

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

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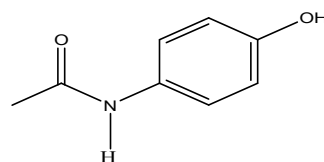
**Abstract:** A sensitive, selective, accurate and precise stability-indicating high-performance liquid chromatography method has been developed for the quantitative determination of Paracetamol (PCT) and Etoricoxib (ETO) in tablet formulation. The good chromatographic separation between drugs was achieved 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.

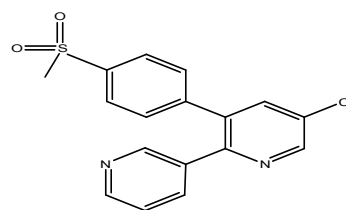
## INTRODUCTION

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 inflammation<sup>1</sup>. Analysis of PCT tablet was reported by spectrophotometrically, HPLC and HPTLC<sup>2-6</sup>. ETO analysis was published by spectrophotometrically, RP-HPLC and HPLC-Mass spectroscopy<sup>7-13</sup>. The UV spectroscopy and RP-HPLC method were also developed for the analysis of these drugs in combined dosage form<sup>14-15</sup>. 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



Etoricoxib

## **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 5 $\mu$ m particle size as a stationary phase. Rheodyne injector with a 20  $\mu$ l 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  $\mu$ g/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  $\mu$ g/ml of PCT and 1.2  $\mu$ g/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  $\mu$ g/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  $\mu$ g/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  $\mu$ g/ml and 2.4  $\mu$ g/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  $\mu$ g/ml of PCT and 1.2  $\mu$ g/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  $\mu$ g/ml of PCT and 1.2  $\mu$ g/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  $\mu$ g/ml of PCT and 1.2  $\mu$ g/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  $\mu$ g/ml of PCT and 1.2  $\mu$ g/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 30% H<sub>2</sub>O<sub>2</sub> 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

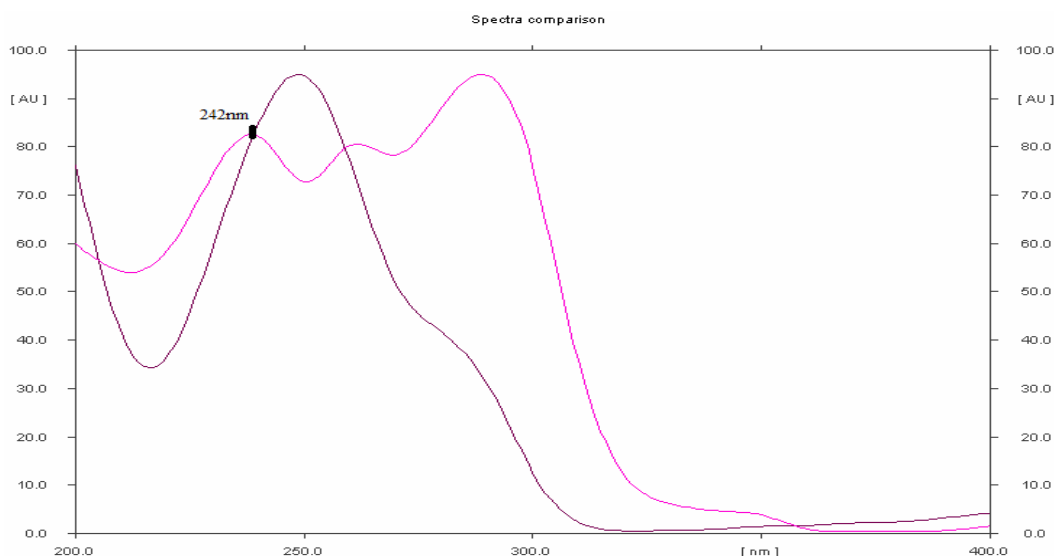
PCT and 1.2 µg/ml ETO was prepared by appropriate dilution. The 0.1 mL of sample was withdrawn after 2 & 3 hr from the same flask and dilution were made with mobile phase to get final concentration of 10 µg/ml of PCT and 1.2 µg/ml ETO.

#### With reflux

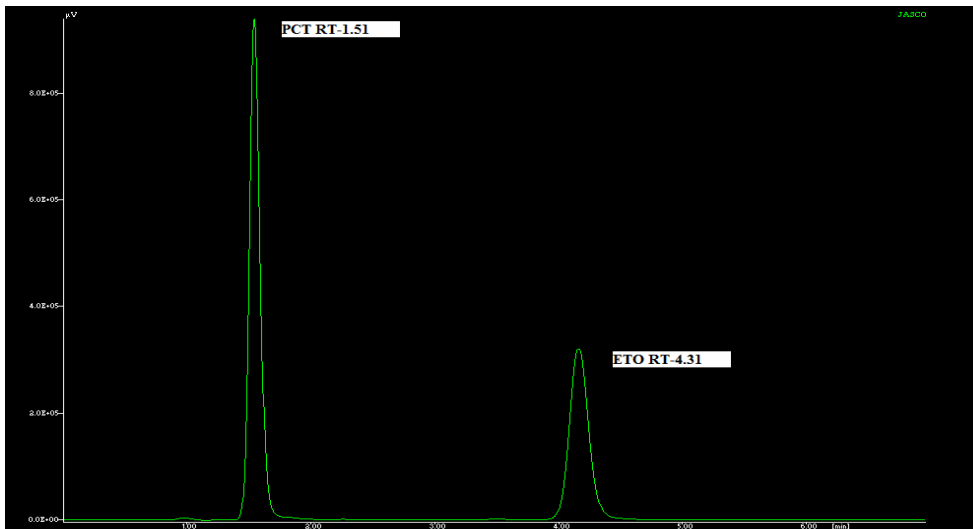
Twenty tablets were weighed and powdered, quantity equivalent to 500mg of PCT and 60mg ETO was transferred to 25 mL volumetric flask, to this 25 mL aqueous 30% H<sub>2</sub>O<sub>2</sub> added and the solution was reflux for 1hr. 0.1 mL of this solution was transferred to 100mL volumetric flask and volume 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 BY DRY HEAT

Twenty tablets were weighed 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**



**Fig 1: A typical HPLC chromatogram of paracetamol and etoricoxib in Bulk**

**Table 1: Accuracy study \***

Recovery level	% Recovery found		% RSD	
	PCT	ETO	PCT	ETO
80	98.91	99.07	1.82	1.53
100	100.28	102.37	0.54	1.23
120	101.13	99.17	1.20	0.93

\* n = 3 , RSD- Relative standard deviation

**Table 2: System suitability parameters**

Sr no.	Parameters	PCT	ETO
1	Theoretical plate	89325	70891
2	Tailing Factor	0.2	0.04
3	Capacity factor	5.6	1.62

**Table 3: Robustness and ruggedness study**

Sr. No.	Variables	PCT(mean±SD)		ETO (mean±SD)	
		Rt	TF	Rt	TF
1	pH	1.51±0.01	0.25 ±0.06	4.24±0.06	0.04 ±0.04
2	Mobile phase	1.66±0.02	0.73 ±0.21	4.32±0.12	0.47 ±0.14
3	Flow rate	1.53±0.02	0.40 ±0.14	4.48±0.14	0.51 ±0.17

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

Marketed Formulation	Label claim (mg/tab)		Amount found (mg/tab ± RSD)		Label claim (% ± RSD)	
	PCT	ETO	PCT	ETO	PCT	ETO
Nucoxia-P	500	60	493.89 ±0.32	59.24 ±1.45	99.76 ±0.32	101.37 ±1.45

\* 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**

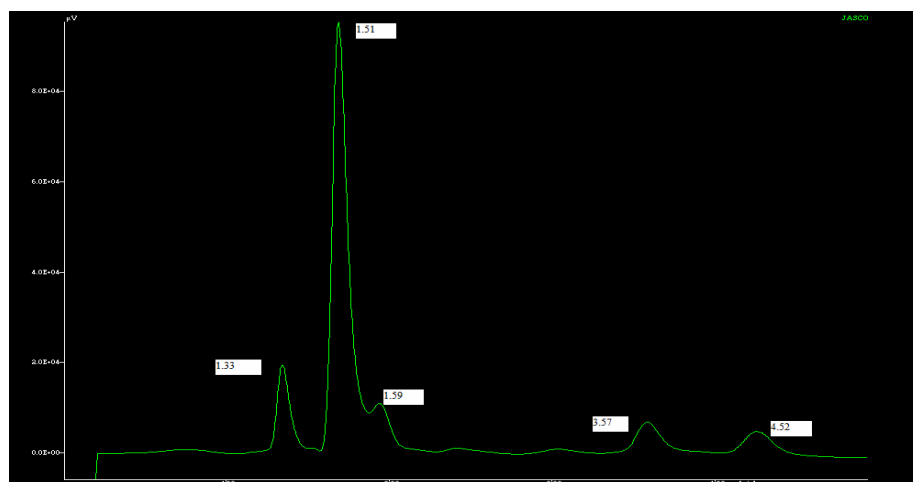
System suitability parameters such as tailing factor, theoretical plates and capacity factor were determined (Table 2).

#### **ROBUSTNESS AND RUGGEDNESS**

The robustness of the HPLC method, was determined by purposefully altering the conditions such as the composition mobile phase ( $\pm 1\%$  ACN), pH ( $\pm 0.2$ ), and flow rate ( $\pm 0.2$  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)

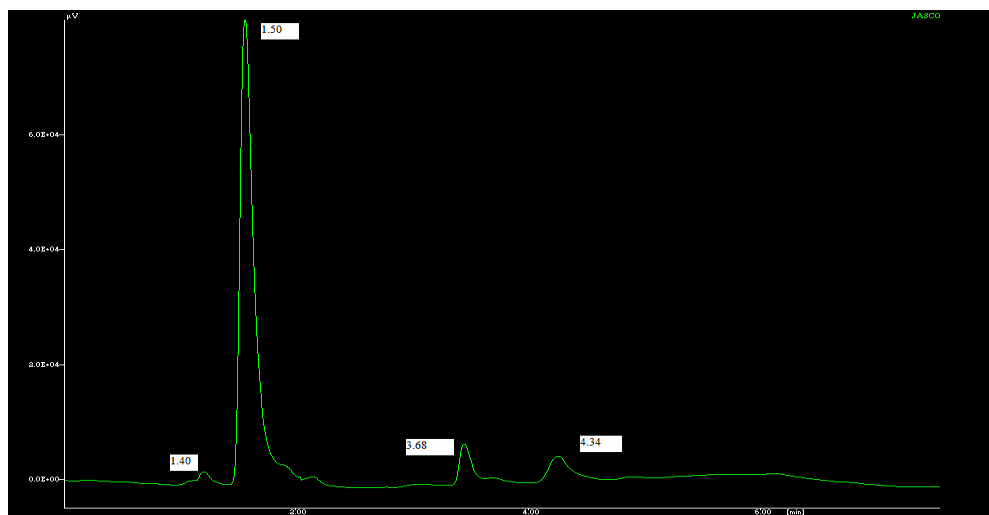


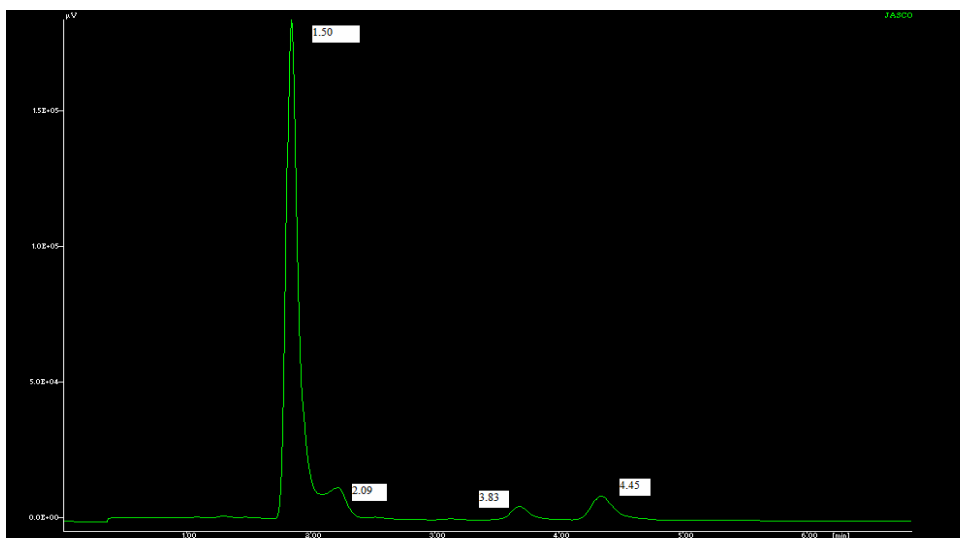
**Fig. 2: A typical HPLC chromatogram of PCT and ETO in 0.1 N NaOH (3hr)**

**Table 5: Percentage degradation of PCT and ETO in tablet**

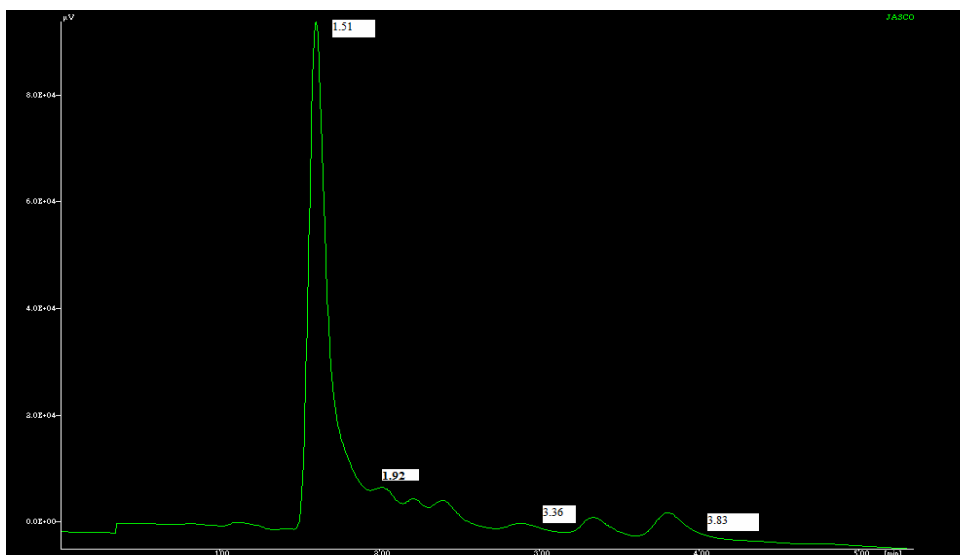
Stress degradation condition			% Degradation $\pm$ RSD*	
Reagent	Temp.	Time in hr	PCT	ETO
Base catalysed (0.1 N NaOH)	Ambient	1	1.21 $\pm$ 0.45	2.23 $\pm$ 0.63
		2	1.64 $\pm$ 1.02	2.60 $\pm$ 0.86
		3	3.82 $\pm$ 1.34	3.25 $\pm$ 1.28
Base catalysed (0.1 N NaOH)	Reflux	1	17.03 $\pm$ 0.65	2.83 $\pm$ 1.31
		2	18.36 $\pm$ 0.98	3.05 $\pm$ 1.59
		3	21.25 $\pm$ 1.42	6.25 $\pm$ 1.83
Acid catalysed (0.1 N HCl)	Ambient	1	1.96 $\pm$ 1.32	1.17 $\pm$ 0.65
		2	3.13 $\pm$ 1.76	2.93 $\pm$ 0.60
		3	3.96 $\pm$ 0.68	4.74 $\pm$ 1.12
Acid catalysed (0.1 N HCl)	Reflux	1	6.13 $\pm$ 0.63	2.80 $\pm$ 1.32
		2	10.30 $\pm$ 0.94	3.49 $\pm$ 0.98
		3	11.68 $\pm$ 1.10	5.74 $\pm$ 1.45
Oxidation (30% $H_2O_2$ )	Ambient	1	2.34 $\pm$ 1.76	1.27 $\pm$ 0.65
		2	5.13 $\pm$ 1.53	2.30 $\pm$ 0.90
		3	7.28 $\pm$ 1.70	3.29 $\pm$ 1.21
Oxidation (30% $H_2O_2$ )	Reflux	1	5.79 $\pm$ 0.54	6.83 $\pm$ 1.38
		2	10.16 $\pm$ 0.73	9.27 $\pm$ 1.73
		3	12.95 $\pm$ 0.96	10.27 $\pm$ 1.52
Dry Heat	100 $^{\circ}$ C in oven	1hr	15.11 $\pm$ 1.74	3.21 $\pm$ 1.58

n=3\*

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



**Fig. 4:** A typical HPLC chromatogram of PCT and ETO in 30 % H<sub>2</sub>O<sub>2</sub>(3hr)



**Fig. 5:** A typical HPLC chromatogram of PCT and ETO by dry heat

### STRESS DEGRADATION OF FORMULATION

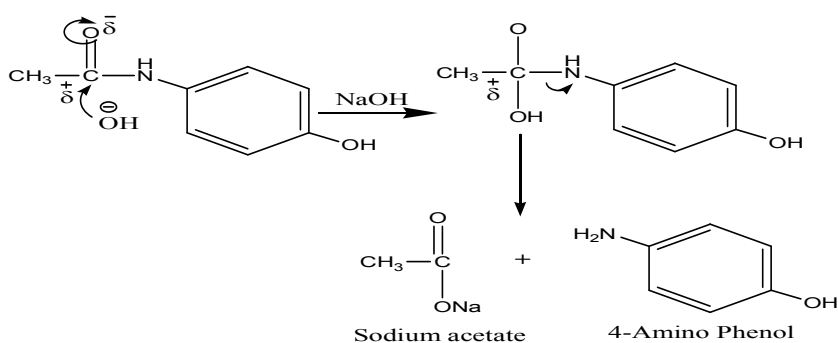
The degradation in 0.1 N sodium hydroxide was found to be less than 4% after 3 hr at ambient temperature for PCT and ETO where as 21. 25 % and 6.25% degradation was obtained for PCT and ETO respectively at reflux temperature after 3 hr. (Fig 2)

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)

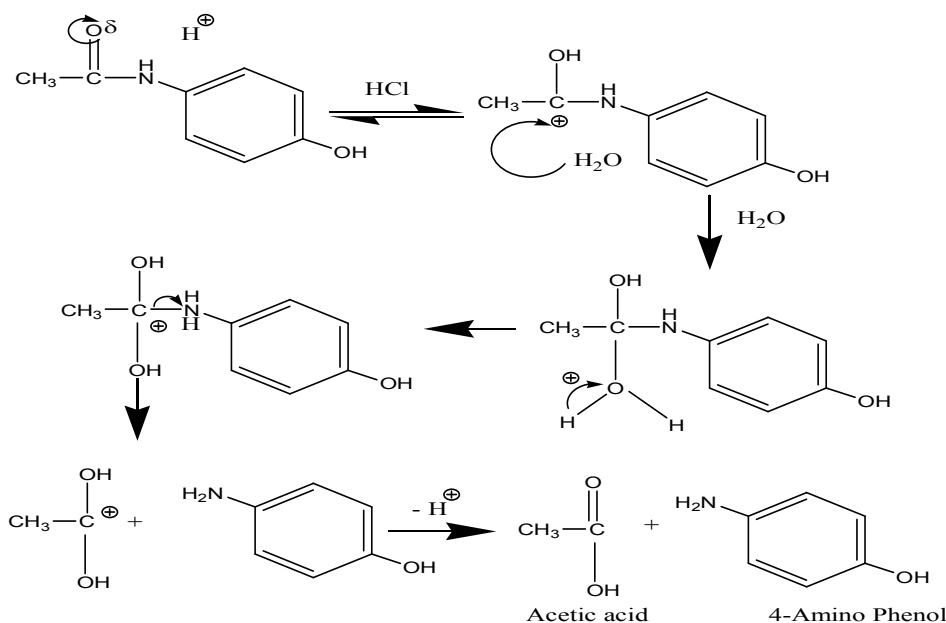
Oxidation degradation study in 30% hydrogen peroxide gave around 7.28 and 3.29% degradants at ambient temperature where as 12.95 and 10.27 % degradants at reflux temperature at end of 3 hr (Fig 4).

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.

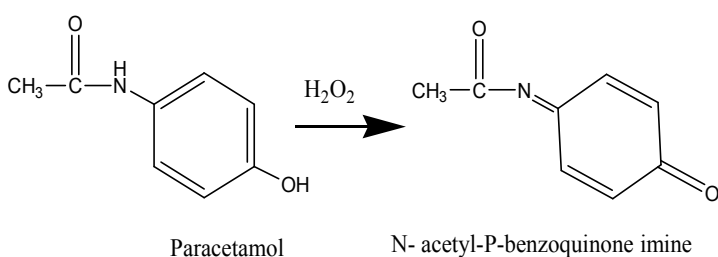
The degradation study reveals great amount of degradation of PCT and ETO. The proposed degradation product of base and acid catalysed degradant is 4- amino phenol where as oxidation degradation leads to N-acetyl-p-benzoquinoneimine product.<sup>16,17</sup>



**Fig 6: Degradation pathway of PCT in 0.1 N alkali**



**Fig 7: Degradation pathway of PCT in 0.1N hydrochloric acid**



**Fig 8: Degradation pathway of PCT in hydrogen peroxide**

## 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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