transaminase, glutamate pyruvate transaminase, alkaline phosphatase (ALP) and serum bilirubin (total and direct) were determined (Table 1). Liver was excised quickly fixed in 10% formalin and then fixed in bovine solution, they were processed for paraffin embedding following the standard micro technique. Sections of liver were stained with haematoxylin-eosin and were observed microscopically for any histopathological changes.

The mean value ± SEM was calculated for each parameter, each parameter was analyzed separately using ANOVA followed by Dunnets 't' test. It is revealed that the alcoholic and aqueous extract contains flavonoids, sterols, tannins, carbohydrates and glycosides (flavonoidal). Results revealed that both the alcoholic and aqueous extract of leaves of *Nyctanthes arbor-tristis* Linn exhibited an ability to counteract the CCl₄-induced hepatotoxicity by decreasing the elevated enzyme levels in the blood compared to the CCl₄ group (P<0.01).

Histopathology of liver from normal control group shows prominent central vein, normal arrangement of hepatic cells. Microscopical examination of carbon tetrachloride treated liver section shows various degrees of pathological changes starting from centrilobular necrosis of hepatic cells and central lobular fatty regeneration. Liver section of standard treated and from *Nyctanthes* treated groups shows moderate protection in CCl₄-induced liver damage.

Since the results of hepatoprotective activity showed a significant decrease in the elevated levels of serum enzymes and histopathological results showed a significant regeneration of hepatocytes. Thus, from the studies we may conclude that the ethanolic and aqueous extracts of the leaves of *Nyctanthes arbor-tristis* Linn. can be used as hepatoprotective. The results were also comparable with standard drug.

**REFERENCES**


**Table 1:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bilirubin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>158.2±3.499</td>
<td>227.4±7.054</td>
<td>12.32±1.930</td>
<td>0.74±0.04</td>
</tr>
<tr>
<td>CCl₄</td>
<td>0.7</td>
<td>273.2±3.455*</td>
<td>297.4±16.567*</td>
<td>15.22±0.205</td>
<td>1.4±0.07*</td>
</tr>
<tr>
<td>Standard</td>
<td>1.0</td>
<td>160.8±2.131**</td>
<td>226.6±2.441**</td>
<td>12.72±0.159</td>
<td>0.78±0.02**</td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>5.0</td>
<td>161.4±3.076**</td>
<td>229.4±3.541**</td>
<td>12.94±0.139</td>
<td>0.84±0.02**</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>5.0</td>
<td>160.2±1.844**</td>
<td>236.2±3.736**</td>
<td>12.88±0.164</td>
<td>0.84±0.02**</td>
</tr>
</tbody>
</table>

All values are expressed as Mean ±SEM, (n=6), *P<0.01 when compared with control, **P<0.01 when compared with carbon tetrachloride.
Roxithromycin\(^1\) is a macrolide antibiotic which acts on gram-positive bacteria and gram-negative bacteria. Chemically\(^1,2\) it is \((3R, 4S, 5S, 6R, 7R, 9R, 11S, 12R, 13S, 14R)-4-[[2, 6-dideoxy-3-C-methyl-3-O-methyl-a-L-\(\text{ribo\text{-}hexopyranosyl}\)oxy]-14-ethyl-7,12,13-trihydroxy-10-[(E)-[2-methoxy ethoxy]methoxy]limino]-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-b-D-\(\text{xylo\text{-}hexopyranosyl}\)oxy]oxacyclotetradecan-2-one. It is used in respiratory tract infections\(^2\) like pharyngitis, pneumonia, chronic bronchitis and bronchopneumonia. The recommended dosage for roxithromycin is 150-300 mg per day.

Roxithromycin is official in British Pharmacopoeia\(^3\) and European Pharmacopoeia\(^4\) and it is assayed by high-performance liquid chromatographic method. Literature survey reveals that roxithromycin is estimated in pharmaceuticals and biological fluids by spectrophotometric\(^4,7\) methods, HPLC\(^8-11\) and microbiological methods\(^12\). These methods are too expensive and time consuming. The present work describes a simple, economical, accurate and reproducible spectrophotometric method for estimation of roxithromycin in pharmaceutical formulations. In the proposed method, roxithromycin is oxidized with potassium permanganate (excess potassium permanganate is decolourized with oxalic acid) to liberate formaldehyde, which is determined in situ using acetyl acetone in the presence of ammonium acetate to give a yellow-coloured chromogen with absorption maxima at 412 nm. The proposed method was successfully applied for determination of roxithromycin in its pharmaceutical dosage forms.

Double-beam Shimadzu 160A UV/Vis spectrophotometer having two matched quartz cells with 1 cm light path was employed for spectral measurement. Thermoc_MOUNT

In a 10 ml volumetric flask, standard roxithromycin solution (2.0 ml) and glacial acetic acid solution (1.0 ml) were pipetted successively. The potassium permanganate solution (0.2 ml) was added. The reaction mixture was heated on water bath at 37° for 10 min. Excess of potassium permanganate was neutralized with oxalic acid. The reagent solution (2.0 ml) was added to it and mixed thoroughly. The reaction mixture was heated on water bath at 37° for 1 min and cooled and the volume was adjusted up to the mark with water. Absorbance of the coloured solution was scanned on Shimadzu UV-visible spectrophotometer from 600 nm to 200 nm against reagent blank. Maximum absorbance was obtained at 412 nm (fig. 1).

Standard solutions of roxithromycin (0.4, 0.8, 1.0, 2.0, 3.0 ml, 250 µg/ml) were pipetted out into a series of 10 ml volumetric flasks and analysed as above. Absorbance of the coloured solution was measured at 412 nm. It was found that the Beer’s law is obeyed in the concentration range of 10-75 µg/ml with regression coefficient of 0.9987. No significant difference was found between the proposed method and the reported method when two-tailed t-tests are applied. Various reaction parameters, such as concentration of potassium permanganate and reagent, time required for oxidation and maximum colour intensity, were optimized. The method was validated and can be used successfully to assay roxithromycin in its pharmaceutical dosage form, viz, tablets.

Ammonium acetate (30 g) was dissolved in water (50 ml). Freshly distilled acetyl acetone (1.0 ml) was added and the final volume was adjusted to 100 ml with water and stored in a refrigerator. Freshly prepared reagent was used in the study. Potassium permanganate (250 mg) was dissolved and diluted to 100 ml with water. Oxalic acid (10 g) was dissolved and diluted to 100 ml with water. Roxithromycin (250 mg) was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved in glacial acetic acid (20 ml, 3 M) and diluted to 100 ml with distilled water. An aliquot (5.0 ml) was further diluted in water (50 ml) to obtain the final concentration of 250 µg/ml.

In a 10 ml volumetric flask, standard roxithromycin solution (2.0 ml) and glacial acetic acid solution (1.0 ml) were pipetted successively. The potassium permanganate solution (0.2 ml) was added. The reaction mixture was heated on water bath at 37° for 10 min. Excess of potassium permanganate was neutralized with oxalic acid. The reagent solution (2.0 ml) was added to it and mixed thoroughly. The reaction mixture was heated on water bath at 37° for 1 min and cooled and the volume was adjusted up to the mark with water. Absorbance of the coloured solution was scanned on Shimadzu UV-visible spectrophotometer from 600 nm to 200 nm against reagent blank. Maximum absorbance was obtained at 412 nm (fig. 1).

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Twenty tablets were weighed accurately and powdered. The powder equivalent to 25 mg roxithromycin was dissolved in glacial acetic acid (20 ml, 3 M), sonicated for 15 min and filtered through Whatman No. 41 filter paper. The residues were washed thoroughly with distilled water. The filtrate and washing were combined in 100 ml volumetric flask and diluted to mark with the same solvent to produce the final concentration of 250 µg/ml. The solution (2.0 ml) was analysed as above. Amount of roxithromycin was computed from the calibration curve (Table 2).

It was known that α-amino alcohol, in which the amine group is primary or secondary, liberates formaldehyde on periodate oxidation. The liberated formaldehyde is determined in situ using acetyl acetone in the presence of ammonia, which gives a yellow-coloured chromogen (3, 5- diacetyl-1, 4- dihydrolutidine) with absorption maximum at 412 nm.

In the proposed method, roxithromycin is oxidized with potassium permanganate (excess potassium permanganate is decolourized with oxalic acid) to liberate formaldehyde, which is determined in situ using acetyl acetone in the presence of ammonium acetate to give a yellow-coloured chromogen with absorption maximum at 412 nm. The colour is found to be stable for at least 2 h.

In the proposed method, various parameters, such as concentration of potassium permanganate and reagent, time required for oxidation and maximum colour intensity, were studied and optimized to obtain maximum colour intensity. The optical characteristics of roxithromycin such as Beer’s law limit, Sandell’s sensitivity and molar extinction coefficient were determined. The linear regression equation for determination of roxithromycin is

\[ y = 0.0097x + 0.0258 \] with correlation coefficient 0.9987. The RSD was found to be 0.14-2.86% (Table 3).

For recovery study, known amounts of pure drug were added to the previously analysed pharmaceutical preparations and the mixtures were analysed by the proposed method. The percent recovery was calculated, which was found to be 99.96-101.37% for roxithromycin. The analysis was carried out in triplicate – for three pharmaceutical dosage forms, i.e., tablets. The results of analysis of pharmaceutical dosage forms are shown in Table 2. Good recovery confirmed the accuracy and specificity of the proposed method and the lack of interference from the common excipients, film-coating materials and colorant used in the manufacture of tablets. The developed method was also compared with the reported method and no significant difference was observed. This method is particularly useful for routine in-process quality control for its pharmaceutical preparations, i.e., tablets.

**ACKNOWLEDGEMENTS**

The authors are thankful to Torrent Pharmaceuticals Ltd.,
Simultaneous Spectrophotometric Determination of Atorvastatin Calcium and Amlodipine Besylate in Tablets

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Two simple, accurate and precise methods for simultaneous estimation of atorvastatin calcium and amlodipine besylate in combined dosage form have been described. First method employs formation and solving of simultaneous equations using 245 nm and 363 nm as two analytical wavelengths. Second is dual wavelength method, which uses the difference of absorbance value at 259.9 nm and 354 nm for estimation of atorvastatin calcium and absorbance at 363 nm for amlodipine besylate. Fifty percent methanol was used as solvent, in which atorvastatin calcium and amlodipine besylate shows linearity in the range of 0-40 µg/ml and 0-20 µg/ml, respectively. Standard deviation was <1.5 in the assay of tablets. Methods were validated as per ICH norms and accuracy, precision, repeatability and robustness was found to be within the acceptable limit.

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TABLE 3: OPTICAL CHARACTERISTICS OF THE PROPOSED METHOD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength for measurement (nm)</td>
<td>412</td>
</tr>
<tr>
<td>Beer’s Law limit (µg/ml)</td>
<td>10-75</td>
</tr>
<tr>
<td>Molar absorptivity (l/mole/cm)</td>
<td>8.980×10³</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg/ml/cm²/0.001 abs. unit)</td>
<td>9.32×10⁻²</td>
</tr>
<tr>
<td>Regression equation (Y)</td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0097</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0258</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9987</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
</tr>
<tr>
<td>Intra day precision (%)</td>
<td>0.79-2.75</td>
</tr>
<tr>
<td>Inter day precision (%)</td>
<td>0.95-2.86</td>
</tr>
<tr>
<td>Relative standard deviation (%)</td>
<td>0.1399</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>99.96-101.37</td>
</tr>
</tbody>
</table>

"Means Y=a+bC, where 'C' is concentration in µg/ml and Y is absorbance unit. "Means five replicate samples. "Means five replicate samples.

Ahmedabad, for supplying gift sample of roxithromycin.

REFERENCES


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