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Novel RP-HPLC Method Development and Validation of Budesonide Suppository

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Abstract: In the current study, a simple and cost-effective stability-indicating RP-HPLC method was developed and validated to estimate Budesonide from bulk and pharmaceutical dosage forms.0.1% formic acid and methanol (15:85 v/v) were employed as the mobile phase with an injection volume of 20µl, and detection was carried out at 244 nm. The developed method was validated as per ICH Q2 guidelines. The RT of Budesonide was determined to be 4.3 ± 0.328 minutes, providing a reliable marker for its identification. The method was found to be linear between 2-12 µg/ml concentration with (R^2) of 0.997. This demonstrates the method's ability to measure varying concentrations of Budesonide accurately. Additionally, the percentage recovery of Budesonide was approximately 100%, confirming the accuracy of the developed method. The parameters for the system's suitability have also been found to be within acceptable limits. Force degradation studies reinforced the method's selectivity and sensitivity in detecting Budesonide under various degradation scenarios. In conclusion, our proposed RP-HPLC method provides a sensitive, accurate, and precise way to analyze Budesonide in bulk and pharmaceutical dosage forms.

Keywords: Budesonide; validation; suppository; stability-indicating;RP-HPLC;ICH guidelines.

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1. Introduction

Budesonide is a drug widely used to manage inflammatory conditions, particularly those affecting the airways and gastrointestinal tract. Its chemical structure is characterized by the presence of 11β,21-dihydroxy-16α,17α-(butylidenebis(oxy))pregna-1,4-diene-3, 20-dione (Figure 1).

Figure 1. Chemical Structure of Budesonide

When Budesonide is administered, it interacts with intracellular glucocorticoid receptors (GRs) within the body. By binding to these receptors, Budesonide triggers the

expression of genes responsive to glucocorticoids. These genes are responsible for producing various anti-inflammatory mediators, including specific anti-inflammatory cytokines like interleukin 10 (IL-10) and lipocortins[1]. Budesonide is a corticosteroid class of medications with a broad spectrum of activity. It manages and treats inflammatory diseases, mainly affecting the airways and gastrointestinal tract[2]. It is an immunosuppressant glucocorticosteroid generally used to ameliorate chronic inflammation [3]. Budesonide reduces adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) and macrophage inflammatory protein-2 (MIP-2), reducing inflammation and preventing infiltration of immune cells, making it effective in treating inflammatory conditions and promoting healing [4]. In addition to its use in managing respiratory conditions, Budesonide is also used to treat inflammatory bowel disease (IBD) and gastric irritation associated with ulcerative colitis[5].

One study focuses on the estimation of photodegradation impurities using LC-MS and NMR spectroscopy [6]. Another work presents an HPLC method for the simultaneous analysis of Budesonide and its synthesized hemiesters in a formulation [7]. In the literature, few analytical methods are available for determining Budesonide in pure form and dosage forms [8-15]. Considering this information, the current study aims to fill this analytical gap by presenting a simple and stability-indicating HPLC method for quantifying Budesonide [16,17]. The method follows the guidelines set by the International Council for Harmonisation (ICH) [18,19]. The analysis includes bulk drug samples and suppository formulations, ensuring the method's applicability to real-life dosage forms. In the current study, a simple and cost-effective stability-indicating RP-HPLC method was developed and validated to estimate the Budesonide from bulk and pharmaceutical dosage forms.

2. Materials and Methods

2.1. Chemicals and reagents.

Budesonide was obtained as a gift sample from Cipla Ltd. Mumbai, India. In-house HPLC-grade water collected from the Milli-Q system was utilized in the experiments. All of the chemicals and reagents used in the study were HPLC grade.

2.2. Instrumentation.

The method development and validation were conducted using an HPLC system consisting of a JASCO model PU 2080 Plus pump and a Rheodyne sample injection port with a 20 µl loop. The system was equipped with a JASCO UV 2075 Plus detector. Borwin chromatography software (version 1.5) was employed for data analysis and control of the HPLC system.

2.3. Preparation of mobile phase.

It was prepared using a mixture of 0.1% formic acid and methanol (15:85 v/v). Both solutions were mixed properly, passed with 0.45 µm nylon filter twice, and sonicated aimed at 15 mins and degas.

2.4. Standard stock solution preparation.

Budesonide, 10 mg, was dissolved in 10 ml of methanol to give a stock solution of 1000 µg/ml. Further dilutions were performed with methanol to get the final 100 µg/ml

concentration. Additional dilutions were made as required, using methanol as the diluent to obtain the desired concentrations for calibration and analysis purposes.

2.5. Sample preparation (Suppository formulation).

The suppositories (20 in number, each containing 4 mg of Budesonide) were weighed and crushed. A 10 mL volumetric flask was filled with crushed suppositories equal to 10 mg of the drugs. Methanol was added to get the concentration of 1000 µg/ml. The resulting solution was sonicated and filtered using a 0.45 µ filter. 1 mL stock solution was pipette out and added into a 10 mL volumetric flask, and volume was made with methanol. The mobile phase was further diluted to get a 4 µg/ml concentration. Before analysis, the resultant solution was filtered using a 0.45 µ membrane filter.

2.6. Assay (Formulation Content Analysis).

The analysis of the suppository formulation was performed following the sample solution preparation method mentioned earlier. The procedure was repeated six times. Each time, the sample solution was prepared as described and injected into the HPLC system. The corresponding peak area for Budesonide was recorded. The concentration of Budesonide was determined using the linear equation. Additionally, the percentage recovery of Budesonide was calculated based on the known concentration of Budesonide in the suppository formulation. By repeating this procedure for six samples and analyzing their concentrations and recovery percentages, a comprehensive assessment of the Budesonide content in the suppository formulation could be obtained.

2.7. Chromatographic conditions.

The method development experiments were performed on the Agilent Zorbax C18 column (250 × 10mm; 5 µm). The temperature of the column was maintained at 25°C, with an injection volume of 20µl. The mobile phase was composed of a 15:85 v/v combination of 0.1% formic acid and methanol. The solutions were analyzed at 244 nm, and the flow rate was maintained at 1.0 mL/min.

2.8. Validation of analytical method.

As per ICH Q2 guidelines, the analytical method was validated [17]. The following validation parameters were considered.

2.8.1. Specificity.

The term "specificity" refers to the ability to evaluate the analyte of interest without interference from any specific components present in the sample [20]. The specificity of the analytical method was determined using a peak purity profile study. It was validated by comparing the Budesonide chromatograms to a blank chromatogram. This includes impurities, degradants, or excipients that may be present in the sample matrix. In a specific test method, the measurement or detection of the analyte is selective and accurate, allowing for reliable determination of its concentration or presence without being influenced or affected by other components in the sample.

2.8.2. System suitability.

System suitability tests are used to confirm that the chromatographic system is operating satisfactorily [21]. This investigation used six replicate injections of the standard at the working concentration to calculate the retention time, the number of theoretical plates, and the peak tailing factor (T).

2.8.3. Linearity.

A stock solution of Budesonide (1000 µg/ml) was prepared, and from this solution with appropriate dilutions with methanol, 100 µg/ml solution was prepared in a volumetric flask. Six different concentrations ranging from 2-12 µg/ml were prepared with serial dilutions, and linearity was determined between concentration and peak area.

2.8.4. System precision.

Intra and inter-day precision studies were carried out to evaluate the precision of the developed method. In the intra-day study, three different concentrations were analyzed in a single day, while in the interday study, the same concentrations were analyzed in three consecutive days, and finally, % RSD was determined.

2.8.5. Accuracy.

Recovery studies were conducted by adding the standard drug to the sample solution. The sample solution, with a basic concentration of 4 µg/ml, was spiked at three different levels, around 50%, 100%, and 150%. Three determinations were performed at each level. The percent mean recovery was determined using the linearity equation.

2.8.6. Robustness.

It was assessed by analyzing the samples under altered conditions, specifically by varying the mobile phase composition, detection wavelength (within a range of ± 1 nm), and flow rate (within a range of ± 0.05 ml/min). The changes in these operational parameters were carefully observed, and their effects on the peak areas were recorded.

2.8.7. Forced degradation study.

Through this study, the stability of the method under various stress situations was estimated. The sample solution was subjected to thermal stress (60°C for 24 hours), UV stress (exposed to UV light with a minimum illumination of 200-watt hr/m^2, followed by exposure to cool white fluorescence light of at least 1.2 million Lux-Hr for 7 days), acid (0.1N HCl), base (0.1N NaOH), peroxide (3% H_2O_2). These stress conditions were employed to evaluate the potential degradation of the sample solution.

3. Results and Discussion

3.1. Optimization of chromatographic condition.

The parameters mentioned in Table 1 were found to be optimized chromatographic conditions, which showed excellent separation of the peak. To minimize interference from methanol in the chromatogram, the optimal wavelength for the detection of Budesonide was

determined to be 244 nm. The total run time was controlled at 10 minutes. Figure 2 shows the standard solution chromatogram. Table 1 represents the optimized chromatographic conditions for Budesonide.

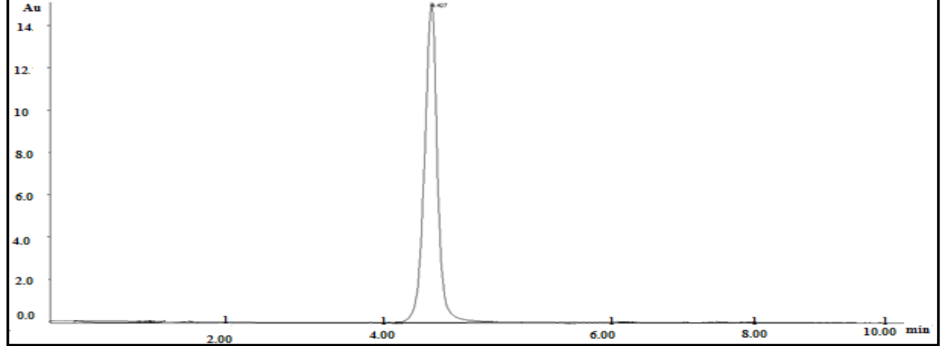

Figure 2. Chromatogram of the standard solution (10 µg/ml).

Table 1. Optimized chromatographic conditions.

Sr. No	Parameter	Condition
1	HPLC Instrument	JASCO model PU 2080
2	Column	Agilent Zorbax C18 RP (250 x 10 mm, 5 micron)
3	Wavelength	244 nm
4	Mobile Phase	0.1% formic acid: methanol (15:85 v/v)
5	Diluent	Methanol
6	Run time	10 minutes
7	Injection Volume	20 µl
8	Column Oven temperature	25°C
9	Flow Rate	1 mL/min

3.2. Method validation.

The validation of the method followed the guidelines set forth by ICH for the validation of analytical methods [18-19]. Various parameters, including system suitability, specificity, linearity, accuracy, precision, ruggedness, and robustness, were considered during validation. These validations aimed to establish the reliability and effectiveness of the method in producing accurate and precise results consistently.

3.2.1. System suitability.

System suitability test results are presented in Table 2. The peak retention time for Budesonide was determined to be 4.3 ± 0.328 min. The number of theoretical plates obtained for the Budesonide peak in all working standard injections was 5730, which exceeded the minimum requirement of 2000. Furthermore, the tailing factor was measured at 1.02, indicating a symmetrical peak shape. The observed peak area was 1679664. These results demonstrate the system's effectiveness, as it met the acceptable criteria for system suitability parameters, including high theoretical plates and a low tailing factor.

Table 2. System suitability test results of Budesonide.

Parameters	Budesonide
RT (min)	4.3± 0.328
Number of Theoretical plates (N)	5730
Tailing factor (T)	1.02
Area	1679664

3.2.2. Specificity.

This study involves the assessment of the purity of individual peaks in a chromatographic analysis. These studies aim to determine whether a peak of interest is pure or contains any impurities or co-eluting compounds. The peak purity was more than 996 (Table 3), suggesting no impurity or degradation product interference with the peak.

Table 3. Specificity of the analytical method.

Drug	Purity tail	Purity front
Budesonide	998.74	997.88

3.2.3. Linearity.

The calibration curve of Budesonide concentration versus peak area was found to be linear (R^2=0.997, equation y= 162581.2 x + 18867.99) within the concentration range of 2-12 µg/mL (Table 4), which was found within the limit. Hence, the method is said to be linear. The linear calibration curve of Budesonide is presented in (Figure 3).

Table 4. Linearity and regression analysis results.

Replica	Concentrations of Budesonide (µg/ml)					
	2	4	6	8	10	12
	Peak Area					
1	365580	688989.4	924307.7	1337582	1650003	1976831
2	373981.4	681707	935887.1	1294137	1679664	2004349
3	369079.8	685531.9	932636	1301344	1625814	1959477
4	372112.9	685878.5	936927	1292555	1643956	1969148
5	371313	698985.4	943907.7	1332584	1664703	1983214
6	364316.8	679189.4	934107.7	1274315	1666663	2048920
AVG	369397.317	686713.600	934628.867	1305419.500	1655133.833	1990323.167
STDEV	3809.001	6921.702	6383.274	24695.045	19168.421	32443.074
% RSD	1.031	1.008	0.683	1.892	1.158	1.630

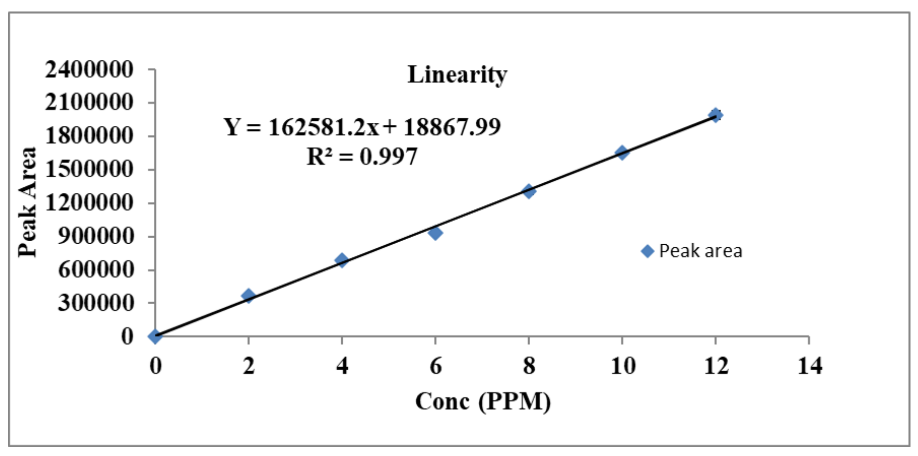

Figure 3. Linearity calibration curve for Budesonide.

3.2.4.System precision.

System precision studies are important to method validation and quality control processes [22]. They help to assess the reliability and repeatability of the measurement system, ensuring that it provides consistent and accurate results over time. These studies also aid in identifying potential sources of variation and evaluate the effectiveness of any corrective actions taken to improve the system's precision [23-24].

The repeatability of the developed method was assessed by calculating the % RSD for three assay results. The % RSD values obtained were less than 2%, indicating that the

developed method is precise in terms of repeatability. The assay results and corresponding % RSD values are presented in Table 5. Furthermore, the intermediate precision, also known as inter-day precision or ruggedness, was evaluated by different analysts to determine the method's precision under different conditions. The % RSD values obtained for the intermediate precision study were less than 2%, indicating that the developed method is precise and rugged. The results and corresponding % RSD values for the intermediate precision study are presented in Table 6. The % RSD values below 2% indicate that the method demonstrates good precision and reproducibility within the same day (repeatability) and across different days or operators (intermediate precision/ruggedness). These results provide confidence in the reliability and consistency of the developed method for accurate and precise measurements.

Table 5. Intra-day precision of Budesonide (Repeatability).

Theoretical Conc (μg/ml)	Peak Area	% Assay	AVG	STDEV	% RSD
4	672504.644	100.509			
4	665185.109	99.384	99.956	0.563	0.563
4	669029.466	99.975			
6	989023.534	99.453			
6	990661.998	99.621	99.795	0.455	0.456
6	997394.359	100.312			
8	1344406.8	101.914			
8	1300739.78	98.556	99.861	1.799	1.802
8	1307983.47	99.113			

Table 6. Inter-day precision of Budesonide (Ruggedness).

Theoretical Conc (μg/ml)	PeakArea	% Assay	AVG	STDEV	% RSD
4	679029.466	101.513			
4	679377.86	101.566	101.204	0.582	0.575
4	672654.644	100.532			
6	987394.359	99.286			
6	981707.28	98.703	99.143	0.388	0.391
6	988873.534	99.438			
8	1327983.47	100.651			
8	1319149.99	99.972	100.343	0.344	0.343
8	1324817.17	100.407			

3.2.5. Accuracy.

Accuracy study is an essential component of method validation, which assesses the closeness of results obtained by an analytical method to the valid or accepted reference values[25]. It provides information about the systematic errors or biases present in the method and ensures that the method produces accurate and reliable results.

Table 7. Results of accuracy studies for Budesonide.

Concentration Level%	Standard(μg/ml)	Sample(μg/ml)	Amount Recovered(μg/ml)	Mean ± % RSD
50	2	4	5.957	99.891 ± 0.533
			6.005	
			6.018	
100	4	4	8.048	100.149 ± 0.933
			8.062	
			7.926	
150	6	4	9.987	100.731 ± 0.823
			10.152	
			10.080	

The percent recovery should be close to 100% for accurate results with a low percent relative error or bias. A high percent recovery indicates that the method tends to overestimate the analyte concentration, while a low percent recovery suggests underestimation. The accuracy of the developed

method was assessed by comparing the recovery values to the accepted limits of 98%-102%. The recovery values obtained from the accuracy study were found to fall within this required range. This indicates that the method demonstrates good recovery values and is considered accurate. The accuracy of the study results is presented in Table 7.

3.2.6. Robustness.

The robustness study in analytical method validation is crucial for establishing the method's reliability, identifying critical parameters, and ensuring the method's consistent performance under practical conditions[25]. It provides valuable information to users, analysts, and regulatory bodies, further enhancing the method's credibility and usability. The results indicated that deliberate alterations to the analytical parameters did not significantly impact the areas of the peaks of interest. This observation suggests that the method is robust, as the areas of the relevant peaks were not significantly altered due to minor changes in the standard parameters. The robustness study results are presented in Tables 8 to 10.

Table 8. Change in wavelength.

Sr. No	Change in wavelength	RT	Theoretical plates	Area
1	243 (Decrease)	4.5	5721	1679600
2	244 (Normal)	4.3	5730	1679664
3	245 (Increase)	4.4	5710	1679700

Table 9. Change in mobile phase composition.

Sr. No	Mobile phase composition	RT	Theoretical plates	Area
1	0.1% formic acid: methanol (20:80 v/v) (Decrease)	4.6	5735	1678955
2	0.1% formic acid: methanol (15:85 v/v) (Normal)	4.3	5730	1679664
3	0.1% formic acid: methanol (10:90 v/v) (Increase)	4.5	5742	1679665

Table 10. Change in flow rate.

Sr. No	Change in wavelength	RT	Theoretical plates	Area
1	0.95 mL/min (Decrease)	4.4	6021	1678890
2	1 mL/min (Normal)	4.3	5730	1679664
3	1.05 mL/min (Increase)	4.5	6256	1678900

3.2.7.Forced degradation study.

This study is crucial in the development and validation processes of the method. It involves subjecting the drug substance or drug product to various stress conditions to assess its stability, identify potential degradation pathways, and evaluate the robustness of the analytical method [26]. The stress conditions used in a forced degradation study are designed to simulate potential degradation pathways that the drug substance or product may encounter during storage, transportation, or use. By subjecting the drug to these conditions, any potential degradation products and impurities can be identified, characterized, and quantified. The analytical method used for the forced degradation study should be able to accurately separate, detect, and quantify the drug substance and its degradation products. It is essential to evaluate the stability-indicating capability of the analytical method to ensure that it can distinguish between the intact drug and any degradation products formed.

The results of the forced degradation study demonstrated that the developed method is suitable for studying the stability of Budesonide under different forced degradation conditions. During the study, specific stress conditions were applied to the Budesonide sample, and the resulting chromatograms were analyzed. One degradation product was detected specifically under acidic stress conditions among the various stress conditions employed. This indicates

that Budesonide is susceptible to degradation when exposed to acidic conditions, leading to the formation of the identified degradation product. Figure 4-8 represents the degradation of the chromatogram.

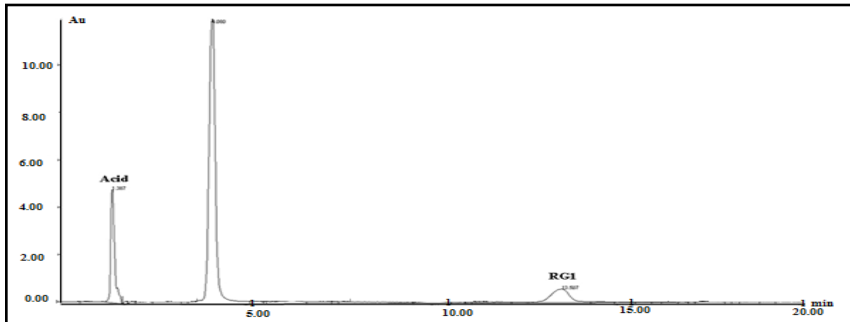

Figure 4. Chromatogram of Budesonide after acid degradation.

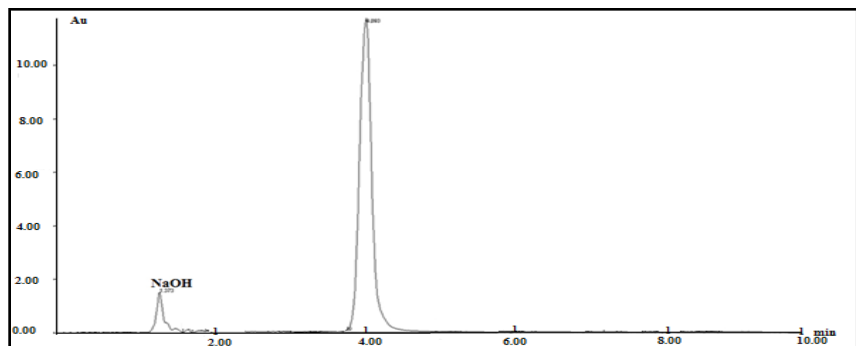

Figure 5. Chromatogram of Budesonide after alkaline degradation.

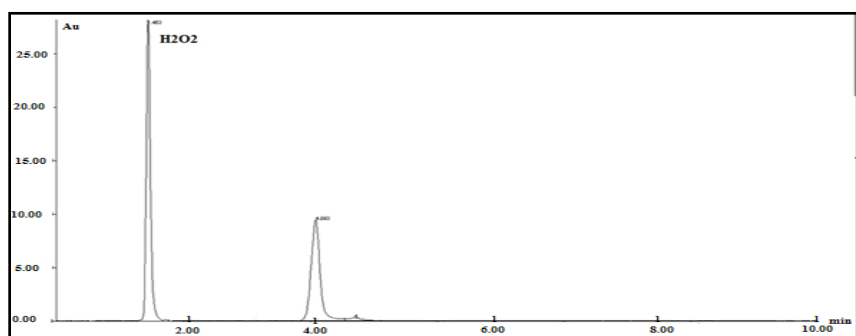

Figure 6. Chromatogram of Budesonide after oxidation with 30% v/v H_2O_2.

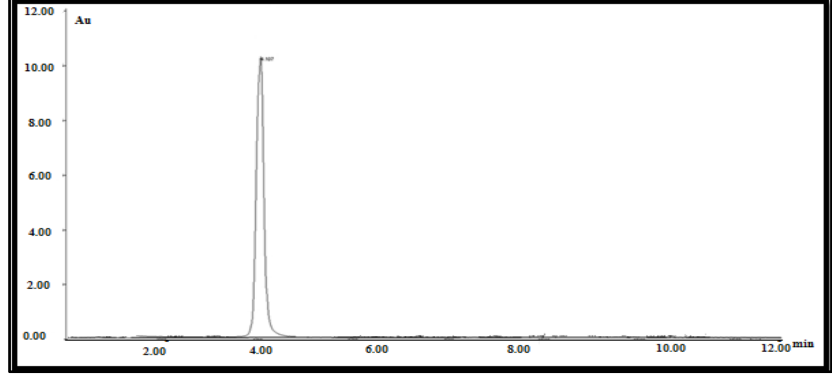

Figure 7. Chromatogram of Budesonide after thermal degradation.

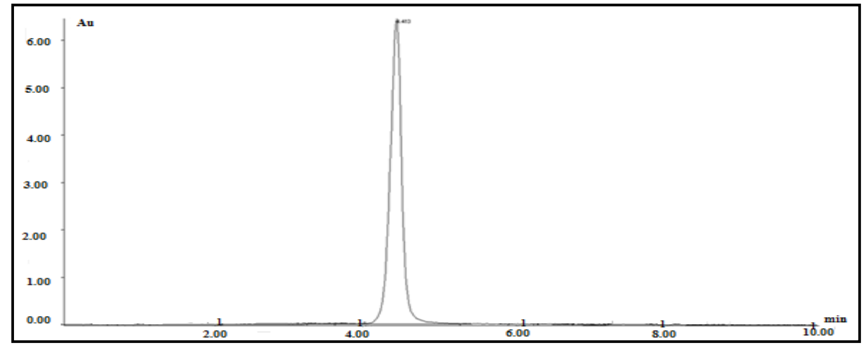

Figure 8. Chromatogram of Budesonide after Photolytic degradation.

3.2.8. Assay.

The developed and validated analytical method of Budesonide was applied to determine the assay of in-house suppositories and check its workability. The assay of the Budesonide from suppository formulation ranged between 99.9 and 101.8 %(Table 11), which is within acceptable limit criteria.

Table 11. Assay results of Budesonide.

Sr no.	Peak Area	Amount Recovered(µg/ml)	%Recovery
1	672607.482	4.021	100.525
2	668928.935	3.998	99.959
3	674499.734	4.033	100.816
4	681039.567	4.073	101.822
5	670149.623	4.006	100.147
6	676304.387	4.044	101.094
Mean	673921.621	4.029	100.727
S.D	4417.212	0.027	0.679
%RSD	0.655	0.674	0.674

4. Conclusions

The developed RP-HPLC method for quantitatively estimating Budesonide in Suppositories demonstrated several favorable attributes. Firstly, the method proved to be economical as it required less consumption of the organic phase. Secondly, a good linear relationship was observed between the 2 to 12 µg/ml drug concentration range. The precision of the method was confirmed through satisfactory inter-day and intra-day precision results, indicated by low % RSD values below 2%. Accuracy studies further supported the method's reliability, with mean recoveries falling within the range of 99.981% to 100.731%. The method's robustness was established as no variations were observed in the system suitability results, even after deliberate changes in the mobile phase composition, detection wavelength, and flow rate. Degradation studies conducted under different conditions validated the method's suitability for studying the stability of Budesonide, with the detection of one degradation product under acidic stress conditions. The proposed method successfully separated the compound from its degradants and accurately estimated the active content. Overall, the developed RP-HPLC method demonstrated its suitability for quantitative estimation of Budesonide in Suppositories, meeting the requirements of specificity, accuracy, precision, linearity, ruggedness, and robustness per the ICH guidelines.

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Conflicts of Interest

The authors declare no conflict of interest.

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