SIMULTANEOUS DETERMINATION OF BECLOMETHASONE DIPROPIONATE AND FORMOTEROL FUMARATE IN ROTACAP DOSAGE FORM USING TWO DIFFERENT SPECTROPHOTOMETRIC METHODS

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ABSTRACT
Two simple, rapid and precise UV spectroscopic methods namely Simultaneous equation (method 1), Q-absorbance ratio (method 2) and have been developed for the simultaneous determination of Beclomethasone dipropionate and Formoterol fumarate. In method 1, both the drugs exhibit good linearity over the concentration range of 10-50µg/ml and 1 to 5µg/ml of BD and FF at 239.2nm ($r^2$=0.998 and 0.998 respectively) and 213nm ($r^2$=0.999 and 0.998 respectively) wavelengths respectively. Method 2 involves the formation of Q-absorbance equation using the absorptivity values at 218.3nm ($\lambda_{max}$ BD)($r^2$=0.998 and 0.999 respectively) and 239.2nm and Beer’s Lambert’s law was obeyed over the concentration range of 10-50µg/ml and 1-5µg/ml respectively. The proposed methods were validated according to ICH guidelines for evaluation of accuracy, precision, sensitivity etc. In conclusion, the proposed methods are novel, simple, accurate, precise, sensitive, rapid and economically viable methods that do not require any prior separation procedure. The proposed two methods hold potential for simultaneous determination of BD and FF in rotacap formulation.

Keywords: Beclomethasone dipropionate, Formoterol fumarate, Spectrophotometric method, Simultaneous equation method, Q-ratio method, Validation.
INTRODUCTION
Asthma is an inflammatory condition in which there is recurrent reversible airways obstruction in response to irritant stimuli. Reversibility of airways obstruction in asthma contrasts with COPD. Asthmatic patients experience intermittent attacks of wheezing, shortness of breath—with difficulty especially in breathing out, and sometimes cough.\(^{(1)}\)

Beclomethasone dipropionate is 9α-Chloro-11β-hydroxy-16β-methyl-3, 20-dioxopregn-1,4-diene-17, 21 dipropionate. Beclomethasone dipropionate is an orally inhaled adrenocortico-steroid which suppresses bronchial inflammation, increases peak expiratory flow rate and prevents episodes of acute asthma. It has long onset of action and long duration of action. It also used to control bronchial asthma in patients requiring chronic treatment, prophylaxis & treatment of allergic & vasomotor rhinitis. BD is a highly hydrophobic steroidal drug which is formulated as an aqueous suspension for nebulizer therapy, DPI & MDI form.\(^{(3-6)}\).

Formoterol fumarate is \((\pm)-N\{-2-hydroxy-5-[(1RS)-1-hydroxy-2-\{[(2RS)-1-(4-methoxyphenyl) propan-2-yl] amino\}-ethyl\} phenyl\} formamide fumarate. Formoterol fumarate is a long-acting selective beta2-adrenergic receptor agonist (beta2-agonist). Formoterol fumarate use in management of asthma and chronic obstructive pulmonary disease (COPD). It works like other beta2-agonists, causing bronchodilatation by relaxing the smooth muscle in the airway so as to treat the exacerbation of asthma.\(^{(7-10)}\)

![Chemical structures of (a) Beclomethasone dipropionate (b) Formoterol fumarate](image)

Fig. I: Chemical structures of (a) Beclomethasone dipropionate (b) Formoterol fumarate

From a detailed literature review, it was revealed that different analytical methods for Beclomethasone dipropionate High Performance Thin Layer Chromatographic Method, UV - Spectroscopic Methods, High Performance Liquid Chromatographic Methods and Methods
for Estimation from Biological Fluids in bulk drug and/or individual dosage form and/or combined dosage form (with other drugs) are existing.\(^{[14-23]}\).

Non-aqueous Titration (Potentiometric), Colorimetric, UV–Spectroscopic, High Performance Liquid Chromatographic and Methods for Estimation from Biological Fluids for Formoterol fumarate dihydrate in bulk drug and/or individual dosage form and/or combined dosage form (with other drugs) are existing.\(^{[15-38]}\)

According to detailed survey of analytical literature, None of analytical method reported for simultaneous estimation of Formoterol fumarate dihydrate and Beclomethasone dipropionate in their combined dosage form using simultaneous equation method and Q-ratio method.

**MATERIALS AND METHODS**

**Instrument**

A Shimadzu model UV-1800 double beam UV-visible Spectrophotometer, attached to a computer software UV probe 2.34, with a spectral width of 1 nm and pair of 1 cm matched quartz cells was used. Shimadzu analytical balance was used throughout practical. Class ‘A’ volumetric glassware were used.

**Materials**

Reference standard of Beclomethasone dipropionate API gifted by Dial Pharmaceutical Ltd., Ahmedabad, Gujarat, India. Reference standard of Formoterol fumarate API purchased from Vamsi Labs Ltd., Solapur, Maharashtra, India.

Marketed formulation contains 200 µg of Beclomethasone dipropionate and 6µg of Formoterol fumarate dihydrate in Rotacap form (Fullform-200 Cipla Pvt. Ltd).

**Preparation of Standard stock solution**

Accurately weighed standard drug of BD (10mg) and FF (10mg) were transferred to a separate 100ml volumetric flask, dissolved in 10 ml MeOH by shaking manually for 5 min. The Volume was adjusted with the same up to mark to give final concentrations of BD (100µg/ml) and FF (100µg/ml).

**Preparation of Calibration curve (method 1)**

From the Standard solutions of BD (1, 2, 3, 4 and 5 ml) and standard solutions of FF (0.1, 0.2, 0.3, 0.4 and 0.5 ml)was pipette out in to a separate series of 10 ml volumetric flask. The volume was adjusted to the mark with MeOH and mixed.
The absorbances of the solutions were measured at 239.2 nm and 213.0 nm against taking MeOH as a blank. The absorptivity coefficients of each drug at both wavelengths were determined and substituted in their equation to obtain concentration of both drugs. The concentration of each compound in the mixture was calculated from the following simultaneous equations\(^{(12)}\),

\[
C_{BD} = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2} \quad 1.
\]

\[
C_{FF} = \frac{A_1ax_2 - A_2ax_2}{ax_2ay_1 - ax_1ay_2} \quad 2.
\]

Where, \( C_{BD} \) and \( C_{FF} \) are concentration of BD and FF respectively; \( A_1 \) and \( A_2 \) are absorbance of mixture at 239.2 nm and 213 nm respectively; \( ax_1 \) and \( ax_2 \) are absorptivity coefficient of BD at 239.2 nm and 213 nm respectively; \( ay_1 \) and \( ay_2 \) are absorptivity coefficient of FF at 239.2 nm and 213 nm respectively.

**Preparation of Calibration curve (method 2)**

From the Standard solutions of BD (1, 2, 3, 4 and 5 ml) and standard solutions of FF (0.1, 0.2, 0.3, 0.4 and 0.5 ml) was pipette out into a separate series of 10 ml volumetric flask. The volume was adjusted to the mark with MeOH and mixed.

The over line spectrum of BD and FF, one wavelength was selected for the estimation of both drugs, which is known as iso-absorptive point (at 218.3 nm) and one was \( \lambda_{max} \) of one drug. The dilutions of standard and sample solutions were prepared. The Absorptivity values were determined at 218.3 nm. The method employs Q values and the concentrations of drugs in sample solution were determined by using following formula\(^{(13)}\),

\[
C_X = \frac{(Q_M - Q_Y) \times A_1}{(Q_X - Q_Y) \times aX_1} \quad \text{AND} \quad C_Y = \frac{A_1}{aX_2 - C_X}
\]

Where, \( A_1 \) & \( A_2 \) are the absorbance of the mixture at 218.3 nm & 239.2 nm respectively; \( aX_1 \) and \( aY_1 \) are absorptivities of BD and FF respectively at 218.3 nm; \( aX_2 \) and \( aY_2 \) are absorptivities of BD and FF respectively at 239.2 nm; \( QM=A_2/A_1 \); \( QX= aX_2/ aX_1 \) and \( QY= aY_2/ aY_1 \).

**VALIDATION OF METHODS\(^{(13)}\)**

Proposed methods were validated in accordance with ICH guidelines Q2 (R1) for evaluation of various parameters; linearity, limit of detection, limit of quantification, precision and accuracy.
Linearity
Calibration curves were plotted over a concentration range of 10-50 µg/ml and 1-5 µg/ml for BD and FF respectively. The calibration curves were constructed by plotting absorbances Vs concentrations.

2.2 Method precision (repeatability)
The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of BD and FF (40 µg/ml and 1.2 µg/ml respectively) without changing the parameters for the simultaneous equation method.

2.3 Intermediate precision (reproducibility)
The intraday and interday precisions of the proposed method was determined by analyzing corresponding responses in triplicate on the same day and on 3 different days, different concentrations of standard solutions of BD (35, 40 and 45 µg/ml) and FF (1.0, 1.2 and 1.35 µg/ml). Results were reported in terms of %RSD.

2.4 LOD and LOQ
The limit of detection (LOD) and limit of quantification (LOQ) of the drug was derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline:

The LOD may be expressed as:

$$\text{LOD} = \frac{3.3 \sigma}{S}$$

Where, $\sigma =$ the standard deviation of the response

$S =$ the slope of the calibration curve

The LOQ may be expressed as:

$$\text{LOQ} = \frac{10 \sigma}{S}$$

Where, $\sigma =$ the standard deviation of the response

$S =$ the slope of the calibration curve
2.5 Accuracy (Recovery study)

The accuracy of the methods was determined by calculating recoveries of BD and FF by the standard addition method. Known amounts of standard solutions of BD and FF were added at 80, 100 and 120% levels to prequantified sample solutions of BD and FF (40 and 1.2 µg/ml respectively). The amounts of BD and FF were estimated by applying the obtained values to the simultaneous equation method.

2.6 Analysis of BRM and TIM in Pharmaceutical dosage form

In pharmaceutical dosage form, both drugs BD and FF in ratio of 33:1. The absorbance was measured at 239.2 nm and 213.0 nm (Simultaneous equation) and 218.3 and 239.2 nm (Q-ratio) for quantification of BD and FF, respectively. The amounts of BD and FF present in sample solutions were determined by fitting the response into the simultaneous equation and Q-ratio method for BD and FF.

RESULTS AND DISCUSSION

Simultaneous equation method

The standard solutions of BD and FF were prepared separately in MeOH. They were scanned in the wavelength range of 200-400 nm. Data were recorded at an interval of 1 nm. Maximum absorbance was obtained at 239.2 nm and 213.0 nm for BD and FF, respectively. Select these two analytical wavelengths for determination of BD and FF, respectively shown in figure II. These two wavelengths can be employed for the determination of BD and FF without any interference from their combined pharmaceutical dosage form.

Figure II Overlain spectra of BD and FF (10 µg/ml in MeOH)
Q-ratio method
The standard solutions of BD and FF were prepared separately in MeOH. They were scanned in the wavelength range of 200-400nm. Data were recorded at an interval of 1 nm. The overlaid spectrum of BD and FF, one wavelength was selected for the estimation of both drugs, which is known as iso absorptive point (at 218.3 nm) shown in figure III and one was \( \lambda_{\text{max}} \) of one drug. The dilutions of standard and sample solutions were prepared. The Absorptivity values were determined at 218.3 nm. The method employs Q values and the concentrations of drugs in sample solution were determined by using the formula.

\[
Q = \frac{A_{\text{test}}}{A_{\text{stand}}} \cdot \frac{C_{\text{stand}}}{C_{\text{test}}}
\]

Validation of the proposed method:
The proposed methods have been validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). The calibration curves were constructed for the proposed methods according to their respective concentration ranges and were found to be linear over the concentration range for BD and FF with acceptable regression coefficient as shown in Table I for two proposed methods.

Linearity
Linear correlation was obtained between absorbance versus concentrations of BD and FF in the ranges of 10-50\( \mu \)g/ml and 1-5\( \mu \)g/ml, respectively for both the methods. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression.
Table I: Regression analysis data for the proposed methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>239.2</td>
<td>213</td>
</tr>
<tr>
<td>BD</td>
<td>239.2</td>
<td>213</td>
</tr>
<tr>
<td>FF</td>
<td>218.3</td>
<td>239.2</td>
</tr>
<tr>
<td>Linearity (µg/ml)</td>
<td>10-50</td>
<td>10-50</td>
</tr>
<tr>
<td>BD</td>
<td>10-50</td>
<td>10-50</td>
</tr>
<tr>
<td>FF</td>
<td>10-50</td>
<td>10-50</td>
</tr>
<tr>
<td>Regression equation</td>
<td>0.026x +</td>
<td>0.026x +</td>
</tr>
<tr>
<td>(Y= mx + c)</td>
<td>0.0027</td>
<td>0.0027</td>
</tr>
<tr>
<td>Slope= m</td>
<td>0.042x</td>
<td>0.042x</td>
</tr>
<tr>
<td>Intercept=c</td>
<td>0.133x</td>
<td>0.133x</td>
</tr>
<tr>
<td>Correlation Coefficient ($r^2$)</td>
<td>0.9986</td>
<td>0.9986</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.022</td>
<td>0.063</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.0676</td>
<td>0.192</td>
</tr>
</tbody>
</table>

* Average of five determination, LOD=Limit of detection, LOQ=Limit of quantification

Table I give LOD and LOQ value of BD and FF. The precision data for the both methods is given in Table II. Recovery study performed by spiking the standard solution at 80,100 and 120%, less than 2 % RSD indicate the recovery study was acceptable (Table-III).

Table II: Precision data for BD and FF by proposed methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method I</th>
<th>Method II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability (%)</td>
<td>0.610</td>
<td>1.058</td>
</tr>
<tr>
<td>BD</td>
<td>0.600</td>
<td>1.670</td>
</tr>
<tr>
<td>FF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precision (%)</td>
<td>0.216-0.339</td>
<td>1.21-1.82</td>
</tr>
<tr>
<td>Interday (n=3)</td>
<td>0.249-0.263</td>
<td>0.599-1.290</td>
</tr>
<tr>
<td>Intraday (n=3)</td>
<td>0.216-0.339</td>
<td>0.792-1.476</td>
</tr>
</tbody>
</table>

* Number of determination

Table III: Recovery data for BD and FF by Proposed methods

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount present in mixture (µg/ml)</th>
<th>Amount Added (%)</th>
<th>% Recovery ± %RSD</th>
<th>Amount present in mixture (µg/ml)</th>
<th>Amount Added (%)</th>
<th>% Recovery ± %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD</td>
<td>40</td>
<td>80%</td>
<td>99.49 ± 0.522</td>
<td>40</td>
<td>80%</td>
<td>98.85 ± 0.231</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>100%</td>
<td>99.90 ± 0.577</td>
<td>40</td>
<td>100%</td>
<td>99.16 ± 0.148</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>120%</td>
<td>100.31 ± 0.503</td>
<td>40</td>
<td>120%</td>
<td>99.41 ± 0.221</td>
</tr>
<tr>
<td>FF</td>
<td>1.2</td>
<td>80%</td>
<td>98.43 ± 0.231</td>
<td>1.2</td>
<td>80%</td>
<td>98.64 ± 0.239</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>100%</td>
<td>99.16 ± 0.148</td>
<td>1.2</td>
<td>100%</td>
<td>98.73 ± 0.314</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>120%</td>
<td>99.41 ± 0.221</td>
<td>1.2</td>
<td>120%</td>
<td>99.37 ± 0.791</td>
</tr>
</tbody>
</table>

* Average of three determination
Assay of pharmaceutical dosage form (Rotacap) by proposed methods
The proposed validated methods were successfully applied for the determination of BD and FF in their combined dosage forms. Results are given in table IV. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine analysis of Beclomethasone dipropionate and Formoterol fumarate in pharmaceutical dosage form.

Table IV: Assay for the Pharmaceutical dosage (Rotacap) form for proposed methods

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Amount taken</th>
<th>Amount found</th>
<th>% label claim</th>
<th>Amount found</th>
<th>% label claim</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BD</td>
<td>FF</td>
<td>BD</td>
<td>FF</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>1.2</td>
<td>39.44</td>
<td>1.19</td>
<td>98.60</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>1.2</td>
<td>39.32</td>
<td>1.2</td>
<td>98.30</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>1.2</td>
<td>39.41</td>
<td>1.19</td>
<td>98.52</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>1.2</td>
<td>39.13</td>
<td>1.18</td>
<td>97.82</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>1.2</td>
<td>39.56</td>
<td>1.21</td>
<td>99.90</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>1.2</td>
<td>39.85</td>
<td>1.22</td>
<td>99.62</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>39.45</td>
<td>1.21</td>
<td>98.79</td>
<td>100.1</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.242</td>
<td>0.019</td>
<td>0.801</td>
<td>0.740</td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td>0.613</td>
<td>1.57</td>
<td>0.810</td>
<td>0.738</td>
</tr>
</tbody>
</table>

CONCLUSION
Based on the results, it can be concluded that the method has linear response in the range of 10 – 50 and 1 – 5 µg/ml for Beclomethasone dipropionate and Formoterol fumarate. Less than 2 %RSD indicate that UV-spectroscopic methods are accurate and precise.

The result of the analysis rotacap by the proposed method is highly reproducible and reliable and is in good agreement with prepared ratio of the drugs. The additive usually present in the rotacap of the assayed samples did not interfere with determination of Beclomethasone dipropionate and Formoterol fumarate.

The method can be used for the routine analysis of for Beclomethasone dipropionate and Formoterol fumarate in combined rotacap dosage form.

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