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Estimation of Betamethasone in Pharmaceutical Dosage Forms by Visible Spectrophotometry

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Simple, accurate, precise and reproducible methods have been developed and validated for the estimation of betamethasone in pharmaceutical dosage forms by visible spectrophotometry. **Method-A** is based on the reduction of molybdate in acidic medium by betamethasone, producing one or two more of the possible reduced species which have a characteristic intense blue colour (molybdenum blue) chromogen exhibiting λ_{max} at 670 nm. **Method-B** is based on the oxidation of α-ketol portion of betamethasone to formaldehyde and then converted into its phenyl hyrdazone and then a red colour is developed under the action of oxidizing agent [hexacyano ferrate(III)] in acid medium exhibiting λ_{max} at 520 nm. The results of analysis have been validated statistically and recovery studies confirm the accuracy of the proposed methods. The methods proposed are applied to various pharmaceutical dosage forms as well.

Key Words: Betamethasone, Molybdenum blue, Recovery studies and chromogen.

INTRODUCTION

Betamethasone [BMS][1] is 9-fluoro-11β, 17a-21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione is a synthetic adreno-corticosteroid, for dermatologic use. Betamethasone, an analog of prednisolone, has a high degree of corticosteroid activity and a slight degree of mineral corticoid activity. It is also used as antiinflammatory and immunosuppressive agent. Few analytical techniques have been reported for the determination of betamethasone in bulk and pharmaceutical formulations including spectrophotometric methods[2,3] pharmacodynamics and parmacokinetics of betamethasone[4] and chromatographic methods like LC/MS, HPLC, *etc*.[5-14]. Existing analytical techniques revealed that little attention was paid in developing visible spectrophotometric methods by exploring thoroughly the useful functional groups in betamethasone. The present paper describes two visible spectrophotometric methods based on the reactivity of different functional groups present in the drug with the given reagents. The developed methods proposed by the author are extended to pharmaceutical formulations as well.

EXPERIMENTAL

All spectral measurements were made on ELICO SL-159 digital UV-visible spectrophotometer.

All chemicals used are of analytical grade. Ammonium molybdate, sodium metaperiodate and phenyl hydrazine hydrochloride were purchased from Loba chemie, Mumbai, H_2SO_4 from Qualizens, Mumbai, $K_3Fe(CN)_6$, from SD fine chemicals, Mumbai and NaOH from BDH, Mumbai.

The pharmaceutical formulation analyzed was betacortil (Pfizer, Mumbai). A tablet contains 1.0 mg of betamethasone and excipients are lactose, starch maize, gelatin, magnesium stearate, purified water.

Method-A: Ammonium molybdate solution (Loba; 2 %, 1.618×10^{-2} M): Prepared by dissolving 2 g of ammonium molybdate in 100 mL of distilled water. Conc. H_2SO_4 (Qualigens): Used as it is.

Method-B: $NaIO_4$ solution (Loba; 0.855 %, 4.00×10^{-2} M): Prepared by dissolving 855 mg of sodium metaperiodate ($NaIO_4$) in 100 mL of 0.3 M HCl. Phenyl hydrazine hydrochloride (PHH) (Loba; 1.0 %, 6.90×10^{-2} M): Prepared by dissolving 1.0 g of phenyl hydrazine hydrochloride in 100 mL of distilled water and filtered. $K_3Fe(CN)_6$ solution (SD-Fine; 2.0 %, 6.00×10^{-2} M): Prepared by dissolving 2.0 g of potassium ferricyanide in 100 mL distilled water. NaOH solution (BDH, 0.4 %, 0.1 M): Prepared by dissolving 400 mg of sodium hydroxide (NaOH) to 100 mL distilled water and standardized.

Preparation of standard drug solution: Pure betamethasone (100 mg) was accurately weighted and dissolved in 10 mL of methanol transferred to a standard 100 mL volumetric flask. The final volume was made up to the mark with methanol.

Assay of betamethasone pharmaceutical dosage forms: Tablets of the betamethasone were weighed and powdered and a quantity of the powder equivalent to 100 mg was dissolved in 25 mL of methanol shaken well and filtered. The filtrate was diluted to 100 mL to get 1 mg/mL solution of drug in formulations. The general procedure was then followed in the concentration ranges mentioned above for the assay of betamethasone. A sensitive UV method (CH_3OH as solvent) has been developed and adopted it as a reference method to compare the results obtained by proposed methods.

Recommended procedures

Method-A: Aliquots of standard betamethasone solution (0.5-2.5 mL, 200 µg/mL) were delivered in to a series of 20 mL calibrated tubes. To each tube 1.0 mL of 1.618×10^{-2} M ammonium molybdate reagent and 4.0 mL of conc. H_2SO_4 were added to each tube and the contents were heated for 20 min in boiling water bath. After cooling, the volume was made up to 20 mL with distilled water. The resulting absorbance was measured at 670 nm against a reagent blank.

Method-B: Aliquots of standard betamethasone solution (0.5-2.5 mL, 200 µg/mL) were transferred into a series of 25 mL-calibrated tubes. Then 0.5 mL of $NaIO_4$ solution was added to each tube and the volume made upto 5 mL with distilled water. After keeping the tubes for 0.5 h at room temperature, 1.5 mL of NaOH, 2.0 mL of phenyl hydrazine hydrochloride solution and 1.0 mL of $K_3Fe(CN)_6$ solutions were added successively and shaken well. The tubes were kept in ice water for 5 min later 5.0 mL of conc. HCl was added. Finally the solution in each tube was made up to 25 mL with ethanol. The absorbance was measured after 15 min at 520 nm against reagent blank.

Validation method

Selectivity: The selectivity of the two methods proposed was investigated by interferences between betamethasone and the excipients.

Linearity: For the proposed spectrophotometric methods, the working solutions were prepared by dilution of the stock solution of betamethasone to reach the concentration range of 5-25 and 4-20 µg/mL for the **method-A** and **method-B**, respectively. Calibration curves were set up by plotting absorbance *versus* respective drug concentrations.

Precision: The precision of proposed two methods were estimated by the relative standard deviation of six determinations of betamethasone in its tablet dosage forms.

Accuracy: The accuracy of the proposed methods was verified by adding known amounts of betamethasone standard solution to known amounts of various excipients. To determine the accuracy of the proposed methods, different amount of bulk samples of betamethasone with in Beer's law limits were taken and analyzed by the proposed methods. The results are recorded in Table-1.

Limit of detection: The limit of detection was estimated as 3.3 and 10 times standard deviation intercept/slope ratio.

RESULTS AND DISCUSSION

Both methods described for the determination of betamethasone in tablet were specific. No interferences with excipients were encountered.

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS FOR BETAMETHASONE

Parameter	**Method-A**	**Method-B**
λ_{max} (nm)	670	520
Beer's law limits (µg/mL)	5-25	4-20
Detection limit (µg/mL)	0.3191	0.0903
Molar absorptivity (1 mol^{-1} cm^{-1})	1.03×10^4	1.09×10^4
Sandell's sensitivity (µg cm^{-2}/0.001 absorbance unit)	0.1119	0.1081
Optimum photometric range (µg/mL)	4.5-23.0	5.5-18.5
Regression equation (Y = a + bc) slope (b)	2.596×10^{-2}	2.772×10^{-2}
Standard deviation on slope (S_b)	1.665×10^{-4}	6.2915×10^{-5}
Intercept (a)	5.2×10^{-2}	3.3×10^{-3}
Standard deviation on intercept (S_a)	2.762×10^{-3}	8.347×10^{-4}
Standard error on estimation (S_e)	2.633×10^{-3}	7.958×10^{-4}
Correlation coefficient (r)	0.9999	0.9999
Relative standard deviation (%)	0.4448	0.4839
Range of error (confidence limits) (%)		
0.05 level	0.4669	0.5081
0.01 level	0.7323	0.7968
Limit of detection (LOD)	0.3192	0.0903

*Average of six determinations considered.

For quantification, linear calibration curves were obtained over the working concentration range of 5-25 and 4-20 µg/mL for the **method-A** and **method-B**, respectively for spectrophotometric determination of betamethasone. The equations of calibration curves were obtained using the least square regression equation. The slope and the correlation coeffient (γ) were presented in Table-1. Beer's law limits, molar absorptivity and Sandell's sensitivity for betamethasone with each one among mentioned reagents were calculated. The optical characteristics are presented in Table-1. Correlation coeffient for the linear fit were 0.9999 for the two proposed methods. The results indicate a good linearity between the absorbance and the concentration of betamethasone.

The precision of each one among two proposed spectrophotometric methods were ascertained separately from the absorbance value obtained by actual determination of six replicates of a fixed amount of betamethasone in total solution. The percent relative standard deviation and percent range of error (at 0.05 % level and 0.01% level confidence limits) were calculated for the proposed methods and are presented in Table-1. Limit of detection of each method were given in Table-1.

Commercial formulations containing betamethasone were successfully analysed by each proposed method. The values obtained by the proposed and reference methods for formulations were compared statistically by the *t*- and F-tests found not to differ significantly. Recovery studies were conducted by analyzing each pharmaceutical formulation in the first instance for the active ingredient by the proposed methods. The results are incorporated in Table-2.

Conclusion

The proposed methods were found to be simple, selective and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing betamethasone showed no interference from the common

TABLE-2
ASSAY OF BETAMETHASONE IN PHARMACEUTICAL FORMULATIONS

Formulations*	Amount taken (mg)	Amount found by proposed methods**		Reference method	Percentage recovery by proposed methods***	
		Method-A	**Method-B**		**Method-A**	**Method-B**
Tablet-I	0.5	0.495 ± 0.04 F = 2.25 t = 0.10	0.489 ± 0.03 F = 2.94 t = 0.109	0.492 ± 0.06	100.61 ± 0.13	99.39 ± 0.12
Tablet-II	1.0	0.998 ± 0.05 F = 3.24 t = 0.15	0.985 ± 0.08 F = 1.27 t = 0.18	0.994 ± 0.09	100.40 ± 0.21	199.09 ± 0.16

*Tablets two different pharmaceutical companies. **Average + standard deviation of six determinations, the *t*- and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95 % confidence limit, F = 5.05, t = 2.262. ***Recovery of 10 mg added to the pre-analyzed pharmaceutical formulations (average of three determinations).

excipients. Hence, the methods are suitable for rapid and not expensive for the determination of betamethasone in the quality control laboratories.

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