UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION
FOR ESTIMATION OF ETODOLAC IN BULK

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Sample, cost effective, accurate, precise and rapid UV Spectrophotometric method was developed for the estimation of etodolac in pure form. Absorbance maximum of etodolac was estimated at 279.5 nm in methanol and water (1:9V/V). The recovery studies ascertained the accuracy of the proposed method and the results were validated as per ICH guidelines. The drug exhibited the linearity in the concentration range of 10-60µg/ml with correlation coefficient of R^2 0.999. The % recovery of the drug for the proposed method was found to be 100.6%. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.21µg/ml and 0.65µg/ml respectively. The apparent molar absorptivity and sandell’s sensitivity were found to be 2.16 mol^{-1}cm^{-1} and 0.143µg/cm^2 respectively.

INTRODUCTION:

Etodolac is chemically 2-{1,8-diethyl-1,h,3h,4h,9h-pyran[3,4b]indol-1-yl}acetic acid, it is non steroidal anti inflammatory drug used for the management of mild to moderate pain, fever, and inflammation. it is licensed for the treatment of inflammation and pain caused by osteoarthritis and rheumatoid arthritis. etodolac blocks the cyclo-oxygenase enzyme (cox) which form prostanoids, it lower the concentration of prostaglandins. which results in inflammation , pain fever are reduced. etodolac is generally avoided during pregnancy and nursing methods. nasid’s may cause adverse cardiovascular effects in foetus during pregnancy. . a survey of the literature reveals that there is no method available for the determination of etd in pure form and pharmaceutical formulations by oxidation-reduction reactions. a survey of the literature reveals that there are very few reported methods for the determination of etd in biological fluids, pharmaceutical formulations and in presence of its enantiomer. the aim of the present study was to develop a simple , accurate and validated uv spectrophotometric method for etodolac.

Fig 1: Structure of Etodolac

MATERIALS AND METHODS

Elico SL-159, UV-Visible spectrophotometer with matched cuvettes were used for the estimation. Ultrasonicator and electronic balance (Wensar ISO 9001-2000 certified) used for the experiment. Glass ware and filter paper (Whatmann No: 1) were used for the experiment.
Methodology:

Selection of solvent

The solubility of etodolac was determined in various solvents as pharmacopeia standard. Solubility test was carried out in different solvents like distilled water, methanol, chloroform, dimethyl sulfoxide, and aqueous polyethylene glycol. From the solubility studies, it was found that Etodolac was soluble in methanol and distilled water (1:9).

PREPARATION OF STANDARD SAMPLE:

The standard stock solution of etodolac was prepared by transferring accurately weighed 30mg of drug to 50ml volumetric flask and dissolving it with water and methanol (1:9) to get a concentration of 3000μg/ml. The solution was diluted accordingly to a concentration of 300μg/ml and was kept as the stock solution. The prepared stock solution was diluted with water and methanol to get working standard solutions of concentration 10-70μg/ml.

Determination of λ max:

The standard solution of etodolac (30μg/ml) was scanned in the wavelength region of 190 to 370 nm and the spectrum was recorded. Solvent methanol and water (1:9) was used as blank. It was observed that λ max was to be 279.5 nm by plotting a graph between absorbance vs. wavelength.

VALIDATION:

The objective of method validation is to demonstrate from the method is suitable for its intended purpose. The method was validated for linearity, precision, accuracy, LOD, LOQ, molar absorptivity, and Sandell’s sensitivity as per ICH guidelines.

LINEARITY:

The standard stock solution, the various dilutions in the conc. Of 10μg/ml, 20μg/ml, 30μg/ml, 40μg/ml, 50μg/ml &60μg/ml were prepared. The solution was scanned at 279.5nm and absorbance was recorded.

ACCURACY:

The accuracy of the proposed method was tested by recovery studies at 80%, 100% and 120% according to ICH guidelines by adding a known amount of pure drug to the pre-analysed formulation of concentration 15μg/ml. From above solution the mean was calculated according to the formula. From the mean, the standard deviation was calculated.

\[ \text{The Mean value} = \text{The sum of the absorbance/total absorbance} \]

\[ s = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} (x_i - \bar{x})^2} \]

From standard deviation percent relative standard deviation was also calculated.

\[ \%\text{RSD} = \frac{\text{SD}}{\text{MEAN}} \times 100 \]

The % recovery also calculated according to the below formula.

\[ \%\text{Recovery} = \frac{\text{Amount formula}}{\text{Amount found}} \times 100 \]

PRECISION:

Precision was calculated by preparing six solutions of same concentration which is the middle concentration level among the linearity range mean. S.D was calculated for these 6 concentrations. The precision value was found to be 1.014%, which was found to be with in limits i.e < 2.

Methanol and Water (1:9) was taken as blank and 6 readings were recorded at wavelength of 279.5nm then LOD, LOQ was calculated by following formula.

\[ \text{Limit of detection (LOD)} = 3.3 \times \text{SD} \]

\[ \text{Limit of quantification (LOQ)} = 10 \times \text{SD} \]

LOD of ETODOLAC = 0.212
LOQ of ETODOLAC = 0.657

SANDELL’S SENSITIVITY:

Determination of Sandell’s Sensitivity

Standard stock solution of etodolac: Sandell’s Sensitivity was calculated from the linearity data for their respective absorbance values. From this observation table, the mean of observations was taken and Sandell’s Sensitivity was calculated by the following formula:
Fig 2: Absorption maximum of Etodolac by UV-Spectrophotometer

Table No: 1 Linearity data for Etodolac.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.074</td>
</tr>
<tr>
<td>20</td>
<td>0.148</td>
</tr>
<tr>
<td>30</td>
<td>0.232</td>
</tr>
<tr>
<td>40</td>
<td>0.296</td>
</tr>
<tr>
<td>50</td>
<td>0.370</td>
</tr>
<tr>
<td>60</td>
<td>0.460</td>
</tr>
</tbody>
</table>

Fig 3: Linearity plot of Etodolac
Table no 2: ACCURACY DATA

<table>
<thead>
<tr>
<th>Samples</th>
<th>%Recovery</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1: 50%</td>
<td>101.78</td>
<td>Mean =0.114 SD =0.01 %RSD =0.877%</td>
</tr>
<tr>
<td>S2: 100%</td>
<td>98.26%</td>
<td>Mean =0.198 SD =0.001 %RSD =0.505%</td>
</tr>
<tr>
<td>S3: 120%</td>
<td>101.89%</td>
<td>Mean =0.3186 SD =0.0015 %RSD =0.470%</td>
</tr>
</tbody>
</table>

Table No 3: Precession Data

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Absorbance</th>
<th>Amount present</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.135</td>
<td>10.0036</td>
<td>Mean=0.134667 SD=0.001366 %RSD=1.014%</td>
</tr>
<tr>
<td>30</td>
<td>0.137</td>
<td>10.0310</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.135</td>
<td>10.0036</td>
<td></td>
</tr>
<tr>
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<td>30</td>
<td>0.134</td>
<td>10.0036</td>
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</tr>
<tr>
<td>30</td>
<td>0.133</td>
<td>10.0588</td>
<td></td>
</tr>
</tbody>
</table>

Table No: 3 Sandell’s Sensitivity Data

<table>
<thead>
<tr>
<th>S.no</th>
<th>Concentration(µg/ml)</th>
<th>absorbance</th>
<th>Sensivity</th>
<th>Mean sensivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.074</td>
<td>0.144</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.148</td>
<td>0.139</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.232</td>
<td>0.131</td>
<td></td>
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<td>4</td>
<td>40</td>
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<td>5</td>
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<td>6</td>
<td>60</td>
<td>0.46</td>
<td>0.146</td>
<td>0.143</td>
</tr>
</tbody>
</table>

MOLAR ABSORPTIVITY:

Molar Absorpitivity was calculated by using the following formula.

\[
\text{slope/}\text{path length}\] = \log \left(\frac{Y_2-Y_1}{X_2-X_1}\right)

Where slope = \log \left(\frac{y_2-y_1}{x_2-x_1}\right)

Slope/path length =2.16/1 =2.16 mol\(^{-1}\)cm\(^{-1}\)

RESULTS AND DISCUSSION:

The method developed and valied as per ICH guidelines. The method was validated in terms of linearity, precision, accuracy, LOD, LOQ, sandell’s sensitivity and molar absorptivity. Detection wavelength was selected at 279.5 nm linearity in response was observed in 10-60µg/ml having R\(^2\)=0.999. (R\(^2\) not less than 0.996). The precision results show % RSD =1.014%(less than 2) each level clearly that the method is precision enough for the analysis of etodolac. The accuracy of the method was checked by recovery studies. The LOD =0.212 and LOQ=0.657 indicate
sensitivity of the method. The sandell’s sensitivity for the developed method was found to be 0.143 and the molar absorptivity was found to be 2.16 mol⁻¹cm⁻¹.

CONCLUSION:

A validation UV spectrophotometric method has been developed for the estimation of etodolac in bulk as well as pharmaceutical dosage form. In this proposed method the linearity was observed in the concentration range of 10-60µg/ml with correlation coefficient R²=0.99 for ETODOLAC at 279.5 nm. The developed was found to be simple, accurate, precise, specific, reproducible and linear over the concentration range studies. The proposed method can be used for routine analysis of etodolac. The method was validated as per ICH guidelines.

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REFERENCES:

12. Pirkle WH and Murray PG: The separation of the enantiomers of a variety of non-steroidal anti-inflammatory drugs (NSAIDs) as their


20. Pharco Pharmaceuticals personal communications, February 2004


28. British Pharmacopoeia. 2004


32. Ficarra R, Ficarra P, Calauro ML, Costantino D: Quantitative high-performance liquid chromatographic determination of etodolae in
34. Giachetti C, Gas chromatography-mass spectrometry determination of etodolac in human plasma following single epicutaneous administration. Biomed Chromatogr. 1994, 8: 180-