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

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Received: 22 May 2023, Revised and Accepted: 04 July 2023

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_{26}H_{24}O_{7}. 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 a TLC scanner III, Linomat S applicator; software (winCATS [version 1.4.3]), microfilter syringes (Hamilton [100 μL]), 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 (Newtonic, 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), water 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 °C. 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% H₂O₂ and volume made up to the mark with methanol and refluxed for 4 h at 80 °C. 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 60 mg 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/m² 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.

**Thermal degradation**

The bulk drug was exposed to thermal stress by placing it in an oven at 80 °C 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 WinCAT 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition used for analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary phase</td>
<td>Merck’s TLC aluminum plates pre-coated with silica Gel G60 F254</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Ethylacetate: toluene (7:3 v/v)</td>
</tr>
<tr>
<td>Band length</td>
<td>6 mm</td>
</tr>
<tr>
<td>Saturation time</td>
<td>20 min</td>
</tr>
<tr>
<td>Detection wavelength</td>
<td>246 nm</td>
</tr>
<tr>
<td>Rf value</td>
<td>0.48±0.03</td>
</tr>
</tbody>
</table>

Table 1: Optimized chromatographic parameters

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

**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.

Table 2: Summary of forced degradation studies for budesonide

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Degradation conditions</th>
<th>% Recovery</th>
<th>Rf</th>
<th>Rf of degradation product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acidic condition (1 N HCL for 4 h at 80 °C for Reflux)</td>
<td>91.36</td>
<td>0.45</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Alkali condition (1 N NaOH for 0 min)</td>
<td>16.47</td>
<td>0.48</td>
<td>0.81</td>
</tr>
<tr>
<td>3</td>
<td>Photo stability: (1) UV (200 watt h meter square) (2) cool white fluorescent light (1.2 million reflux h)</td>
<td>86.71</td>
<td>0.49</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Oxidative condition (30% H₂O₂ for 4 h at 80 °C for reflux)</td>
<td>9.18</td>
<td>0.45</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Thermal condition (80 °C for 8 h)</td>
<td>84.86</td>
<td>0.45</td>
<td>-</td>
</tr>
</tbody>
</table>

The correlation coefficient was found to be 0.9931 with an equation of y=2.1247x+4.4043. 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%.
Table 3: Summary of validation parameters

<table>
<thead>
<tr>
<th>S. No</th>
<th>Validation parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Specificity</td>
<td>Specific</td>
</tr>
<tr>
<td>2</td>
<td>Linearity and range</td>
<td>$y = 2.1247x + 4404.3$</td>
</tr>
<tr>
<td></td>
<td>$R^2 = 0.9931$</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Repeatability % (RSD)</td>
<td>0.39%</td>
</tr>
<tr>
<td>4</td>
<td>Intermediate % (RSD)</td>
<td>0.35%</td>
</tr>
<tr>
<td>5</td>
<td>Accuracy</td>
<td>% Recovery</td>
</tr>
<tr>
<td>6</td>
<td>LOD</td>
<td>28.04 ng/band</td>
</tr>
<tr>
<td>7</td>
<td>LOQ</td>
<td>84.96 ng/band</td>
</tr>
<tr>
<td>8</td>
<td>Robustness</td>
<td>Robust</td>
</tr>
</tbody>
</table>

Table 4: Specificity studies

<table>
<thead>
<tr>
<th>Name</th>
<th>Retention factor</th>
<th>Peak purity r (s, m)</th>
<th>Peak purity r (m, e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>0.48</td>
<td>0.9954</td>
<td>0.9968</td>
</tr>
<tr>
<td>Sample</td>
<td>0.48</td>
<td>0.9963</td>
<td>0.9979</td>
</tr>
</tbody>
</table>

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: $LOD = 3.3\times\sigma/S$ and $LOQ = 10\times\sigma/S$, respectively, where $\sigma$ 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.
It was observed that there were no marked changes in the peak Robustness 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.

**ACKNOWLEDGEMENTS**

The authors would like to thank NATCO Pharmaceutical Hyderabad for providing API, as well as the principal and management of AISSMS College of Pharmacy, Pune, for providing the necessary infrastructure and institutional facilities to carry out this work.

**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 INTEREST**

Declared none.

**AUTHORS FUNDING**

Nil.

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