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RESEARCH ARTICLE

FORCED DEGRADATION STUDY OF PARACETAMOL IN TABLET FORMULATION USING RP-HPLC

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This study describes the development of stability indicating RP-HPLC method for paracetamol (PCT), an analgesic and antipyretic. In order to investigate the stability of drug, a stress testing of drug sample by exposing it to variety of forced degradation conditions has been recommended. PCT was subjected to stress degradation under different conditions recommended by International Conference on Harmonization (ICH). Stress testing methods are screening methods to be used to understand the degradation chemistry of a drug and therefore do not need to be validated to the extent of final control methods. The sample so generated was used to develop a stability indicating high performance liquid chromatographic method for PCT. The chromatographic separation of PCT and its degradation products was done on C_{18} column. The mobile phase containing mixture of acetonitrile and methanol in ratio 60:40 was found to be most satisfactory at a flow rate of 1 ml/min. Detection was carried out using single wavelength detector at 230 nm.

Key words: Forced degradation study, Paracetamol, RP-HPLC, ICH guideline.

INTRODUCTION

Paracetamol (PCT) (4-hydroxylacetanilide) (**Figure 1**) is used in the treatment of pain and inflammation (Indian Pharmacopoeia, 1996). It is commonly used for the relief of headaches, other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post surgical pain and providing palliative care in advanced cancer patients (SIGN guideline, 2008). Analysis of PCT tablet was reported by UV spectroscopy, HPLC and HPTLC. The UV spectroscopy and RP-HPLC method were also developed for the analysis of PCT in combined dosage form (Likhar and Gupta, 2010; Gupta *et al* 2010; Shukla *et al* 2011). The pharmaceutical products are prone to undergo degradation in various physical and chemical conditions and yield 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. In continuation of efforts made for RP-HPLC/UV spectrophotometric method developments for determination of drugs (Patil *et al* 2011; Prasanthi *et al* 2011; Shah *et al* 2011), the present work was aimed at the development of forced degradation study of PCT in tablet formulation using RP-HPLC as per ICH guideline.

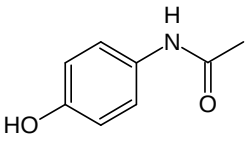

Fig. 1. Structure of paracetamol (PCT)

MATERIALS AND METHODS

Materials

Acetonitrile (HPLC grade), potassium dihydrogen phosphate, orthophosphoric acid, hydrogen peroxide (30%) and HPLC grade water were obtained from Merck Ltd. The tablet formulation was purchased from a local pharmacy. Shimadzu HPLC, LC-20 AD comprising of SPD20A dual wavelength detector and rheodyne injector fitted with 20 microlitre capacity loop was used in present study. Separation and quantitation was done using reverse phase Varian C-18 Phenomenex Luna (250 × 4.6 mm) column.

Methods

RP-HPLC assay procedure for tablets

Chromatographic conditions:

The acetonitrile : disodium hydrogen ortho phosphate in the ratio of 65:35 was used as the mobile phase with a pH of 3.0 which was adjusted by using ortho phosphoric acid. The solution was injected with a flow rate of 1.5 ml/min.

Preparation of standard solution:

A standard solution (1 mg/ml) of paracetamol was prepared in methanol. This solution (0.5 ml) was further diluted to 10 ml with mobile phase.

Preparation of sample:

Ten tablets were weighed and average weight of each tablet was calculated. The tablets were ground to fine powder. Amount of powder equivalent to 100 mg of paracetamol was taken and dissolved in 100 ml of methanol and sonicated for 20 min for the purpose of degassing. From this, 0.5 ml was taken and diluted to 10 ml with mobile phase. The tablet sample solution was injected and chromatogram was obtained. The peak area of the PCT was calculated. Using the regression equations and peak areas of the sample, the amount of PCT in the sample was calculated and finally, the amount of paracetamol per tablet was determined.

Stress degradation of formulation

Preparation of standard solution:

Standard stock solution containing 8 mg/ml of PCT was prepared in separate 100 ml volumetric flask using methanol. A stock solution containing PCT was prepared using methanol. Working solution was prepared by diluting the stock solution mobile phase to contain 0.8-8 μg/ml for paracetamol (**Table 1**). These solutions were used to obtain the calibration graph by plotting peak area *vs* concentration and regression equations were shown (**Figure 2**).

Table 1. Standard values of paracetamol pure drug

Concentration (μg ml^{-1})	Retention time (min)	Area (cm^{-1})
0.8	2.863	31982
1.6	2.602	39096
2.4	2.593	42509
3.2	2.589	49110
4.0	2.594	56639
4.8	2.598	65848
5.6	2.595	73250
6.4	2.600	77694
7.2	2.597	83718
8.0	2.593	93052

Sample preparation:

Twenty tablets of PCT was weighed and powdered. The powder equivalent to 8 mg of PCT was weighed and transferred into 100 ml volumetric flask. Drug extracted into acetonitrile and methanol, vortexed and filtered through a 0.22 μm nylon filter. From this solution further dilutions were made using mobile phase to get a final concentration of 8 μg/ml of PCT. 20 μl of solution was injected into HPLC system to obtain chromatograph for standard drug solutions (n=6) and samples solutions (n=6). Concentrations of PCT in the formulations were calculated by comparing the peak area of sample with that of standard. Forced degradation studies of both drugs were carried out under conditions of acid/base/neutral hydrolysis, temperature conditions and oxidation.

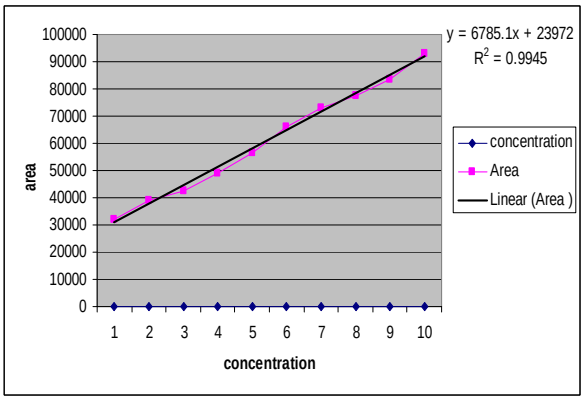

Fig. 2. Standard graph of different conc. of pure paracetamol (PCT)

Acidic degradation studies:
One ml of HCl (0.1 M) was added to 9 ml of drug solution and allowed to stand for 24 h.

Alkali degradation studies:
One ml of sodium hydroxide solution (0.1 M) was added to 9 ml of drug solution and allowed to stand for 24 h.

Oxidative studies:
One ml of hydrogen peroxide solution (3% *v/v*) was added to 9 ml of drug solution. This solution was allowed to stand for 24 h.

Temperature stress studies:
The solution containing drug was maintained at 50°C for 24 h. The solutions were left to equilibrate to room temperature and an aliquot of samples were withdrawn and diluted with mobile phase to get concentration equivalent to 8 µg/ml of PCT. 20 µg/ml solution was injected into HPLC system and analyzed under chromatographic conditions.

RESULTS AND DISCUSSION

Method development

The mobile phase consisting of acetonitrile and disodium hydrogen orthophosphate in composition of (65:35 *v/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 2.594 min for PCT. The analysis was carried at 230 nm in UV where Paracetanol showed good absorbance. The chromatographic analysis time was less than 4 min.

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.

Forced degradation study

The study was carried out by exposure of tablet powder to dry heat at 100°C for 1 h. There were many degradation peak observed in chromatogram with significant degradants of 15.11 and 13.21% for PCT (**Figure 3, 4**). **Table 2** shows the data of degradation study in various experimental conditions. The degradation study revealed great amount of degradation of PCT. The proposed mechanism of degradation of PCT in different experimental conditions was shown in **Figure 5-7**.

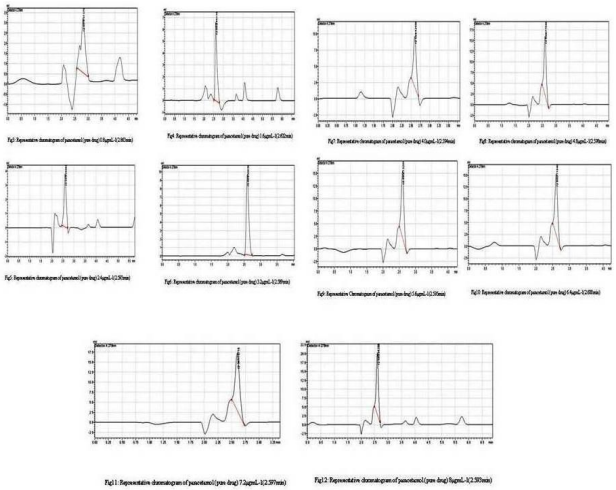

Fig. 3. Standard HPLC chromatograms of different conc. of pure PCT

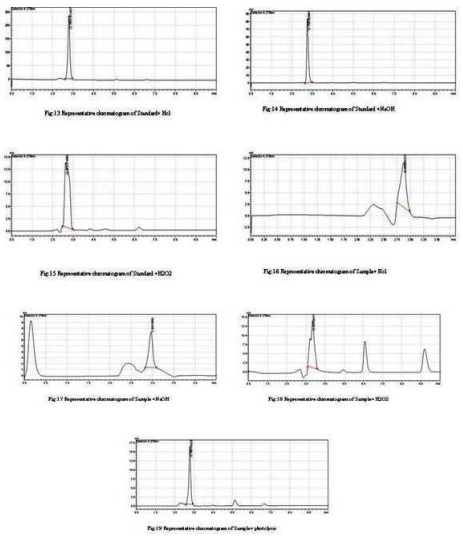

Fig. 4. HPLC chromatograms of the forced degradation samples of PCT in HCl, NaOH, H_2O_2, exposure to light

Table 2. Evaluated parameters of standard and sample under degradation conditions

Degradation conditions	Retention time	Area	Height
Standard + HCl	2.795	1612431	236340
Standard + NaOH	2.758	500841	83232
Standard + H_2O_2	2.633	214654	12969
Sample + HCl	2.869	68273	9600
Sample + NaOH	2.962	38662	6003
Sample + H_2O_2	3.333	189771	12760
Photolysis	2.780	109226	15872

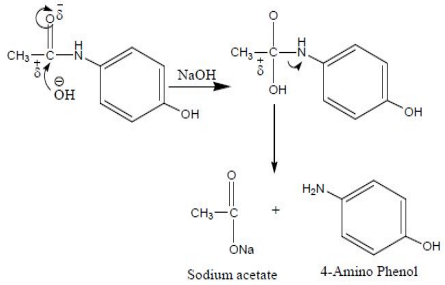

Fig. 5. Degradation pathway of PCT in 0.1 N alkali

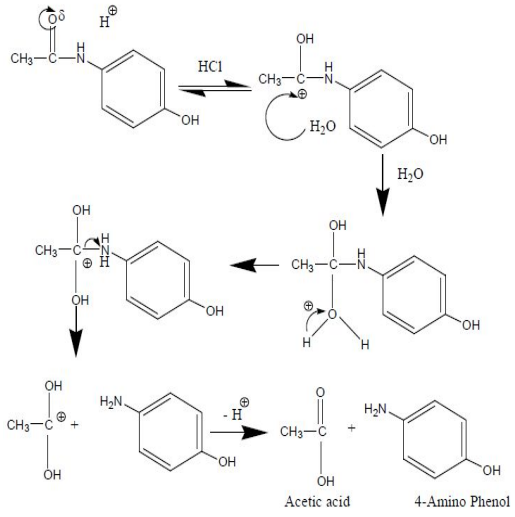

Fig. 6. Degradation pathway of PCT in 0.1 N HCl

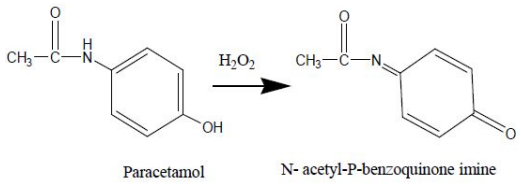

Fig. 7. Degradation pathway of PCT in 0.1 N H_2O_2

The proposed degradation product of the base and acid catalyzed degradants is 4-aminophenol where as the oxidation degradation leads to the *N*-acetyl-*p*-benzo quinone imine product (Vieira *et al* 2003; Cekic *et al* 2005).

CONCLUSION

The proposed method was simple, sensitive, accurate, precise and reproducible and hence can be used for routine analysis of paracetamol. The method was stability indicating and can be used in determination of paracetamol (PCT) in the presence of its degradants. The degradation study in basic and acidic conditions revealed the formation of 4-aminophenol as degradant product and *N*-acetyl-*p*-benzo quinine imine degradant in 30% hydrogen peroxide oxidation.

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