SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TOLPERISONE HYDROCHLORIDE AND DICLOFENAC SODIUM IN SYNTHETIC MIXTURE

Patel Satish A, Hariyani Kaushik P*
Department of Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat Vidyanagar – 384012, Mehsana, Gujarat, India

ABSTRACT
The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of Diclofenac sodium and Tolperisone hydrochloride in bulk and synthetic mixture. The method is based on the simultaneous equations for analysis of both the drugs using methanol as solvent. Diclofenac sodium has absorbance maxima at 281 nm and Tolperisone hydrochloride has absorbance maxima at 255 nm in methanol. The linearity was obtained in the concentration range of 2-20 μg/ml and 2-20 μg/ml for Diclofenac sodium and Tolperisone hydrochloride, respectively. The concentrations of the drugs were determined by using simultaneous equations at both the wavelengths. The mean recovery was 100.6 ± 0.41 and 99.64 ± 0.50 for Diclofenac sodium and Tolperisone hydrochloride, respectively. The method was successfully applied to laboratory prepared synthetic mixture because no interference from the mixture excipients was found. The suitability of this method for the quantitative determination of Diclofenac sodium and Tolperisone hydrochloride was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of Diclofenac sodium and Tolperisone hydrochloride in combination. The results of analysis have been validated statistically and by recovery studies.

KEY WORDS: Diclofenac sodium, Tolperisone hydrochloride, Recovery, Simultaneous equations method, Validation.

INTRODUCTION
Tolperisone (TOL) is chemically 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one (Figure 1) is a well known antispasmodic drug. It is official in Japanese Pharmacopoeia (JP). JP describe potentiometric method for its estimation. Literature survey reveals HPLC and UV methods for estimation of TOL in single dosage form. Literature survey also reveals HPLC and UV spectrophotometry methods for determination of TOL with other drugs in combination. Diclofenac sodium (DIC) is chemically 2-[2,6dichlorophenylamino] benzene acetic acid sodium salt (Figure 2). Diclofenac sodium (DIC) is official in IP and BP. IP and BP describes liquid chromatography method for its estimation. Literature survey reveals HPLC and UV and UV methods for determination of DIC in single dosage form. Literature survey also reveals HPLC and UV spectrophotometry method for the determination of DIC with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of TOL and DIC in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of TOL and DIC in synthetic mixture or dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on simultaneous equations for simultaneous estimation of both drugs in their combined synthetic mixture.

MATERIALS AND METHODS

Apparatus
A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 nm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe 2.0 system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials
TOL and DIC bulk powder was kindly gifted by Torrent Research Centre, Gandhinagar, India and Acme Pharmaceuticals Ltd., Ahmedabad, Gujarat, India respectively. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard stock solutions
An accurately weighed quantity of standard TOL (10 mg) and DIC (10 mg) powder were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 μg/ml each of TOL and DIC.

Methodology
The working standard solutions of TOL and DIC were prepared separately in methanol having concentration of 10 μg/ml. They were scanned in the wavelength range of 200-400 nm against methanol as blank. Maximum absorbance was obtained at 255 nm and 281 nm for TOL and DIC, respectively. These two wavelengths can be employed for the determination of TOL and DIC without any interference from the other components in their synthetic formulations.

Validation of the proposed method
The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines. 18

Linearity (calibration curve)
The calibration curves were plotted over a concentration range of 2-20 μg/ml for TOL and 2-20 μg/ml for DIC. Accurately measured standard solutions of TOL (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 2.0 ml) and DIC (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 2.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbances of the solutions were measured at 255 and 281 nm against methanol as blank. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.
Method precision (repeatability)
The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions \( (n = 6) \) for TOL and DIC \((6 \, \mu \text{g/ml} \) for both drugs) without changing the parameter of the proposed spectrophotometry method.

Intermediate precision (reproducibility)
The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of TOL and DIC \((4, 6, 8 \, \mu \text{g/ml} \) for TOL and \(4, 6, 8 \, \mu \text{g/ml} \) for DIC). The result was reported in terms of relative standard deviation \((\% \text{ RSD})\).

Accuracy (recovery study)
The accuracy of the method was determined by calculating recovery of TOL and DIC by the standard addition method. Known amounts of standard solutions of TOL and DIC were added at 50, 100 and 150 \% level to prequantiﬁed sample solutions of TOL and DIC \((7.5 \, \mu \text{g/ml} \) TOL and 2.5 \( \mu \text{g/ml} \) DIC). The amounts of TOL and DIC were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for ﬁve times.

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 \((S/N, \text{i.e., 3.3 for LOD and 10 for LOQ})\) using the following equations designated by International Conference on Harmonization (ICH) guidelines\(^{18}\):

\[
\text{LOD} = \frac{3.3 \times \sigma}{S} \\
\text{LOQ} = \frac{10 \times \sigma}{S}
\]

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

Analysis of TOL and DIC from synthetic mixture
Tolperisone \((75 \, \text{mg})\) and diclofenac \((25 \, \text{mg})\) standard drug powder were accurately weighed and then mixed with commonly used formulation excipients like starch, lactose, magnesium stearate and talc. The synthetic mixture was then transferred to 100 ml volumetric ﬂask containing 50 ml methanol and sonicated for 20 min. The solution was ﬁltered through Whatman ﬁlter paper No. 41 and the volume was adjusted up to the mark with methanol. This solution \((0.2 \, \text{ml})\) was taken in to a 10 ml volumetric ﬂask and the volume was adjusted up to mark with methanol to get a ﬁnal concentration of TOL \((15 \, \mu \text{g/ml})\) and DIC \((5 \, \mu \text{g/ml})\). The responses of the sample solution were measured at 255 nm and 281 nm for quantitation of TOL and DIC, respectively. The amounts of the TOL and DIC present in the sample solution were determined by solving the respective simultaneous equations for TOL and DIC as follows.

\[
\begin{align*}
\text{Cx} &= (2A_aY_1 - A_1 aY_2) / (aX_1 aX_2 - aY_1 aX_1) \\
\text{Cy} &= (A_1 aX_2 - A_2 aX_1) / (aY_1 aX_2 - aY_2 aX_1)
\end{align*}
\]

Where,

\( A_1 \) and \( A_2 \) are absorbances of mixture at 255 nm and 281 nm; \( aX_1 \) and \( aY_1 \) are absorptivities of TOL and DIC respectively at 255 nm; \( aX_2 \) and \( aY_2 \) are absorbivities of TOL and DIC respectively at 281 nm.

RESULTS
The standard solutions of TOL and DIC were scanned separately in the UV range and zero-order spectra for TOL and DIC were recorded. Maximum absorbance was obtained at 255 nm and 281 nm for TOL and DIC, respectively. These two wavelengths can be employed for the determination of TOL and DIC without any interference from the other drug in their combined synthetic mixture. Overlain zero-order absorption spectrum of TOL and DIC in methanol is shown in (Figure 3). Linear correlation was obtained between absorbances and concentrations of TOL and DIC in the concentration ranges of 2-20 \( \mu \text{g/ml} \) and 2-20 \( \mu \text{g/ml} \), respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The RSD values of TOL were found to be 0.79 and 0.80 \% at 255 and 281 nm, respectively. The RSD value of DIC was found to be 0.72 and 0.44 \% at 255 and 281 nm, respectively. Relative standard deviation was less than 2 \%, which indicates that proposed method is repeatable. The low RSD values of interday \((0.31-0.89\%) \) and \(0.33-0.58\%) \) for TOL at 255 and 281 nm, respectively and 0.30-0.53\% and 0.50-0.65\% for DIC at 255 and 281 nm, respectively) and intraday \((0.29-0.82\%) \) and \(0.44-0.77\%) \) for TOL at 255 and 281 nm, respectively and 0.44-0.98\% and 0.43-0.78\% for DIC at 255 and 281 nm, respectively) variation for TOL and DIC, reveal that the proposed method is precise. LOD and LOQ values for TOL were found to be 0.26 and 0.23 \( \mu \text{g/ml} \) and 0.79 and 0.70 \( \mu \text{g/ml} \) at 255 and 281 nm, respectively. LOD and LOQ values for DIC were found to be 0.22 and 0.14 \( \mu \text{g/ml} \) and 0.66 and 0.43 \( \mu \text{g/ml} \) at 255 and 281 nm, respectively. These data show that method is sensitive for the determination of TOL and DIC. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.64 \( \pm \) 0.5 and 100.6 \( \pm \) 0.41 for TOL and DIC, respectively (Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine TOL and DIC in their combined synthetic mixture. The results obtained for TOL and DIC were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of TOL and DIC in synthetic mixture as well as in pharmaceutical dosage forms.

DISCUSSION
The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of TOL and DIC in Synthetic mixture. The method utilizes easily available and cheap solvent for analysis of TOL and DIC hence the method was also economic for estimation of TOL and DIC from Synthetic mixture. The common excipients and other additives are usually present in the Synthetic mixture do not interfere in the analysis of TOL and DIC in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

ACKNOWLEDGEMENT
The authors are thankful to Torrent Research Centre, Gandhinagar, India and Acme Pharmaceutical Ltd., Ahmedabad, India for providing gift sample of TOL and DIC, respectively for carry out the research work. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar – 384012, Mehsana, Gujarat, India for providing all the facilities to carry out the research work.

REFERENCES

Table 1: Regression analysis data and summary of validation parameters for TOL and DIC

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TOL</th>
<th>DIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>255</td>
<td>281</td>
</tr>
<tr>
<td>Beer’s law limit (µg/ml)</td>
<td>2.20</td>
<td>2.20</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 0.063x -</td>
<td>y = 0.01x -</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.021</td>
<td>0.01</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.063</td>
<td>-0.002</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.26</td>
<td>0.32</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.79</td>
<td>0.79</td>
</tr>
<tr>
<td>Repeatability (% RSD, n = 6)</td>
<td>0.49</td>
<td>0.99</td>
</tr>
</tbody>
</table>

RSD = Relative standard deviation. LOD = Limit of detection. LOQ = Limit of quantification S. D. is standard deviation

Table 2: Recovery data of TOL and DIC

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken (µg/ml)</th>
<th>Amount added (%)</th>
<th>% Recovery ± S. D. (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOL</td>
<td>7.5</td>
<td>50</td>
<td>99.92 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>100</td>
<td>99.80 ± 0.84</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>150</td>
<td>99.19 ± 0.13</td>
</tr>
<tr>
<td>DIC</td>
<td>2.5</td>
<td>50</td>
<td>100.3 ± 0.63</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>100</td>
<td>101.5 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>150</td>
<td>100.1 ± 0.35</td>
</tr>
</tbody>
</table>

S. D. = Standard deviation. n = Number of determinations.

Table 3: Analysis of TOL and DIC in synthetic mixture

<table>
<thead>
<tr>
<th>Synthetic mixture</th>
<th>Label claim (mg)</th>
<th>Amount found (mg)</th>
<th>% Label claim ± S. D. (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TOL</td>
<td>DIC</td>
<td>TOL</td>
</tr>
<tr>
<td>I</td>
<td>150</td>
<td>50</td>
<td>150.04 ± 0.78</td>
</tr>
</tbody>
</table>

S. D. = Standard deviation. n = Number of determinations.
Figure 1: Chemical structure of Tolperisone (TOL)

Figure 2: Chemical structure of Diclofenac Sodium (DIC)

Figure 3: Overlain absorption spectra of TOL (255 nm) and DIC (281 nm) in methanol

Source of support: Nil, Conflict of interest: None Declared