

Forced Degradation Studies and Impurity Characterisation of Endoxifen in Drug and Formulation

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ABSTRACT

Background: This research concentrates on creating analytical methods for Endoxifen that are robust, precise, and capable of indicating stability. The objective of the study is to establish validated analytical techniques, perform systematic forced degradation studies, and identify impurities generated under stress conditions in both bulk drug substances and pharmaceutical formulations. **Materials and Methods:** The active metabolite of tamoxifen is Endoxifen, which is structure complex and also prone to hydrolysis and oxidation degradation. To enable reliability in providing method development and offer impurity profiling, high-purity Endoxifen reference material was unavoidable. The two standards of references were placed in sealed containers of which there were regulated environmental conditions as recommended by the manufacturers. **Results:** Linearity: The method was proven to be highly linear across the tested concentration ranges, with a regression analysis showing a correlation coefficient nearing one. Precision: The precision was observed within regulatory acceptance limits, demonstrating a highly consistent chromatographic response. Repeatability testing yielded an excellent RSD value of 0.42%. Intraday precision RSD varied between 0.01% and 1.43%, and interday precision RSD ranged from 0.33% to 1.85%. Accuracy: Accuracy studies were successfully performed at 80%, 100%, and 120% target concentration levels. The recovery levels for these concentrations averaged 98-100%, 99-101%, and 99-102%, respectively. Specifically, the mean recoveries were recorded as 99.96%±0.95 for the 80% level, 99.00%±0.36 for the 100% level, and 101.10%±0.22 for the 120% level. Forced degradation studies of endoxifen in acidic, basic and oxidative conditions have revealed that endoxifen is particularly sensitive to acidic, basic and oxidative degradation, but relatively more temporal events of stability when endoxifen is under thermal and photolytic conditions. **Conclusion:** The endoxifen in comparison to the high chemical stressor sensitivity was not degraded as much by thermal and photolytic conditions. Although some degree of degradation was also observed under high temperatures and in the presence of light, it was very low when compared to the level of acidic, basic and oxidative stresses. This means that the level of thermolabile or photo sensibility of endoxifen is not as active as thymoquinone. Despite its complex molecular structure, endoxifen appears to make endoxifen relatively resistant to degradation by energy-based mechanisms, such as heat and light reactions. Due to this, the influence of physical stressors on endoxifen degradation is not so loud as the one of chemical stressors.

Keywords: Force Degradation, ICH Guideline, Impurity, Method Developments, Stability.

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INTRODUCTION

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug. The FDA and ICH Guidance state the requirements of stability testing data to understand how the quality of a drug substances and drug products changes with time under the influences of

various environmental factors. The behaviour of thymoquinone also demonstrated erratic degradation behaviour in various strains with which the drug was subjected to and is the result of the chemical form of the drug being a quinone-based molecule (Kelly *et al.*, 2017). These observations reveal that thymoquinone is particularly sensitive to high temperatures and light that could lead to structure rearrangement, and photochemical reactions. This very distinction proves the stability-signalling nature of the developed approach to an analytical method and its versatility to stability testing and profiling of impurities. These degradation products were detected under some severe stress factors which included oxidative, thermal, photolytic stress, and the force of quinone structure of thymoquinone to redox, photochemical



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reaction. The structural clarification of the degradation products of thymoquinone was explained through the mass spectrometry and nuclear magnetic resonance spectroscopy. The stability behaviour of endoxifen displaying in the present study indicate typical degradation profile that convey impressively varied profile as it is the case in also largely affected by chemical stressor and not physical stress conditions. The present differing behaviour can provide worth in comprehending about the relative chemical stability of endoxifen and with the immediate implication on the designing of formulations, the designing of the analytical methods and the storage recommendations.

The extreme degradation of endoxifen disclosed its high acidity and alkalinity, which is an indication that the molecule has sensitive functional groups, when it comes to change of the pH. Endoxifen is a compound, whose ionizable groups and heteroatom-based functional group might be protonated or deprotonated at extremely polarized pH (Iriventi *et al.*, 2020). In such ionization, certain chemical bonds might also be destabilized such that hydrolytic dissociation or restructuring might occur. During acidic reaction, the susceptible functional groups could attain an increased electrophilicity where the functional group could be hydrolyzed. In addition to this, the selection of excipients has to be selected carefully to exclude such excipients that are likely to alter the microenvironmental pH or that react with endoxifen in a manner that will lead to degradation. Protection against the oxidative environment is another significant problem in the development and packaging of endoxifen containing products. Antioxidant may be included where required to aid in the reduction of oxidative degradation (Barzani *et al.*, 2025). It may also be improved by packaging the products in material that is less oxygen-permeable or airtight containers to protect the products. The above measures can particularly be applied to the stability and effectiveness of endoxifen formulations in the long term. the chemical stress factors which influence stability behaviour of endoxifen are largely acidic, basic and oxidative stresses with moderate insensitivity to thermal and photolytic stress. Such findings can be very beneficial in the formulation, package selection, and storage suggestions. The research links itself with questioning the significance of the assessment of the stability of pharmaceutical products that comprise endoxifen-based on their own features and the aspects that the existence of the necessity to practice pH regulation and protection against oxidative conditions so as to preserve the quality, safety, and effectiveness of the chosen pharmaceutical products.

MATERIALS AND METHODS

Material

Instruments Specification

- **Instrumentation:** HPLC LC-2010 CHT Liquid Chromatography.

- **Detector:** UV-PDA detector LC-2010 CHT.
- **Column:** Zodiac C18 (150 mm X 4.6 mm), 5 μ Make: Zodiac Life sciences.
- **Chromatographic software:** Lab Solution.

Reagents and Materials Used

- Acetonitrile HPLC Merck, Rankem.
- Water HPLC Merck, Rankem.
- Ortho-phosphoric acid AR Merck, Rankem.
- Methanol HPLC Merck, Rankem.

Chromatographic Parameters

- **Column:** Zodiac C18 (150 mm $\times$ 4.6 mm), 5 μ make: Zodiac Life sciences.
- **Flow rate:** 1.0 mL/min.
- **Wave length:** 278 nm.
- **Injection Volume:** 10 μ L.
- **Temperature:** 30°C.
- **Diluent:** can.
- **Buffer*:** Trifluoro acetic acid 1 mL was added in 1000 mL water, mixed well. Filtered through 0.45 μ nylon filter and degassed before used. (0.1%).
- **Mobile phase:** Buffer: ACN (78:22) pH 3.5.

Stock Solution

Accurately weighed quantity of Endoxifen 20 mg was transferred into 100 mL volumetric flask and final volume of solution was made 100 mL with mobile phase to get stock solution containing 200 μ g/mL of Endoxifen. 50 mL of aliquot from above stock solution was further diluted to 100 mL with mobile phase.

Preparation of Sample Solution

Powder equivalent to 10 mg of Endoxifen was weighed and transferred in a 100 mL volumetric flask and sufficient mobile phase was added to dissolve the drug. It was sonicated for 20 min and final volume was made to the mark with mobile phase. The solution was filtered through 0.45 μ membrane filter. 10 mL of aliquot from above stock solution was further diluted to 100 mL with mobile phase. The sample solution of 10 mcg/mL was assayed as per proposed method and % assay was calculated.

METHODOLOGY DEGRADATION STUDY OF ENDOXIFEN

Acid Degradation

To 5 mL stock Endoxifen, 2 mL of 2 N Hydrochloric acid was added and refluxed for 8 hr at 60°C. The acidic degradation was performed under dark condition to exclude the possible photolytic degradation. The degradation sample was cooled at room temperature and neutralized the sample with same strength of NaOH. Suitable aliquots of resultant degradation samples were pipetted out and made up the volume with the mobile phase.

Base Degradation

To 5 mL stock solution of Endoxifen, 2 mL of 2 N Sodium hydroxide was added and refluxed for 8 hr at 60°C. The basic degradation was performed under dark condition to exclude the possible photolytic degradation. The degradation sample was cooled at room temperature and neutralized the sample with same strength of HCl. Suitable aliquots of resultant degradation samples were pipetted out and made up the volume with the mobile phase.

Oxidative Degradation

To 5 mL stock solution of Endoxifen, 2 mL of 5% Hydrogen peroxide (H_2O_2) was added separately. The solutions were refluxed for 6 hr at room temperature. The oxidative degradation

performed under dark condition to exclude the possible photolytic degradation. The degradation sample was cooled at room temperature. Suitable aliquots of resultant degradation samples were pipetted out and made up the volume with the mobile phase.

Thermal Degradation

For dry heat degradation the sample were placed in the oven at 80°C for 8 hr under the dark condition and then cooled at room temperature. Degradation sample were subjected to analysis after its dilution (10 µg/mL) with the mobile phase.

Photolytic Degradation

For Photolytic degradation samples were kept in Photolytic chamber for exposure of 1.2 Mill. Lux light and samples were subjected to analysis (10 µg/mL) after dilution with mobile phase. (10 µg/mL).

RESULTS AND DISCUSSION

Forced Degradation of Endoxifen

Endoxifen was much different when compared to thymoquinone with a higher sensitivity of the compound to stress conditions in the chemical environment. It also shows in Table 1 that the highest percentage of degradation was observed in the acidic, basic and oxidative environments with the percentage of degradation being

Table 1: Forced Degradation Results for Endoxifen.

Parameter	Standard Area	Endoxifen Peak Area (mV) After Degradation	% Degradation
Acid Degradation	697438	620162	11.08
Base Degradation		582849	16.43
Peroxide Degradation (Oxidative)		577200	17.24
Thermal Degradation		644502	7.59
Photolytic Degradation		633832	9.12

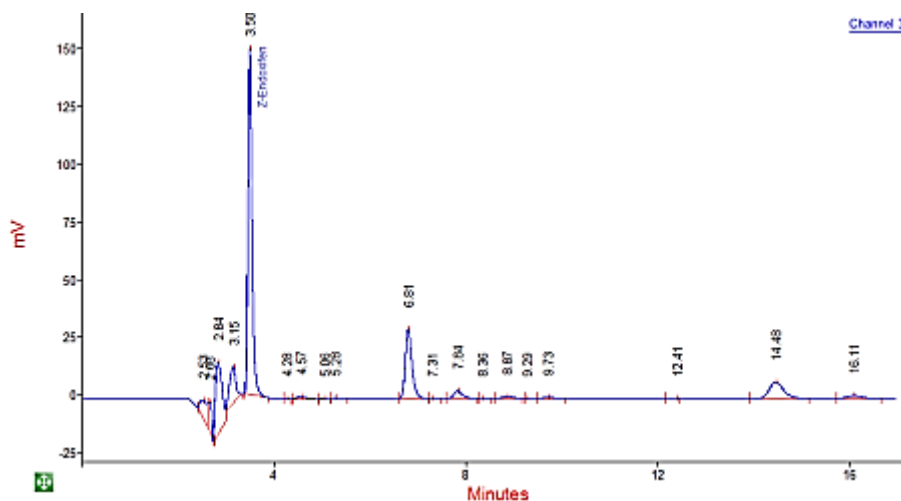


Figure 1: Acid Degradation.

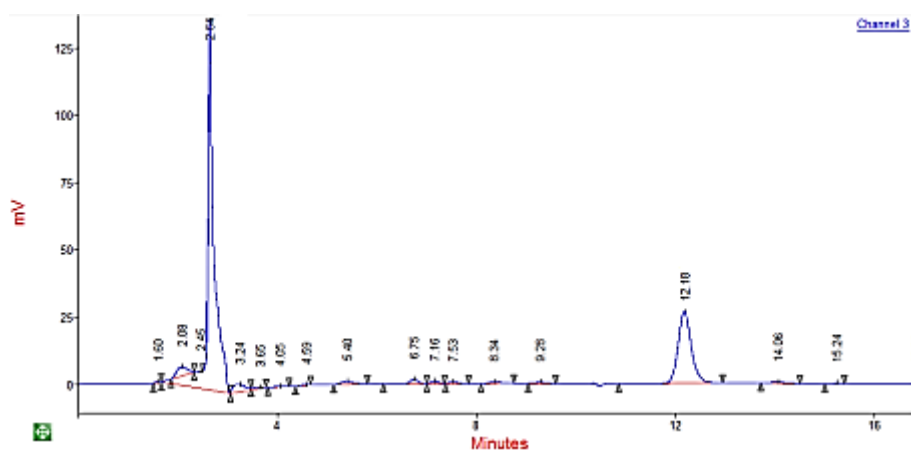


Figure 2: Base Degradation.

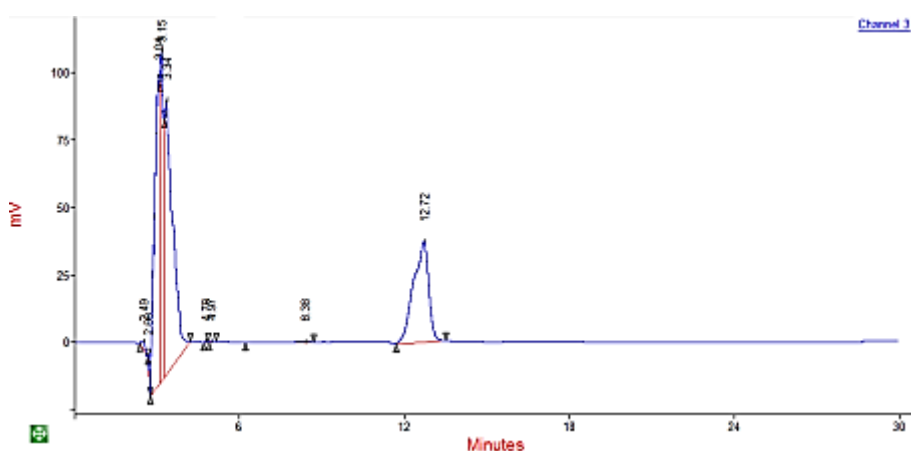


Figure 3: Photolytic Degradation.

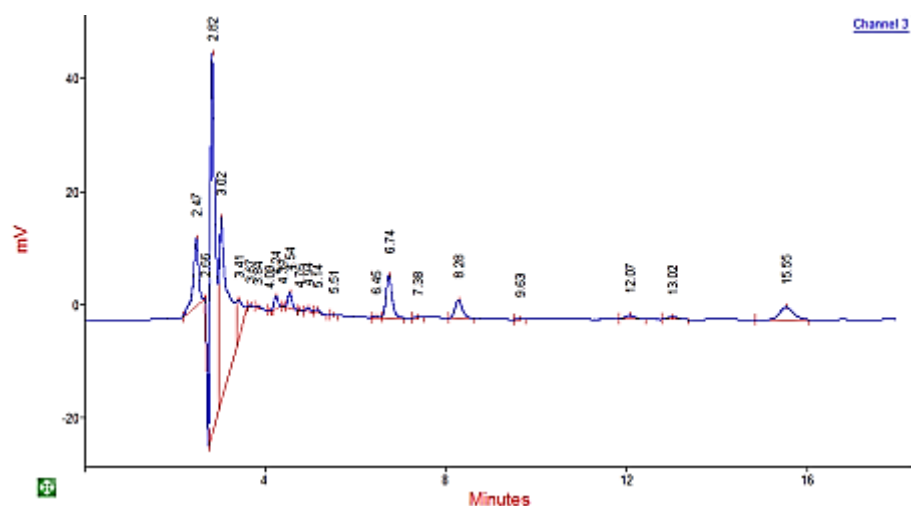


Figure 4: Hydrogen peroxide (Oxidative degradation).

11.08%, 16.43% and 17.24% respectively (Table 1). The findings revealed that Endoxifen was heavily prone to alteration in pH and conditions of oxidation which can most likely be explained by the fact that functional groups are likely to exhibit vulnerability to hydrolytic and oxidative reactions (Pathan *et al.*, 2011). The

photolytic and thermo stress produced comparatively low level of degradation at 7.59% level and 9.12% level of degradation of Endoxifen. The chromatographic data of Endoxifen in various stress states are presented in that indicates the formation of various degradation peaks that were distinctly spotted by the

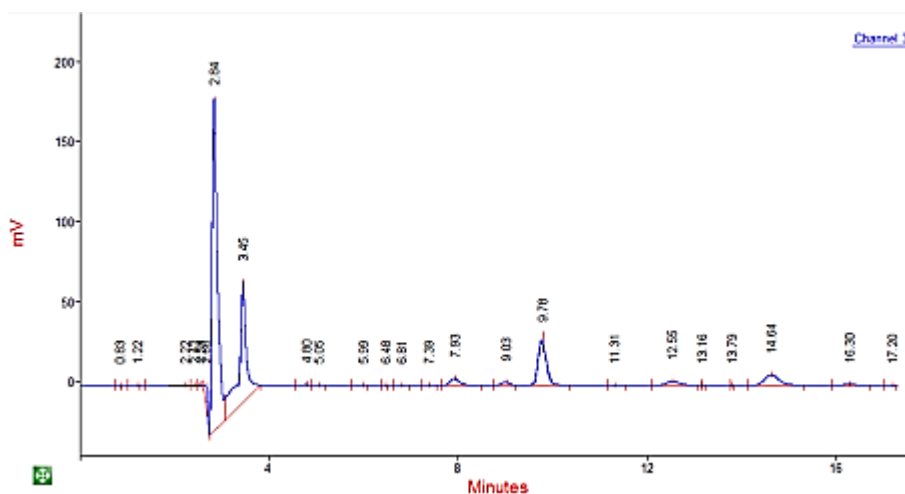


Figure 5: Thermal Degradation.

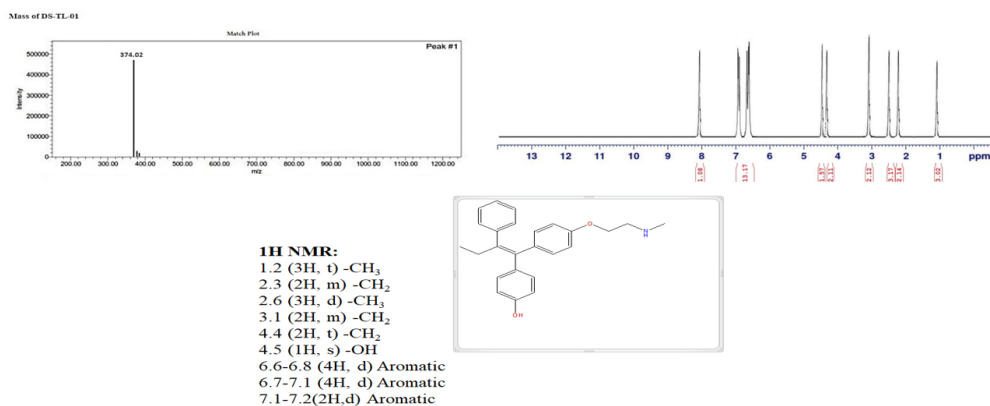


Figure 6: Endoxifen Impurity NMR Interpretation.

4-Hydroxy Tamoxifen

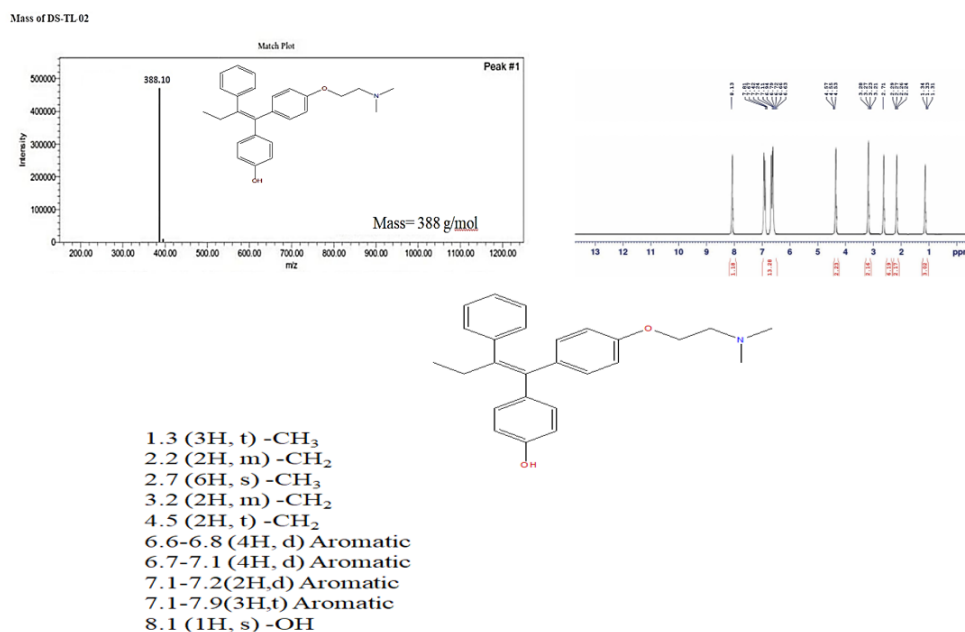


Figure 7: Endoxifen Impurity of 4-Hydroxy Tamoxifen NMR Interpretation.

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