IJBPAS, October, 2024, 13(10): 5148-5166

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR CONCURRENT DETERMINATION OF MUPIROCIN AND BECLOMETHASONE DIPROPIONATE IN PHARMACEUTICAL FORMULATION AND PERFORM FORCED DEGRADATION STUDY

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Received 24th Sept. 2023; Revised 25th Oct. 2023; Accepted 16th Jan. 2024; Available online 1st Oct. 2024

https://doi.org/10.31032/IJBPAS/2024/13.10.8420

ABSTRACT

Simple, specific, accurate, precise and reproducible method has been developed and validated for the simultaneous estimation of both drugs in their combined dosage form. In RP-HPLC, analysis is carried out using Methanol: Buffer pH 4 (65:35, v/v) mobile phase and Phenomenex Gemini ODS C18 column (200 mm x 4.6 mm, 5.0 µ particle size) as stationary phase with detection wavelength of 215 nm. Linearity was obtained in the concentration range of 40-120 µg/ml and 0.5-1.5 µg/ml for Mupirocin and Beclomethasone dipropionate respectively. The % recoveries of the both the drugs were found to be 99.45- 99.86% and 100.10-100.39% respectively. LOQ were found to be 29.11µg/ml and 0.297µg/ml at 215 nm for Mupirocin and Beclomethasone dipropionate respectively.

Methods were statistically validated for accuracy, precision, specificity, LOQ and robustness. Also carried out a Force degradation study according to ICH guidelines and can be used for analysis of combined dosage form.

Keywords: RP-HPLC, Mupirocin, Beclomethasone dipropionate

INTRODUCTION

Mupirocin, recognized by its commercial names Bactroban and Centany, is classified as a monoxycarbolic acid and serves as a topical antibacterial agent. Originally sourced from Pseudomonas fluorescens, it was developed by Beecham **[18]**. Its chemical nomenclature is 9-[(E)-4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-[[(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2-yl]methyl]oxan-2-yl]-3-methylbut-2-enoyl]oxynonanoic acid, with a molecular composition represented by C26H44O9. Mupirocin boasts a molecular weight of 500.6 and is designated with the CAS number 12650-69-0 **[23]**. This compound presents as a nearly white powder, exhibiting solubility in acetone and ethanol, along with minor solubility in water **[15-17]**. Its melting point resides within the range of 77 to 78°C **[10-12]**.

Mupirocin showcases bacteriostatic attributes when present at lower concentrations and transitions into bactericidal effects as concentrations increase. This topical application demonstrates efficacy particularly against Gram-positive bacteria, including the formidable MRSA strain **[31]**. The composition of Mupirocin encompasses diverse pseudomonic acids, with a predominant contribution from pseudomonic acid A (PA-A), constituting more than 90% of the mixture **[19]**. The underlying mechanism of action involves Mupirocin's reversible binding to bacterial isoleucyl-tRNA synthetase, an enzyme pivotal in catalyzing the conversion of isoleucine and tRNA into isoleucyl-tRNA **[20]**. This binding event hampers the enzyme's proper functioning, consequently impeding the synthesis of bacterial protein and RNA, thereby orchestrating inhibitory effects **[10-12]**.

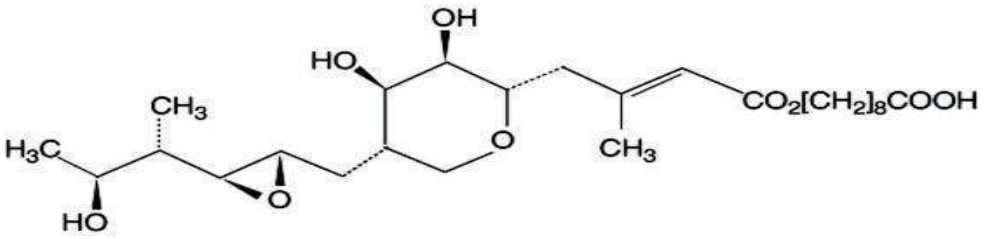

Figure 1: Structural formula of Mupirocin

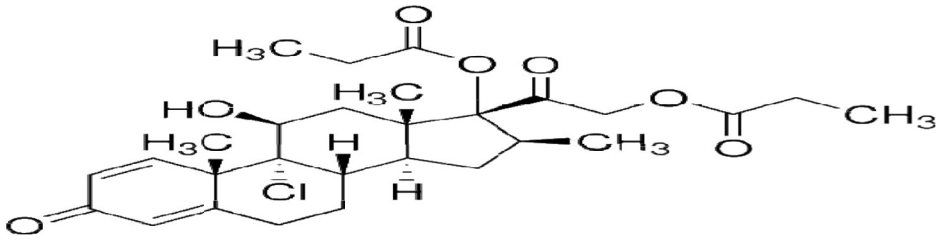

Figure 2: Structural Formula of Beclomethasone dipropionate

Beclomethasone dipropionate belongs to the Anti-inflammatory and Anti-bacterial drug category. Chemically, it is denoted as 9-chloro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate, with a molecular formula of C28H37ClO7. The molecular weight of Beclomethasone dipropionate is 521.1 **[23-24]**, and it exists in the form of a white or nearly white to off white crystalline powder. It is soluble in acetone and ethanol, while being insoluble in water **[25-28]**. The melting point of Beclomethasone dipropionate lies within the range of 117-120°C **[13-15]**.

Beclometasone dipropionate, sometimes spelled as beclomethasone dipropionate, is a type of steroid medication. It is marketed under various brand names, including Qvar. This medication comes in different forms, such as inhalers, creams, pills, and nasal sprays. The inhaled variant is utilized for the prolonged control of asthma. The cream formulation can be employed to address dermatitis and psoriasis. Additionally, the pills have been utilized in the treatment of ulcerative colitis **[16-17]**.

Unbound corticosteroids have the ability to traverse cell membranes and attach themselves with a strong affinity to specific cytoplasmic receptors. This interaction leads to various outcomes, such as the prevention of white blood cell infiltration at the inflammation site, disruption of the activities of agents involved in the inflammatory response, dampening of humoral immune reactions, and the decrease of both edema and scar tissue **[28]**.

MATERIAL AND METHODS

Chemical and reagents

The reference standards for Mupirocin and Beclometasone dipropionate, which were utilized throughout the experiment, were provided as gift samples by Glenmark Pharmaceuticals Ltd., located in Mumbai, Maharashtra, India. The commercially available formulation, Supirocin B plus® ointment, produced by Glenmark Pharmaceuticals Ltd. in Mumbai, India, containing 2% Mupirocin and 0.025% Beclometasone dipropionate, was obtained from the market.For the experiment, methanol and acetonitrile of analytical reagent (AR) and

HPLC grades were used as solvents, procured from Merck Specialties Pvt. Ltd. in India.

Apparatus

The HPLC system used in the experiment was the Shimadzu Corporation's LC 20 AT model, equipped with an SPD-20A diode array detector (UV-visible). The system operated on an Isocratic setup with a back pressure of 5000 psi and a Flow is maintained at a rate of 1 milliliter per minute. Injection of samples was done using a Rhenodyne valve with a 20 µl fixed loop injection. The chromatographic separation was executed employing a Phenomenex Gemini ODS-C18 column.

For weighing purposes, a highly sensitive electronic balance with the model name AX 200, manufactured by SHIMADZU CORPORATION in Japan, was employed.

Preparation of Standard stock Solution

To prepare individual stock solutions of Mupirocin and Beclomethasone dipropionate, a meticulous process was followed. Specifically, 80 mg of Mupirocin was accurately weighed and transferred into a 100 ml volumetric flask. The substance was then dissolved, and methanol was incrementally added until the flask reached its intended mark, achieving a stock solution with an 800 mcg/ml concentration for Mupirocin. In a parallel manner, a precise amount of 1 mg of Beclomethasone dipropionate was weighed and introduced into a 100 ml volumetric flask. Following dissolution and successive methanol dilution up to the calibrated level, a stock solution was generated, featuring a concentration of 10 mcg/ml for Beclomethasone dipropionate.

Preparation of Working Standard Solution of Mupirocin

An 800 mcg/ml solution of Mupirocin was prepared by diluting the stock solution with a volume of 10 ml using the mobile phase. Subsequently, this solution was further diluted in the mobile phase to achieve a series of concentrations: 40, 60, 80, 100, and 120 mcg/ml of Mupirocin solution.

Preparation of Working Standard Solution of Beclomethasone Dipropionate

A solution containing Beclomethasone dipropionate at a concentration of 10 mcg/ml which prepared by diluting the stock solution to a volume of 10 ml using the mobile phase. This solution was then subjected to additional dilution within the mobile phase to achieve a series of concentrations: 0.5, 0.75, 1, 1.25, and 1.5 mcg/ml of Beclomethasone dipropionate solution.

Sample Preparation

Weigh an amount of ointment equivalent to 80 mg of Mupirocin or 1 mg of Beclomethasone dipropionate and place it into a 100 ml volumetric flask. Add 60 ml of methanol to

the flask and shake the mixture for a duration of 15 minutes. Subsequently, subject this solution to sonication for 10 minutes at a temperature of 60°C. After allowing the solution to cool down, bring the volume up to 100 ml by adding methanol. Filter this solution using a suitable filter paper for your intended purpose.

System Suitability Parameter of Developed Method [1-3]

A volume of 20 µl, either containing the standard or the sample, was introduced into the column for analysis. The chromatographic separation was carried out using a mixture of Phosphate buffer in water at pH 4.0 and Methanol (in a ratio of 35:65 %v/v), which had been subjected to sonication for a duration of 30 minutes. Detection of compounds was performed at a wavelength of 215 nm. The chromatogram acquisition was terminated once a complete separation was achieved. Subsequently, the chromatogram was recorded under the optimized chromatographic conditions that had been finally established. To ensure the suitability of the system for the proposed method, key parameters such as Retention times (Rt), theoretical plates (N), resolution (RS), and tailing factor (AS) were evaluated. This step was taken to confirm that the system met the necessary criteria for the successful implementation of the method.

RESULTS AND DISCUSSION

Selection of detection wavelength

Both Mupirocin and Beclomethasone dipropionate exhibited absorbance at a wavelength of 215 nm. Hence, the wavelength of 215 nm was chosen for the purpose of quantifying and analyzing both of these drugs.

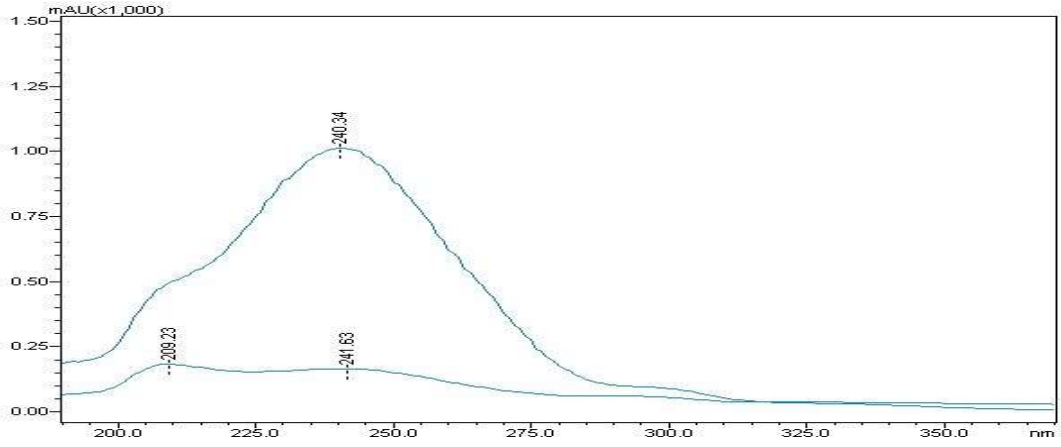

Figure 3: Mupirocin- Beclomethasone dipropionate HPLC wavelength determination

Selection of Mobile Phase

The choice of the mobile phase was made considering factors such as the best separation according to the Indian Pharmacopoeia (I.P.), peak purity index, peak symmetry, and theoretical plate performance. Several trials were conducted to arrive at the optimal mobile phase.

Ultimately, a mobile phase comprising a combination of buffer (pH 4) and methanol in a ratio of 35:65 v/v was selected as the most suitable mobile phase for the analysis **[21-25]**.

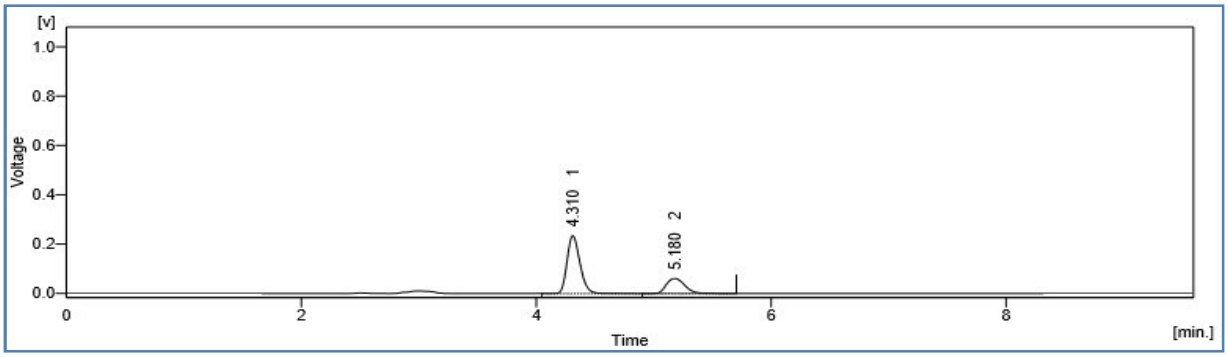

Figure 4: Chromatogram of standard solution containing 80mcg/ml MUP and 1mcg/ml BEC using mobile phase Buffer (pH 3.5): Methanol (35:65)

Method Validation [1-2,4-5]

Linearity and Range

A linear relationship was established by correlating peak areas with the concentration of Mupirocin within the concentration range of 40-120 mcg/ml, and for Beclomethasone dipropionate, the correlation was established within the range of 0.5-1.5 mcg/ml. The corresponding regression parameters are outlined in the provided **Table 1**, while the calibration curves for Mupirocin and Beclomethasone dipropionate at a wavelength of 215nm are depicted in the accompanying **Figure 5**.

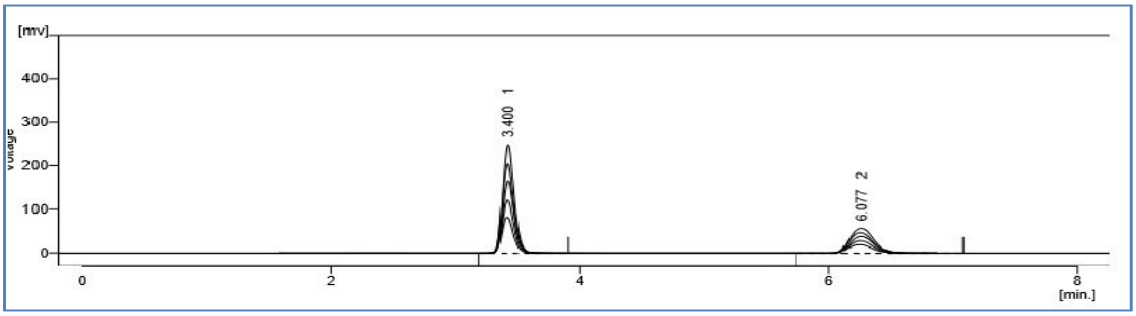

Figure 5: Calibration curves for Mupirocin and Beclomethasone dipropionate at a wavelength of 215nm

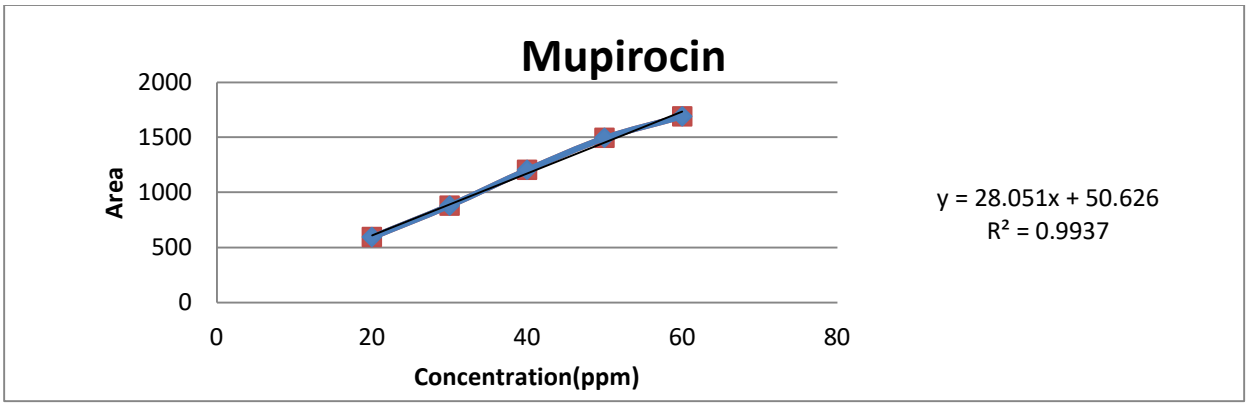

Figure 6: Linearity graph of Mupirocin

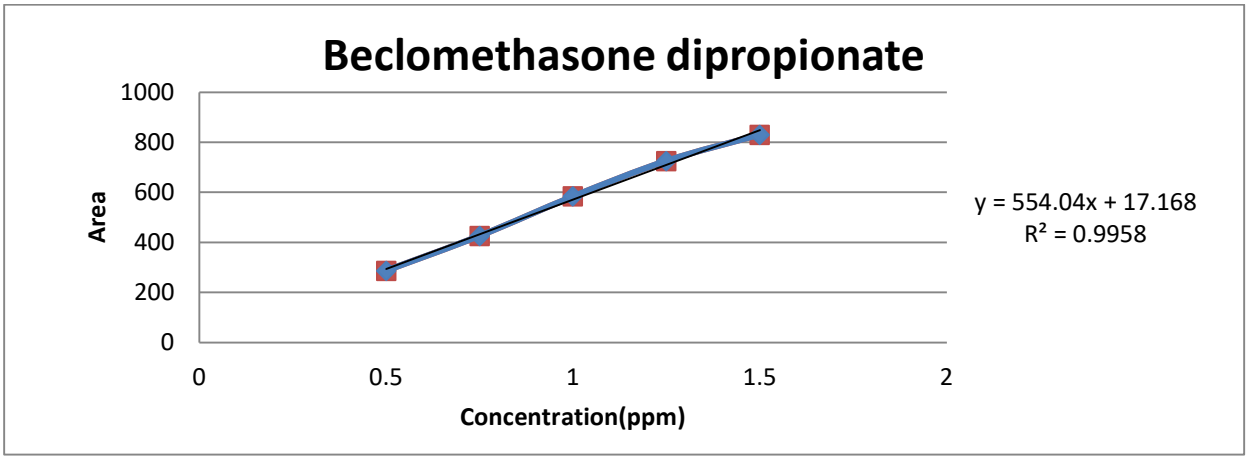

Figure 7: Linearity graph of Beclomethasone dipropionate

Table 1: Linearity of Mupirocin and Beclomethasone dipropionate

Mupirocin Conc. (mcg/ml)	Beclomethasone dipropionate Conc. (mcg/ml)	MupirocinMean area ±SD	Beclomethasonedipropionate Mean area ±SD
20	0.5	594.675 ±8.4	287.885 ±6.3
30	0.75	880.374 ±4.7	426.877 ±2.7
40	1	1203.485 ±2.9	584.364 ±7.3
50	1.25	1494.662 ±7.4	726.083 ±2.5
60	1.5	1690.063 ±8.2	830.832 ±2.8
Correlation coefficient		0.99683765	0.997877184
Slope of regression line		28.05	554.0
Y-intercept		50.62	17.16

Discussion:

Mupirocin and Beclomethasone dipropionate exhibited a linear response within the concentration range of 40-120 mcg/ml and 0.5-1.5 mcg/ml, respectively. The correlation coefficient values obtained were 0.9968 for Mupirocin and 0.9978 for Beclomethasone dipropionate. This indicates a strong correlation between the concentrations and the response for both substances. Thus, the established concentration ranges for Mupirocin and Beclomethasone dipropionate

were 40-120 mcg/ml and 0.5-1.5 mcg/ml, respectively.

LOD and LOQ

The drug's limit of detection (LOD) and limit of quantification (LOQ) were determined through the application of the visual method or the calculation of the signal-to-noise ratio. Specifically, the values 3.3 for LOD and 10 for LOQ were employed, as defined by the guidelines established by the International Conference on Harmonization (ICH). This methodology aligns with the recommended approach for assessing the lower detection limit and the lowest quantifiable concentration of the drug compound.

LOD= 3.3*σ/s

LOQ= 10* σ/s

Where, σ =standard deviation of the response, s= slope of calibration curve.

Table 2: LOD and LOQ of Mupirocin and Beclomethasone dipropionate

For Mupirocin		For Beclomethasone dipropionate	
SD	**40.82**	**SD**	**16.50**
Slope	**14.02**	**Slope**	**554**
LOD	**9.60**	**LOD**	**0.098**
LOQ	**29.11**	**LOQ**	**0.29**

Precision

Repeatability, as well as intra-day and inter-day precision, were assessed in terms of the % relative standard deviation (% RSD). The experiment was conducted thrice within a single day for evaluating intra-day precision and repeated on three distinct days for inter-day precision. The calculated average % RSD values for both intra-day and inter-day measurements, across all three methods employed for the determination of the drugs, were consistently found to be below 2%.

Repeatability

Table 3: Determination of repeatability for Mupirocin and Beclomethasone Dipropionate

Drug	Target conc. (µg/ml)	Area	Mean±SD	%RSD
Mupirocin	**80**	**989.818**	**991.55 ± 5.043**	**0.508**
	80	**982.158**		
	80	**993.838**		
	80	**995.827**		
	80	**992.841**		
	80	**994.83**		
Beclomethasone dipropionate	**1**	**481.34**	**480.06 ± 6.985**	**1.455**
	1	**482.298**		
	1	**465.968**		
	1	**484.255**		
	1	**482.781**		
	1	**483.769**		

Intraday Precision

Table 4: Intraday Precision of Mupirocin and Beclomethasone Dipropionate

Drug	Targetconc. (µg/ml)	Area	Mean ±SD	%RSD
Mupirocin	40	483.756	487.861 ±3.56	0.730
	40	489.668		
	40	490.159		
	80	974.684	983.135 ±7.46	0.759
	80	988.848		
	80	985.874		
	120	1462.73	1474.182 ±10.48	0.711
	120	1483.30		
	120	1476.507		
Beclomethasone dipropionate	0.5	237.234	235.79 ±3.102	1.31
	0.5	232.238		
	0.5	237.924		
	1	479.865	474.59 ±8.711	1.83
	1	464.543		
	1	479.387		
	1.5	723.661	717.07 ±10.781	1.50
	1.5	704.632		
	1.5	722.931		

Inter day precision

Table 5: Interday Precision of Mupirocin and Beclomethasone Dipropionate

Drug	Target conc. µg/ml)	Area	Mean ±SD	%RSD
Mupirocin	40	482.597	487.80 ±4.51	0.92
	40	490.158		
	40	490.649		
	80	976.456	985.37 ±7.72	0.78
	80	989.839		
	80	989.841		
	120	1463.002	1477.53 ±12.58	0.85
	120	1484.792		
	120	1484.8		
Beclomethasone dipropionate	0.5	237.472	237.12 ±1.24	0.52
	0.5	235.734		
	0.5	238.159		
	1	480.347	475.56 ±9.15	1.92
	1	465.007		
	1	481.331		
	1.5	724.385	722.53 ±4.53	0.62
	1.5	717.359		
	1.5	725.849		

Discussion: Result obtained reveals that % RSD of Mupirocin and Beclomethasone dipropionate were within acceptance criteria given in ICH i.e. less than 2. So, the proposed method for estimation of Mupirocin and Beclomethasone dipropionate is precise in nature.

Accuracy

The accuracy of the methodology was validated through a recovery study, which involved conducting standard additions at

three different levels (80%, 100%, and 120%) of the label claim. This study was conducted in triplicate. The calculated percentage recoveries ranged from 98% to 102%, and the presence of low standard deviation (SD) values further substantiated the accuracy of the method.

Base Concentration: 20 mcg/ml Mupirocin (MUP) and 0.5 mcg/ml Beclomethasone dipropionate (BEC).

Table 6: Recovery study of Mupirocin and Beclomethasone dipropionate

% Spiking	DRUG	Conc.of testtaken (mcg/ml)	Conc.of std added(mcg /ml)	Totalconc. (mcg/ml)	Amt. of drug recovered (mcg/ml)	% Recovery	Mean % recovery ±SD
80%	MUP	40	16	36	15.811	98.82	99.86 ±0.953
		40	16	36	16.110	100.68	
		40	16	36	16.013	100.081	
	BEC	0.5	0.4	0.9	0.396	99.16	100.39 ±1.135
		0.5	0.4	0.9	0.405	101.39	
		0.5	0.4	0.9	0.402	100.62	
100 %	MUP	40	20	40	19.720	98.60	99.45 ±0.761
		40	20	40	20.011	100.05	
		40	20	40	19.943	99.71	
	BEC	0.5	0.5	1	0.497	99.47	100.12 ±0.661
		0.5	0.5	1	0.503	100.79	
		0.5	0.5	1	0.500	100.09	
120 %	MUP	40	24	44	23.95	99.80	99.61 ±0.307
		40	24	44	23.82	99.26	
		40	24	44	23.94	99.70	
	BEC	0.5	0.6	1.1	0.603	100.59	100.10 ±0.494
		0.5	0.6	1.1	0.597	99.60	
		0.5	0.6	1.1	0.600	100.12	

Discussion: The results obtained demonstrate that the percentage recovery of both Mupirocin and Beclomethasone dipropionate falls within the acceptable range stipulated by the International Conference on Harmonization (ICH), which is between 98% and 102%.

Robustness

Table 7: Robustness Study for Mupirocin and Beclomethasone Dipropionate

Drugs	Condition	Mean Area [a]	Retention time (min)	Theoretical plates	Tailing factor	%RSD (Peak Area)
CHANGE IN FLOW RATE						
MUP	0.8 ml/min	1024.16	3.5	7027	1.4	1.62
	1.0 ml/min	993.82	3.4	7352	1.3	0.75
	1.2 ml/min	968.34	3.3	6836	1.4	0.84
BEC	0.8 ml/min	496.34	6.2	4923	1.3	1.76
	1.0 ml/min	483.27	6.0	4790	1.3	1.83
	1.2 ml/min	469.88	5.9	4870	1.3	0.93
CHANGE IN MOBILE PHASE BUFFER PH CHANGE						
MUP	pH3.8	1016.12	3.4	7207	1.4	1.12
	pH4	993.82	3.3	7352	1.3	0.75
	pH4.2	947.21	3.2	6884	1.3	1.28
BEC	pH3.8	491.58	6.2	4881	1.3	1.70
	pH4	483.27	6.0	4790	1.3	1.83
	pH4.2	458.07	5.8	4829	1.3	1.79

Discussion: The results indicate that the percent relative standard deviation (% RSD) of Mupirocin and Beclomethasone dipropionate, when subjected to changes in experimental conditions, remained within the acceptable limits outlined by the International Conference on Harmonization (ICH) guidelines, which specifies a threshold of less than 2%. This confirms that the proposed method for estimation is highly precise.

Assay

Assay results for combined formulation of Mupirocin and Beclomethasone dipropionate.

Table 8: Assay results for combined formulation of Mupirocin and Beclomethasone dipropionate

Drug	serialno	Label claim (w/w)	Result (w/w)	% Assay	Avg % Assay ± SD	%RSD
MUP	1	2	1.956	97.8129	98.3770 ± 0.491	0.4991
	2	2	1.974	98.7087		
	3	2	1.972	98.6095		
BEC	1	0.025	0.0244	97.9384	97.1914 ± 1.379	1.4195
	2	0.025	0.0238	95.5992		
	3	0.025	0.0245	98.0365		

Discussion: The assay results obtained demonstrate that the percent relative standard deviation (% RSD) and the average percentage assay of Mupirocin and Beclomethasone dipropionate, when present in their combined dosage form, fall within the acceptable limits specified by the International Conference on Harmonization (ICH) guidelines. This signifies that the combined dosage form successfully passes the assay test.

Stability Study: Force Degradation Study [6, 8]

Experimental Work

A validated RP-HPLC method was successfully developed, characterized by its speed, precision, accuracy, and specificity, enabling the simultaneous estimation of Mupirocin and Beclomethasone dipropionate. Furthermore, this RP-HPLC method was extended to encompass a comprehensive stability-indicating assay approach, facilitating the concurrent determination of Mupirocin and Beclomethasone dipropionate within the drug product.

To establish the method's stability-indicating nature, a series of forced degradation studies were conducted on standards of the drugs, the drug product itself, and placebo samples. These studies encompassed various stress conditions, including acid and base hydrolysis, oxidative stress, and thermal stress. Notably, thermal degradation was performed on the drug product in its solid state.

The stress studies were meticulously conducted with a range of severity for each stress condition, ensuring that degradation

levels spanning 10% to 30% were achieved. This rigorous approach underscores the ability of the method to identify and quantify the degradation products resulting from different stress conditions.

Forced Degradation Studies of Bulk Drug And Synthetic Mixture

To confirm the stability-indicating nature of the newly developed analytical method, a series of forced degradation studies were carried out on both the active pharmaceutical ingredient (API) and the pharmaceutical formulation. These studies involved subjecting the substances to diverse and intentional stress conditions, aimed at simulating potential degradation pathways.

Under conditions such as acid and base hydrolysis, oxidative stress, and thermal stress, the API and the pharmaceutical formulation were exposed to conditions that could lead to degradation. The goal of these studies was to trigger the formation of potential degradation products.

By analyzing the response of the analytical method to these degradation products, the method's capability to accurately identify and quantify degradation under various stress conditions is assessed. This provides evidence of the method's effectiveness in detecting potential instability in both the API and the final pharmaceutical formulation, hence establishing its status as a stability-indicating method.

Preparation of Standard Stock Solution And Sample Stock Solution

To evaluate the stability-indicating property and specificity of the proposed method, both the active pharmaceutical ingredient (API) and the pharmaceutical formulation underwent a comprehensive set of preparations.

For the API and pharmaceutical formulation, individual stock solutions were meticulously prepared. A stock solution of standard Mupirocin at a concentration of 800 µg/ml and a stock solution of Beclomethasone dipropionate at 10 µg/ml were each prepared in a suitable diluent. Additionally, a stock solution of the drug product was created, containing 800 mcg/ml of Mupirocin and 10 mcg/ml of Beclomethasone dipropionate, also in the chosen diluent.

From these prepared solutions, a quantity of 10 ml was extracted and transferred into a 100 ml volumetric flask. Diluent was then added to reach the mark, resulting in a solution containing 80 mcg/ml of Mupirocin and 1 mcg/ml of Beclomethasone dipropionate.

These meticulously prepared stock solutions, along with necessary dilutions, were employed in the subsequent forced degradation studies. This process provided a

reliable way to investigate the proposed method's capability to indicate stability, while also ensuring specificity in identifying degradation products under a variety of stress conditions.

1. **Acid degradation from the standard stock solution**

For acid-induced decomposition investigations, the following procedure was carried out:

An aliquot of 1 ml was withdrawn from the prepared stock solution and transferred into a 10 ml volumetric flask. Subsequently, 2 ml of a 0.1 N hydrochloric acid solution was added and thoroughly mixed. This mixture was then subjected to reflux conditions for a duration of 4 hours, utilizing a 250 ml round-bottom flask and maintaining a temperature of 70 ºC.

Upon completion of the stipulated time period, the content in the flask was allowed to cool down to room temperature. Following this, the solution's volume was adjusted using the chosen diluent to attain target concentrations of 80 mcg/ml for Mupirocin and 1 mcg/ml for Beclomethasone dipropionate. This approach aimed to imitate and assess the possible effects of acid hydrolysis on the stability and potential degradation of the substances being studied.

2. **Alkali degradation from the standard stock solution**

To investigate the impact of basic decomposition, the following procedure was conducted:

A 1 ml aliquot was withdrawn from the prepared stock solution and transferred into a 10 ml volumetric flask. Subsequently, 2 ml of a 0.1 N sodium hydroxide (NaOH) solution was introduced and thoroughly mixed. The resulting mixture was then subjected to reflux conditions for a duration of 4 hours, employing a 250 ml round-bottom flask and maintaining the temperature at 70 ºC.

After the specified time interval, the content within the flask was allowed to cool to room temperature. Following this, the solution's volume was adjusted using the chosen diluent to achieve target concentrations of 80 mcg/ml for Mupirocin and 1 mcg/ml for Beclomethasone dipropionate. This protocol aimed to replicate and evaluate the potential effects of basic hydrolysis on the stability and potential degradation of the substances being studied.

3. **Oxidative degradation from the standard stock solution**

To examine the influence of oxidative decomposition, the following procedure was carried out:

A 1 ml sample was drawn from the prepared stock solution and transferred into a 10 ml volumetric flask. Subsequently, 2 ml of a 3%

hydrogen peroxide (H2O2) solution was introduced and thoroughly mixed. The resultant mixture was then subjected to reflux conditions for duration of 4.5 hours, utilizing a 250 ml round-bottom flask and maintaining the temperature at 70 ºC.

After the specified duration elapsed, the content within the flask was allowed to cool to room temperature. Subsequently, the solution's volume was adjusted using the chosen diluent to attain target concentrations of 80 mcg/ml for Mupirocin and 1 mcg/ml for Beclomethasone dipropionate. This protocol aimed to simulate and evaluate the potential effects of oxidative stress on the stability and potential degradation of the substances being studied.

4. Thermal degradation from the standard stock solution

Investigate the impact of thermal degradation, the following procedure was carried out:

A 1 ml portion was taken from the prepared stock solution and transferred into a 10 ml volumetric flask. Subsequently, this volumetric flask was placed in an oven and stored at a temperature of 110°C for duration of 3 hours.

Upon completion of the 3-hour thermal exposure, the flask was removed from the oven, and the solution within was allowed to cool down to room temperature. Following this, the solution's volume was adjusted using the selected diluent to achieve desired concentrations of 80 mcg/ml for Mupirocin and 1 mcg/ml for Beclomethasone dipropionate. This protocol aimed to replicate and evaluate the potential effects of thermal stress on the stability and potential degradation of the substances being studied.

5. Photolytic degradation from the standard stock solution

To examine the impact of photo degradation, the following procedure was carried out:

A 1 ml portion was withdrawn from the prepared stock solution and transferred into a 10 ml volumetric flask. Subsequently, this volumetric flask was exposed to sunlight for duration of 4 hours.

Following the 4-hour exposure to sunlight, the flask was taken indoors, and the solution inside was allowed to return to room temperature. After this, the volume of the solution was adjusted using the chosen diluent to achieve the desired concentrations of 80 mcg/ml for Mupirocin and 1 mcg/ml for Beclomethasone dipropionate. This process aimed to replicate and assess the potential effects of photostress on the stability and potential degradation of the substances being studied.

Table 9: Summary of Forced Degradation Study for API

Stress type	Stress condition	MUP Area ofpeak	% Degradation	BEC Area ofpeak	% Degradation
Control Sample	NA	1010.322	NA	451.783	NA
Acid stress	0.1N HCL 2ml 4hrs	762.262	24.55	359.127	20.508
Base stress	0.1N HCL 2ml 4hrs	686.845	32.02	336.194	25.585
Peroxidestress	3% H_2O_2 2ml 4.5hrs	808.362	19.99	354.177	21.604
Thermal degradation	At 110°C for 3hrs	707.047	30.02	290.151	35.776
Photolytic degradation	At Sun light for 4hrs	859.756	14.90	388.762	13.949

Table 10: Summary of Forced Degradation Study for PharmaceuticalFormulation

Stress type	Stress condition	MUP Area ofpeak	% Degradation	BEC Area ofpeak	% Degradation
Control Sample	NA	1010.322	NA	451.783	NA
Acid stress	0.1N HCL 2ml 4hrs	771.234	23.66	363.28	19.589
Base stress	0.1N HCL 2ml 4hrs	707.055	30.02	327.191	27.577
Peroxidestress	3% H_2O_2 2ml 4.5hrs	797.991	21.02	362.648	19.729
Thermal degradation	At 110°C for 3hrs	739.565	26.80	294.373	34.841
Photolytic degradation	At Sun light for4hrs	848.538	16.01	390.123	13.648

CONCLUSION AND SUMMARY

An RP-HPLC method was developed to quantify Mupirocin (MUP) and Beclomethasone dipropionate (BEC) within their combined dosage form.

In this RP-HPLC method, an isocratic liquid chromatography analysis was carried out on a Phenomenex Gemini ODS C18 column (200 mm x 4.6 mm, 5µ). The mobile phase used consisted of Methanol and Buffer pH 4, in a ratio of 65:35 (v/v). The flow rate was maintained at 1.0 mL/min. Detection and quantification were achieved using a UV detector set at a wavelength of 215 nm.

The obtained retention times were 3.36 minutes for Mupirocin and 6.02 minutes for Beclomethasone dipropionate. These retention times serve as critical markers for identifying and quantifying the respective substances during the analysis.

The analytical method underwent validation following the guidelines set by the International Council for Harmonization (ICH). The correlation coefficient was determined to be 0.996 for MUP and 0.997 for BEC, indicating a strong linear relationship between the measured concentrations and the actual concentrations. The recovery of MUP

ranged from 99.45% to 99.86%, while for BEC it ranged from 100.10% to 100.39%, affirming the accuracy of the method.

The limit of quantification was established at 29.11 µg/ml for MUP and 0.297 µg/ml for BEC, demonstrating the minimum concentration levels that can be reliably quantified using this method. Through rigorous assessment, the method was verified to be accurate, precise, specific, selective, repeatable, and reproducible, ensuring its suitability for intended analyses.

Table 11: Summary of Validation Parameters of RP-HPLC method

Parameters	Mupirocin	Beclomethasone dipropionate
Correlation coefficient	0.9968	0.9978
LOD	9.60	0.098
LOQ	29.11	0.297
% Recovery	99.45-99.86	100.10-100.39
Repeatability (%RSD) (n=6)	0.508	1.455
Precision(%RSD) Intra-day (n=3) Inter-day (n=3)	 0.71-0.75 0.78-0.92	 1.31-1.83 0.52-1.92
Specificity	Specific	Specific
Robustness	Robust	Robust

Following the ICH guidelines, a comprehensive stability study was conducted, encompassing conditions of acidity, alkalinity, oxidation, thermal stress, and photolysis. The chromatographic analysis revealed that all peaks corresponding to degraded products were distinctly separated from the peaks of the original drugs, exhibiting different retention times. The method's capacity to effectively distinguish between the drug and its degradation products established its role as a stability-indicating method.

Due to its ability to successfully separate the drug and its degradation products, this method was applied for the estimation of Mupirocin and Beclomethasone dipropionate within a synthetic mixture. The results endorse its suitability for routine analysis, underscoring its reliability and robustness in determining the quantities of these compounds.

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