METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF TOLPERISONE HCL IN BULK AND IN TABLET DOSAGE FORM AND ITS STRESS DEGRADATION STUDY USING SPECTROPHOTOMETRIC METHOD

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ABSTRACT
A simple, rapid, and specific spectrophotometric method was developed and validated for the determination of Tolperisone HCL in bulk and its dosage form. The drug was estimated by using methanol as solvent for this study, which is determined by spectrophotometrically at 254nm. The Beer-lamberts law was obeyed in a concentration range of 3-15 μg/ml with correlation coefficient of 0.9991. The precision and accuracy of the developed method was confirmed by repeatability and recovery studies validated statistically. The LOD and LOQ of Tolperisone HCL were found to be 0.007 μg/ml and 0.021 μg/ml respectively. The percentage recovery study of the drug for the proposed method was found in the range of 99.75 - 100.24 % w/v for Tolperisone HCL. The proposed method is recommended for routine analysis since it is rapid, simple, precise and accurate. Tolperisone HCL was subjected to stress conditions (acid hydrolysis, alkali hydrolysis, oxidation, thermal stress and photolytic degradation) and the stressed samples were analysed by use of the method. Degradation was observed in alkali, oxidation, and Thermal stress condition. The drug was stable under the other stress conditions investigated.

Keywords: Recovery Studies, Spectrophotometric, Stress degradation, Tolperisone HCL.

INTRODUCTION
Tolperisone hydrochloride chemically 2- methyl –1- ( 4 –methyl phenyl ) – 3- ( 1-piperidyl ) propane-1 one is a piperidine derivative and the structure is shown in fig-1. Tolperisone HCL is an aryl alkyl β-aminoketone having an asymmetric carbon atom α to the carbonyl group. It has higher muscle relaxant activity than the dextrorotatory enantiomer. It exhibits membrane stabilizing potency, which is characteristic of anti arrhythmic and local anesthetic agents.

It is a Centrally Acting Muscle Relaxant which is used in the treatment of different pathological conditions like Low back pain syndrome, Pain-central stoke spasticity,
Neuropathyism, Trapezitis and Periarthritis. Tolperisone hydrochloride is official in Japan pharmacopoeia. The literature survey revealed that there are some analytical methods reported for Tolperosine HCL either individually like UV-visible spectrophotometric method, HPTLC, or in combination with other drugs by RP-HPLC and also reported on biological fluids. The present work is an attempt to develop a Simple, accurate, precise and validated UV Spectrophotometric method for the estimation of Tolperisone HCL in bulk and Tablet dosage form.

![Structure of Tolperisone HCl](image)

**Figure 1**
Structure of Tolperisone HCl

### MATERIALS AND METHODS

**Instrumentation**

An UV – Visible spectroscopy instrument of model SL218 (ELICO) which is a double beam instrument with 1.5 cm slit, matched with 1cm path length quartz cells were used for this experiment. Digital balance (Shimadzu AX 200) was employed for the estimation.

**Chemicals and Reagent**

Tolperisone HCL reference standard was provided by Themis Medicare Ltd - Vapi. Tablet formulations containing Tolperisone HCL (TOLIFAST 150mg - Lupin Ltd) was used for this estimation.

**Preparation of Standard stock solution of Tolperisone HCL**

Accurately weighed quantity of Tolperisone HCL 50 mg was transferred into 50 ml volumetric flask dissolved and diluted up to mark with methanol. This will give a stock solution having strength of 1000 g/ml.

**Preparation of working standard solution of Tolperisone HCL**
100 g/ml of Tolperisone HCL solution was prepared by diluting 10 ml of stock solution to 100 ml with methanol.

**METHOD DEVELOPMENT:**

**Determination of wavelength for measurement**

1 ml of working standard of Tolperisone HCL was diluted to 10 ml to get 10 g/ml. Solution was scanned between 200-400 nm. Wavelengths was selected from the spectra of Tolperisone HCL.

**Calibration curve for Tolperisone HCL**

Calibration curve for Tolperisone HCL consisted of different concentration of standard solution ranging from 3-15 g/ml. This solution prepared from working standard solution of Tolperisone HCL by pipette out 0.3, 0.6, 0.9, 1.2, 1.5 ml & dilute up to mark with methanol in 10 ml volumetric flasks. Absorbance was measured at selected wavelengths. Calibration curve was plotted at selected wavelengths.

**Assay of tablet formulation**

Twenty tablets of Tolperisone HCL was weighed and powdered. The tablet powder equivalent to 10 mg of Tolerisone HCL was transferred to a 100 ml volumetric flask, sonicate to dissolved and diluted up to mark with methanol. The solution was filtered through Whatman filter paper no.41 and first few drops of filtrate were discarded. 1.5 ml of this solution was diluted to 10 ml with methanol. Absorbance of the resulting solution was measured at respective wavelengths against methanol.

**METHOD VALIDATION**

**Linearity**

For both drugs, appropriate dilutions of standard stock solutions were analysed as per the developed method. Calibration curve was plotted in the concentration range of 3-15 g/ml for Tolperisone HCL.

**Accuracy**

Accuracy was confirmed by recovery study as per ICH guidelines at three different concentration levels 80%, 100%, 120% by replicate analysis (n=3). To a preanalyzed sample solution, standard drug solutions were added and then percentage of drug content was calculated. The results of accuracy study were reported in Table 2.
Precision
The precision of analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogeneous samples. The relative standard deviation for six replicates of sample solution was less than 2.0%, which met the acceptance criteria established for spectrophotometric.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The LOD and LOQ of Tolperisone HCL by proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma / S$ and $10 \sigma / S$ respectively, where $S$ is the slope of the calibration curve and $\sigma$ is the standard deviation of response.

DEGRADATION STUDIES:
The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Tolperisone HCL using the method developed.

Standard Solution (1 mg/ml)
Standard solution was prepared by transferring about 10 mg of Tolperisone HCL into a 10 ml volumetric flask, dissolved in and diluted to volume with methanol.

Acid Hydrolysis (0.1 N HCL at Room Temperature)
From standard solution, 1.0 ml was transferred into a 10 ml volumetric flask and add 0.5 ml of 0.1 N Hydrochloric acid solution. The solutions were kept at room temperature for 24 hours, then neutralized with 0.5 ml of 0.1 N sodium hydroxide solution and diluted to 10 ml with methanol to achieve the concentration 100 µg/ml. From that solution, 15 µg/ml solution prepared and analysed at 254 nm against methanol.

Alkali Hydrolysis (0.1 N NaOH at Room Temperature)
From standard solution, 1.0 ml was transferred into a 10 ml volumetric flask and add 0.5 ml of 0.1 N Sodium hydroxide solution. The solutions were kept at room temperature for 24 hours, then neutralized with 0.5 ml of 0.1 N Hydrochloric acid solution and diluted to 10 ml with methanol to achieve the concentration 100 µg/ml. From that solution, 15 µg/ml solution prepared and analysed at 254 nm against methanol.
Peroxide degradation (3% H₂O₂ at Room Temperature)
From standard solution, 1.0 ml was transferred into a 10 ml volumetric flask and add 0.5 ml of 3% H₂O₂ solution. The solutions were kept at room temperature for 24 hours, then diluted to 10 ml with methanol to achieve the concentration 100 µg/ml. From that solution, 15 µg/ml solution prepared and analyzed at 254 nm against methanol.

Thermal degradation
The drug was placed in a controlled-temperature oven at 70°C for 24 hr. accurately weighed Tolperisone HCL (10 mg) was transferred in 10 ml of volumetric flask and dissolved in methanol (10 ml). The aliquot (1 ml) was diluted with methanol up to 10 ml to achieve concentration of Tolperisone HCL 100 µg/ml. From that solution, 15 µg/ml solution prepared and analysed at 254 nm against methanol.

Photolytic degradation
The drug was irradiated with UV radiation at 254 nm for 24 hrs. Accurately weighed TOLP (10 mg) was transferred in 10 ml of volumetric flask and dissolved in methanol (10 ml). The aliquot (1 ml) was diluted with methanol up to 10 ml to achieve concentration of Tolperisone HCL 100 µg/ml. From that solution, 15 µg/ml solution prepared and analysed at 254 nm against methanol.

RESULTS AND DISCUSSION
Selection of Wavelength
The UV scan of the standard solution (10 g/ml) between 200-400 nm showed the absorbance maxima at 254 nm for Tolperisone HCL. 

![Figure 2](Spectrum of Tolperisone HCL(10 µg/ml))
Calibration Curve

The overlain spectrum of Tolperisone HCL is shown in Figure 2.

![Figure 3](image)

**Figure 3**
Overlaid spectra of Tolperisone HCL (3-15 µg/ml)

Validation:

Linearity

The linearity range for Tolperisone HCL was found to be in the range of 3-15 µg/ml. As shown in Figure 4.

![Figure 4](image)

**Figure 4**
Linearity for Tolperisone HCL (3-15 µg/ml)
Accuracy

Accuracy of the method was confirmed by recovery study from test formulation at three level of standard addition. % Recovery for TOLP was 99.75-100.24%. The results are shown in Table 1.

**TABLE 1: RECOVERY DATA FOR TOLPERISONE HCL FROM TABLET FORMULATION**

<table>
<thead>
<tr>
<th>% Level</th>
<th>Amount of Drug in Sample (µg/ml)</th>
<th>Amount of Std. drug added (µg/ml)</th>
<th>Mean % Recovered± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>15</td>
<td>12</td>
<td>99.75 ± 1.38</td>
</tr>
<tr>
<td>100</td>
<td>15</td>
<td>15</td>
<td>100.24 ± 0.53</td>
</tr>
<tr>
<td>120</td>
<td>15</td>
<td>18</td>
<td>99.85 ± 0.47</td>
</tr>
</tbody>
</table>

Precision

% RSD for intraday and interday precision was found to be less than 2% (Table 2), it indicate that method is precise.

**TABLE 2: RESULT OF INTERMEDIATE PRECISION**

<table>
<thead>
<tr>
<th>Intraday precision* Mean ± SD</th>
<th>Interday Precision* Mean ± SD</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.93 ± 0.13</td>
<td>14.97 ± 0.14</td>
<td>0.88</td>
</tr>
</tbody>
</table>

*Mean of Six determination

Limit of Detection (LOD)

LOD for Tolperisone HCL was found to be 0.007 g/ml.

Limit of quantitation (LOQ)

LOD for Tolperisone HCL was found to be 0.021 g/ml.
Assay of tablet formulation

Applicability of the proposed method was tested by analyzing the commercially available tablet formulation of Tolperisone HCL. Results are shown in Table 3. The assay result was comparable to labelled value of drug in tablet dosage form.

**TABLE 3: DATA FROM THE ANALYSIS OF TABLET FORMULATION (N=3)**

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Label Claim (mg/tab)</th>
<th>Amount Found (mg/tab)</th>
<th>% Assay ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOLIFAST</td>
<td>150.00</td>
<td>149.30</td>
<td>99.53% ± 0.64</td>
</tr>
</tbody>
</table>

Degradation study:

**Acid Hydrolysis (0.1 N HCL)**

In acidic hydrolytic condition, no degradation product was detected. It was observed that the absorbance value decreased (Figure 5).

**Figure 5**

Overlain spectra of Tolperisone HCL (A) Standard solution (15 µg/ml) (B) 1 N HCL after 24 hr.

**Alkali hydrolysis (0.1 N NaOH)**

In alkali hydrolytic condition, no degradation product was detected. It was observed that the λmax shift and the absorbance value decreased (Figure 6).
Figure 6
Overlain spectra of Tolperisone HCL (A) Standard solution (15 µg/ml) (B) 0.1 N NaOH after 24 hr.

Peroxide Degradation
In oxidation condition, no degradation product was detected. It was observed that the \( \lambda_{\text{max}} \) shift and the absorbance value decreased (Figure 7).

Figure 7
Overlain spectra of Tolperisone HCL (A) Standard solution (15 µg/ml) (B) 3% \( \text{H}_2\text{O}_2 \) after 24 hr.
Thermal degradation (70°C)
In thermal degradation condition, no degradation product was detected. It was observed that $\lambda_{\text{max}}$ shift and the absorbance value decreased (Figure 8).

![Figure 8](image)

Overlain spectra of Tolperisone HCL (A) Standard solution (15 µg/ml)
(B) Thermal stress after 24 hr.

Photolytic degradation (UV light-254nm)
In thermal degradation condition, no degradation product was detected. It was observed that the absorbance value decreased (Figure 9).

![Figure 9](image)

Overlain spectra of Tolperisone HCL (A) Standard solution (15 µg/ml)
(B) Photolytic stress after 24 hr.
The stress degradation studies showed that Tolperisone HCL undergoes degradation in alkaline, oxidation and thermal degradation conditions whereas it is relatively stable when exposed to acidic and photolytic conditions. Summary of the results of stress degradation studies of Tolperisone HCL are shown in the Table 4.

**TABLE 4: RESULTS OF DEGRADATION STUDIES OF TOLPERISONE HCL**

<table>
<thead>
<tr>
<th>Stress condition/State</th>
<th>Exposure Time</th>
<th>$\lambda_{\text{max}}$</th>
<th>% Drug remaining after degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 N HCL/Solution</td>
<td>24 hr</td>
<td>254 nm</td>
<td>92.58%</td>
</tr>
<tr>
<td>0.1 N NaOH/Solution</td>
<td>24 hr</td>
<td>254 nm</td>
<td>51.57%</td>
</tr>
<tr>
<td>3% H$_2$O$_2$/Solution</td>
<td>24 hr</td>
<td>253 nm</td>
<td>89.57%</td>
</tr>
<tr>
<td>Thermal Stress/70°C/Solid</td>
<td>24 hr</td>
<td>253 nm</td>
<td>81.17%</td>
</tr>
<tr>
<td>UV light/254nm/Solid</td>
<td>24 hr</td>
<td>254 nm</td>
<td>83.12%</td>
</tr>
</tbody>
</table>

**TABLE 5: DATA OF OPTICAL CHARACTERISTICS FOR TOLPERISONE HCL**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observed Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>254 nm</td>
</tr>
<tr>
<td>Beer’s Law Limit (µg/ml)</td>
<td>3-15 µg/ml</td>
</tr>
<tr>
<td>Molar absorptivity (lit./mole/cm)</td>
<td>$1.5603 \times 10^4$</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg cm$^2$/0.001 absorbance unit)</td>
<td>0.0180</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.9991</td>
</tr>
<tr>
<td>Regression equation $y = mx + c$</td>
<td></td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.0539</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.0044</td>
</tr>
<tr>
<td>Parameters</td>
<td>Observed Value</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Linearity Range</td>
<td>3-15 µg/ml</td>
</tr>
<tr>
<td>Intraday Precision (%RSD)</td>
<td>0.88%</td>
</tr>
<tr>
<td>Interday Precision (%RSD)</td>
<td>0.95%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>99.75-100.24%</td>
</tr>
<tr>
<td>LOD</td>
<td>0.007 µg/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.021 µg/ml</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The method developed is a simple, accurate, precise and economical and convenient method for the analysis of Tolperisone HCL in bulk and tablet dosage form using UV-spectrophotometry. The proposed method utilizes inexpensive solvent and applied for routine analysis in laboratory. The proposed method is also useful for determination of Tolperisone HCL stability in sample of pharmaceutical dosage forms.

**ACKNOWLEDGEMENT**

I Mr. Kirtan Gohil is very much thankful to DR. M.R. Patel, Head of Dept. (Pharmaceutics), B. M. Shah College of Pharma. Edu. and Research, Modasa for providing the standard drug sample for doing this work. The authors are grateful to the management of B. M. Shah College of Pharma. Edu, and Research, Modasa for providing the required facilities.

**REFERENCES:**


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