4595	0.91	9560
4	99.4	4579	0.91	9677
5	99.7	4599	0.95	9482
6	99.3	4573	0.93	9624
Mean	99.3	4587	–	–
SD	0.33	10.63	–	–
RSD (%)	0.33	0.23	–	–

Values are expressed as mean ± standard deviation of six samples (n = 6)

the peak of blank and placebo solution to the main peak of dexamethasone, chromatograms of blank, placebo and sample solution as shown in Fig. 3.

Accuracy: Known amount of dexamethasone was spiked in placebo at about 50, 100 and 150 % of test concentration, each accuracy level were prepared in triplicate. The amount of dexamethasone recovered was quantified as per developed method. The percentage recovery was calculated from the amount found and actual amount added. The results are shown in Table-2. The overall recovery of dexamethasone in the samples was in between 98 to 102 % (RSD < 2 %), which is satisfactory for quantification of dexamethasone in finished product.

Linearity: Standard stock solution was used to prepare all linearity levels. All linearity levels were injected in duplicate into the chromatographic system. Correlation coefficient value was calculated and observed within acceptance criteria *i.e.* correlation coefficient (R^2) 0.995. A graph was plotted with concentration (μg/mL) of dexamethasone on X-axis and peak areas of dexamethasone on Y-axis. The results are tabulated in
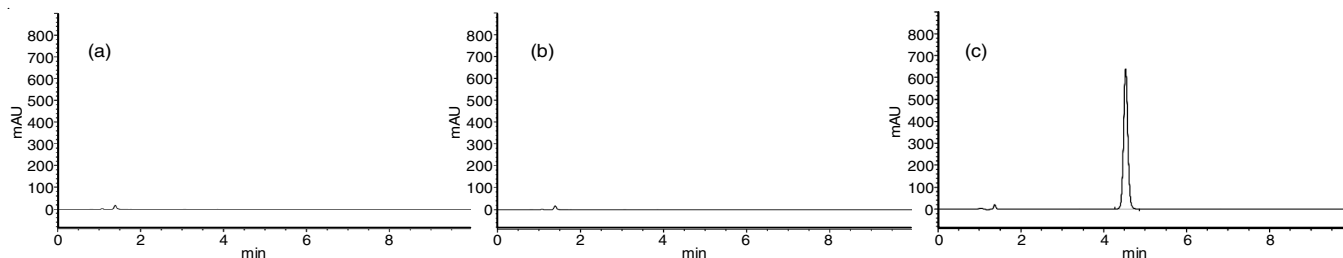

Fig. 3. HPLC chromatogram (a) blank, (b) placebo and (c) sample solution

TABLE-2
ACCURACY EVALUATION FOR QUANTIFICATION OF DEXAMETHASONE

Sample No.	Accuracy level (%)	Actual amount of API added (mg)	Amount of dexamethasone found (mg)	Recovery (%)	Mean	SD	RSD (%)
1	50	12.4747	12.3991	99.4			
2	50	12.4648	12.2637	98.4	99.0	0.55	0.56
3	50	12.4946	12.4045	99.3			
1	100	25.0488	24.8199	99.1			
2	100	25.0190	24.7792	99.0	99.2	0.30	0.30
3	100	24.9395	24.8334	99.6			
1	150	37.1955	37.3625	100.4			
2	150	37.2651	37.3977	100.4	100.5	0.10	0.10
3	150	37.2154	37.4248	100.6			

Values are expressed as mean ± standard deviation of replicate (n = 3)

Table-3. Excellent linear response has been observed with correlation coefficient (R^2) values of 0.999.

TABLE-3
RESULTS OF LINEARITY

Spike level (%)	Concentration (mcg/mL)	Average area (n = 2)
50	49.72	2324
80	79.55	3664
100	99.44	4587
120	119.33	5507
150	149.16	6866
Slope		45.77
Y-Intercept		38.37
Correlation coefficient®		0.99998

Robustness: Robustness performed by injecting blank and standard solution in duplicate, robustness of the method was verified by deliberately slight change in the chromatographic conditions. All the variations observed are found satisfactory (Table-4).

Solution stability: Solution stability has been performed for standard solution and sample solution at test concentration, monitored upto 12 and 10 h, respectively and found within the acceptable limit (< 2 %) (Table-5).

TABLE-5
SOLUTION STABILITY OF DEXAMETHASONE STANDARD AND SAMPLE SOLUTIONS

Time (h)	Standard solution		Sample solution	
	Area	% Deviation w.r.t. initial	Area	% Deviation w.r.t. initial
Initial	4594	NA	4579	NA
12 h	4561	0.72	4604	0.55
10 h	–	–	4593	0.24

Forced degradation: Forced degradation performed on 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 calculate percen-

tage assay of dexamethasone peak. The strategy used for forced degradation to produce desire amount of degradation *i.e.* 2-20 % and satisfactory degradation observed. On the basis of chromatograms generated from all forced degradation experiments, the degradation at each specific condition is shown in Table-6.

All the stress conditions applied were enough to achieve degradation. Under acidic and alkali stress conditions, dexamethasone was degraded up to 5.2 and 13.0 %, respectively. Under the exposure of oxidative, thermal and humidity stress conditions, dexamethasone was degraded upto 6.2, 2.7 and 2.2 %, respectively. Hence, it has been observed that dexamethasone is highly sensitive to alkali stress condition. The degradation products of dexamethasone were effectively separated by the developed method. Hence, this developed method is considered to be extremely selective and specific for future use.

Conclusion

A new and simple stability indicating RP-HPLC method for the routine sample analysis of dexamethasone in dexamethasone finished product was developed and validated. Present developed method do not use any buffer in mobile phase, which directly proportional to cost of stationary phase. 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 from excipients in a very short analytical run time. Solution stability and forced degradation study of dexamethasone proves that the method stability indicating and can be used for routine as well as stability sample analysis of finished product. This method can also be used for preparative, characterization, dissolution, content uniformity and blend uniformity of dexamethasone finished product.

CONFLICT OF INTEREST

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

TABLE-4
ROBUSTNESS EXPERIMENT

Robustness parameter	Retention time (min)	Tailing factor	Theoretical plates	% RSD of standard solution
Wavelength (238 nm)	4.50	0.91	9872	0.11
Wavelength (242 nm)	4.50	0.91	10179	0.09
Flow rate (0.90 mL/min)	4.98	0.92	10126	0.15
Flow rate (1.10 mL/min)	4.10	0.93	9567	0.10
Column temperature (25 °C)	4.52	0.93	9584	0.15
Column temperature (29 °C)	4.49	0.94	9514	0.12

TABLE-6
RESULTS OF FORCED DEGRADATION

Sample condition	Peak purity factor	Assay % of label claim	% Degradation with respect to control sample
Control sample (untreated sample)	Within threshold limit	99.0	NA
Acid treated (2 m l 0.1 N HCl)	Within threshold limit	93.8	5.2
Alkali treated (1 mL 0.01 N NaOH)	Within threshold limit	86.0	13.0
H_2O_2 treated (2 mL of 5 % H_2O_2 treated)	Within threshold limit	92.8	6.2
Thermal treated (sample exposed to 60 °C for 15 h)	Within threshold limit	96.4	2.7
Humidity treated (sample exposed to 75 % RH for 15 h)	Within threshold limit	96.9	2.2

REFERENCES

1. A.M. Watts, A.W. Cripps, N.P. West and A.J. Cox, *Front. Pharmacol.*, **10**, 294 (2019);
   https://doi.org/10.3389/fphar.2019.00294

2. C.H. Gill, P.N. Kulkarni, A.S. Nipate, C.K. Jadhav, A.V. Chate and A.M. Dodake-Supekar, *J. Drug Deliv. Ther.*, **8(6-s)**, 236 (2018);
   https://doi.org/10.22270/jddt.v8i6-s.2222

3. C.A.C. Jessurun, A.F.C. Hulsbergen, L.D. Cho, L.S. Aglio, R.D.S.N. Tewarie and M.L.D. Broekman, J. Neurooncol., 144. 249 (2019);
   https://doi.org/10.1007/s11060-019-03238-4

4. S. Shaikh, H. Verma, N. Yadav, M. Jauhari and J. Bullangowda, ISRN Anesthesiol., **2012**, Article ID 985495 (2012);
   https://doi.org/10.5402/2012/985495

5. Dexamethasone USP monograph, USP29-NF24-Page 643;
   http://ftp.uspbpep.com/v29240/usp29nf24s0_m23280.html

6. T. Thamaraikani, S. Mounika and S. Vijith, *Int. Res. J. Pharm. App. Sci.*, **2**, 5 (2012).

7. International Conference on Harmonization ICH guidelines, Q6A, Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances (2000);
   https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-test-procedures-acceptance-criteria-new-drug-substances-new-drug-products-chemical_en.pdf

8. G.M.S. Gonçalves and P.M.B.G.M. Campos, *Braz. J. Pharm. Sci.*, **45**, 1 (2009);
   https://doi.org/10.1590/S1984-82502009000100002

9. International Conference on Harmonization ICH-Q2 (R1), Guideline on Validation of Analytical Procedure: Text and Methodology (2005).

10. International Conference on Harmonization ICH guidelines, Q1A (R2): Stability Testing of New Drug Substances and Products (revision2) (2003).

11. H. Brummer, *Life Sci. Technol. Bull.*, **31**, 1 (2011).

12. R. Maheshwaran, *Pharm. Technol.*, **36**, 73 (2012).

13. European Pharmacopeia 7.0, European Directorate of Quality Medicines (2011).

14. M. Blessy, R.D. Patel, P.N. Prajapati and Y.K. Agrawal, *J. Pharm. Anal.*, **4**, 159 (2014);
    https://doi.org/10.1016/j.jpha.2013.09.003