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Simultaneous Estimation Of Betamethasone & Mupirocin In Its Dosage Form

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ABSTRACT

The present study describes the development and validation of a simple, rapid, and cost-effective UV spectroscopic method for the simultaneous estimation of Betamethasone and Mupirocin in pharmaceutical dosage forms. Owing to the overlapping spectra of the two drugs, the method was developed using the simultaneous equation approach (Vierordt's method), based on absorbance measurements at two selected wavelengths corresponding to the λ max of Betamethasone and Mupirocin.

Betamethasone and Mupirocin exhibited maximum absorbance at approximately 240 nm and 220 nm, respectively, in the chosen solvent system. The method was validated according to ICH guidelines for parameters including linearity, accuracy, precision, and specificity. Linearity was observed over the concentration range of 2–18 µg/mL for both Betamethasone and Mupirocin, with correlation coefficients exceeding 0.999. Accuracy studies demonstrated recovery values within acceptable limits, while precision studies yielded %RSD values below 2%, confirming the method's reproducibility.

Overall, the developed UV spectroscopic method is simple, accurate, and suitable for the routine analysis of Betamethasone and Mupirocin in combined dosage forms without the need for prior separation.

KEYWORDS: UV spectrophotometric, Probe Sonication, Betamethasone and Mupirocin validation.

INTRODUCTION

Mupirocin is a bacterially derived antibiotic that is of primary use as a topical agent for the treatment of bacterial skin infections and to eliminate Staphylococcus aureus, including methicillin-resistant Staphylococcus aureus (MRSA), from the nasal passages.

chemically known (2S,3R,4S,5S)-5-[(2S,3R,4E,6S)-3-[(2S)-2-Butanoyl] oxy-6-[(1R,2S,3S)-3-ethyl-2-hydroxycyclopropyl]-1-oxo-4-heptenyl] tetrahydrofuran-2,3,4-triol

Betamethasone is a very strong glucocorticoid steroid with immunosuppressive and anti-inflammatory characteristics. It is used most often to treat autoimmune and inflammatory diseases, as well as dermatological conditions. Its chemical and drug properties are described below as (11β,16β)-9-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione

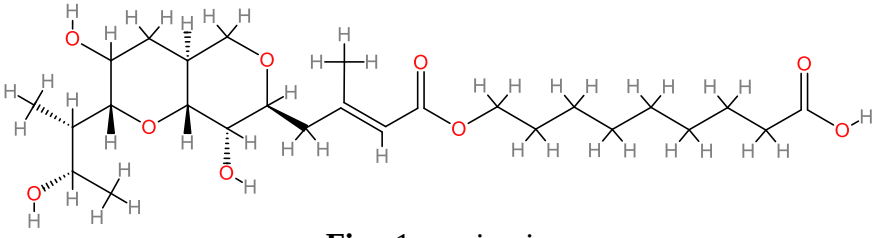

Fig : 1 mupirocin

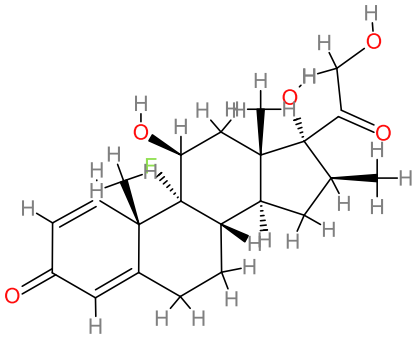

Fig : 2 Betamethasone

Mupirocin kills skin-surface bacteria (*Staphylococcus aureus*), while *betamethasone* calms inflammation, redness and itching. Together they shorten healing time of small eczematous or impetiginized lesions.

MATERIALS AND METHOD:

Betamethasone and mupirocin pure powder were gift sample supplied from Cerata Pharmaceuticals LLP is a leading manufacturer of Active Pharmaceutical Ingredients (API) Ahmedabad, Gujrat India. Formulation Supirocin-B Ointment 5gm (Label claim: betamethasone-0.05% w/w + mupirocin-2% w/w) was manufactured by Glenmark Pharmaceutical Pvt. Ltd. and purchased from local store in Gwalior,

India UV-Visible spectrophotometer (PerkinElmer Lambda 25) was employed with a spectral band width of 1 nm and a wavelength accuracy of 0.3nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells).

SELECTION OF SOLVENT AND WAVELENGTH:

The solubility of Betamethasone and Mupirocin was evaluated in different solvents, including ethanol, water, and methanol. Their UV absorption spectra were then recorded in these media. Among the tested solvents, ethanol showed the highest absorbance for both drugs. Based on the obtained spectra figure

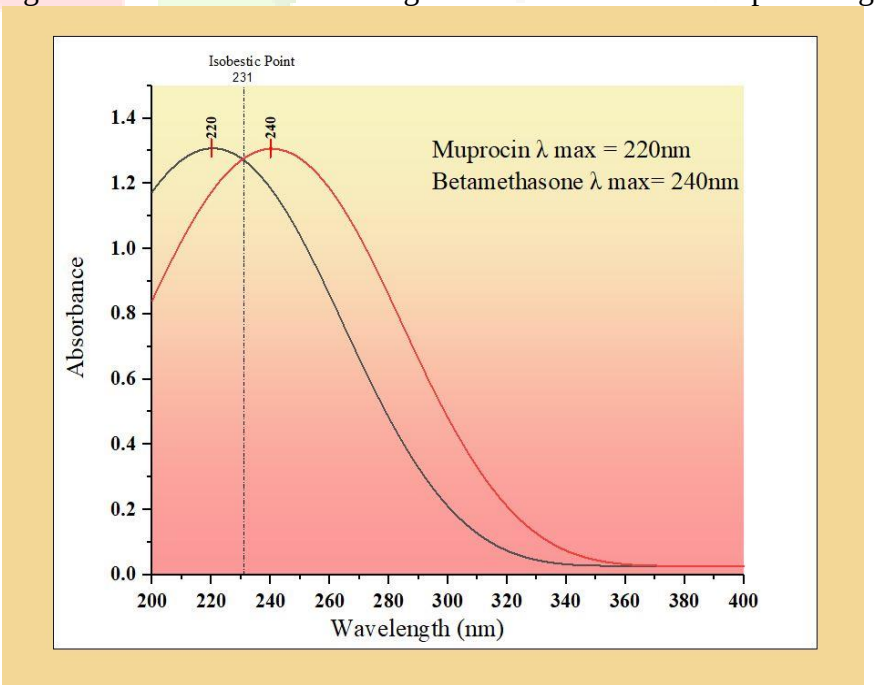

Fig: 3 UV spectra Mupirocin and betamethasone (2-12µg/ml)

PREPARATION STANDARD STOCK SOLUTIONS:

The laboratory Prepare the stock solution of mupirocin and betamethasone

Betamethasone 10mg was separately weighed and transferred to 100ml volumetric flask for stock solution from the concentration of 100 µg/ml after the initial addition of ethanol, the solution was subjected to sonication for 10min. additional ethanol was then added to reach a final volume of 100ml.

The prepare stock solution is being for mupirocin

Initially, 10 mg of Mupirocin (MUP) was accurately weighed and transferred into a 100 mL volumetric flask. The drug was dissolved in ethanol and sonicated for 10 minutes to ensure complete solubilization. The solution was then diluted with ethanol to obtain a final volume of 100 mL

Preparation of a working standard solution for betamethasone

For the preparation of Betamethasone (BETA) samples, a stock solution of 100 µg/mL was first prepared. From this, aliquots were transferred into separate 10 mL volumetric flasks to obtain concentrations of 0.2, 0.6, 0.8, 1.0, and 1.2 µg/mL Each solution was diluted to volume with ethanol, yielding final concentrations of 2, 6, 8, 10, and 12 µg/mL, respectively. The absorbance of these solutions was subsequently recorded

Preparation of a working standard solution for betamethasone

Aliquots were withdrawn from a 100 µg/mL standard stock solution of Mupirocin (MUP) to prepare concentrations of 0.2, 0.6, 0.8, 1.0, and 1.2 µg/mL each aliquot was transferred into a separate 10 mL volumetric flask, and methanol was added to achieve final MUP concentrations of 2, 6, 8, 10, and 12 µg/mL, respectively. The absorbance of each resulting solution was subsequently measured.

Preparing the sample solution

Approximately 1 g of the ointment was accurately weighed and transferred into a centrifuge tube, followed by dilution to 10 mL with the selected solvent. The mixture was heated at 70 °C for about 8 minutes with intermittent mixing to facilitate extraction. After heating, the sample was centrifuged at 1000 rpm for 10 minutes, and the supernatant containing the analyte was carefully collected. An aliquot of this layer was filtered through 0.4 µm filter paper. From the filtrate, 1 mL was withdrawn and diluted to 10 mL with solvent. Subsequently, 1 mL of this solution was again diluted to 10 ml. the absorbance of the final solution was measured at 220 nm and 240 nm for UV spectrophotometric validation

Simultaneous equation method (SE)

Standard stock solutions of Mupirocin (MUP) and Betamethasone (BETA), each at a concentration of 100 µg/mL, were prepared in ethanol. From these, working solutions of 10 µg/mL were obtained by appropriate dilution with ethanol. The UV spectra of the prepared solutions were recorded over the wavelength range of 200–400 nm.for quantitative analysis, the Simultaneous Equation (SE) method was applied, which relies on the spectral properties of the drugs. From the overlapped spectra, two wavelengths were chosen: 220 nm for MUP and 240 nm for BETA.

To establish the calibration, stock solutions of MUP and BETA were further diluted to prepare a series of solutions in the concentration range of 2–12 µg/ml. The UV spectra of these solutions were recorded, and absorbance values were measured at 220 nm and 240 nm. These absorbance readings were used to calculate the absorptivity values (the ratio of absorbance to concentration, expressed as g/mL per 100 ml) for both drugs at the selected wavelengths.

Finally, the average absorptivity values at each wavelength were applied in the SE method to determine the concentrations of MUP and BETA in the prepared solutions

Sample solution of analysis

The simultaneous equations were constructed based on the absorbance values of MUP and BETA at their respective λ max (220 nm and 240 nm). The general form of the equations is given as

Here, $\mathbf{C_{MUP}}$ represents the concentration of MUP, while $\mathbf{C_{BETA}}$ denotes the concentration of BETA. The absorptivity values of MUP at 220 nm and 240 nm are expressed as $\mathbf{ax_1}$ and $\mathbf{ax_2}$, respectively. Similarly, the absorptivity values of BETA at 243 nm and 265 nm are represented as $\mathbf{ay_1}$ and $\mathbf{ay_2}$. The absorbance of the sample solution, measured at 220 nm and 240 nm, is denoted by **A1** and **A2**, respectively.

$$A1 = ax_1bcx + ay_1bcy$$
$$A2 = ax_2bcx + ay_2bcy$$

LINEARITY:

The calibration curves demonstrated linearity in accordance with Beer's law over the concentration range of 2–18 µg/mL for both Mupirocin (Figure 4) and Betamethasone (Figure 5). The relationship between absorbance and concentration was evaluated using least-squares linear regression analysis to obtain the calibration equations and correlation coefficients. The regression data, summarized in Table 1, confirmed a strong linear correlation between concentration and absorbance for both drugs.

TABLE 1: Linearity

Parameters	**Betamethasone**	**Mupirocin**
Linearity range	2-18µg ml	2-18µg ml
Correlation coefficient	0.999	0.998
Slope	0.00254	0.00129
Intercept	0.157862	0.122379725

TABLE 2: Linearity data of mupirocin and betamethasone

Sr no	**con (µg/ml)**	**absorbance of mupirocin (220nm)**	**absorbance of betamethasone (240nm)**
1	2	**1.1251**	1.1631
2	6	1.1301	**1.1731**
3	10	1.1351	1.1831
4	14	1.1401	1.1931
5	18	1.1459	1.2039

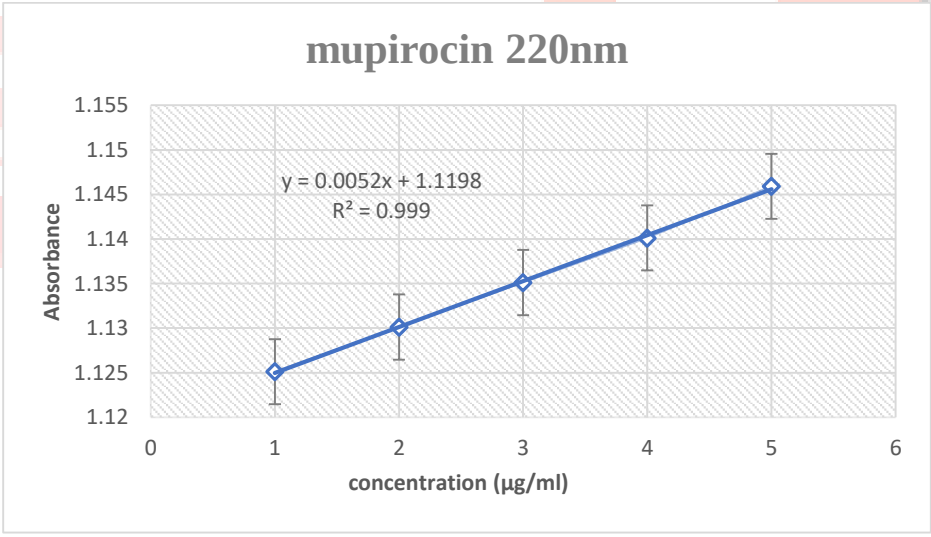

Fig: 4 Calibration curve of mupirocin at 220nm

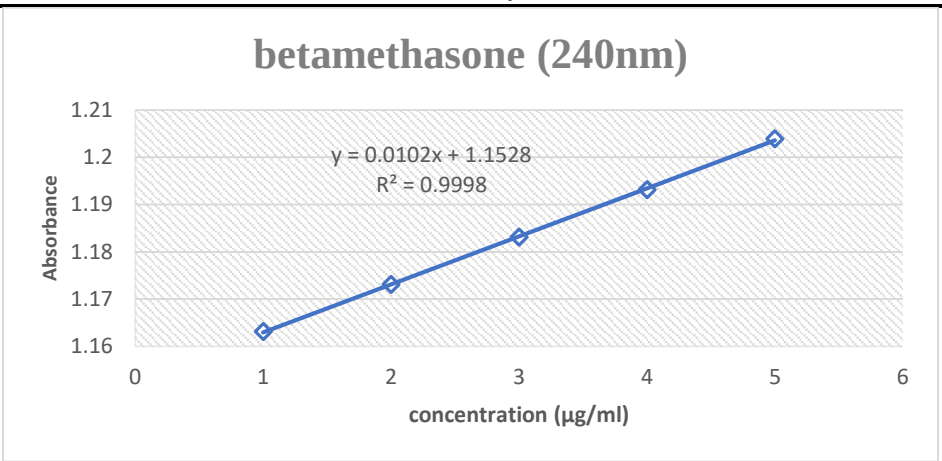

Fig: 5 Calibration curve of betamethasone at 240nm

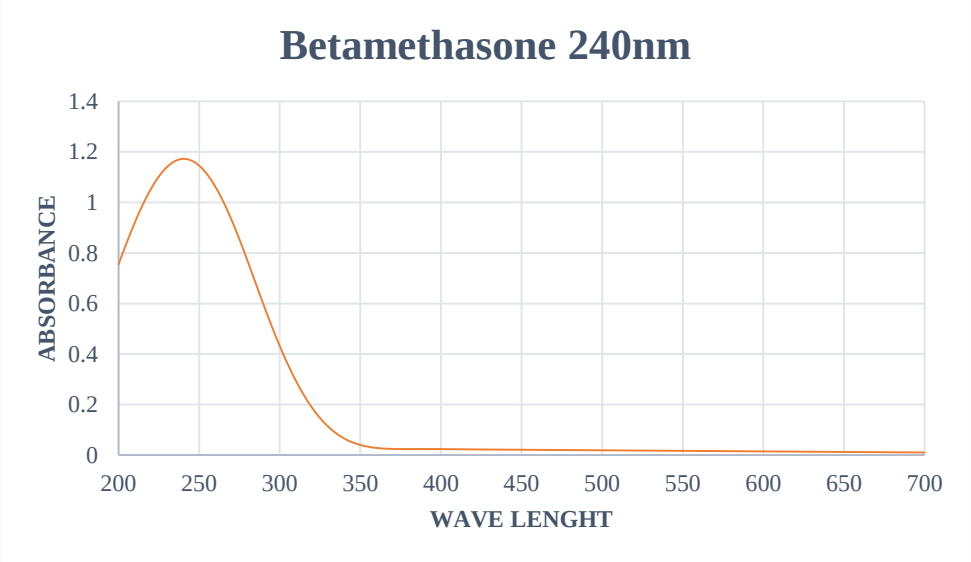

Fig: 6 betamethasone (6µg /ml)

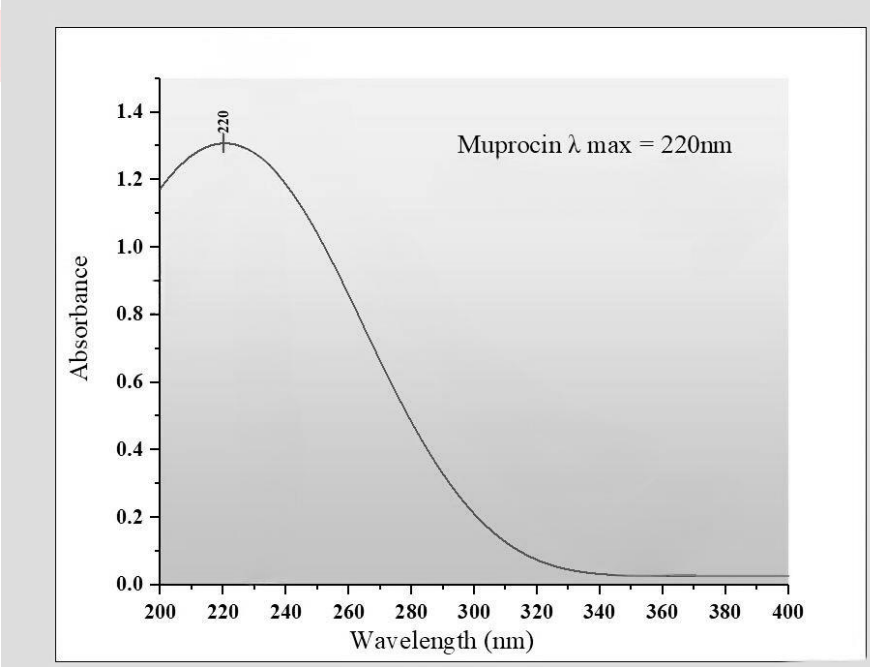

Fig: 7 mupirocin (2µg /ml)

Precision

The precision of the method was evaluated by assessing both intraday and inter-day variations. For the intraday study, the concentrations of Mupirocin and Betamethasone were analyzed three times within the same day at 1-hour intervals. For the inter-day study, the analysis was repeated on three consecutive days. The results were statistically evaluated, and the Relative Standard Deviation (%RSD) was found to be less than 2%, indicating excellent repeatability and precision of the method. The detailed results are presented in Table 2

TABLE 2: Precision Studies mean of three observations

Drug	**Concentration (µg/ml)**	**ntraday precision % RSD**	**nter-day precision % RSD**
Betamethasone	6-8	0.049	.29
Mupirocin	2-5	0.050	.01

LIMIT OF DETECTION (LOD) & LIMIT OF QUANTITATION (LOQ)

The LOD and LOQ for Mupirocin and Betamethasone were determined mathematically using the following formulas: LOD=3.3 σSandLOQ=10 σS\text{LOD} = \frac{3.3 \, \sigma}{S} \quad \text{and} \quad \text{LOQ} = \frac{10 \, \sigma}{S}LOD=S3.3σandLOQ=S10σ

where σ\sigmaσ is the standard deviation of the response and SSS is the slope of the calibration curve for the respective analyte. The LOD values for Mupirocin and Betamethasone were found to be 2.0 µg/mL and 6.0 µg/mL, respectively, while the LOQ values were 4.0 µg/mL for Mupirocin and 5.0 µg/mL for Betamethasone

ROBUSTNESS& RUGGEDNESS

Repeatability of the method was evaluated by performing the procedure multiple times over a short period under identical conditions. The pharmaceutical preparation, recovery studies, and intraday precision all exhibited low %RSD values, indicating excellent repeatability. Robustness for Mupirocin (MUP) and Betamethasone (BETA) was assessed by making slight variations in the analytical wavelength (±0.5 nm), and the resulting %RSD values confirmed the consistency and reliability of the method

ACCURACY

To evaluate the accuracy and reliability of the proposed method, recovery studies were performed. Known amounts of standard Mupirocin and Betamethasone were added to an equivalent quantity of the formulation powder at 50%, 100%, and 150% levels. The mixtures were then analyzed using the proposed method, and the percent recovery (%Recovery) along with the relative standard deviation (%RSD) were calculated. The results, summarized in Table 3, demonstrate the method's accuracy and suitability for quantitative analysis

TABLE 3: Recovery Studies Data of Mupirocin and Betamethasone

Level	**%Recovery**		**%RSD**	
	Mupirocin	**Betamethasone**	**Mupirocin**	**Betamethasone**
50%	98.14	98.6	0.119	0.201
100%	99.65	99.45	0.211	0.210
150%	101.23	100.58	0.231	0.248

TABLE: 4 Statistical data of repeatability of mupirocin and betamethasone

Concentration	**Mupirocin(220nm)**	**Concentration**	**Betamethasone(240nm)**
2 µg/ml	1.125	6 µg/ml	1.173
	1.126		1.174
	1.125		1.173
Mean	1.125		1.173
SD	50		33.169
%RSD	0.051		0.049

Analysis of formulation

For the assay, 5 g of ointment, each containing 100 mg of Mupirocin and Betamethasone, was accurately weighed. The average weight was dissolved in ethanol, and the volume was adjusted to obtain the desired concentrations. The absorbance of the resulting solutions was measured at 220 nm and 240 nm (Figure 3). The recorded absorbance values are summarized in Table 5. The concentrations of Mupirocin and Betamethasone were then calculated using the simultaneous equations described below.

Table 5: Analysis of Formulation

Drug	Amount (mg/ointment)		%label claim	% RSD
	Labeled	Estimated		
Mupirocin	100	98.22	99.9	0.49
Betamethasone	100	97.89	99.8	0.90

RESULTS AND DISCUSSION

The estimation of Mupirocin and Betamethasone was performed using the simultaneous equation method with a UV spectrophotometer. Linearity was assessed over the concentration range of 2–18 µg/mL for both drugs, and the method was found to obey Beer's law. For Mupirocin at 220 nm, the slope, intercept, and correlation coefficient were 0.00129, 1.1224, and 0.998, respectively. For Betamethasone at 240 nm, the corresponding values were 0.00254, 1.1579, and 0.999. Recovery studies were conducted to confirm the reproducibility and reliability of the method by adding known amounts of standard drugs to the formulation and analyzing them according to the established procedure

CONCLUSION

The developed UV spectrophotometric method is simple, precise, accurate, linear, reproducible, and repeatable for the estimation of Betamethasone and Mupirocin in pharmaceutical dosage forms, with no interference from excipients. This method can be reliably applied for the routine analysis of both drugs in pharmaceutical formulations

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