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THE ICH GUIDANCE PRESCRIBED: STRESSED DEGRADATION STUDY OF PARACETAMOL AND DEVELOPMENT OF A VALIDATED METHOD BY UV SPECTROPHOTOMETRY IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract: A simple, accurate, precise and cost effective UV-Visible spectrophotometric method have been developed and validated for the estimation of paracetamol in bulk and pharmaceutical dosage forms and extended towards investigating stress degradation behavior by exposing it to variety of forced degradation conditions recommended by ICH. Degradation was moderate in acidic (25 %- 37 %) and alkaline conditions (23 %-26 %). Complete under oxidative stress while 3.3 % in the thermal conditions; however it stable upon neutral and photolytic exposure. The method was specific to paracetamol and also selective to its degradation products. The proposed method is suitable for quality control of formulation without interference of the excipients.

Keywords: Paracetamol, UV-Visible spectrophotometric, forced degradation, ICH

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INTRODUCTION

Paracetamol (PCM) is a centrally and peripherally acting one of the most commonly used non-opioid analgesic and antipyretic [1]. Its determination in pharmaceutical dosage forms and in biological fluids (overdose monitoring) remains the area of great interest. This makes PCM a drug of choice in the management of many diseases and thus there is requirement for a simple, sensitive, accurate and a precise method for its estimation which also emphasizes on the forced degradation aspects of this active pharmaceutical ingredient (API).

There are many methods available in literature till date pertaining to the assay of PCM in diverse types of samples including single estimation in pharmaceutical preparations, simple titrimetric method, HPLC, potentiometry [2] and simultaneous UV spectrophotometric estimation in combinations has been reported by many analyst. Forced degradation study utilizing UV spectrophotometric method has not been reported in bulk and in pharmaceutical formulation for PCM, henceforth, the purpose of present study was to develop a cost effective UV spectrophotometric method for PCM encompassing all the parameters in accordance with ICH guidelines [3, 4, 5, 6].

MATERIALS AND METHODS:

Materials:

Drug:

Paracetamol was kindly supplied by Triveni Chemicals, Vapi, Gujarat, India and was used without further purification.

Marketed formulation:

Dolo 500 (Micro Labs Ltd, Bangalore, India) was purchased from an open market for this study which contains PCM IP 500 mg. Paracetamol tablets containing 500 mg Paracetamol and the inactive ingredient used in drug matrix were obtained from market.

Reagent and chemicals:

Methanol was used as a solvent which was procured from LobaChemi (Laboratory reagents and fine chemicals Ltd) and HCl, NaOH, and H_2O_2 from Central Drug House (P) Ltd. Highly pure water was prepared by using Millipore Milli Q plus purification system available in the lab facility. All other reagents were of analytical grade.

Instrumentation:

Digital balance: Mettler Toledo, Sonicator: Eneritech (Ultra Sonicator), Photo stability chamber (Thermolab), Hot air oven: Macro Scientific works, Double beam UV-Visible spectrophotometer (Shimadzu-1700) with UV probe 2.31 software.

Methods:

Diluent preparation:

Methanol and water (15:85, v/v) was used as a diluent as per I.P 2010.

Preparation of standard stock solution:

10 mg PCM was dissolved in 15 ml methanol and sonicated for 15 min and final volume was adjusted up to 100 ml with double distilled water. This solution (100 µg/ml) was labeled as standard stock. 5 ml of this standard stock solution was pipetted out and volume was adjusted to 50 ml (10µg/ml) with diluent and filtered through 0.45 mm nylon filter.

Absorption maxima determination:

Standard solution was scanned for PCM in the spectrum mode 400 to 200 nm. λmax of PCM was found to be 244.34 nm [Figure 1]. A standard calibration curve was plotted [Figure 2].

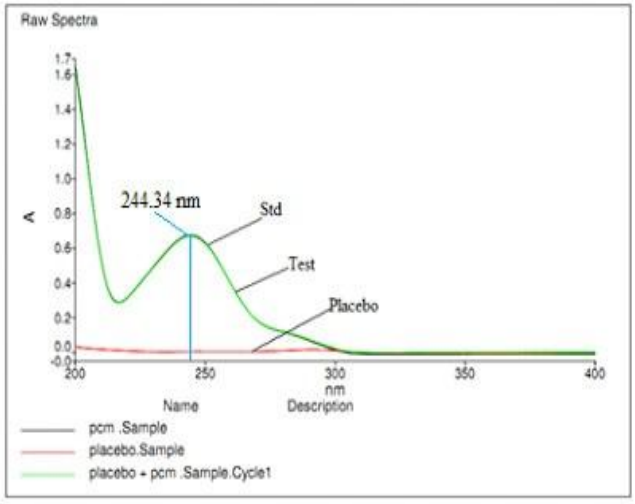

Figure 1: UV showing PCM standard, test sample and placebo

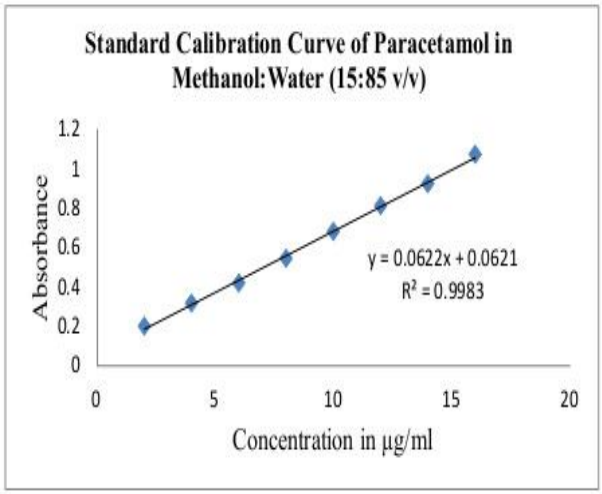

Figure 2 Standard calibration curve of PCM

Test preparation:

10 tablets were weighed and powdered. Powdered tablet equivalent to 100 mg of PCM was weighed and transferred into 100 ml volumetric flask containing 15 ml of methanol and sonicated well for 15 mins thereafter volume was adjusted with 85 ml of distilled water (1000 µg/ml) and subsequently solution was filtered. 1 ml of solution was withdrawn and transferred into 100 ml volumetric flask, adjusted with diluent up to 100 ml (10 µg/ml). The absorbance was measured against blank solution. The drug content in each tablet was estimated by using the standard calibration curve.

Validation of the method:

Linearity:

The aliquots of concentrations ranging from 1- 20 µg/ml were prepared in triplicate. The linearity was evaluated by the least square regression method.

System suitability:

Six replicate reading of standard preparation (10 µg/ml) were taken and %RSD of standard reading were taken for same.

Specificity:

Specificity was assessed by spiking a pre-weighed quantity [7] of PCM with excipients and absorbance was measured to determine quantity of PCM.

Accuracy:

Accuracy was ascertained by known spiking of standard at three different levels (80%, 100%, and 120%) to the sample formulation (10µg/ml).

Precision:

Six replicates, of three different concentrations were scanned within the entire linearity range and % RSD were reported for repeatability (intraday) and intermediate precision (inter-day) [8].

LOD and LOQ:

The limit of detection (LOD) for the lowest concentration of an analyte, while limit of quantification (LOQ) for the lowest concentration of calibration curve was reported [Figure 3].

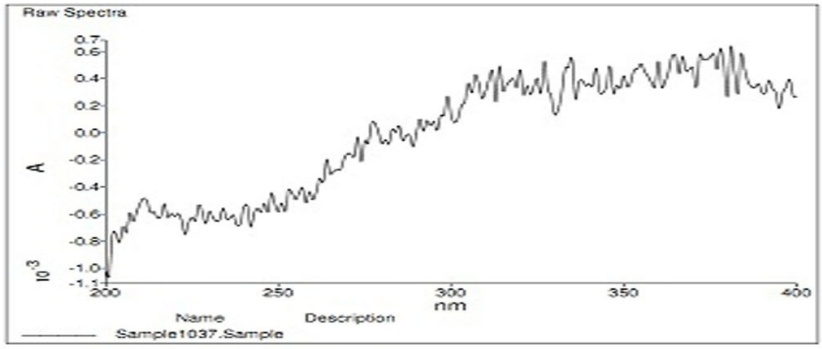

Figure 3 UV Spectrum of Blank

Robustness:

Three replicates, of three different concentrations were scanned by changing the λ max ± 5%. A system change was also done to check the robustness.

Ruggedness (Reproducibility):

The same procedure was carried by another analyst and the results were compared with the previous procedure.

Forced degradation studies:

Acidic and alkaline degradation:

10 mg of bulk drug was accurately weighed and transferred in a 10 ml clean volumetric flask. The drug was solubilized by adding few drops of methanol and then rest volume was made up using 0.1 M HCl, 1 M HCl, 2 M HCl and 5 M HCl respectively. These solutions were refluxed for 6 hrs at 50 °C in a water bath. Initially at 0 h , 0.1 ml of this solution was taken and diluted to 10 ml with diluent, such continuous samplings were done hourly and subsequent absorbance was measured and percentage degradation was calculated in terms of percentage. A similar procedure was repeated using 0.1 N NaOH, 1 N NaOH, 2 N NaOH and 5 N NaOH respectively for estimation of alkali degradation.

Neutral and oxidative degradation:

10 mg of bulk drug was accurately weighed and transferred in a 10 ml clean volumetric flask. The drug was solubilized by adding few drops few drops of methanol and then rest volume was made up using distilled water. This solution was then refluxed for 6 hrs at 50 °C in a water bath and another such sample was kept at 30 °C. Initially at 0 h , 0.1 ml of this solution was taken and diluted to 10 ml with diluent, such continuous samplings were done hourly and subsequent absorbance was measured and percentage degradation was calculated. Similar procedure was repeated using 3% H_2O_2 and 30% H_2O_2, oxidative degradation was estimated.

Thermal degradation:

A specified amount of bulk drug was weighed in a cleaned petridish and placed into the oven at 50 °C for 6 h, hourly 10 mg of bulk drug was transformed into 10 µg/ml solution with diluents and subsequent absorbance was measured and percentage degradation was calculated.

Photolytic degradation:

10 mg of bulk drug was accurately weighed and transferred in a 10 ml clean volumetric flask. The volume was adjusted up to the mark with diluent. This solution was placed in the photostability chamber for 30 days at an illumination point of 7000 lux [10]. Sampling was carried out at 0 h with 0.1 ml of solution diluted to 10 ml with diluent, another at 30^{th} day, subsequent absorbance was measured and percentage degradation was calculated.

RESULT AND DISCUSSION:

Validation of the developed stability-indicating method:

The developed and validated method was aimed to utilize UV spectrophotometric capabilities present in industrial setup [9] for enumerating the stress degradation study along with qualitative and quantitative determination of PCM in pharmaceutical preparations. The absorbance maxima of PCM was found to be 234.34 nm [Figure 1 and Table 1] and

linearity was observed in the concentration range of 2-16 µg ml^{-1} for all validated methods [Table 1]. A percent assay for PCM by above validated methods was found in the range of 98.95–99.80% indicating specificity of the method [Figure 1]. Standard deviation was found to be less than ± 2.0 and the % RSD was found to be less than ±1.0 indicating ruggedness [Table 1], accuracy [Table 2], precision [Table 3], and robustness [Table 4(a) and 4(b)] of the method. The percentage recovery for PCM was found to be in the range of (100.12–101.30) % [Table 2]. The LOD and LOQ were found to be 0.021 and 0.065 respectively [Table 1].

Table 1 Optical Characteristics of PCM

Optical characters*	Values
Absorbance maxima	244.34 nm
Linearity	2-16 µg ml^{-1}
Regression equation (Y*)	0.062x + 0.062
Slope (a)	0.062
Intercept (b)	0.062
Correlation coefficient (r)	0.998
LOD	0.021
LOQ	0.065
Ruggedness	[1] *9.8612 ± **0.0615; ***0.6239 [2] *9.7795 ± **0.0971; ***0.993

Analyst [1]; Analyst [2]; *Calculated concentration (µg ml^{-1}) ± **S.D.; ***RSD % (n = 3)

Table 2 Recovery studies data

Actual Concentration ($\mu g\ ml^{-1}$)	Calculated concentration ($\mu g\ ml^{-1}$) ± S.D.; RSD % (n = 3)	Recovery* (%)
8	8.023 ± 0.028; 0.028	100.12
12	12.261 ± 0.021; 0.021	101.30
16	16.119 ± 0.029; 0.029	100.49

*Results are the percentage recovery after assay of the formulation.

Table 3 Precision Results Showing Repeatability data

Actual Concentration ($\mu g\ ml^{-1}$)	Intra-day measured concentration ($\mu g\ ml^{-1}$) ± S.D.; RSD % (n = 6)	Inter-day measured concentration ($\mu g\ ml^{-1}$) ± S.D.; RSD % (n = 6)
6	6.702 ± 0.055; 0.831	6.091 ± 0.087; 1.43
10	10.045 ± 0.068; 0.676	9.862 ± 0.126; 1.277
14	13.899 ± 0.025; 0.183	14.201 ± 0.124; 0.875

Table 4(a) Robustness data (change of λ_{max})

Actual Concentration ($\mu g\ ml^{-1}$)	Calculated concentration ($\mu g\ ml^{-1}$) ± S.D.; RSD % (n = 3)	
	λ_{max} = 249.34 nm	λ_{max} = 239.35 nm
10	8.413 ± 0.0107; 0.1274	8.539 ± 0.0061; 0.0720
12	10.085 ± 0.0154; 0.152	10.021 ± 0.238; 2.375
14	11.827 ± 0.348; 2.94	11.614 ± 0.109; 0.938

Table 4(b) Robustness data (change of system)

	Calculated concentration (µg ml^{-1}) ± S.D.; RSD % (n = 3)	
Actual Concentration (µg ml^{-1})	Equipment -1: Perkin Elmer (UV/VIS Spectrometer Model-Lambda 25)	Equipment -2: Shimadzu (UV-Spectrophotometer Model-UV-1700)
10	9.8612 ± 0.0615; 0.6239	9.8413 ± 0.0629; 0.6391

Degradation behavior:

The main cause of appearance of impurities in drug substance or product is due to its degradation. The chemical instability of the drug substance under hydrolytic, oxidative, thermal and photolytic conditions which may be encountered during manufacture, isolation, purification, drying, storage, transportation, and/or formulation has been demonstrated as under a variety of ICH recommended test conditions [11, 12].

The drug rapidly degraded in strongly acidic conditions over a short period of time. On heating at 50 °C in 0.1 M (6 h), 1 M (6 h), 2 M (6 h) and 5 M (6 h) HCl, the height of the drug peak decreased, without corresponding rise in a new peak [Figure 4 and Table 5]. Highest degradation was observed with increase in molarity due to catalyzation of ionisable functional groups present in the molecule. The proposed degradation product of the acid catalyzed degradant is 4-aminophenol [17] [Figure 5].

Acidic conditions:

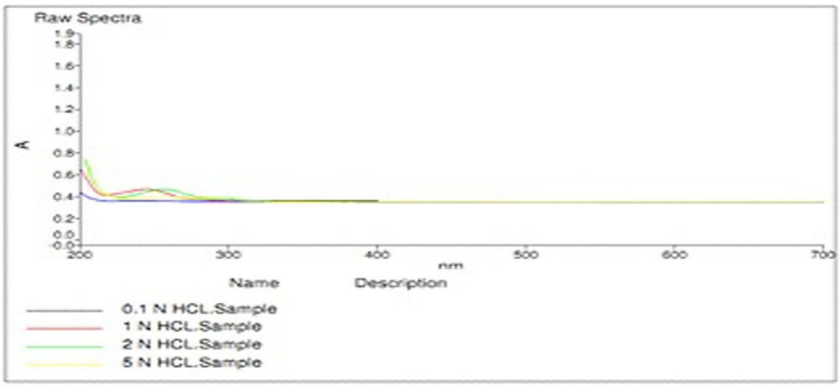

Figure 4 Acid degradation spectrums at 0 hr and at 6 hr

Table 5 Degradation studies data

Name	Time in hr		Absorbance	concentration in µg/ml	% Degradation
Acid	0	---------	0.67481	9.88403226	0
	6	0.1 M HCl	0.51564	7.31677419	25.97379286
		1 M HCl	0.49803	7.03274194	28.84744048
		2 M HCl	0.49786	7.03	28.87518154
		5 M HCl	0.44583	6.19080645	37.36557824
Alkali	0	-------	0.67441	9.87758065	0
	6	0.1 N NaOH	0.53151	7.57274194	23.33404092
		1 N NaOH	0.52826	7.52032258	23.86473114
		2 N NaOH	0.52511	7.46951613	24.37909244
		5 N NaOH	0.51136	7.24774194	26.62432031
Neutral	0	30 °C	0.66561	9.735645161	0
	6	30 °C	0.66593	9.740806452	0.029821
	0	50 °C	0.66561	9.735645161	0
	6	50 °C	0.66553	9.734354839	0.013254
Thermal	0	----------	0.67481	9.88403226	0
	6		0.65432	9.55354839	3.343613844
Oxidative	0	----------	0.67485	9.88467742	0
	6	3% H_2O_2	----------	Complete degradation	Complete degradation
		30% H_2O_2	----------	Complete degradation	Complete degradation

Photolytic	0	----------	0.67311	9.8566129	0
	6		0.67112	9.82451613	0.325636956

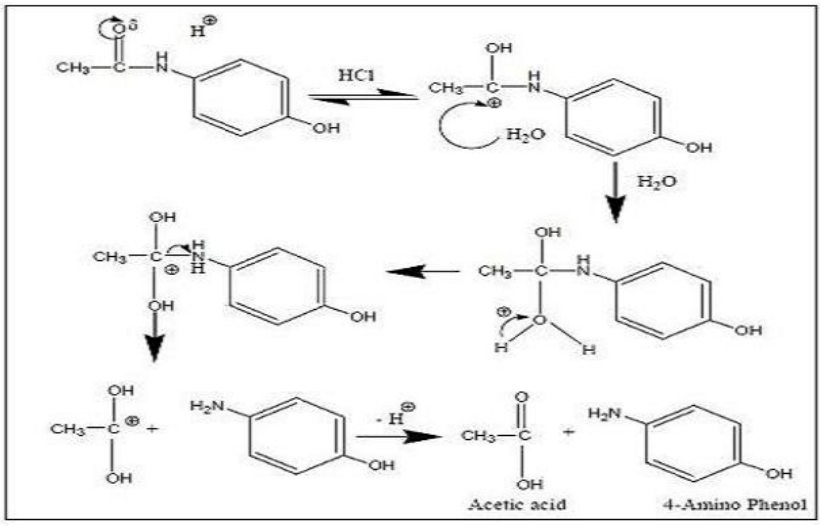

Figure 5 Degradation pathway of PCM in acid [17].

Degradation in alkali:

The drug gets slowly degraded in strongly alkali conditions over a period of time. The lower part of the dip of UV spectra flattened indicating the loss of functionality of certain groups of the compound. The extent of degradation was not so profound upon increasing the normality of the alkali [Figure 6 and Table 5]. The proposed degradation product of the alkali catalyzed degradant is 4-aminophenol [17] [Figure 7].

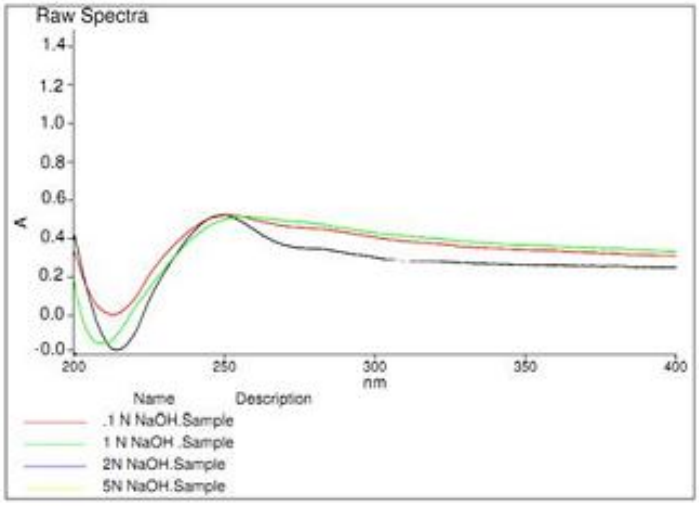

Figure 6 Alkali degradation spectrums at 0 hr and at 6 hr

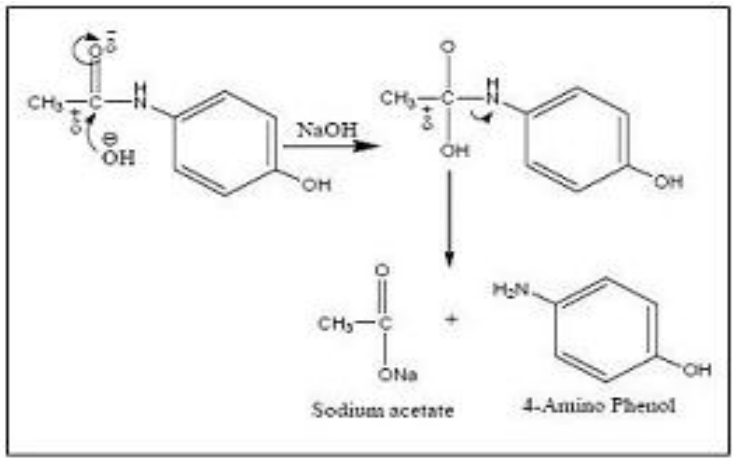

Figure 7 Degradation pathway of PCM in alkali [17].

Neutral condition:

No degradation was seen over a period of 6 h at 30 °C and at 50 °C. There was no loss of the absorption maxima peak of PCM [Figure 8 and Table 5].

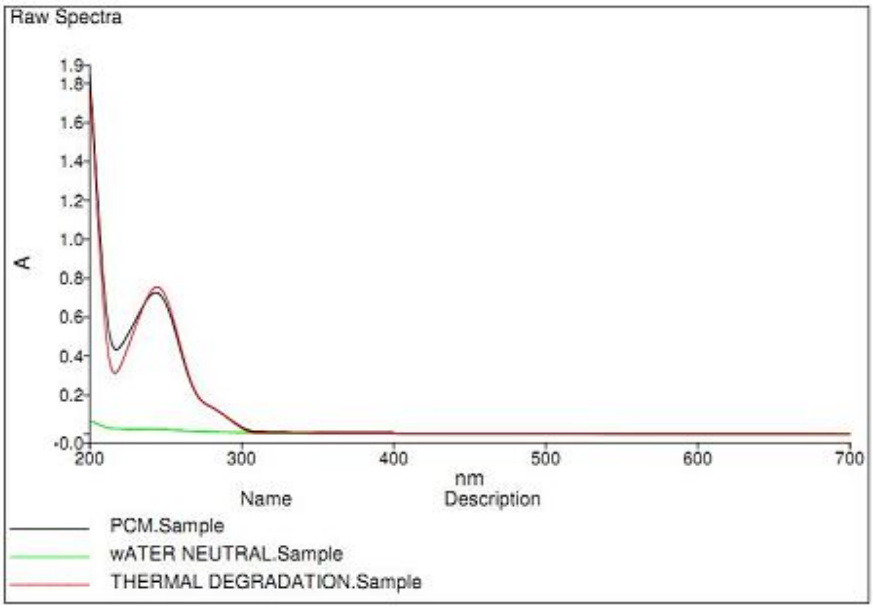

Figure 8 Neutral and thermal degradation spectrum at 0 hr and at 6 hr

Thermal degradation:

There was mild degradation upon thermal stressing of PCM. But there was prominent degradation noticed as the temperature was raised to 70 °C. There was complete loss of the absorption maxima peak and slight shift in the dip was observed [Figure 8 and Table 5].

Oxidative degradation:

The drug was found to degrade in hydrogen peroxide at room temperature. The peaks were also found to disappear with time [Figure 9 and Table 5]. The mechanism of oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations. Amines, sulphides and phenols are susceptible to electron transfer oxidation to give N-oxides, ydroxylamine, sulphones and sulphoxide [13]. Oxidation degradation leads to the N-acetyl-p-benzoquinone imine product [17] [Figure 10].

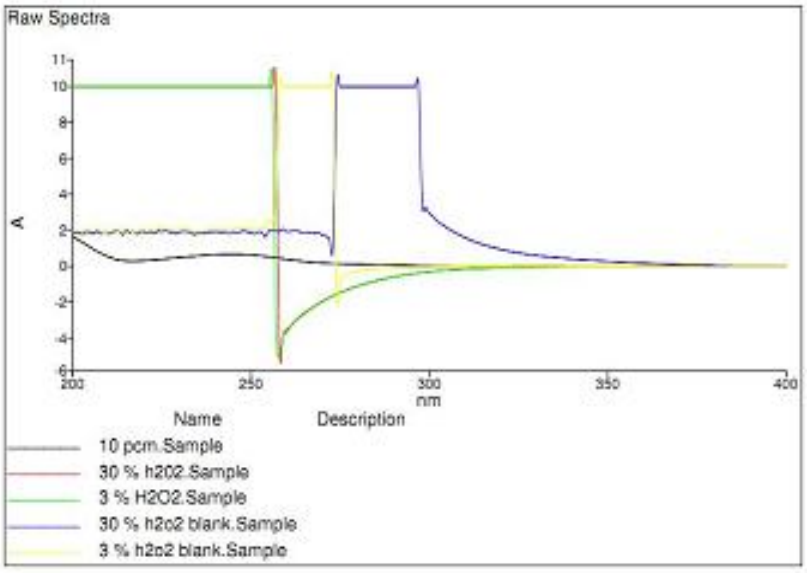

Figure 9 Oxidative degradation spectrums at 0 hr and at 6 hr

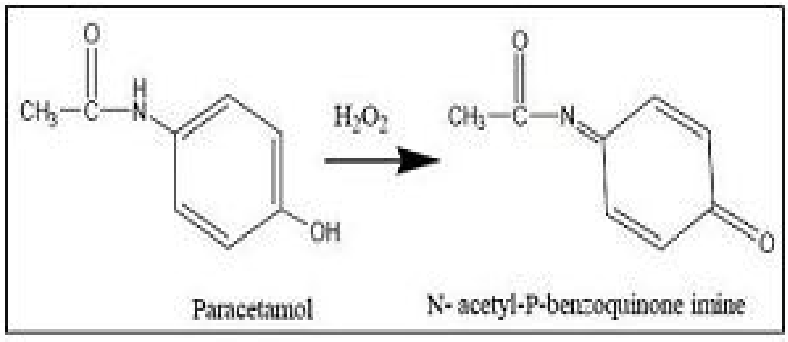

Figure 10 Degradation pathway of PCM in H_2O_2 [17].

Photolytic degradation:

The degradation spectrum suggested fractional degradation of PCM on exposure at 7000 lux for a period of 30 days [Figure 11 and Table 5].

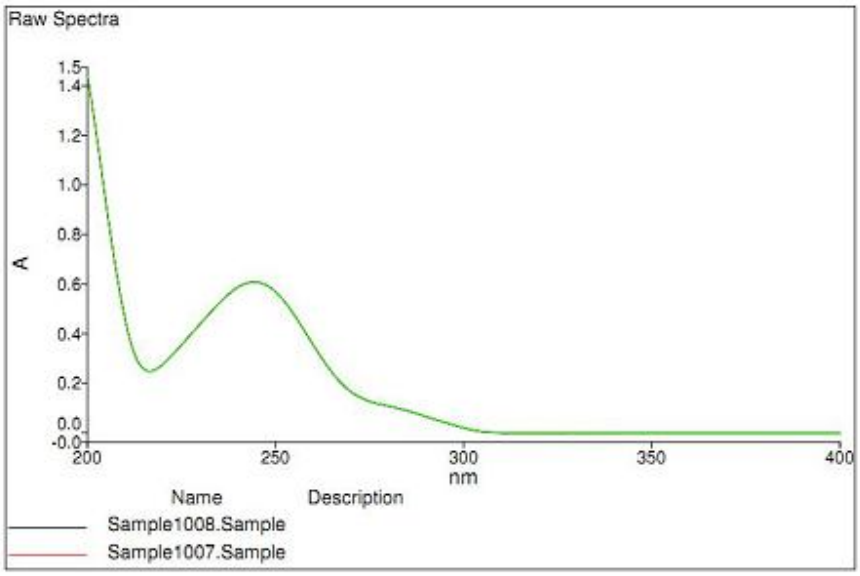

Figure 11 Photolytic degradation spectrums at 0 hr and after 30 days.

CONCLUSION:

This concluding study is a rare example where forced degradation and all parameters of analytical method validation studies were done under various suggested conditions as per ICH. In literature, most of the studies on development of stability-indicating assays involve either forced degradation studies under only one or two conditions [14–16]. The degradation study results showed that the PCM was stable at neutral, thermal and photolytic conditions, while unstable at oxidative stress. There was moderate degradation under acidic and alkaline stress with proposed common degradation product of 4-aminophenol. The developed method is simple, accurate, precise, specific, selective, robust and rugged. The method was specific and selective to degradation products. It is thus, proposed for analysis of the drug and degradation products in stability samples in industry [18].

CONFLICTS OF INTEREST:

Declared none.

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