ORIGINAL ARTICLE

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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Abstract

A straightforward, specific, precise, and reproducible technique has been developed and validated for concurrently determining both drugs in their combined dosage form. The analysis employs reverse phase high-performance liquid chromatography with a mobile phase of Methanol: Buffer pH 4 (65:35, v/v) and a Phenomenex Gemini ODS C18 column (200 mm × 4.6 mm, 5.0 µ particle size) as the stationary phase, detecting at a wave length of 215 nm. Linearity was established in the concentration range of 40–120 µg/mL for mupirocin (MUP) and 0.5–1.5 µg/mL for beclomethasone dipropionate (BEC). The %recoveries for both drugs ranged from 99.45% to 99.86% for MUP and 100.10% to 100.39% for BEC. The limits of quantification (LOQ) were determined as 29.11 µg/mL and 0.297 µg/mL at 215 nm for MUP and BEC, respectively. The methods underwent statistical validation for accuracy, precision, specificity, LOQ, and robustness. In addition, a force degradation study following ICH guidelines was conducted, affirming the suitability of the method for analyzing the combined dosage form.

Key words: Beclomethasone dipropionate, mupirocin, reverse phase high-performance liquid chromatography

INTRODUCTION

Mupirocin (MUP), known under commercial names such as Bactroban and Centany, falls under the category of monoxycarbolic acids and functions as a topical antibacterial agent. Originally isolated from Pseudomonas fluorescens, it was developed by Beecham.[18] The chemical nomenclature for MUP 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 formula of $C_{26}H_{44}O_9$ and structural formula of MUP show in Figure 1. MUP has a molecular weight of 500.6 and is identified by the CAS number 12650-69-0.[23] It appears as a nearly white powder, displaying solubility in acetone and ethanol, with limited solubility in water.[15-17] The melting point of this compound falls within the range of 77–78°C.[10-12]

MUP exhibits bacteriostatic properties at lower concentrations, shifting to bactericidal effects with increasing concentrations. This topical application is particularly effective against Gram-positive bacteria, including the challenging methicillin-resistant

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Staphylococcus aureus strain.[31] The composition of MUP includes various pseudomonic acids (PAs), with PA-A being the predominant contributor, making up over 90% of the mixture.[19] The mechanism of action involves MUP reversibly binding to bacterial isoleucyl-transfer RNA (tRNA) synthetase, a crucial enzyme in catalyzing the conversion of isoleucine and tRNA into isoleucyl-tRNA.[20] This binding event disrupts the proper functioning of the enzyme, hindering the synthesis of bacterial protein and RNA, thereby exerting inhibitory effects.[10-12] Beclomethasone dipropionate (BEC) is classified as an anti-inflammatory and anti-bacterial drug. Its chemical designation is 9-chloro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate, with a molecular formula of C28H37ClO7 structural formula of BEC as shown in Figure 2. The molecular weight of BEC is 521.1,[23,24] and it presents as a white or nearly white to off-white crystalline powder. It demonstrates solubility in acetone and ethanol, while remaining insoluble in water.[25-28] The melting point of BEC falls within the range of 117–120°C.[13-15]

BEC, alternatively spelled as BEC, is a steroid medication available under various brand names, including Quar. It is offered in diverse forms, including inhalers, creams, pills, and nasal sprays. The inhaled version is prescribed for the sustained management of asthma.[7,9] Cream formulations are applied to treat dermatitis and psoriasis. Furthermore, the oral pills have been employed in the treatment of ulcerative colitis.[16,17]

Corticosteroids that are not bound exhibit the capability to cross cell membranes and bind strongly to specific cytoplasmic receptors. This binding interaction results in several effects, including the inhibition of white blood cell infiltration at the site of inflammation, interference with the activities of agents involved in the inflammatory response, suppression of humoral immune reactions, and reduction of both edema and scar tissue.[28-30]

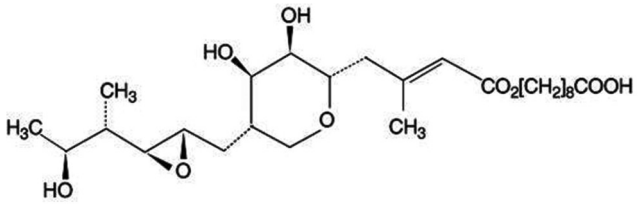

Figure 1: Structural formula of mupirocin

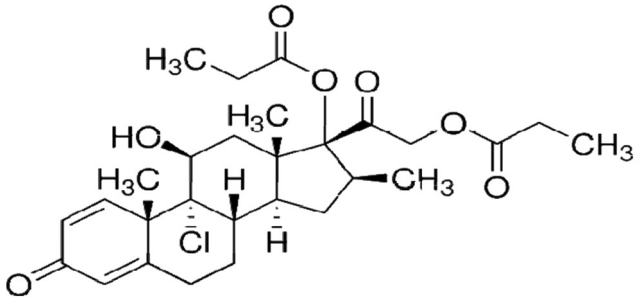

Figure 2: Structural formula of beclomethasone dipropionate

MATERIAL AND METHODS

Chemical and reagents

The reference standards for MUP and BEC, employed throughout the experiment, were generously provided as gift samples by Glenmark Pharmaceuticals Ltd., based in Mumbai, Maharashtra, India. The commercially available formulation, Supirocin B plus® ointment, manufactured by Glenmark Pharmaceuticals Ltd. in Mumbai, India, and containing 2% MUP and 0.025% BEC, was sourced from the market. Methanol and acetonitrile of analytical reagent (AR) and high-performance liquid chromatography (HPLC) grades, essential solvents for the experiment, were procured from Merck Specialties Pvt. Ltd. in India.

Apparatus

The experimental HPLC setup utilized the LC 20 AT model from Shimadzu Corporation, featuring an SPD-20A diode array detector (ultraviolet [UV]-visible). The system operated in an Isocratic mode with a back pressure of 5000 psi, maintaining a flow rate of 1 mL/min. Sample injections were conducted using a Rheodyne valve with a fixed 20 µL loop injection. The chromatographic separation was carried out using a Phenomenex Gemini ODS–C18 column.

For precise weighing, an AX 200 model highly sensitive electronic balance, manufactured by SHIMADZU CORPORATION in Japan, was employed in the experiment.

Preparation of standard stock solution

To prepare separate stock solutions for MUP and BEC, a systematic procedure was adhered to. Specifically, 80 mg of MUP was accurately weighed and transferred to a 100 mL volumetric flask. The substance was then dissolved, and methanol was incrementally added until the flask reached its designated mark, resulting in a stock solution with a concentration of 800 mcg/mL for MUP. In a similar fashion, a precise quantity of 1 mg of BEC was weighed and introduced into a 100 mL volumetric flask. Following dissolution and successive dilution with methanol up to the calibrated level, a stock solution was prepared, featuring a concentration of 10 mcg/mL for BEC.

Preparation of working standard solution of MUP

To create an 800 mcg/mL MUP solution, the stock solution was diluted with a 10 mL volume of the mobile phase. Following this, the resulting solution underwent additional dilution in

the mobile phase, generating a set of concentrations: 40, 60, 80, 100, and 120 mcg/mL for the MUP solution.

Preparation of working standard solution of BEC

A solution with a BEC concentration of 10 mcg/mL was formulated by diluting the stock solution to a 10 mL volume using the mobile phase. Subsequently, this solution underwent further dilution within the mobile phase to produce a sequence of concentrations: 0.5, 0.75, 1, 1.25, and 1.5 mcg/mL for the BEC solution.

Sample preparation

Measure an amount of ointment corresponding to 80 mg of MUP or 1 mg of BEC and transfer it to a 100 mL volumetric flask. Add 60 mL of methanol to the flask and shake the mixture for 15 min. Next, subject the solution to sonication for 10 min at a temperature of 60°C. Once the solution has cooled, adjust the volume to 100 mL by adding methanol. Filter the solution using an appropriate filter paper for your specific application.

System suitability parameter of developed method.[1-3]

A 20 µL volume, containing either the standard or the sample, was introduced into the column for analysis. Chromatographic separation utilized a mixture of phosphate buffer in water at pH 4.0 and Methanol (in a ratio of 35:65 %v/v), which underwent sonication for 30 min. Compound detection was conducted at a wavelength of 215 nm. Chromatogram acquisition concluded upon achieving complete separation. Subsequently, the chromatogram was recorded under the established and optimized chromatographic conditions. To ensure the system's suitability for the intended method, crucial parameters such as Retention times (Rt), theoretical plates (N), resolution (RS), and tailing factor (AS) were assessed. This step was undertaken to verify that the system met the requisite criteria for the successful implementation of the method.

RESULTS AND DISCUSSION

Selection of detection wave length

MUP and BEC demonstrated absorbance at a wavelength of 215 nm. Consequently, this specific wavelength was selected for the quantification and analysis of both these drugs. MUP -BEC HPLC wavelength determination is shown in Figure 3.

Selection of mobile phase

The selection of the mobile phase took into account criteria such as achieving optimal separation in accordance with the Indian Pharmacopoeia, peak purity index, peak symmetry,

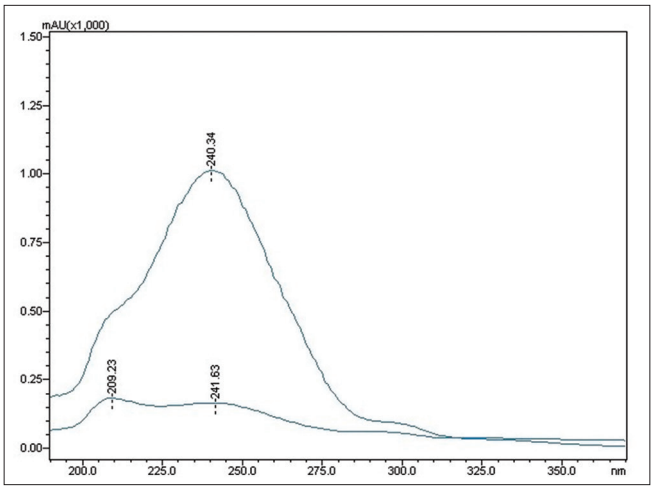

Figure 3: Mupirocin-beclomethasone dipropionate high-performance liquid chromatography wavelength determination

and N performance. Multiple trials were conducted to determine the optimal mobile phase. Ultimately, a mobile phase composed of a mixture of buffer (pH 4) and methanol in a ratio of 35:65 v/v was identified as the most suitable for the analysis.[21-25] Chromatogram of standard solution containing 80 mcg/mL MUP and 1 mcg/mL BEC using mobile phase Buffer (pH 3.5): Methanol (35:65) is show in Figure 4.

Method validation[1,2,4,5]

Linearity and range

A linear correlation between peak areas and the concentration of MUP was established in the concentration range of 40–120 mcg/mL, and for BEC, the correlation was established within the range of 0.5–1.5 mcg/mL. The associated regression parameters are detailed in the provided table, while the calibration curves for MUP and BEC at a wave length of 215 nm are illustrated in the accompanying Figures 5-7 and Table 1.

Discussion

MUP and BEC demonstrated 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 MUP and 0.9978 for BEC, indicating a robust correlation between the concentrations and the response for both substances. Therefore, the established concentration ranges for MUP and BEC were confirmed as 40–120 mcg/mL and 0.5–1.5 mcg/mL, respectively.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for the drug were established using either the visual method or signal-to-noise ratio calculation. In adherence to the guidelines set by the International

Table 1: Linearity of mupirocin and beclomethasone dipropionate

Mupirocin Conc (mcg/mL)	Beclomethasone dipropionate Conc. (mcg/mL)	Mupirocin mean area±SD	Beclomethasone dipropionate 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

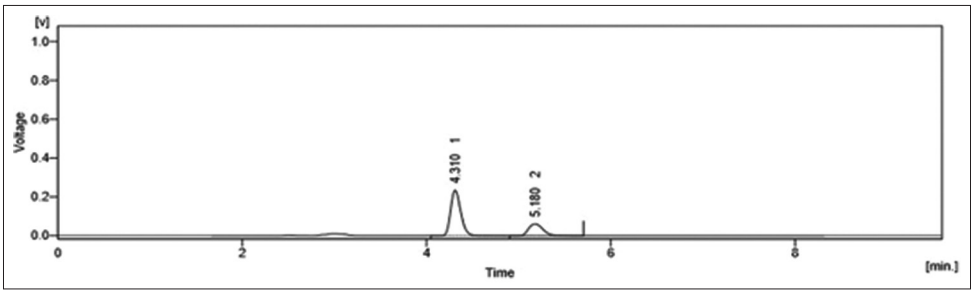

Figure 4: Chromatogram of standard solution containing 80 mcg/mL mupirocin and 1 mcg/mL beclomethasone dipropionate using mobile phase Buffer (pH 3.5): Methanol (35:65)

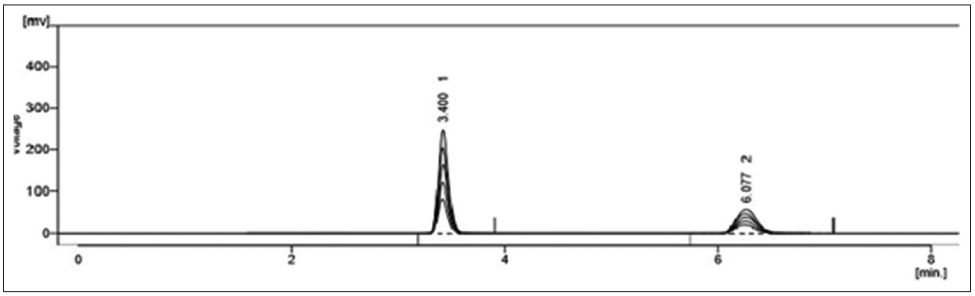

Figure 5: Linearity chromatograms for mupirocin and beclomethasone dipropionate

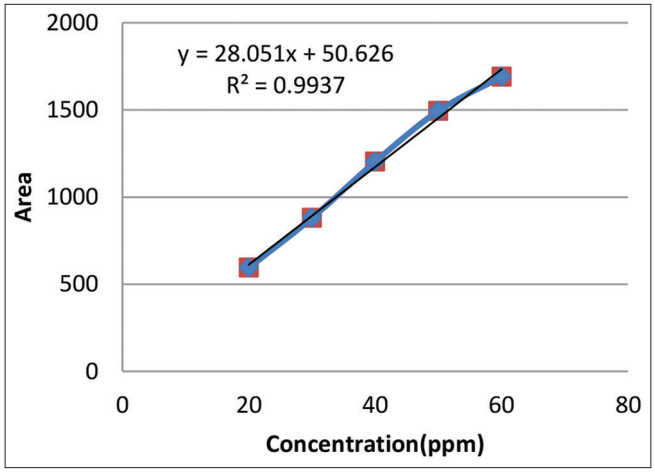

Figure 6: Linearity graph of mupirocin

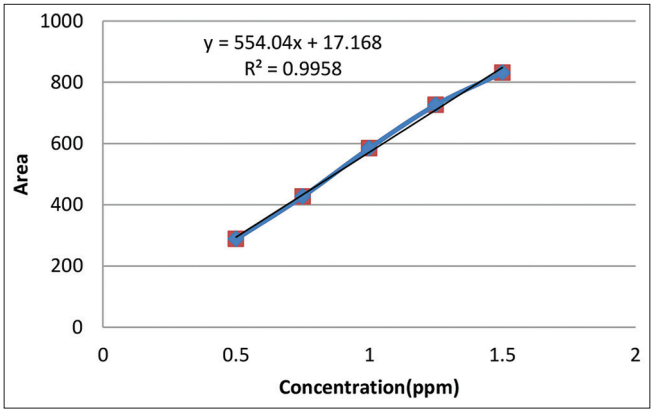

Figure 7: Linearity graph of beclomethasone dipropionate

Council on Harmonization (ICH), the values chosen were 3.3 for LOD and 10 for LOQ. This approach aligns with the recommended methodology for evaluating the lower detection limit and the minimum quantifiable concentration of the drug compound. LOD and LOQ of MUP and BEC data are show in Table 2.

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

LOD: Limit of detection, LOQ: Limit of quantification, SD: Standard deviation

$$LOD = 3.3*\sigma/s$$

$$LOQ = 10*\sigma/s$$

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

Precision

Repeatability, along with intra-day and inter-day precision, was evaluated based on the % relative SD (percent RSD [% RSD]). The experiment was performed three times within a single day to assess intra-day precision and repeated on three separate days for inter-day precision. The average % RSD values, calculated across all three methods used for determining the drugs, consistently remained below 2% for both intra-day and inter-day measurements. The determination of repeatability for MUP and BEC, Intraday Precision of MUP and BEC, Interday Precision of MUP and BEC, respectively, is show in Tables 3-5.

Discussion

The results indicate that the % RSD values for both MUP and BEC fall within the acceptance criteria specified by ICH, i.e., <2%. Consequently, it can be concluded that the proposed method for estimating MUP and BEC is precise.

Accuracy

The accuracy of the methodology was confirmed via a recovery study, where standard additions were carried out at three distinct levels (80%, 100%, and 120%) of the label claim. This study, conducted in triplicate, yielded calculated percentage recoveries within the range of 98% to 102%. The low SD values observed further support the accuracy of the method. Recovery study of MUP and BEC is show in Table 6.

Base concentration

20 mcg/mL MUP and 0.5 mcg/mL BEC.

Discussion

The obtained results indicate that the percentage recovery for both MUP and BEC falls within the acceptable range specified by the International Conference on Harmonization(ICH), i.e., between 98% and 102%.

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		

RSD: Relative standard deviation, SD: Standard deviation

Table 4: Intraday precision of mupirocin and beclomethasone dipropionate

Drug	Target conc. (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		

RSD: Relative standard deviation, SD: Standard deviation

Robustness

Robustness study for MUP and BEC is show in Table 7.

Table 5: Inter-day 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		

RSD: Relative standard deviation, SD: Standard deviation

Discussion

The results indicate that the % RSD of MUP and BEC, 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 MUP and BEC are show in Table 8.

Discussion

The assay results obtained demonstrate that the % RSD, and the average percentage assay of MUP and BEC, 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 reverse phase (RP)-HPLC method has been successfully developed, characterized by its speed, precision, accuracy, and specificity, allowing for the simultaneous estimation of MUP and BEC. In addition, this RP-HPLC method has been extended to include a comprehensive stability-indicating assay approach, enabling the simultaneous determination of MUP and BEC within the drug product.

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

The stress studies were conducted meticulously, with a range of severity for each stress condition, ensuring the achievement of degradation levels spanning 10–30%. This rigorous approach underscores the method's capability to identify and quantify degradation products resulting from different stress conditions.

Forced degradation studies of bulk drug and synthetic mixture

To affirm the stability-indicating nature of the newly developed analytical method, a series of forced degradation studies were conducted on both the active pharmaceutical ingredient (API) and the pharmaceutical formulation. These studies involved intentionally subjecting the substances to diverse stress conditions, aiming to simulate potential degradation pathways.

The API and the pharmaceutical formulation were exposed to conditions such as acid and base hydrolysis, oxidative stress, and thermal stress, where the goal was to induce potential degradation. These studies were designed to provoke the formation of potential degradation products.

By analyzing how the analytical method responded to these degradation products, the method's ability to accurately identify and quantify degradation under various stress conditions was evaluated. This assessment provides evidence of the method's efficacy in detecting potential instability in both the API and the final pharmaceutical formulation, thereby establishing its status as a stability-indicating method.

Preparation of standard stock solution and sample stock solution

To assess the stability-indicating property and specificity of the proposed method, both the API and the pharmaceutical formulation underwent a thorough set of preparations.

For the API and pharmaceutical formulation, individual stock solutions were carefully prepared. A stock solution of standard MUP at a concentration of 800 µg/mL and a stock solution of BEC at 10 µg/mL were each created in an appropriate diluent. In addition, a stock solution of the drug

Table 6: Recovery study of mupirocin and beclomethasone dipropionate

% Spiking	Drug	Conc. of test taken (mcg/mL)	Conc. of std added (mcg/mL)	Total conc. (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	

MUP: Mupirocin, BEC: Beclomethasone dipropionate, RSD: Relative standard deviation, SD: Standard deviation

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 inflow 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	pH 3.8	1016.12	3.4	7207	1.4	1.12
	pH 4	993.82	3.3	7352	1.3	0.75
	pH 4.2	947.21	3.2	6884	1.3	1.28
BEC	pH 3.8	491.58	6.2	4881	1.3	1.70
	pH 4	483.27	6.0	4790	1.3	1.83
	pH 4.2	458.07	5.8	4829	1.3	1.79

MUP: Mupirocin, BEC: Beclomethasone dipropionate, RSD: Relative standard deviation

product was formulated, containing 800 mcg/mL of MUP and 10 mcg/mL of BEC, also in the chosen diluent.

From these prepared solutions, a volume 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 MUP and 1 mcg/mL of BEC.

These meticulously prepared stock solutions, along with the necessary dilutions, were utilized in the subsequent forced degradation studies. This approach provided a reliable means to investigate the proposed method's capability to indicate stability while ensuring specificity in identifying degradation products under various stress conditions.

Table 8: Assay results for combined formulation of mupirocin and beclomethasone dipropionate

Drug	Serial no	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		

MUP: Mupirocin, BEC: Beclomethasone dipropionate, RSD: Relative standard deviation, SD: Standard deviation

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 4 h, utilizing a 250 mL round-bottom flask and maintaining a temperature of 70°C.

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 MUP and 1 mcg/mL for BEC. This approach aimed to imitate and assess the possible effects of acid hydrolysis on the stability and potential degradation of the substances being studied.

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 solution was introduced and thoroughly mixed. The resulting mixture was then subjected to reflux conditions for a duration of 4 h, employing a 250 mL round-bottom flask and maintaining the temperature at 70°C.

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 MUP and 1 mcg/mL for BEC. This protocol aimed to replicate and evaluate the potential effects of basic hydrolysis on the stability and potential degradation of the substances being studied.

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 solution was introduced and thoroughly mixed. The resultant mixture was then subjected to reflux conditions for duration of 4.5 h, utilizing a 250 mL round-bottom flask and maintaining the temperature at 70°C.

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 MUP and 1 mcg/mL for BEC. This protocol aimed to simulate and evaluate the potential effects of oxidative stress on the stability and potential degradation of the substances being studied.

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 h.

Upon completion of the 3-h 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 MUP and 1 mcg/mL for BEC. This protocol aimed to replicate and evaluate the potential effects of thermal stress on the stability and potential degradation of the substances being studied.

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 h.

Table 9: Summary of forced degradation study for API

Stress type	Stress condition	MUP area of peak	% Degradation	BEC area of peak	% Degradation
Control sample	NA	1010.322	NA	451.783	NA
Acid stress	0.1NHCL 2mL 4h	762.262	24.55	359.127	20.508
Base stress	0.1NHCL 2 mL 4h	686.845	32.02	336.194	25.585
Peroxide stress	3%H_2O_2 2 mL 4.5 h	808.362	19.99	354.177	21.604
Thermal degradation	At 110°C for 3h	707.047	30.02	290.151	35.776
Photolytic degradation	At Sunlight for 4 h	859.756	14.90	388.762	13.949

MUP: Mupirocin, BEC: Beclomethasone dipropionate, API: Active pharmaceutical ingredient, H_2O_2: Hydrogen peroxide

Table 10: Summary of forced degradation study for pharmaceutical formulation

Stress type	Stress condition	MUP area of peak	% Degradation	BEC area of peak	% Degradation
Control sample	NA	1010.322	NA	451.783	NA
Acid stress	0.1NHCL 2mL 4h	771.234	23.66	363.28	19.589
Base stress	0.1NHCL 2mL 4h	707.055	30.02	327.191	27.577
Peroxide stress	3%H_2O_2 2 mL 4.5 h	797.991	21.02	362.648	19.729
Thermal degradation	At110°C for 3 h	739.565	26.80	294.373	34.841
Photolytic degradation	At Sunlight for 4h	848.538	16.01	390.123	13.648

MUP: Mupirocin, BEC: Beclomethasone dipropionate, H_2O_2: Hydrogen peroxide

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)	0.71–0.75	1.31–1.83
Inter-day (*n*=3)	0.78–0.92	0.52–1.92
Specificity	Specific	Specific
Robustness	Robust	Robust

RP-HPLC: Reverse phase high performance liquid chromatography, RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limits of quantification

Following the 4-h 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 MUP and 1 mcg/mL for BEC. This process aimed to replicate and assess the potential effects of photo stress on the stability and potential degradation of the substances being studied. Summary of forced degradation study for API is show in Table 9 and summary of forced degradation study for pharmaceutical formulation is show in Table 10.

CONCLUSION AND SUMMARY

An RP-HPLC method was developed to quantify MUP and 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 × 4.6 mm, 5 µ). 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 wave length of 215 nm.

The obtained Rts were 3.36 min for MUP and 6.02 min for BEC. These Rts 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. Summary of validation parameters of RP-HPLC method show in Table 11.

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 Rts. 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 MUP and BEC 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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