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Research Article

A NOVEL VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF BETAMETHASONE USING OXIDATION APPROACH

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ABSTRACT
A simple, sensitive, rapid and accurate spectrophotometric method was developed and validated for the estimation of betamethasone in bulk and pharmaceutical dosage forms. The method was based on the formation of bluish green chromophore with 0.04 % of sodium dichromate and concentrated sulphuric acid showing the absorption at 615 nm. The proposed method has permitted the quantification of betamethasone over linearity in the range of 5 – 30 µg/mL. The method was validated as per ICH guidelines.
Keywords: Spectrophotometry, sodium dichromate, betamethasone, chromophore.

INTRODUCTION

Betamethasone is chemically, (8S,9R,10S,11S,13S,14S,16S ,17R)-9-fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta [a]phenanthren-3-one having the chemical formula $C_{22}H_{29}FO_5$ with average molecular weight 392.4611. It is a synthetic glucocorticoid used topically as an anti inflammatory and administered orally as a replacement therapy for adrenal insufficiency. Betamethasone and its derivatives: betamethasone sodium phosphate and betamethasone acetate are used as anti inflammatory and immunosuppressant agents. Betamethasone is combined with a mineralocorticoid to manage adrenal insufficiency and is used in the form of betamethasone benzoate, betamethasone dipropionate or betamethasone valerate for the treatment of inflammation due to corticosteroid-responsive dermatoses. Betamethasone and clotrimazole are used together to treat cutaneous tinea infections[1]. The official methods for determination of betamethasone dipropionate in different pharmaceutical dosage forms were prescribed in the United States Pharmacopoeia (USP). Past studies showed different analytical methods of betamethasone using spectrophometry[2,3], voltammery in biological fluids[4], HPLC[5-9], stability studies[10,12], reverse phase sequential injection chromatography[13] and LC-MS[14]. Estimation of betamethasone was performed in human plasma[15], milk[16] and urine[17]. The determination of related substances was reported for betamethasone alone and in combination with other drugs of marketed formulations[18]. In the present communication, we have developed a simple visible spectroscopic method with considerable precision, accuracy and sensitivity for the estimation of betamethasone in bulk and pharmaceutical dosage forms at 615 nm wavelength.

EXPERIMENTAL

Reagents and materials

The pure standard of betamethasone was obtained as a gift sample from Active Health Care Ltd., Vadodara, India. The purity of the standard found 99.85 % and it was established by spectral confirmation. A UV-visible spectrophotometer (Model 6505, Jenway, UK, Serial No: 2177 and 2182) with 1 cm quartz matched cells was used for spectral measurements. All the chemicals used were of analytical grade from BDH, Germany. Commercial betamethasone tablets were purchased from the market.

Preparation of sodium dichromate solution

Sodium dichromate (0.04 %) was prepared with 40 mg of accurately weighed sodium dichromate is dissolved and diluted to 100 mL using distilled water in a 100 mL volumetric flask.

Determination of absorption maximum

Different concentrations of betamethasone (10, 20, 40 and 100 µg/mL) were scanned against the reagent blank.

Optimization of beers law limit

Different trials were made to optimize the beers law limit by preparing different concentrations of betamethasone along with sodium dichromate and concentrated sulphuric acid and the prepared colored solutions were subjected for the measurement of absorbance. The standard plot was constructed using the concentration and absorbance values. From the standard plot the linearity range or beers law limit was optimized.

Preparation of working standard solution

The standard solution of betamethasone was prepared by dissolving 100 mg of betamethasone in 100 mL of distilled water to obtain 1 mg/mL solution. From the above stock solution 10 mL was taken and diluted to 100 mL with distilled water to get a concentration about 100 µg/mL.

Construction of linearity

From the working standard solution, aliquots ranging from 0.5 – 3.0 mL were transferred to each of six 10 mL volumetric flasks. To the flasks 2 mL each of 0.04 % sodium dichromate solution and concentrated sulphuric acid were added. The flasks were kept aside for 3 minutes for color development. The appropriate volume of distilled water was added to each flask to make the total volume to 10 mL to obtain the concentrations of 5, 10, 15, 20, 25 and 30 µg/mL.

The absorbance of final bluish green color chromophore was measured at 615 nm against the reagent blank. A calibration graph was plotted and regression equation was calculated.

Preparation of sample stock solution

Aliquots of working standard solution (100 µg/mL) of the drug ranging from 0.5 – 3.0 mL (50 – 300 µg/mL) were transferred to a series of 10 mL volumetric flasks. Then 2 mL each of 0.04 % sodium dichromate and concentrated hydrochloric acid were added and kept aside for 3 minutes for color development. The appropriate volume of distilled water was added and the volume was made up to 10 mL. The absorbance of the bluish green colored chromophore of any concentration of the linearity range was measured at 615 nm against the reagent blank. The amount of betamethasone present in the sample solution was computed from the calibration curve.

Table 1: Optical characteristics of betamethasone

Parameter	Values
λ_{max} (nm)	615
Beer's law (µg/mL)	5–30
Regression equation[a] a. Slope b. Intercept c. Correlation coefficient	 0.013 0.002 0.999
Molar extinction coefficient (1 $mole^{-1}.cm^{-1}$)	596.37
Sandell's sensitivity ($\mu g/cm^2$/0.001-absobance unit)	0.128
% Range of errors 95 % Confidence interval[b] 99 % Confidence interval[c]	 ± 0.0285 ± 0.0126
% RSD[d]	± 0.319

[a]Y= a+bc, where c is the concentration of analyte and Y is the absorbance unit; [b]p<0.05 considered significant; [c]p<0.01 considered significant; [d]RSD=relative standard deviation

Table 4: Assay of betamethasone in commercial formulations

Brand name	Label claim (mg/tab)	Amount estimated* (mg/tab)	% Amount estimated
Betnesol	0.5	0.496 ± 0.014	99.2
Acticard	0.5	0.502 ± 0.009	100.4

*Mean of five values

Validation of method

To study the accuracy, reproducibility of the proposed method, the recovery studies were carried out by the addition of 10, 20 and 20 µg/mL of standard drug solution to pre analyzed samples. The intra- and inter day variations of the method were established using six samples of three concentrations (10, 20 and 20 µg/mL) and they were analyzed on the same day and on three different days over a period of two weeks.

Assay of marketed formulation

Two different brands of betamethasone tablet formulations containing 0.5 mg were selected for the study. Twenty tablets were accurately weighed and powdered. A weight of the powder equivalent to 25 mg of betamethasone was transferred into a 25 mL volumetric flask and 10 mL of distilled water was added and shaken on a mechanical shaker for 10 minutes. The volume was made up to 25 mL distilled water and filtered through whatmann filter paper (0.4 µ). A 10 mL of the solution was further diluted to 100 mL to get the concentration of about 100 µg/mL.

Table 2: Recovery of the method

Amount added (mg)	Amount recovered* (mg)	% Recovery
10	10.05 ± 0.03	100.5
20	19.92 ± 0.08	99.6
30	30.09 ± 0.17	100.3

*Mean of three values

Table 3: Precision of method

Concentration (µg/mL)	Observed concentrations			
	Intra-day*	% cv	Inter-day*	% cv
10	10.09	0.48	9.96	0.71
20	19.88	0.32	19.71	0.55
30	30.17	0.41	30.09	0.68

*Mean of six values, cv = coefficient of variation

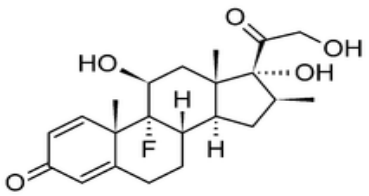

Figure 1: Chemical structure of betamethasone

RESULTS AND DISCUSSION

The optimum conditions were established by changing one parameter at a time and keeping the others fixed and by observing the effect produced on the absorbance of the colored species. Various parameters involved in the color development such as the concentration of the reagents, volume and time involved for maximum color development were optimized. The bluish green colored chromophore at λ_{max} 615 nm was formed due to the oxidation of the drug by sodium dichromate in the presence of hydrochloric acid. The optical characteristics such as beer's law limit, molar extinction coefficient, sandell's sensitivity were determined. The statistical parameters: correlation coefficient, slope, intercept were calculated from the regression equation. Standard deviation, relative standard deviation was calculated for the proposed method and the results are shown in Table 1. To study the accuracy, reproducibility of the proposed method, the recovery studies were carried out by the addition of the standard drug solution to pre analyzed samples. Results of recovery studies were found within the range and are presented in Table 2. The intra- and inter-day variations of the method were established using six samples of three concentrations and the results were lying within the limits as shown in Table 3. The assay of betamethasone in two marketed tablets was determined using the developed method employing optimized spectrophotometric conditions and it was found more accurate and reliable. The results are presented in Table 4. There was no interference of excipients found during the assay of commercial formulations.

CONCLUSION

A simple, rapid and sensitive visible spectroscopic method for betamethasone was successfully developed with reasonable precision and accuracy which makes it as choice for routine quality control analysis. There was no interference of excipients presented in tablet formulations throughout the experimental process which reflects the reliability of the developed method.

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