CHAPTER - V

'ALUATION OF VARIUOS CHROMOGENIC REAGENT IN SPECTROPHOTOMETRIC ANALYSIS OF BETAMETHASONE.

5.01. Drug Profile

Betemethasone [355] is 9-Fluoro-11β, 17a-21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione is a synthetic adrenocorticosteroid, 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 anti-inflammatory and immunosuppressive agent. The characteristics, therapeutic importance, chemical names, structure, analytically useful functional groups and commercially available formulations of BMS are presented in (**Tables 5.01, P.245; 5.02, P.246 & 5.03, P.247**). Few analytical techniques have been reported for the determination of Betamethasone in bulk and pharmaceutical formulations including extractive spectrophotometric method [356] and Chromatographic methods including LC/MS, HPLC, etc. [357-365]. Existing analytical techniques reveal that little attention was paid in developing visible spectrophotometric methods by exploring thoroughly the useful functional groups in Betamethasone. The present chapter describes five visible spectrophotometric methods based on the reactivity of different functional groups present in the drug with the given reagents. The developed five methods are extended to pharmaceutical formulations as well.

5.02. EXPERIMENTAL:

All spectral measurements were made on ELICO SL 159 digital UV-visible spectrophotometer. All chemicals used are of analytical grade.

i) Preparation of reagents:

Method M_1

m- Dinitro Benzene (MDNB) (0.5%, $2.97x10^{-2}M$)	:	Prepared by dissolving 500mg of MDNB in 100mL of distilled water.
NaOH Solution (0.1M)	:	Prepared by dissolving 400mg of NaOH in 100mL distilled water and standardized.

Method- M_3

INH solution (0.8%, $5.83x10^{-3}M$)	:	Prepared by dissolving 800mg of INH in 100mL of methanol containing 1% conc. HCl.

Method M_9

MBTH solution (Loba, 0.5%, $2.14x10^{-2}M$)	:	Prepared by dissolving 500mg of MBTH in 100mL distilled water.
HCL solution (1.0M)	:	Prepared by dissolving 8.6mL of conc. HCl to 100mL distilled water and standardized.
NaOH solution (BDH,4%,0.1M)	:	Prepared by dissolving 400mgs of NaOH to 100mL distilled water and standardized.

Method M_{12}

$NaIO_4$ solution (Loba; 0.855%, $4.00x10^{-2}M$)	:	Prepared by dissolving 855mg of $NaIO_4$ in in 100mL of 0.3M HCl.
PHH	:	Prepared by dissolving 1.0g of PHH in 100mL

(Phenyl Hydrazine Hydrochloride) (Loba; 1.0%, $6.90x10^{-2}M$)		of distilled water and filtered.
$K_3Fe(CN)_6$ solution (Sd-fine;.2.0%,$6.00x10^{-2}M$)	:	Prepared by dissolving 2.0g of $K_3Fe(CN)_6$ in 100mL distilled water.
NaOH solution (BDH, 0.4%, 0.1M)	:	Prepared by dissolving 400mgs of NaOH to 100mL distilled water and standardized.

Method M_{18}

AM Solution (Loba; 2%, $1.618 \times 10^{-2}M$)	:	Prepared by dissolving 2gms of ammonium molybdate in 100mL of distilled water.
Conc.H_2SO_4 (Qualigens)	:	Used as it is.

ii. Preparation of standard drug solution:

Betamethasone (pure) (100mg) was accurately weighted and dissolved in 10.0mL of methanol transferred to a standard 100mL volumetric flask. The final volume was made up to the mark with methanol.

Assay of Betamethasone pharmaceutical dosage forms:

Ten tablets of the Betamethasone drug were weighed and powdered, and a quantity of the powder equivalent to 100mg was dissolved in 25.0mL of methanol shaken well and filtered. The filtrate was diluted to 100mL to get 1mg/mL solution of drug in formulations. The general procedure was then followed in the concentration ranges mentioned above for the assay of Betamethasone. The author has developed a sensitive UV method (CH_3OH as solvent) and adopted it as a reference method (Fig. 5.16 & 5.17,P.262) to compare the results obtained by proposed methods.

Table - 5.01

Structural features of Betamethasone

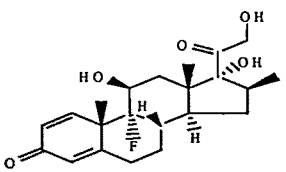
Sl. No	Generic Name	Category	Chemical Name	Structure	Analytical important moieties/functional groups
5	Betamethasone (BMS)	Anti-inflammatory / Immunosuppressive agent	9-fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydrocyclopenta [a]phenanthren-3-one.		$\Delta^{1,4}$- 3- Keto group and α- ketol group.

Table - 5.02

Physico chemical characteristic and therapeutic importance of Betamethasone

Category	Characteristics	Mode of action and therapeutic use
Anti-inflammatory / Immunosuppressive agent	**Molecular formula:** $C_{22}H_{29}FO_5$ **Formula Weight:** 392.46g/moles **Appearance:** White, odorless, crystalline powder. **Solubility:** Practically insoluble in water and soluble in Methanol.	Betamethasone is an synthetic adrenocorticosteroid, 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 anti-inflammatory and immunosuppressive agent.

Table - 5.03

Commercially available formulations

S.No.	Proprietary name	Pharmaceutical concerned	Formulations	Other ingredients usually present (active)	Inactive
5)	Betacortril Betacortril forte	Pfizer Pfizer	Tab - 0.5 mg Tab - 1.0 mg	------	Lactose, Starch maize, Gelatin, Magnesium stearate, Purified water.

Recommended procedures:

After systematic and detailed study of the various parameters involved, as described under results and discussions the following procedures [Methods M_1 (MDNB-NaOH), M_3 (INH-H^+), M_9(MBTH- Fe(III)), M_{12}(IO_4^-/PHH+$K_3Fe(CN)_6^{-3}$) and M_{18} (AM-H_2SO_4) were recommended for the assay of BMS in bulk samples and pharmaceutical formulations.

Method-M_1

Aliquots of standard Betamethasone (BMS) solution (0.5-2.5mL, 200µg/mL) were transferred into a series of 10mL calibrated tubes. Then 0.5mL of 0.5% MDNB solution and 0.5mL of 0.1M NaOH were added to each tube and kept for 5 minutes in dark. Then 4.0mL of benzene was added shaken well and waited for 5 minutes in dark. Subsequently 1.0mL each of ethanol and pyridine were added. Finally the solution in each tube was made up to 10mL with methanol. The absorbance was measured after 5 minutes at 490nm against similar reagent blank. The amount of BMS was computed from its calibration graph. (Fig. 5.06, P.259).

Method-M_3

Delivered aliquots of standard BMS solution (0.5-2.5mL, 100µg/mL) into a series of 10mL calibrated tubes. Then 2.0mL of 0.8% INH solution was added to each tube and heated for 10minutes at 60°C. The solutions in each tube were cooled and make up to 10mL with methanol. The absorbance was measured at 445nm against the reagent blank. The amount of BMS was computed from its calibration graph. (Fig. 5.07, P.259).

Method-M_9

Aliquots of standard BMS solution (0.5–2.5mL, 100μg/mL) were transferred into a series of 25mL calibrated tubes. Then 0.5mL of $2.14x10^{-2}$M of MBTH solution, 0.5mL of 0.1M NaOH were added to each tube and the contents were heated for 10min. in a water bath at 100°C and cooled for 5min. in a water bath at 15°C, then 0.5mL of 1.0M HCl and 2.0mL of 1.53×10^{-2}M of Fe (III) solution were added successively and kept aside for 1hr. Finally the solution in each tube was made up to 10mL with distilled water. The absorbance was measured at 620nm against a similar reagent blank. The amount of BMS was computed from its calibration graph. (Fig. 5.08, P.260).

Method-M_{12}

Aliquots of standard BMS solution (0.5–2.5mL, 200μg/mL) were transferred into a series of 25mL-calibrated tubes. Then 0.5mL of $NaIO_4$ solution was added to each tube and the volume made upto 5mL with distilled water. After keeping the tubes for 30min. at room temperature, 1.5mL of NaOH, 2.0mL of PHH solution and 1.0mL of $K_3Fe(CN)_6$ solutions were added successively and shaken well. The tubes were kept in ice water for 5min.Later 5.0mL of Conc.HCl was added. Finally the solution in each tube was made up to 25mL with ethanol. The absorbance was measured after 15min. at 520nm against reagent blank. The amount of BMS was computed from its calibration graph. (Fig. 5.09, P.260).

Method-M_{18}

Aliquots of standard BMS solution (0.5-2.5mL, 200μg/mL) were delivered in to a series of 20mL calibrated tubes. To each tube 1.0mL of 1.618×10^{-2}M AM reagent and 4.0mL of Conc.H_2SO_4 were added to each tube and the contents

were heated for 20min. in boiling water bath. After cooling, the volume was made up to 20mL with distilled water. The resulting absorbance was measured at 670nm against a reagent blank. The amount of BMS was computed from to appropriate calibration graph (Fig 5.10; P. 260).

5.03. RESULTS AND DISCUSSION:

a. Spectral charteristics:

In order ascertain the λ_{max} (optimum wavelength of maximum absorption) of the colored species formed in each of two spectrophotometric methods, specified amounts of Betamethasone (40μg/mL, 10μg/mL, 8μg/mL, 16μg/mL, and 20μg/mL for methods M_1, M_3, M_9, M_{12} and M_{18} respectively) in final dilution were taken and the colors were developed separately following above mentioned procedures individually. The absorption spectra were scanned on a spectrophotometer in a wavelength region 300-700nm against a corresponding reagent blank. The reagent blank absorption spectrum of each method was also recorded against appropriate solvent.

b. Optimum conditions fixation in procedures:

Method M_1:

The method involves the complex formation reaction between BMS and MDNB. The optimum conditions were fixed basing on the study of effects of various parameters, such as volume of (2.97×10^{-2}M) MBNB solution, volume of NaOH solution, volume of solvents used initially and subsequently for final dilution and the stability of colored species after dilution. The absorbances were measured at 490nm and the results are incorporated in (Table 5.04, P. 252).

Method M_3:

The method involves the condensation reaction between BMS and INH. The optimum conditions were fixed basing on the study of effects of various parameters, such as volume of (5.83×10^{-3}M) INH solution, heating time and temperature, volume of solvents used initially and subsequently for final dilution and the stability of colored species after dilution. The absorbance was measured at 445nm and the results are incorporated in (Table 5.05, P. 253).

Method M_9:

The method involves the reaction of BMS with MBTH in the presence of an oxidant, Fe (III) solution. The optimum conditions in this method were fixed, basing on the study of the effects of various parameters such as MBTH and alkali to be added to accompanied by heating (time and temperature), acid and oxidant required after cooling, the waiting time for maximum color development and solvent used for final dilution on the intensity and stability of the colored species formed. The optimum conditions developed and actual conditions chosen for the procedure are incorporated in (Table 5.06, P. 254).

Table 5.04

Optimum conditions established in method M_1 for BMS

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	480-500	490	
Effect of volume of (2.97×10^{-2}M) MDNB solution.	0.2-0.6mL	0.5mL	0.5mL of (2.97×10^{-2}M) MDNB solution was necessary for covering broad range of Beer's law limits.
Effect of volume of NaOH (0.1M) solution.	0.2-1.0mL	0.5mL	Minimum amount of 0.2mL of NaOH solution was necessary to maintain alkali conditions.
Waiting period	4-15min. in dark	5min in dark	
Volume of Benzene	4.0mL	4.0mL	
Solvent used for final dilution for maximum absorbance	Ethanol (1.0mL) Pyridine (1.0mL)	Ethanol (1.0mL) Pyridine (1.0mL)	
Waiting period for development of color	2-10min.	5min.	5min. was sufficient for development of color.
Solvent for final dilution	Methanol	Methanol	Methanol has been found to be suitable for final dilution to give better absorbance values.
Stability period after final dilution	Immedaiate-30min.	Within 30min.	After the stability period, the absorbance of colored species decreased slowly.

Table 5.05

Optimum conditions established in method M_3 for BMS

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	420-460	445	
Effect of volume of (5.83×10^{-3}M) of INH in methanol containing 1% HCl and heating time and temperature.	1.0-3.0mL, 5-15min., 60°C	2.0mL, 10min., 60°C	
Solvent for final dilution	Methanol	Methanol	Methanol has been found to be suitable for final dilution to give better absorbance values.
Stability of colored species after final dilution.	Immediate – 50 min.	10min.	After the stability period the absorbance of colored species decreased slowly.

Table 3.06

Optimum conditions established in method M_9 for BMS

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	610-630	620	
Nature of oxidant for color development	Fe(III) solution	Fe(III) solution	Other oxidants such as Ce (IV), Cr (VI), CAT, IO_4^- and $S_2O_8^{2-}$ were used instead Fe (III), resulting in the decrease in absorbance was noticed.
Volume of (2.14×10^{-2}M) of MBTH on color development.	0.2 - 0.8mL	0.5mL	<0.2mL decreased the absorbance of the test solution. The absorbance of the colored species remained stable with rise in volume of MBTH up to 0.8mL.
Effect of volume of (1.0M) NaOH solution.	0.2 – 1.0mL	0.5mL	Minimum amount of 0.2mL of NaOH solution was necessary to maintain alkaline conditions.
Temperature and time.	Boiling water bath, 5-20min.	Boiling water bath, 10min.	Heating on a boiling water bath for 10min. has been preferred.
Effect of volume of (1.0M) HCl solution.	0.2-1.0mL	0.5mL	Minimum amount of 0.2mL of HCl solution was necessary to maintain acidic conditions.
Volume of (1.53×10^{-2}M) Fe (III) solution required for color development.	1.5-2.5mL	2.0mL	<1.5mL of Fe (III) solution decreased the absorbance of test solution and >2.5mL of Fe (III) solution increased the blank absorbance.
Effect of order of addition of reagents on color development.	BMS, MBTH, and Fe(III) solution	BMS, MBTH, and Fe(III) solution	BMS, Fe (III) solution followed by MBTH order of addition, decreases the final color to considerable extent.
Nature of solvent for final dilution.	Distilled water	Distilled water	Other water miscible solvents like methanol, ethanol, propane-1-ol and acetonitrile were found not to enhance the intensity of the final colored product.
Waiting period.	50-70min.	60min.	
Stability period after final dilution.	Immediate - 60min.	5min.	After the stability period, the intensity of the colored species was found to decrease with time after 60min.

Table 5.07

Optimum conditions established in method M_{12} for BMS

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	510-530	520	
Volume of (4.0×10^{-2}M) $NaIO_4$ solution	0.3-0.8mL	0.5mL	Beyond the upper and lower limits low absorbance was observed either in lower or upper Beer's law limits.
Time and temperature.	25-35min. at Laboratory temperature	30min. at Laboratory temperature	30min. was necessary to complete oxidation.
Volume of (6.90×10^{-2}M) PHH solution.	1.5-2.5mL	2.0mL	1.5mL of PHH solution was found necessary for color development.
Volume of Hexacyano ferrate (III) solution	0.5-1.5mL	1.0mL	Addition of <0.5mL results in erratic values especially in upper region of Beer's law limits.
Time and temperature prior to the addition of Conc.HCl.	5min. 0-5°C	5min. in ice bath.	Minimum cooling for 5min. in ice bath has been found to be necessary.
Addition of Conc.HCl.	4-6mL	5mL	4.0mL of Conc. HCl was found necessary for maximum color development.
Solvent for final dilution	Ethanol	Ethanol	Ethanol was found to be the best solvent for final dilution.
Stability period after final dilution.	Immediate-30min.	15min.	

Table 5.08

Optimum conditions established in method M_{18} for BMS

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	660-680	670	
Effect of volume of 1.61×10^{-2}M of AM solution.	0.7-1.3mL	1.0mL	1.0mL of 1.61×10^{-2}M of AM solution was necessary for covering broad range of Beer's law limits.
Effect of volume of Conc. H_2SO_4 on color development	3.0-5.0mL	4.0mL	<3.0mL of conc. H_2SO_4 results in low absorbance values and >5.0mL has no additional value.
Effect of the order of addition of reagent on color development	BMS, AM solution, Conc. H_2SO_4	BMS, AM solution, Conc. H_2SO_4	The change in the order of addition has no effect.
Effect of temperature and time	Boiling water bath 20-30min.	Boiling water bath 20min.	It was found that boiling water bath was necessary for uniform temperature and maximum color development. Heating on a boiling water bath for 20min. is necessary for maximum color development
Solvent for final dilution	Ethanol	Ethanol	The absorbance of the test solution decreased when water was used instead of ethanol for final dilution.
Stability period after final dilution	5min-24 hours	5min.	

Fig. 5.01: Absorption spectrum of BMS with MDNB-NaOH (M_1)

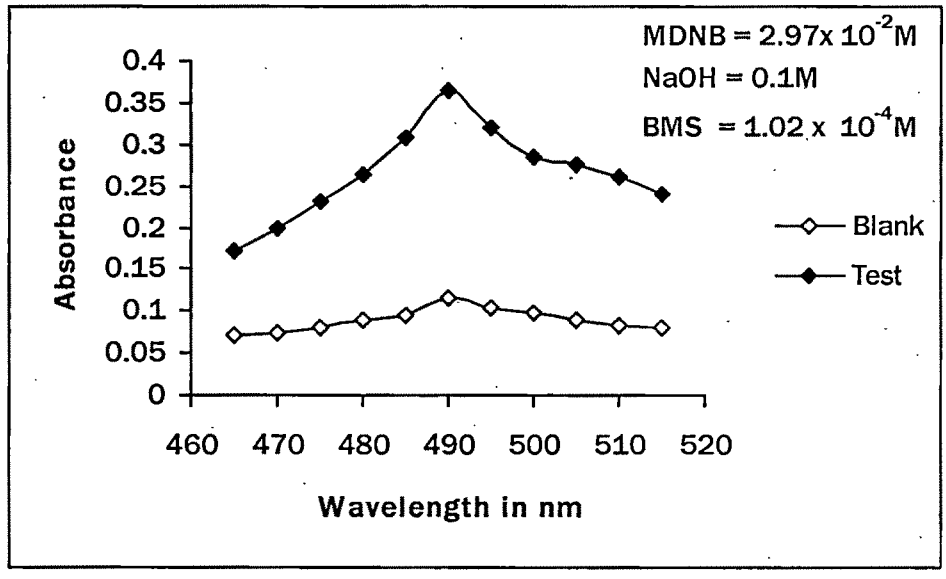


Fig. 5.02: Absorption spectrum of MIRT with INH (M_3)

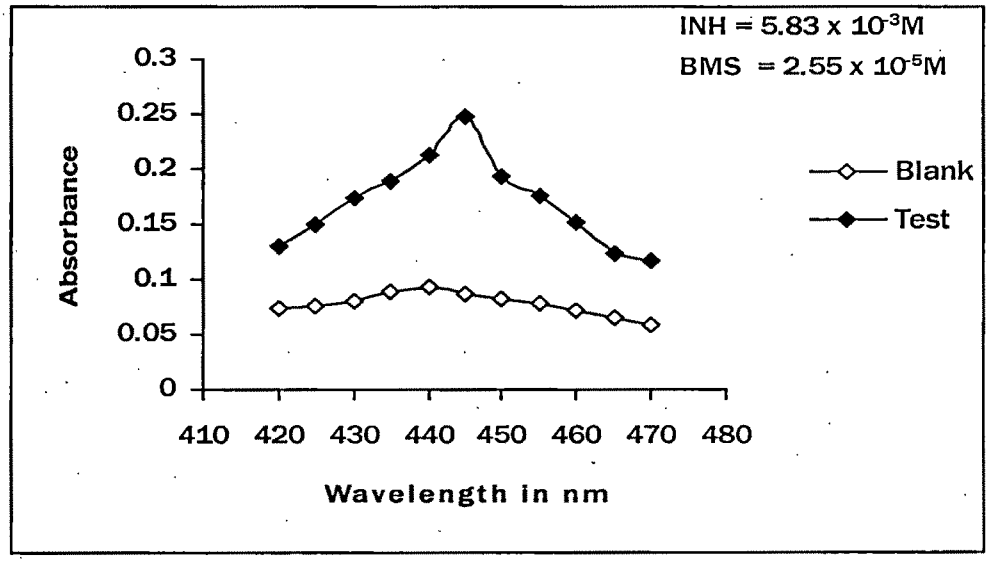


Fig. 5.03: Absorption spectrum of MIRT with MBTH – $FeCl_3$ (M_9)

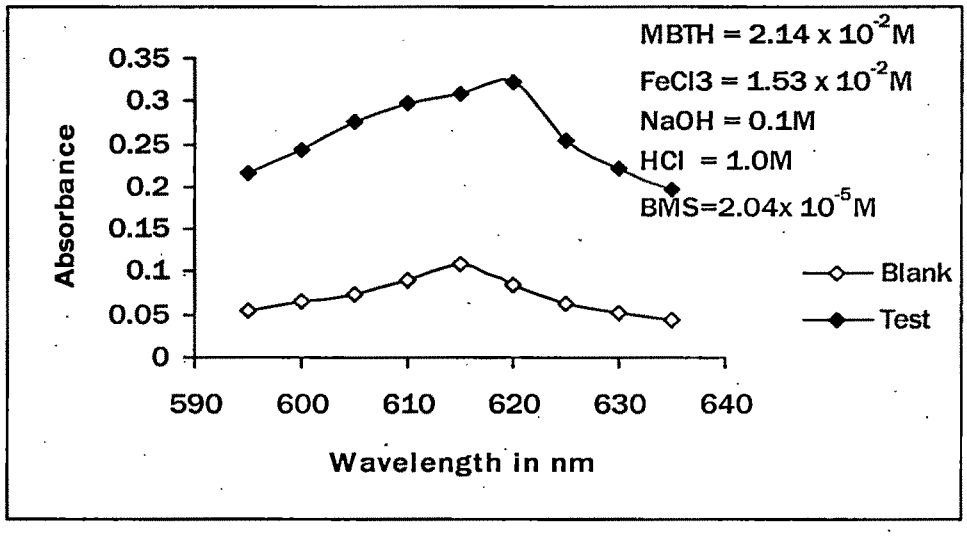


Fig. 5.04: Absorption spectrum of MIRT with IO_4^-/PHH–$[Fe(CN)_6]^{-3}$(M_{12})

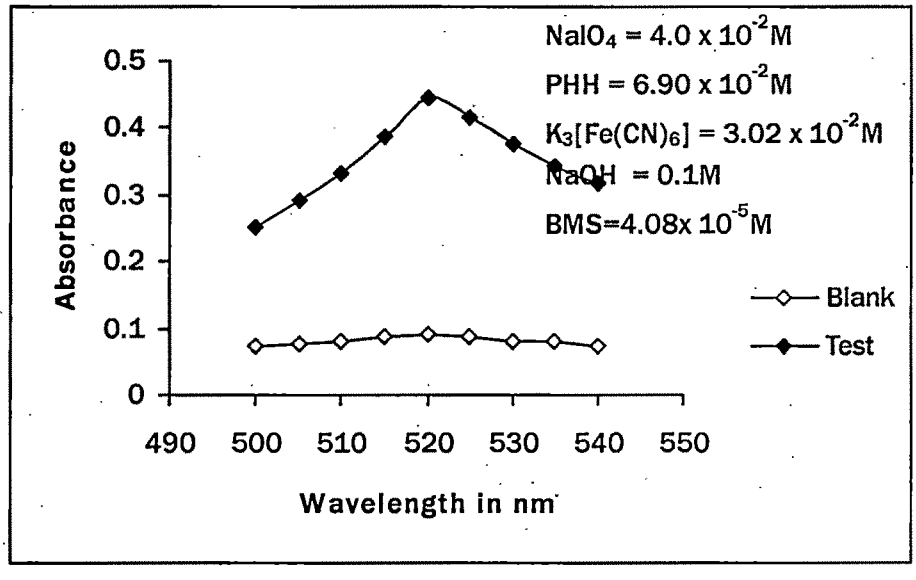
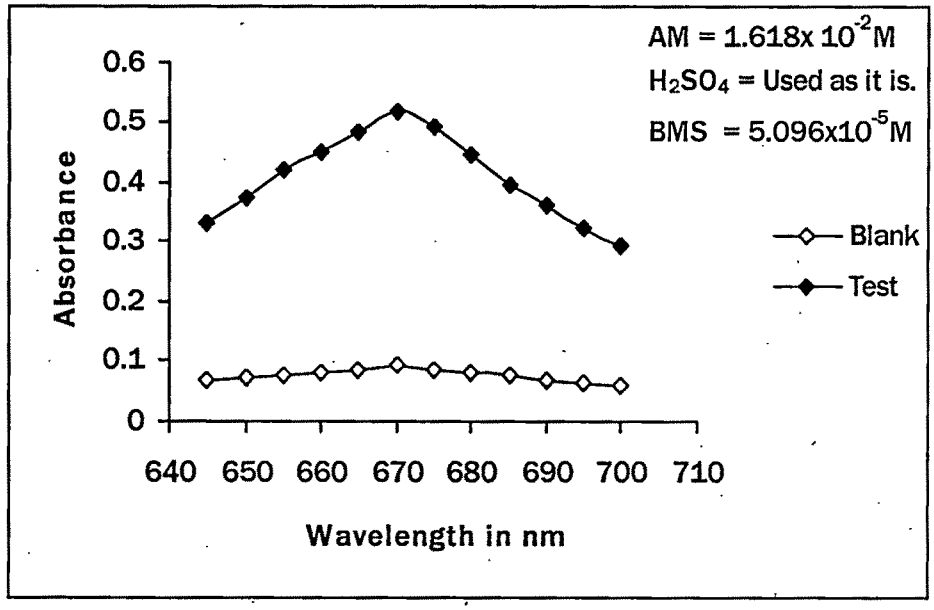


Fig. 5.05: Absorption spectrum of MIRT with AM – H_2SO_4 (M_{18})



6: Beer's Law plot of MIRT with MDNB-NaOH (M_1)

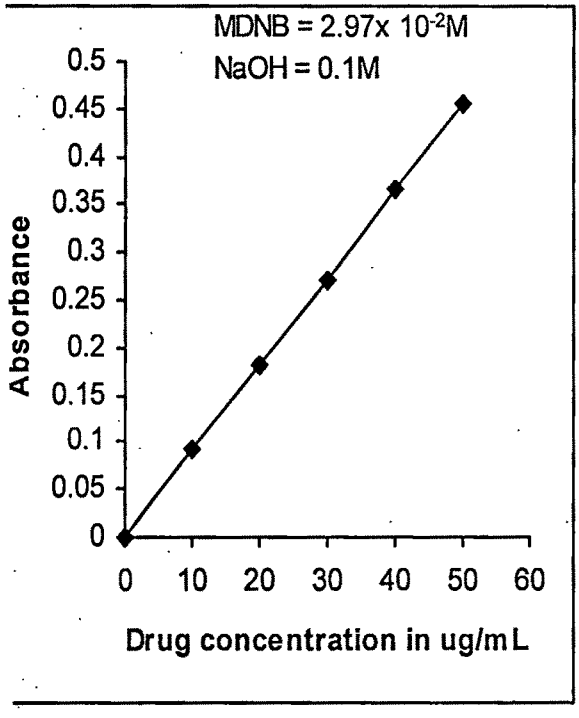
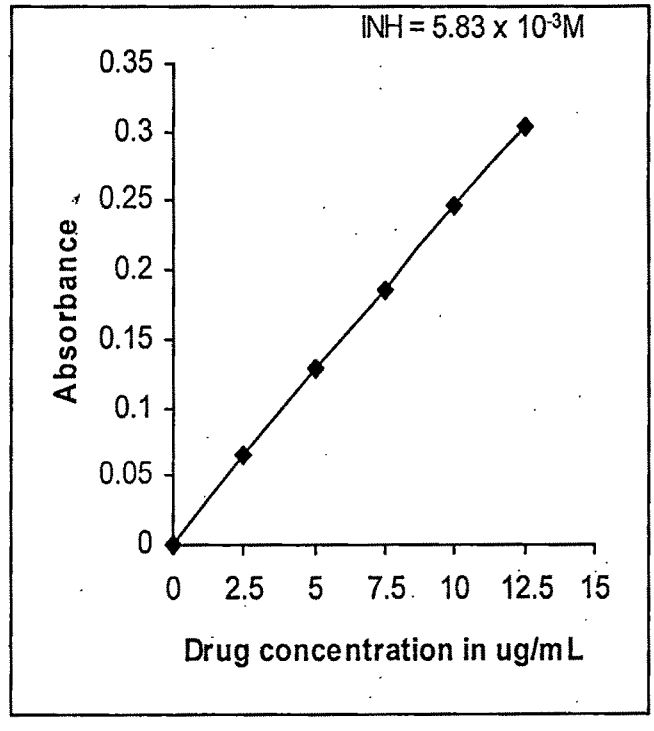


Fig. 5.07: Beer's Law plot of MIRT with INH (M_3)



: Beer's Law plot of MIRT with MBTH – Fe(III) (M_9)

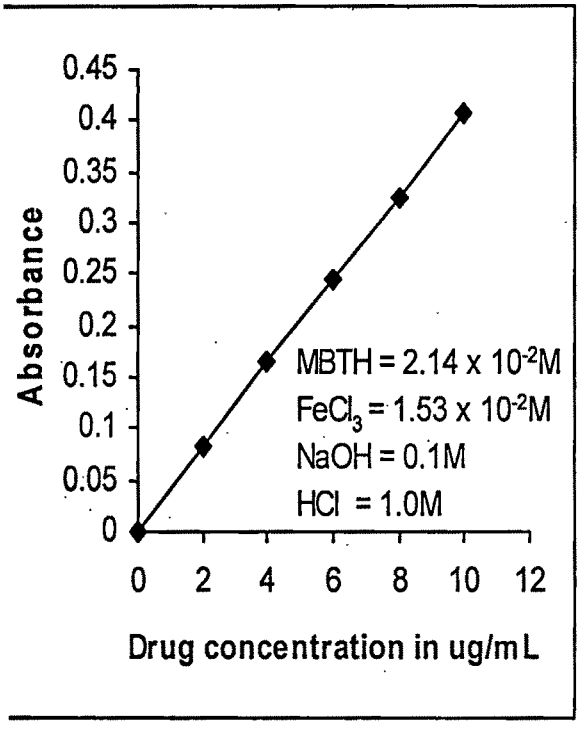


Fig. 5.09: Beer's Law plot of MIRT with IO_4^-/PHH-$[Fe(CN)_6]^{-3}$ (M_{12})

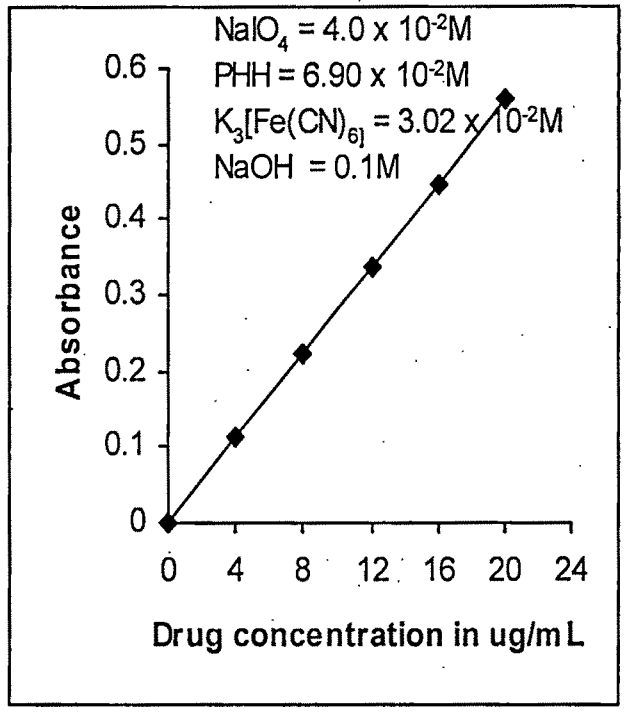


Fig. 5.10: Beer's Law plot of MIRT with AM – H_2SO_4 (M_{18})

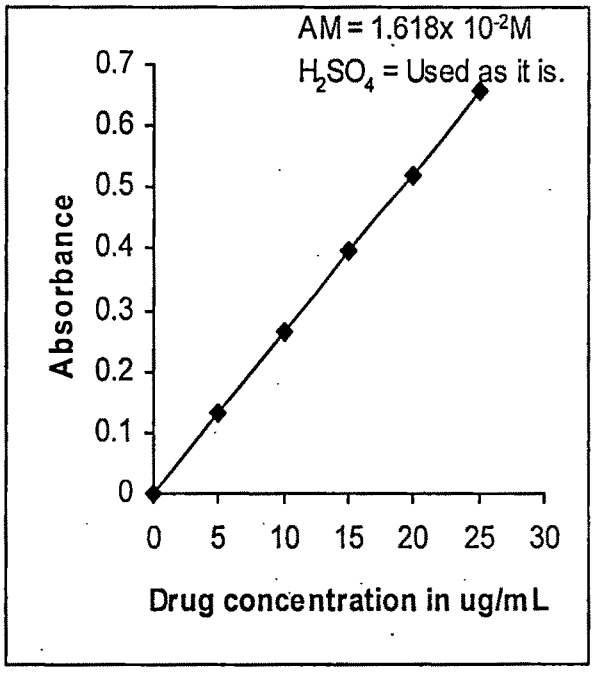


Fig. 5.11: Ringbom plot of BMS with MDNB (M_1)

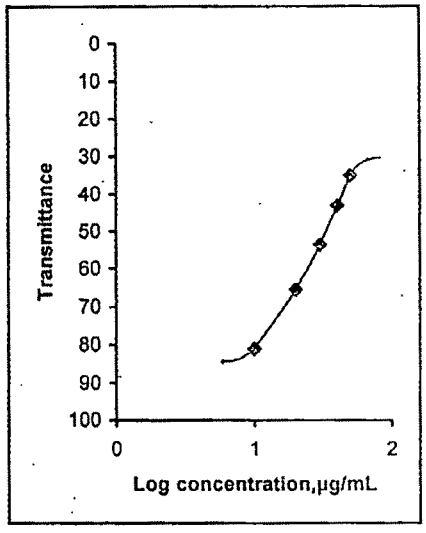


Fig. 5.12: Ringbom plot of BMS with INH (M_3)

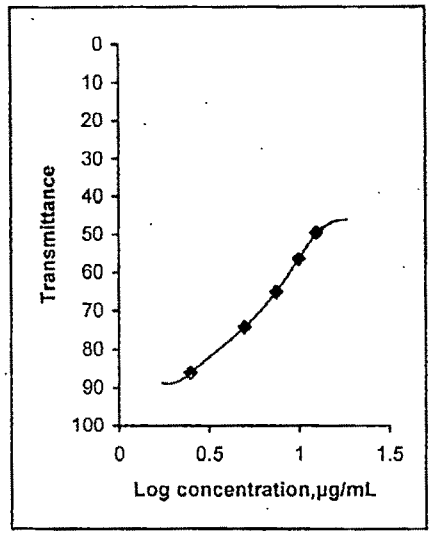


Fig. 5.13: Ringbom plot of BMS with MBTH – Fe(III) (M_9)

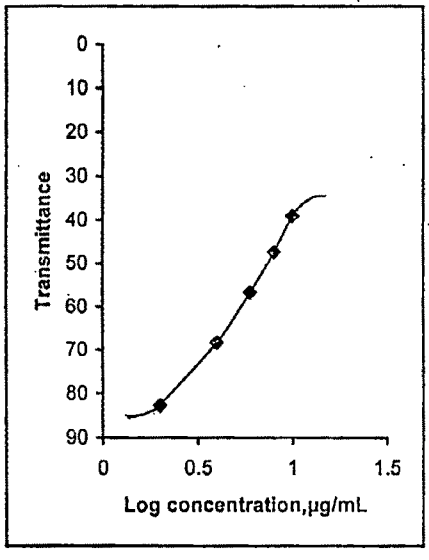


Fig. 5.14: Ringbom plot of BMS with PHH –IO_4(M_{12})

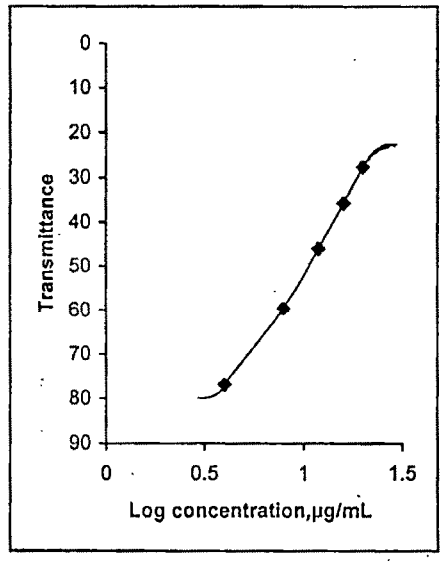


Fig. 5.15: Ringbom plot of BMS with AM – $H_2SO_4(M_{18})$

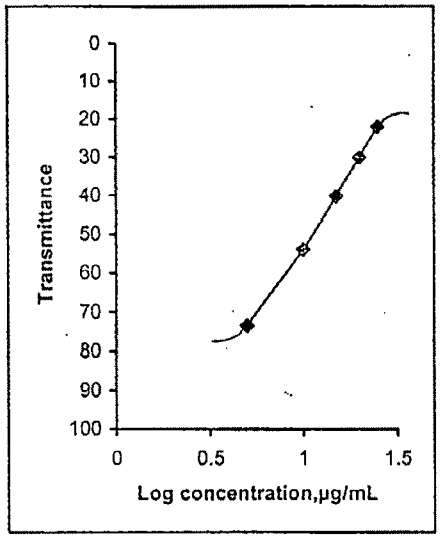


Fig. 5.16: Absorption spectra of BMS in CH_3OH (UV reference method)

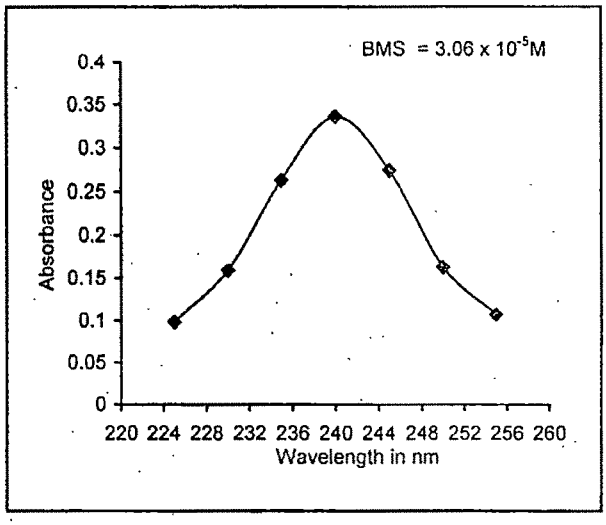
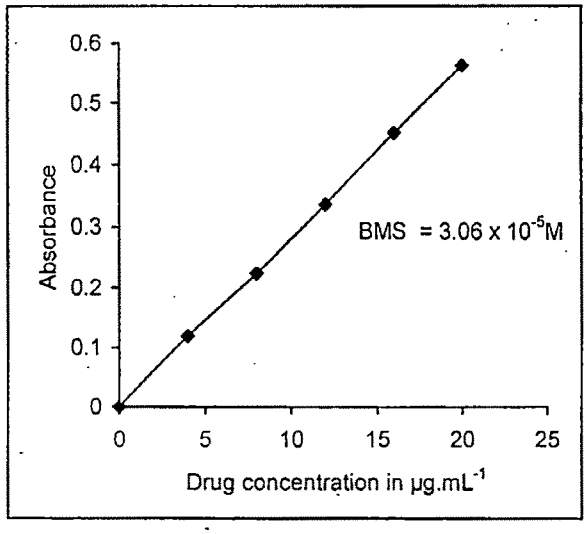


Fig. 5.17: Beer's law plot of BMS in CH_3OH (UV reference method)



Method M_{12}:

In developing this method, the effect of various parameters likes strength and volume of $NaIO_4$, time and temperature required for oxidation, volume and strength of reagents [PHH and $K_3Fe(CN)_6$] ,volume of Conc.HCl, solvent for final dilution in developing color of maximum stability and intensity were studied. The results are incorporated in (Table 5.07, P. 255).

Method M_{18}:

The method involves the reaction of the BMS with AM in acid medium. The effect of various parameters, such as concentration and volume of AM, nature and strength of acid, order of addition of reagents, solvent for final dilution were studied and the optimum conditions developed and are recorded in (Table 5.08, P.256).

c. Optical characteristics:

In order to test whether the colored species formed in the above methods adhere to Beer's law the absorbance at appropriate wavelength of a set of solution containing different amount of Betamethasone and specified amount of reagents were noted against appropriate reagent blanks. The Beer's law was obeyed for the above mentioned methods. Least square regression analysis was carried out for the slope intercept and correlation coefficient. 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 5.09 P.268).

d. Precision & Accuracy:

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 5.09 P.268).

e. Accuracy:

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 5.10; P.269).

f. Interference studies:

The effect of wide range of excipients and other active ingredients usually present in the formulations for the assay of BMS in methods M_1, M_3, M_9, M_{12}, and M_{18} under optimum conditions were investigated. The commonly used execipients and other active ingredients usually present in formulations did not interfere even if they were present in amount than they usually exist.

g. Analysis of formulations

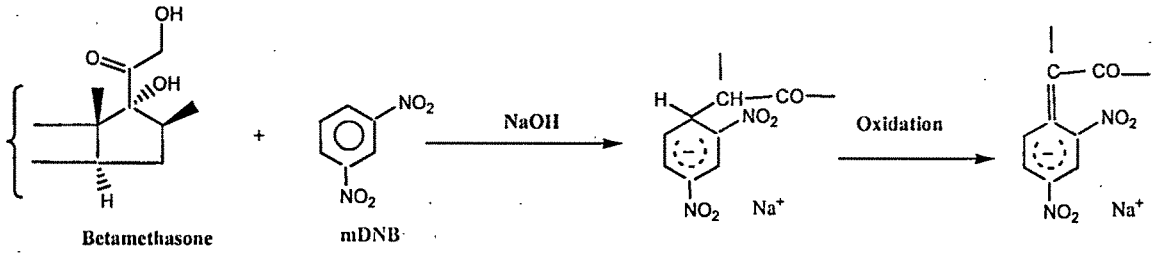
Commercial formulations containing Betamethasone were successfully analysed by each proposed method. The values obtained by the proposed and reference methods for formulations were compared statistically by the t- and F- tests found not to differ significantly. Recovery studies were conducted by analyzing each

pharmaceutical formulation in the first instance for the active ingredient by the proposed methods. The results are incorporated in (Table 5.10; P.269).

h. Nature of colored species:

Method M_1:

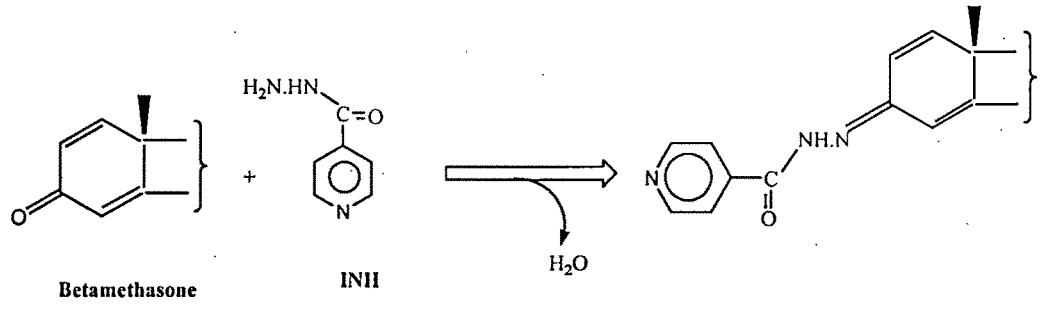
In this Method, m-dinitrobenzene in alkaline medium to give Meisenheimer like σ-complex with the α-Ketol group present in the Betamethasone, which in then oxidized by the excess reagent (Zimmermann regent) to a colored product (Scheme-5.01) which exhibiting λ_{max} at 490nm.



Scheme-5.01

Method M_3:

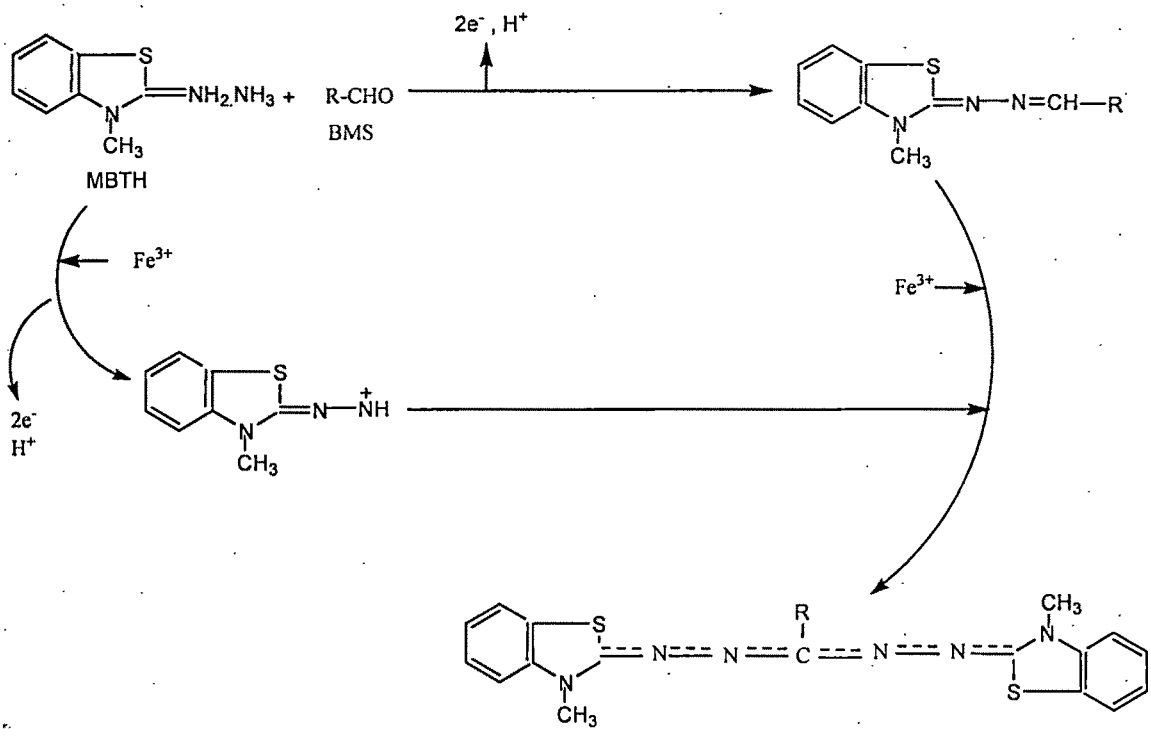
In this Method, Isonicotinic acid hydrazide (INH) give colored hydrazone with $\Delta^{1,4}$-3-keto steroid (Betamethasone) drug .The colored species formation may be represented as under (Scheme-5.02).



Scheme-5.02

Method M_9:

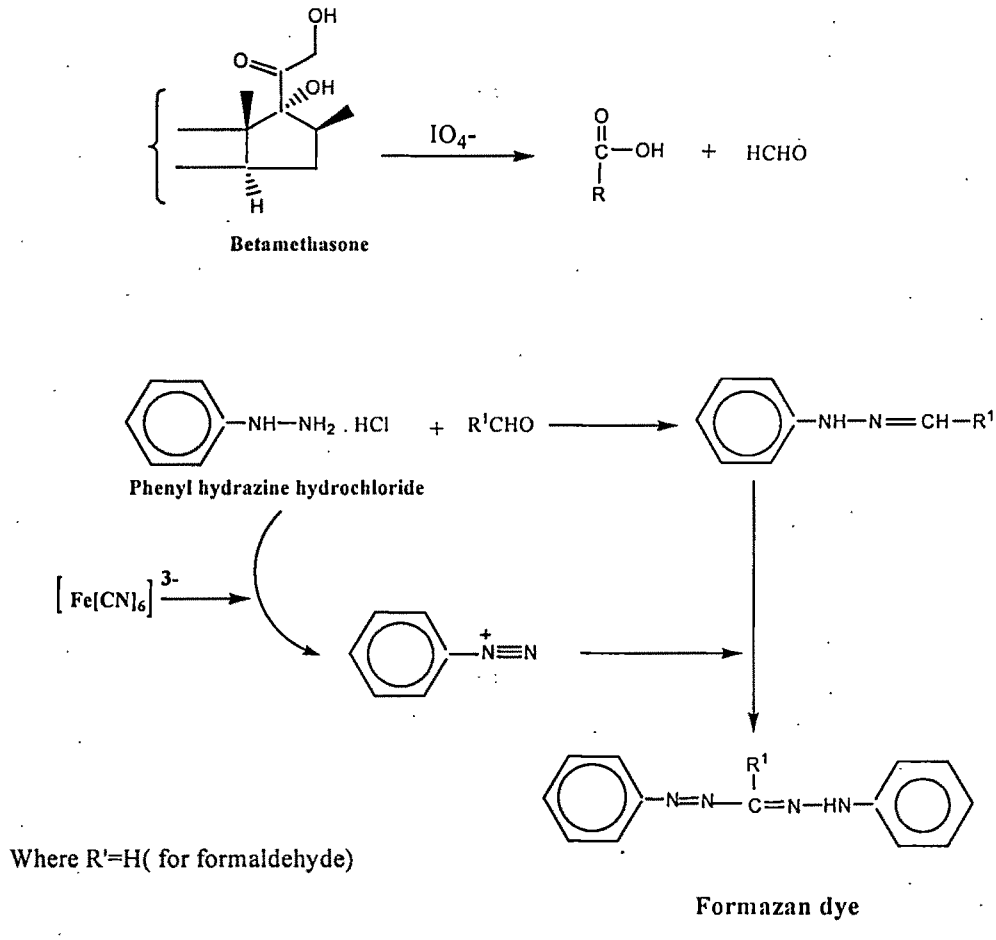
Betemethasone possesses α-Ketol group. The keto group in it undergoes condensation with MBTH in alkali medium initially followed by development as formazan with the diazonium salt generates in the reaction medium by the oxidation of the excess reagent. It may be postulated that, at least with ketoses, the hydrazone initially formed is converted into osazone thus giving rise to an aldehyde-hydrazone group, which then react according to the usual scheme as represented in scheme-5.03.



Scheme-5.03

Method M_{12}:

In this method formaldehyde formed from α-ketol portion of betamethasone through oxidation, is first converted into its phenyl hydrazone, then a red color is developed under the action of the oxidizing agent [(hexacyanoferrate (III)] in acid medium. The colored species formation may be represented as under scheme-5.04.



Scheme-5.04

Method M_{18}:

In this method tetrahedral anion MoO_4^{-2} in aqueous medium, which is not strongly oxidizing form, on acidification with Conc.H_2SO_4 exist as isopolyanionic species as a result of polymerization and condensation reactions having an arrangement of Mo_6 octahedral as exemplified by $Mo_7O_{24}^{-6}$ and $Mo_8O_{26}^{-4}$.The α-ketol group present in the Betamethasone probably effects the reduction of 1,2 or 3 oxygen atoms from exemplified molybdate, thereby producing one or two more of possible reducing species which have a characteristic intense blue color(Molybdenum blue).

Table 5.09

Optical and regression characteristics, precision and accuracy of the proposed methods for BMS

Parameter	M_1	M_3	M_9	M_{12}	M_{18}
λ_{max} (nm)	490	445	620	520	670
Beer's law limits (μg/mL)	10 - 50	2.5 - 12.5	2 - 10	4 - 20	5 - 25
Detection limit (μg/mL)	0.6575	0.2768	0.1162	0.0903	0.3191
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	3.53×10^3	9.73×10^3	3.22×10^4	1.09×10^4	1.03×10^4
Sandell's sensitivity (μg.cm^{-2}/0.001 absorbance unit)	0.2286	0.1145	0.0838	0.1081	0.1119
Optimum photometric range (μg/mL)	14.5 - 47.5	4.9 - 11.6	3.5-8.7	5.5 - 18.5	4.5 - 23.0
Regression equation (Y=a+bc) slope (b)	9.08×10^{-3}	2.384×10^{-2}	4.045×10^{-2}	2.772×10^{-2}	2.596×10^{-2}
Standard deviation on slope (S_b)	6×10^{-5}	2.653×10^{-4}	2.36×10^{-4}	6.2915×10^{-5}	1.665×10^{-4}
Intercept (a)	2×10^{-4}	7.4×10^{-3}	2.1×10^{-3}	3.3×10^{-3}	5.2×10^{-2}
Standard deviation on intercept (S_a)	1.989×10^{-3}	2.2×10^{-3}	1.567×10^{-3}	8.347×10^{-4}	2.762×10^{-3}
Standard error on estimation (S_e)	1.897×10^{-3}	2.097×10^{-3}	1.494×10^{-3}	7.958×10^{-4}	2.633×10^{-3}
Correlation coefficient (r)	0.9999	0.9998	0.9999	0.9999	0.9999
Relative standard deviation (%)	0.5897	0.7559	0.7146	0.4839	0.4448
% Range of error (confidence limits)					
0.05 level	0.6191	0.7935	0.7502	0.5081	0.4669
0.01 level	0.9709	1.2445	1.1766	0.7968	0.7323

* Average of six determinations considered

Table 5.10

Assay of BMS in Pharmaceutical Formulations

Formulations*	Amount taken (mg)	Amount found by proposed Methods**					Reference method	Percentage recovery by proposed methods***				
		M_1	M_3	M_9	M_{12}	M_{18}		M_1	M_3	M_9	M_{12}	M_{18}
Tablet I	0.5	0.489±0.03 F=4.0 t=0.12	0.488±0.04 F=2.25 t=0.14	0.496±0.05 F=1.44 t=0.13	0.489±0.035 F=2.94 t=0.109	0.495±0.04 F=2.25 t=0.10	0.492 ±0.06	99.39±0.23	99.19±0.31	100.81±0.14	99.39±0.12	100.61±0.13
Tablet II	1.0	0.986±0.05 F=3.24 t=0.19	0.989±0.06 F=2.25 t=0.115	0.988±0.07 F=1.65 t=0.13	0.985±0.08 F=1.27 t=0.18	0.998±0.05 F=3.24 t=0.15	0.994±0.09	99.19±0.21	99.50±0.13	99.39±0.28	99.09±0.16	100.40±0.21

* Tablets from 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 10mg added to the preanalysed pharmaceutical formulations (average of three determinations)

5.04. CONCLUSIONS

The proposed five 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 excipients. Hence, the methods could be considered for the determination of Betamethasone in the quality control laboratories.

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