ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF BETAMETHASONE BY USING UV SPECTROPHOTOMETRY

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ABSTRACT:

A simple, rapid, precise and cost effective UV-Visible spectrophotometric method has been developed for estimation of Betamethasone in its crude form and in pharmaceutical dosage form. Betamethasone shows maximum absorbance at 234nm. The method was carried out by using Sulphuric acid as a solvent. Beers law obeyed in the concentration range of 5-30µg/ml and correlation coefficient was found to be 0.998. The proposed method was statistically validated for precision, accuracy, ruggedness, robustness, limit of detection, limit of quantification as per the ICH guidelines. Hence this method can be successfully applied for routine analysis of Betamethasone in crude form and also in pharmaceutical dosage form.

KEYWORDS: Betamethasone; Sulphuric acid; UV-spectroscopy; validation.

INTRODUCTION

Its molecular formula is C22H29F05 and molecular weight is 392.5g/mol. It is freely soluble, in sulphuric acid, water and slightly soluble in chloroform, Methanol. Betamethasone is a 50% orally effective and topically active drug. Initially Betamethasone described as an anti Inflammatory agent but it also shows some new applications, especially in the treatment of psoriasis.

According to the literature survey, few analytical methods were reported for the estimation of Betamethasone with other drug combinatons. The other methods were also proposed for its determination includes RP-HPLC and LC. The present investigation is to develop a simple, rapid, precise and cost-effective UV method for method development and validation of Betamethasone in crude form and in pharmaceutical dosage form.

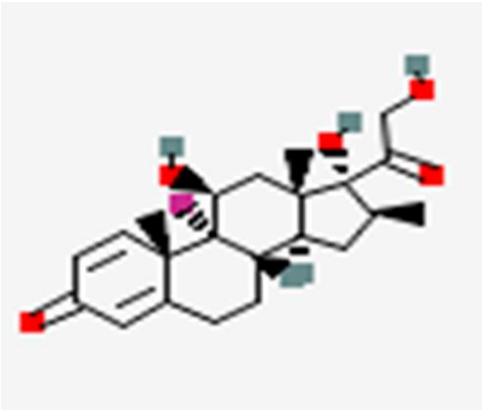

Figure 1: Structure of BETAMETHASONE

MATERIALS AND METHODS:

Single pan electronics balance-sartorious G412, UV visible double beam spectroph-otometer (systronics-2203 smart), matches quartz cells corresponding to 1cm path length. Betamethasone was taken as a gifted sample from sun pharmaceuticals pvt. Ltd.

Reagents: Sulphuric acid, Betamethasone, Reference Standard.

Preparation of standard stock solution: The standard stock solution was prepared by dissolving 50mg of drug in30ml of sulphuric acid to produce a 50ml µg/ml. From the above solution, 1ml of stock solution is withdrawn and diluted with 50ml of sulphuric acid to produce 20µg/ml concentration.

Determination of λmax:

20µg/ml concentration of Betamethasone was prepared and scanned under UV from 220-300nm. The λmax of the drug was found at 234nm.

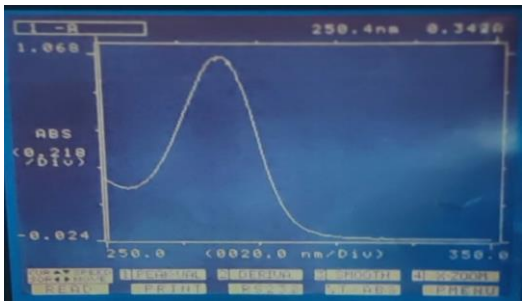

Figure 2: Determination of λmax BETAMETHASONE

Beer's law concentration range: The stock solution was suitably diluted with sulphuric acid to get a concentration range from20µg/ml and their absorbance was measured at 348.7nm. Using the absorbance values against concentration, calibration curve was plotted. From the graph it was found that, Betamethasone obeys beer's law between 5-30µg/ml.

Table 1: Linearity data for BETAMETHASONE

S.No.	Concentration (µg/ml)	Absorbance
1	5	0.136
2	10	0.284
3	15	0.445
4	20	0.594
5	25	0.72
6	30	0.879
	slope	= **0.030749**
	Correlation coefficent	= **0.998**

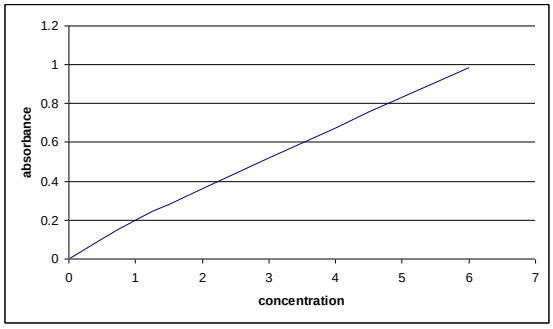

Figure 3: Linearity graph of BETAMETHASONE

Preparation of sample solution: 10 tablets were finely powdered. An accurately weighed quantity of powder equivalent to 50 mg of Betamethasone was transferred to 50ml standard flask. This was diluted with 50ml sulphuric acid to give 50µg/ml. fro m this 4ml of sample solution was taken and diluted with 20ml of sulphuric acid to give 20 µg/ml concentration. The solution was filtered and absorbance value of sample solutions was recordered at 234nm.

Method validation

Validation is defined as the establishing evidence which provides high degree of assurance that a specific process will consistently produce a product meeting its determined specification quality characterristics. The following parameters used for validation studies are

Precision: The closeness of agreements between a series of measurements, multiple sampling of homogenous samples under prescribed condition, precision is of two types:

- Repeatability
- Reproductibility

Repeatibility (System precision): 4µg/ml concentration solution of Betamethasone was prepared whose absorbance measured six times for which relative standard deviation was calculated.

Reproducibility (Method precision): Six individual preparations of Betamethasone was prepared with a concentration of 20µg/ml, whose absorbance was measured at 234 nm.

Solution stability: 20µg/ml concentration solution of Betamethasone was prepared and the solutiuon whose absorbance was measured for every half an hour for 90min and the solution were found to be stable upto 90min.

Limit of detection: The detection limit of an individual analytical procedure is the lowest amount of in a sample which can be detected but not necessarily quantitated as an exact value.

$$\textbf{LOD} = \frac{3.3 \times S.D}{slope}$$

Table 2: Results for LOD

S.No.	Parameter	Betamethasone
1	Slope	0.0385
2	Standard deviation	0.01948
3	LOD	1.6697

Limit of quantitation: The limit of quantitation of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with precision and accuracy.

$$\text{LOQ} = \frac{\mathbf{10} \times \boldsymbol{S.D}}{\boldsymbol{slope}}$$

Table 3: Results of LOQ

S.No.	Parameter	Betamethasone
1	Slope	0.0385
2	Standard deviation	0.01948
3	LOQ	5.059

Accuracy: Accuracy of the method is the closeness of the measured value to the true value for the sample. Accuracy is usually by recovery studies.

Recovery studies are performed by spiking pure powdered drug into the sample solution. The spiked samples are prepared at a concentration range of 90%, 100%, 110%.

Procedure: The sample solution was prepared to get a concentration range of 20µg/ml into which 50mg pf pure powdered drug was added to get 90%, 100%, 110% concentration range. The percentage recovery was calculated for these concentrations from absorbance obtained.

The percentage recovery can be calculated by using the following formula;

$$\textbf{Percentage recovery} = \frac{amount\ obtained}{amount\ added} \times 100$$

The percentage recovery for the spiked preparation should be within 98 - 102%.

Ruggedness: The extent to which is turned precision should be eastablished depends on the circumstances which the procedure is intended to be used. Intermediate precision expresses within laboratory variation i.e., different days, different analysts, and different equipments.

Procedure: The procedure followed for this is the same followed in the method precision was repeated on two different days by two different analyst. The result for

the intermediate precision recorded in the table.

Acceptance criteria: The relative std. deviation for the preparation should not be more than 2%.

Table 4: Results for formulation 0.5mg

S.No.	Conc. (µg/ml)	Label claim (mg)	Amount present	% of label claim	% deviation
1	15	20	0.302	101.55	0.0155
2	20	20	0.409	96.97	0.030
3	25	20	0.492	99.27	0.0073

Table 5: Results for system precision

S.No.	Conc. (µg/ml)	Absorbance
1	20	0.592
2	20	0.567
3	20	0.572
4	20	0.568
5	20	0.574
6	20	0.577
	Average	**0.575**
	Std. deviation	**0.00833**
	% RSD	**1.44**

Table 7: Results of method precision

S.No.	Conc. (µg/ml)	Absorbance	Average
1	20	0.628	0.529
		0.636	
2	20	0.594	0.517
		0.596	
3	20	0.622	0.530
		0.623	
4	20	0.625	0.527
		0.625	
5	20	0.596	0.529
		0.595	
6	20	0.632	0.535
		0.628	
		Average	**0.527**
		Std. deviation	**0.00595**
		% RSD	**1.129**

Table 8: results for % recovery studies

S.No.	Conc. (µg/ml)	Mg found	Mg added	% recovery
1	90	2.48	2.5	98.6
2	100	2.52	2.5	101.6
3	110	2.50	2.5	92.2

Table 9: Ruggedness parameters

Analyte	Conc. (µg/ml)	%SD	% RSD
Analyte-1	20	0.0056	1.12
Analyte-2	20	0.0083	1.44

Table 10: robustness parameters

S.No.	Conc. (µg/ml)	Wavelength (nm)	Absorbance
1	20	225	0.315
2	20	226	0.318
3	20	227	0.317

Robustness: Robustness of the method is its ability to remain unaffected by small changes in parameters such as changes in wavelength, changes in p^H, changes in temperature etc.

Robustness examines the effect of operation parameters on the analytical method.

Procedure: 20µg/ml concentration of Betamethasone was prepared. Absorbance was measured at two different wavelengths closer to the λmax of the drug.

Acceptance criteria: No change in the absorbance

Results and Discussions: Under UV spectral analysis, the absorption maxima (λmax) of Betamethasone was observed at 234nm and tabulated in table-2. Obeyance to beer's law was confirmed by the linearity of the calibration curve of Betamethasone, which represented in figure-3.

Betamethasone showed linearity in the concentration range of 1-6µg/ml and are given in table-3

The quantitative estimation was carried out in capsule formulation by taking concentrations of 20µg/ml and tabulated in table-4.

the validation of the studies the proposed method was further confirmed by recovery studies. The recovery data is given in tabl-e-8. The recovery values vary from 98 – 102%. This serves as a good index of accuracy of the method.

Conclusion: On the basis of our experimental results, we conclude that the UV spectrophotometric method developed for the determination of Betamethasone was found to be rapid, precise, accurate and cost effective. Hance this method can be used for the routine analysis of Betamethasone in bulk and pharmaceutical dosage forms.

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