A simple, precise and economical procedure for the simultaneous estimation of Mesalazine and Prednisolone in combined dosage form has been developed. Mesalazine and Prednisolone are widely aimed for bacterial cure and are recommended for patients with inflammation of the digestive tract ulcerative colitis and mild-to-moderate Crohn’s disease. The present method involves the solving of simultaneous equations (Vierodt’s method). Mesalazine and Prednisolone were found to have absorption maxima at 320 and 246 nm respectively in phosphate buffer (pH 7.4). Both drugs show that very less amount of both drugs can be effectively detected by this method. The recoveries of Mesalazine and Prednisolone from the standard mixture solution were found to be 99.04% and 99.92% respectively. The recovery results indicated that Mesalazine and Prednisolone could be quantified by this procedure simultaneously in combined dosage form without the interference of common excipients.

**Keywords:** Simultaneous estimation, Mesalazine, Prednisolone, Spectrophotometric method

**ABSTRACT**

A simple, precise and economical procedure for the simultaneous estimation of Mesalazine and Prednisolone in combined dosage form has been developed. Mesalazine and Prednisolone were found to have absorption maxima at 320 and 246 nm respectively in phosphate buffer (pH 7.4). Both these drugs obeyed Beer’s law in the concentration range of 2-20 μg/ml. The high values of correlation coefficients (r²) indicated good linearity of calibration curve for both the drugs. Sandell’s sensitivity pg/cm²/0.001/abs unit of Mesalazine and Prednisolone was found to be sufficient and this show that very less amount of both drugs can be effectively detected by this method. The recoveries of Mesalazine and Prednisolone from the standard mixture solution were found to be 99.04% and 99.92% respectively. The recovery results indicated that Mesalazine and Prednisolone could be quantified by this procedure simultaneously in combined dosage form without the interference of common excipients.

**INTRODUCTION**

Spectrometry deals with instruments based on the absorption or emission of electromagnetic radiation as a result of its interaction with matter. Absorption spectrometry is the quantification of electromagnetic radiation absorbed by atoms, molecules or ions of specific wavelength. The amount of absorption depends on the wavelength of radiation and the structure of compound. The absorption of radiation is due to subtraction of energy from the radiation beam when electrons in orbital of lower energy are excited into orbital of higher energy. Since this is a electron transition phenomenon, UV is sometime called electronic spectroscopy. The technique of UV visible spectrophotometry is very frequently employed in pharmaceutical analysis. It involves the measurement of the amount of ultraviolet (190-380 nm) or visible (380-800nm) radiation absorbed by a substance in solution by an instrument which measures the ratio or a function of the ratio of the intensity of two beams of light in UV-visible region. The basis of all spectrophotometric methods for multicomponent sample analysis is the property that (a) The absorbance of a solution is the sum of absorbances of individual components or (b) the measured absorbance is the difference between total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell. The various spectrophotometric methods which are used for estimation of drug in combine dosage form include simultaneous equation method, absorbance ratio method, geometric correction method, orthogonal polynomial method, difference spectrophotometry derivative spectrophotometry absorption correction method, multicomponent method of analysis and two wavelength quantitation method.

**SIMULTANEOUS EQUATION METHOD OR VIERODT’S METHOD**

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λmax different from the other, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt’s method), provided certain criteria apply. The information required is (a) The aborbities of X at and λ1, and λ2, are aX1 and aX2 respectively. (b) The absorbities of Y at and λ1, and λ2, are aY1 and aY2 respectively. (c) The absorbances of the diluted sample at λ1 and λ2 are A1 and A2 respectively. Let Cx and Cy be the concentrations of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that at λ1 and λ2, the absorbance of the mixture is the sum of the individual absorbance of X and Y.

\[
\begin{align*}
\text{At} \lambda_1 & \quad A_1 = aX_1 \cdot b \cdot Cx + aY_1 \cdot b \cdot Cy \quad \text{-------- (1)} \\
\text{At} \lambda_2 & \quad A_2 = aX_2 \cdot b \cdot Cx + aY_2 \cdot b \cdot Cy \quad \text{-------- (2)} \\
\end{align*}
\]

For measurements in 1 cm cells b=1

Rearrange eq. (2)

\[
Cy = \frac{A_2 - aX_1 \cdot Cx}{aY_2} \quad \text{-------- (3)}
\]

Substituting for Cy in eq. (1) and rearranging

\[
\begin{align*}
C_x = & \quad \frac{A_2 - A_1 \cdot aY_2}{aX_1} \\
\end{align*}
\]

As an exercise one needs to drive modified equation containing a symbol b for path length for application in situations where A1 and A2 are measured in cells other than 1 cm path length. Criteria for obtaining maximum precision based upon absorbance ratios have been suggested that place limits on the relative concentration of the components of the mixture. The criteria are that the ratios

\[
\begin{align*}
\frac{A_{X_1}}{aX_1} \quad \text{and} \quad \frac{A_{Y_2}}{aY_2}
\end{align*}
\]

should lie outside the range 0.1-2.0 for the precise determination of X and Y respectively. These criteria are satisfied only when the λmax of two component are reasonably dissimilar. An additional criterion is that the two components don’t interact chemically thereby negating the initial assumption that the total absorbance is the sum of individual absorbances. The additivity of the absorbance should...
always be confirmed in the development of a new application of this technique.

**MATERIALS AND METHOD**

Mesalazine and Prednisolone were generously supplied as a gift samples by M/s Glaxo Smith Kline, Bombay (India) and Torrent Pharmaceuticals, Ahmedabad India respectively. All other chemicals and reagents used were of analytical grade.

**Apparatus and conditions**

A double beam GBC Cintra-10 UV/Visible spectrophotometer with data processing capacity was used. Absorption and overlain spectra of both test and standard solutions were recorded over the wavelength range of 200-400 nm using 1 cm quartz cell at a scanned speed of 100 nm/min and fixed slit width of 3 nm.

**Preparation of standard stock solution**

Stock solutions (1000 µg/ml) of Mesalazine and Prednisolone were prepared by dissolving separately 100 mg of drug in minimum quantity of dimethyl formamide (DMF) and finally diluted with PBS (pH 7.4) to make up the volume up to 100 ml. The maximum absorbance (λ<sub>max</sub>) of Mesalazine and Prednisolone were obtained at 332 nm and 246 nm, respectively for simultaneous estimation of Mesalazine and Prednisolone. A series of standard drug solutions in concentration range of 5-20 µg/ml were prepared by diluting appropriate volumes of the standard stock solutions. The scanning for solution of Mesalazine and Prednisolone were carried out in the range of 200-400 nm against PBS (pH 7.4) solution as blank for obtaining the overlain spectra that was used in the analysis. Absorbance and absorptivity of series of standard solutions were recorded at selected wavelengths.

The molar absorption coefficient equation was determined for the two drugs using calibration curve equations. Further, the molar absorption coefficient was determined by using the equation:

\[
\varepsilon = \frac{E_{1\%}^{1cm} \times \text{Molecular weight}}{10}
\]

Sandell’s Sensitivity = \( \frac{\text{Molecular weight}}{\text{Molar Absorptivity}} \)

![Fig. 1: Overlaid spectra of Mesalazine and Prednisolone in PBS (pH 7.4)](image1)

![Fig. 2: Regressed standard curve of Mesalazine at λ<sub>max</sub> 332 nm](image2)
Development of simultaneous equation

Simultaneous equation was developed using the following set of equations:

At 332 nm \[ A_1 = a_1bc_x + a_ybc_y \] \hspace{1cm} (1)
At 246 nm \[ A_2 = a_2bc_x + a_ybc_y \] \hspace{1cm} (2)

Where \(C_x\) and \(C_y\) are concentration of Mesalazine and Prednisolone respectively. \(A_1\) and \(A_2\) are absorbance at 332 nm and 246 nm respectively; \(a_{x1}\) and \(a_{x2}\) are absorption coefficient of Mesalazine at 332 nm and 246 nm respectively; \(a_{y1}\) and \(a_{y2}\) are absorption coefficient of Prednisolone at 332 nm and 246 nm respectively; \(b=1\) (for measurement in 1cm cells). Substituting the values of \(a_{x1}, a_{x2}, a_{y1},\) and \(a_{y2}\) the equation could be rearranged as:

\[ A_1 = 0.0346c_x + 0.0375c_y \]
\[ A_2 = 0.0140c_x + 0.0178c_y \]

Where \(C_x\) and \(C_y\) are the concentration in µg/ml.

Recovery studies

In order to check the accuracy, reproducibility and precision of the proposed method, recovery study was carried out by taking standard mixture solution of both Mesalazine and Prednisolone and absorbance was determined at 320 and 246 nm respectively.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Conc. (µg/ml)</th>
<th>Mesalazine Absorbance</th>
<th>Predictionsolone Absorbance</th>
<th>(E_{1%}^{1\text{cm}}) Mesalazine</th>
<th>(E_{1%}^{1\text{cm}}) Predictionsolone</th>
<th>Mean (a_{x1})</th>
<th>Mean (a_{y1})</th>
<th>(E_{1%}^{1\text{cm}}) Mean (a_{x1})</th>
<th>(E_{1%}^{1\text{cm}}) Mean (a_{y1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>0.1804</td>
<td>360.80</td>
<td>0.0064</td>
<td>0.0129</td>
<td>0.0018</td>
<td>0.0168</td>
<td>0.2397</td>
<td>479.40</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>0.2345</td>
<td>335.00</td>
<td>0.0099</td>
<td>0.0141</td>
<td>0.0022</td>
<td>0.0209</td>
<td>0.2922</td>
<td>417.43</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>0.3278</td>
<td>364.00</td>
<td>0.011</td>
<td>0.0132</td>
<td>0.0029</td>
<td>0.0267</td>
<td>0.3576</td>
<td>397.33</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>0.3966</td>
<td>305.80</td>
<td>0.015</td>
<td>0.0142</td>
<td>0.0033</td>
<td>0.0339</td>
<td>0.4073</td>
<td>370.27</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>0.4751</td>
<td>316.30</td>
<td>0.018</td>
<td>0.0145</td>
<td>0.0039</td>
<td>0.0391</td>
<td>0.4591</td>
<td>353.15</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>0.5409</td>
<td>360.00</td>
<td>0.021</td>
<td>0.0144</td>
<td>0.0045</td>
<td>0.0401</td>
<td>0.5070</td>
<td>338.00</td>
</tr>
<tr>
<td>7</td>
<td>17</td>
<td>0.6225</td>
<td>366.80</td>
<td>0.024</td>
<td>0.0145</td>
<td>0.0050</td>
<td>0.0433</td>
<td>0.5604</td>
<td>329.65</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>0.6832</td>
<td>359.80</td>
<td>0.026</td>
<td>0.0141</td>
<td>0.0056</td>
<td>0.0487</td>
<td>0.6101</td>
<td>321.11</td>
</tr>
</tbody>
</table>

Mean \(a_{x1}\) 346.06 Mean \(a_{y1}\) 0.0140 Mean \(a_{y1}\) 0.0329 Mean \(a_{y1}\) 375.79

<table>
<thead>
<tr>
<th>S.No</th>
<th>Conc. (µg/ml)</th>
<th>Mesalazine Absorbance</th>
<th>Predictionsolone Absorbance</th>
<th>(E_{1%}^{1\text{cm}}) Mesalazine</th>
<th>(E_{1%}^{1\text{cm}}) Predictionsolone</th>
</tr>
</thead>
</table>

Table 2: Optical characteristics of Mesalazine and Prednisolone

<table>
<thead>
<tr>
<th>Optical characteristics</th>
<th>Mesalazine</th>
<th>Prednisolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{max}) (nm)</td>
<td></td>
<td>320</td>
</tr>
<tr>
<td>Beer lamber’s law limits (µg/ml)</td>
<td>5-50</td>
<td>2-20</td>
</tr>
<tr>
<td>Molar absorptivity (Lmol⁻¹cm⁻¹)</td>
<td>5.29 x 10²</td>
<td>13.54 x 10²</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg.cm²/ 0.001 abs unit)</td>
<td>0.2899</td>
<td>0.2661</td>
</tr>
<tr>
<td>Regression equation</td>
<td>(y = 0.0368x - 0.0086)</td>
<td>(Y=0.0005X + 0.0707)</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.0368</td>
<td>0.0005</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.0086</td>
<td>0.0707</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9983</td>
<td>0.9969</td>
</tr>
</tbody>
</table>

Fig. 3: Regressed standard curve of Prednisolone at \(\lambda_{max}\), 246nm
Table 3: Recovery studies

<table>
<thead>
<tr>
<th>Drug in standard mixture (µg/ml)</th>
<th>%Recovery Mesalazine</th>
<th>Prednisolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesalazine</td>
<td>99.04±0.24</td>
<td>99.30±0.18</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>99.72±0.11</td>
<td>99.22±0.21</td>
</tr>
<tr>
<td>Mesalazine</td>
<td>98.57±0.39</td>
<td>101.01±0.61</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>98.77±0.55</td>
<td>99.92±0.84</td>
</tr>
</tbody>
</table>

* Results are shown as mean ± S.D (n=3)

Sensitivity

Sensitivity of drug viz. Mesalazine and Prednisolone was separately evaluated by estimating Sandell's sensitivity (µg/cm²/0.001Abs unit) to determine the minimum amount of substance that can be quantified in column of unit cross section.

RESULTS AND DISCUSSION

Absorption maxima and linearity

Overlain spectra of the drugs depicted occurrence of two peaks at 246nm and 332nm. Mesalazine and Prednisolone showed linearity with absorbance in the range 2-20 µg/ml at their respective maxima, which were validated by least square method. Coefficient of correlation was found to be 0.99B3 for Mesalazine and 0.9987 for Prednisolone. The observations are presented in Table 1, Fig.2 and Fig.3. The optical characteristics and the statistical analysis of the experimental data, the regression equation from the calibration graphs along with standard error of the slopes and intercepts and regression correlation coefficient for the ultraviolet spectrophotometric method are shown in Table 2.

The absorptivity were found approximately same for all the concentrations hence both drugs obeyed Beer's law in indicated concentration range. The high values of correlation coefficients (r²) also indicate good linearity of calibration curve for both the drugs. A study of spectra of Mesalazine and Prednisolone in Phosphate buffer saline (pH 7.4) shows that at 332nm Mesalazine shows maximum absorbance whereas Prednisolone shows almost zero absorbance. Similarly Prednisolone illustrated maximum absorbance at 246nm and Mesalazine depicted almost zero absorbance. This indicates that there is considerable difference in absorbance peaks of both the drugs and hence there is no interference.

Recovery studies and sensitivity

The recovery of Mesalazine and Prednisolone from the standard mixture solution was found to be 99.04% and 99.92% respectively (Table 3). The recovery results indicated that the method is accurate, reproducible and Mesalazine and Prednisolone could be quantified by this procedure simultaneously. Sandell's sensitivity for Mesalazine was 0.2899 and for Prednisolone was 0.2661 µg.cm⁻²/0.001 abs respectively (Table 2).

CONCLUSION

The proposed simultaneous equation method is very simple that can be performed by use of any spectrophotometer and does not require any costly instrument equipped with special package. It also shows good linearity values and sensitivity. The results demonstrated that simultaneous equation method by GBC Cintra-10 UV/Visible spectrophotometer could be useful for technique for determination of Mesalazine and Prednisolone when they are given in same dosage form.

ACKNOWLEDGEMENT

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