Application of Stability Indicating HPLC Method for Quantitative Determination of Etoricoxib and Paracetamol in Pharmaceutical Dosage Form

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Abstract

A stability indicating reversed-phase HPLC method has been developed and subsequently validated for simultaneous estimation of etoricoxib (ETX) and paracetamol (PCT) from their combination product. The proposed RP-HPLC method utilizes a Phenomenex® C18, 5µm, 250mm X 4.6mm i.d. column, mobile phase consisting of acetonitrile, methanol and water in the proportion of 60:15:25 (v/v/v) and UV detection at 236.0 nm using a UV detector. Separation was completed within 10 minutes. ETX, PCT and their combination drug product were exposed to thermal (60C), humidity (75% RH), hydrolytic (acidic 1 N HCl for 24 h at 50 °C, alkaline 1 N NaOH kept for 24 h at 50°C) and oxidative stress conditions (3% H_2O_2 for 24h at 50°C), the stressed samples were analyzed by the proposed method. The described method was linear over a range of 8.3-41.5 µg mL^{-1} for PCT and 1-5 µg mL^{-1} for ETX with correlation coefficients values of 0.9999 and 0.9993, respectively. The mean recoveries were 99.69±0.52 and 99.66±1.29 for PCT and ETX, respectively. The proposed method can be useful in the quality control of combination drug products.
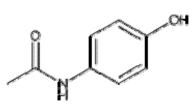
1. Introduction

Etoricoxib [1], (5-chloro-2-(6-methyl pyridine-3-yl)-3-(4-methylsulfonyl phenyl) pyridine) (Fig.1), is a relatively new non-steroidal anti-inflammatory drug with high selectivity in cyclooxygenase-2-inhibitory activity. It is indicated to relieve the signs and symptoms of osteoarthritis, ankylosing spondylitis and acute gouty arthritis. Literature survey reveals that many methods are reported for its estimation alone in biological fluids [2-4] and Pharmaceutical formulations [5-8].

Paracetamol, N-(4-Hydroxyphenyl) acetamide (Fig.2), Literature survey reveals that many HPLC methods are reported for its estimation in combination with other drugs in pharmaceutical formulations [9-12]. But no methods are reported for the stability indicating HPLC analysis in the present drug combination. Hence the present study reports a simple and less time consuming method of analysis.
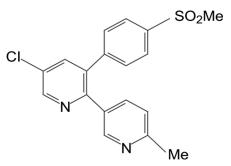
Fig. 1. Structure of Paracetamol

Fig. 2. Structure of Etoricoxib

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enables recommendation of storage conditions, retests periods, and shelf lives to be established. The two main aspects of drug product that play an important role in shelf life determination are assay of active drug, and degradants generated, during the stability study. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines [13] and USP 26 [14]. Although stability indicating methods have been reported for assay of various drugs in drug products, most of them describe assay procedures for drug products containing only one active drug substance. Only few stability indicating methods are reported for assay of combination drug products containing two or more active drug substances. The objective of this work was to develop an analytical LC procedure, which would serve as stability indicating assay method for combination drug product of PCT and ETX in presence of their degradation products formed under thermal, humidity, hydrolytic and oxidative stress conditions.

2. Experimental

2.1. Chemicals and Materials

Paracetamol and Etoricoxib was gift sample from Cadila Pharma. Acetonitrile, methanol (E-Merck Limited) and double distill water was used. All other chemicals used during the experimentation were of analytical grade.

2.2. Instrumentation

Shimadzu 1100 high performance liquid chromatographic system was used for this experiment. Shimadzu 1100 system equipped with binary gradient pump LC10 ADvP, SPD10 UV-VIS detector controlled by spinchrom software and rheodyne manual injector. The Phenomenex ODS C18 column (250 x 4.6mm), 5μm was used as stationary phase.

2.3. Mix Standard solution

Standard stock solutions having concentration of 832 μg mL^{-1} of PCT and 100 μg mL^{-1} of ETX were prepared in methanol. Aliquot potions were diluted with mobile phase to get the final concentration of 16.6 μg mL^{-1} of PCT and 2 μg mL^{-1} of ETX, respectively.

2.4. Construction of Calibration curve

Aliquots of mixed standard stock solution (L) were diluted in range 1.0 mL to 5.0 mL in a 50.0 mL volumetric flask with mobile phase and volume was made up to mark with

mobile phase to obtain concentration ranging from 8.3 - 41.5 µg mL^{-1} for PCT and 1- 5 µg mL^{-1} for ETX.

2.5. Analysis of dosage form

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of tablet powder equivalent to 41.6 mg of Paracetamol (~ 5.0 mg of Etoricoxib) was transferred to 50.0 mL volumetric flask, dissolved by sonication for 30 min in sufficient quantity of methanol, and volume was made up to mark with methanol. The content was filtered through whatman filter paper (no.41). A 10.0 mL portion was diluted to 50.0 mL with mobile phase. A 5.0 mL portion of the solution was further diluted to 50.0 mL with mobile phase. A small portion of sample solution was filtered through 0.45 µm nylon filter and used for injection on HPLC. The results presented in Table 1 indicate the suitability of the method for routine analysis of PCT and ETX from their combination drug products (Fig. 3).

Table 1. Analysis of PCT and ETX by proposed method (n=5)

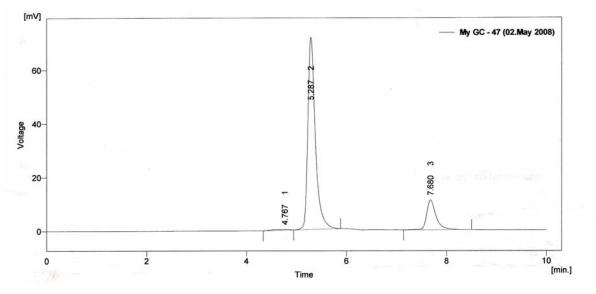| Drug	Labeled Amount (mg)	Amount found (mg)	Assay (%) ± CV (%)
PCT	500	503.450	100.69 ± 0.94
ETX	5	4.995	99.90 ± 1.08

Fig.3 Chromatogram of PCT and ETX from marketed formulation

2.6. Forced degradation studies

Forced degradation of each drug substances and the drug product was carried out under thermolytic, acid/base hydrolytic and oxidative stress conditions. Thermal and photo-degradation of drug substances and drug product was carried out in solid state. After the degradation these solutions were diluted with mobile phase to achieve a concentration of 16.6 µg mL^{-1} of PCT and 2 µg mL^{-1} of ETX (on label claim basis for marketed formulation).

Acid hydrolysis of drug product in solution state was conducted by adding 1 mL of 1N hydrochloric acid. Base hydrolysis of drug product in solution state was conducted by adding 1 mL of 1 N sodium hydroxide solution. For oxidative stress, sample solutions of drug product in 3% hydrogen peroxide, all the samples were kept at 50 °C for 24 h.

For thermal stress, samples of drug product were placed in a controlled-temperature oven at 60 °C for 24 h. For humidity stress, samples of drug product were kept in 75% RH for 24 h.

3. Results and Discussion

3.1 Development of validated stability indicating method

To develop a precise, accurate, specific and suitable stability indicating RP-HPLC method for the simultaneous estimation of PCT and ETX, different mobile phases were employed and proposed chromatographic condition was found appropriate for the quantitative determination in presence of degradation products. The optimum mobile phase consisted of acetonitrile, methanol and water, selected because it was found to ideally resolve the peaks of PCT (*t*R_5.47 min) and ETX (*t*R_7.65 min), with clear line separation in presence of their degradation products at effluent flow rate of 1.0 mL min^{-1}. UV detection wavelength at 236 nm, injection volume 20 μL, ambient temperature for column and HPLC system was found to best for analysis.

3.2.1 Alkaline condition

The drug showed about 20% degradation in 1 N NaOH kept for 24 h at 50 °C. PCT shows the presence of three additional peaks in chromatogram at RT of 3.453, 3.980 and 4.770 respectively as they appear in chromatogram. For ETX, the area percent was increased indicating that drug has undergone some degradation but no additional peaks were seen in chromatogram (Fig. 4)
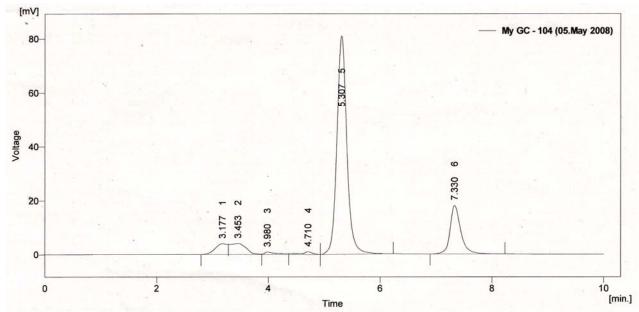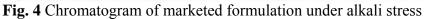
3.2.2 Acidic condition

On treating the drug under 1N HCl for 24h at 50°C, around 20% degradation was seen for PCT while ETX showed slight increase in area percent but there was no corresponding rise in degradation product peaks (Fig. 5)
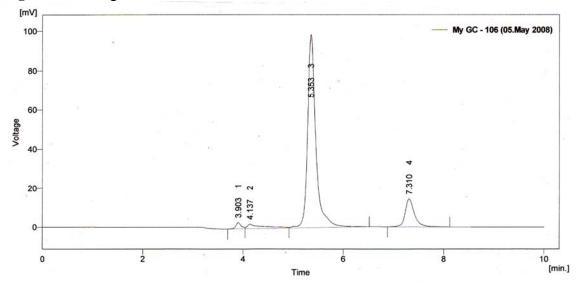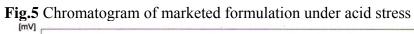
3.2.3 Oxidative studies

Around 10% degradation was seen for PCT while ETX showed slight increase in area percent on exposure of the drug to 3% H_2O_2 for 24h at 50°C, showing that it was stable against oxidative stress (Fig.6)

The proposed mechanism for the hydrolysis or oxidation of the drugs is shown in (Fig. 7)
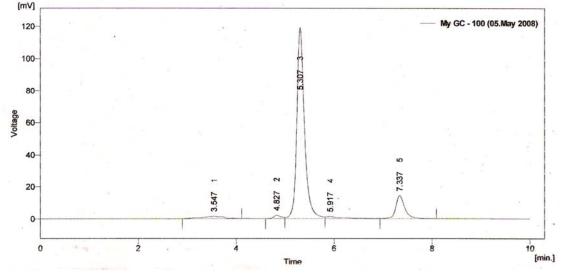
Fig. 4 Chromatogram of marketed formulation under alkali stress

Fig.5 Chromatogram of marketed formulation under acid stress

Fig.6 Chromatogram of marketed formulation under oxide stress

NaOH Hydrolysis

CH3COOH

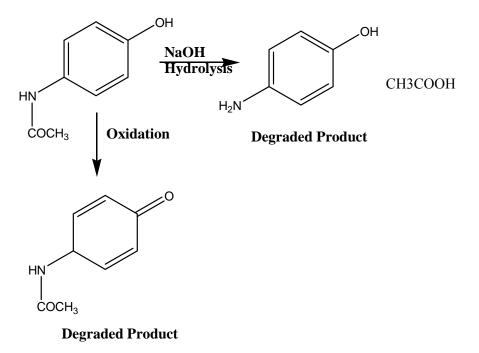**Degraded Product**

Oxidation

Degraded Product

Fig.7 Proposed Mechanism for Hydrolysis (NaOH) and Oxidation stress

3.2.4 Solid state studies

There was no significant degradation of solid PCT and ETX on exposure to dry heat at 60°C for 24h, which indicated that the drug was stable against thermal stress (Fig.8). Stable behavior of both drugs was also observed on exposure of solid drug to the conditions of humidity (75% RH) for 24 h (Fig.9). Both the drug showed increased area percent in above said conditions.
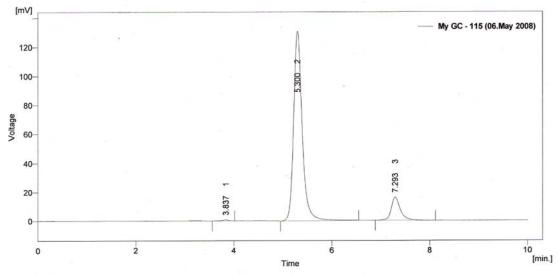

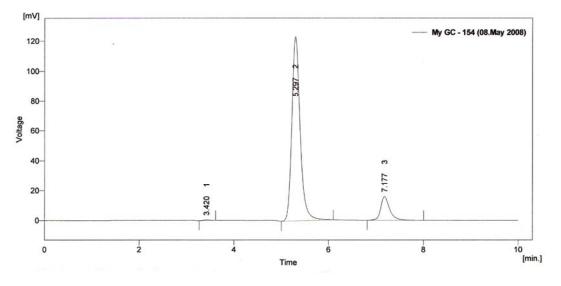**Fig.8** Chromatogram of marketed formulation under Heat at 60°C

Fig.9 Chromatogram of marketed formulation under RH (75%)

3.3. Method validation

The described method has been validated, apart from specificity, for response function, accuracy, and intermediate precision. The nominal concentrations of standard and test solutions for PCT and ETX were 2 µg mL^{-1} and 16.6 µg mL^{-1}, respectively. Response function was determined by preparing standard solution at five different concentration levels ranging form 8.3 to 41.6 µg mL^{-1} for PCT and 1 to 5 µg mL^{-1} for ETX. The correlation coefficients were found to be around 0.9999 for both the drugs. Repeatability of measurements of peak area was carried out using seven replicates of same concentration (16.6 and 2 µg mL^{-1} for PCT and ETX respectively). Characteristic parameters for regression equation and system suitability are given in Table 2.

Accuracy of the method was determined by performing the recovery experiment. This experiment was performed at four levels, in which sample stock solutions were spiked with standard drug solution. Three replicate samples of each concentration level were prepared and the % recovery at each level and mean % recovery were determined Table 3. The mean recovery was 99.69 and 99.66% for PCT and ETX, respectively.

Table 2. Regression characteristics and system suitability parameters

Parameters	PCT	ETX
Retention Time (min)	5.472	7.650
Asymmetry	1.42	1.35
Resolution	---	5.07
Theoretical plates/meter	20542	21813
Linearity range (µg mL^{-1})	8.3-41.6	1-5
Correlation coefficient (r)	0.9999	0.9993

Table 3. Recovery data of the proposed HPLC method

Drug	Level	Amount of pure drug spiked	Amount of pure drug recovered	Recovery (%)
PCT	I	24.4	4.112	100.29
	II	39.9	4.572	100.96
	III	64.3	5.073	101.45
	IV	84.3	5.772	101.27
ETX	I	5.1	5.18	101.56
	II	10.1	10.45	103.46
	III	15.3	15.85	103.59
	IV	20.0	20.40	102.00

Precision of estimation of PCT and ETX by proposed method was ascertained by replicate analysis of homogeneous samples of capsule powder. Intermediate precision of the method was studied by intra- and inter-day variation of the method was carried out. The low % RSD values of within a day for PCT and ETX revealed that the proposed method is precise. A batch of tablets was analysed by two different analysts on different days using proposed method, %RSD values were found to be less than 2% indicating ruggedness of the method.

Robustness of the proposed method was ascertained by deliberately changing the mobile phase composition, detection wavelength and flow rate of the mobile phase. The results of system suitability parameters under deliberate change were found to be well within the range.

4. Conclusion

Based on results, obtained from the analysis of forced degraded samples using described method, it can be concluded that there is no other co-eluting peak with the main peaks and the method is specific for the estimation of PCT and ETX in presence of degradation products and impurities. The method has linear response in stated range and is accurate and precise. The % RSD for ruggedness (analyst to analyst) was found to be 0.386 and 0.374 for PCT and ETX. Though no attempt was made to identify the degradation products, described method can be used as stability indicating method for assay of PCT and ETX in their combination drug product.

5. Acknowledgements

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