RESEARCH PAPER

Forced Degradation Studies of Oral Thin Film of Betahistine Dihydrochloride

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Abstract

The present study aimed to evaluate the stability profile of Betahistine Dihydrochloride in its pure active pharmaceutical ingredient (API) form and as an Oral Thin Film (OTF) formulation through forced degradation studies under various stress conditions. Degradation studies were performed under acidic (0.1N HCl), alkaline (0.1N NaOH), oxidative (3% H_2O_2), and thermal (100°C) conditions to assess the intrinsic stability of the drug and to establish the stability-indicating capability of the developed analytical method. Samples were analyzed at predetermined time intervals up to 48 hours. The results demonstrated that Betahistine Dihydrochloride is susceptible to all applied stress conditions, with oxidative degradation showing the highest extent of degradation (20.76% for API and 13.23% for OTF at 48 hours). Acidic and thermal stress also produced significant degradation, with 17.64% and 18.23% degradation observed for the API, respectively. Alkaline degradation was comparatively moderate (16.56% at 48 hours). In all stress conditions, the OTF formulation exhibited lower degradation than the pure API, indicating a protective effect of the polymeric film matrix. Chromatographic analysis confirmed clear separation of degradation products from the parent drug peak, demonstrating the specificity and stability-indicating nature of the analytical method. Overall, the study concludes that Betahistine Dihydrochloride is particularly susceptible to oxidative and thermal stress, while the OTF formulation offers improved stability compared to the pure drug. These findings emphasize the importance of appropriate storage conditions and provide essential data for formulation development and stability assessment in accordance with ICH guidelines.

Keywords: Betahistine dihydrochloride; Oral thin film; Forced degradation; Stability-indicating HPLC; Oxidative degradation; ICH guidelines

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I. Introduction

Betahistine dihydrochloride is a histamine analogue widely prescribed for the management of Ménière's disease, vertigo, and vestibular disorders. It acts primarily as an H_1 receptor agonist and H_3 receptor antagonist, thereby improving microcirculation in the inner ear and reducing endolymphatic pressure. Due to its short biological half-life (approximately 3–4 hours) and extensive first-pass metabolism, frequent dosing is often required to maintain therapeutic efficacy. These limitations highlight the need for alternative drug delivery systems that enhance patient compliance and ensure rapid onset of action.[1-4]

Oral thin films (OTFs) have emerged as a novel drug delivery platform designed to disintegrate rapidly when placed on the tongue, releasing the drug for buccal or sublingual absorption. OTFs offer several advantages, including improved patient compliance (particularly in geriatric and pediatric populations), avoidance of first-pass metabolism (in part), rapid drug release, accurate dosing, and ease of administration without water. For drugs like Betahistine dihydrochloride, which are indicated for vertigo where nausea and difficulty in swallowing are common, oral thin films represent a clinically meaningful formulation strategy.[5-6]

Despite formulation advancements, the stability of pharmaceutical products remains a critical determinant of their safety, efficacy, and shelf life. Drug degradation can occur through various chemical pathways such as hydrolysis, oxidation, photolysis, and thermal decomposition. The formation of degradation products not only reduces therapeutic potency but may

also result in toxic or irritant impurities. Therefore, understanding the intrinsic stability of both the active pharmaceutical ingredient (API) and the finished dosage form is essential during formulation development.[7-9]

Forced degradation studies, also referred to as stress testing, are systematic stability investigations performed under exaggerated conditions to accelerate chemical degradation. According to International Council for Harmonisation (ICH) guidelines Q1A(R2) and Q1B, stress testing helps in:

- Identifying likely degradation pathways
- Establishing degradation mechanisms
- Developing and validating stability-indicating analytical methods
- Determining degradation products
- Supporting formulation development and packaging selection

Typically, stress conditions include acidic and alkaline hydrolysis, oxidative degradation (commonly using hydrogen peroxide), thermal stress, and photolytic exposure. The goal is to achieve controlled degradation, generally in the range of 10–20%, which is sufficient to generate detectable degradation products without complete destruction of the drug substance. This range ensures that the analytical method can distinguish the intact drug from its degradation products while maintaining measurable assay values.

Betahistine dihydrochloride, being a dihydrochloride salt of a histamine analogue, may be particularly susceptible to hydrolytic and oxidative stress due to the presence of amine functional groups. Additionally, the polymeric matrix and plasticizers used in oral thin film formulations may influence the stability profile of the drug. Interactions between excipients and the API under stress conditions can either enhance stability or accelerate degradation. Therefore, it is essential to compare the degradation behavior of the pure API with that of the formulated oral thin film.[10-11]

Stability-indicating High-Performance Liquid Chromatography (HPLC) plays a crucial role in forced degradation studies. A properly validated HPLC method must demonstrate specificity, peak purity, resolution between degradation products and the main drug peak, and acceptable mass balance. Photodiode array (PDA) detection further ensures the absence of co-eluting impurities and confirms the method's stability-indicating capability.

Although Betahistine dihydrochloride has been extensively studied for its pharmacological efficacy, limited literature is available regarding its systematic forced degradation behavior in oral thin film dosage forms. Understanding its degradation kinetics under acidic, alkaline, oxidative, and thermal conditions is essential to predict long-term stability and to optimize formulation strategies.[12]

Therefore, the present study aims to:

1. Evaluate the intrinsic stability of Betahistine dihydrochloride under hydrolytic, oxidative, and thermal stress conditions.
2. Compare degradation behavior between the pure API and the oral thin film formulation.
3. Establish the stability-indicating capability of the validated HPLC analytical method.
4. Identify the most critical degradation pathways affecting formulation stability.

The outcomes of this study provide valuable insights into degradation mechanisms, formulation robustness, and quality assurance of Betahistine dihydrochloride oral thin films. Such data are essential for regulatory submission, product development, and ensuring patient safety throughout the product's intended shelf life.[13-14]

II. Materials and Methods

Materials

The active pharmaceutical ingredient (API) used in this study was obtained as a working standard with certified purity (>99%). Hydrochloric acid (HCl), sodium hydroxide (NaOH), and hydrogen peroxide (H_2O_2, 30% w/v) of analytical reagent (AR) grade were procured from a certified chemical supplier. Methanol and acetonitrile (HPLC grade) were used as diluents and mobile phase components. Potassium dihydrogen phosphate and orthophosphoric acid were used for preparation of buffer solutions. Ultrapure water was obtained from a Milli-Q purification system.

All chemicals and reagents were used without further purification. Glassware was calibrated prior to use.

Methodology for Forced Degradation Studies[15-17]

Forced degradation studies were carried out to evaluate the intrinsic stability of the drug substance and to establish the stability-indicating capability of the analytical method. Stress studies were conducted under hydrolytic (acidic and alkaline), oxidative, and thermal conditions in accordance with ICH Q1A(R2) and Q1B guidelines. The objective was to achieve controlled degradation in the range of approximately 10–20%, sufficient to generate degradation products without complete destruction of the drug.

1. Hydrolytic Degradation Studies

Hydrolytic stress testing was performed under acidic and alkaline conditions to evaluate susceptibility of the drug to pH-mediated degradation.

Acidic Degradation

An accurately weighed quantity of the drug (equivalent to 10 mg) was transferred into a 10 mL volumetric flask and dissolved in a small volume of methanol. The volume was adjusted with 0.1 N HCl to obtain a final concentration of 1000 µg/mL. The solution was kept at room temperature (25 ± 2°C) for 24 hours.

If significant degradation (>20%) was observed at 0.1 N HCl, the acid strength was reduced (e.g., 0.01 N HCl) or exposure time shortened. In case of negligible degradation, the sample was refluxed at 60°C for 2–4 hours to accelerate hydrolysis.

After completion of the stress period, the solution was neutralized using 0.1 N NaOH, diluted appropriately with mobile phase, filtered through a 0.45 µm membrane filter, and analyzed using the validated stability-indicating HPLC method.

Alkaline Degradation

Similarly, 10 mg of drug was dissolved and made up to volume with 0.1 N NaOH. The solution was stored at room temperature for 24 hours.

If complete degradation was observed, the alkali strength was reduced or the reaction temperature was lowered. After the specified time, the solution was neutralized with 0.1 N HCl, diluted suitably, filtered, and analyzed.

Hydrolytic degradation is highly influenced by pH and temperature, as both parameters affect the rate constant (k) of the reaction.

2. Oxidative Degradation Studies

Oxidative stress studies were performed using hydrogen peroxide as the oxidizing agent.

An accurately weighed quantity of drug (10 mg) was dissolved in methanol and treated with 3% w/v hydrogen peroxide to obtain a final concentration of 1000 µg/mL. The solution was stored at room temperature for 24 hours protected from light.

Depending on the extent of degradation, the concentration of hydrogen peroxide was varied between 0.1% and 3% w/v. The target degradation level was maintained at 10–20%.

After the stress period, the solution was diluted appropriately with mobile phase, filtered through a 0.45 µm membrane filter, and analyzed.

Oxidative degradation involves electron transfer mechanisms resulting in formation of reactive intermediates such as free radicals, anions, or cations, leading to structural modification of the drug molecule.

3. Thermal Degradation Studies

Thermal degradation studies were performed to evaluate the effect of elevated temperature on the stability of the drug substance.

Approximately 50 mg of the drug was placed in a clean, dry glass Petri dish and kept in a hot air oven maintained at 60°C ± 2°C for 24–72 hours. Additional studies were performed at 40°C and 80°C to assess temperature-dependent degradation.

For long-term thermal stress, samples were stored at 70°C for up to 7 days under both dry and humid conditions (75% RH).

After completion of the stress period, samples were dissolved in suitable diluent to obtain a concentration of 1000 µg/mL, filtered, and analyzed.

The rate of thermal degradation was interpreted using the Arrhenius equation:

$$K = Ae^{-E_a/RT}$$

Where:

- **K** = specific reaction rate constant
- **A** = frequency factor
- **Ea** = activation energy
- **R** = gas constant (1.987 cal/mol·K)
- **T** = absolute temperature (Kelvin)

Thermal degradation may proceed through mechanisms such as pyrolysis, hydrolysis, decarboxylation, isomerization, rearrangement, or polymerization. Extreme temperatures (>80°C) were avoided where non-representative degradation pathways could occur.

4. Sample Analysis

All stressed samples were analyzed using a validated stability-indicating HPLC method. Separation was achieved on a C18 column using an appropriate mobile phase at a specified flow rate (e.g., 1.0 mL/min) and detection wavelength corresponding to maximum absorbance (λmax) of the drug.

Peak purity was assessed using a photodiode array detector to confirm the absence of co-eluting impurities. Mass balance was evaluated to ensure that the sum of assay and degradation products was close to 100%.

III. Results and Discussion

Forced Degradation Studies

Acid degradation-

Acid decomposition studies were performed by refluxing 1ml of stock solution was transferred in to 10ml of volumetric flask. 2 ml of 0.1N HCL solutions was added and mixed well and put for 6 hrs at 70ºC 250ml round bottom flask. After time period the content was cooled at RT. Then the volume was adjusted with diluents to get 1mg/ml for Betahistine Dihydrochloride.

Table No. 1: Acid Degradation Data of Betahistine Dihydrochloride API and OTF

Acid Degradation		
Time Frame	**% Degradation (Betahistine Dihydrochloride API)**	**% Degradation (Betahistine Dihydrochloride OTF)**
Initial (0 hr)	0 %	0 %
3 hrs	1.34 %	2.75 %
6 hrs	4.53 %	3.53 %
12 hrs	5.64 %	5.45 %
24 hrs	11.22 %	8.12 %
48 hrs	17.64 %	13.64 %

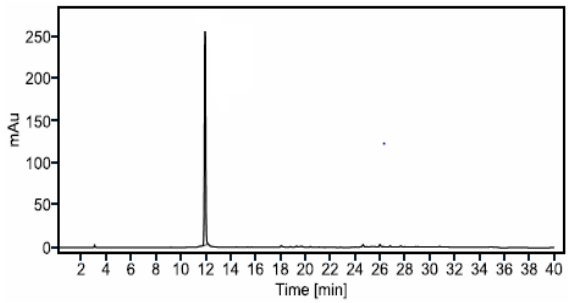

Fig. 1: Chromatogram of Acid Degradation of Betahistine Dihydrochloride API at 48 hr

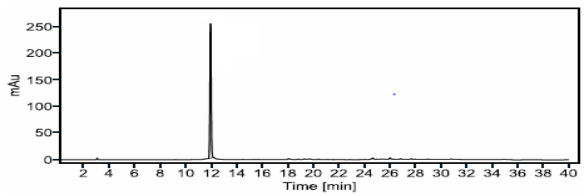

Fig. 2:Chromatogram of Acid Degradation of Betahistine Dihydrochloride OTF at 48 hr

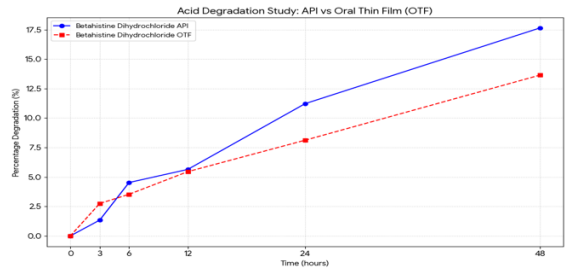

Fig. 3: Schematic Curve of Acid Degradation of Betahistine Dihydrochloride

The observed degradation rates may be influenced by various factors, including the composition of the OTF, pH of the acid used, and storage conditions. Further studies or experiments might be required to investigate the reasons behind the differences in degradation between the API and the OTF. This could involve analyzing the specific components in the OTF and their interaction with the acidic environment. Based on these results, appropriate storage and handling recommendations can be made to ensure the stability of Betahistine Dihydrochloride products, particularly the OTF formulation, which seems to be more sensitive to acidic conditions.

Base degradation-

Base decomposition studies were performed by refluxing 1ml of stock solution was transferred into 10ml of volumetric flask. 2 ml of 0.1N NaOH solutions was added and mixed well and put for 4 hrs at 70ºC 250ml round bottom flask. After time period the content was cooled to RT. Then the volume was adjusted with diluents to get 1 mg/ml for Betahistine Dihydrochloride.

Table No. 2 : Base Degradation Data of Betahistine Dihydrochloride API and OTF

Base Degradation		
Time Frame	**% Degradation (Betahistine Dihydrochloride API)**	**% Degradation (Betahistine Dihydrochloride OTF)**
Initial (0 hr)	0 %	0 %
3 hrs	1.42%	1.75 %
6 hrs	2.56%	1.34 %
12 hrs	3.23%	3.24 %
24 hrs	4.54 %	5.75 %
48 hrs	16.56 %	15.34 %

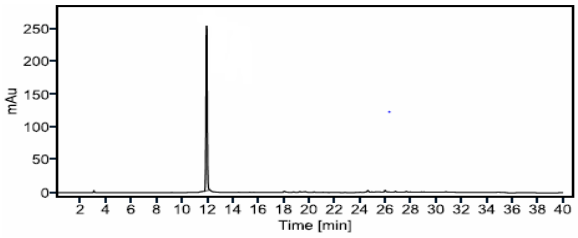

Fig. 4 :Chromatogram of Base Degradation of Betahistine Dihydrochloride API at 48 hr

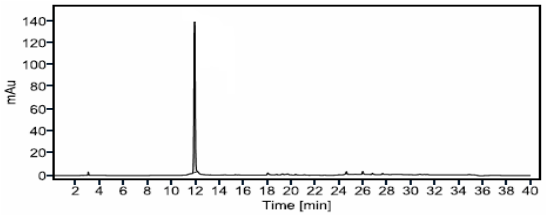

Fig. 5: Chromatogram of Base Degradation of Betahistine Dihydrochloride OTF at 48 hr

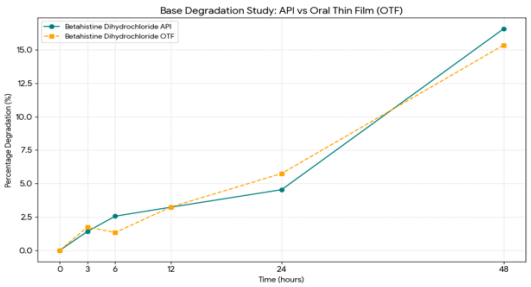

Fig. 6: Schematic Curve of Base Degradation of Betahistine Dihydrochloride

In summary, this data demonstrates that the Betahistine Dihydrochloride API and the OTF formulation are both susceptible to base degradation. While the OTF initially appears to offer some protection against degradation in the early hours, this effect diminishes over time. The API degrades faster, but the differences become less pronounced as time progresses. These findings highlight the importance of proper storage and handling of both the API and the OTF to ensure their stability and efficacy over their shelf-life. Further studies may be needed to explore the causes of degradation and to develop strategies for improving the stability of both the API and the OTF.

Oxidation degradation-

Oxidative decomposition studies were performed by refluxing 1ml of stock solution was transferred in to 10ml of volumetric flask. 2ml of 3% H_2O_2 solutions was added and mixed well and put for 6 hrs at 70ºC 250ml round bottom flask. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 1 mg/ml for Betahistine Dihydrochloride.

Table No. 3 Oxidative Degradation Data of Betahistine Dihydrochloride

Oxidative Degradation		
Time Frame	**% Degradation (Betahistine Dihydrochloride API)**	**% Degradation (Betahistine Dihydrochloride OTF)**
Initial (0 hr)	0 %	0 %
3 hrs	1.65 %	1.76 %
6 hrs	3.34 %	2.23 %
12 hrs	6.65 %	3.44 %
24 hrs	11.23 %	7.73 %
48 hrs	20.76 %	13.23 %

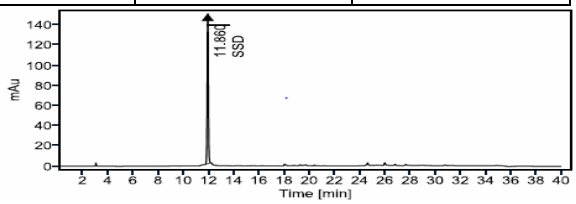

Fig. 7:Chromatogram of Oxidation Degradation of Betahistine Dihydrochloride API at 48 hr

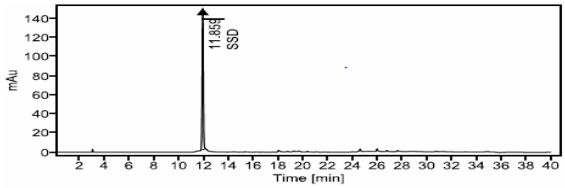

Fig. 8 :Chromatogram of Oxidation Degradation of Betahistine Dihydrochloride at 48hr

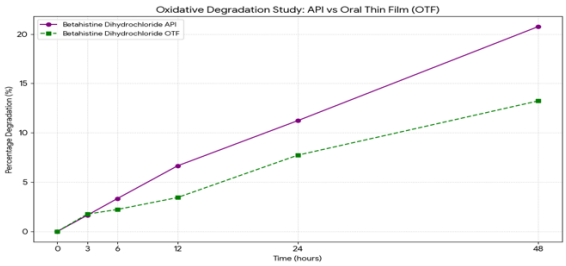

Fig. 9:Schematic Curve of Oxidation Degradation of Betahistine Dihydrochloride

The provided data shows the oxidative degradation of Betahistine Dihydrochloride, both in its pure form (API) and as a OTF formulation, over a 48-hour period. In the initial hours, minimal degradation is observed in both forms. As time progresses, the API exhibits a gradual increase in degradation, reaching 20.50% after 48 hours, indicating susceptibility to oxidation. The OTF, on the other hand, demonstrates a slower rate of degradation, reaching 13.34% at the 48-hour mark, likely due to the protective formulation. These results underscore the importance of proper storage and handling of the API to maintain its stability. It's noteworthy that the OTF formulation appears to offer some protection against oxidative degradation, which can be advantageous for its pharmaceutical use. Further studies and stability testing may be needed to understand the implications for product shelf life and

quality.

Thermal degradation-

1ml of stock solution was transferred in to 10ml of volumetric flask. This solution was put in oven 100ºC for 8 hrs. Then the volume was adjusted with diluent to get 1 mg/ml for Betahistine Dihydrochloride.

Table No. 4: Thermal Degradation Data of Betahistine Dihydrochloride

Thermal Degradation		
Time Frame	**% Degradation (Betahistine Dihydrochloride API)**	**% Degradation (Betahistine Dihydrochloride OTF)**
Initial (0 hr)	0 %	0 %
3 hrs	1.54 %	1.65 %
6 hrs	2.23 %	2.24 %
12 hrs	5.54 %	3.65 %
24 hrs	12.65 %	6.76 %
48 hrs	18.23 %	16.23 %

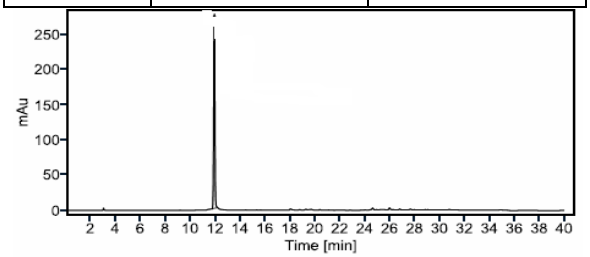

Fig. 10: Chromatogram of Thermal Degradation of Betahistine Dihydrochloride API at 48 hr

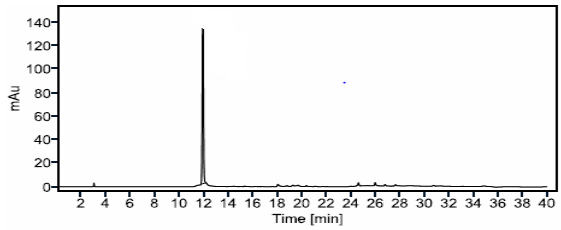

Fig. 11: Chromatogram of Thermal Degradation of Betahistine Dihydrochloride OTF at 48hr

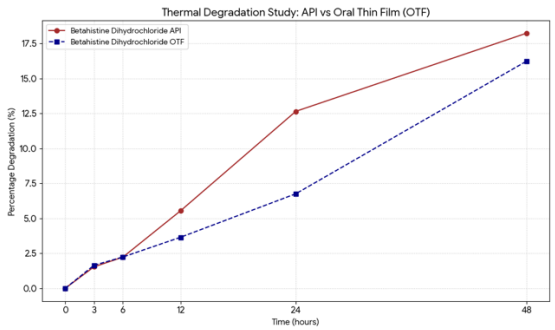

Fig. 12: Schematic Curve of Thermal Degradation of Betahistine Dihydrochloride

Forced degradation studies were performed to evaluate the intrinsic stability of Betahistine Dihydrochloride in both its pure API form and Oral Thin Film (OTF) formulation under acidic, alkaline, oxidative, and thermal stress conditions. The purpose of these studies was to determine degradation behavior, compare stability between API and formulation, and establish the stability-indicating capability of the analytical method. The results demonstrated that Betahistine Dihydrochloride undergoes measurable degradation under all applied stress conditions, with the extent varying depending on the type of stress.

Under acidic conditions using 0.1N HCl at 70°C, gradual degradation was observed in both API and OTF over the 48-hour period. The API showed 17.64% degradation at 48 hours, whereas the OTF exhibited slightly lower degradation of 13.64%. Minimal degradation occurred during the initial hours, followed by a steady increase after 12 hours, indicating acid sensitivity of the drug. The comparatively lower degradation in the OTF suggests that the polymeric film matrix may provide a mild protective barrier against direct acid exposure, possibly by limiting drug interaction with the acidic medium or creating a modified microenvironment within the film.

In alkaline degradation studies using 0.1N NaOH at 70°C, both API and OTF demonstrated moderate susceptibility. At 48 hours, the API showed 16.56% degradation while the OTF showed 15.34%. Degradation was slower in the early hours but increased significantly after 24 hours. The results indicate that Betahistine Dihydrochloride is moderately sensitive to alkaline hydrolysis. Although the OTF showed slightly better stability initially, the protective effect diminished over prolonged exposure, suggesting limited resistance of the formulation against strong alkaline stress.

Oxidative degradation using 3% hydrogen peroxide at 70°C produced the highest level of degradation among all stress conditions. The API exhibited 20.76% degradation at 48 hours, whereas the OTF showed

13.23%. The progressive increase in degradation from 6 to 48 hours confirms that the drug is highly susceptible to oxidative stress. The lower degradation observed in the OTF formulation may be attributed to restricted oxygen diffusion through the polymer matrix or possible stabilizing effects of excipients. Among all conditions tested, oxidative stress was identified as the most critical degradation pathway for Betahistine Dihydrochloride.

Thermal degradation studies conducted at 100°C also demonstrated significant degradation over time. At 48 hours, the API showed 18.23% degradation, while the OTF showed 16.23%. The results indicate that the drug is temperature sensitive and undergoes accelerated degradation at elevated temperatures. Although the OTF provided slightly better thermal stability, the difference between API and formulation was not substantial, suggesting that prolonged exposure to high temperatures should be avoided.

Comparative analysis of all stress conditions revealed that the order of degradation for the API was oxidative > thermal > acidic > alkaline. In all cases, the OTF formulation exhibited slightly lower degradation compared to the pure API, indicating that the film matrix contributes to improved stability. The chromatograms obtained at 48 hours showed well-resolved degradation peaks separated from the main drug peak, confirming that the analytical method is stability-indicating and capable of detecting degradation products effectively. The overall degradation levels achieved (approximately 10–20%) fall within the acceptable range recommended for forced degradation studies.

IV. Conclusion

The forced degradation studies confirm that Betahistine Dihydrochloride is susceptible to acidic, alkaline, oxidative, and thermal stress conditions, with oxidative degradation being the most pronounced. The Oral Thin Film formulation demonstrated comparatively improved stability over the pure API under all tested conditions, suggesting a protective influence of the polymeric matrix. However, significant degradation observed under prolonged stress highlights the necessity for appropriate storage conditions, including protection from heat, light, and oxidative environments. The developed analytical method successfully separated degradation products from the intact drug, establishing its stability-indicating nature. Overall, the study provides valuable insights into the degradation behavior of Betahistine Dihydrochloride and supports the suitability of the OTF formulation with proper storage and handling measures to ensure product stability and shelf life.

Conflict of Interest:

We declare that we do not have conflict of interest.

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