THE SIMULTANEOUS ESTIMATION OF TOLPERISONE HYDROCHLORIDE AND DICLOFENAC SODIUM IN TABLET DOSAGE FORM BY UV SPECTROPHOTOMETRIC METHODS


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ABSTRACT
Two methods for simultaneous estimation of Tolperisone Hydrochloride and Diclofenac Sodium in combined tablet dosage form have been developed using Methanol as a solvent. The first UV spectrophotometric method was a determination using the simultaneous equation method at 254 nm and 282 nm. The second UV spectrophotometric method is the Q – analysis (absorption ratio) method, which involves the formation of absorbance equation at 278 nm (isoabsorptive point) and at 254 nm the maximum absorption of Tolperisone Hydrochloride. The linearity ranges for Tolperisone Hydrochloride and Diclofenac sodium were 6-18 μg/ml and 2-6 μg/ml respectively. The accuracy of the methods was assessed by recovery studies was found to be 101.11 ± 1.28 and 101.96 ± 1.92 for simultaneous equation method and 101.7 ± 1.71 and 101.17 ± 1.59 for Q analysis (absorption ratio) method for Tolperisone Hydrochloride and Diclofenac Sodium respectively. These methods are simple, accurate and rapid; those require no preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

Keywords: Diclofenac Sodium, Tolperisone Hydrochloride, Q–analysis spectrophotometric method, simultaneous estimation method.

INTRODUCTION
Tolperisone hydrochloride is chemically 2RS)-2-Methyl - 1 - ( 4-methylphenyl )- 3 - piperidin - 1 - yl propan -1-one monohydrochloride a piperidine derivative centrally acting muscle relaxant which is used in the treatment of different pathological conditions like acute and chronic muscle spasm, neurological conditions and orthopedic manipulation, myelopathy, encephalomyelitis, spondylosis, spondylarthrosis, cervical and lumbar syndrome, arthrosis of the large joints obliterating artherosclerosis of the extremity vessels, diabetical angthromboangitis obliterans TOL is official in Japanese pharmacopoeia

Chemically Diclofenac sodium is, Sodium 2-[(2,6-dichlorophenyl)-amino]phenyl acetate used as analgesic and anti-inflammatory drug used in the treatment of rheumatoid arthritis, osteoarthritis and alkylosing spondylitis and also for a variety of
non-rheumatic inflammatory conditions\[2\]. Diclofenac sodium is official in Japanese Pharmacopoeia, British Pharmacopoeia\[4\], United States Pharmacopoeia\[5\] and Indian Pharmacopoeia\[6\]. The review of literature revealed that various analytical methods involving spectrophotometry\[7\], HPLC\[8\], HPTLC\[9\], have been reported for TOL in single form and in combination with other drugs. Several analytical methods have been reported for DFS in single form and in combination with other drugs including spectrophotometry\[10\], HPLC\[11\], LC-MS\[12\], HPTLC\[13\]. But there is no spectrophotometric methods have been reported. Hence it was decided to develop and validate a sensitive, accurate and rapid spectrophotometric methods for determination of TOL and DFS in bulk and pharmaceutical dosage form so as to fulfill the requirements of routine quality control analysis.

MATERIALS AND METHODS

Chemicals and Reagents

Tolperisone hydrochloride and Diclofenac sodium API was kindly gifted by Zydus Healthcare, Ahmedabad, Gujarat, India. The commercial fixed dose combination Tolpidol-D was procured from the local market. All other chemicals used were of analytical grade. Methanol and glass wares were employed throughout the work.

Apparatus

A shimadzu double beam UV-1800 Spectrophotometer(Japan) was used to measure absorbance of all the solutions. A Ohaus electronic weighing analytical balance, and a ultrasonic bath was used in the study.

Preparation of standard stock solutions

An accurately weighed quantity of TOL (100 mg) and DFS (100 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of TOL (1000 g/ml) and DFS (1000 g/ml). Accurately pippeted 10 ml of both the solutions were transferred to 100ml of volumetric flask and diluted to the mark with methanol to obtain solution having concentration 100 g/ml of TOL and DFS

Analysis of TOL and DFS in combined Dosage Form (Tablet)

Twenty tablets were accurately weighed and average weight was calculated. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to
150 mg TOL & 50mg DFS was dissolved in 10 ml methanol and sonicated for 20 min and volume was made up to 100ml. The solution was filtered through Whatman filter paper No 41 and aliquot portion of filtrate was diluted to produce solution having concentration of 30 g/ml of TOL and 10 g/ml of DFS. The absorbance of sample solution was measured at selected wavelengths and the concentrations of the two drugs were estimated using equations (1) and (2) for simultaneous equation method and equations (3) and (4) for absorbance ratio method. The analysis procedure was repeated six times and the results are depicted in Table 2.

**Method 1:**
The standard solutions of TOL (10 g/ml) and DFS (10 g/ml) were scanned separately in the UV range of 200-400 nm to determine $\lambda_{\text{max}}$ of both the drugs. The $\lambda_{\text{max}}$ of TOL and DFS were found to be 254 nm and 282 nm respectively (Fig.1). Five standard solutions having concentration 6, 9, 12, 15 and 18 g/ml for TOL and 2, 3, 4, 5, and 6 g/ml for DFS were prepared in methanol using the solutions having concentration 100 g/ml. The absorbance of resulting solutions was measured at 254 nm and 282 nm and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of TOL and DFS in sample solution was determined by solving the respective simultaneous equations generated by using absorptivity coefficients and absorbance values of TOL and DFS at these wavelengths. The absorbance and absorptivities values at the particular wavelength were substituted in the following equations to obtain the concentration.

\[
C_x = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \quad \text{(1)}
\]

\[
C_y = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2} \quad \text{(2)}
\]

$A_1, A_2$ -- absorbance of the mixture,
Method 2:
The standard solutions of TOL (10 g/ml) and DFS (10 g/ml) were scanned in the UV range of 200-400 nm to determine isoabsorptive point. The isoabsorptive point was found to be 278nm (Figure 1). Five standard solutions having concentration 6, 9, 12, 15, and 18 g/ml for TOL and 2, 3, 4, 5, and 6 g/ml for DFS were prepared in methanol using the solutions having concentration 100 g/ml. The absorbance of resulting solutions was measured at 278nm (isoabsorptive point) and 254nm (λmax of TOL) and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of TOL and DFS in sample solution was determined by solving the respective Q-analysis equations generated by using absorptivity coefficients and absorbance values of TOL and DFS at these wavelengths. The absorbance and absorptivities values at the particular wavelength were substituted in the following equations to obtain the concentration.

For TOL,
C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \times A / ax_1 \tag{3}

For DFS,

C_y = \frac{Q_m - Q_x}{Q_y - Q_x} \times A / ay_1 \tag{4}

Where,

Absorbance of sample at 260 nm
\[ Q_m = \frac{A}{Absorbance \ of \ sample \ at \ 254 \ nm} \]

Absorptivity of TOL at 260 nm
\[ Q_x = \frac{Absorptivity \ of \ TOL \ at \ 254 \ nm}{Absorptivity \ of \ TOL \ at \ 260 \ nm} \]

Absorptivity of DFS at 260 nm
\[ Q_y = \frac{Absorptivity \ of \ DFS \ at \ 260 \ nm}{Absorptivity \ of \ DFS \ at \ 254 \ nm} \]

A = Absorbance of sample at isoabsorptive point,
ax_1 = Absorptivities of TOL at isoabsorptive point.
ay_1 = Absorptivities of DFS at isoabsorptive point.

Validation of the proposed method:
The proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines\textsuperscript{[14]}.

Linearity (Calibration curve)
The calibration curves were plotted over a concentration range of 6-18 g/ml and 2-6 g/ml for TOL and DFS respectively for Simultaneous equation and same for TOL and DFS respectively for Q-analysis. Accurately pipetted standard solutions of TOL (6, 9, 12, 15 & 18 ml) and DFS (2, 3, 4, 5 & 6 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with methanol for simultaneous equation
Method and same for Q-analysis Method. The absorbances of the solutions were measured at 254 and 282 nm against methanol as blank for simultaneous equation Method. The absorbances of the solutions were measured at 254 and 278 nm against methanol as blank for Q-analysis Method. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

**Precision**

The intraday and interday precision of the proposed methods was determined by analyzing the corresponding responses 4 times on the same day and on 3 different days by standard solutions of TOL and DFS for both methods.

**Accuracy (recovery study)**

The accuracy of the method was determined by calculating recovery of TOL and DFS by the standard addition method. Known amounts of standard solutions of TOL and DFS were added at 50, 100 and 150 % level to prequantified sample solutions of TOL and DFS. The amounts of TOL and DFS were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times for both methods.

**Limit of detection and Limit of quantification**

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

\[
LOD = 3.3 \times \sigma / S
\]

\[
LOQ = 10 \times \sigma / S
\]

Where, \( \sigma \) = the standard deviation of the Intercept of Calibration curve and \( S \) = slope of the calibration curve.

**RESULTS AND DISCUSSION**

The overlain spectra of TOL and DFS exhibit \( \lambda_{max} \) of 254 nm and 282 nm for TOL and DFS respectively which are quite separated from each other. Additionally, one is isoabsorptive point was observed at 278 nm. This wavelength was selected for simultaneous estimation of TOL and DFS for Q value analysis and it is assumed to be
sensitive wavelength. Standard calibration curves for TOL and DFS were linear with correlation coefficients (r) values in the range of 0.9965 – 0.9999 at all the selected wavelengths. The calibration curves were repeated three times in a day and the average % RSD was found to be 1.78 for TOL and 1.63 for DFS. The accuracy of the methods was confirmed by recovery studies.

**TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETER OF THE CALIBRATION CURVES**

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Beer’s law limit (μg/ml)</th>
<th>Regression equation (y = a + bc)</th>
<th>Correlation coefficient (r²)</th>
<th>LOD (μg/ml)</th>
<th>LOQ (μg/ml)</th>
<th>Precision(%, RSD, n=3) Interday</th>
<th>Precision(%, RSD, n=3) Intraday</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOL DFS</td>
<td>TOL DFS</td>
<td>TOL DFS</td>
<td>TOL DFS</td>
<td>TOL DFS</td>
<td>TOL DFS</td>
<td>TOL DFS</td>
<td>TOL DFS</td>
</tr>
<tr>
<td>254 282</td>
<td>3-18</td>
<td>$y = 0.073x - 0.004$</td>
<td>0.999</td>
<td>0.063</td>
<td>0.19</td>
<td>2.0 0.5</td>
<td>2.3 0.5</td>
</tr>
<tr>
<td>254 282</td>
<td>2-6</td>
<td>$y = 0.010x - 0.004$</td>
<td>0.999</td>
<td>0.29</td>
<td>0.47</td>
<td>2.3 0.6</td>
<td>2.3 0.6</td>
</tr>
<tr>
<td>254 282</td>
<td>3-18</td>
<td>$y = 0.013x - 0.018$</td>
<td>0.999</td>
<td>0.22</td>
<td>0.47</td>
<td>2.6 0.8</td>
<td>2.6 0.8</td>
</tr>
<tr>
<td>254 278</td>
<td>3-18</td>
<td>$y = 0.013x - 0.018$</td>
<td>0.999</td>
<td>0.077</td>
<td>0.47</td>
<td>2.3 0.8</td>
<td>2.3 0.8</td>
</tr>
<tr>
<td>254 278</td>
<td>2-6</td>
<td>$y = 0.047x - 0.020$</td>
<td>0.999</td>
<td>0.63</td>
<td>0.64</td>
<td>2.3 0.1</td>
<td>2.1 0.1</td>
</tr>
<tr>
<td>254 278</td>
<td>3-18</td>
<td>$y = 0.080x - 0.009$</td>
<td>0.998</td>
<td>0.212</td>
<td>0.47</td>
<td>2.7 0.8</td>
<td>2.7 0.8</td>
</tr>
<tr>
<td>254 278</td>
<td>2-6</td>
<td>$y = 0.011x - 0.002$</td>
<td>0.997</td>
<td>0.22</td>
<td>0.47</td>
<td>2.1 0.1</td>
<td>2.1 0.1</td>
</tr>
<tr>
<td>254 278</td>
<td>3-18</td>
<td>$y = 0.016x - 0.033$</td>
<td>0.999</td>
<td>0.080</td>
<td>0.47</td>
<td>2.6 0.8</td>
<td>2.6 0.8</td>
</tr>
</tbody>
</table>

**IC Value – 4.01**
TABLE 2: RESULTS OF ANALYSIS OF THE TABLET

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>Simultaneous equation method % ± SD(n=5)</th>
<th>Q-Absorbance method % ± SD(n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOL</td>
<td>100.98 ± 1.25</td>
<td>99.25 ± 1.31</td>
</tr>
<tr>
<td>DFS</td>
<td>98.21 ± 1.13</td>
<td>97.25 ± 1.28</td>
</tr>
</tbody>
</table>

TABLE 3: RESULTS OF THE RECOVERY STUDIES

<table>
<thead>
<tr>
<th>Level of Recovery</th>
<th>Amount of pure drug added(ml)</th>
<th>Simultaneous equation method % recovery</th>
<th>Q-Absorbance method % recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TOL (100 g/ml)</td>
<td>DFS (100 g/ml)</td>
<td>TOL</td>
</tr>
<tr>
<td>80</td>
<td>3</td>
<td>1.5</td>
<td>99.77</td>
</tr>
<tr>
<td>100</td>
<td>6</td>
<td>3</td>
<td>101.25</td>
</tr>
<tr>
<td>120</td>
<td>9</td>
<td>4.5</td>
<td>102.33</td>
</tr>
<tr>
<td>Mean % Recovery</td>
<td>101.1167</td>
<td>101.9667</td>
<td>101.7</td>
</tr>
<tr>
<td>SD</td>
<td>1.285198</td>
<td>1.920738</td>
<td>1.716858</td>
</tr>
</tbody>
</table>

CONCLUSION

The proposed Simultaneous equation method provides simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of TOL and DFS in combined dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The proposed method can be used for routine analysis and quality control assay of TOL and DFS in combined dosage form.

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REFERENCES


6. Indian pharmacopoeia, Govt. of India ,Ministry of health & family welfare, the controller & publication, Delhi, Vol-II; 2007:402-403.


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