DEVELOPMENT OF FIRST DERIVATIVE SPECTROSCOPY METHOD FOR ESTIMATION OF BUDESONIDE AND FORMETEROL IN COMBINED DOSAGE FORM

Neema M. Gurjar*, A. K. Seth, Aarti Zanwar, Jitendra Patel, Gajanan Deshmukh

Department of Pharmacy Sumandeep Vidyapeeth Vadodara (Gujarat)-391760

ABSTRACT

Sensitive, precise, accurate and simple UV spectrophotometric methods have been developed for simultaneous estimation of Budesonide (BUD) and Formeterol (FRL) in dry powder inhaler dosage form. The method employed first order derivative spectroscopy in which derivative amplitudes were measured at selected wavelengths (269.4 nm for BUD and 284.6 nm for FRL). Linearity was observed in the concentration range of 2-26 µg/ml for BUD and 10-100 mcg/ml for FRL. The method was found to be accurate (mean percentage accuracy 100.07 ) for budesonide and (mean percentage accuracy 100.06) for formeterol and with %RSD 0.41 and 0.96 for BUD and FRL (API) respectively (for intraday) and % RSD 0.42 and 0.84 for BUD and FRL (combined dosage form) respectively (for intraday) and 0.78 and 0.48 for BUD and FRL (API) respectively (for inter-day) and 0.61 and 0.45 for BUD and FRL (combined dosage form) respectively (for interday). The proposed methods have been applied successfully to the analysis of cited drugs in pharmaceutical formulations. The methods were validated as per ICH guidelines.

Keywords: Budesonide, Formeterol, first order derivative spectroscopy.

INTRODUCTION

Budesonide is a synthetic corticosteroid indicated for the treatment of mild to moderate Asthma as well as for mild to moderate active Crohn's disease involving the ileum and/or the ascending colon. The analog possesses greater potency than the natural hormone. The chemical name is 16, 17-(butylidenebis (oxy))-11, 21-dihydroxy-, (11-β, 16-α)-pregna-1, 4-diene-3, 20-dione.\[1\]

Formoterol is a long-acting selective beta2-adrenergic receptor agonist (beta2-agonist). Inhaled formoterol fumarate acts locally in the lung as a bronchodilator. The chemical name is N-[2-hydroxy-5-[1-hydroxy-2-[1-(4-methoxyphenyl)propan-2-ylamino]ethyl] phenyl]formamide.\[2\]

Some analytical methods for the quantitative determination of Budesonide and Formeterol in pharmaceutical formulations are described in the literature like high
performance liquid chromatography assay method\cite{3}, method available for spray drying technology\cite{4-6}, spectrophotometric determination in combined dosage form\cite{7}, pharmacokinetics\cite{8-10}.

No simultaneous derivative spectrophotometric method has been reported for both the components in a combined dosage forms. The aim of this paper was to develop the spectroscopy method for estimation of BUDESONIDE & FORMOTEROL in combined dosage form.

**MATERIAL & METHOD**

1. PREPARATION OF CALIBRATION CURVE OF BUD IN METHANOL: CHLOROFORM (9:1)

   The spectroscopic determination of BUD is based on the 1\textsuperscript{st} derivative UV spectra of BUD giving maxima at 269.4 nm in methanol: chloroform (9:1) mixture.

   **Reagents**

   Freshly prepared methanol: chloroform (9:1) mixture.

   Stock solution of BUD: 1 mg/ml solution of BUD was prepared in methanol: Chloroform (9:1) mixture.

   **Method**

   Appropriate aliquots of the stock solution of BUD were transferred to 10ml volumetric flasks and were diluted up to the mark with methanol: chloroform (9:1) mixture. The absorption maxima ($\lambda_{\text{max}}$) were determined by scanning 10 $\mu$g/ml solution against reagent blank on UV-Visible Spectrophotometer (UV-1700, Shimadzu) and taking its derivative. The absorption of all the prepared solutions was then measured at the absorbance maxima, 269.4 nm against the reagent blank in the derivative plot. The readings were recorded in triplicate. Mean value ($n=3$) along with the standard error of mean (SEM) are recorded in Table1. The regressed values of absorption were plotted graphically against the concentrations, as shown in Figure1.

   Stability of the solutions of BUD in methanol: chloroform (9:1) mixture used for preparing the calibration curve, was ascertained by observing the changes in the absorbance of the solution at the analytical wavelength, over a period of 48hr at room temperature.
2. PREPARATION OF CALIBRATION CURVE OF FRL IN METHANOL: CHLOROFORM (9:1) MIXTURE

The spectroscopic determination of FRL is based on the UV spectra of FRL giving absorption maxima at 284.6 nm in methanol: chloroform (9:1) mixture.

Reagents

- Freshly prepared methanol: chloroform (9:1) mixture.
- Stock solution of FRL: 1 mg/ml solution of FRL was prepared in methanol: chloroform (9:1) mixture.

Method

Appropriate aliquots of the stock solution of FRL were transferred to 10ml volumetric flasks and were diluted up to the mark with methanol: chloroform (9:1) mixture. The absorption maxima (λ_{max}) was determined by scanning 30 µg/ml solution against reagent blank on UV-Visible Spectrophotometer (UV-1601, Shimadzu). The absorption of all the prepared solutions were then measured at the absorbance maxima, 284.6 nm against the reagent blank. The readings were recorded in triplicate. Mean value (n=3) along with the standard error of mean (SEM) are recorded in Table 2. The regressed values of absorption were plotted graphically against the concentrations, as shown in Figure 2.

Stability of the solutions of FRL in methanol: chloroform (9:1) mixture used for preparing the calibration curve, was ascertained by observing the changes in the absorbance of the solution at the analytical wavelength, over a period of 48hr at room temperature.

3. ANALYTICAL METHOD FOR ESTIMATION OF BUD IN THEIR COMBINED DOSAGE FORM.

For estimation of BUD in combined dosage form, zero-crossing first derivative UV spectroscopic method was used which was similar to method described earlier in 1. Absorption wave length of 267.4 nm was taken under consideration where FRL gave no interference or zero absorbance. BUD showed good linearity in presence and absence of FRL at that wavelength. So that wavelength can be used for estimation of BUD in combined dosage form.
4. ANALYTICAL METHOD FOR ESTIMATION OF FRL IN THEIR COMBINED DOSAGE FORM.

For the estimation of FRL in combined dosage form, zero order UV spectroscopic methods were used which were similar to methods described in 2. Absorption wavelength of 295.2 nm was taken where BUD gave no interference or zero absorbance. FRL showed good linearity in presence and absence of BUD at that wave length. So that wave length can be used for estimation of FRL in combined dosage form and in diffusion medium.

RESULT & DISCUSSION:

The proposed methods for simultaneous estimation of BUD and FRL in combined dosage form were found to be accurate, simple and rapid which can be well understood from validation data as given in Table 9-11. The % R.S.D. as indicated in Table 11 was found to be less than 2, which indicates the validity of methods. Linearity was observed by linear regression equation method for BUD and FRL in different concentration range. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity.

TABLE 1: CALIBRATION CURVE OF BUD IN METHANOL: CHLOROFORM (9:1) MIXTURE

<table>
<thead>
<tr>
<th>Conc.(µg/ml)</th>
<th>Absorbance (+SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-0.0223 (0.001528)</td>
</tr>
<tr>
<td>5</td>
<td>-0.0573 (0.001528)</td>
</tr>
<tr>
<td>8</td>
<td>-0.087 (0.003)</td>
</tr>
<tr>
<td>11</td>
<td>-0.126 (0.003606)</td>
</tr>
<tr>
<td>14</td>
<td>-0.1503 (0.002082)</td>
</tr>
<tr>
<td>17</td>
<td>-0.1823 (0.002082)</td>
</tr>
<tr>
<td>20</td>
<td>-0.2193 (0.002082)</td>
</tr>
<tr>
<td>23</td>
<td>-0.2473 (0.003215)</td>
</tr>
<tr>
<td>26</td>
<td>-0.2723 (0.002517)</td>
</tr>
</tbody>
</table>
TABLE 2: OPTICAL CHARACTERISTICS OF BUD IN METHANOL: CHLOROFORM (9:1) MIXTURE.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ&lt;sub&gt;max&lt;/sub&gt;</td>
<td>269.4 nm</td>
</tr>
<tr>
<td>Solvent</td>
<td>MeOH:CHCL&lt;sub&gt;3&lt;/sub&gt; (9:1)</td>
</tr>
<tr>
<td>Range</td>
<td>2-26 µg/ml</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = -0.0107x - 0.0024</td>
</tr>
<tr>
<td>Regression Coefficient (R&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.9990</td>
</tr>
</tbody>
</table>

TABLE 3: CALIBRATION CURVE OF FRL IN METHANOL: CHLOROFORM (9:1) MIXTURE FOR THE ESTIMATION OF FRL (N=3)

<table>
<thead>
<tr>
<th>Conc. (mcg/ml)</th>
<th>Absorbance (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.1046 (0.002082)</td>
</tr>
<tr>
<td>20</td>
<td>0.2106 (0.001155)</td>
</tr>
<tr>
<td>30</td>
<td>0.3163 (0.001528)</td>
</tr>
<tr>
<td>40</td>
<td>0.4306 (0.001528)</td>
</tr>
<tr>
<td>50</td>
<td>0.5413 (0.001155)</td>
</tr>
<tr>
<td>60</td>
<td>0.622 (0.001)</td>
</tr>
<tr>
<td>70</td>
<td>0.7336 (0.001528)</td>
</tr>
<tr>
<td>80</td>
<td>0.865 (0.002)</td>
</tr>
<tr>
<td>90</td>
<td>0.9686 (0.000577)</td>
</tr>
<tr>
<td>100</td>
<td>1.0826 (0.002517)</td>
</tr>
</tbody>
</table>
TABLE 4: OPTICAL CHARACTERISTICS OF FRL IN METHANOL: CHLOROFORM (9:1) MIXTURE.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$</td>
<td>284.6nm</td>
</tr>
<tr>
<td>Solvent</td>
<td>MeOH:CHCL$_3$ (9:1)</td>
</tr>
<tr>
<td>Range</td>
<td>10-100 mcg/ml</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$y=0.0108x-0.0071$</td>
</tr>
<tr>
<td>Regression Coefficient ($R^2$)</td>
<td>0.9991</td>
</tr>
</tbody>
</table>

TABLE 5: CALIBRATION CURVE OF BUD IN METHANOL: CHLOROFORM (9:1) FOR THE ESTIMATION OF BUD IN COMBINED DOSAGE FORM. (N=3)

<table>
<thead>
<tr>
<th>Conc.(µg/ml)</th>
<th>Absorbance (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-0.021 (0.02)</td>
</tr>
<tr>
<td>5</td>
<td>-0.055 (0.023)</td>
</tr>
<tr>
<td>7</td>
<td>-0.087 (0.016)</td>
</tr>
<tr>
<td>11</td>
<td>-0.122 (0.019)</td>
</tr>
<tr>
<td>14</td>
<td>-0.15 (0.025)</td>
</tr>
<tr>
<td>17</td>
<td>-0.181 (0.019)</td>
</tr>
<tr>
<td>20</td>
<td>-0.215 (0.021)</td>
</tr>
<tr>
<td>23</td>
<td>-0.245 (0.018)</td>
</tr>
<tr>
<td>26</td>
<td>-0.27 (0.023)</td>
</tr>
</tbody>
</table>
Figure 3
Calibration curve of BUD in methanol: chloroform (9:1) mixture.

Figure 4
Zero crossing First order spectra of BUD and FRL

TABLE 6: OPTICAL CHARACTERISTICS OF BUD IN METHANOL:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave length</td>
<td>267.4 nm</td>
</tr>
<tr>
<td>Solvent</td>
<td>MeOH:CHCl (9:1)</td>
</tr>
<tr>
<td>Range</td>
<td>2-26 µg/ml</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = -0.0103x - 0.0062</td>
</tr>
<tr>
<td>Regression Coefficient (R²)</td>
<td>0.9975</td>
</tr>
</tbody>
</table>
### TABLE 7: CALIBRATION CURVE OF FRL IN METHANOL: CHLOROFORM (9:1) FOR THE ESTIMATION OF FRL IN COMBINED DOSAGE FORM. (N=3)

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.084 (0.008)</td>
</tr>
<tr>
<td>20</td>
<td>0.175 (0.014)</td>
</tr>
<tr>
<td>30</td>
<td>0.262 (0.014)</td>
</tr>
<tr>
<td>40</td>
<td>0.355 (0.018)</td>
</tr>
<tr>
<td>50</td>
<td>0.448 (0.015)</td>
</tr>
<tr>
<td>60</td>
<td>0.517 (0.021)</td>
</tr>
<tr>
<td>70</td>
<td>0.607 (0.016)</td>
</tr>
<tr>
<td>80</td>
<td>0.714 (0.015)</td>
</tr>
<tr>
<td>90</td>
<td>0.805 (0.013)</td>
</tr>
<tr>
<td>100</td>
<td>0.899 (0.013)</td>
</tr>
</tbody>
</table>

**Figure 5**
Calibration curve of FRL in methanol: chloroform (9:1) mixture.

**Figure 6**
First order spectra of FRL

IC Value – 4.01
TABLE 8: OPTICAL CHARACTERISTICS OF FRL IN METHANOL:
CHLOROFORM (9:1) MIXTURE.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave length</td>
<td>295.2 nm</td>
</tr>
<tr>
<td>Solvent</td>
<td>MeOH:CHCL$_3$ (9:1)</td>
</tr>
<tr>
<td>Range</td>
<td>10-100 μg/ml</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 0.009x - 0.0077</td>
</tr>
<tr>
<td>Regression Coefficient (R$^2$)</td>
<td>0.9991</td>
</tr>
</tbody>
</table>

TABLE 9: ACCURACY FOR BUDESONIDE

<p>| For Budesonide (API and in combined dosage forms) |</p>
<table>
<thead>
<tr>
<th>Theoretical Concentration (μg/ml)</th>
<th>Calculated Concentration (μg/ml)</th>
<th>% Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.05</td>
<td>100.19</td>
</tr>
<tr>
<td>6</td>
<td>16.08</td>
<td>100.26</td>
</tr>
<tr>
<td>26</td>
<td>25.91</td>
<td>99.78</td>
</tr>
</tbody>
</table>

TABLE 10: ACCURACY FOR FORMETEROL

<p>| For Formeterol (API and in combined dosage forms) |</p>
<table>
<thead>
<tr>
<th>Theoretical Concentration (μg/ml)</th>
<th>Calculated Concentration (μg/ml)</th>
<th>% Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10.06</td>
<td>100.20</td>
</tr>
<tr>
<td>50</td>
<td>50.17</td>
<td>100.35</td>
</tr>
<tr>
<td>100</td>
<td>99.78</td>
<td>99.65</td>
</tr>
</tbody>
</table>

TABLE 11: INTRADAY AND INTERDAY PRECISION

<table>
<thead>
<tr>
<th>Drug</th>
<th>Intraday precision (API)</th>
<th>Interday precision (API)</th>
<th>Intraday Precision (FORMULATION)</th>
<th>Interday precision (FORMULATION)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>%RSD</td>
<td>SD</td>
<td>%RSD</td>
</tr>
<tr>
<td>BUD</td>
<td>0.40</td>
<td>0.41</td>
<td>0.75</td>
<td>0.78</td>
</tr>
<tr>
<td>FRL</td>
<td>0.94</td>
<td>0.96</td>
<td>0.45</td>
<td>0.48</td>
</tr>
</tbody>
</table>

CONCLUSION
From above mentioned experimentation it was found that analytical methods for estimation of both BUD and FRL in their individual and combined dosage form showed good linearity, accuracy and precision. So these methods can be used for further study.
ACKNOWLEDGEMENT

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For Correspondence:
Neema .M. Gurjar
Department of Pharmacy
Sumandeep Vidyapeeth Vadodara
(Gujarat)-391760
Email: neema2287@yahoo.com