INTRODUCTION
Naphthaquinones have a variety of applications in the field of pharmacols, cosmetols and agro ecosystems. Juglone, a naphthaquinone derivative is the important component of family Juglandaceae. It possesses insecticidal, herbicidal and allelochemical properties. Traditionally, it has been used as a natural dye as ink and hair dyes. Bioactivities of juglone and related naphthaquinones are reported. Sedative effect of juglone was studied. It possesses allelochemical properties, antifungal and antimicrobial activity. In order to exploit juglone various methods have been reported in the literature for the analysis. Very few reports are available on investigation of electronic transitions. Literature survey revealed the shifts in the UV-Vis absorption of the molecules with the specific arrangement of groups. UV-Vis spectroscopic method has been developed for the standardization of isolated compound from different extracts of stem bark of Juglans regia. The method was tested and validated for various parameters according to the ICH (International Conference on Harmonization). The study was performed for the stability of the compound under neutral conditions with respect to time. Confirmation of chromophores present in the molecule and position of functional groups was studied.
Simple and accurate analysis of juglone is performed using UV-vis spectrophotometer. Standard solutions of juglone (1-10mg/ml) in methanol are employed to get the calibration curve at $\lambda_{\text{max}} = 423$ nm. The spectral shifts for juglone were studied with reagents like NaOMe, NaOAc and AlCl₃. The analytical parameters such as molar extinction coefficient and conductivity were performed under the same conditions.

EXPERIMENTAL
The compound (CAS No. 481-39-0) procured from Aldrich Chemical Company Mumbai, Maharashtra, India was referred as standard for UV-VIS experiment. All other chemicals and solvents used were of spectroscopic grade. UV-VIS spectra were recorded on Schimadzu UV-1700 Therma spectrophotometer. The IR Spectra (4000-350cm⁻¹) were recorded on Shimadzu FTIR 8400S spectrophotometer. Statistical tests were performed on validation data.

A standard solution of isolated bioactive compound in methanol (0.1mg /ml) was used as stock solution. Anhydrous Sodium acetate (5%), Aluminium chloride (5%) and Sodium methoxide solution (5%) solutions were used.

**Determination of $\lambda_{\text{max}}$**
A solution of 10μg/ml was prepared from the standard solution (100μg/ml). The maximum absorbance was recorded by taking the care of solvent error.

**Calibration curve**
The working standards in the concentration range of 10-100 μg/ml were used. The absorbance was measured at 423 nm against solvent blank and calibration curve was plotted and from it validation parameters were calculated (Table 1).

**Stability Profile**
Period over which absorbance value at 423 nm of juglone in methanol remained stable was investigated using above concentrations at 15 min intervals (Table 3).

**Shifts in absorbance**
Spectral shifts for juglone were studied with reagents NaOMe, NaOAc and AlCl₃. The stock solution (0.1mg/ml) of standard was used along with above mentioned concentrations of reagents according to standard spectral methods. The shifts in absorption with respect to $\lambda_{\text{max}}$ of the standard were recorded and comparative spectral peaks scan for different spectral reagents were noted (Fig 1). Analytical parameters were recorded under same conditions (Table 2).

RESULTS AND DISCUSSION
The standard solution displays broad bands of absorption at 694 nm, 423 nm and 244 nm of which 423 nm. is selected for study. By addition of sodium acetate to test sample, the absorption maxima shifts to broad plateu region showing peaks at 477nm, 467nm causing bathochromic shift. The addition of sodium methoxide shows larger bathochromatic shift at 526 nm as it is a base, so hydroxyl group ionizes which is usually used to detect C-5 hydroxy group. By the addition of aluminium chloride bathochromic shift at 489 nm is seen due to formation of stable complex between ortho-hydroxyl group and keto function of naphthaquinone molecule. Although addition of all reagents shows bathochromic shift, intensity of the absorption has been reduced as compared to standard causing hypochromic shift. It is maximum with aluminium chloride and minimum with sodium methoxide due to distortion in geometry.

Infrared spectra of juglone exhibited band at 3403 cm⁻¹ that assigned to hydroxyl group. Characteristic bands for naphthaquinone compound 1715cm⁻¹ and 1655 cm⁻¹ were assigned to the free carbonyl group stretch and the conjugated carbonyl group respectively. Band at 1475cm⁻¹ was probably assigned to a skeletal ring stretches. In neutral compound juglone it shows intramolecular hydrogen bonding. It is proved by $^1$HNMR where a singlet for one proton is observed at δ 11.89. As intramolecular H-bonding is always stronger than intermolecular H-bonding, so it is very difficult to break by solvent. There is no effect on O-H frequency i. e. on the polarization of –O-H bonding by addition of sodium methoxide or sodium acetate.
Addition of AlCl₃ indicates the stronger bond formation of –O-H. The bond becomes more tighter which is indicated by –O-H frequency. Here more energy is required for normal polarization of –O-H bond. This shows that C-1 carbonyl has formed a coordinate covalent bond with AlCl₃. Due to such bond formation C-2, C-3 conjugated double bond electron cloud gets shifted towards C-1. which affects/increases deficient character of C-4 carbonyl carbon that is intramolecularly bonded to hydroxyl proton. Thus –O-H bond becomes tighter, it requires more energy, resulting in the higher frequency 3414 cm⁻¹. The change in characteristic bands for hydroxyl and carbonyl group is shifted to higher frequency indicates complex formation.

Table 1: Validation Parameters
by calibration curve

<table>
<thead>
<tr>
<th>Validation Parameters</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ max</td>
<td>423 nm</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y=0.004x +0.0707</td>
</tr>
<tr>
<td>Slope</td>
<td>0.004</td>
</tr>
<tr>
<td>Y intercept</td>
<td>0.0707</td>
</tr>
<tr>
<td>Range</td>
<td>10-100 (μg/ml)</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.989</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.973</td>
</tr>
</tbody>
</table>

Table 2: λ max, ε, mΩ⁻¹ for Juglone with various reagents

<table>
<thead>
<tr>
<th>S. No</th>
<th>Reagents</th>
<th>Absorption Maxima (λ max)</th>
<th>Molar Extinction Coefficient (ε)</th>
<th>Conductivity mΩ⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Juglone (J)</td>
<td>422</td>
<td>821.35</td>
<td>0.33</td>
</tr>
<tr>
<td>ii</td>
<td>J+NaOAc</td>
<td>467</td>
<td>547.83,555.94</td>
<td>6.33</td>
</tr>
<tr>
<td>iii</td>
<td>J+NaOMe</td>
<td>526</td>
<td>368.37</td>
<td>4.85</td>
</tr>
<tr>
<td>iv</td>
<td>J+AlCl₂</td>
<td>489</td>
<td>290.54</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Table 3: System precision study/Stability profile (n=9)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Conc. (μg/ml)</th>
<th>Absorbance at 422 nm at time intervals in minutes Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>0.129±0.001</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.163±0.001</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.198±0.002</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>0.229±0.003</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>0.272±0.001</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>0.300±0.002</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>0.347±0.001</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
<td>0.397±0.002</td>
</tr>
<tr>
<td>9</td>
<td>90</td>
<td>0.407±0.003</td>
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CONCLUSION
Juglone (5-Hydroxy 1,4-Naphthaquinone) has been isolated from the acetone extract of stem bark. The UV-Vis spectral study of juglone definitely is helpful for further method development and validation study.
in plant extracts of medicinal value as well as drugs. This method can be utilized for detection as well as validation of known and isolated compounds. This simple, accurate, economic, reproducible method is reported for the first time for this compound.

ACKNOWLEDGEMENT
Authors are thankful to The Head, Dept. of Chemistry, and The Principal, S. P. College, Pune-411030, for providing experimental facilities and all cooperation.

REFERENCES