ABSTRACT
The present study deals with UV spectrophotometric method development & validation for estimation of Paracetamol & Aceclofenac in bulk drug & tablet dosage form by viedort’s method & first order UV derivative spectrophotometry. The viedort’s method involves measurement of absorbance at $\lambda$max of Paracetamol & Aceclofenac at 248 nm & 276 nm respectively. The linearity of paracetamol and aceclofenac was found to be 3-30 $\mu$g/ml and 2-20 $\mu$g/ml respectively. The % recovery of Paracetamol & Aceclofenac was found out to be 99.72 % & 99.69 % respectively. First order UV derivative spectrophotometry ($D_1$ method), the zero crossing method was chosen as paracetamol could be easily analyzed without any interference from Aceclofenac and vice-versa.
Paracetamol was determined by measurement of its $D_1$ amplitude at the zero crossing point of Aceclofenac at (276 nm), While Aceclofenac was determined by measurement of its $D_1$ amplitude at zero crossing point of Paracetamol at (248 nm) Result of tablet analysis indicated that there was no interference of the common excipients used in the tablet formulation. Validation of this method is done as per ICHQ2B guidelines. The proposed method is highly sensitive and successfully applied for the analysis of Aceclofenac and Paracetamol in tablet formulation.

Key words: - Paracetamol, Aceclofenac, Derivative spectroscopy, Validation.
INTRODUCTION
Aceclofenac [(2, 6-Dichlorophenylamino) phenyl] acetoxy acetic acid is a phenyl acetic acid derivative that shows analgesic properties and good tolerability profile in a variety of painful conditions.\textsuperscript{1-3} It is used in the treatment of rheumatic disorders and soft tissue injuries. Aceclofenac inhibits the cyclooxygenase enzyme and thus exerts its anti-inflammatory activity by inhibition of prostaglandin synthesis. This effect seems to be correlated to the appearance of acute protocolitis associated with nonsteroidal anti-inflammatory drug therapy.\textsuperscript{6, 7} Since no spectrophotometric method is reported for simultaneous estimation of in combination using methanol & phosphate buffer. Paracetamol and Aceclofenac in Combined Tablet Dosage Formulation. Hence simple, sensitive, reliable and rapid spectroscopic methods have been developed for the determination of Paracetamol and Aceclofenac in combined tablet dosage form. Determinations were performed on Shimadzu UV-Visible double beam recording spectrophotometer (Model UV-1700). The stability of the solution was found to be 72 hrs. The method was validated for accuracy, precision, repeatability as per ICH Guidelines. This method can be used commercially for routine estimation of various compounds in pharmaceutical dosage forms.

MATERIALS AND METHODS
Apparatus
UV/Visible spectrophotometer (Shimadzu Model 1700) was employed with spectral bandwidth of 1nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). Mettler M80 analytical balance was used for weighing. Analytical grade reagents and solvents were used for the study; Combined Aceclofenac and Paracetamol tablets were procured from the local market.

Preparation of standard and sample
Selection of Common Solvent
Methanol: distilled water was selected as a common solvent for developing spectral characteristics of drugs. The selection was made after assessing the solubility and stability of both the drugs in different solvent.

Pharmaceutical Preparation
A commercial pharmaceutical preparation (Lekace-P tablets, JB Chemicals, India, was used for analysis. Each tablet contains 100 mg of aceclofenac and 325 mg of Paracetamol.
Preparation of Standard Solutions
Standard stock solution containing Aceclofenac (ACE) and Paracetamol (PARA) was prepared by dissolving 100 mg of Aceclofenac and Paracetamol separately in mixture of 10 ml of methanol to get standard stock solution of 1000 µg/ml respectively by sonicating for 15 min and 1 ml was pipette out and further volume was made up to 10 ml with phosphate buffer to obtain concentration of 100 µg/ml. Further dilutions were made in phosphate buffer from stock solution to get concentrations of 1-10 µg/ml of ACE & 2-20 µg/ml of paracetamol.

Procedure for Determining the Sampling Wavelength for Simultaneous Analysis
By appropriate dilution of two standard drug solutions of 100 µg/ml working standard solutions of drug were prepared by appropriate dilutions were prepared in phosphate buffer, were scanned in entire UV range to determine λmax solutions containing 10 µg /ml of Aceclofenac and 10 µg/ml of Paracetamol were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for both the drugs. Aceclofenac and Paracetamol showed absorbance maxima at 274 nm and 248 nm respectively. Individual and overlain spectra for both the drugs are shown in Fig. No.1 to 3.

Selection of Method and Wavelength
Viedort’s method was used to determine Aceclofenac and Paracetamol. the method developed for simultaneous estimation of Aceclofenac and Paracetamol, the wavelengths were selected from the overlain spectra shown in Fig. No.3. For Aceclofenac the wavelength selected was 274 nm and 248 nm for Paracetamol respectively.

Procedure for Determination of Absorptivity
By appropriate dilution of two standard drug solutions with methanol: Glass distilled water, six different solutions containing 10 µg/ml of Aceclofenac and 10 µg/ml of Paracetamol were prepared separately and scanned in the range of 200-400 nm. The absorbances were recorded at the selected wavelengths and the absorptivity values were determined for Aceclofenac and Paracetamol. The absorptivity values for Aceclofenac and Paracetamol are given in Table. No. 1. Absorptivity values determined for Aceclofenac at 274 nm and 248 nm were 390.24 and 150.62, while respective values for Paracetamol were 209.21 and 1013.34

Derivation of Equations
From the absorptivity values determined for Aceclofenac and Paracetamol the simultaneous
equation is derived for determination of Aceclofenac and Paracetamol in pure drug mixed standards and in its pharmaceutical formulation.

\[ A_1 = 390.24 \times C_1 + 209.21 \times C \] 
\[ \text{at 274nm (1)} \]

\[ A_2 = 150.62 \times C_1 + 1013.34 \times C_2 \] 
\[ \text{at 248 nm (2)} \]

Hence,

\[ A_1 - 150.62 \times C_1 \] 
\[ C_2 = \frac{-1013.34}{1013.34} \times A_1 \] 
\[ \text{at 248 nm (3)} \]

\[ A_1 - 0.2065 \times A_2 \] 
\[ C_1 = \frac{-359.14}{359.14} \times A_1 \] 
\[ \text{at 274nm (4)} \]

**Sample Preparation**

Marketed tablet formulations containing 100 mg of Aceclofenac and 325 mg of Paracetamol were analyzed by this method. From the triturate of 20 tablets, an amount equivalent to 100 mg of Aceclofenac was weighed and transferred to a flask and make up volume with methanol to get stock solution of 1000 µg/ml. Further dilutions was made with phosphate buffer to get a stock solution containing 100 µg/ml of Aceclofenac and 325 µg/ml of Paracetamol. Solutions were ultrasonicated for 10 min. The solution was filtered through Whatmann filter paper no. 41 after appropriate dilutions, the absorbance was measured and the concentration of each analyte was determined with the equations generated. The statistical data obtained after replicate determinations (n = 5) is shown in Table No. 2.

**Validation**

The proposed method has been statistically validated for accuracy, precision, repeatability and reproducibility. The results of validation data has been represented in Table No.4 to Table no.7.

**RESULTS AND DISCUSSION**

The proposed method for simultaneous estimation of Aceclofenac and Paracetamol utilizes the spectrum mode of analysis of Schimadzu 1700 spectrophotometer. The method utilizes 274 nm and 248 nm as analytical wavelength for estimation of Aceclofenac and Paracetamol. The method employing simultaneous equation is very simple method and can be employed for routine analysis of Aceclofenac and Paracetamol. Once the absorptivity values are determined very little time is required for analysis, as it would only require determination of
absorbance’s of the sample solution at two selected wavelengths and few simple calculations. The accuracy of the method was determined by investigating the recovery of the two drugs using spiked concentrations of the standard drug. The results indicated excellent recoveries ranging from 100.49 to 101.33 % for the two drugs. Precision for tablet analysis was determined by analysis of tablets containing Aceclofenac and Paracetamol. Result of tablet analysis indicated that there was no interference of the common excipients used in the tablet formulation.

Fig. 1: UV spectra of Aceclofenac

Fig. 2: UV spectra of Paracetamol

Fig. 3: Overlain spectra of ACE and PARA

Fig. 4: ZERO order spectra of ACE and PARA
Recovery Studies

To study the accuracy of the proposed method, recovery studies were carried out at three different levels 80%, 100% and 120% by addition of known amount of Aceclofenac and Paracetamol to a known concentration of the commercial tablet.

<table>
<thead>
<tr>
<th>Level of % recovery</th>
<th>% mean recovery*</th>
<th>Standard deviation</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACE</td>
<td>PARA</td>
<td>ACE</td>
</tr>
<tr>
<td>80</td>
<td>98.42</td>
<td>99.66</td>
<td>1.08</td>
</tr>
<tr>
<td>100</td>
<td>99.24</td>
<td>99.74</td>
<td>1.04</td>
</tr>
<tr>
<td>120</td>
<td>100.64</td>
<td>101.34</td>
<td>1.16</td>
</tr>
</tbody>
</table>

Table 1: Validation As Per ICH

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method I</th>
<th>Method II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACE (274nm)</td>
<td>PARA (248nm)</td>
</tr>
<tr>
<td>Linearity range(µg/ml)</td>
<td>1-10 µg/ml</td>
<td>3.25-32.5 µg/ml</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.999</td>
<td>0.998</td>
</tr>
<tr>
<td>Interday</td>
<td>0.26</td>
<td>0.07</td>
</tr>
<tr>
<td>Intraday</td>
<td>0.54</td>
<td>0.12</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0342</td>
<td>0.1124</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0017</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

CONCLUSION

Excellent statistical parameters and recovery data indicate that the method can be employed for efficient, rapid, accurate and precise analysis of the two drugs from multicomponent formulation. The result of analysis clearly indicates absence of interference from the
excipients in the formulation.

REFERENCES