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Forced Degradation and Stability Indicating Studies for Clomifene Citrate Tablet

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pharmaceutical medications must be stable to preserve their efficacy and safety over the period of their shelf lives. Studies on force degradation are crucial for identifying probable degradation pathways and identifying extreme stresses that may lead to the breakdown of the pharmaceutical product. This report offers a thorough analysis of the force degradation tests performed on Clomifene citrate tablets. Evaluation of the effects of several stress factors, including temperature, humidity, light, and mechanical forces, on the stability of clomifene citrate is the main goal. To replicate actual storage and handling situations, the study used stress testing criteria specified by the International Conference on Harmonization (ICH). According to the study, temperature and humidity have the biggest roles in the degradation of Clomifene citrate tablets. In this article the FDS was performed by using a stainless-steel column (250 mm x 4.6 mm) packed with *Butylsilane C4* (5µm) L26, detector 233nm, mobile phase; mobile Phase was prepared by mixing 550 volumes of methanol, 450 volumes of water and 3 volumes of triethylamine. Then adjust the pH to 2.5 with phosphoric acid. Flow rate; 1 mL/mint. The quantification and hyphenation of the instrumental analysis were successfully accomplished using the developed method.

Keywords: Pharmaceutical medications; clomifene citrate tablets; drug substance; degradation.

1. INTRODUCTION

A synthetic nonsteroidal substance called clomifene citrate has made a name for itself as a leading therapeutic treatment for female infertility. To ensure their therapeutic efficacy and patient safety, Clomifene citrate tablets must maintain their quality and stability throughout their shelf life, as is the case with any pharmaceutical product [1]. The resilience and vulnerability of pharmaceutical formulations under various stress circumstances are critically evaluated by force degradation studies, a type of stress testing [2]. The pharmaceutical sector depends greatly on force degradation studies to evaluate a medicinal substance's stability and potential breakdown mechanisms. It is a systematic study carried out under various stresses to imitate and speed up any degradation processes the drug substance may experience over the course of its shelf life. The study offers useful understanding of the mechanisms of degradation, impurity development, and degradation products, all of which are essential for guaranteeing the drug's safety, effectiveness, and quality [3].

The force degradation study tries to detect any potential degradation pathways and contaminants that may emerge by exposing the medicinal ingredient to stress conditions such as temperature, humidity, light, pH, and oxidative conditions [4]. It aids in comprehending the kinetics of degradation, the creation of degradation products, and the impact of various conditions on the stability of the therapeutic ingredient [5]. The study offers vital information for formula creation, packing issues, and creating suitable storage conditions [6].

The degradation products created throughout the investigation are analyzed and characterized using analytical techniques such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), spectroscopy, and other analytical methods. These methods provide structural clarification, quantification, and identification [7].

2. MATERIALS AND METHODS

2.1 Instruments

The chromatographic HPLC system (Agilent infinity-II 1260 series) was used for the development of stability indicating research and forced degradation for Clomifene Citrate Tablets. Other equipment used were oven, weight balance, Photo stability Chamber and Accelerated Stability Chamber [8].

2.2 Materials Required

Clomifene Citrate (working standard) and Clomifene citrate Tablets were provided by Pacific Pharmaceuticals Ltd. Lahore, Pakistan. HPLC grade methanol were purchased from Mark, Germany. Analytical grade Hydrochloric acid, Hydrogen peroxide, Sodium Hydroxide and Triethylamine were purchased from sigma Aldrich chemicals, Mark Germany [9].

2.3 Chromatographic Conditions

The chromatographic analysis has been performed using a stainless-steel column 250 mm x 4.6 mm packed with *butylsilane* C4 (5µm) L26. The HPLC mobile phase were prepared by mixing 550 volumes of methanol, 450 volumes water and 3 volume Triethylamine [10]. Then the pH was adjusted to 2.5 with H_3PO_4. 233 nm detection wavelength and 25°C column temperature were used. HPLC flow rate was adjusted at 1.0 mL/mint with 50 μL injection volume [11].

2.4 Standard Preparation for Clomifene Citrate (working standard)

In a 100 mL flask, 50 mg of clomifene citrate (the working standard) was accurately weighed, 50 mL of mobile phase was added, and the mixture were sonicated for 15 minutes [12].

2.5 Hydrolysis

Water is reacted with a chemical at varying pH levels in a process known as hydrolysis, which is a typical degradative process [13]. During forced degradation, the drug interacted with the water in both acidic and basic conditions [14].

2.5.1 Acid hydrolysis (0.1 M HCl)

2.5.1.1 Sample solution-1 (No Acid, no base)

20 clomifene citrate pills were crushed to a fine powder. 100 mL of volumetric flask containing 305 mg of powder or 50 mg of clomifene citrate was weighed. After that, 50 mL of mobile phase was added. 15 minutes of sonication produced volume with mobile phase [15].

2.5.1.2 Sample solution-2 (0.1M HCl)

20 tablets of clomifene citrate were taken and fine powder was made. Then 305 mg of powder (equivalent to 50mg of clomifene citrate) was weighed in 100mL of volumetric flask. Then 50mL of 0.1 M HCl was added and sonicated for 15 minutes. Then volume was made with 0.1M HCl [16].

2.5.1.3 Sample solution-3 (0.1M NaOH)

20 tablets of clomifene citrate were taken and fine powder was made. Then 305 mg of powder (equivalent to 50mg of clomifene citrate) was weighed in 100mL volumetric flask. Then 50mL of 0.1 M NaOH was added and sonicated for 15 minutes. Then volume was made with 0.1M NaOH [17].

2.5.1.4 Sample solution-4 (0.1M HCl with No API)

Placebo sample of clomifene citrate tablets on lab scale were prepared by mixing excipients of 20 tablets. Then 255mg of powder (without API) were taken in 100mL flask and 50mL of 0.1M HCl was added and sonicated for 15 minutes. Then volume was made with 0.1M HCl [17].

2.5.1.5 Sample solution-5 (0.1M NaOH with No API)

Placebo sample of clomifene citrate tablets were prepared on lab scale by mixing excipients of 20 tablets. Then 255mg of powder (without API) was taken in 100mL flask and 50mL of 0.1M NaOH was added and sonicated for 15 minutes. Then volume was made with 0.1M NaOH [18].

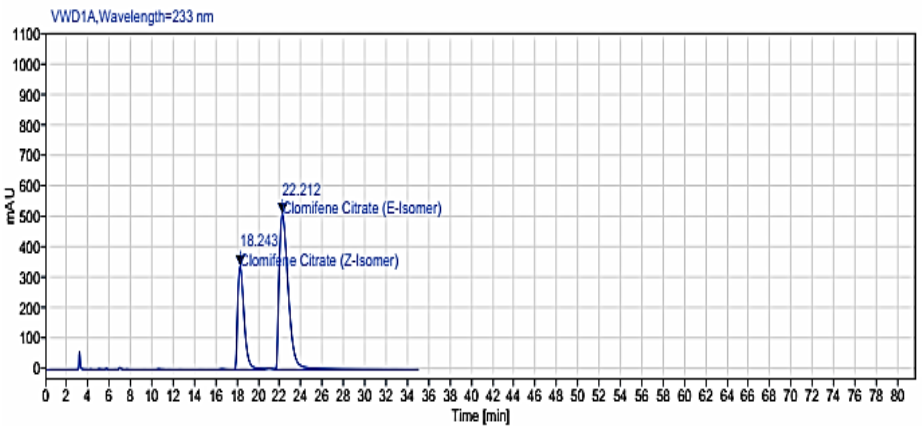

Fig. 1. Chromatogram for Soln-1 on Hydrolysis

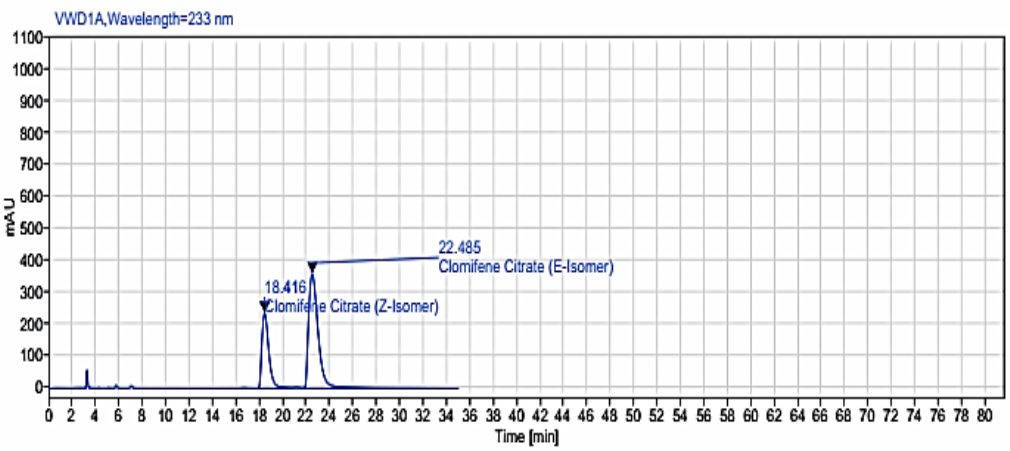

Fig. 2. Chromatogram for soln-2 on hydrolysis

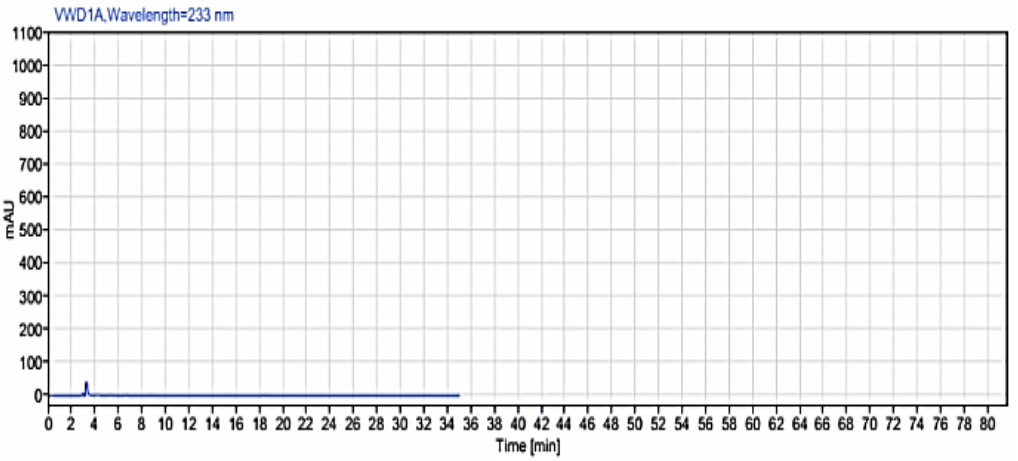

Fig. 3. Chromatogram for soln-3 on hydrolysis

**Comment: No Peak detection was observed because Clomifene Citrate (API) was not soluble in 0.1M NaOH.*

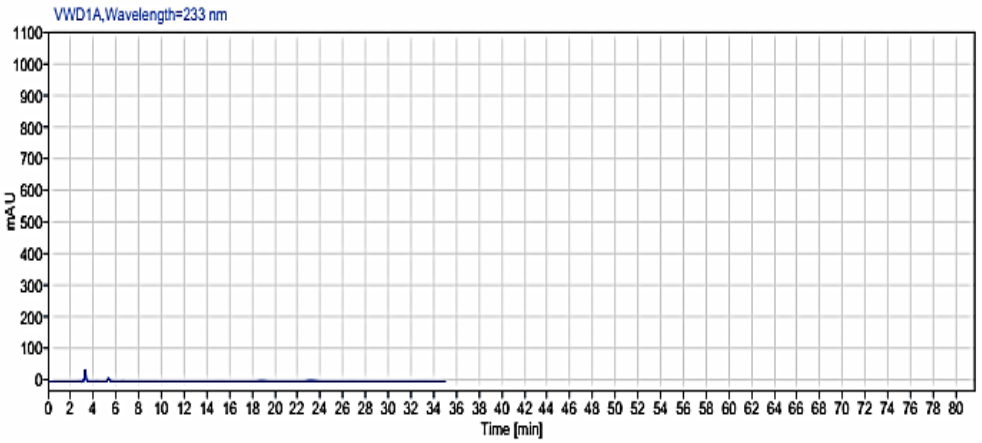

Fig. 4. Chromatogram for soln-4 on hydrolysis

**Comment: No Peak detection was observed because Clomifene Citrate (API) was not added.*

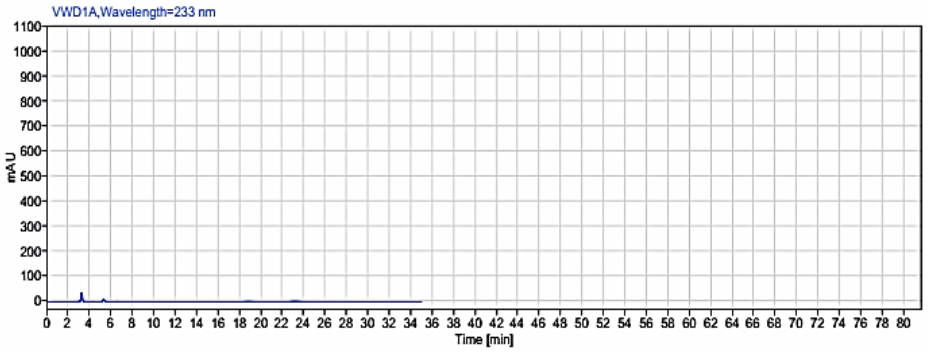

Fig. 5. Chromatogram for soln-5 on hydrolysis

**Comment: No Peak detection was observed because Clomifene Citrate (API) was not added.*

2.5.1.6 Sample solution-6 (0.1M NaOH, API without matrix)

50mg of Clomifene Citrate (working standard) was accurately weighed in 100mL flask. 50mL of 0.1M NaOH was added and sonicated for 15 minutes. Then volume was made with 0.1M NaOH [19].

2.5.1.7 Sample solution-7 (0.1M HCl, API without matrix)

50mg of Clomifene Citrate (working standard) was weighed in 100mL flask. 50mL of 0.1M HCl was added and sonicated for 15 minutes. Then volume was made with 0.1M HCl [20].

2.5.1.8 Sample solution-8 (Neutral pH 7.0)

20 tablets of clomifene citrate were taken and fine powder was made. Then 305 mg of powder (equivalent to 50mg of clomifene citrate) was weighed in 100mL volumetric flask. Then 50mL of phosphate buffer solution (pH 7.0) was added and sonicated for 15 minutes. Then made volume with mobile phase [21].

2.5.1.9 Sample solution-9 (Neutral pH 7.0, API without matrix)

50mg of Clomifene Citrate (working standard) was accurately weighed in 100mL flask. 50mL of phosphate buffer solution (pH 7.0) was added and sonicated for 15 minutes. Then made volume with phosphate buffer solution pH 7.0 [22].

2.5.1.10 Sample Solution-10 (Neutral pH 7.0 with No API)

Placebo sample of clomifene citrate tablets on lab scale was prepared by mixing excipients of 20 tablets. Then 255mg of powder (without API) was taken in 100mL flask and 50mL of buffer solution (pH 7.0) was added and sonicated for 15 minutes. Then made volume with buffer solution pH 7.0 [23].

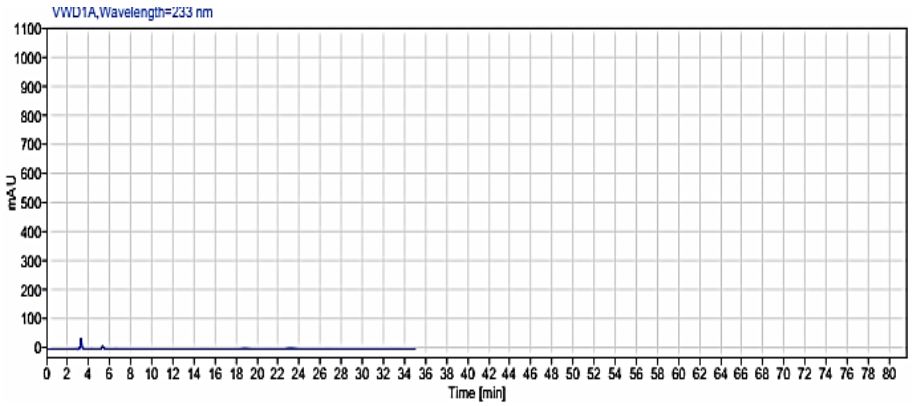

Fig. 6. Chromatogram for soln-6 on hydrolysis

**Comment: No Peak detection was observed because Clomifene Citrate (API) was not soluble in 0.1M NaOH.*

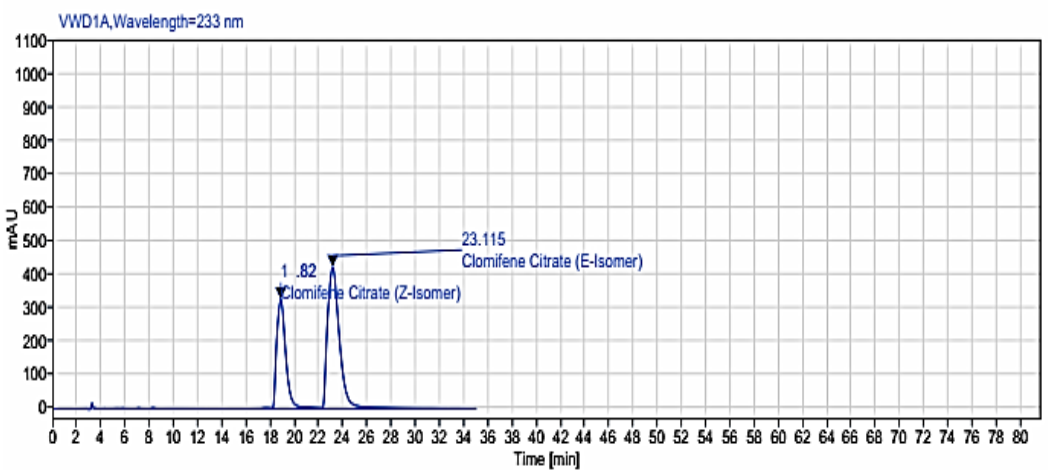

Fig. 7. Chromatogram for soln-7 on hydrolysis

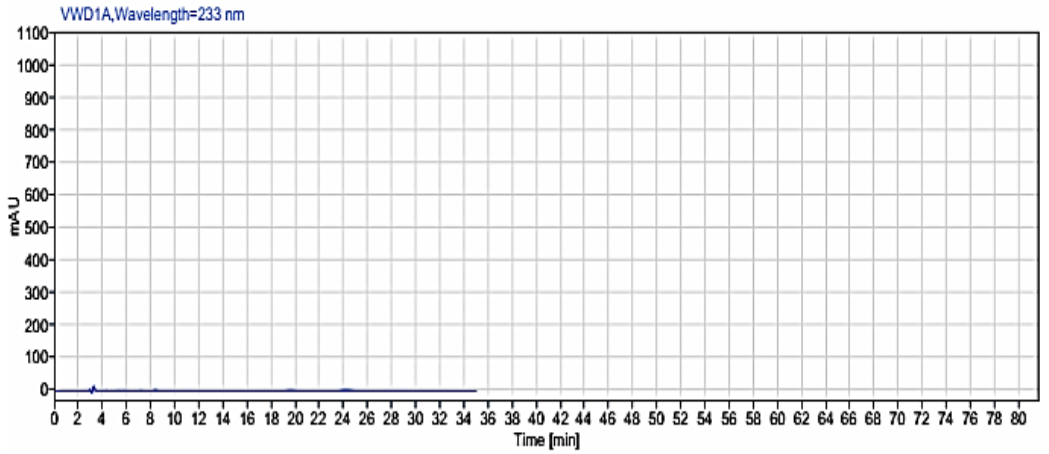

Fig. 8. Chromatogram for soln-8 on hydrolysis

**Comment: No Peak detection was observed because Clomifene Citrate (API) was not soluble in Neutral pH 7.0.*

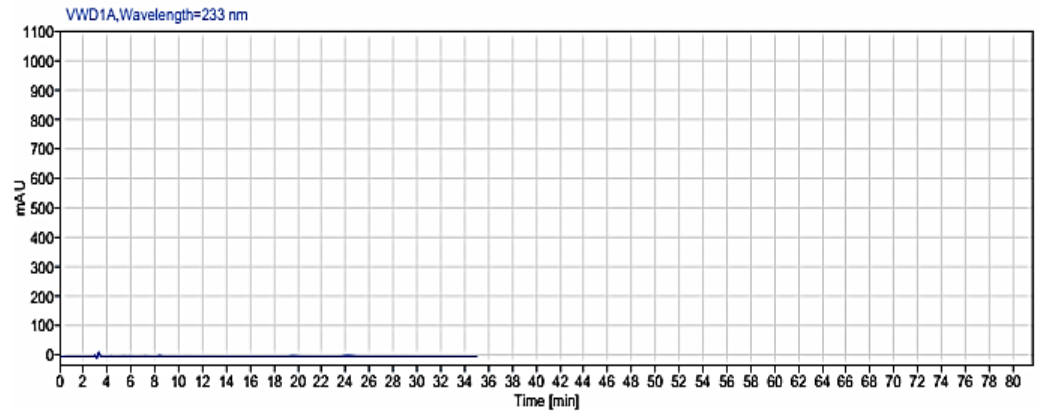

Fig. 9. Chromatogram for soln-9 on hydrolysis

Comment: No Peak detection was observed because Clomifene Citrate (API) was not soluble in Neutral pH 7.0.

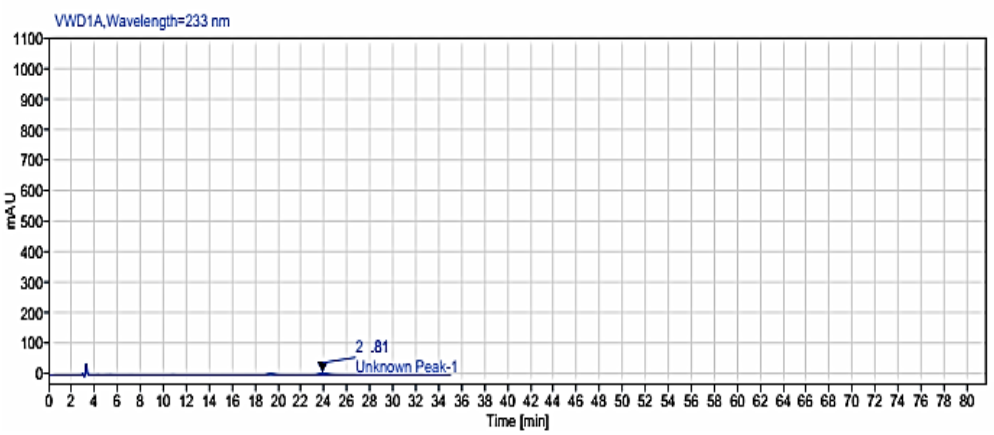

Fig. 10. Chromatogram for soln-10 on hydrolysis

Comment: No Peak detection was observed because Clomifene Citrate (API) was not added

2.6 Oxidation

Hydrogen peroxide is widely used to oxidize the pharmaceutical compounds in forced degradation investigations. 3.0% hydrogen peroxide solution was used for research on oxidation degradation [24].

2.6.1 Sample solution-1 (without Oxidation)

20 tablets of clomifene citrate were taken and fine powder was made. Then 305 mg of powder (equivalent to 50mg of clomifene citrate) was weighed in a 100 mL volumetric flask. Then 50 mL of mobile phase was added and sonicated for 15 minutes. After that made volume of the flask with mobile phase [25].

2.6.2 Sample solution-2 (with Oxidation)

20 tablets of clomifene citrate were taken and fine powder was made. Then 305 mg of powder (equivalent to 50mg of clomifene citrate) was weighed in 100 mL volumetric flask. Then 50 mL of 3.0% v/v H_2O_2 was added and sonicated for 15 minutes [26]. Then made volume with 3.0% v/v H_2O_2.

2.6.3 Sample solution-3- placebo (with Oxidation)

Placebo sample of clomifene citrate tablets on lab scale was prepared by mixing excipients of 20 tablets. Then 255 mg of powder (without API) was taken in 100 mL flask and 50mL of 3.0% v/v H_2O_2 was added and sonicated for 15 minutes. Then made volume with 3.0% v/v H_2O_2. [27]

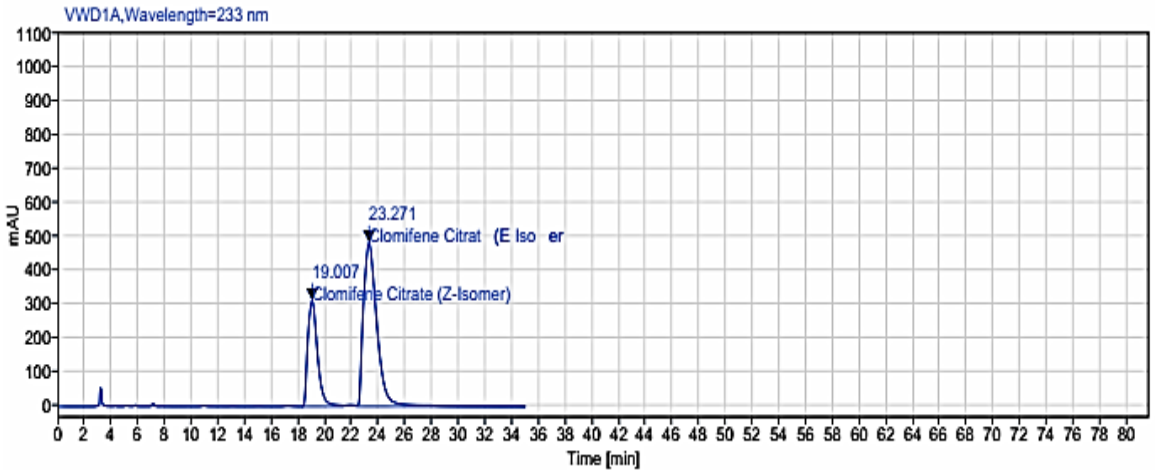

Fig. 11. Chromatogram for soln-1 on oxidation

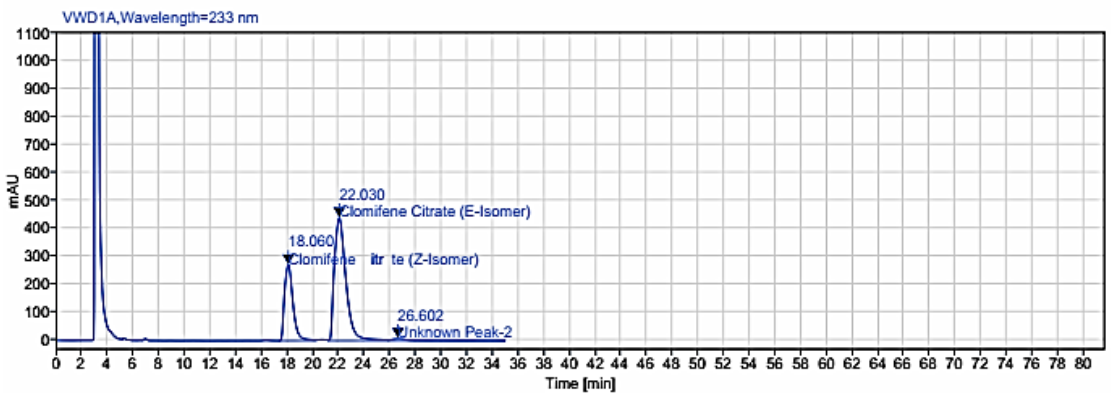

Fig. 12. Chromatogram for soln-2 on oxidation

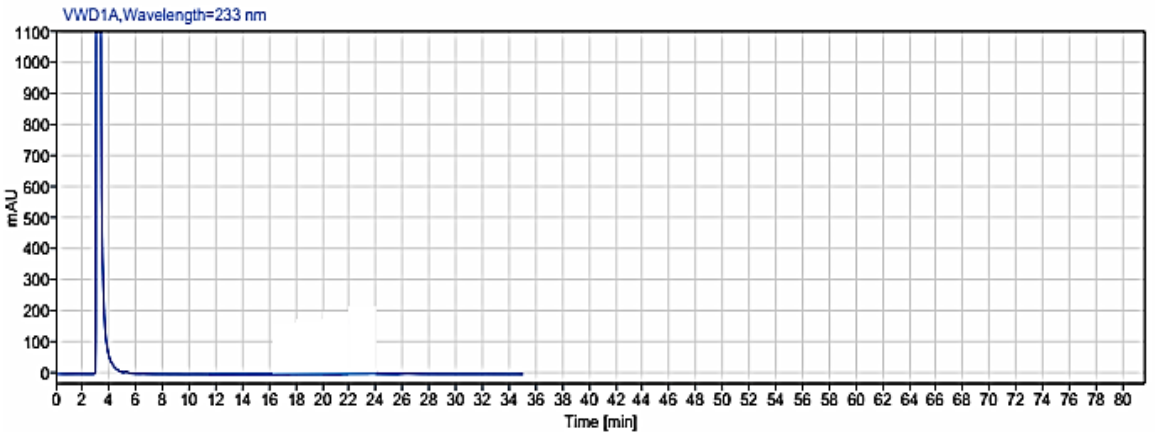

Fig. 13. Chromatogram for soln-3 on oxidation

**Comment: No Peak detection was observed because Clomifene Citrate (API) was not added.*

2.7 Photolytic Degradations

The photo stability analysis of pharmaceutical compounds must be evaluated to demonstrate that exposure to light does not affect in an unacceptably undesirable change [28]. When a pharmaceutical material is exposed to UV or fluorescent light, photo stability studies reveal the main degradants that are created. The range between 300 and 800 nm is considered to be the most common wavelength of light to cause photolytic degradation [29].

2.7.1 Sample preparation-1

The Clomifene citrate tablet samples were exposed to light at a minimum of 200 W h/m2 and 1.2 million lux hours. Fine powder was made out of 20 Clomifene citrate tablets. In a 100 mL volumetric flask, 305 mg of powder (or 50 milligrammes of clomifene citrate) was weighed. 50 mL of mobile phase was added and sonicated for 15 minutes [30].

2.7.2 Sample preparation-2: (API without matrix)

50mg of Clomifene Citrate (working standard) was accurately weighed in 100 mL flask. 50mL of mobile phase was added and sonicated for 15 minutes. Then volume was made with mobile phase [30].

2.7.3 Sample preparation-3 placebo

Placebo sample of Clomifene citrate tablets on lab scale was prepared by mixing excipients of 20 tablets. Then 255 mg of powder (without API) was weighed in 100 mL flask. 50mL of mobile phase was added and sonicated for 15 minutes. Then made volume with mobile phase [31].

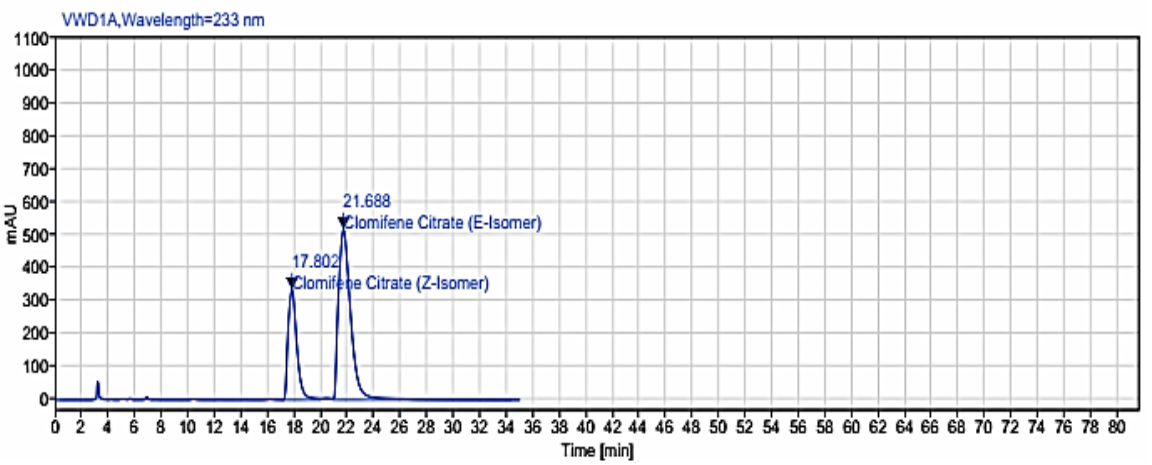

Fig.14. Chromatogram for soln-1 on photo degradation

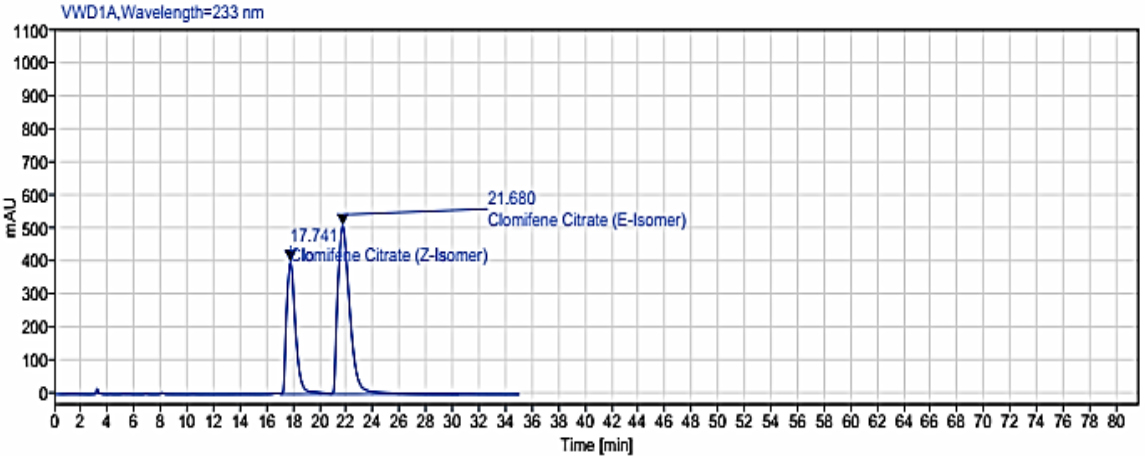

Fig.15. Chromatogram for soln-2 on photo degradation

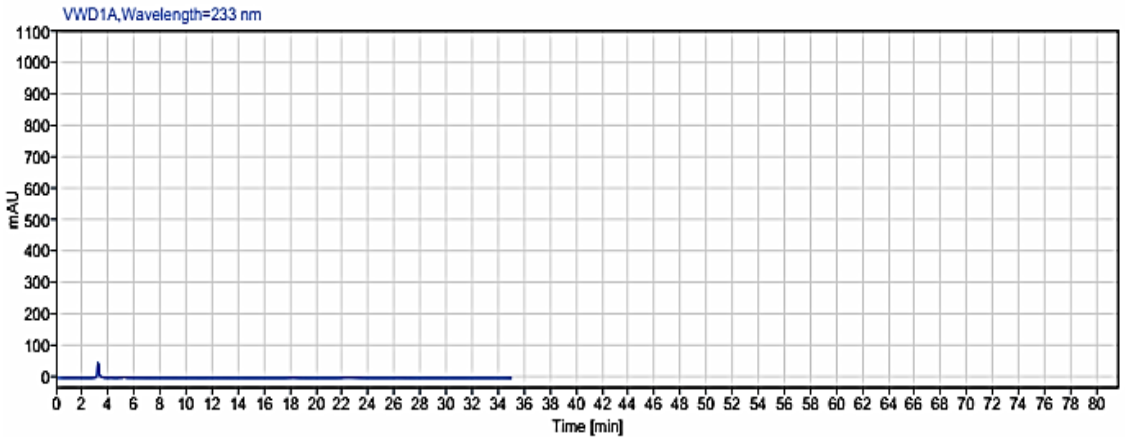

Fig. 16. Chromatogram for soln-3 on photo degradation

**Comment: No Peak detection was observed because Clomifene Citrate (API) was not added.*

2.8 Thermal Degradation

It is best to undertake thermal deterioration under more difficult conditions (such as dry heat and moist heat) [32].

2.8.1 Sample preparations-1

Samples of clomifene citrate tablets were exposed to dry and wet heat to see the effect of temperature and humidity on the product. 20 tablets of clomifene citrate were taken and fine powder was made [33]. Then 305 mg of powder (equivalent to 50mg of clomifene citrate) was weighed in 100 mL volumetric flask. 50 mL of mobile phase was added and sonicated for 15 minutes and made volume with mobile phase [31].

2.8.2 Sample preparation-2: (API without matrix)

50mg of Clomifene Citrate (working standard) was accurately weighed in 100 mL flask. 50mL of mobile phase was added and sonicated for 15 minutes. Then made volume with mobile phase [34].

2.8.3 Sample Preparation-3 Placebo

Placebo sample of Clomifene citrate tablets was prepared on lab scale by mixing excipients of 20 tablets. Then 255 mg of powder (without API) was taken in 100 mL flask. 50mL of mobile phase was added and sonicated for 15 minutes. Then made volume with mobile phase [35].

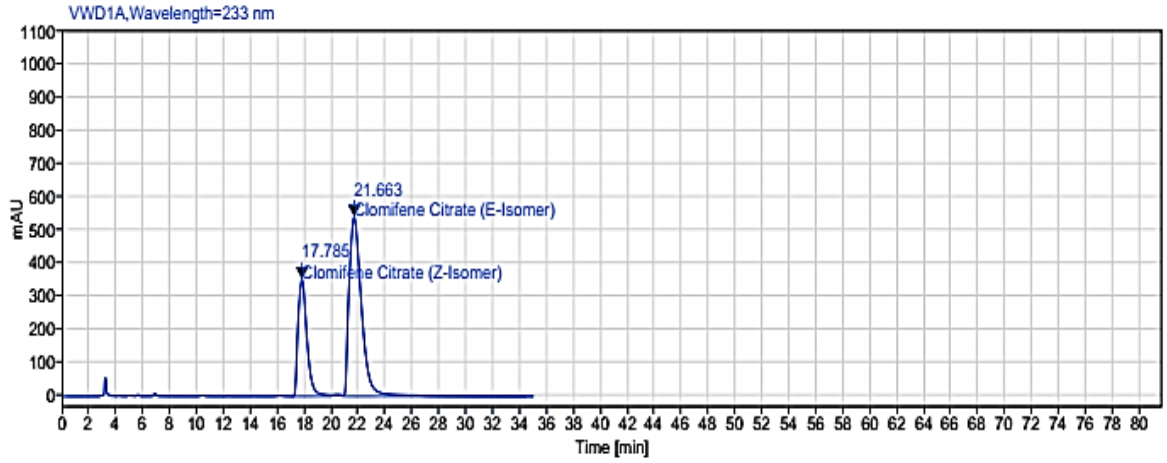

Fig. 17. Chromatogram for soln-1 on thermal degradation

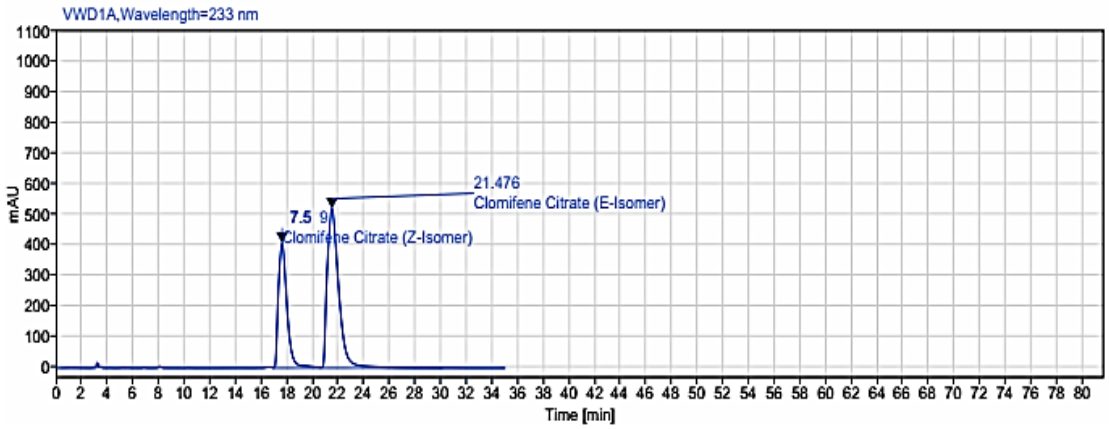

Fig. 18. Chromatogram for soln-2 on thermal degradation

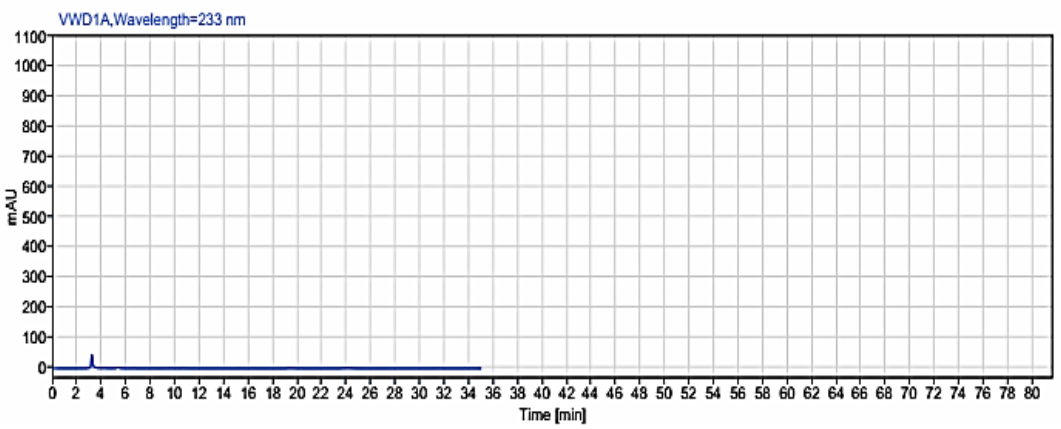

Fig. 19. Chromatogram for soln-3 on thermal degradation
**Comment: No Peak detection was observed because Clomifene Citrate (API) was not added.*

3. RESULTS AND DISCUSSION

3.1 Thermal Stress Analysis

The forced degradation study aimed to evaluate the susceptibility of Clomifene citrate tablets to various stress conditions, including acid, base, oxidation, photolysis, and thermal stress. The results revealed significant degradation under both acidic and alkaline conditions. These findings suggest that Clomifene citrate is sensitive to pH extremes, potentially leading to the formation of acidic and basic impurities during manufacturing and storage processes. The identification of these degradation products is essential in understanding the stability profile of the drug substance and guiding appropriate storage conditions.

Thermal stress testing was conducted to assess the impact of elevated temperatures on Clomifene citrate tablets. The results demonstrated a temperature-dependent degradation pattern, with higher temperatures leading to accelerated degradation. This observation indicates that appropriate temperature control during storage and transportation is crucial in maintaining the drug substance's stability. Additionally, the identification of thermal degradation products through chromatographic analysis provides valuable insights into potential degradation pathways, allowing for the formulation of more stable drug products.

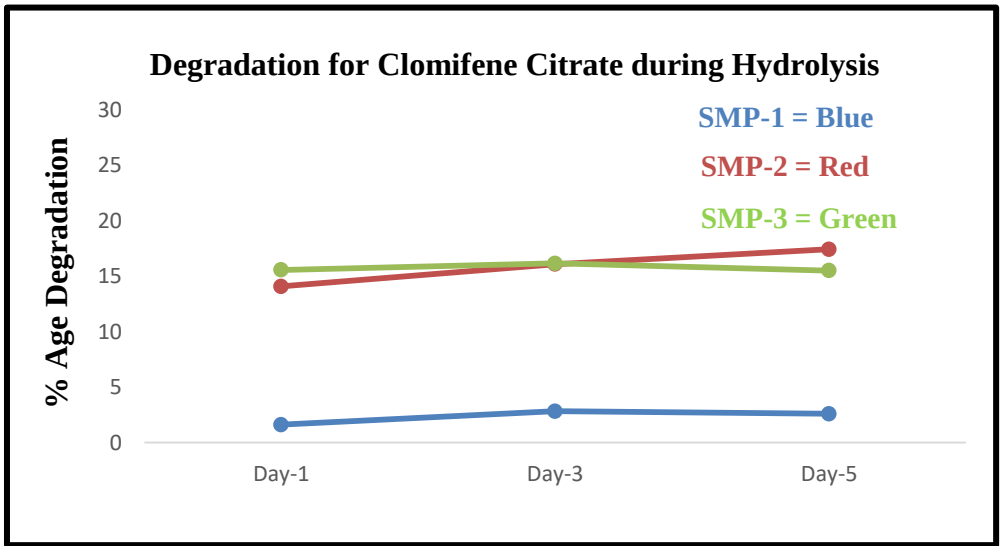

Fig. 20. Graphical representation of degradation for clomifene citrate during hydrolysis parameter

Table 1. Conditions and results used for hydrolysis

Degradation	Solution types	Storage conditions	Sampling interval (Days)	% Age degradation
Hydrolysis	Sample Solution-1	40 °C	1st 3rd 5th	1.62 2.83 2.60
	Sample Solution-2	40 °C	1st 3rd 5th	14.07 16.08 17.42
	Sample Solution-3	40 °C	1st 3rd 5th	- - -
	Sample Solution-4	40 °C	1st 3rd 5th	- - -
	Sample Solution-5	40 °C	1st 3rd 5th	- - -
	Sample Solution-6	40 °C	1st 3rd 5th	- - -
	Sample Solution-7	40 °C	1st 3rd 5th	15.56 16.15 15.50
	Sample Solution-8	40 °C	1st 3rd 5th	- - -
	Sample Solution-9	40 °C	1st 3rd 5th	- - -
	Sample Solution-10	40 °C	1st 3rd 5th	- - -

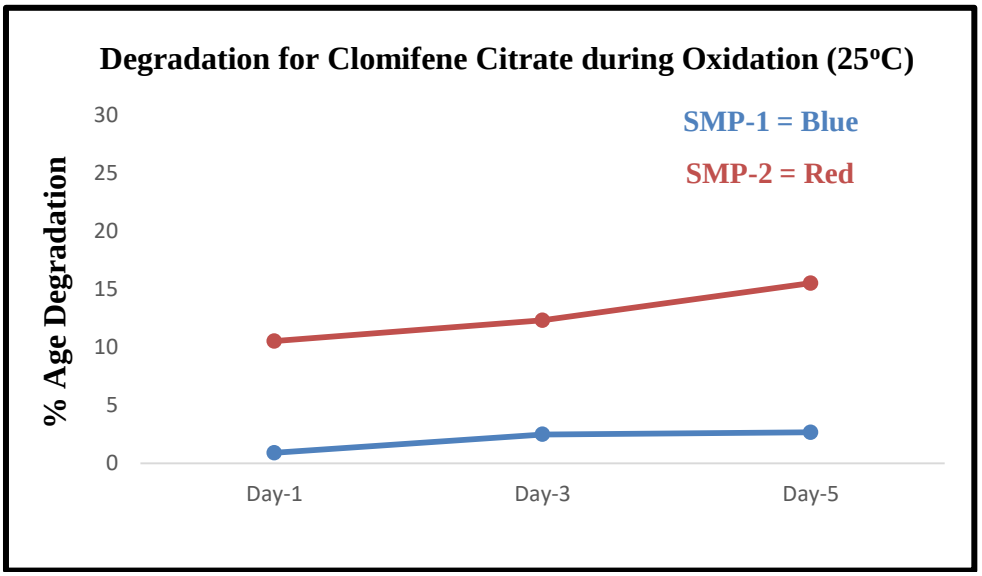

Fig. 21. Graphical representation of degradation for clomifene citrate during oxidation (25°C) parameter

Table 2. Conditions and results used for oxidation (25 °C)

Degradation	Solution types	Storage conditions	Sampling interval (Days)	% Age degradation
Oxidation	Sample Solution-1	25 °C	1st	0.90
			3rd	2.48
			5th	3.66
	Sample Solution-2	25 °C	1st	10.51
			3rd	12.30
			5th	15.50
	Sample Solution-3	25 °C	1st	-
			3rd	-
			5th	-

Table 3. Conditions and results used for Oxidation (40 °C)

Degradation	Solution types	Storage conditions	Sampling interval (Days)	% Age degradation
Oxidation	Sample Solution-1	40 °C	1st	-
			3rd	6.88
			5th	10.88
	Sample Solution-2	40 °C	1st	-
			3rd	16.52
			5th	15.24
	Sample Solution-3	40 °C	1st	-
			3rd	-
			5th	-

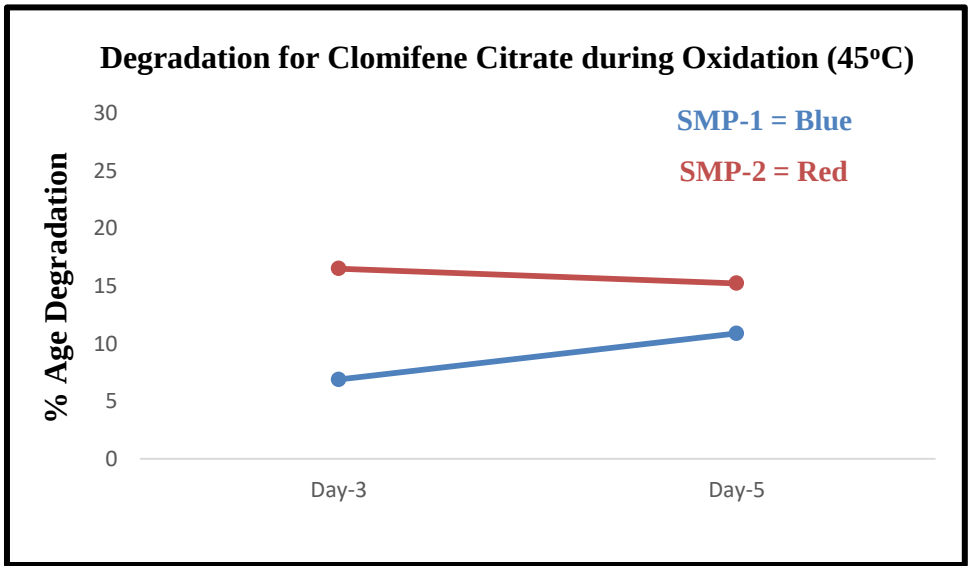

Fig. 22. Graphical representation of degradation for clomifene citrate during oxidation (45oC) parameter

3.2 Photolytic Degradation

The study also investigated the photolytic stability of Clomifene citrate tablets under exposure to UV light. The results revealed a moderate degree of degradation, with the drug substance being susceptible to photolysis. This indicates the need for light-protective packaging during storage to minimize photochemical degradation. The identification of photodegradation products is essential in understanding the drug's photosensitivity and in devising appropriate packaging solutions to ensure product quality and efficacy.

3.3 Stability Indicating Method Development

In the context of the stability-indicating study, a validated high-performance liquid chromatography (HPLC) method was developed to separate and quantify Clomifene citrate from its degradation products. The method exhibited excellent linearity, accuracy, and precision, making it suitable for stability testing and routine quality control. The use of a stability-indicating method is essential in accurately assessing the drug substance's stability over time and determining its shelf life.

Table 4. Conditions and results used for photolysis

Degradation	Solution types	Storage conditions	Sampling interval (Days)	% Age degradation
Photolysis	Sample Solution-1	NLT 1.2 million lux hour	1st	0.99
			3rd	2.98
			5th	3.83
	Sample Solution-2	NLT 1.2 million lux hour	1st	0.80
			3rd	5.04
			5th	10.39
	Sample Solution-3	NLT 1.2 million lux hour	1st	-
			3rd	-
			5th	-

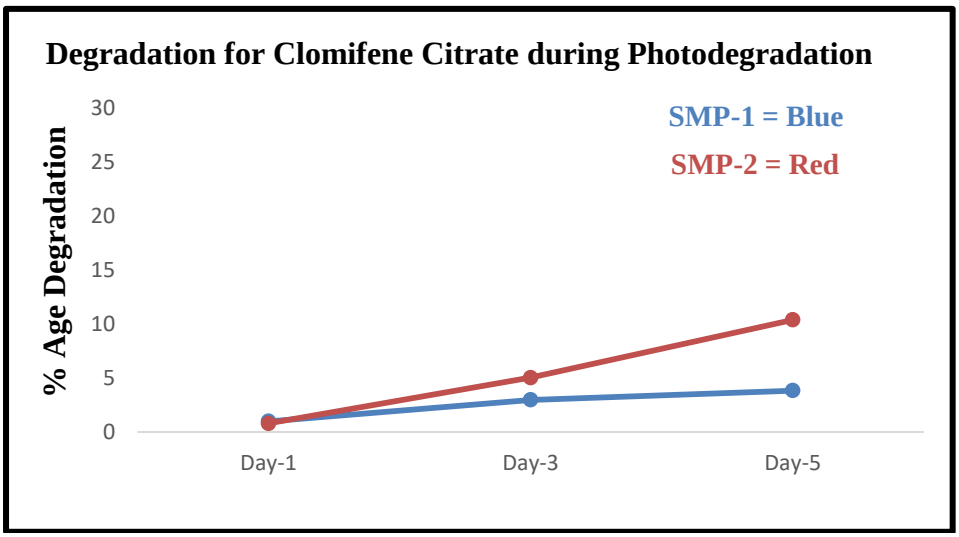

Fig. 23. Graphical representation of degradation for clomifene citrate during photolysis parameter

Table 5. Conditions and results used for Thermolysis

Degradation	Solution Types	Storage Conditions	Sampling interval (Days)	% age Degradation
Thermolysis	Sample Solution-1	50 °C / 75% Humidity	1st	1.79
			3rd	2.03
			5th	3.48
	Sample Solution-2	50 °C / 75% Humidity	1st	1.00
			3rd	4.69
			5th	7.97
	Sample Solution-3	50 °C / 75% Humidity	1st	-
			3rd	-
			5th	-

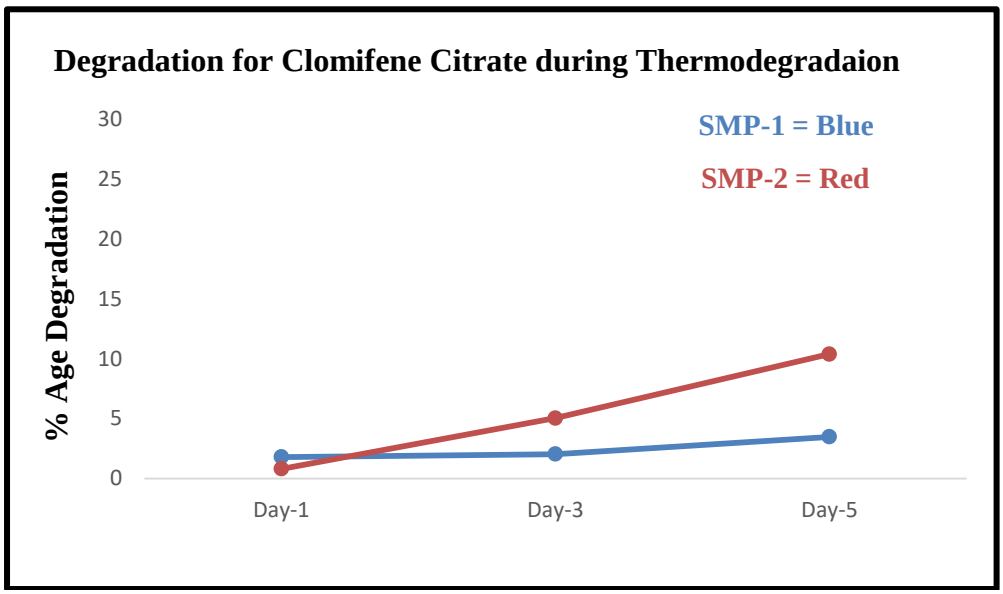

Fig. 24. Graphical representation of degradation for clomifene Citrate during thermolysis parameter

4. CONCLUSION

The Clomifene citrate tablets force degradation experiment provided significant understandings of the stability and likely breakdown mechanisms of the formulation. The study included thorough testing under a range of stress conditions, such as temperature, humidity, light, and pH to demonstrate the tablets' susceptibility to disintegration. According to the study's findings, Clomifene citrate tablets degraded when exposed to acidic and alkaline environments as well as high temperatures. Understanding the stability profile of the formulation and choosing the best storage situations to preserve the consistency and potency of the drug depend heavily on this knowledge. According to the results, it is advised to store Clomifene citrate tablets in a cool, dry location away from harsh light and temperature changes to reduce the risk of product deterioration and assure product quality for the duration of the stated shelf life. Overall, the force degradation study contributed useful information to the knowledge of the stability of Clomifene citrate tablets, aiding in formulation optimization, packaging concerns, and making sure the efficacy and safety of the product.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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