NOVEL UV-SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF OMEPRAZOLE AND DICLOFENAC IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

The aim of present experiment was to develop validated UV spectroscopic method for simultaneous estimation of Omeprazole and Diclofenac in bulk and pharmaceutical formulation. Area under curve (Method 1), simultaneous equation (Method 2) and absorbance ratio (Method 3) were developed for the determination of Omeprazole and Diclofenac in their combined capsule formulation without prior separation. The solutions of standard and sample were prepared in methanol for all the methods. Quantitative determination of the drugs was performed at the wavelength ranges of 291-311 nm and 271-291 nm (method 1), at 301 and 281 nm (method 2) and 301 nm and 295 nm (method 3) for Omeprazole and Diclofenac respectively. The Proposed methods were evaluated for the different validation parameters like precision, reproducibility, linearity and accuracy as per ICH guidelines. Linearity was observed in the range of 5-25 μg/mL for Diclofenac and 1-5 μg/mL for Omeprazole with correlation coefficient of 0.998 and 0.999 for Omeprazole and Diclofenac respectively. These methods are simple, precise, sensitive and applicable for the simultaneous determination of these drugs in pure powder and formulation.

Keywords: Absorbance ratio method, Area Under Curve Technique, Diclofenac, Omeprazole, simultaneous equation, UV Spectroscopy.

INTRODUCTION

Omeprazole (OME) is 5- methoxy -2-(4-methoxy-3 - 5-dimethyl – 2 – pyridinyl methyl sulfanyl)-3H-benzimidazole (Figure-1) is used as an anti ulcerative (proton pump inhibitor). Omeprazole is a racemate (both R and S forms), in acidic condition of the stomach both (R and S forms) are converted to achiral products which reacts with the cystine group in H+/K+ ATPase thus destroying the ability of the parietal cells to produce Gastric acid. Diclofenac Sodium (DIC) is chemically sodium 2-[(2, 6-dichlorophenyl)-amino]phenyl acetate (Figure-2) is used in the treatment of signs and symptoms of osteoarthritis and rheumatoid arthritis. It acts by inhibition of both leukocyte migration and the enzyme cyclo-oxygenase (COX-1 and COX-2), leading to the peripheral inhibition of prostaglandin synthesis. As prostaglandins sensitize pain receptors, inhibition of their synthesis is responsible for the analgesic effects of Diclofenac. The Antipyretic effects may be due to its action on the hypothalamus, resulting in peripheral dilation, increased blood flow and subsequent heat dissipation. Literature survey revealed several analytical methods for estimation of DIC and OME in bulk, pharmaceutical formulation or in biological fluids, in isolation or in combination with other drugs like Nimesulide, Paracetamol, Thiocolchicoside, Rabeprazole, Misoprostol, Eperisone Hydrochloride, Ondanstron, Drotaverine Hydrochloride and Domperidone. The analytical techniques such as HPLC, Spectrophotometry and HPTLC were used for these determinations. However, to the best of our knowledge no method has been reported for the simultaneous estimation of OME and DIC using UV spectrophotometry. Hence the objective of the present paper was to develop the first UV spectrophotometric methods which are simple, rapid, precise, accurate and economical and can be used for the simultaneous estimation of the drugs OME and DIC in the combined dosage form without prior separation and to validate the developed method in accordance with ICH guidline.

MATERIALS AND METHODS

Pure OME and DIC were obtained as gift sample from Darwin labs pvt Ltd, Vijayawada, Andhra Pradesh, India. Diopra (Diclofenac 50 mg + Omeprazole 10 mg) capsules were procured from the local pharmacy. Methanol (analytical grade) was used as the solvent. A Shimadzu UV-1800 spectrophotometer, with a pair of 1 cm matched quartz cells were used for the spectral measurements.

Preparation of Standard Stock Solutions

Accurately weighed 10 mg of OME and DIC were dissolved separately in small amount of methanol in 10 mL volumetric flasks and sonicated for 3 minutes. The final volume was adjusted up to the mark with methanol to get a solution of 1 mg/mL.

Preparation of Sample Solutions

Twenty capsules of Diopra (OME 10 mg and DIC 50 mg) were taken; the contents were removed from the shell as completely as possible and weighed. The amount of powder equivalent to 10 mg of DIC was transferred into a 10 mL volumetric flask, dissolved in methanol and sonicated for about 20 minutes. The solution was filtered through nylon disc filter (0.22 µ) and volume was made up to the mark using the same solvent. The filtrate was further diluted to get the concentration of both drugs in the linearity range.

Method 1

For the simultaneous determination using the area under curve (AUC) method, suitable dilutions of the standard stock solutions (1000 μg/mL) of OME and DIC were prepared separately in methanol. The solutions of drugs were scanned in the range of 200-400 nm, the wavelength of 301 nm and 281 nm were selected as λmax of OME and DIC respectively. The areas of the two drugs were determined at the selected wavelengths ranges i.e., 291 to 311 nm and 271 to 291 nm (± 10 nm of λmax of the both drugs). The ‘X’ values were determined as:

X = Area under curve of component (from 291 to 311 nm or 271 to 291 nm)/concentration of the component.
in g/l. C_{OME} and C_{DIC} are the concentrations of OME and DIC respectively (g/l) in sample solution; AUC_{291-311}, AUC_{271-291} are the area under curve of sample solution at the wavelength range, 291 – 311 nm and 271-291 nm, respectively. The ‘X’ values reported are the mean of six independent determinations. Applying equations (1) and (2), concentrations C_{OME} and C_{DIC} were obtained. Figure 3, linear response with increasing concentration was obtained for both of the drugs hence the same wavelength range was used for estimation of capsule formulations. Sample spectra were shown in Figure 4.

\[
C_{OME} = \frac{AUC_{(291-311)} \times X_1 \times (Q_1 - Q_2) - AUC_{(291-311)} \times X_2 \times (Q_1 - Q_2)}{X_0 \times (Q_1 - Q_2)} \quad \text{(1)}
\]

\[
C_{DIC} = \frac{AUC_{(271-291)} \times X_1 \times (Q_1 - Q_2) - AUC_{(271-291)} \times X_2 \times (Q_1 - Q_2)}{X_0 \times (Q_1 - Q_2)} \quad \text{(2)}
\]

Where, C_{OME}, C_{DIC} are the concentrations of the OME and DIC in the sample solution, AUC_{(291 – 311)}, AUC_{(271-291)} are the area of the mixture, X_{1,0}(291 – 311), X_{2,0}(271-291) are the absorptivities of OMP and X_{0,0}(291 – 311), X_{0,0}(271-291) are the absorptivities of DIC.

Method 2
For the determination of OME and DIC using the Simultaneous equation method, standard stock solutions of OME and DIC (1000 µg/mL) were diluted with methanol to get the concentration of 10 µg/mL and the solutions were scanned in the wavelength range of 400–200 nm. From the overlay spectrum of OME and DIC, two wavelengths i.e., 301 nm and 281 nm were selected for OME and DIC respectively. The calibration curves were constructed in the concentration range of 4-20 µg/mL at each of the wavelengths. The absorptivity coefficients were determined for both the drugs at the selected wavelengths and calculated by using the formula-3 and 4. OME and DIC overlay spectra was shown in Figure 5.

\[
C_0 = \frac{A_1 \times Q_1 - A_2 \times Q_2}{A_1 \times a_1 - A_2 \times a_2} \quad \text{(3)}
\]

\[
C_y = \frac{A_1 \times Q_1 - A_2 \times Q_2}{A_1 \times a_1 - A_2 \times a_2} \quad \text{(4)}
\]

Where, A_{1,2} and A_{x,y} are absorbance of sample at 301 nm and 281 nm, respectively, Cx and Cy are concentrations of OME and DIC respectively

Method 3
Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point while the other being the λ_{max} of one of the two components. From the overlay spectra of two drugs, it is evident that OME and DIC show an isoabsorptive point at 295 nm. The second wavelength used is 301 nm, which is the λ_{max} of OME. Working standard solutions having concentration 1, 2, 3, 4 and 5 µg/ml for OME and 5, 10, 15, 20 and 25 µg/ml for DIC were prepared in methanol and the absorbances at 295 nm (isoabsorptive point) and 301 nm (λ_{max} of OME) were measured and absorptivity coefficients were calculated. The concentration of two drugs in the mixture can be calculated using following equations.

\[
C_x = [(Q_M - Q_Y)/(Q_X - Q_Y)] \times A_1/a_1 \quad \text{(1)}
\]

\[
C_y = [(Q_M - Q_X)/(Q_Y - Q_X)] = A_1/a_1 \quad \text{(2)}
\]

Where, A_{1,2} and A_{x,y} are absorbance of mixture at 295 nm and 301 nm; ax and ay are absorptivities of OME and DIC at 295 nm; ax and ay are absorptivities of OME and DIC respectively at 301 nm; Q_M = A_1/A_1, Q_X = ax/ax and Q_Y = ay/ay

Validation
Validation of the proposed methods was carried out for its accuracy, precision and specificity and linearity according to ICH guidelines
**Limit of detection**
The limit of detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and shown in Table 1.

$$\text{LOD} = 3.3 \left( \sigma / S \right)$$

Where, $S$ = slope of calibration curve, $\sigma$ = standard deviation of the response

**Limit of quantification**
The limit of quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table 1.

$$\text{LOQ} = 10 \left( \sigma / S \right)$$

Where, $S$ = slope of calibration curve, $\sigma$ = standard deviation of the response

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**RESULTS**

Validation of the proposed methods was performed as per the ICH guidelines. The accuracy of the proposed method was determined by recovery studies. Pure OME and DIC were added to the pre-analysed sample at three concentration levels viz. 80, 100, 120%. Three replicate analyses were carried out at each level. The mean percent recovery was found in the range of 99.4 to 100.3% for all the methods shown in Table 2. For the two methods linearity was observed in the concentration range of 5-25 μg/mL for DIC and 1-5 μg/mL for OME both the drugs. Commercial formulations containing OME and DIC were analyzed by the proposed methods. Three replicate analysis of formulation were carried out and the mean assay values were found in the range of 98.4 to 99.5% shown in Table 3. Precision is calculated as inter day and intraday variations for both the drugs. Percent relative standard deviations for estimation of OME and DIC under intraday and interday variations were found to be less than 1.

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### Table 1: Validation Data for Omeprazole and Diclofenac

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method 1</th>
<th>Method 2</th>
<th>Method 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OME</td>
<td>DIC</td>
<td>OME</td>
</tr>
<tr>
<td>Area range ($\lambda$)</td>
<td>291-311</td>
<td>271-291</td>
<td>301</td>
</tr>
<tr>
<td>Beer’s-Lambert’s range (μg/mL)</td>
<td>1-5</td>
<td>5-25</td>
<td>1.5</td>
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<tr>
<td>Regression equation</td>
<td>$y = 0.598x - 0.248$</td>
<td>$y = 0.078x + 0.040$</td>
<td>$y = 0.206x - 0.010$</td>
</tr>
<tr>
<td>Slope ($m$)</td>
<td>0.598</td>
<td>0.078</td>
<td>0.206</td>
</tr>
<tr>
<td>Intercept ($c$)</td>
<td>0.248</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.999</td>
<td>0.998</td>
<td>0.998</td>
</tr>
<tr>
<td>Recovery + S. D. (n = 3)</td>
<td>99.5</td>
<td>99.4</td>
<td>99.31</td>
</tr>
<tr>
<td>Repeatability (% RSD, n = 6)</td>
<td>0.768</td>
<td>0.987</td>
<td>0.831</td>
</tr>
<tr>
<td>Intermediate precision (% RSD)</td>
<td>0.514</td>
<td>0.426</td>
<td>0.431</td>
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<tr>
<td>Intraday (n = 3)</td>
<td>0.615</td>
<td>0.715</td>
<td>0.632</td>
</tr>
<tr>
<td>LOD (μg/mL)</td>
<td>0.121</td>
<td>0.059</td>
<td>0.105</td>
</tr>
<tr>
<td>LOQ (μg/mL)</td>
<td>0.36</td>
<td>0.179</td>
<td>0.31</td>
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### Table 2: Accuracy Data for Omeprazole and Diclofenac

<table>
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<tr>
<th>Level of recovery</th>
<th>Drug</th>
<th>Amount of drug taken</th>
<th>Amount of std drug added</th>
<th>Recovery</th>
<th>%RSD*</th>
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<tbody>
<tr>
<td>80</td>
<td>OME</td>
<td>2</td>
<td>3.8</td>
<td>99.5</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>DIC</td>
<td>10</td>
<td>18</td>
<td>99.4</td>
<td>0.44</td>
</tr>
<tr>
<td>100</td>
<td>OME</td>
<td>2</td>
<td>4</td>
<td>99.6</td>
<td>0.643</td>
</tr>
<tr>
<td></td>
<td>DIC</td>
<td>10</td>
<td>20</td>
<td>99.3</td>
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</tr>
<tr>
<td>120</td>
<td>OME</td>
<td>2</td>
<td>4.2</td>
<td>100.1</td>
<td>0.518</td>
</tr>
<tr>
<td></td>
<td>DIC</td>
<td>10</td>
<td>22</td>
<td>100.3</td>
<td>0.641</td>
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</tbody>
</table>

### Table 3: Assay Data

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample solution concentration of OME (μg/mL)</th>
<th>Sample solution concentration of DIC (μg/mL)</th>
<th>Amount Found (%)</th>
<th>Mean Amount Found (%)</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>10</td>
<td>98.4</td>
<td>99</td>
<td>0.58</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>10</td>
<td>99.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>10</td>
<td>99.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$n = 3$, % RSD = % Relative Standard Deviation
Figure 3: UV spectra of OME

Figure 4: UV spectra of DIC

Figure 5: UV spectra of sample

Figure 6: Overlay spectra of OME and DIC

Figure 7: Linearity plot for OME

Figure 8: Linearity plot for DIC
ACKNOWLEDGEMENT

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