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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHIC METHOD FOR BUDESONIDE

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ABSTRACT

Objective: A stability-indicating high-performance thin-layer chromatographic method has been developed for budesonide.

Methods: Chromatographic separation was achieved on aluminum plates pre-coated with silica gel 60 F_{254} as the stationary phase using ethyl acetate: toluene (7:3) v/v as the mobile phase. The densitometric evaluation was carried out at 246 nm. The developed method of stability-indicating was validated as per the ICH Q2 (R1) guidelines. Stress degradation studies such as hydrolysis under different pH conditions, photolytic degradation, thermal degradation, and oxidative degradation were performed as per ICH Q1A (R2) and Q1B guidelines.

Results: The Rf value of budesonide was found to be 0.48±0.03. The response in terms of peak area was linear over the concentration range of 500–2500 ng/band, with the regression coefficient value greater than 0.99. The LOD and LOQ were 28.04 ng/band and 84.96 ng/band, respectively.

Conclusion: This method can conveniently be used for quantitative analysis of budesonide on a routine basis.

Keywords: High-performance thin-layer chromatographic, Forced degradation, Stability indicating, Validation, Budesonide.

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INTRODUCTION

Budesonide is a glucocorticoid that is a mix of the 22R and 22S epimers used to treat inflammatory conditions of the lungs and intestines such as asthma, chronic obstructive pulmonary disease (COPD), Crohn's disease, and ulcerative colitis. It is also available in extended-release capsules that are indicated for the treatment and maintenance of mild to moderate Crohn's disease [1]. Various inhaled budesonide products are indicated for prophylactic therapy in asthma and reduce exacerbations of COPD. A budesonide nasal spray is available over the counter for symptoms of hay fever and upper respiratory allergies. Extended-release capsules are indicated to induce remission of mild-to-moderate ulcerative colitis, and rectal foam is used for mild-to-moderate distal ulcerative colitis. In addition, a delayed-release capsule formulation of budesonide is indicated to reduce proteinuria in adults with IgA nephropathy at risk of rapid disease progression. Budesonide is a corticosteroid. It works by preventing inflammation (swelling) in the lungs, which makes the asthma attack less severe. Inhaled budesonide may be used with other asthma medicines, such as bronchodilators, which are also used to open up narrowed breathing passages in the lungs. This drug is available in the following dosage forms: Suspension powder and tablet. The chemical formula of budesonide is $C_{23}H_{34}O_6$. Budesonide is a corticosteroid used to treat Crohn's disease, asthma, COPD, hay fever, allergies, and ulcerative colitis [2]. The stress study proposed in this method is based on guidance by Bakh et al. [3]. A detailed literature search indicates that there are a few RP-HPLC methods [4-12], a few high-performance thin-layer chromatographic (HPTLC) methods [13-15] and a few LC-MS methods [16] developed for this drug. However, degradation products in stability studies of budesonide were not reported in any of these papers. To ensure a better stability-indicating method, the proposed work was carried out, and degradation products were significantly visible. Also, the mobile phase used in the proposed work (ethyl acetate: toluene) indicates that the method developed is simple and economical as compared to the methods found in other literature where four to five component mobile phases were used.

METHODS

Instrumentation

Instruments that are used in this method are the HPTLC system (CAMAG), comprising of a TLC scanner III, Linomat 5 applicator, software (winCATS [version 1.4.3]), microliter syringes (Hamilton [100 uL]), TLC plates (Merck's aluminum TLC plate pre-coated with silica gel 60F_{254}), and a twin-trough glass chamber. Others are the UV-visible spectrophotometer (JASCO [Model-V730]), electronic balance (Shimadzu [Model ATR-224R]), sonicator (PRAMA [Model SM15 US]), hot air oven (BIOMEDICA), and a photo-stability chamber (Newtronic, Model-IC DAC version 1.2).

Chemicals

Budesonide was received as a gift sample from NATCO Pharmaceuticals, along with other chemicals and reagents such as chloroform (HPLC grade), methanol (AR grade), ethyl acetate 99.5% (AR grade), toluene 99.5% (AR grade), and glacial acetic acid 99.8% (HPLC grade). HCl (AR grade) and 30% v/v H_2O_2 (AR grade) are purchased from LOBA CHEMIE PVT. LTD., Mumbai.

Preparation of standard stock solution

For the preparation of the standard solution, an accurately weighed 25 mg of budesonide was transferred to the 25 mL volumetric flask. After that, the volume was made up to the mark with methanol to get the standard stock solution of budesonide (1000 µg/mL).

Selection of analytical wavelength

A solution of budesonide of strength (25 µg/mL) was prepared using methanol, and the UV spectrum was recorded.

Optimization of chromatographic conditions

Chromatographic separation of budesonide drug was performed on aluminum plates pre-coated with silica gel 60 F_{254} (10 cm×10 cm with 250 µm layer thickness). Samples were applied to the plate as a band of 6 mm in width using a 100-µL syringe with a Linomat applicator. The mobile phase was composed of ethyl acetate: toluene (7:3) v/v.

A 10 cm×10 cm twin trough glass chamber was used for the linear ascending development of the TLC plate with 20 min saturation conditions; the migration distance was 80 mm. Densitometric scanning was performed at 246 nm, operated by software, and the slit dimensions were 4×0.45 mm. Chromatographic conditions such as saturation time, band length, detection wavelength, stationary phase, and mobile phase were optimized and summarized in Table 1. The standard densitogram of budesonide (1000 ng/band) is shown in Fig. 1.

Forced degradation studies

The degradation conditions were as per ICH guidelines Q1A (R2). The strength of the reagent and the time of exposure were optimized to obtain 10–30% degradation. The optimized conditions are as follows [17].

Acid hydrolysis

For sample preparation, 1 mL of budesonide stock solution (1000 µg/mL) was mixed with 1 ml of 1 N HCl, and the volume was made up to the mark with methanol and refluxed for 4 h at 80 ËšC. The resultant solution (100 µg/mL) was applied to the TLC plate and developed using an optimized mobile phase.

Hydrolysis under basic pH

For sample preparation, 1 mL of budesonide stock solution (1000 µg/mL) was mixed with 1 ml of 1 N NaOH, and the volume was made up to the mark with methanol. The resultant solution (100 µg/mL) was applied to the TLC plate and developed using an optimized mobile phase.

Oxidative degradation

For sample preparation, 1 mL of budesonide stock solution (1000 µg/mL) was mixed with 1 mL of 30% H_2O_2 and volume made up to the mark with methanol and refluxed for 4 h at 80 ËšC. The resultant solution (100 µg/mL) was applied to the TLC plate and developed using an optimized mobile phase.

Photolytic degradation

For sample preparation, accurately weighed 60mg of budesonide and was transferred into a clean petri dish with a cover and exposed to UV light until the exposure of 200 watt-h/m^2 and white cool fluorescent light up to the exposure of 1.2 million Lux h [18]. After completion of the required illumination, the sample was removed. Appropriately, weighed and diluted to get 100 µg/mL. The resultant solution was applied to the TLC and analyzed under optimized chromatographic conditions.

Table 1: Optimized chromatographic parameters

Parameter	Condition used for analysis
Stationary phase	Merck's TLC aluminum plates pre-coated with silica Gel G60 F_{254}
Mobile phase	Ethyl acetate: toluene (7:3 v/v)
Band length	6 mm
Saturation time	20 min
Detection wavelength	246 nm
Rf value	0.48±0.03

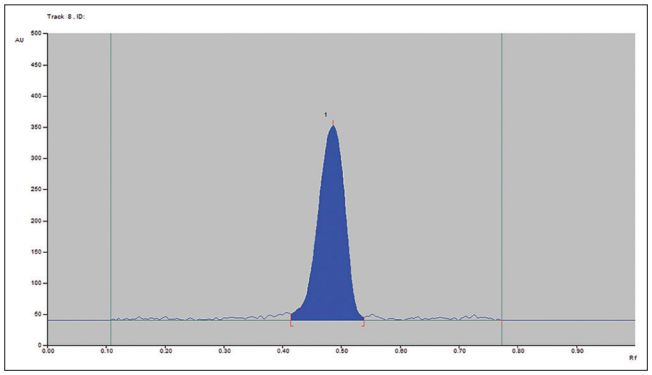

Fig. 1: Representative 3D densitogram of budesonide (2000 ng/band, Rf =0.48)

Thermal degradation

The bulk drug was exposed to thermal stress by placing it in an oven at 80 ËšC for 8 h. A sample was taken from the oven, cooled to room temperature, weighed, and dissolved in methanol to provide a final concentration of 100 µg/mL of budesonide, which was then applied to HPTLC and evaluated.

Validation of the analytical method

The developed HPTLC method for budesonide was validated as per the ICH guidelines ICH Q2 (R1) in terms of linearity and range, accuracy, specificity, limit of detection (LOD), limit of quantitation (LOQ), repeatability, and intermediate precision and robustness [19].

Specificity

Peak purity profiling studies were carried out for evaluating the specificity of the method. Peak purity for the drug peak of all degradation conditions as well as the assay was monitored using Win CAT software. It compares the UV spectrum at the peak start, midpoint, and peak end.

Linearity and range

Appropriate volumes of working solution of budesonide (100 µg/mL) were applied on the TLC plate (5, 10, 15, 20, and 25 µL), thus leading to spotted amounts in the range of 500–2500 ng/band. The plate was developed, and this procedure was repeated five times. The 3D densitogram is shown in Fig. 2 for linearity. The calibration curve was obtained by plotting the amount of drug spotted (ng/band) versus the peak area is shown in Fig. 3.

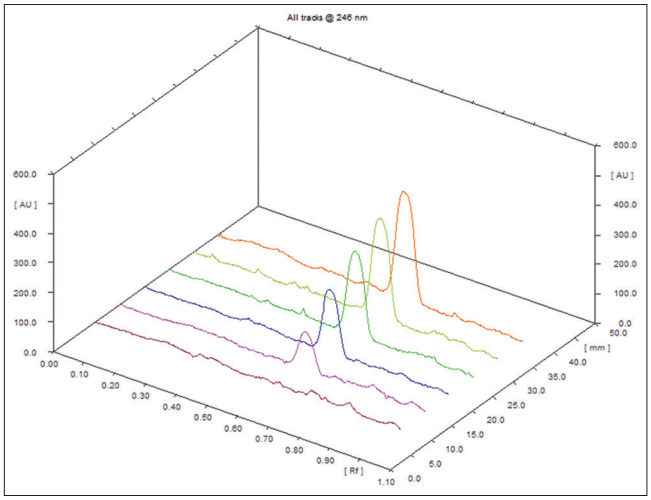

Fig. 2: 3D densitogram of linearity (500–2500 ng/band)

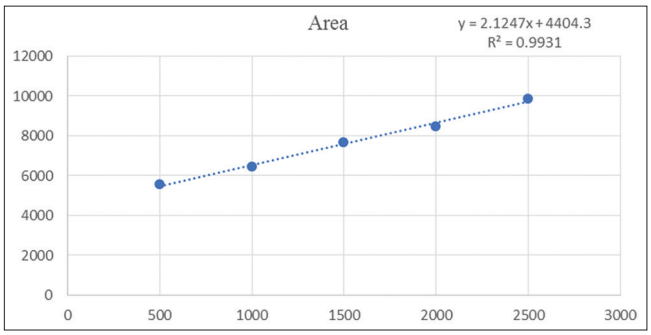

Fig. 3: Calibration curve

Assay

A marketed product of budesonide soft gelatin capsule was used for the assay. For sample preparation, an accurately weighted powdered marketed preparation (3 mg budesal) capsule was used, which is equivalent to 10 mg of drug content and was diluted appropriately to 1000 µg/mL. The solution was filtered and sonicated. 2 replicates of the sample solution (100 µg/mL) were prepared from the 1000 µg/mL stock solution. After sonication and filtration, a 10 µL volume of each sample solution was applied to the TLC plate. The plate development was done in the mobile phase and scanned at 246 nm. Peak area was recorded, and % recovery was calculated.

Accuracy

The accuracy of the method was determined by the standard addition method. The marketed product of budesonide capsule (assay solution) was analyzed by adding a known amount of the standard drug at 80, 100, and 120% levels. 2 replicates of 3 concentrations (1800 ng/band, 2000 ng/band, and 2200 ng/band) were evaluated, and % recovery was calculated.

Precision

The method's precision was demonstrated by intraday (repeatability) and interday (intermediate) precision studies. For intraday precision, six replicates of the standard solution (100 µg/mL) were spotted on the TLC plate on the same time interval. In an interday precision study, application of six replicates of the standard solution (100 µg/mL) was spotted on the TLC plate on 3 consecutive days. The % RSD was calculated, and the values were found to be <2%.

LOD and LOQ

The LOD and LOQ were calculated using equations: LOD= 3.3×σ/S and LOQ=10×σ/S, respectively, where σ is the standard deviation and S is the slope of the calibration curve.

Robustness

The robustness of the developed method was evaluated by small but deliberate changes in mobile phase ratio, saturation time, the effect of time from spotting to development and time from development to scanning, detection wavelength, and mobile phase volume was changed by ±0.2 mL, and saturation time was varied by ±5 min, i.e., 15 min and 25 min. The detection wavelength was varied by ±2 nm. One factor at a time was varied at a concentration of 500 ng/band for budesonide to study the effect of each factor on the peak area of the drug.

RESULTS AND DISCUSSION

The methanolic solution showed maximum absorbance at 246 nm. The UV spectrum is shown in Fig. 4.

Forced degradation studies

The stability-indicating property of the developed method was confirmed by forced degradation studies that were carried out in accordance with ICH guidelines Q1A (R2) as shown in Table 2.

The drug was found to be susceptible to all stress conditions except photolytic conditions. Only in the basic hydrolytic condition, we got a degradation product at Rf 0.81 densitogram, as shown in Fig. 5. The spectral scanning overlay is shown in Fig. 6.

Method validation

The summary of the validation parameters and their results are shown in Table 3.

Specificity

Specificity was monitored by peak purity studies for both the standard and sample, and it was found to be more than 0.995, as shown in Table 4. The values indicate that the method is specific.

Linearity and range

Linearity was determined by plotting the amount spotted versus the peak area. Linearity was observed in the range of 500–2500 ng/band.

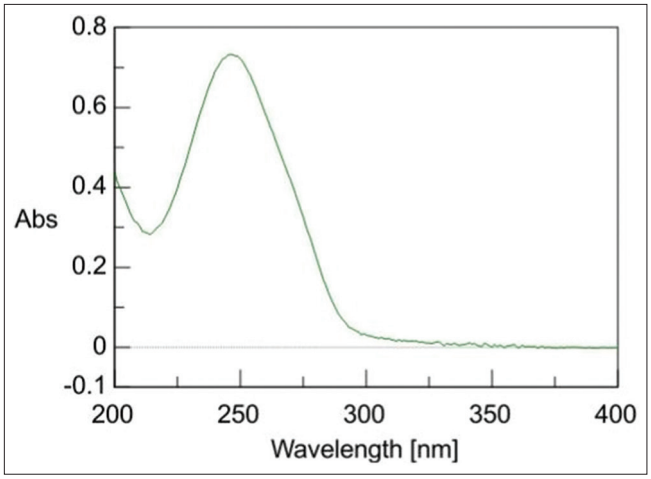

Fig. 4: UV spectrum of budesonide (25 µg/mL)

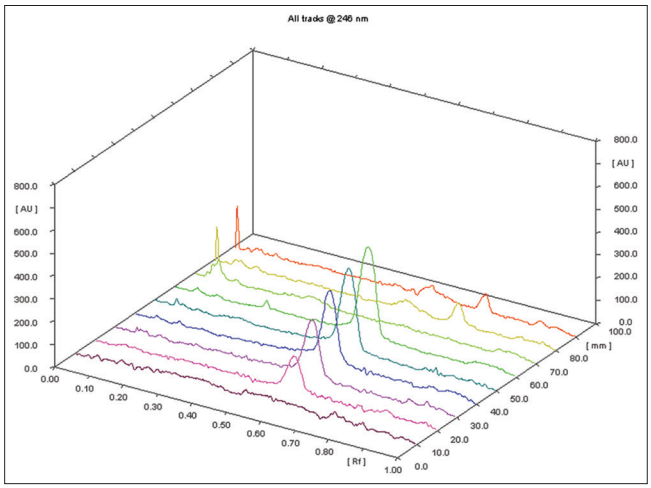

Fig. 5: 3D densitogram of base degradation (track1-blank, track 2, 3, 4, 5, 6-standard 500–2500 ng/band, track 7-blank, track 8, 9-stress solution)

Table 2: Summary of forced degradation studies for budesonide

S. No.	Degradation conditions	% Recovery	Rf	Rf of degradation product
1	Acidic condition (1 N HCL for 4 h at 80 ËšC for Reflux)	91.36	0.45	-
2	Alkali condition (1 N NaOH for 0 min)	16.47	0.48	0.81 0.82
3	Photo stability: (1) UV (200 watt h meter square)	86.71	0.49	-
	(2) cool white fluorescent light (1.2 million reflux h)	89.31	0.48	-
4	Oxidative condition (30% H_2O_2 for 4 h at 80 ËšC for reflux)	90.18	0.45	-
5	Thermal condition (80 ËšC for 8 h)	84.86	0.45	-

The correlation coefficient was found to be 0.9931 with an equation of y=2.1247×+4404.3. The 3D densitogram of linearity is shown in Fig. 2. The calibration curve is shown in Fig. 3.

Assay

The assay was carried out using a marketed formulation (capsule). The drug content in the capsule was found to be 101.09%.

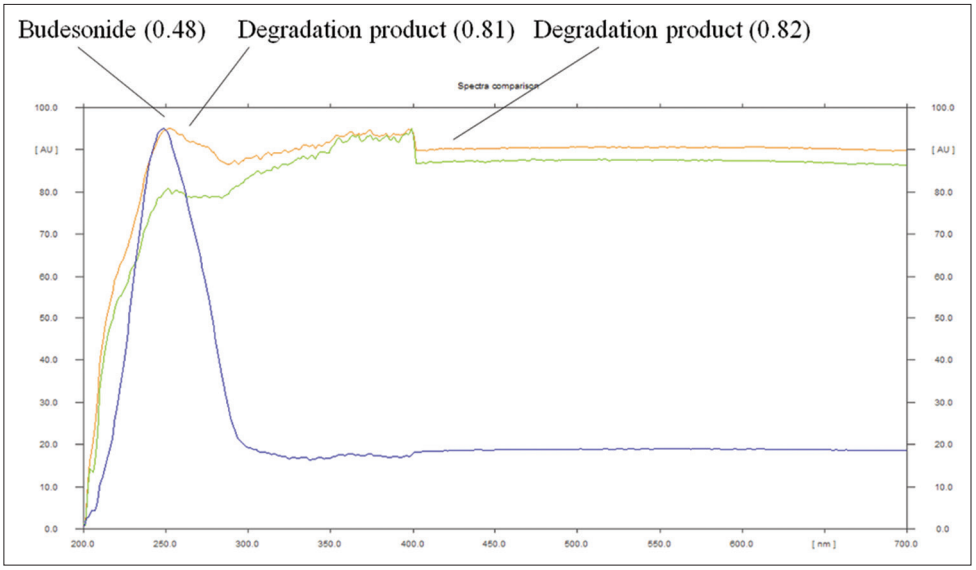

Fig. 6: 3D densitogram of spectral overlay for base degradation product

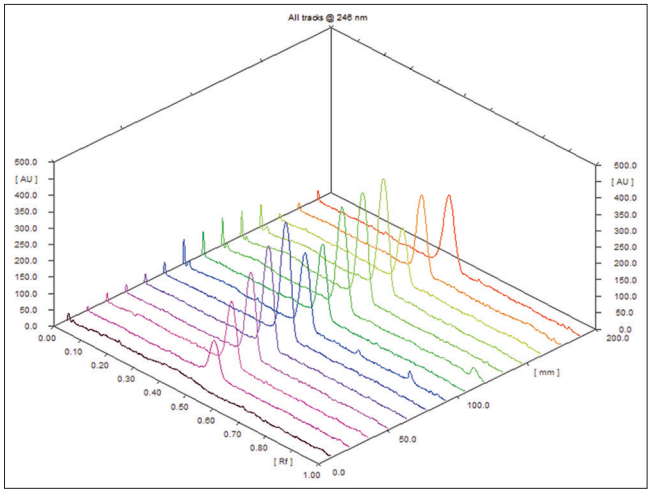

Fig. 7: 3D densitogram of accuracy study (track 1-blank, track 2, 3, 4, 5, 6-standard 500–2500 ng/band, track 7, 8–1000 ng/band track-9- 80% level, track-10–100% level, track-11–120% level track- 12-standard 800 ng/band, track 13,14-standard 1200 ng/band)

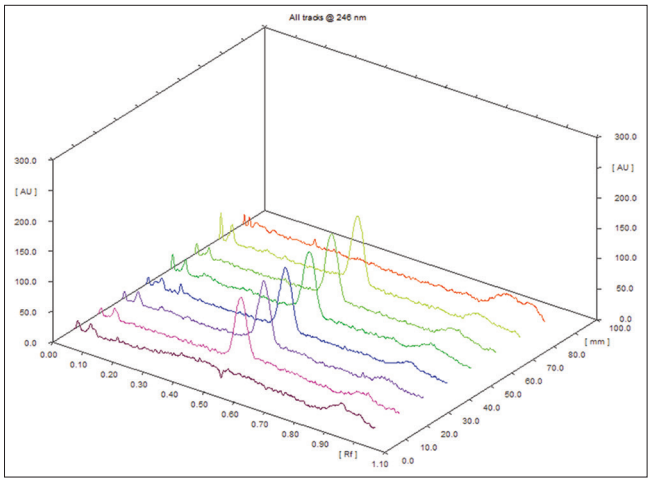

Fig. 8: 3D densitogram of precision (6 replicate of 500 ng/band)

Table 3: Summary of validation parameters

S. No	Validation parameters	Result
1	Specificity	Specific
2	Linearity and range	y=2.1247× + 4404.3 R^2=0.9931
3	Repeatability % (RSD) Intermediate % (RSD)	0.39% 0.35%
4	Assay	101.09%
5	Accuracy 1. 80% level 2. 100% level 3. 120% level	% Recovery 101.07% 102.61% 100.70%
6	LOD	28.04 ng/band
7	LOQ	84.96 ng/band
8	Robustness	Robust

Table 4: Specificity studies

Name	Retention factor	Peak purity r (s, m)	Peak purity r (m, e)
Standard	0.48	0.9954	0.9968
Sample	0.48	0.9963	0.9979

Accuracy

The mean recovery was found to be 101.07% for budesonide, which indicated that the proposed method is accurate for the estimation of the drug in capsule dosage form. The percent recovery for budesonide was found to be in the range as shown in Table 5. The representative 3D densitogram is shown in Fig. 7.

Precision

Repeatability and intermediate precision were performed. The RSD was found to be 0.39 and 0.35, respectively. The representative 3D densitogram is shown in Fig. 8.

LOD and LOQ

The LOD and LOQ were calculated using equations: LOD=3.3×σ/S and LOQ=10×σ/S, respectively, where σ is the standard deviation and S is the slope of the calibration curve. LOD and LOQ were found to be in range, i.e., 28.04 ng/band and 84.96 ng/band, respectively.

Table 5: Accuracy (%recovery)

S. No	Amount spotted from marketed formulation (ng/band)	Amount of Std. Added (ng/band)	Total Amount of the drug (ng/band)	Amount recovered	%recovery
1	1000	800	1800	1819.42	101.07%
2	1000	1000	2000	2152.21	102.61%
3	1000	1200	2200	2313.53	100.70%

Table 6: Robustness

S. No	Parameter	Robust condition	% RSD
1	Mobile phase composition	6.8:3.2 v/v	0.28
	(Ethyl acetate: toluene 7:3 v/v, ±0.2 mL)	7.2:2.8 v/v	0.35
2	Saturation time	15 min	0.43
	(10±5 min)	25 min	0.49
3	Time from application to development	Immediately after application	0.19
		After 2 h	0.40
4	Time from development to scanning	Immediately after development	0.35
		After 2 h	0.37
5	Change in wavelength (246±2 nm)	244 nm	0.60
		248 nm	0.48

Robustness

It was observed that there were no marked changes in the peak areas, which confirmed that the developed method was robust. For results of robustness, see Table 6.

DISCUSSION

As per the literature survey, it was observed that many reported methods have a mobile phase consisting of four to five components, making it prone to poor reproducibility. The proposed method is economical as well as simple, especially in terms of binary mobile phase preparation. Also, a few of the literature papers have not reported a degradation product under alkaline conditions; only one work by Panchal *et al*. has reported complete degradation of budesonide under alkaline conditions and a well-resolved product of degradation. Our results concur with their work; the stress study proposed in this method shows degradation product peaks under alkaline hydrolytic conditions that are well resolved from the drug peak. This rapid method can help detect possible degradations under alkaline hydrolytic conditions.

CONCLUSION

This developed HPTLC method is simple, rapid, and stable, indicating routine quantitative analysis of budesonide as a bulk drug and in the dosage form without interference of commonly used excipients. The developed method was validated as per ICH guidelines. Budesonide was found to be relatively stable under all stress conditions except the alkaline hydrolysis condition. The peak purity value was found within the limit, confirming specificity and stability and indicating the nature of the developed method. Thus, this method can conveniently be used for quantitative analysis of budesonide on a routine basis.

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AUTHORS CONTRIBUTION

MCD designed the work. PDM contributed for the analysis and data collection parts of the work. MCD and PDM contributed to the interpretation of the results.

CONFLICTS OF INTERST

Declared none.

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Nil.

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