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Research Article

DEVELOPMENT AND VALIDATION OF A RAPID HPLC METHOD FOR DETERMINATION OF DEXAMETHASONE IN BULK AND PHARMACEUTICAL DOSAGE FORM

Thamaraikani Tamilanban, **Mounika. S**, Vijith. S
Deparment of Pharmacology, Bharat institute of Education and Technology (BIET), Chennai, Tamilnadu, India.
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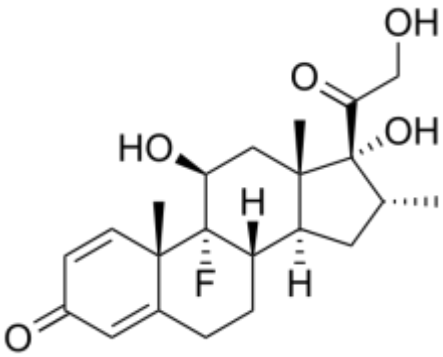
Corresponding Author's email: sadinenimounika1989@gmail.com

Abstract: A simple and reliable reversed-phase high performance liquid chromatographic (HPLC) method was developed and validated for dexamethasone. The method was employed on a thermo hypersil BDC column (250 mm × 4.6 mm, 5 µm) at ambient temperature. The mobile phase consisted of buffer(0.1Mpotassium dihydrogen phosphate adjust the PH2.3 with ortho phosphoric acid: methanol (60:40) at a flow rate of 1 ml/min. The detection wavelength was set at 226 nm and 10 µL of sample was injected into the HPLC system. Dexamethasone was used as the internal standard. The retention time for internal standard is 4.189mins. The method was linear and correlation coefficient is found to be1.000 The method presented the requisite accuracy, selectivity, sensitivity and precision and showed good results. The proposed method was successfully used for analysis of the drug

Key words: HPLC, Validation, Dexamethasone, RP-HPLC

INTRODUCTION

Dexamethasone belongs to the class of steroidal anti-inflammatory, immunosuppressant drug[1]. The IUPAC name of Dexamethasone is (1R,2S,10S,11S,13R,14R,15S,17S)-1-fluoro-14,17-dihydroxy-14-(2-hydroxyacetyl)-2,13,15-trimethyltetracyclo[8.7.0.0^{2,7}.0^{11,15}]hepta deca-3,6-dien-5-one. It has a molecular weight of 392.4611 and the molecular formula $C_{22}H_{29}FO_5$. [2.] The drug is manufactured in pharmaceutical formulation, profound search from data and literature available, it reveal that many methods have been reported including LC-MS[3,5], ultraviolet spectrophotometry, high performance liquid chromatography[4], Very few reports have appeared dealing with the estimation of dexamethasone by HPLC method[6] . Taking simplicity, cost-effectiveness, in the sector of chromatographic techniques for pharmaceutical analysis into account, HPLC method was developed.



Dexamethasone

Instrumentation

The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software and thermo hypersil BDC column (250mmx4.6mm,particle size 5µm).

Chemicals and Reagents

Dexamethasone was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Methanol of HPLC grade were purchased from E.Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

HPLC conditions

The mobile phase consisting of potassium dihydrogen phosphate buffer (pH 2.3 adjusted with orthophosphoric acid) and methanol (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 60:40v/v was pumped into the column at a flow rate of 1.0ml/min. The detection was monitored at 226nm and the run time was 10min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

Preparation of standard solution:

An accurately weighed quantity, 12.8 mg of dexamethasone was transferred into 100ml of volumetric flask and add 30ml of water and

sonicate for 15 mins make up the volume with water.

Preparation of sample preparation:

An accurately weigh 8 tablets and calculate average weight of those tablets and crushed.transfer the tablet powder equivalentl weight of 1736mg of standard drug.into 50ml of volumetric flask add 25ml of water and sonicate for 30mins and filter through the 0.45µm filter paper and make up the volume with water.Transfer above solution 5ml into 25 ml volumetric flask and make up the volume with water.

Validation of proposed method

Selectivity of the method was assessed on the basis of elution of dexamethasone using the above mentioned chromatographic conditions. To study the specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters has been validated for the determination of dexamethasone. The results are furnished in Table-3.

Specificity

The specificity was established by preparing a dexamethasone standard at 0.5% level of test concentration and injected 6 times into HPLC system as per the test procedure.

Linearity

The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits.

Precision

The precision of the assay was determined in terms of intra-day and inter-day precision. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.) obtained by multiplying the ratio of standard deviation to mean with 100. The results are furnished in Table-4.

Limit of detection (LOD) and limit of quantitation (LOD)

The LOD and LOQ for dexamethasone were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of dexamethasone

Accuracy

The accuracy of the HPLC method was assessed by adding known amounts of sample solutions of dexamethasone at 50%, 100% and 150% of the specification were prepared in triplicate to the test solutions and injected into the HPLC system as per the proposed method. The results are furnished in Table-5.

RESULTS AND DISCUSSION

By applying the proposed method, the retention time of dexamethasone in a typical chromatogram was found to be 4.189min, which indicates a good base line. Linearity range was observed. A typical chromatogram was found to be 4.106min, for linearity which indicates a good base line.

The regression equation of dexamethasone concentration over its peak area ratio was found to be Y=17939X+10567 (r=1.000) where Y is the peak area ratio and X is the concentration of dexamethasone(µg/ml). The proposed HPLC method was also validated for intra-day and inter-day variation. the coefficient of variation in the peak area of drug for three replicate injections was found to be less than 1%. Also, the inter-day variation on three different days was found to be less than 1%. The number of theoretical plates was found to be 5877, which indicates efficient performance of the column. To optimize the chromatographic conditions, various combinations of potassium dihydrogen phosphate buffer with methanol were tested. The use of potassium dihydrogen phosphate buffer with methanol in the ratio of 60:40v/v resulted in peak with good shape.No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by proposed HPLC method.

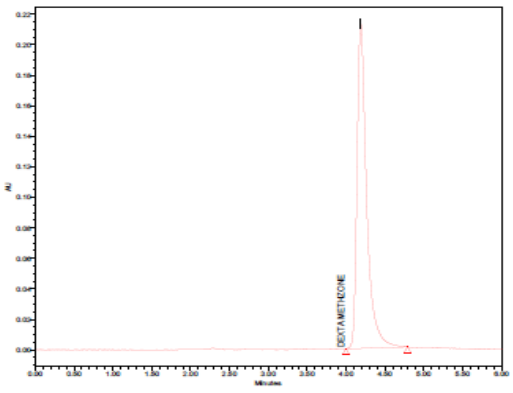


Fig.1 chromatogram of Dexamethasone

Table 1Calibration of proposed HPLC method for the estimation of dexamethasone:

Concentration	Peak ratio
50	909899
75	1352969
100	1802249
125	2256795
150	2700340

Table2 System suitability parameters

S No	Parameters	Result
1	Correlation coeficient	0.999
2	Theoretical plates	6398
3	Tailing factor	1.662
4	LOD	0.00028µg/ml
5	LOQ	0.00094µg/ml

Table 3 Accuracy results of Dexamethasone

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	%recovery	mean
50%	557.50	909441	39.510	39.39	100	99
50%	548.80	905107	39.604	39.20	99	
50%	547.10	900531	39.481	39.01	99	
50%	548.50	908792	39.582	39.36	99	
50%	547.90	907394	39.539	39.30	99	
50%	548.20	907556	39.560	39.31	99	
100%	1094.1	1817414	78.955	78.72	100	100
100%	1095.9	1816299	79.085	78.67	99	
100%	1094.4	1815739	78.976	78.65	100	
150%	1642.80	2708338	118.551	117.31	99	99
150%	1642.80	2723656	118.537	117.97	100	
150%	1643.10	2716258	118.573	117.65	99	
150%	1643.70	2714427	118.616	117.57	99	
150%	1643.25	2701037	118.584	116.99	99	
150%	1644.10	2707291	118.645	117.26	99	

Table 4 Precision results for dexamethasone

S NO	Sample weight	Area	%Assay
1	1095	1809480	99
2	1094	1806694	99
3	1096	1800155	99
4	1095	1808754	99
5	1096	1803737	99
6	1094	1806107	99

Table5 Robustness of Dexamethasone

S No	Sample name	change	Name	RT	Area	Tailing	Platecount
1	Flow1	1.2ml/min	Dexamethasone	5.055	2163572	1.625	6565
2	Flow2	0.8ml/min	Dexamethasone	3.432	1478761	1.605	5725
3	Temp1	50^{o}C	Dexamethasone	5.067	2271640	1.610	6855
4	Temp2	40^{o}C	dexamethasone	4.973	2300198	1.636	6904

Table6 LOD and LOQ

	Sample name	inj	name	RT	Area
1	LOD	1	Dexamethaone	4.117	74307
2	LOQ	1	dexamethasone	4.112	147574

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the determination of dexamethasone in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of dexamethasone in pure and its pharmaceutical dosage forms.

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