Available online on 15.05.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

© 2011-18, publisher and licensee JDDT, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited

Open Access Research Article

Development and Validation of RP-HPLC Method for Desoximetasone in Bulk and Cream Formulation

Ashish Gorle*, Jayashri Mahajan, Prathamesh Bhave

Department of Pharmaceutics, R C Patel Institute of Pharmaceutical Education and Research Shirpur, Dhule, Maharashtra 425405, India

ABSTRACT

Desoximetasone chemically is 9-fluoro-IIβ21-dihydroxy-I6a-methylpregna-I.4-diene-3.The precise mechanism of the anti-inflammatory activity of topical steroids in the treatment of steroid-responsive dermatoses, in general, is uncertain. So, in present investigation chromatographic methods were developing use RP-HPLC for estimation of Desoximetasone in bulk and in cream formulation and method validation according to ICH guidelines. The main objective of this study was to develop a simple and reproducible method for desoximetasone by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). In this work the desoximetasone separation was carried out by using C18 cosmosile column (250mmx4.6mm particle size 5µm). By using 0.1% orthophosphoric acid pH adjusted up to 3 at uv detection of 240nm.The mobile phase was used at various ratio for gradient elution the ratio of mobile phase was 20:80 v/v. Methanol and water used for mobile phase and flow rate was being set at 1mL/min. The linearity of proposed method was found in range of r =0.9989. Statistically validation parameters such as linearity, accuracy, precision, LOD and LOQ were checked.

Keywords: Desoximetasone, RP-HPLC, Method validation.

Article Info: Received 24 March 2019; Review Completed 25 April 2019; Accepted 01 May 2019; Available online 15 May 2019

Cite this article as:

Gorle A, Mahajan J, Bhave P, Development and Validation of RP-HPLC Method for Desoximetasone in Bulk and Cream Formulation, Journal of Drug Delivery and Therapeutics. 2019; 9(3):154-159

http://dx.doi.org/10.22270/jddt.v9i3.2630

***Address for Correspondence:**

Dr Ashish Prakash Gorle, Department of Pharmaceutics, R.C. Patel Institute of Pharmaceutical Education & Research, Karwandnaka, Shirpur, Dhule, Maharashtra, 425405, India.

INTRODUCTION

Quantitative determination plays important role in several scientific field and instruments for chemical analysis are highly developed. Quantification depends on comparison of signals obtained for the sample with those of analyte standard with known concentration and composition. Analyte compounds are not easily available for every compound. For accurate measurements and analytical standards need to have high purity and stability. Purity is very essential for analytical standard compound and variety of chemical research area [1]. Spectroscopy in ultraviolet and visible special region is widely used for quantitative estimation of organic, inorganic and biological species. Ultra violet (UV) and absorption of visible radiation is related with excitation of electrons in both atoms and molecules from lower to higher energy level. UV spectroscopy in which light of ultra violet region is absorbed by molecule.

Derivative Spectrophotometric Method [2]

Derivative spectrometry in the UV-Vis region is applicable method in extracting qualitative and quantitative data by overlapping bands of the analyte and interferences.

Derivative spectroscopy plays very important role in multi-component analysis of mixture by UV-Vis molecular absorption spectrometry under computer controlled.

Derivative spectrophotometry it is an analytical technique it is consist of in the different normal spectrum by mathematical transformation of spectral curve into a derivative. This method Eliminates the influenced of background or matrix and provides more accurate fingerprints than traditional or direct absorbance spectra. This method improves resolution bands.

Area Under Curve technique

AUC method (area under curve) this method is use when there is no sharp peak and spectra occurred. This method involves calculation of integrated value of absorbance within two selected wavelengths. λ1 and λ2. Area calculation processing items find out with the help of area bound with curve and horizontal axis. The horizontal axis is selected with help of wavelength range in which area has to be calculated. According to repeated observation of linearity within area under curve and concentration on this basis wavelength range is selected. The zero order and first order

spectrum were used to calculate AUC. And the calibration curve was plotted by concentration versus AUC.

Concept of chromatography [3]

Chromatography is important biophysical method that enables the separation, identification, and purification of component of mixture for qualitative and quantitative analysis. Chromatography is based on principle where the mixture of substances is separated according to their affinity towards stationary phase and mobile phase is used for elution of separated substance.

It is based on the two phases

1. Stationary phase:

This phase is consisting of solid phase or liquid phase coated on surface of solid phase.

2. Mobile phase:

This phase is consisting of liquid or gaseous component.

Technique of chromatography

1. Ion exchange chromatography

2. Column chromatography

3. Affinity chromatography

4. Thin layer chromatography

5. Paper chromatography

6. Gas chromatography

7. Dye-ligand chromatography

8. Hydrophobic interaction chromatography

9. Pseudo affinity chromatography

10. High pressure liquid chromatography (HPLC)

Types of chromatography

a. Adsorption chromatography

b. Partition chromatography

c. Size exclusion chromatography

d. Ion exchange chromatography

a) **Adsorption chromatography[4]**

The substances in the mixture of analyte are interacting with solid stationary phase surface and this substance is displaced with eluent.

b) Partition chromatography

Partition chromatography it is a thermodynamic distribution between two liquids like phases. In this study taking consideration of relative polarities of stationary and mobile phase. Partition chromatography is divided into normal phase and reverse phase chromatography in normal phase chromatography, the nature of mobile phase is nonpolar and the nature of stationary phase. Polar sample are those on which column packing not more than less polar material. In the reversed phase chromatography, mobile phase is polar liquid, such as mixtures of water and methanol or acetonitrile and stationary phase is nonpolar (hydrophobic) in nature. In this more nonpolar samples strongly retained.

c) Size exclusion chromatography

In this chromatography solute are separated according to their molecular size. It includes stationary phase is solid with controlled pore size.

d) Ion exchange chromatography

It includes solid stationary phase containing anionic and cationic group on the surface. And in which the solute particles of opposite charges are attracted.

In the analysis of pharmaceutical high-performance liquid chromatography (HPLC) and High-performance thin layer chromatography (HPTLC) methods are widely used due to simplicity, precision, accuracy and reproducibility of results.

Terms in chromatography [5]

In chromatography the various terms are involved as follows.

- The peak maximum is the highest point of peak.
- The injection point is that point in which sample is placed on column.
- The dead point is position of peak maximum of unretained solute.
- The dead volume **(**V_0) is the volume of mobile phase passed thought the column between the injection point and the dead point. Thus, $V_0 = Qt_0$ where Q is the flow rate in ml/min and t_0 is dead time.
- The retention time (t_R) it is time interval between the injection point and the peak maximum**.** Each solute has a characteristic retention time.
- The retention volume (V_R) is the volume of mobile phase passed through the column between the injection point and the peak maximum Thus, $V_R = Qt_R$ where Q is the flow rate in ml/min and t_R is retention time. Each solute will also have a characteristic retention volume.
- The corrected retention time(t'_R) is the time elapsed between the dead point and the peak maximum
- The corrected retention value (V'_R) is the volume of mobile phase transfer through the column between the dead point and the peak maximum It will also be the retention volume minus the dead volume Thus, $V_R=V_R-V_0$=Q where Q is the flow rate in ml/min.
- The peak height **(**it is the distance between the peak maximum and the base line geometrically produced beneath the peak.
- The peak width at half height ($W_{0.5}$) is the distance between each side of a peak measured at half of the peak height. There is no significance and peak width measured at half height with chromatographic theory.
- The peak width at the base (W_b) is the distance between the intersections of the tangents drawn to the sides of the peak and the peak base geometrically produced
- The peak width has significance in chromatographic theory and peak width is equivalent four standard deviation.

The standard analytical procedure of newer drug may not be available in pharmacopoeia hence it is essential to developed analytical method validation such as linearity, accuracy, precision, sensitivity.[8] The chemical name of Desoximetasone is 9a-fluro-11ß,21-dihydroxy-16a-methyl pregna-1,4diene 3,20-dione. It is used as anti-inflammatory agent corticosteroid and dermatological preparations [6]. It is topical corticosteroids represent the treatment of choice for numerous different inflammatory skin diseases, in particular atopic eczema. [10] In present study the new method was

developed methanol as a solvent in HPLC. It is the simple method for desoximetasone separation from mixture of compound.

Drug profile

Desoximetasone Structure [7]

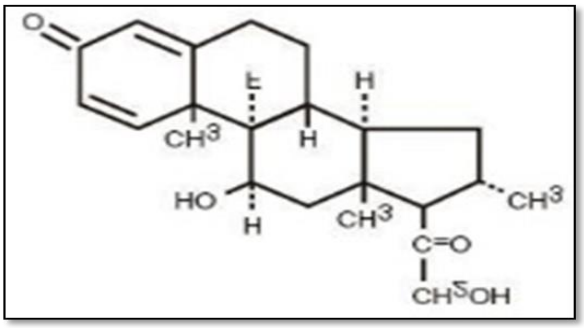

Figure 1: Desoximetasomne Structure

MATERIALS AND METHOD [9]

Methanol and orthrophosphoric acid were used as HPLC grade. All reagents were used as analytical grade and reverse osmosis, ultra-pure water, water purification system, sonicator, filtration assembly and pure drug of desoximetasone drug.

Chromatographic system

The RP-HPLC system composed of Agilent 1200 series instant pilot software: Chemstation Plus and certified for pharmaceutical QA/QC. It constitutes micro vacuum degasser G1379B, binary pump G1312B, variable wavelength detector SL G1315C, thermostatted column compartment G1316B with C18 column [4.6x250mm, pore size 5µm], high performance auto sampler G1367C, thermostat for high performance auto sampler G1330B. It is most flexible configuration for the maximum in gradient and low flow rate accuracy and precision, high-speed, multiwavelength and full spectral UV-visible detection for peak purity analysis and spectral confirmation.

Chromatographic condition

The mobile phase prepared by using two solvent containing methanol and water filter through 0.5µm filter paper (pH adjusted to 3 with 0.1% orthrophosphoric acid). Degassed the mixture with sonication. The detection was carried out at 240nm by using variable wavelength detector.

Preparation of standard stock solution

Stock solution was prepared by dissolving 10mg of desoximetasone in 10 ml of methanol that gives concentration of 100µg/ml of desoximetasone.

Preparation of mobile phase

For the preparation of mobile phase methanol and water were used. The ratio of mobile phase was 20:80v/v. filtrated the mobile phase by using 0.5µm size of filter paper. Then sonicated that mixture for 15 min. and added 0.1% orthrophosphoric acid. And these mixture of solvent used as mobile phase.

Optimization of Method

The main aim of the HPLC method is to develop and validation of desoximetasone. There are different mobile phases were tried for the method development but good resolution observed with methanol and water. PH adjusted up to the 3 by using orthrophosphoric acid. C18 column (250mmx4.5mm particle size 5µm) was used.

Table 1: Optimization of Method

Chromatographic Mode	Chromatographic Condition
HPLC system	Agilent
Pump	Gradient
Detector	Wavelength variable detector
Data processor	Chemstation software
Stationary phase	C_{18} column cosmosile (250mmx4.6mmx5µm)
Mobile phase	Water: Methanol (20:80v/v) pH adjusted up to 3 with OPA)
Detection of wavelength	240nm
Flow rate (mL/min)	1.0
Sample size	20µl.

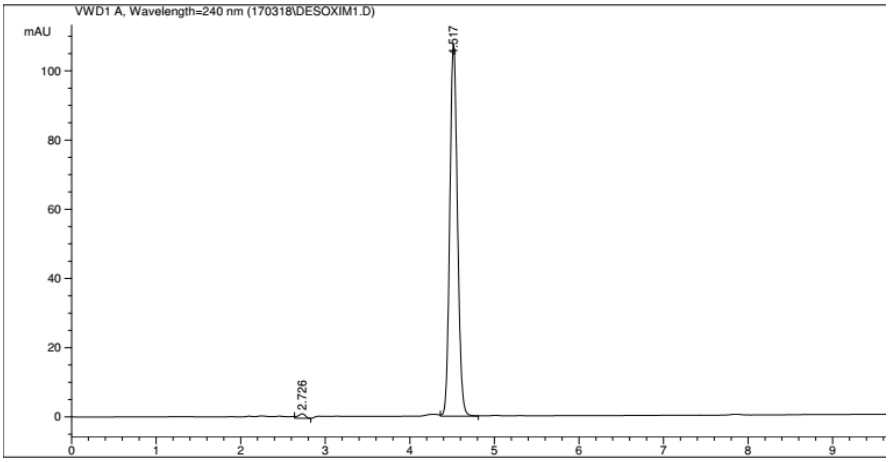

Figure 2: Chromatogram of desoximetasone showing retention time 4.5 min

Validation of method [13]

Validation of optimize method was carried out by following parameters.

Linearity

From stock solution of desoximetasone 0.3-1.8ml concentration were taken in 10 ml. volumetric flask diluted up to the mark with mobile phase such that final concentration of desoximetasone in the range of 3-18µg/ml. volume of 20µl of each sample solution was injected with the help of syringe all measurement was repeated for three times for each concentration and standard calibration curve were plotted peak area vs concentration.

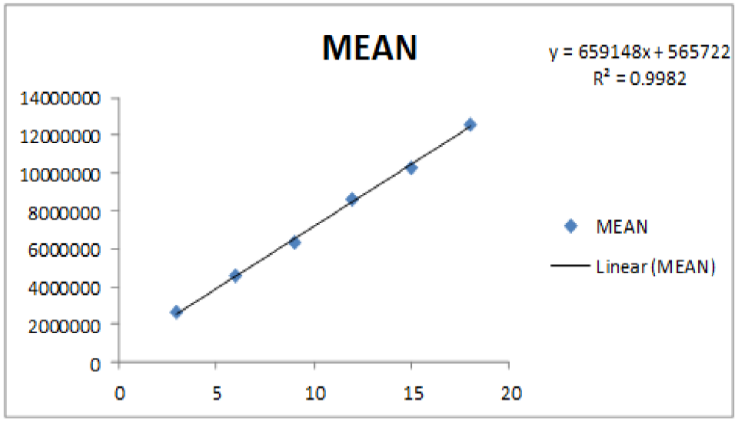

Figure 3: Linearity curve of Desoximetasone

Table 2: Linearity study of desoximetasone

Parameters	Desoximetasone
Linearity range (µg/ml)	3-18
Slope	659148
Intercept	565722
Correlation coefficient(r^2)	0.9982

Analysis of bulk formulation

Accurately weighed 10 mg of desoximetasone dissolved in 10 ml of methanol. And the solution was diluted with the prepared mobile phase and this method is subjected for the proposed method amount of desoximetasone calculated by linear regression equation and the same concentration was repeated for six times.

Table 3: Analysis of bulk formulation Study

Sr.no	Amount taken	Amount Found (µg/ml)	% Amount Found ± SD	% RSD
1.	12	11.99	99.22 ±0.60	0.603

Analysis of cream formulation [11]

Determined the content of desoximetasone in marketed cream (label claim 0.25% of desoximetasone 10mg/ml) 10gm cream was weighed and average weight was calculated. The drug was dissolved in 100ml methanol and the extraction was sonicated for 15 min and centrifuge at 300rpm.Then 1ml solution from it was diluted with 10 ml mobile phase. The resulting solution was injected in HPLC and drug peak area was noted. The peak area regression equation and amount of desoximetasone in sample was calculated.

Table 4: Analysis of cream formulation Study

Sr.no	Amount taken	Amount Found (µg/ml)	% Amount Found ± SD	% RSD
1	12	11.96	99.69 ± 1.93	1.94

Accuracy

Accuracy was done by recovery study using standard addition of method at 80%,100%, and 120% level known amount of desoximetasone standard was added to reanalyzed sample and subjected them to the proposed HPLC method. Results are shown in below.

Table 5: Recovery study

Sr. no	Initial amount (µg/ml)	Amount of standard drug added (µg/ml)	Amount of drug added %	Amount Recovered (µg/ml)	%Drug Recovered Mean ± SD	% RSD
1	9	7.2	80	16.21	99.85 ± 0.27	1.69
2	9	9.0	100	18.07	100.40 ±0.14	0.82
3	9	10.8	120	19.77	99.87 ±0.19	0.99

Precision

The precision study was carried out by including the repeatability study Inter day and intraday precision. Repeatability study was carried out by injecting 2µg/ml. concentration for six times and intraday precision was carried out by analyzing one concentration in different time period of same day. Inter day precision study was carried out by injecting the one concentration in different day of varies in time period.

Table 6: Precision study

Precision	Amount taken (µg/ml)	Amount Found (µg/ml)	% Amount Found (µg/ml) Mean ± SD	% RSD
Repeatability (n=6)	12	11.99	99.94 ±0.78	0.78
Intraday (n=6)	9	11.90	99.28 ± 2.88	1.31
	12	11.94	99.77 ±2.83	1.52
	15	11.95	99.81 ±2.81	1.58
Inter day (n=6)	9	11.89	99.20 ±2.90	1.48
	12	11.95	99.81 ±2.85	1.27
	15	11.96	99.99 ±2.78	1.82

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of detection is the lowest amount of analyte in sample which can be detected but not necessarily quantitated as an exact value. Formula for LOD is 3.3σ/s and LOQ is the lowest amount of analyte in sample that can be quantitatively determined by suitable accuracy and precision. Formula for LOQ is 10 σ/s.

Table 7: Sensitivity Study

LOD (µg/ml)	0.31
LOQ (µg/ml)	0.95

Table 8: Ruggedness Study

The ruggedness analysis was done by two different analysts on the environmental condition. These analyses were done by six time of same concentration by different analyst.

Amount taken (µg/ml)	Analyst –I (n=6)			Analyst –II (n=6)		
	Amount found	% Amount found ± SD	% RSD	Amount found	% Amount found ± SD	% RSD
12	11.94	99.54 ± 1.38	1.39	12.02	100.18 ± 0.79	0.65

CONCLUSION

RP-HPLC method it is a simple precise and accurate method was developed and validated for routine analysis for desoximetasone in bulk and cream formulation. The proposed method could be successfully applied for the routine analysis and quality control of dosage form containing desoximetasone.

REFERENCES

1. Ohira et. al, Kaneda S.I., Matsuzaki K., Mori T., Mori S., M. and Toda, K., 2018. Universal HPLC detector for hydrophilic organic compounds by means of total organic carbon detection. *Analytical chemistry*.

2. Willard, Metritt H. H.et.al, Dean L. L., Settal J, A., Instrumental methods of Analysis, 7thedn., CBS Publishers and Distributers, 1986, p.118.

3 Coskun O.et.al, Yerleskesi T., Binasi D., Separation techniques: Chromatography, North Clin Istanb. 2016; 3(2):156–160.

4. Skoog West D. A., Holler D. M., F. J and Crouch S. R., Fundamental of analytical chemistry, 8thedn, Thomson Brooks/Cole, 2007, pp.1-5, 355, 906-909.

5. Scott P., Principles and Practice of Chromatography, Chrome-ED Book Series; pp.1-2, 12-14.

6. Srinivas P., Sudhakarbabu K. And Sreeramulu, J., Identification, characterization of degradation component in desoximetasone pharmaceutical dosage forms and its quantification in the presence

of process related impurities. Journal o K. f Liquid Chromatography & Related Technologies, 2012; 35(8):1114-1129.

7. Heel R.C., Brogden R.N., Speight T.M. and Avery G.S., Desoxymethasone: A Review of its Pharmacological Properties and Therapeutic Efficacy in the Treatment of Dermatoses. *Drugs*, 1978; *16*(4):302-321.

8. Gali H. and Yerragunta V., Development and validation of RP-HPLC method for simultaneous estimation of naproxen and esomeprazole in pharmaceutical dosage form. Asian Journal of Research in Chemistry, 2016; 9(8):366.

9. Saravanan V, Revathi R, Meera N, Method Development and Validation for the Simultaneous Estimation of Lycopene and Ubidecarenone By RP-HPLC in Combined Pharmaceutical Dosage Form. Journal of Drug Delivery and Therapeutics, 2016; 6(5):46-51.

10. Borelli, Gassmueller C., Fluhr J., Nietsch J.W., K.H., Schinzel K.H., S. and Korting, H.C., Activity of different desoximetasone preparations compared to other topical corticosteroids in the vasoconstriction assay. *Skin pharmacology and physiology*, 2008; *21*(3):181-187.

11. Sheliya, K., Shah, K. and Kapupara, P., Development and validation of analytical method for simultaneous estimation of mometasone furoate, hydroquinone and tretinoin in topical formulation by RP-HPLC. Journal of Chemical and Pharmaceutical Research, 2014'6(4):934-9408.

12. Hadad, G.M., Emara, S. and Mahmoud, W.M., Development and validation of a stability-indicating RP-HPLC method for the determination of paracetamol with dantrolene or/and cetirizine and pseudoephedrine in two pharmaceutical dosage forms. *Talanta*, 2009; *79*(5):1360-1367.

13. ICH, Q2B, Harmonised tripartite guideline, Validation of analytical procedure: Methodology, International conference on harmonization, Geneva, Switzerland, March 1996.

14. Vladimirov S., Čudina O., Agbaba D., Jovanović M., and Živanov-Stakić D., Spectrophotometric determination of desoximetasone in ointment using 1,4-dihydrazinophthalazine. Journal of pharmaceutical and biomedical analysis, 1996; 14(8-10):947-950.

Journal of Drug Delivery & Therapeutics
JDDT