Development and Validation of First-Order Derivative Spectrophotometry for Simultaneous Determination of Indacaterol Maleate and Glycopyrronium Bromide in Pharmaceutical Dosage Form

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ABSTRACT

A simple, accurate, reliable and reproducible first order derivative method was developed for the simultaneous determination of Indacaterol maleate (IND) and Glycopyrronium bromide (GLY) in pharmaceutical formulation. Methanol was selected as a solvent for estimation of IND and GLY. The linearity was carried out by using the concentration range 11-66 μg/ml for IND (250 nm ZCP of GLY) and 5-30 μg/ml for GLY (244 nm ZCP of IND) respectively. The correlation coefficient of IND and GLY was found to be 0.999 and 0.999 respectively. At zero crossing point (ZCP) of IND (244 nm) GLY showed a measurable derivative absorbance, whereas at zero crossing point (ZCP) of GLY (250nm) IND showed a appreciable derivative absorbance value. Precision study showed that %RSD was within range of acceptable limits (< 2%). The % recovery for IND and TAZO was found to be 99.16-100.20% and 99.68-100.33% respectively. The percentage assay was found to be 99.56% and 100.20% for IND and GLY. The result of analysis has been validated as per ICH guideline. This method has applied successfully for determination of IND and GLY in its pharmaceutical formulation.

Keywords: Indacaterol maleate, Glycopyrronium bromide, UV Spectrophotometry, First order derivative Spectrophotometry

INTRODUCTION

Indacaterol Maleate (IND) is chemically known as 2-[(5,6-Diethyl-2,3-dihydro-1H-inden-2-yl)amino]-1-hydroxyethyl]-8-hydroxyquinolin-2(1H)-one (Figure 1).

Figure1. Chemical Structure of Indacaterol Maleate

IND stimulate adrenergic β₂ receptors in the smooth muscle of the airways. IND prevents airway spasms caused by chronic obstructive pulmonary disease (COPD). This drug is indicated for the treatment of COPD. This causes relaxation of the muscle, thereby increasing the diameter of the airways, which becomes constricted in asthma and COPD [1,2,3]. Glycopyrronium bromide (GLY) is a chemically 1, 1 -dimethylpyrrolidin-1-iium-3-yl 2-cyclopentyl-2-hydroxy phenyl acetate bromide (Figure 2).

Figure2. Chemical Structure of Glycopyrronium bromide
GLY is a synthetic anticholinergic agent with a quaternary ammonium structure. It reduces secretions in the mouth, throat, airways, and stomach before surgery [4,5]. It was used along with other medicines to treat peptic ulcers. The combination of Indacaterol Maleate and Glycopyrronium Bromide mainly used as β₂ adrenoceptor agonist and anticholinergic agent with a quaternary ammonium structure and widely used in COPD [6].

The deep literature review revealed that various analytical methods like spectrophotometric, HPLC, HPTLC, stability indicating HPLC, LC-MS and other methods are reported for estimation of IND and GLY individually and in combined with other dosage form and in biological fluids but none of the analytical method is reported for simultaneous estimation of both the drugs in combined pharmaceutical dosage form [7-13]. Therefore, there is a challenge to develop UV spectrophotometric method for the simultaneous estimation of Indacaterol maleate and Glycopyrronium bromide. The present study was involved in a research effort aimed at developing and validating a simple, specific, accurate, economical, and precise First order derivative UV spectrophotometric method for the simultaneous estimation of two drugs in pharmaceutical dosage form.

MATERIALS AND METHODS

Reagents and Chemicals

Analytically pure IND and GLY were used. IND was procured from Cipla Pvt Ltd., Mumbai, (Maharashtra, India). And GLY was procured from Vav Life Sciences Pvt Ltd. Mumbai (Maharashtra, India). Marketed formulation containing 110 mcg of IND and 50 mcg of GLY was purchased from Local Market. Methanol was purchased from Merck Specialities Pvt Ltd., Mumbai, India.

Instrument

A shimadzu UV/Vis 1800 double beam spectrophotometer is used with wavelength accuracy (±0.5 nm), 1 cm matched quartz cells and UV probe 2.33 software was used for all the spectral measurements. Calibrated analytical Balance Denver SI234, Germany, was used for weighing purpose.

Preparation of Standard Stock Solution

The stock solution having 1000μg/ml concentration of IND and GLY were prepared separately by dissolving accurately weighed 100 mg of both drugs in 100 ml methanol. The working standard stock solution of 100μg/ml concentration of IND and GLY were prepared separately by diluting 10 ml of standard stock solution of respective drug with methanol in 100ml volumetric flask up to the mark.

Selection of Analytical Wavelength

The solution having 10 μg/ml concentrations of IND and GLY were prepared separately by diluting 1 ml of standard working stock solution of respective drug with methanol in 10 ml volumetric flask up to the mark. These solutions were scanned in UV range 200-400 nm. The λmax of IND and GLY were found to be 258 nm and 222 nm respectively in normal UV spectra shown in figure 3. All zero order spectrums (D₀) were converted to first derivative spectrum (D₁) using delta lambda 4 and scaling factor 2.0. It was observed that IND shows ZCP at 244nm and GLY shows a measurable dA/dλ, whereas at ZCP of GLY (250nm), IND showed a measurable dA/dλ. Hence the wavelengths 250 nm and 244 nm were selected as analytical wavelengths for determination of IND and GLY first order derivative method, respectively. Figure 3 and 4 shown the zero order and first order UV spectra of IND and GLY.

Figure 3. Overlain zero order UV spectra of IND (10 μg/ml) and GLY (10 μg/ml)
Method Validation

The proposed method was validated according to the ICH Guideline Q2 (R1) [14]. The method has been validated in terms of Linearity, Precision, Accuracy, Limit of detection (LOD) and Limit of quantification (LOQ).

Calibration Curve (Linearity) and Range

Appropriate volume of aliquot from IND and GLY standard stock solution was transferred to volumetric flask of 10 ml capacity separately. The volume was adjusted to the mark with Methanol to give a solution containing 11-66 μg/ml IND and 5-30 μg/ml GLY individually. All D₁ spectrums were recorded using above spectrophotometric condition. D₁ absorbances at 250 nm and 244 nm were recorded for IND and GLY respectively. Calibration curve were constructed by plotting average absorbance versus concentration for both drugs.

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the known amount of marketed formulation at 3 different concentration levels 50, 100, and 150% taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured.

Precision

The intraday and interday precision study was carried out by estimating different concentrations of (22, 33, 44 μg/ml) IND and (10,15,20 μg/ml) GLY, three times on the same day and on three different days while repeatability was carried out by estimating test concentration of (22 μg/ml) IND and (10 μg/ml) GLY, six times. The results are reported in terms of % RSD.

Detection Limit and Quantitation Limit

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 σ/S and 10 σ/S criterions, respectively;

Where; σ is the standard deviation of y-intercepts of regression lines S is the slope of the calibration curve

Assay of Pharmaceutical Formulation

Commercially available marketed formulation (Capsule) containing both IND and GLY (Lupitum) were used for the study. Twenty Capsules (each containing 110 mcg IND and 50 mcg GLY) were accurately weighed and finely powdered. A quantity of powder equivalent to 11 mg of IND and 5 mg of GLY was weighed and transferred to 100 ml volumetric flask. This stock solution was prepared in Methanol, sonicated for 15 min, the volume was adjusted up to the mark with same solvent. Then solution was filtered through whatman filter paper No. 41. This stock solution contains IND 110 μg/ml and GLY 50 μg/ml. From this stock solution, 2 ml of solution was taken and diluted up to 10 ml with methanol which contains 22 μg/ml of IND and 10 μg/ml of GLY. All the determinations were carried out in triplicate. The absorbance of the prepared solutions was measured at ZCP of IND and ZCP of GLY and then the concentration of both the drug was calculated using calibration curve equation. The amount of the drug found in dosage form was shown in Table 5.
RESULTS AND DISCUSSION

The first order derivative method is useful for routine analysis of IND and GLY in Pharmaceutical formulation. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The zero order and first order spectra for IND and GLY were recorded and shown in the figure 3 and 4. The zero crossing point was found at the wavelength of 244 nm and 250 nm for IND and GLY respectively.

Linearity and Range

The Beer- Lambert’s concentration range was found to be 11-66 μg/ml for IND and 5-30 μg/ml for GLY at 250 nm and 244 nm respectively. Straight line equations were obtained from mean of five sets and the calibration curves was shown in figure 5 and 6. The correlation coefficient was found to be 0.999 for IND and 0.999 for GLY for proposed method.

![Figure 5. Linearity graph for first order derivative spectra of IND (11-66μg/ml)](image)

![Figure 6. Linearity graph for first order derivative spectra of GLY (5-30μg/ml)](image)

Precision

Precision was determined by studying repeatability, intraday and interday precision. The standard deviation and Relative standard deviation (%RSD) were calculated for both the drugs. The % RSD for proposed method were found to be not more than 2.0% which indicates good intermediate precision which was shown in Table 1 and 2.
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Table 1. Intraday precision data of IND and GLY

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Absorbance ± SD (n = 3)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND</td>
<td>GLY</td>
<td>IND</td>
</tr>
<tr>
<td>22</td>
<td>10</td>
<td>0.0477 ± 0.00010</td>
</tr>
<tr>
<td>33</td>
<td>15</td>
<td>0.0574 ± 0.00051</td>
</tr>
<tr>
<td>44</td>
<td>20</td>
<td>0.0641 ± 0.00015</td>
</tr>
</tbody>
</table>

Table 2. Interday precision data of IND and GLY

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Absorbance ± SD (n = 3)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND</td>
<td>GLY</td>
<td>IND</td>
</tr>
<tr>
<td>22</td>
<td>10</td>
<td>0.0476 ± 0.0007</td>
</tr>
<tr>
<td>33</td>
<td>15</td>
<td>0.0573 ± 0.0005</td>
</tr>
<tr>
<td>44</td>
<td>20</td>
<td>0.0641 ± 0.0001</td>
</tr>
</tbody>
</table>

LOD and LOQ

The proposed method was evaluated statistically. The method was successfully applied to capsule formulation. The values of LOD and LOQ were 2.88 µg/ml and 5.15 µg/ml for IND and 0.70 µg/ml and 2.14 µg/ml for GLY respectively. The summary of validation parameter was shown in Table 3.

Table 3. Summary of Validation Parameter

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IND</th>
<th>GLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (µg/ml)</td>
<td>11-66</td>
<td>5-30</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y=0.0007x+0.0353</td>
<td>Y=0.0016x+0.0021</td>
</tr>
<tr>
<td>slope</td>
<td>0.0007</td>
<td>0.0016</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0353</td>
<td>0.0021</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9990</td>
<td>0.9990</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>2.88</td>
<td>0.70</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>5.15</td>
<td>2.14</td>
</tr>
<tr>
<td>Repeatability (n=6) % RSD</td>
<td>0.47</td>
<td>1.78 %</td>
</tr>
<tr>
<td>Intraday (n=3) % RSD</td>
<td>1.19–0.81 %</td>
<td>0.28–1.40 %</td>
</tr>
<tr>
<td>Interday(n=3) % RSD</td>
<td>1.41–1.442%</td>
<td>0.30–0.82 %</td>
</tr>
</tbody>
</table>

Accuracy

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of IND and GLY to reanalyzed respective solutions of commercial Dosage form. The accuracy of IND and GLY was carried out as percent recovery was shown in Table 4 and average recovery was found in the range of 99.16% - 100.20 % for IND and 99.68 % - 100.33 % for GLY.

Table 4. Results of Recovery Studies

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Conc. of drug</th>
<th>Recovery Level present</th>
<th>Amount added (µg/ml)</th>
<th>Total amount of drug (µg/ml)</th>
<th>% Recovery ± SD (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND</td>
<td>22 µg/ml</td>
<td>50 %</td>
<td>11</td>
<td>33.06</td>
<td>100.20 ± 1.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 %</td>
<td>22</td>
<td>43.89</td>
<td>99.76 ± 0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 %</td>
<td>33</td>
<td>54.53</td>
<td>99.16 ± 1.17</td>
</tr>
<tr>
<td>GLY</td>
<td>10 µg/ml</td>
<td>50 %</td>
<td>5</td>
<td>14.95</td>
<td>99.68 ± 1.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 %</td>
<td>10</td>
<td>20.06</td>
<td>100.33 ± 1.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 %</td>
<td>15</td>
<td>25.03</td>
<td>100.13 ± 1.66</td>
</tr>
</tbody>
</table>

Analysis of the Marketed Formulation

The method was successfully applied to capsule formulation. The results are shown in Table 5. The drug content was found to be 99.56% and 100.20% for IND and GLY respectively. It may therefore be inferred that degradation of IND and GLY had not occurred in the marketed formulations that were analyzed by this method. This method can be used for routine analysis of IND and GLY in pharmaceutical dosage form.
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Table 5. Results of Recovery Studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label Claim (μg/ml)</th>
<th>Amount found (μg/ml)</th>
<th>% Drug found ± SD (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND</td>
<td>110</td>
<td>110.71</td>
<td>100.14 ± 0.729</td>
</tr>
<tr>
<td>GLY</td>
<td>50</td>
<td>50.31</td>
<td>100.405 ± 0.188</td>
</tr>
</tbody>
</table>

CONCLUSION

First order derivative method was developed for simultaneous estimation of IND and GLY in their combined formulation without prior separation. Spectra of IND were overlapped by GLY and derivatization was used as a powerful tool for simultaneous determination. Method was found to be accurate, sensitive and precise as can be reflected from validation data. Developed methods were successfully applied for estimation of IND and GLY in pharmaceutical formulation.

ACKNOWLEDGEMENT

The authors express their sincere thanks to, Cipla Pvt. Ltds., Mumbai and Vav Life Sciences Pvt Ltd., Mumbai, India for supplying the gift samples of Indacaterol maleate and Glycopyrronium bromide, respectively. Authors also extend their thanks to the management, Pioneer Pharmacy Degree College, Vadodara for providing the facilities to carry out the present research work.

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novel analytical method and validation research programs.

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