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Development and validation of UV method for identification and qualification of Betamethazone in API and drug formulation including stability studies.

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Abstract: . :

Betamethasone is a corticosteroid that is available as a pill, by injection, and as an ointment, cream, lotion, gel, or aerosol for the skin, and a foam for the scalp.A rapid, simple, selective and precise UV-Visible Spectrophotometric method has been developed for the determination of Betamethazone in bulk forms and dosage formulations. The spectrophotometric detection was carried out at an absorption maximum of 241nm using ethanol as solvent. The method was validated for specificity, linearity, accuracy, precision, robustness and ruggedness. The detector response for the Betamethazone was linear over the selected concentration range 2 to 40µg/ml with a correlation coefficient of 0.9999. The accuracy was between 91.2%. The precision of 10 µg/ml sample preparation three times in a day (intraday) was 0.1325%. The Limit of Detection (LOD) and Limit of Quantification (LOQ) are 0.84 and 2.55 µg/ml, respectively. The recovery of Betamethazone was about 101.84%. The results demonstrated that the excipients in the commercial formulation did not interfere with the method and can be conveniently employed for daily routine quality control analysis of Betamethazone in bulk drug, marketed formulations.

Key words: Betamethazone, Validation, UV spectrophotometric method.

INTRODUCTION:

Betamethasone is a corticosteroid that is available as a pill, by injection, and as an ointment, cream, lotion, gel, or aerosol for the skin, and a foam for the scalp. When given by injection, anti-inflammatory effects begin in around two hours and last for seven days. It is used as a topical cream to relieve skin irritation, such as itching and flaking from eczema. It is used as a treatment for local psoriasis, as betamethasone dipropionate and salicylic acid, or as the combination calcipotriol/betamethasone dipropionate. Betamethasone sodium phosphate is used orally and via injection with the same indications as other steroids. Many betamethasone-based pharmaceuticals include the steroid as the valerate ester.

In a randomized controlled trial betamethasone was shown to reduce some of the ataxia (poor coordination) symptoms associated with ataxia telangiectasia (A-T) by 28-31%.

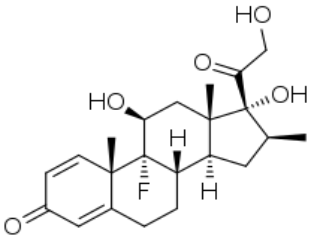


Figure 1. Structure of Betamethazone

Betamethasone is also used to stimulate fetal lung maturation in order to prevent infant respiratory distress syndrome (IRDS) and to decrease the incidence and mortality from intracranial hemorrhage in premature infants.

UV Spectroscopy:

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Any molecule has either n, π or σ or combination of these electrons. These bonding (σ and π) and non-bonding electrons absorb the characteristic radiation and undergoes transition from ground state to excited state. By the characteristic absorption peaks and the nature of the electron present the molecular structure can be elucidated UV spectroscopy obeys the Beer-Lambert law, Beer law: This law can be stated as follows: "When a beam of monochromatic radiation is passed through a solution of absorbing substances, the intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substances exponentially". I=I0*e-k1 *c Where, I0 = intensity of light incident upon sample cell I = intensity of light leaving sample cell C = molar concentration of solute K1=constant Lambert's law: This law can be stated as follows "When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light".

$$I=I0*e-k2 *l$$

Where,

I0 = intensity of light incident upon sample cell
I = intensity of light leaving sample cell
L = length of sample cell (cm.)

K2=constant After combining equation 1 and 2 and deriving we get the following equation 3 of Beer Lambert law as: A = log (I0/I) = ƐCl
Where, A = absorbance
I0 = intensity of light incident upon sample cell
I = intensity of light leaving sample cell
C = molar concentration of solute L = length of sample cell (cm.)
Ɛ = molar absorptivity

Instrumentation:
Double beam UV spectrophotometer; Model: SL 210; Make: ELICO. The data was obtained using Spectra Treats the analysis was performed using UV SL120 using UV detector used for method development and validation. The output signal was checked and integration of data was performed using spectral threats. Software on a computer. The diluents are filtered through 0. 25µm.detection was detection was monitored at 241nm.

2.MATERIALS AND METHODS:

Betamethazone (ointment) was collected from market with drug equivalent. All the other reagents and chemicals used were of analytical grade.

2.1 Instrument

Double beam UV Visible Spectrophotometer

2.2 Chemicals:

Betamethazone (ointment) was collected from market with drug equivalent. All the other reagents and chemicals used were of analytical grade.

3.METHOD DEVELOPMENT

3.1 Preparation of Standard Stock Solution

Accurately weighed 10 mg of standard Betamethazone was dissolved in 10 ml of ethanol (standard stock solution). From this standard stock solution, prepare the aliquots of different concentration by suitable dilutions varying in between 2 and 40 µg/ml using ethanol and distilled water. These diluted solutions were checked for Linearity, Precision, Accuracy, Robustness, Limit of Quantification (LOQ) and Limit of Detection (LOD).

3.2 Method optimization

Selection and Optimization of Solvent .As reported in literature, the solvent have a profound influence on the shape and quality. The choices of solvents for ultra violet method development are: ethanol, methanol, acetone, etc. Various solvents were checked and ethanol was found to fulfill all the conditions relating to quality and non-interference of peak at the specified wavelength.

3.3 Selection of wavelength

In order to determine the wavelength of absorption maxima (ʎmax) of Betamethazone, aliquot of 1000 µg/ml solution was prepared by taking weighed amount of drug (10 mg) in 10 ml of ethanol and scanned by UV-Visible spectrophotometer in the wavelength range of 200-400 nm against ethanol as a blank. The resulting spectrum and absorption curve showed characteristic maximum absorption at 241 nm for Betamethazone. The wavelength at which maximum absorption observed is 241 nm, which is selected for further analysis.

4. METHOD VALIDATION

Method validation was performed as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) (ICH, 2005)18,19 and all the parameters were evaluated.

4.1. Linearity

The linearity of this method was checked at concentrations ranging between 2-40 µg/ml. The curve of absorbance v/s concentration of Betamethazone was found to linear. The investigated concentrations followed Beer's Lambert law20.

4.2.Precision

The precision of the UV method was performed by intermediate precision (inter-day) and repeatability (intra-day).

4.3.Assay:

Preparation of standard solution: 10mg of Bumetanide drug was accurately weighed and transferred into 10ml of volumetric flask and the volume was made up to the mark with distilled water to get concentration of 1000ppm.From this 0.1 ml was pipetted out and transferred into 10ml of volumetric flask and the volume was made up to the mark to get 10ppm solution and its absorbance was measured at 222nm.

Preparation of test solution: 10 tablets were weighed and powdered. Powdered tablet equivalent to 10 mg of Bumetanide was weighed accurately and it was taken into 10ml volumetric flask then volume was made up to the mark with distilled water. From the above solution 0.1 ml of solution was pipetted out and taken in 10ml volumetric flask. The volume was made up to 10ml to get 10ppm solution and its absorbance was measured at 222nm.

The % Assay is calculated by using the following formula:
% Assay=(absorbance of the sample/absorbance of the standard)×(concentration of the standard/concentration of the sample))×100

4.4.Accuracy

Accuracy is defined as closeness between the actual (true) value and value obtained by repeating test method for a number of times. Accuracy may be expressed as % Recovery by the assay of known analyte which is added. It gives exact measure of the analytical method. The preanalyzed samples were spiked with extra 50, 100 and 150% of the standard Betamethazone (10 µg/ml) and the mixtures were analyzed using UV visible spectrophotometer.

4.5 LOD and LOQ

The Detection Limit (DL) is the lowest concentration of analyte present in a sample, which can be analyzed but not necessarily quantitated. The Quantitation Limit (QL) is the lowest concentration of analyte present in a sample, which can be quantitatively analyzed with acceptable precision and accuracy.

The limit of detection and limit of quantification were assessed based on the technique of signal-to-noise ratio10 using the Equations (1) and (2).

$QL = 10\ \sigma / S$ (1)

$DL = 3.3\ \sigma / S$ (2)

Where, σ is the standard deviation of the intercept of the calibration plot and
S is the slope of the calibration curve.

4.6.Robustness:
6 aliquots of 4ppm of standard solution was prepared and it was scanned at wavelength at (±)1nm of λmax. The absorbance was noted down

4.7.Ruggedness:
4ppm standard solution was prepared and scanned for 6 times by different analyst and different instruments.

5. Forced Degradation Studies:

Forced degradation studies are also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used. But these guidelines are very general in conduct of forced degradation and do not provide details about the practical approach towards stress testing (16-19) . Knowledge of the stability of molecule helps in selecting proper formulation and package as well as providing proper storage conditions and shelf life, which is essential for regulatory documentation.

Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule Although forced degradation studies are a regulatory requirement and scientific necessity during drug development, it is not considered as a requirement for formal stability program. A forced degradation study is an essential step in the design of a regulatory compliant stability program for both drug substances and products, and formalized as a regulatory requirement in ICH Guideline Q1A in 1993.

Acid degradation: From the 10ppm of drug solution,1 ml of the 40ppm solution was taken into 10ml volumetric flask to that added 1 ml of 0.1 N HCl kept for 24 hours at room temperature. After 24 hours neutralize the solution with 1 ml of 0.1N NaOH and measured its absorbance at 222nm

Alkali degradation: From the 40ppm of drug solution,1 ml of 10ppm solution was taken into 10 ml volumetric flask to that added 1ml of 0.1N NaOH Was kept for 24 hours at room temperature. After 24 hours neutralize with 1 ml of 0.1 N HCl and Measured its absorbance at 222nm

Photolytic degradation: 10mg of drug powder was exposed to UV light in UV chamber for 3hrs by placing the drug in Petri dish. After 3hrs Sample was diluted to get concentration of 40 µg/ml and absorbance was measured at 222nm

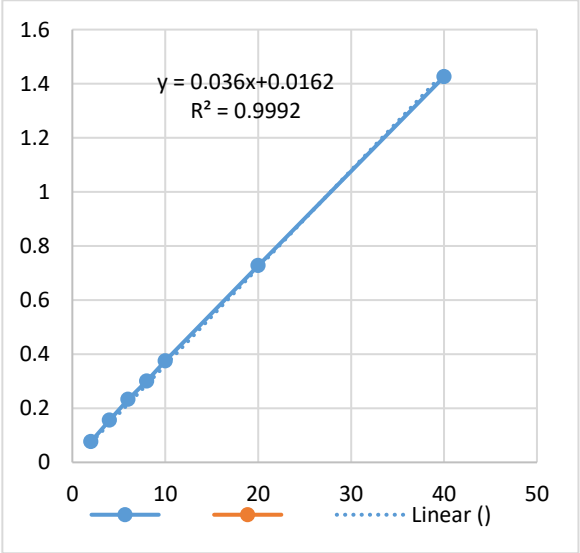
Thermal degradation: Drug was exposed to dry heat 40ºC in oven at for 3hrs by placing the drugs in Petri dish. Weighed 10mg of drug and diluted to get a final concentration of 40 µg/ml. Measure the absorbance at 222nm and calculate the percentage of Degradation.

Peroxide degradation: From the 10ppm of drug solution, 1 ml of the drug solution was taken into 10 ml volumetric flask to that added 1 ml of 3% hydrogen peroxide solution kept for 24 hours at room temperature After 24 hours dilute with water to get concentration of 10 µg/ml and measured its absorbance at 222nm

6. Results and Discussion:

6.1 Linearity:

s.no	Concentration ppm	Absorbance nm
1.	2	0.0765
2.	4	0.1558
3	6	0.2327
4	8	0.3004
5	10	0.3749
6	20	0.7273
7	40	1.4255



S. No.	Parameters	results
1.	Absorbance maximum(nm)	241
2.	Linearity and range(µg/ml)	2-40µg/ml
3.	Slope	0.036
4.	Correlation coefficient	0.9992
5.	Y-intercept	0.0162

Table 1: Results of linearity study of Bumetanide.

6.2 Precision:

%RSD = (SD of measurement/mean value of measurement) x 100

Concentration	Intraday precision (%RSD)	Inter-day precision (%RSD)	
4µg/ml	0.129%	Day 1	Day 2
		0.08940%	0.1528%

Table 2: Results of precision studies.

6.3 Accuracy:

Level	Amount of standard added (µg/ml)	Pre-analysed sample (µg/ml)	% Recovery
50%	10	10	98.56%
100%	15	10	99.04%
150%	25	10	98.18%

Table 3: Results of accuracy studies (50%, 100%, 150%).

6.4 Robustness:

S.no	Concentration	Wavelength	%RSD
1.	4µg/ml	240nm	0.078%
2.	4µg/ml	241nm	0.039%
3.	4µg/ml	242nm	0.037%

Table 4: Results of robustness studies.

6.5 Ruggedness:

Concentration	Analyst	% RSD	Instrument	SD
5µg/ml	Analyst 1 Analyst 2	0.7144%	Instrument 1 (ELICO)	0.0000471
	Analyst 2	0.1744%	Instrument 2 (SYSTRONIC)	0.0000451

Table 5: Results of ruggedness studies.

6.6 LOD:

LOD = 3.3 x SD/ slope.

The LOD of the proposed method was found to be 0.0234µg/ml.

6.7 LOQ:

LOQ = 10 x SD/ slope.

The LOQ of the proposed method was found to be 0.07054µg/ml.

6.8 Assay:

The % Assay is calculated by using the following formula:

% Assay=(absorbance of the sample/absorbance of the standard)×(concentration of the standard/concentration of the sample))×100

COMPENDIAL METHOD:

Absorbance of the sample = 0.1154

Absorbance of the standard = 0.1005

Concentration of the standard = 4ppm

Concentration of the sample =

(absorbance of the sample/absorbance of the std) ×concentration of the std

0.1558/0.1045×4

= 4.5ppm

%Assay =

(absorbance of the sample/absorbance of std) × (concentration of std/con of the sample) ×100

= (0.1154/0.1005) x (4/4) x100

=

= 114.8%

Intercept method:

Y= mx+c

0.1154=0.036x+0.0162

0.1154=0.036x+0.0162

0.1154=0.036x+0.0162

0.1154=0.036x+0.0162

0.1154-0.0162=0.036x

0.0992=0.036x

x=0.0992/0.036

=3

%Assay =(obtained con/original con) x100

= (3/4) x100

=0.75x100

=75%

7. FORCED DEGRADATION STUDIES:

CONDITIONS	ACTUAL ABSORBANCE		DEGRADED ABSORBANCE	DEGRADED ABSORBANCE
	1.1730(40ppm)		BLANK -Ethanol	BLANK (Acid+base)
0.1N HCL			0.082	0.017
1N HCL			0.110	0.048
0.1 N NaoH			0.142	0.050
1N NaoH			0.150	0.065
3%HYDROGEN PEROXIDE				0.758
BENCH TOP				0.4425
TEMPERATURE				NO DEGRADATION

Table 6: Results of forced degradation studies.

8. CONCLUSION:

A simple rapid method with cost effectively and less economically method was developed and validated by using UV-Visible spectrophotometry.

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