Stability indicating Rp-Hplc Method for Simultaneous Estimation Paractamol and Etoricoxib in Tablet formulation

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Abstract: A sensitive, selective, accurate and precise stability-indicating high-performance liquid chromatography method has been developed for the quantitative determination of Paracetamol (PCT) and Etoricoxib (ETO) in tablet formulation. The good chromatographic separation between drugs was achieved in the mobile phase of phosphate buffer (0.2M, pH 5): acetonitrile (60:40v/v). The detection of analytes was carried out in UV at 242 nm. The linearity for the PCT and ETO in the range of 5-30 and 1-6 μg/ml was obtained with correlation coefficients of 0.991 and 0.998 respectively. The retention time were found to be 1.51 and 4.31 min for PCT and ETO respectively. Forced degradation study showed a significant degradation of PCT and ETO in 0.1N sodium hydroxide, 0.1N hydrochloric acid and 30% hydrogen peroxide solution.

Keywords - paracetamol, etoricoxib, RP-HPLC, stability indicating method.

INTRODUCTION

Etoricoxib (ETO) 5-chloro-6'-methyl-3(4'-methyl sulfonyl phenyl)-2,3'-bipyridine and paracetamol (PCT) (4-hydroxyl acetanilide) are used in the treatment of pain and inflammation1. Analysis of PCT tablet was reported by spectrophotometrically, HPLC and HPTLC2,6. ETO analysis was published by spectrophotometrically, RP-HPLC and HPLC-Mass spectroscopy7,13. The UV spectroscopy and RP-HPLC method were also developed for the analysis of these drugs in combined dosage form14,15. The pharmaceutical products are prone to undergo degradation in various physical and chemical conditions and yield of impurities which adversely affect the performance of drug substance. Hence, it has been mandated by regulatory agencies of various countries to submit the stability indicating data of the drug substance and drug product before approval for commercialization of products. Hence, it is necessary to develop stability indicating method for analysis of drug substance, drug product and their impurities. The present work aims at the development of stability indicating RP-HPLC method for PCT and ETO in tablet formulation as per ICH guideline.

Paracetamol

Etiricoxib
EXPERIMENTAL

CHEMICALS AND REAGENTS
PCT and ETO reference standard were obtained from Glenmark Pharma Private Ltd, Nashik, as a gift samples. Acetonitrile (HPLC grade), potassium dihydrogen phosphate (AR), orthophosphoric acid (AR), 30% hydrogen peroxide and water (HPLC grade) were obtained from Merck Ltd. The tablet formulation Nucoxia-P (500mg Paracetamol and 60mg Etoricoxib was purchased from a local medical shop.

INSTRUMENTATION
An isocratic HPLC (JASCO) with LC-2000, UV-2075 plus intelligent pump UV detector, PU-2080 was used. The analysis was carried out on RP-C18 X-Terra (150mm x 3.5mm) column with 5µm particle size as a stationary phase. Rheodyne injector with a 20 µl loop was used for the injection of sample solution and the mobile phase. The HPLC system was equipped with JASCO Borwin software version 1.2

PREPERATION OF SOLUTION
PREPERATION OF STANDARD SOLUTION
PCT (30mg) and ETO (10mg) were weighed independently and transferred seperately to 10mL volumetric flask. The drug was dissolved in mobile phase (phosphate buffer pH 5: acetonitrile (60:40v/v)) and dilution was made to the mark. From this solution the various working solution of concentration 5-30 and 1-6 μg/ml PCT and ETO respectively were prepared.

PREPERATION OF MIXED STANDARD SOLUTION
A mixed standard solution was prepared from above stock solution by proportionate addition of both the stock solution to get the final concentration of 10 μg/ml of PCT and 1.2 μg/ml of ETO.

PREPERATION OF TABLET SOLUTION
Twenty tablets were accurately weighed and finely powdered. A quantity of powder equivalent to 500mg of PCT and 60mg for ETO were transferred to 100mL volumetric flask and dissolved in mobile phase. The solution was ultrasonicated for 15min at room temperature and diluted to mark with mobile phase. The solution was filtered through Whatmann filter paper. This stock solution was diluted with mobile phase to get the final concentration of 10 and 1.2 μg/ml of PCT and ETO respectively.

METHOD DEVELOPMENT

CHROMATOGRAPHIC CONDITION
The mobile phase consists of phosphate buffer (pH 5) and acetonitrile (60:40v/v), was selected for analysis. The concentration range was determined by injecting the solution to HPLC system & it was found to be linear in the range of 5-30 and 1-6 μg/ml PCT and ETO respectively. The method was validated as per ICH guideline for linearity, range, precision, accuracy, LOD, LOQ, ruggedness and robustness.

ASSAY OF TABLET FORMULATION
The solution of tablet (label content 500mg of PCT and 60mg of ETO each) sample powder was prepared in mobile phase. After appropriate dilution, the final concentration was made 20 μg/ml and 2.4 μg/ml of PCT and ETO respectively. These sample solution were injected six different times.

STRESS DEGRADATION OF FORMULATION
DEGRADATION UNDER BASE CATALYZED HYDROLYTIC CONDITION
At ambient temperature
Twenty tablets were weighed and powdered, quantity equivalent to 500mg of PCT and 60mg ETO was transferred to 25 mL volumetric flask, to this 25 mL 0.1N aqueous NaOH added and the solution was kept for 1hr. After 1hr, 0.1 mL of this solution was transferred to 100mL volumetric flask and volume was made up mark with mobile phase. The solution was filtered and from this solution a final concentration of 10 μg/ml of PCT and 1.2 μg/ml ETO was prepared by appropriate dilution. The 0.1 mL of sample was withdrawn after 2 & 3 hr from the same flask and dilution were made with mobile phase to get final concentration of 10 μg/ml of PCT and 1.2 μg/ml ETO.

With reflux
Twenty tablets were weighed and powdered, quantity equivalent to 500mg of PCT and 60mg ETO was transferred to 25 mL volumetric flask, to this 25 mL aqueous 0.1N NaOH added and the solution was reflux for 1hr. 0.1 mL of this solution was transferred to 100mL volumetric flask and volume was made up mark with the mobile phase. The solution was filtered and from this solution a final concentration of 10 μg/ml of PCT and 1.2 μg/ml ETO was prepared by appropriate dilution. Heating was continued and 0.1 mL of sample was withdrawn after 2 & 3 hr from the same flask and dilution were made with mobile phase to get final concentration of 10 μg/ml of PCT and 1.2 μg/ml ETO.

DEGRADATION UNDER ACID CATALYZED HYDROLYTIC CONDITION
At ambient temperature
Twenty tablets were weighed and powdered, quantity equivalent to 500mg of PCT and 60mg ETO was transferred to 25 mL volumetric flask, to this 25 mL 0.1N HCl added and the solution was kept for 1hr. After 1hr, 0.1 mL of this solution was transferred to
100mL volumetric flask and volume was made up mark with mobile phase. The solution was filtered and from this solution a final concentration of 10 μg/ml of PCT and and 1.2 μg/ml ETO was prepared by appropriate dilution. The 0.1 mL of sample was withdrawn after 2 & 3 hr from the same flask and dilution were made with mobile phase to get final concentration of 10 μg/ml of PCT and 1.2 μg/ml ETO.

**With reflux**

Twenty tablets were weighed and powdered, quantity equivalent to 500mg of PCT and 60mg ETO was transferred to 25 mL volumetric flask, to this 25 mL aqueous 0.1N HCl added and the solution was reflux for 1hr. 0.1 mL of this solution was transferred to 100mL volumetric flask and volume was made up mark with the mobile phase. The solution was filtered and from this solution a final concentration of 10 μg/ml of PCT and 1.2 μg/ml ETO was prepared by appropriate dilution. Heating was continued and 0.1 mL of sample was withdrawn after 2 & 3 hr from the same flask and dilution were made with mobile phase to get final concentration of 10 μg/ml of PCT and 1.2 μg/ml ETO.

**OXIDATIVE DEGRADATION**

**At ambient temperature**

Twenty tablets were weighed and powdered, quantity equivalent to 500mg of PCT and 60mg ETO was transferred to 25 mL volumetric flask, to this 25 mL aqueous 30% H₂O₂ added and the solution was kept for 1hr. After 1hr, 0.1 mL of this solution was transferred to 100mL volumetric flask and volume was made up mark with the mobile phase. The solution was filtered and from this solution a final concentration of 10 μg/ml of PCT and 1.2 μg/ml ETO was prepared by appropriate dilution. Heating was continued and 0.1 mL of sample was withdrawn after 2 & 3 hr from the same flask and dilution were made with mobile phase to get final concentration of 10 μg/ml of PCT and 1.2 μg/ml ETO.

**DEGRADATION BY DRY HEAT**

Twenty tablets were weighed and powdered and transferred to china dish and it was kept in oven at 100°C for 1hr. After 1hr, the quantity of powder equivalent to 500mg of PCT and 60mg of ETO was transferred to 25 mL volumetric flask, to this 25 mL of mobile phase was added and solution was ultrasonicated for 15min. From this, 0.1ml of this solution was transferred to 100mL volumetric flask and volume was made up mark with the mobile phase. The solution was filtered and final concentration of 10 μg/ml of PCT and 1.2 μg/ml ETO was prepared by appropriate dilution.

![Spectrum 1: UV absorbance overlay spectrum of paracetamol and etoricoxib](image_url)
Fig 1: A typical HPLC chromatogram of paracetamol and etoricoxib in Bulk

### Table 1: Accuracy study *

<table>
<thead>
<tr>
<th>Recovery level</th>
<th>% Recovery found</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCT</td>
<td>ETO</td>
</tr>
<tr>
<td>80</td>
<td>98.91</td>
<td>99.07</td>
</tr>
<tr>
<td>100</td>
<td>100.28</td>
<td>102.37</td>
</tr>
<tr>
<td>120</td>
<td>101.13</td>
<td>99.17</td>
</tr>
</tbody>
</table>

*n = 3, RSD- Relative standard deviation

### Table 2: System suitability parameters

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Parameters</th>
<th>PCT</th>
<th>ETO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Therotical plate</td>
<td>89325</td>
<td>70891</td>
</tr>
<tr>
<td>2</td>
<td>Tailing Factor</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>Capacity factor</td>
<td>5.6</td>
<td>1.62</td>
</tr>
</tbody>
</table>

### Table 3: Robustness and ruggedness study

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Variables</th>
<th>PCT(mean±SD)</th>
<th>ETO(mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rt</td>
<td>TF</td>
</tr>
<tr>
<td>1</td>
<td>pH</td>
<td>1.51±0.01</td>
<td>0.25±0.06</td>
</tr>
<tr>
<td>2</td>
<td>Mobile phase</td>
<td>1.66±0.02</td>
<td>0.73±0.01</td>
</tr>
<tr>
<td>3</td>
<td>Flow rate</td>
<td>1.53±0.02</td>
<td>0.40±0.04</td>
</tr>
</tbody>
</table>

### Table 4: Quantitative analysis of PCT and ETO in tablet by HPLC *

<table>
<thead>
<tr>
<th>Marketed Formulation</th>
<th>Label claim (mg/tab)</th>
<th>Amount found (mg/tab ± RSD)</th>
<th>Label claim (% ± RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucoxia-P</td>
<td>PCT</td>
<td>ETO</td>
<td>PCT</td>
</tr>
<tr>
<td>500</td>
<td>60</td>
<td>493.89 ±0.32</td>
<td>59.24 ±1.45</td>
</tr>
</tbody>
</table>

*n = 6, RSD- Relative standard deviation
RESULTS AND DISCUSSION

METHOD DEVELOPMENT

The mobile phase consisting of phosphate buffer (pH 5.0) and acetonitrile in composition of (60: 40v/v) was selected. Acetonitrile was selected because of its favourable UV transmittance, low viscosity, low back pressure and it provides good chromatographic resolution between drugs. The buffer helps in obtaining sharp peaks and produces good resolution with retention time 1.51 and 4.31 min for PCT and ETO respectively. The analysis was carried at 242nm in UV where both drugs showed good absorbance (Spectrum 1). The chromatographic analysis time was less than 10 min. The typical HPLC chromatograms of PCT and ETO in bulk were shown in fig 1.

METHOD VALIDATION

LINEARITY, RANGE AND CALIBRATION

Response to PCT and ETO was linear in the concentration ranges of 5-30 and 1-6 µg/ml. The equations of calibration curves for PCT and ETO by HPLC method, were $y = 26780x - 16569$, $y = 81130x + 28345$ respectively. The correlation coefficients were 0.999 and 0.998 for PCT and ETO respectively was obtained.

PRECISION (REPETABILITY)

Precision of the method was tested by performing intra-day and inter-day studies. For intra-day studies, triplicate of samples were analyzed within same day. For inter-day validation, analysis was carried out on three separate days. %RSD of 1.12 & 1.06 of PCT and ETO for intraday whereas %RSD of 1.23 and 1.51 of PCT and ETO respectively for interday study was obtained.

ACCURACY

Accuracy of method was evaluated by the percent recovery study at 80, 100 and 120% levels. The recoveries were verified by estimation of drugs in triplicate at each specified level. Results of recovery study were given in Table 1.

SPECIFICITY

The specificity of the method was checked for the interference of impurities and excipients in the analysis of drug solution under optimized chromatographic condition. No interference was observed during analysis between drugs and excipients in tablet. Hence the method was found to be specific.

SENSITIVITY

The sensitivity PCT and ETO measurement was estimated as the limit of detection (LOD) and limit of quantitation (LOQ). The LOD of 1.04 and 0.69 µg/ml in PCT and ETO and LOQ of 4.18 and 2.23 µg/ml to PCT and ETO respectively was obtained.

SYSTEM SUITABILITY PARAMETERS

System suitability parameters such as tailing factor, theoretical plates and capacity factor were determined (Table 2).

ROBUSTNESS AND RUGGEDNESS

The robustness of the HPLC method, was determined by purposefully altering the conditions such as the composition mobile phase (± 1 % ACN), pH (± 0.2), and flow rate (± 0.2 ml/min) of the mobile phase. The results of study showed that the method is robust (Table 3).

ASSAY OF TABLET FORMULATION

Results of tablet analysis showed that the method is accurate and precise. The purity of sample was found to be 99.76 and 101.37% w/w for PCT and ETO respectively. Analysis was performed on six replicates(Table 4).

Fig. 2: A typical HPLC chromatogram of PCT and ETO in 0.1 N NaOH (3hr)
### Table 5: Percentage degradation of PCT and ETO in tablet

<table>
<thead>
<tr>
<th>Stress degradation condition</th>
<th>Temp.</th>
<th>Time in hr</th>
<th>PCT</th>
<th>ETO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base catalysed (0.1 N NaOH)</td>
<td>Ambient</td>
<td>1</td>
<td>1.21±0.45</td>
<td>2.23±0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1.64±1.02</td>
<td>2.60±0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3.82±1.34</td>
<td>3.25±1.28</td>
</tr>
<tr>
<td>Base catalysed (0.1 N NaOH)</td>
<td>Reflux</td>
<td>1</td>
<td>17.03±0.65</td>
<td>2.83±1.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>18.36±0.98</td>
<td>3.05±1.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>21.25±1.42</td>
<td>6.25±1.83</td>
</tr>
<tr>
<td>Acid catalysed (0.1 N HCl)</td>
<td>Ambient</td>
<td>1</td>
<td>1.96±1.32</td>
<td>1.17±0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3.13±1.76</td>
<td>2.93±0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3.96±0.68</td>
<td>4.74±1.12</td>
</tr>
<tr>
<td>Acid catalysed (0.1 N HCl)</td>
<td>Reflux</td>
<td>1</td>
<td>6.13±0.63</td>
<td>2.80±1.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>10.30±0.94</td>
<td>3.49±0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>11.68±1.10</td>
<td>5.74±1.45</td>
</tr>
<tr>
<td>Oxidation (30%H₂O₂)</td>
<td>Ambient</td>
<td>1</td>
<td>2.34±1.76</td>
<td>1.27±0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5.13±1.53</td>
<td>2.30±0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>7.28±1.70</td>
<td>3.29±1.21</td>
</tr>
<tr>
<td>Oxidation (30%H₂O₂)</td>
<td>Reflux</td>
<td>1</td>
<td>5.79±0.54</td>
<td>6.83±1.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>10.16±0.73</td>
<td>9.27±1.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>12.95±0.96</td>
<td>10.27±1.52</td>
</tr>
<tr>
<td>Dry Heat</td>
<td>100°C in oven</td>
<td>1hr</td>
<td>15.11±1.74</td>
<td>3.21±1.58</td>
</tr>
</tbody>
</table>

n=3

*Fig. 3: A typical HPLC chromatogram of PCT and ETO in 0.1 N HCl (3hr)*
STRESS DEGRADATION OF FORMULATION

The degradation in 0.1 N sodium hydroxide was found to be less than 4% after 3 hr at ambient temperature for PCT and ETO where as 21.25% and 6.25% degradation was obtained for PCT and ETO respectively at reflux temperature after 3 hr. (Fig 2)

In 0.1N hydrochloric acid degradation study at ambient temperature showed 3.96 and 3.74 % of degradation at the end of 3 hr and 11.68 and 5.74% PCT and ETO respectively at the end of 3 hr of the reflux conditions. (Fig 3)

Oxidation degradation study in 30% hydrogen peroxide gave around 7.28 and 3.29% degradants at ambient temperature where as 12.95 and 10.27 % degradants at reflux temperature at end of 3 hr (Fig 4).

The study was carried out by exposure of tablet powder to dry heat at 100°C for 1 hr. There were many degradation peak observed in chromatogram with significant degradants of 15.11 and 13.21% for PCT and ETO respectively (Fig 5). The table 5 showed the data of degradation study in various experimental conditions.

The degradation study reveals great amount of degradation of PCT and ETO. The proposed degradation product of base and acid catalysed degradant is 4- amino phenol where as oxidation degradation leads to N-acetyl-p-benzoquinoneimine product.
CONCLUSION
The proposed method is simple, sensitive, accurate, precise and reproducible and hence can be used for routine analysis of paracetamol and etoricoxib in bulk and formulation. The proposed method is stability indicating and can be used in determination of PCT and ETO in the presence of their degradants. The degradation study in basic and acidic condition reveals the formation of 4-amino phenol as degradant product and N-acetyl-p-benzoquinoneimine degradant in 30% hydrogen peroxide oxidation.

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